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Editorial

In spite of the remarkable increase of global awareness on HIV, there is still a lot to do to stop the AIDS epidemic. There is urgent need for more action to move towards the target to achieve Universal Access to HIV prevention, treatment, care and support.

Many migrants are unaware of AIDS and continue to remain so even after testing HIV positive. A general absence of support services and treatment for sexually transmitted infections, including HIV, throughout the migration cycle is evident in all countries of SAARC.

As stated in this issue "Knowing is not enough" – Migrant workers spouses vulnerability to HIV" by Aryal N et al, a comprehensive response to reducing the HIV vulnerability of migrant and mobile populations and their spouses in South Asia requires an appropriate, balanced, and integrated regional migration management system that effectively links policy with enforcement to ensure that the rights of migrants are protected throughout the migration cycle.

Lack of referral systems and support services in most destination countries is a major impediment in addressing HIV among mobile populations.

There have been several reported cases of migrants who tested positive for HIV and were prohibited from migrating and, further, who were not informed of their HIV test result or given counseling or referral. As a result, these prospective migrants remained unaware of their status and were likely to infect their partner or spouse unknowingly.

People living in informal settlements and deprived areas are vulnerable to economic and social marginalization and are more likely to lack access to basic health services while also having a greater need for HIV services.

Recent advances in science, accumulated implementation experience, stronger institutions, political commitment, civil society and community activism, global solidarity and associated resources offer an opportunity to end the AIDS epidemic as a public health threat by 2030. This goal is reflected in the UNAIDS Fast-Track approach, which requires rapidly scaling up and focusing the implementation and delivery of proven, high-impact HIV prevention and treatment services: an approach that urban leaders are increasingly adopting.

The steady trend towards urbanization will influence virtually every facet of human endeavor in the coming years, including the global movement to end the AIDS epidemic as a public health threat by 2030. Towards this aim, the world has embraced a series of Fast-Track Targets for 2020, including that 90% of people living with HIV should know their status, 90% of people who know their HIV-positive status should receive treatment and 90% of people on treatment should have suppressed viral loads (90–90–90 treatment target).The Fast-Track Targets also call for the reduction in new HIV infections and for the elimination of HIV-related stigma and discrimination.

To achieve these targets, the SAARC TB & HIV/AIDS Centre has been coordinating the efforts of Member States in combating HIV/AIDS.

FAMILY BURDEN AND HEALTH RELATED QUALITY OF LIFE OF HIV INFECTED INDIVIDUALS IN MADURAI, SOUTH INDIA

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ABSTRACT

Introduction: With the advent of Highly Active Antiretroviral Therapy (HAART) in 1996, HIV-infected patients are living longer and are concerned not only with treatment's ability to extend their life but also with the quality of the life they are able to lead, because, efficacy of treatment is strongly related to meaningful outcome i.e., better Quality of Life. Especially Health related quality of life has not been studied well. Hence, this study was necessitated with the objectives to evaluate Health Related Quality of Life (HRQoL) in HIV infected persons on ART. The secondary objectives were to assess the family burden experienced by the families of HIV infected, and measure influence of family burden on overall quality of life.

Methodology: The HIV infected individuals who were started on treatment six months prior to date of interview were considered for the study The SF36 (Short Form with 36 questions) was used to evaluate function and mental Health while Pai and Kapur's Family Burden Interview schedule was used to assess family burden. Interview schedule was pre-tested on 10 HIV infected individuals for consistency. Data analysis was performed using SPSS version 11 (SPSS inc. Chicago, IL, USA). Pearson product moment Correlation were computed to explore the relationships of SF36 with SLI, Family Burden and BMI. Further, Independent student "t" – test was performed to see the association between HRQoL and gender.

Results: Of 91 participants interviewed 51.6% were women. Median age (years) of the respondents was 33. The overall mean score for Physical health was 45.13 SD (12.40) and for Mental health 56.91 SD (15.52). Age of HIV infected persons had significant influence in scores in social functioning (p-value .015), emotional well being scores (.015), and Mental health (.010). Socio life Index was directly related to physical health, mental health, Vitality, social functioning and emotional scores on HRQoL. Physical health score was negatively affected by the Family burden score. Similarly, BMI status of the respondents correlated with Mental health, Body Pain, Vitality and Role emotional scores of HRQoL scale SF 36.

Conclusion: Socio Life Index and BMI appear to be the two important predictors of HRQoL. Therefore, special attention may be required to HIV infected persons with lower SLI and BMI. Nutritional supplements, in addition to ART drugs, may be provided to ensure some improvements in physical functioning.

Key words: People Living with HIV/AIDS, ART, Health Related Quality of Life, Family Burden

INTRODUCTION

With the advent of Highly Active Antiretroviral Therapy (HAART), in 1996 , HIV-infected patients are living longer and are concerned not only with treatment's ability to extend their life but also with the quality of the life they are able to lead, because better quality of life (QoL) is an important indicator for efficacy of treatment. Further, poor QoL is said to intervene in drug regularity and often associated with poorer treatment adherence.[1] In order to obtain full benefit of ART, near perfect and sustained adherence to treatment is critical. Unfortunately, non-adherence is common among individuals treated with HAART. Several studies have shown varying levels of adherence: Non-

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adherence to ART in adult population varied from 33% to 88%.[2] Studies report that more than 10% of patients report missing one or more medication doses on any given day, and more than 33% report missing doses in the past two to four weeks.[3] Studies also indicate that consistent non-adherence can lead to inadequate suppression of viral replication, continued destruction of CD4 cells, progressive decline in immune function and disease progression. Non-adherence is also an important reason for the emergence of viral resistance to one or more antiretroviral medications.[4,5]

One of the important predictors to sustain treatment adherence is the quality of life of persons infected with HIV. Many studies have reported that the QOL of the patients living with HIV/AIDS were significantly inferior to those of general population[6,7,8,9] particularly, women with HIV/AIDS experience considerable distress.[10] In fact, for most HIV infected individuals, HIV illness itself is a stressor and tends to cause emotional disturbances. Holmes et al report that financial worries were directly related to low adherence[11] as according to estimates HIV causes[12] Indian Rs. 3447 billion economic losses and Indian Rs. 64204 billion as productivity loss. Increased hospital admission, forced to sell their means of production to cover the high economic burden of treatment and their cost associated with HIV/AIDS. That means, not only the presence of HIV infected but also death of HIV infected could lead to family burden to the households. Combination of these factors have great impact on the well being of the patient and impair their quality of life and thereby influencing ART drug adherence itself. Hence, it is increasingly important for health research workers to better understand and improve the quality of life in this group. HRQoL assessment is relatively new index for health measurement. HRQoL takes into consideration those aspects of life that are directly affected by the health status.

Though there are different instruments made available to measure the QoL, the researchers adopted SF36 questionnaire which is used in a number of health outcome studies including HIV/AIDS. SF36 helps to measure the relative burden of the disease and differentiate health benefit produced by the treatment.

This scale has been tested on HIV patients in India and has been validated.[13] In the present study, we evaluate HRQoL in HIV individuals on ART. This study also assessed the family burden experienced by their families and socio-life index and tried to correlate with SF36 score to measure family burden on overall quality of life.

METHODOLOGY

Settings: National AIDS Control Organization (NACO) since the introduction of ART, has increased the number of centers providing free Anti Retroviral Treatment (ART) centres across india from 54 to 91 with another 9 more centers getting operational soon. At these 91 ART centers, medicines for treating 85000 patients have been made available. One of the first centers to come in South of Chennai is the ART centre located at Govt Rajaji Hospital, Madurai. Being a new centre and catering services to 6000 odd HIV infected individuals add significant to any study that address quality of life relating to HIV infected in this region.

Study Population: ART centre located in Government Rajaji Hospital, Madurai has 2352 patients on ART during the first quarter of year 2008 which include 1461 male, 725 women 2 Trans gender and 164 children. On an average, daily 10 to 15 new cases and 90 to 120 old cases attend for pre-treatment assessment and drug collection respectively. The HIV infected individuals put on treatment within six months prior to date of interview were considered for the study and those who gave consent for participation in the study were included in the sample.

Data collection tools: A structured interview schedule was used to collect information on the socio-demographic characteristics such as age, gender, education, occupation, marital status, spouse HIV status and income etc., A brief HIV questionnaire was administered on study participants and one of their family members. Anthropometry was performed to asses BMI. **SF36:** The SF36[14] (Short Form with 36 questions) is a well-documented, self-administered QoL scoring system that includes eight independent scales and two main dimensions. This tool is widely used and has been validated. Physical Function, Role-Physical, Bodily Pain and General Health are grouped as one to measure physical health. Likewise, Vitality, Social Functioning, Emotional well being and Mental Health are grouped as one to measure Mental Health. All questions are scored

on a scale from 0 to 100, with 100 representing the highest level of functioning possible. A higher scale score on SF-36 indicates better quality of life.

English version of SF36 scale was translated to Tamil and then back translated to English. Compared with the original version and modifications were made wherever necessary to ensure equivalence in meaning between the English and translated version.

Standard of Living Index (SLI): SLI is calculated based on the definitions used in the National Family Health Survey (NFHS-I). The factors considered are type of house, availability and type of toilet facility, main fuel used for cooking, source of drinking water, availability of separate room for cooking, ownership of house, ownership of land, ownership of livestock and ownership of other durable goods. Scoring system is used to classify the patients into 3 groups (scores 0-14 for a low SLI, 15-24 for a medium SLI and 25-67 for a high SLI).

Family Burden Interview Schedule (FBIS): Pai and Kapur's Family Burden Interview Schedule[15] was used to assess family burden. The FBIS assesses the burden placed on families of psychiatric patients living in the community setting. This scale measures objective and subjective aspects of burden and it contains six general categories of burden, each having two to six individual items for further investigation. Subcategories include: financial burden, effects on family routine, effects on family leisure, effects on family interaction, effects on physical health of family members and effects on mental health of other family members. Each item is rated on a three-point scale, where 0 is no burden and 2 is severe burden.

Data collection and Analysis: Interviews were conducted at the ART centre Govt Rajaji Hospital, Madurai. Participants were informed of the study objectives and procedures prior to data collection. Interviews were conducted in the presence of an attendant. Interview schedule was pre-tested on 10 HIV infected individuals for consistency.

Data were entered in Excel spread sheet followed by data cleaning and recoding. Further data analysis was performed in SPSS version 11 (SPSS inc. Chicago, IL, USA). Univariate analysis was performed to compare demographic and socio-economic characteristics of patients using χ^2 test. Mean differences were measured in Physical, Mental, Overall well being and Family burden score for HIV positive individuals with or without ART. Regression was performed to assess the factors influencing HRQoL of HIV infected individuals. Pearson product moment Correlation were computed to explore the relationships of SF36 with Family Burden and BMI. Further, Independent student "t" – test was performed to see the association between HRQoL and sex.

RESULTS

Demographic and HIV related variables: Of 91 participants interviewed 51.6% were women. Median age of the respondents was 33.Eighty five percent of them were married. Mean age at marriage was 22.75 SD (5.58) years for men and 21.90 SD (5.52) for women. More than 75 percent of respondents (82.4%) and their spouses (74.7%) were literate. Fifty three (53%) of respondents were daily wage earners. Fifty six percent of spouses were HIV positive. Twenty four out of 161 children born to the study participants were HIV positive of which 10 have already died. (Table 1)

Table 1. Socio Demographic Characteristic of the Participants

SN	Factors		Frequency	%	p-value
1	**Sex**	Male	44	48.4	0.753
		Female	47	51.6	
2	**Age in years**	Median age 33 yrs			
		More than 33 yrs	44 yrs	51.6	
		Less than 33 yrs	47 yrs	48.4	
3	**Marital Status**	Married	85	93.4	0.000
		Separated	6	6.6	
4	**Age at Marriage -** Mean age 22.75 SD 5.577				
5	**Family Size (Mean)**	adult	1.69	SD1.040	
		Children	1.81	SD.855	
6	**Education**	Illiterate	16	17.6	0.000
		Literate	75	82.4	
7	**Occupation**	Daily wage	52	57.14	0.000
		Skilled/salaried	21	23.07	
		House maker/ Unemployed	18	19.78	
Spouse					
1	**Spouse Age -** Median age 22.75 yrs				
2	**Spouse age at Marriage** - Mean age 21.90 SD 5.520yrs				
3	**S. Education**	Illiterate	23	25.3	
		Literate	68	74.7	
4	**Spouse Occupation**	Daily wage	53	58.24	
		Skilled/ salaried	11	12.09	
		House maker/ Unemployed	27	29.67	

Mean duration (in months) of illness from the date of diagnosis was 31.58 SD (20.15). Respondents sought HIV screening at different healthcare settings. For example 25.3% respondents sought HIV screening in private hospitals while 3 percent reported to private laboratory for HIV screening. After initial HIV screening respondents reported to have sought treatment at different healthy care settings at the initial stage before reaching government run free ART centers, However majority of the respondents (95.6%) sought screening at Govt settings before branching out to different settings for treatment. On 76.9% occasion, HIV result was disclosed by the Counselors. One man and 4 women did not disclose their HIV status to their spouses.

Health Related Quality of Life and Family Burden: The QoL scores obtained for the 91 participants based on the SF-36 schedule is give in Table 2. Eight dimensions of SF-36 is further summarized under two broad categories ie., Physical health, mental health and cumulative scores are given below:

The overall mean score for Physical health is 45.13 SD (12.40) and for Mental health is 56.91 SD (15.52). However, mean overall well being score was 51.43 SD (12.96). Difference in mean score between gender and, age groups was observed, however, the difference was not statistically different. Respondents with high Socio life Index (SLI) have had higher mean score ie. 55.61 than other groups. HIV infected individuals who were on ART medication had better mean score of 52.77 than those who are not. Likewise, respondents with normal BMI had better mean HRQoL score (55.07). Higher socio Life Index score had positive impact on physical health score (p Value -.001) and mental health (p value- .005) in HRQoL scores. Particularly better SLI score resulted in higher scores in vitality (p value- .005) and emotional well being (p value-.005) as a results respondents reported better social functioning (p value-0.001). (Table 2)

Table 2. HIV results and disclosure related factors

	Factors		Frequency	Percentage	p-value
1	Duration of illness(in Months)	Mean	31.58 SD 20.157		Range 2- 120 Months
2	Place of Treatment (Initial care seeking) seeking	Private	33	36.3	0.000
		Govt	87	95.6	
		Self	1	1.1%	
		Traditional healers	3	3.3	
		Others	1	1.1%	
3	Place of screening	Private Hospital	23	25.3	0.000
		VCTC/Govt	35	38.5	
		VCTC/NGO	3	3.3	
		Private lab	3	3.3	
		Research centre	27	29.7	
4	Result disclosure	Counselor	70	76.9	0.000
		Doctor	16	17.6%	
		Technician	4	4.4%	
		Not informed	1	1.1%	
5	Disclosure to spouse	No	1 (male) 1.1%	4 (female) 4.4% 1.1%	
		Yes	43	47.3%	
6	HIV status of Spouse	Positive	56	61.5	0.000
		Negative	26	28.6	
		Unknown	9	9.9	
7	HIV status of Children I child	No Child	8	8.8	
		Positive	7	7.7	
		Negative	69	75.8	
		Unknown	7	7.7	
	HIV status of Children II child	No Child	32	35.2	
		Positive	10	11.0	
		Negative	46	50.5	
		Unknown	3	3.3	
		Total	91	100.0	
	HIV status of Children III child	No Child	72	79.1	
		Positive	7	7.7	
		Negative	12	13.2	
		Total	91	100.0	

Factors influencing HRQoL infected Individuals: Logistic regression analysis was used to understand the **Factors influencing HRQoL of HIV Individuals.** Barring age other demographic variables did not show any significant Association with SF-36 demission such as Physical, Mental, Overall well being, Family burden and SLI component. Age of HIV infected had significant influence in social functioning (p-value 0.015), emotional well being (0.015) and Mental health (0.010). However, overall mental health (p-value.045) score did not influence the HRQoL score. Respondents receiving ART did not influence HRQoL but for physical health (p-value-0.060) it showed some influence but was not statistically significant. The same way duration of illness had some influence in the physical health (p-value-0.062) not statistically significant. (Table 3)

Table 3. Physical, Mental, Overall well being , Family burden and SLI component score for HIV positive Individuals

Factors	Physical Health	Mental Health	Overall	Family burden	Socio life Index
Sex					
Male	45.07	61.07	54.18	20.00	26.75
Female	45.20	52.93	48.80	17.60	21.94
Age					
Less than 33 years	45.43	55.04	50.37	22.02	18.55
More than 33 years	44.82	58.86	52.55	26.66	18.98
Occupation					
Employed	45.16	56.88	51.47	23.67	19.17
Unemployed	44.50	57.50	50.75	37.25	9.75
Economic status					
Low	51.27	56.60	54.67	20.25	
Moderate	39.95	50.51	45.84	19.16	
High	47.76	63.26	55.61	17.74	
ART					
Yes	45.60	59.16	52.77	19.98	24.77
No	44.70	54.85	50.21	17.62	23.79
BMI					
Underweight (Less than 19),	42.80	53.41	48.41	19.43	22.00
(ii) Normal (20-25)	47.56	61.44	55.07	18.44	26.76
(iii)Over weight (26 - 30)	47.67	48.67	48.00	12.67	25.67

Factors	Physical Health	Mental Health	Overall	Family burden	Socio life Index
		Female	Male		p-value
Family Burden	No burden	45	34	47	0.010
	Severe Burden	2	10	44	
BMI	Undernourished	22	25	47	0.228
	Good Health	25	19	44	

Correlation between Standard Living Index (SLI), Family Burden, BMI and HRQoL: Pearson Correlation test was performed to see the impact of SLI, Family Burden, BMI and HRQoL in table 4 and further details are presented in table 5. Socio life Index was directly related to physical health, mental health, Vitality, social functioning and role emotional scores on HRQoL scale SF 36. Physical health score was negatively affected by the Family burden score. Similarly, BMI status of the respondents correlated with Mental Health, Body Pain, Vitality and Role emotional scores of HRQoL scale SF 36. (Table 4)

Table 4. Pearson Correlation between SLI, Family Burden, BMI and HRQoL

HRQoL	SLI	Family Burden	BMI
Physical Health	0.216*	-.123	0.193
Mental Health	0.391**	-.131	0.232*
Physical Function	-.091	-.037	0.121
Role-Physical	0.115	0.042	-.042
Body Pain	0.110	0.061	0.226*
General Health	0.112	-.296**	-.002
Vitality	0.296**	-.202	0.227*
Social Functioning	0.207*	-.086	0.078
Role Emotional	0.296**	0.090	0.224*
Mental Health	0.174	-.134	0.058

*Significant at .001
** Significant at .005

Association between Sex and HRQoL, Family burden and BMI: Gender difference in various scores was observed in the data analysis. To get a clear picture, we performed independent sample "t" test the significance between the genders. There were significant difference between the genders in Mental health (P-value-.002), Role emotional (p-value-.011) and overall HRQoL score (p-value-.002).Significant difference in the experience of Family burden (p-value-.022) was observed. However, the scores of BMI and SLI did not show significant difference between the genders. (Table5, 6, and 7)

Table 5. Regression analysis on Factors influencing HRQoL infected Individuals

Dependent variable & HRQoL measures	Unstandardized Coefficients	Standardized Coefficients			
	B	Std. Error	Beta	T	p-value
Age					
Social Functioning	-.109	.044	-5.509	-2.489	.015
Role Emotional	-.109	.044	-9.787	-2.487	.015
Mental Health	-.117	.044	-4.274	-2.624	.010
Mental Health (Total)	.392	.193	12.094	2.034	.045
ART					
Physical Health	-.378	.197	-9.322	-1.913	.060
Duration of illness					
Physical Health	.364	.192	9.032	1.893	.062

Table 6. Independent student "t"–test association HRQoL Vs sex

	Overall	Sex	N	Mean	Std. Deviation	t	df	p-value
Physical Function	35.39	M	23	29.13	32.15	-1.057	41	.297
		F	20	39.25	30.32	-1.061	40.708	.295
Role-Physical	41.11	M	23	50.00	49.43	1.090	41	.282
		F	20	35.00	39.24	1.108	40.694	.274
Body Pain	50.14	M	23	53.87	23.49	1.451	42	.154
		F	21	45.19	14.77	1.480	37.460	.147
General Health	48.90	M	23	52.70	16.39	.941	41	.352
		F	20	48.10	15.48	.945	40.697	.350
Vitality	50.44	M	23	55.43	21.37	1.525	42	.135
		F	21	46.19	18.57	1.535	41.907	.132
Social Functioning	79.60	M	23	87.00	20.03	1.993	42	.053
		F	21	72.71	27.27	1.965	36.509	.057
Role Emotional	48.02	M	23	71.04	44.15	2.645	42	.011
		F	21	36.57	42.10	2.650	41.913	.011
Mental Health	57.63	M	23	66.09	15.63	3.330	42	.002
		F	21	51.24	13.78	3.349	41.955	.002
Physical Health	45.13	M	23	48.22	12.27	1.672	41	.102
		F	20	42.60	9.28	1.705	40.274	.096
Mental Health	56.91	M	23	66.43	11.90	3.845	41	.000
		F	20	50.80	14.76	3.787	36.470	.001
Total SF36	51.43	M	23	58.17	11.17	3.372	41	.002
		F	20	46.55	11.40	3.367	39.938	.002
Family Burden		M	23	.2174	.42174	2.360	42	.023
		F	21	.0000	.00000	2.472	22.000	.022
BMI		M	23	18.913	2.5230	-.252	42	.802
		F	21	19.133	3.2497	-.249	37.694	.804
SLI		M	23	27.26	11.577	1.737	42	.090
		F	21	22.05	7.755	1.768	38.667	.085

Table 7. Independent samples t test analysis comparing two groups on pre-intervention family burden

Family Burden subgroup	Sex	Mean	Std. Deviation	t	P-Value	Mean Difference
Financial	M	7.16	3.403	.478	.634	.393
	F	6.77	4.345	.482	.631	.393
Routines	M	2.98	2.226	1.451	.150	.616
	F	2.36	1.811	1.442	.153	.616
Leisure	M	1.91	2.351	1.188	.238	.526
	F	1.38	1.860	1.179	.242	.526
Interaction	M	2.93	2.472	-.287	.775	-.153
	F	3.09	2.611	-.288	.774	-.153
Physical health	M	.66	1.256	.236	.814	.063
	F	.60	1.296	.237	.813	.063
Psychological health	M	2.70	1.564	1.991	.050	.705
	F	2.00	1.794	2.000	.049	.705
Subjective	M	1.66	.526	2.207	.030	.297
	F	1.36	.735	2.230	.028	.297

DISCUSSION

This study perhaps first to document the HRQoL among HIV infected individuals in the region.. The study findings on HRQoL score are consistent with the other studies which reported lower QoL ratings on both physical functioning and psychological well-being components of SF-36 when compared to the general population.[16,17] Infact the score was low compared[18] to HRQoL among patients who underwent treatment for TB (mean score of 74).

Further, there were considerable differences in SF36 mean score between men and women.

Likewise, HIV infected individuals with higher socio-life index and on ART treatment had better mean SF36 score than others. On an average, persons on ART treatment had better mean score in physical health, mental health and overall SF36 score. This highlights the fact that ART does help in improved QoL score among HIV infected ---individuals. Similarly, persons normal BMI had better HRQoL score. However, when we looked at score of eight dimensions of SF36 scale, we found that physical function score did not improve much while social functioning had some improvements, perhaps due to reasons like opportunities for interaction with other HIV infected individuals and health care providers.

The higher discordant couple rate that is 28.6% observed among the study population poses additional emotional risk that could eventually disturb QoL. In many cases this could influence sexual life too. Further disclosure was problematised by 4 women perhaps due to fear of discrimination or anticipated strain in marital life.

Experience of family burden was found more among men, perhaps means that financial hardship caused owing to limitation in physical functioning and loss of employment as men are the main breadwinners in the Indian society. However, this experience of family burden did not influence overall health score on SF36 scale.

On the contrary, BMI level was directly related to mental health, body pain, vitality and emotional well being score on SF36.The same trend was observed in socio life index score too. SLI status of HIV infected individuals influenced, physical health, mental health, vitality, social functioning and role emotional scores of SF36.

The study also had certain limitations. First of all, the sample size was small and unequal in terms of gender and ART medications. Secondly, generalization becomes difficult, as we could contact only the patients who availed free treatment and not those were paying for treatment. Yet these findings assume significance as it throw new insight into HRQoL of patients in resource limited settings like ours where majority can't afford paid services.

CONCLUSION

This study finding clearly demonstrates that overall HRQoL measures are lower among HIV infected individuals irrespective of ART treatment status. Duration of illness and age are the two factors that had some impact on the HRQoL scores. Further, SLI and BMI appear to be the two important predictors of HRQoL. Therefore, special attention may be required to HIV infected persons come form lower SLI and BMI. Nutritional supplements, in addition to ART drugs may be provided to bring some improvements in physical functioning.

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KNOWING IS NOT ENOUGH: MIGRANT WORKERS' SPOUSES VULNERABILITY TO HIV

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ABSTRACT

Introduction: Male migrants and their sexual partners at home are at increased risk of STIs (Sexually Transmitted Infections) including HIV (Human Immunodeficiency Virus). We aimed to assess the knowledge and attitudes of migrants' wives regarding HIV and STIs, and to understand risk perception of HIV due to their husbands' sexual behaviour.

Methodology: A cross-sectional survey among 182 migrants' wives was conducted in two rural villages of Chitwan district in Nepal. The participants were selected through multistage cluster sampling method and data were collected through a questionnaire administered through a face-to-face interview.

Results: Nearly all (94%) of migrants' wives had a good knowledge of HIV, however with some misconceptions. More than two-thirds of the participating migrants' wives were aware about the risk of HIV infection in migrant husbands and subsequent risk of transmitting themselves through sexual intercourse. Nearly half of the participants reported inability to ask their husbands about HIV and STIs even if they had their doubts. Knowledge of HIV and HIV risk associated with migration were statistically significantly higher in younger women, those who were literate and the longer the period of their husbands' migration.

Conclusion: Despite having generally a good knowledge and awareness of HIV and migration induced HIV risk; migrants' wives could not discuss sexual health issues with their husbands, thus increasing their vulnerability to HIV and STIs.

Key words: Culture, Sexual Health, Migration, Gender, South Asia.

INTRODUCTION

HIV prevalence is higher among migrants compared to non-migrants.[1-3] In many countries, migrants account for larger proportion of total HIV infection. For example, in Europe, 49% of the heterosexually acquired HIV infection in 2009 was transmitted abroad.[4] Studies in Africa and Asia demonstrated that female partners of male migrants are also at higher risk of HIV infection.[5-7]

As of July 2015, 26,702 cases of HIV were reported in Nepal.[8] The total number of HIV infection was estimated to be 39,249 and adult HIV prevalence was 0.2%.[9] Migrants working in India and their wives are recognized as most-at-risk populations for HIV in national strategies.[10,11] The trend of seasonal and long-term male labour migration to

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India is common, particularly in the hilly districts of west and far-west region.[12] Between 1.5 to 2 million Nepalese have migrated to India for short-term and long-term work.[13] Resulting is a HIV prevalence of 0.8% among wives of migrants in far- western districts.[14] Earlier in 2008, HIV prevalence of 3.3% was recorded among migrants' wives in west and far-west regions of the country.[15] Against this backdrop, we focuses on two issues:(i) to assess knowledge and attitude of migrants' wives on HIV and STIs; and (ii) to understand knowledge of migrants' wives regarding their husbands risky sexual behaviours and their consequences.

METHODOLOGY

We conducted a cross-sectional survey[16] in two villages of Chitwan district. We used multi-stage cluster samplings; first we randomly selected two villages to meet the target sample size of 180 which was determined considering time and resources. A sampling frame of the migrants' wives was developed. At the second sampling stage, we targeted at least 10 migrants' wives from each of the nine wards of each village. Our inclusion criteria included wives of male migrants of India, of reproductive age (15-45 years) and migration period being at least three months before the study. Inclusion criteria were assessed through self-reported response of the participants. The Nepal Health Research Council (NHRC) awarded research ethical approval.

Data were collected using a structured questionnaire, administered by an interviewer in Spring 2008. The questionnaire included socio-demographic characteristics and questions on awareness and knowledge of STIs (such as syphilis, gonorrhoea, chlamydia) and HIV, and its symptoms. Four local female interviewers were selected as gender can have a significant influence on the responses, particularly in sensitive issues such as sex and HIV.[17,18] Each questionnaire took 30-40 min. Face validity of the questionnaire was ensured with the consultation of relevant experts, whereas reliability was tested by conducting pilot study among 12 non-sampled migrant wives. All interviews were conducted in private location to maintain confidentiality. Data were analysed by Statistical Package for the Social Sciences (SPSS) version 15.0. The associations between socio-demographic variables and selected variables related to knowledge, attitude and risk perception on HIV were examined using Chi-squared tests, at a significance level of $p<0.05$.

RESULTS

In total, 182 wives of migrants participated. Of these, proportion in the youngest age group (15-24 years) was higher (n=54 (30%)), nearly half of them (n=84 (46%)) had completed secondary school and two-thirds (n=122 (67%)) were involved in household and agriculture related work. North India was the prime work destination (n=91 (50%)) and more than one-third (n=69 (38%)) served in the Indian army. Migration duration was more than 18 months for majority (82%), while two-thirds usually came home at least once in a year time.

Overall, participants showed good knowledge of HIV and STIs, but with some misconceptions (Table 1). Likewise, participants displayed modest knowledge of STIs signs and symptoms. Genital sore was commonly known STIs symptoms as reported by nearly three-quarters (n=135 (74%)), whereas lower abdomen pain was known to least number of the participants (n=101 (55%)). Similarly, majority of the participants knew that HIV infection can be transmitted through sexual intercourse, if sex partners are multiple, through injecting drug use and from infected mother to new born child.

Table 1 indicates that slightly more than one-fifth of the participants believed 'HIV infection is the curse of God or sin of former life'. Similarly, nearly half of all participants stated that 'wives cannot ask even if there is a doubt of HIV or STIs on husbands'. Interestingly, just one-quarter of the participants knew about the existence of gay or lesbian relationships.

Many participants had misconceptions about HIV transmission (table 1). A significant proportion of the participants reported that HIV can be transmitted by mosquito/fly bites, by kissing, and by sharing same toilet and clothes. In contrast, participants were found to have good risk perception regarding migration related HIV and STIs. More than two-thirds knew migrant men usually engaged in unprotected sex while staying abroad. Similarly, more than two-thirds perceived that spouses of migrants are vulnerable to HIV in Nepal and believed migrant men are at higher risk of HIV than non-migrant counterparts.

Table 2 shows that younger women (15-29 years) had better knowledge of HIV compared to those over

30, and this difference was statistically significant (p=0.007). Likewise, difference was observed to be strongly significant between literate and illiterate participants (p<0.000), literate ones being more knowledgeable. Similarly, knowledge on HIV was significantly higher among those participants whose husbands usually returned home in more than one year than in those whose husbands came back in less than one year (p=0.015). Knowledge of HIV was not found to be significantly associated with occupation and ethnicity.

Table 1. Knowledge, attitude and risk perception of participants on HIV and STIs

Characteristics	Number (%), N=182
Awareness of STIs	
– HIV	172 (94%)
– Syphilis	142 (78%)
– Chlamydia	120 (66%)
– Gonorrhoea	65 (36%)
Knowledge of STDs sign and symptoms	
– Genital sores	135 (74%)
– Itching in genital area	133 (73%)
– Genital discharge	117 (64%)
– Pain during urination	110 (60%)
– Lower abdomen pain	101 (55%)
– Pain during sexual intercourse	109 (60%)
Mode of transmission	
– HIV infection transmits through sexual intercourse	157 (86%)
– HIV infection can be contracted if sex partners are multiple	155 (85%)
– HIV can be prevented with the use of condoms during intercourse	137 (75%)
– HIV infection can be contracted by injecting drug use	146 (80%)
– HIV infection can be transmitted by infected mother to new born baby	138 (76%)
Misconception about transmission	
– HIV virus can be transmitted by hugging and hand-shaking	42 (23%)
– HIV virus can be transmitted by sharing same room or bed	45 (25%)
– HIV virus can be transmitted by sharing same toilet and clothes	47 (26%)
– HIV virus can be transmitted by kissing	53 (29%)
– HIV virus can be transmitted by mosquito and fly bites	73 (40%)
Attitude on sexual practice, HIV and STIs	
– HIV infection is due to the curse of God or sin of former life	38 (21%)
– HIV virus is not curable and fatal disease	91 (50%)
– There exists a same gender sexual practice	48 (26%)
– Wives cannot ask even if they have a doubt of HIV/STIs on husbands	87 (48%)
– Couple should discuss about STIs and family planning	157 (86%)
– Some women have extra-marital sex while their husbands are away	87 (48%)
Migration related HIV risk perception	
– Migrant men usually engage in unprotected sex abroad	137 (75%)
– Migrant's spouse is vulnerable to HIV infection in Nepal	126 (69%)
– Migrant men are at higher risk of HIV infection than non-migrant men	131 (72%)

Table 2. Association between levels of HIV knowledge and key socio-demographic variables

Variables	Knowledge of HIV				P value
	Good	Some	Poor	None	
Age (yrs)					0.007*
- 15-29	63 (65%)	23 (23%)	6 (6%)	6 (6%)	
- 30-45	54 (64%)	14 (17%)	7 (8%)	9 (11%)	
Education					<0.000**
- Illiterate	7 (23%)	7 (23%)	5 (17%)	11 (37%)	
- Literate	110 (72%)	30 (20%)	8 (5%)	4 (3%)	0.750
Occupation					
- Housework	26 (72%)	5 (14%)	1 (3%)	4 (11%)	
- Housework+	77 (63%)	25 (20%)	10 (8%)	10 (8%)	
Agriculture					
- Labour	7 (54%)	3 (23%)	2 (15%)	1 (8%)	
- Business/ Services	7 (64%)	4 (36%)	0 (0%)	0 (0%)	
Caste / Ethnicity					0.587
- Janajati	58 (66%)	18 (20%)	3 (3%)	9 (10%)	
- Brahmin /Chhetri	48 (63%)	17 (22%)	7 (9%)	4 (5%)	
- Dalit	11 (61%)	2 (11%)	3 (17%)	2 (11%)	
Husband return time					0.015*
- < 1 year	71 (59%)	28 (23%)	8 (7%)	14 (11%)	
- 1-2 years	32 (76%)	4 (10%)	5 (12%)	1 (2%)	
- >3 years	14 (74%)	5 (26%)	0 (0%)	0 (0%)	

*p value significant at 0.5 level, ** p value significant at 0.01 level

Younger age (15-29 years), being literate and long gap in husband's returning time was significantly associated with the perceived risk 'migrant's wives are vulnerable to HIV in Nepal' (Table 3). Occupation and ethnicity did not have significant impact upon this risk perception.

The greater proportion of the literate participants perceived that 'migrant men usually engage in unprotected sex abroad' as compared to illiterate participants (80% vs. 50%), and this difference

Table 3. Association between migration related perceived HIV risk and key socio-demographic variables

Variables	Migrant's wives are vulnerable to HIV in Nepal			Migrant's men usually engage in unprotected sex in abroad			Migrant men are at high risk of HIV than non migrant men		
	Yes	No	P value	Yes	No	P value	Yes	No	P value
Age (yrs)									
– 15-29	75(77%)	23(23%)	0.032*	76(78%)	22(22%)	0.438	78(80%)	20(20%)	0.011*
– 30-45	51(61%)	33(39%)		60(71%)	24(29%)		54 (64%)	30(36%)	
Education									
– Illiterate	11(37%)	19(63%)	<0.000*	15(50%)	15(50%)	0.001**	10(33%)	20(67%)	<0.000**
– Literate	115(76%)	37(24%)		121(80%)	31(20%)		122(80%)	30(20%)	
Occupation									
– Housework	31 (86%)	5 (14%)	0.080	31 (86%)	5 (14%)	0.590	28 (78%)	8 (22%)	0.883
– Housework+	81 (66%)	41(34%)		88 (72%)	34(28%)		87 (71%)	35(29%)	
Agriculture	8 (62%)	5 (38%)		7 (54%)	6 (46%)		9 (69%)	4 (31%)	
– Labour	6 (55%)	5 (45%)		10 (91%)	1 (9%)		8 (73%)	3 (27%)	
– Business/ Services									
Caste/Ethnicity									
– Janajati	63 (72%)	25(28%)		69 (78%)	19(22%)		65 (74%)	23(26%)	
– Brahmin/Chhetri	52 (68%)	24(32%)	0.667	57 (75%)	19(25%)	0.126	56 (74%)	20(26%)	0.520
– Dalit	11 (61%)	7 (39%)		10 (56%)	8 (44%)		11 (61%)	7 (39%)	
Husband return time									
– < 1 years	76 (63%)	45(37%)		86 (71%)	35(29%)		82 (69%)	38(31%)	
– 1-2 years	33 (79%)	9 (21%)		33 (79%)	9 (21%)	0.074	32 (76%)	10(24%)	
– >3 years	17 (89%)	2 (11%)	0.006*	17 (89%)	2 (11%)		17 (89%)	2 (11%)	0.056

**p value significant at 0.05 level, ** p value significant at 0.01 level*

was statistically significant (p=0.001) (table 3). The trend was clearly observed that participants were more likely to perceive this risk accordingly with the increasing gap in returning time of their husbands, but the difference was statistically insignificant (p=0.074).

Literate participants were more likely to perceive that migrant men are at high risk of HIV than non-migrant men (table 3). The difference in proportions between literate and illiterate participants was statistically strongly significant (80% vs. 33%, p<0.0001). Similarly, participants with younger age group (15-29 years) were significantly more likely to perceive this risk as compared to the participants of older age group (30-45 years) (80% vs. 64%, p= 0.011). Occupation and ethnicity did not have any impact for this risk perception. However, participants whose husband's returning time was long were more likely to perceive this risk, p=0.06.

DISCUSSION

Our study shows that the vast majority of the migrants' wives had overall good knowledge of HIV, albeit, with some misconceptions on mode of transmission. Furthermore, we demonstrated that more than two-thirds of the participating migrants' wives were aware about the migration induced HIV risk. Despite this situation, nearly half of them reported that they couldn't ask about husbands' infidelity and risk of STIs and HIV infection even though they had a doubt.

Our findings on knowledge of HIV and prevention method was fairly similar to the result of Integrated biological and behavioural surveillance (IBBS) survey 2010 conducted among 600 wives of migrants in four districts of far-western Nepal. In this survey, 96.3% of participating migrants' wives stated having heard of HIV, 97.5% were aware about condoms but 60.9% of them never used it, and majority of the participants knew the key prevention methods of HIV infection.[14] Likewise, Nepal demographic and health survey (NDHS) 2011 suggested that 86% of Nepalese women were aware of HIV and 79% of the women knew that HIV infection can be prevented by limiting sex to one partner who has no other sexual partner.[19] The high level of knowledge on HIV may be hugely attributed to increasing access of electronic media (especially F.M. radio) in Nepalese communities.

This study noted that 23% to 40% of participating migrants' wives had incorrectly answered the questions related to mode of transmission of HIV. A small study among 150 Nepalese adolescents concluded that the vast majority of the participants

had misconceptions on mode of transmission such as HIV can be transmitted by holding hands, kissing, through air alike cold diseases, mosquito bite etc.[20] Another study among returnee male migrants in far-western Nepal also showed that majority knew the main routes of HIV transmission, but still had misconceptions about other routes.[21]

We found that knowledge of HIV and migration related perceived risk of HIV is significantly associated with younger age, being literate and longer period of husbands' migration. Our findings are corroborated by several other studies conducted in Nepal. For example, a study on rural married women from migrant community in Kailali district documented that 75% of women with some formal education had heard of HIV and STIs as compared to 34% of those with no formal education.[22] Regarding an association with education, NDHS suggested that younger Nepalese women were more likely to have knowledge about HIV and its prevention measures.[19] An association between HIV knowledge and longer migration period of husbands may be because longer stay increases the likelihood of indulging in extra-marital sex [23] and being aware of this situation migrants' wives might become interested to gain knowledge on HIV.

The most important finding is that two-thirds of the participating migrants' wives knew migration induced risk of STIs and HIV, yet 48% of them thought that they should not ask husbands about this. This clearly pointing to a gap between 'knowing and acting' a phenomenon also noted among wives of Tajik migrants.[24] and wives of male Mexican migrants to the United States of America.[25]

The main reason which inhibits migrants' wives of Nepal from negotiating on safer sex is historically and culturally entrenched gender-based discrimination in every domain of life. Nepalese women have limited access to education, employment, health, decision making and thus they were dependent on husband even for sexual direction. Economic dependency is the major factor which prevents wives to discuss on sexuality, HIV related diseases and HIV prevention[26] which is largely true among migrants' wives in our study. The role of 'power' in sexual relationship was demonstrated in a study on South Asian migrant women in Canada where women with high power in relationship had a high level of knowledge on HIV and they could ask their partner to use a condom.[27] Furthermore, in Nepalese context, migrants often gain new social status after returning to home,[21] which may have influences on their sexual dominance over sexual partners. On the other hand, the issue of sexuality is still a taboo in Nepalese society. Nepalese women who initiate discussion about sexuality and issues such as use of condom are apparent to be unfaithful or characterless.[28]

There are several limitations of this study. First, the sample of this study was not a national sample, but from one district. Secondly, more than one-third of the participants fell on one category of migrant wives of Indian army and wives of Nepali migrants going to Middle East and Asia Pacific regions were not included. We could not rule out the possibility that this group of participants were significantly different from other groups in many ways such as higher financial position and increased access to health services and mass media (television, radio). Finally, we could not compare wives of migrants with wives of non-migrants which may be important to notice if migrants' wives have different levels of knowledge, attitude and perception on HIV with associated implications in terms of risk of having HIV.

Our study suggests intensifying the programmes which helps to build conducive environment and encourage migrants' wives to communicate on HIV and STIs risk with their partners and cooperation and support from male migrants is critical. Next, the power of mass media should be fully utilized to dispel the prevalent misconceptions on HIV and STIs. Health care workers at grass-root level should also be adequately sensitized and mobilized for this purpose. Further, most of the HIV related programs are being focused on safer sex practice, but we firmly urge to give attention towards the 'contextual barriers' such as discriminatory cultural practices, uneven power relations in sexual relationships, dearth of awareness on sexual roles and rights of women etc. Finally, a nationally representative study would be important to better understand the knowledge, attitude and perception of HIV among migrants' wives and concomitant sexual health risk. Future research should also include wives of Nepali migrants irrespective of their age and husband's destinations.

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EVALUATION OF GENE XPERT MTB/RIF ASSAY FOR THE DETECTION OF *Mycobacterium tuberculosis* IN SPUTUM OF PATIENTS SUSPECTED OF PULMONARY TUBERCULOSIS VISITING NATIONAL TUBERCULOSIS CENTRE, THIMI, BHAKTAPUR, NEPAL

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ABSTRACT

Introduction: Tuberculosis (TB) is one of the most deadly and common major infectious diseases in developing countries. Rapid and accurate diagnosis of tuberculosis is indispensable to adequately manage the disease and control its transmission. The objective of this study was to evaluate Gene Xpert MTB/RIF Assay for detection of *M. tuberculosis* in sputum of patients suspected of pulmonary tuberculosis and its comparison with traditional conventional methods.

Methodology: A total of 138 patients sputum samples were collected and processed. Gene Xpert MTB/RIF Assay, culture method and smear microscopy were performed under standard guideline inside bio-safety cabinet class II. Data were reported, structured and analyzed using SPSS version 16.00. Study was carried out from June to November 2014.

Results: Assay detected *M. tuberculosis* in 37 (26.81%) samples out of total 138. Of these 37, 10 and 3 were resistance and indeterminate to rifampicin respectively. Culture, Ziehl-Neelsen staining and Auramine staining were positive in 43 (31.16%), 18 (13.04%) and 24 (17.39%) samples respectively. Sensitivity, specificity, Positive predictive value and Negative predictive value of Assay were 76.74%, 95.79%, 89.19% and 90.09% respectively with reference to gold standard culture method.

Conclusions: Assay was found rapid in direct detection of *Mycobacterium tuberculosis* in sputum sample and was also found more sensitive than both Ziehl-Neelsen staining and Auramine staining and especially showed good promise in diagnosis of smear negative specimens.

Key words: *Gene Xpert MTB/RIF Assay*, *M. tuberculosis*, *Pulmonary Tuberculosis*

INTRODUCTION

HIV Tuberculosis (TB) is one of the most deadly and common major infectious diseases in developing and industrialized countries.[1] Tuberculosis in human is most commonly caused by *Mycobacterium tuberculosis* complex (MTBC), which includes *M. tuberculosis*, *M. bovis*, *M. bovis* BCG and *M. africanum* and among them *M. tuberculosis* is predominant.[2] In 2013, an estimated 9.0 million people developed TB and 1.5 million died from the disease.[1] In Nepal, tuberculosis (TB) is a major public health problem. About 45 percent of the total population is infected with TB, of which 60 percent are adult. Every year, 45,000 people develop active TB, of whom 20,580 have infectious pulmonary disease.[3] Rapid and accurate diagnosis of tuberculosis (TB) is indispensable to adequately manage the disease and control its transmission. Acid-fast bacilli (AFB) smear microscopy sensitivity is low, varying between 22 and 80%. And culture takes several weeks to provide microbiological confirmation. Nucleic acid amplification techniques can be used for detection of *M. tuberculosis* results in accurate diagnosis of tuberculosis but requires laborious processing time and dedicated bio-safety conditions.[4]

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Tuberculosis spread has been increasing day by day in low income country like Nepal. This is because of delay in diagnosis which has thwarted the efficient management of the disease control. Rapid diagnostic procedure being simple to operate, cost effective, less bio-hazardous to perform within limited space and with good sensitivity along with specificity is most wanted in a country like Nepal in order to tackle the rapturous distribution of pulmonary tuberculosis. With such problems in consideration and to evolve the diagnostic pattern in new level with the use of rapid technology that has been accredited by WHO recently, this present study has endeavored to evaluate the Gene Xpert MTB/RIF Assay, an automated polymerase chain reaction (PCR) test with culture, Ziehl-Neelsen staining and Auramine staining, especially between Gene Xpert MTB/RIF Assay and Culture followed by different biochemical tests for the identification of *M. tuberculosis*. Gene Xpert MTB/RIF assay detects the presence of MTBC DNA and its susceptibility to rifampin in a single reaction. Mono-resistance to rifampicin is rare; however, 90% of rifampicin resistant isolates also exhibit resistance to isoniazid. Therefore, the detection of rifampicin resistance may serve as a surrogate marker for MDR *M. tuberculosis*. The assay is based on a heminested real-time PCR (RT-PCR) that targets the *rpoB* gene hot spot region. Any deviation from the wild type sequence resulting in a delay in the appearance of the signal exceeding a predetermined *CT* value, between the earliest and latest cycle threshold (*CT*) values is reported as RIF resistant. The test is carried out within 2 h in a disposable cartridge. The only manual step is the mixing of a bactericidal buffer with the sample prior to addition to the cartridge. This pre-amplification step reduces the viability of MTBC organisms, making the assay suitable for use near patients in settings with limited bio-containment facilities.[5]

This study has significant importance in rapid diagnosis of tuberculosis of patients suspected of pulmonary tuberculosis. Additional to diagnosis, this study has importance in direct detection of rifampicin resistance which therefore can provide the basis for prompt treatment of drug resistance tuberculosis. And this study can be valuable in improvising the tuberculosis diagnostic field for the betterment of minimizing the insidious distribution of pulmonary tuberculosis from infected person to healthy individual. Present study with scrupulous details and comprehensive understanding about rapid and effective diagnosis of pulmonary tuberculosis may evolve insight on use of Gene Xpert MTB/RIF Assay throughout the country in achieving the target of treating all the infected patients of tuberculosis and therefore might helps in curbing rapturous distribution of tuberculosis.

METHODOLOGY

Study design, site and setting

The study was a hospital based cross-sectional study carried out in National Tuberculosis Centre, Thimi, Bhaktapur, Nepal. A structured questionnaire was prepared and interview of patients were taken with their informed consent. Data were collected based around patient's personal description and tuberculosis background.

Study population and time period

In this study, patient those who were suspected of pulmonary tuberculosis, willing to participate and responding to study designed format questionnaire were considered as study population. And the study period was carried out from June to November in 2014.

Inclusion/ Exclusion criteria

Patients clinically suspected of pulmonary tuberculosis were involved in this study. And patients recently undergoing anti-tuberculosis treatment, blood stained sputum, sputum with food particles, with saliva in greater amount, leaking, dried or if not fresh collection and patients suspected of extra-pulmonary tuberculosis were excluded from this study.

Sample size

One thirty eight patients were involved in this study, which was determined by sample size calculation using Fischer's formula outlined as $n = [Z^2pq]/d^2$.

Sample collection

Patient morning sputum sample of about 5ml was collected in a clean, wide neck, screw capped and disposable plastic (50 ml Tarson falcon) tube. Samples collected were evaluated directly with naked eyes through transparent tube and selected sample tubes were labeled with patient name and lab number serially and processing of sample was carried out in a bio-safety cabinet II.

Gene Xpert MTB/RIF Assay

1ml sputum sample in falcon tube was mixed with 2 ml sample reagents and vortexed until clear solution was made and left for 15 minutes. And 2ml mixture was transferred into the Xpert MTB/RIF cartridge using sterile dropper. The Xpert MTB/RIF cartridge includes an internal control for sample processing (DNA extraction and for PCR presence inhibitors), afterwards the inoculated cartridge was placed into the Gene Xpert instrument. Results were available in less than 2 hours and interpreted by the Gene Xpert System automatically. For all samples, Assay was carried out only once with meticulous attention.

Smear microscopy and Culture

Sputum sample were processed using standard petroff's method. Freshly prepared NaOH solution was added to the specimen at equal volume, mixed using vortex, and left for 15 minutes for digestion at room temperature. A double amount of sterile phosphate buffer was then added to the mixture and centrifuged for 20 minutes at 300 × g. The supernatant was removed and the sediment was dissolved in few ml of sterile phosphate. And the solution was used for smear microscopy and culture. Smears were prepared, fixed, and Z-N and Auramine staining were performed and observed under standard guidelines. In culture, Lowenstein-Jensen medium was used for inoculation and inoculated LJ media were incubated at 37°C for 8 weeks and examined weekly. Positive cultures of *Mycobacterium isolates* were compared with control H37Rv *M. tuberculosis* and 1-2 weeks fresh colonies on LJ media were used for biochemical tests (viz. Niacin and Catalase tests). Biochemical tests were performed under standard guideline protocol of WHO 2013. And for each sample, replicates were also performed on juxtaposition wise. All the diagnostic tests were performed inside bio-safety cabinet class II.

Ethical consideration

All the suspected pulmonary tuberculosis patients involved in this study were enrolled with their informed consent by counseling them and making them understood the information about the study regarding the confidentiality and implication of the result.

Data analysis

The data were collected, structured and analysis was done using SPSS version 16.0 System. Statistical analysis (i.e. Chi-Square) was employed on determining the association of different variables involved in the study in distribution of pulmonary tuberculosis at 5% level of significance.

RESULT

Out of 138 samples, 37 (26.81%) and 40 (28.98%) were confirmed positive to *M. tuberculosis* in sputum samples using test methods, viz. Gene Xpert MTB/RIF Assay and Culture followed by biochemical tests respectively. And among 26 smears positive, 21 (80.76%) and 23 (88.46%) were confirmed as *M. tuberculosis* detected by Assay and culture respectively. Culture was positive on 43 (31.16%) out of 138 samples. Of these 43 culture positive samples, 33 (76.74%) were found positive by Gene Xpert MTB/RIF Assay. Z-N staining was positive on 18 (13.04%) out of 138 samples. Assay was found positive in all Z-N smear positive specimens except on 2 i.e. 16 (88.89%). Auramine staining was positive in 24 (17.39%) out of 138 samples. And of 24 auramine smear positive specimens, assay was found positive on 20 (83.33%), whereas 4 (16.67%) were negative.

On age wise distribution, 18 (35.29%) out of 51 being highest and 3 out of 27 (11.11%) being lowest number of patients in age group 16-30 and 31-45 were diagnosed positive to PTB respectively. On gender wise distribution 10 (25%) female patient and 27 (27.55%) male patients were diagnosed with pulmonary tuberculosis (PTB). Statistically, there was no significant association in both age wise and gender wise distribution with pattern of pulmonary tuberculosis. On smoking, alcohol consumption and on history of TB wise distribution of pulmonary tuberculosis, 23 (35.39%) out of 65 smokers, 20 (40%) out of 50 who drink alcohol and 15 (46.87%) out of 32 patients with history of TB were diagnosed with PTB respectively. And statistically there was significant association of all variables in distribution with pattern of PTB. Sensitivity, Specificity, Positive predictive value and Negative predictive value of Gene Xpert MTB/RIF Assay with reference to culture in the diagnosis of PTB was 76.74%, 95.79%, 89.19% and 90.09% respectively.

Table 1. Evaluation of Gene Xpert MTB/RIF Assay with conventional methods in detection of *M. tuberculosis*					
Smear microscopy result	Gene Xpert MTB/RIF Assay and Culture result				
	Gene Xpert MTB/RIF Assay Result		Positive culture for MTB detection		No. of Negative culture or culture contaminated
			Niacin test Positive	Catalase test positive	
Smear positive specimens (26)	*M. tuberculosis* detected	21 (80%)	23 (88.46%)	0	0
	M. tuberculosis not detected	5	0	0	0
	Not interpretable	0	0	0	3
Smear negative specimens (112)	*M. tuberculosis* detected	16 (14.28%)	17 (15.19%)	0	0
	M. tuberculosis not detected	96	0	3	0
	Not interpretable	0	0	0	92

Table 2. Distribution of pulmonary tuberculosis by smoking, alcohol consumption and history of TB according to Gene Xpert MTB/RIF Assay					
S. No.	Variable		Total count	Positive (%)	p-value*
1	Smoking	Yes	65	23 (35.39%)	p<0.05
		No	73	14 (19.17%)	
2	Alcohol consumption	Yes	50	20 (40%)	P<0.05
		No	88	17 (19.32%)	
3	History of TB	Yes	32	15(46.87%)	P<0.05
		No	106	22(20.75%)	

*at 5% level of significance

Table 3. Sensitivity, specificity, positive predictive value and negative predictive value of Gene Xpert MTB/RIF Assay in reference to gold standard culture method				
Diagnostic method	Gold standard Culture method			
	Sensitivity (%)	Specificity (%)	Positive Predictive value (%)	Negative Predictive value (%)
Gene Xpert MTB/RIF Assay	76.74%	95.79%	89.19%	90.09%
Z-N Acid Fast Staining	39.53%	98.94%	94.44%	78.33%
Auramine Staining	48.84%	96.84%	87.5%	80.70%

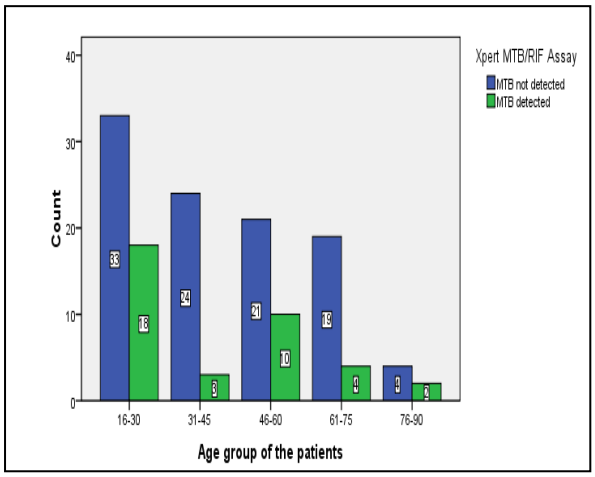

Figure 1. Age wise distribution of pulmonary tuberculosis according to Gene Xpert MTB/RIF Assay

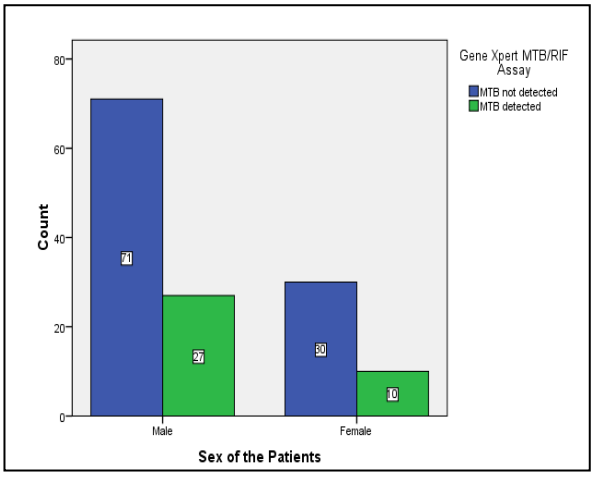

Figure 2. Gender wise distribution of pulmonary tuberculosis according to Gene Xpert MTB/RIF Assay

DISCUSSION

Tuberculosis (TB) has troubled humankind throughout history. Tuberculosis (TB) is an ancient disease that has affected mankind for more than 4,000 years. It is a chronic disease caused by the bacillus *M. tuberculosis* and spreads from person to person through air.[6] In 2013, 6.1 million TB cases were reported by WHO. Of these, 5.7 million were newly diagnosed.[1] Tuberculosis is the most widespread infectious disease in Nepal and poses a serious threat to the health and development of the country.[3] Distribution of tuberculosis is still going on despite DOTS implementation in most parts of the country. This is all because of delay in diagnosis which leads to delay in treatment, as well as inadequate presence of rapid, accurate technique in tuberculosis diagnosis which has also favored the transmission of the disease. And in-order to tackle such problem, present study has evaluated the Gene Xpert MTB/RIF Assay's efficacy in the diagnosis of pulmonary tuberculosis, in which assay was successful in directly detecting *M. tuberculosis* as well as rifampicin susceptibility pattern simultaneously.

This hospital based cross-sectional study was performed, observed and analyzed by being within the scope and objective of the study. This study was successful in diagnosis of pulmonary tuberculosis using Gene Xpert MTB/RIF Assay and in the comparative study with traditional conventional methods such as commonly used smear microscopy (i.e. Ziehl Neelson and Auramine staining) and gold standard culture method. In this study, patients aged above 15 suspected of pulmonary tuberculosis were enrolled since there was difficult in extraction of enough sputum samples from the children and no extra-pulmonary samples were accommodated.

On examination of 138 patient's clinical sputum, assay was successful in identifying *M. tuberculosis* from 37 (26.81%) sputum samples. And simultaneous rifampicin resistance was detected in 10 (7.24%) *M. tuberculosis* while 3 (2.17%) *M. tuberculosis* were rifampicin indeterminate. Assay showed that it was rapid in detection of *M. tuberculosis* directly from clinical sputum and in simultaneous detection of rifampicin resistance. And this finding was in agreement with other studies.[7,8] In general regarding the assay, the basis for the direct detection of *M. tuberculosis* is that probes present within molecular beacon was successful in forming complementary bond with entire 81rpo gene of *M. tuberculosis* with a cycle threshold (CT) of ≤ 38 cycles. Rifampicin resistance is particularly amenable to rapid molecular detection since >95% of all rifampicin resistant strains contain mutations localized within the 81 bp core region of the *M. tuberculosis* RNA polymerase *rpoB* gene, which encodes the active site of the enzyme. Moreover, mutations that occur in this region are highly predictive of rifampicin resistance, whereas susceptible isolates almost always have the same wild-type nucleotide sequence. And the basis for rifampicin indeterminate is when the first probe CT is >34.5 and the last probe CT is >38 cycles.[9] Assay and culture were almost positive to all smear positive specimens while they showed good promise in detection of *M. tuberculosis* in smear negative specimens as well. This finding was supported by other studies.[10,2] And the result was evident because Assay and culture can detect bacilli as few as 131 and from 10-100 cells/ml of sputum respectively whereas smear microscopy is positive if only the bacilli number is at-least 10,000 cells/ml of sputum. Regarding assay negativity in smear positive specimens, because Assay is specific to detect only MTB whereas both staining techniques used in this study, can detect both MTB and Non-tuberculosis mycobacteria in smear specimens. And regarding the assay inability to detect *M. tuberculosis* in sputum specimen which was positive in culture assisted by biochemical test seems to be contrary however this anomaly might have taken place since the analytical limit of detection of the Gene Xpert MTB/RIF assay is reported to be 131 cfu/ml of specimen, based on spiked sputum studies. Culture of concentrated specimens can detect very low concentrations of organisms as low as 10-100 cfu/ml. When testing at the lower limits of any assay, variability is to be expected due to factors such as sampling.[12] And other reason for such contradiction between culture positivity and assay negativity in same specimens occurred because only two available biochemical tests (viz. Niacin and 68ºC heat stable catalase test) were used in the present study for identification without any further molecular characterization of *M. tuberculosis.*

On age wise distribution of pulmonary tuberculosis, age group 16-30 was found highest in PTB

suspects of all age group which was in accordance with previous studies.[6,13] Since this age group people are highly active, their chance of exposure is also high which support evidence of acquiring disease. On gender wise distribution, 10 (25%) female patients and 27 (27.55%) male patients were diagnosed with pulmonary tuberculosis (PTB). This result actually reflects the fact that males are more exposed to the outer environment than females, and there can be higher possibility that male might comes in contact with TB suspected or infected patient directly or indirectly which can lead to transmission of the disease. This result is in corresponds with other studies.[13-15] However, present study showed that there was no significant association of both age and gender wise distribution with pattern of pulmonary tuberculosis. On smoking-wise distribution, smokers were found more susceptible to pulmonary tuberculosis than non-smokers which was in accordance with other studies.[16-18] Smokers are more susceptible to tuberculosis because in cigarette there are more than 1000 chemicals which directly affect lungs affecting the normal functioning of immune cells that may favor the disease establishment in lungs. On alcohol consumption, those who drink alcohol frequently were found having pulmonary tuberculosis than who do not drink and it was in agreement with other studies.[16,19] This may be due to both increased risk of infection related to specific social mixing patterns associated with alcohol use, as well as influence on the immune system of alcohol itself and of alcohol related conditions. And also on patients with history of TB, pulmonary tuberculosis was predominant than those who do not have. This might be due to poor treatment supervision, failure to follow-up smear microscopic observation and patient general conditions may lead to TB recurrence. This finding was in correspondence with previous studies.[20,21]

In present study, Assay showed much higher sensitivity than both Ziehl-Neelsen staining and Auramine staining which was in agreement with other study.[22] But the specificity was lower than both staining technique. Positive predictive value of Assay was lower than Ziehl-Neelsen staining but was higher than Auramine staining. However Negative predictive value of assay was higher than both staining technique. Assay was found to be very sensitive than smear microscopy. Higher sensitivity of Assay compared to smear microscopy is universally evident since assay can detect as few as 131 bacilli per ml in sputum, whereas microscopy is able to detect bacilli if 10,000 cells per ml are present in sputum sample.

However in this study, few limitations were inevitable, because of the certain circumstances such as available HIV patients in the hospital were undergoing anti-retroviral therapy and TB treatment and thus were excluded from the study population. Because of limited time frame provided during the study period and incommodious lab space as well as unavailability of prerequisite factors for accommodating sample size, drug susceptibility test (DST) for both Rifampicin resistant and sensitive specimens were not carried out, which could have averred conformity to the present study. Despite all the reservation, this study was carried out at its best, remaining within the scope and objective of the study, maintaining and following WHO guidelines, ethics and moral values of the research. Moreover, from this study it is recommended that the further research needs to be done regarding the accurateness of Gene Xpert MTB/RIF Assay with reference to advanced molecular methods.

CONCLUSION

Present study has shown that assay can be potential alternative to smear microscopy especially in smear negative specimens despite the fact that assay is expensive than smear microscopy. However assay showed advantage over smear microscopy or even culture method, because of its rapidity, effectiveness and simultaneous detection of rifampicin resistance which leads to early treatment and thus provide aid in control of disease transmission. Gene Xpert MTB/RIF Assay has demonstrated a high capacity for detecting MTB and for predicting multidrug resistance in both smear positive and negative clinical sputum samples. Moreover, assay rapidity, simplicity, low laboriousness make the technique a good candidate for routine use in many clinical laboratories in our country to curb TB transmission given whenever the clinical criteria for its application are settled.

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EFFICACY AND COST OF MOLECULAR IDENTIFICATION OF CLINICAL MYCOBACTERIAL ISOLATES IN A RESOURCE LIMITED SETTING

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ABSTRACT

Introduction: Many molecular methods of identification of mycobacteria are now available. With many molecular consumables now being available at low prices, routine identification in clinical laboratories is now possible, even in low/ middle income settings that have basic molecular facilities. This study was conducted to optimize a low cost PCR-RFLP based identification that can be used in such a laboratory.

Methodology: DNA was extracted from mycobacterial cultures using five methods. Three were heat extraction and two were kit extraction methods. Yield and purity of extracted DNA was evaluated and PCR-RFLP was done on extracts to ensure that the DNA could be used for molecular assays. The method giving the highest yield at low cost was selected and DNA was extracted from 105 mycobacterial cultures from patients diagnosed with pulmonary tuberculosis. *hsp65* PCR and restriction digestion with BstEII and HaeIII enzymes was done to identify mycobacteria, differentiate MTB complex from NTM and identify NTM species. *gyrB*PCR and restriction digestion with RsaI was done to identify MTB complex species. *hsp65*partial sequencing was done to confirm NTM species. Costs for molecular identification were calculated based on consumable cost.

Results: Heat extraction in water (80 ^{0}C for 1 hour) provided a mean DNA yield of 30.07ng/µland mean A260/280 ratio of 1.45. Heat extraction methods gave significantly higher DNA yield compared to the kit extraction methods (ANOVA, $p<0.05$). *hsp65* PCR-RFLP identified 102 isolates as MTB complex and 3 isolates as NTM. *gyrB*PCR-RFLP confirmed the 102 isolates as MTBC and showed that all isolates belonged to the MTB/ *M africanum/ M canettii*group. *Hsp65* partial sequencing identified the NTM as 2 isolates of *M avium*and 1 isolate that could not be identified. An algorithm for PCR-RFLP based identification was developed that allows identification of mycobacterial isolates at low cost (approximately USD 6.00 per sample). The NTM rate in this study population was 2.6%.

Conclusions: Using heat extraction in water, PCR-RFLP based identification of clinical mycobacterial isolates can be established at low cost in a laboratory that has basic molecular facilities.

Key words: Mycobacteria Identification; PCR-RFLP; *hsp65; gyrB*

INTRODUCTION

The methods used for identification of mycobacterial isolates depend on primarily on available laboratory facilities. Several different molecular methods are currently available and these have replaced the more cumbersome and time consuming conventional biochemical tests to a great extent.[1] Both *hsp65* and *gyrB*genePCR restriction digestion are robust methods for identification of Non Tuberculous Mycobacteria (NTM) and *Mycobacterium tuberculosis* complex (MTBC) respectively.[2,3] *Hsp65* restriction digestion with *Bst*EII and *Hae*III enzymes has been used extensively for NTM identification in multiple fields of study, while *gyrB* digestion with *Rsa*I and *Taq*Ihas been successfully used for *M. tuberculosis* (MTB) /*M.africanum* differentiation from *M.bovis/* BCG.[4-13]

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In Sri Lanka, NTM were cultured in approximately 2-3% of all mycobacterial cultures done at the National Tuberculosis Reference Laboratory between 2005 and 2007[14,15] while a recent analysis of bronchoscopy cultures from patients in Kandy by Weeresekera et al. showed that -13-14 % were positive for NTM isolates, including *M.phocaicum*and *M. Smegmatis.*[16] Data on the true (overall) NTM infection rate among patients with pulmonary and extra pulmonary disease in Sri Lanka is not available as cultures are not routinely performed in suspected pulmonary tuberculosis. Even when cultured isolates are available, identification is not routinely done in most local laboratories.

This study was designed to optimize selected molecular assays for basic identification of mycobacteria isolated from clinical samples. Development of identification algorithms that would be suitable for use in a clinical laboratory in the local setting and would provide the maximum species level differentiation in a minimum time and at low cost was targeted. Though many PCR targets have been evaluated for identification of mycobacteria, *hsp65* and *gyrB* were selected for this study as they have been successfully used in other studies and as *hsp65* sequencing provides accurate identification of many NTM species with online sequence databases and identification resources are available.

METHODOLOGY

Sputum samples from patients with suspected pulmonary tuberculosis were cultured on Lowenstein Jensen egg based media for 8-10 weeks. Positive cultures were then stained for acid fastness and molecular identification carried out according to the following protocol.

DNA extraction

DNA was extracted from a standard MTB H37Rv strain isolate and four clinical isolates (n=5) using five different extraction methods (A to E) of which three were heat extraction methods and two were kit extraction methods. The heat extraction methods were developed based on existing literature [17-19] and required a minimum amount of consumables, equipment and time. Kit extractions were done using the Invitrogen Pure Link R Spin Column DNA extraction kit. The first protocol followed was given by the manufacturer for DNA extraction from Gram positive organisms and the second protocol was a general extraction protocol.

Method A: Heat extraction (80 ^{0}C –heat inactivation)

Several colonies were suspended in sterile distilled water by brief vortexing and heated in a water bath to 80 ^{0}C for 1 hour. After centrifugation of the inactivated mycobacterial suspension, 400 µl of the supernatant was transferred into a new micro-centrifuge tube. (Extract A)

The cell pellet was then re-suspended in sterile distilled water by brief vortexing.5 drops of suspension were then cultured on Lowenstien Jensen (LJ) slopes to assess inactivation of cultures.500 µl each of suspension was then transferred into new microcentrifuge tubes (labelled B to E)for the next 4 extraction methods. For methods C, D and E the suspension was centrifuged again and the resulting cell pellet was used.

Method B: Heat extraction (95 ^{0}C)

B tubes were heated on a heat block at 95 ^{0}C for 30 mins. The tubes were then centrifuged at 5000 g for 10 mins and the resulting supernatant was transferred to new tubes. (Extract B)

Method C: Tris EDTA (TE) extraction

The cell pellet in C tubes was re-suspended in 500 µl of 1x TE (Tris EDTA) buffer with brief vortexing. The suspension was heated on a heat block at 95 ^{0}C for 30 mins. The tubes were then centrifuged at 5000 g for 10 mins and the resulting supernatant transferred to new tubes. (Extract C)

Method D: kit extraction – method for Gram positive organisms

DNA was extracted from the cell suspension in D tubes according to the protocol for Gram positive organisms provided by the manufacturer. (Extract D)

Method E: kit extraction – general protocol for non-specified samples

DNA was extracted from the cell suspension in E tubes according to the general protocol provided by the manufacturer. (Extract E)

Preparation of cell lysate: The cell pellet was suspended in 180 µlPureLinkR Genomic Digestion Buffer and 20 µlProtinase K and incubated at 55 °C for 1 hour. 20 µlRNase A (supplied with the kit) was added and incubated at room temperature for 2 mins. The lysate was centrifuged at 13,000g for 5 mins at room temperature to remove any particulate material. The supernatant was transferred to a fresh micro centrifuge tube and 200µLPureLinkR Genomic Binding Buffer supplied with the kit was added to the lysate. This was mixed well by vortexing to yield a homogenous solution. 200 µL 96–100% ethanol was added to the lysate and mixed well by vortexing for 5 seconds to yield a homogenous solution.The other steps for binding; washing and eluting DNA are as specified by the manufacturer.

The DNA content and purity of yield was measured using an automated DNA quantifier. 2µl of each extract was measured with respect to DNA content and Absorbance at 260 and 280nm using the NanoDrop 2000c/2000 UV-vis Spectrophotometer (Thermo Scientific). Mean DNA yield for each extraction method and mean A260/280 ratio were compared. Statistical analysis was performed with Minitab 14 statistical software.

PCR and restriction digestion –*hsp65*and*gyrB*PCR-RFLP

*gyrB*PCR was then performed on all extracts to ensure that extracted DNA could be successfully amplified. Based on the results obtained from above tests, method 'A' was selected for DNA extraction, and extraction was performed on 105 clinical isolates obtained from sputum of patients with pulmonary tuberculosis.

hsp65 PCR was done using the following protocol. GoTaqFlexi DNA polymerase (Promega. USA) (5U/µl) -0.25 µl; primers 10pmol conc. (Tb11: 5'-ACCAACGATGGTGTGTCCAT-3` Tb12: 5' - CTTGTCGAACCGCATACCCT- 3`)- 1.5 µl each; 2.5 mMdNTP -2 µl; 25 mmol $MgCl_2$- 3 µl; template DNA - 5 µl; total reaction volume 25 µl.The 441bp product was visualized on 2% agarose gel. Restriction digestion was done with *Bst*EII (Promega. USA. Ref. R6641) and *Hae*III (Promega. USA. Ref. R6171) enzymes according to established protocols[1]. Digested fragments were visualized on 4% agarose gel with 25bp DNA ladder. RFLP-gel images were analyzed using GelAnalyzer 2010 (Lazar software. Available from http://www.gelanalyzer.com/).*Hsp65 R*estriction digestion patterns were then analyzed using the PRASITE database (available at http://app.chuv.ch/prasite/index.html).

gyrB PCR was performed on all isolates using established protocols[1].Primers Mtubf-5`-TCGGACGCGTATGCGATATC-3`Mtubr-5`-ACATACAGTTCGGACTTGCG-3`. PCR product (1020bp) was visualized on 1% agarose gel with 100bp DNA ladder. *GyrB*positive isolates were confirmed as MTBC and negative isolates were confirmed as NTM. Restriction digestion was done with*Rsa*I (Promega. USA. Ref. R6371) restriction enzyme and digested fragments were visualized on 2% agarose gel with 50bp DNA ladder.

Identification of NTM isolates (*hsp65* PCR positive, *gyrB*PCR negative isolates) was then confirmed by partial sequencing of the *hsp65* product (Macrogen (Seoul, Korea)), manual checking and annotation using BioEdit software (version 7.2.5; Tom Hall, Carlsbad, CA [http:// www.mbio.ncsu.edu/bioedit/bioedit.html]) and aligned using ApE software (v2.0.47; M WayneDavis.[http://biologylabs.utah.edu/jorgensen/wayned/ape/]). The edited sequences were then aligned with sequences available in GenBank using a BLASTN (Basic Local Alignment Search Tool) search (available from http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi.)

Cost per sample was calculated based on the cost of consumables required for identification assuming that an optimal number of samples will be processed at a given time.. Equipment and labour costs were not included in the calculations.

RESULTS

DNA extraction

The minimum, maximum and mean yield of DNA from the five extraction methods used as well as the purity of extracts based on A260/280 ratio are shown in Table 1. Analysis of variance (ANOVA) showed a significant effect of extraction method on the DNA yield. (p=0.001), with an R^2(adjusted) value of 48.79% indicating that approximately 49% of the variability seen in the DNA yield was due to the effect of the extraction method. 2 way-

ANOVA including sample as a factor increased the R^2(adjusted)to 60%, though as a factor it did not have a significant effect on the yield (p=0.093). Extract A had a significantly higher yield than extract D and E (kit extraction). There is no difference in the yield between methods A, B and C.Amplification of a 1020 bp segment of the *gyrB* gene was successfully done from extracted DNA. Figure 1 shows *gyrB* PCR products after successful PCR amplification of DNA extracted by all five methods. All samples were *gyrB* positive and are therefore MTBC. LJ cultures from all 25 extracts gave no growth after 10 weeks incubation. Inactivation of organisms with initial heating to 80 ^{0}C was confirmed.

Table 1. Yield and purity of DNA extracted by 5 methods

Method	No. of sample	DNA yieldng/µl			Standard deviation	A 260/280 (Nano drop 200)
		Min	Max	Mean		
A (80^{0}C heat in water)	5	5.10	31.70	18.40*	10.220	1.540
B (95^{0}C heat in water)	5	2.40	10.60	6.34*	3.270	1.692
C (95^{0}C heat in TE buffer)	5	4.20	23.30	12.80*	8.090	1.880
D (kit-gram+ve protocol)	5	1.30	4.40	2.86	1.155	1.902
E (kit-general protocol)	5	0.50	3.30	1.86	1.234	0.988
*ANOVA – p<0.05						

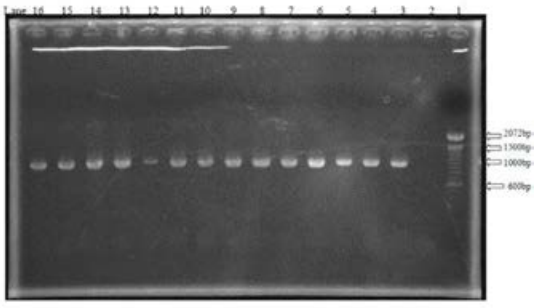
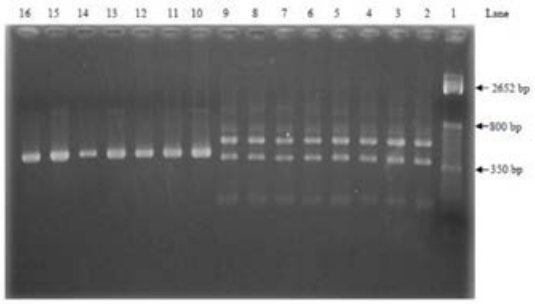


Figure 1. Gel electrophoresis of PCR products (1020 bp) of GyrB gene. Lane 1- 100 bp DNA ladder. Lane 2 - negative control. Lane 3- amplified product from standard TB strain H37Rv, which was used as positive control in all subsequent amplifications. Lanes 8-16 show products from amplification of clinical isolate extracts using methods A to E.

Hsp65 and*gyrB*PCR –RFLP

PCR amplification of the *hsp65* target 441 bp segment was successfully done. All 105 samples tested were positive, indicating that all samples were mycobacteria. (see figure 2.)



(***Figure 2.****GyrBRsaldigestion andhsp65 PCR. Lane 1 – 100 bp DNA ladder.Lane 2 to 9- gyrB- RsaI digestion with 3 bands (100, 385, 560 bp). Lane 10-16 hsp65PCR 441bpamplicon. Lane 10 - positive control –H37Rv. Lanes 11,12 and 13 - PCR products from the isolates that were gyrB negative. Lane 11- M aviumcomplex, lane 12- Mycobacteriumspp, lane 13-Mycobacterium aviumcomplex.)*

Restriction digestion of *hsp65* PCR product from 102 isolates showed the same digestion pattern as the standard H37Rv strain as seen in figure 3. These isolates were therefore identified as belonging to the MBTC. Mixed infection with NTM was unlikely. However the possibility of mixed infection with NTM that had the same RFLP pattern as MTBC organisms could not be excluded. Digestion pattern was different in PCR product from 3 isolates identifying them as NTM.

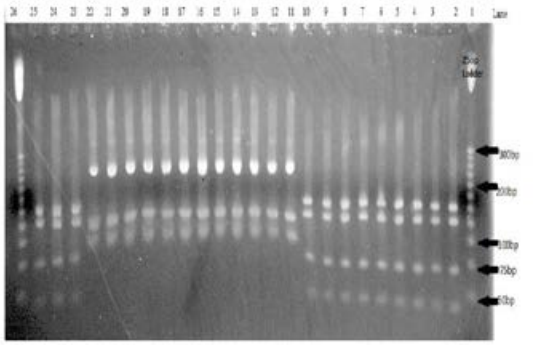


Figure 3. BstEII and HaeIII digestion patterns of hsp65 PCR product from MTBC isolates. Lane 1 and 26 - 25bp DNA ladder. Lane 2 to 10 – HaeIII digestion (4 fragments).Lane 11 to 22-BstEII digestion (3 fragments).Lane 23 to 25-HaeIII digestion.)

GyrB PCR was then performed on all extracts. Of the 105 sample extracts that were amplified, *gyrB* product was positive in 102 samples, confirming

that they were of the MTBC. PCR was negative in 3 samples which qA confirmed by repeat PCR on these extracts and these isolates were therefore identified as NTM. *Rsa*I restriction digestion patterns of the positive isolates are shown in figure 4. The samples showed three bands at 100bp, 380bp and 560bp which are seen in MTB, *M.africanum* and *M. canettii* isolates. These isolates were were therefore identified as belonging to one of these three species, with MTB being the most likely.

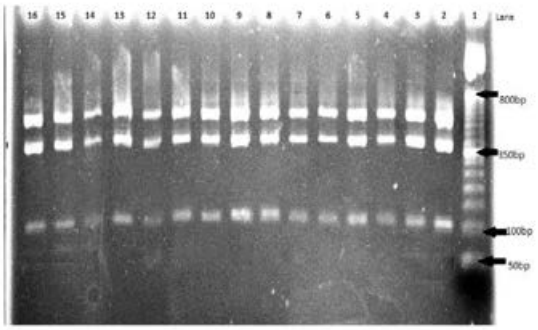


Figure 4. RsaI digestion pattern of GyrB PCR products. Lane 1- 100 bp DNA ladder. Lane 2- digested fragments from MTB H37Rv. Lane 3-16- digestion of products from clinical isolates.

Hsp65 PCR restriction analysis (PRA) patterns obtained from the 3 NTM isolates were entered into PRASITE database and tentatively identified. Two isolates were identified as *M. terrae* and one as *Mavium complex* (MAC) or *M.columbiense* (a member of the MAC described in 2006 [20]MAC-X. All of the seven novel isolates gave a positive result with the MAC-specific AccuProbe (Gen-Probe). However specific identification was not possible as there were a large number of possible species given in the output with very similar digestion patterns.

Based on partial sequences of *hsp65* product and BLASTN search results, these 3 isolates were subsequently identified as two isolates of *M avium* complex (misidentified as *M terrae* in PRA pattern) , and one isolate that could not be specifically identified as the closest matches had only 96% identity (identified as MAC in PRA). These sequences are available in the GENBANK data base. (Accession numbers: *M avium*– GenBank:KJ820768, *M avium* – GenBank:KJ820770, *Mycobacterium species*- GenBank:KJ820769).

In this study, the NTM infection rate in patients diagnosed as having PTB was 2.58%.

Cost

PCR –RFLP based identification of MTBC isolates could be done at approximately LKR 850.00 per sample for consumables (USD 6.00) if optimal numbers of samples are processed simultaneously.

Based on the above results the optimum identification algorithm for routine use in this clinical laboratory for identification of clinical isolates of mycobacterium is shown in figure 5. Using this algorithm would reduce costs further as *hsp65* amplification would only be done if *gyrB*PCR was shown to be negative.

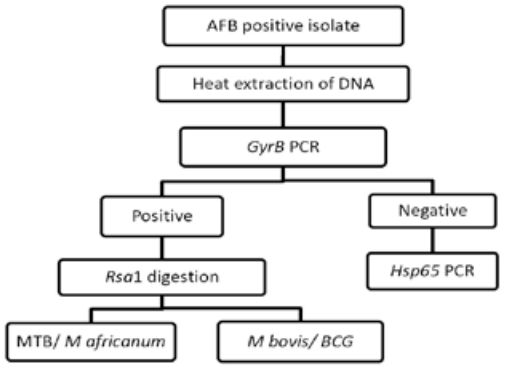


Figure 5. Optimized algorithm for molecular identification of clinical mycobacterial isolates

DISCUSSION

Molecular methods of identification have advantage not only in speed, accuracy and reproducibility, but also in safety as only DNA is required. Cultures can be inactivated safely prior to DNA extraction making molecular methods attractive even in low income settings where safety equipment, protocols and laboratory safety training is minimal. The obstacles for routine use are first, the cost of equipment and consumables, and secondly the availability of technical expertise required for molecular testing.

With many molecular consumables becoming available at lower prices and new economical methods being investigated, a re-evaluation of costs is necessary. In addition, many molecular methods used today are more robust than earlier methods. Conventional PCR and RFLP are easy to perform if optimized protocols are available. This study was designed to optimize an identification algorithm for clinical mycobacterial isolates that can be performed at minimal cost with minimal

expertise in a laboratory in a low-income setting. The research student and technical officer who carried out the work in this study had no previous experience with molecular techniques and learned all required methods and developed the extraction protocols within a short period of time.

GyrB gene PCR was selected as MTBC is the most common infecting organism and this can be detected with a single PCR with no need for additional restriction digestion. Further analysis with RFLP or *Hsp65* PCR can be done only if required. Other gene candidates for differentiation of mycobacterium species are also available including 16S-23S rDNA ISR[16] which were not assessed in this study. The results show that simple heat extraction at 80^0C for 1hour is the adequate as a method of DNA extraction from mycobacterial cultures. The yield is high and of good purity. The efficacy of inactivation of organisms at 80 ^{0}C was also compatible with published literature.[21] As heating for 20 mins has been shown to have variable results, with some studies showing complete inactivation and others showing some positive cultures[19] the safer option of 60 mins was used. Mycobacteria were inactivated by this procedure which rendered the suspensions safe for use. This extraction procedure requires minimum technical ability, equipment or other reagents and can easily be done in any laboratory with basic facilities. However the yield was relatively constant across all samples, whereas heat extraction gave varying results.

It is possible that the kit method will give a constant yield whatever the amount of DNA present in the original sample and therefore may be useful in low DNA samples. However for extraction of DNA from culture, this method shows no advantage. Better yield can probably be obtained using the spin column method by increasing incubation time with lysis digestion buffer and protienase K However a kit method would always be more expensive and require more equipment than the heat extraction method described. As the cell suspension used for method A extraction/ inactivation had a greater volume and higher number of organisms than the other methods B to E (for which equal volumes of the suspension were use) the yield shown here for this method is an overestimate when compared to the other methods. Repeat testing with equal numbers of organisms was not done and is one of the limitations of this study. To ensure that the extracts were all suitable for PCR-RFLP, both *GyrB* PCR and *Rsa*1 digestion were carried out on all 25 extracts. All 25 extracts gave positive PCR bands and digestion bands showing that even the water extracts were of adequate purity.

GyrB PCR has proved an excellent method of identifying MTBC organisms. It is a simple and easy to perform procedure and does not have the disadvantage of gene negative strains that are seen with the IS6110 PCR, which is one of the more widely used PCR methods for TB diagnosis. However, RFLP is necessary to differentiate species and even then it can only differentiate between 4 species of the complex (MTB, *M. bovis, M. africanum* and *M. microti*) when two restriction enzymes are used. It does not have the genotyping power of IS6110 RFLP. This method, therefore, is a practical method for routine use in a clinical laboratory where identification to group level is adequate. Use of a single enzyme that made the important differentiation between MTB and *M bovis/ BCG* was evaluated so costs would be minimal.

*Rsa*Idigestion was successfully performed and based on the band patterns, all 102 samples tested belong to the MTB, *M. africanum, M. canettii* group. The absence of *M. bovis/ BCG* and *M.microti* isolates is not surprising as these strains are rare causes of disease in Sri Lanka.[15] Of the 3 possible species, *M canettii* is unlikely as it is a rare cause of disease in humans. Between MTB and *M.africanum*, the most likely species is MTB.

Hsp65 PCR-RFLP was successfully performed and this accurately differentiated NTM from MTBC. If the digested band pattern is the same as that of the positive control, they are relatively easy to identify and estimate band sizes. However when differing band patterns are seen, which is the case with most NTM, then precise estimation of the band molecular weight is very difficult even with a 4% agarose gel. Using image analyzer software is a useful way of interpreting gel images and with free software available this is now possible at no extra cost.

The PRASITE database generates the closest matches to the given band pattern. However, accurate species level identification with this method

is difficult for two reasons. Firstly, many species differ from each other with only a few base pair differences in fragment length. As image analyzing software is not completely accurate and molecular weights of bands are approximate calculations, identification is an approximation at best. Also, as seen in reference texts, several species can share the same band pattern. Therefore, even if the band molecular weight were accurate, species level identification would not always be possible. However, with this method, the most likely group of organisms can be determined. A drawback of this method is that it compares the band sizes with that of known species. Therefore a new strain would not be identified, but rather each query is put into the closest fitting group. This problem was seen in this study where all three NTM isolates were misidentified using the PRA method.

Hsp65 sequencing is used for accurate species level identification of many bacterial species. Studies have shown that the level of sequence similarity between isolates in a given species is >98.2% with some species like MTB showing 100% similarity in all isolates.[12] Phylogenetic analysis using this sequence has shown great similarity to the phylogenetic tree generated from 16S rRNA analysis.[12] *Hsp65* sequencing has been shown to have a greater resolving ability than 16S rRNA sequencing as well. GenBank contains an extensive database of *hsp65* sequences from different mycobacteria that can be accessed for comparison. Based on the GenBank database and other sources, a web accessible data base of h*sp65* sequences for mycobacterium species identification has also been published.[13] The problems associated with PRA method can be avoided by sequencing of the *hsp65* product, which will give a more accurate species identification. Sequencing costs are approximately the same as PCR-RFLP making this a viable option for the few NTM isolates that are cultured in this population. The NTM isolation rate of 2.58% is compatible with the 3% rate previously described by Elvitigala et al in Sri Lanka (2008).

BLAST analysis showed that two of the isolates were *M.avium*, which is compatible with the figures seen worldwide. *M. avium* is the most common NTM isolated from pulmonary specimens.[22]

An added advantage of using molecular methods is that the DNA extracts can be stored for future extensive study if required. Cost calculations for the optimal algorithm show that molecular identification can be established in a routine laboratory at reasonable cost to the provider/ patient.

This study highlights the following. First, that NTM disease rates (pulmonary) are between 2-3% in this population. Second, that a molecular identification algorithm can be established in a routine clinical laboratory at low cost. This protocol requires only basic equipment such as a thermocycler, electrophoresis apparatus, heat block / water bath, centrifuge and gel documentation system. As there are only a few laboratories (both state and private sector) that provide mycobacterial culture facility, most of these would already be equipped to perform this protocol. Consumables costs etc would be reasonable enough that most patients would be able to afford the test, or if necessary, state sponsoring of identification tests would be feasible. Currently, identification is not done routinely in state laboratories that preform mycobacterial culture. If at all, basic biochemical tests are used for differentiation of MTBC and NTM isolates and though cheap, these tests are time consuming and are not available for the first few months of clinical management of patients. Isolated studies like those mentioned in the introduction provide a glimpse into the NTM disease burden in Sri Lanka, but robust, large scale studies have not been done due to lack of identification data. The importance of having such data and routine identification of isolates is highlighted in studies that show the changing landscape of mycobacterial infections in other countries.[23]including hsp65, rpoB, and 16S-23S rRNA internal transcribed spacer (ITS Utilizing this protocol as a routine in culture laboratories would go a long way to fill this gap. As there are only 3-4 culture laboratories in the TB control programme of Sri Lanka, this method can be used to identify most of the mycobacterial isolates from here.

As we progress towards better TB control measures, improvement of diagnostics and laboratory infrastructure is a must. These improvements need to be targeted towards improving services that are needed in the country, based on local needs, not on foreign models. Improvement does not need to involve large investments. Making maximum use of available resources and implementation of optimised methods that give maximum results is the best way forward.

Limitations

Limitations of this study include the lack of optimization of the kit extraction methods which could potentially yieldhigher amounts of DNA. This method is for use on cultured mycobacteria and not for clinical samples. A further step of optimizing this method for direct DNA extraction from clinical samples was not performed.

CONCLUSIONS

The NTM disease rate in patients diagnosed with pulmonary TB in this cohort was 2.6%. A simple heat extraction in water provides a good DNA yield of adequate purity from mycobacterial cultures that can be used for molecular studies. The simple algorithm developed using PCR-RFLP based identification provides a low cost method for identification of clinical mycobacterial isolates that provides useful information for patient management.

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CLINICO-SOCIAL AND IMMUNOLOGICAL PROFILE OF ANTIRETROVIRAL NAÏVE CHILDREN LIVING WITH HIV IN TERTIARY CARE HOSPITAL, DELHI

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ABSTRACT

Introduction: This study was undertaken to assess the clinical profile of children living with HIV at the time of their enrolment in an ART centre in Delhi. The study also attempts to understand association between clinical staging and immunological profile (CD4 count/percentage) in HIV infected children. The findings of this study may help policy makers to plan better health care of CLHIV in resource constrained country like India. The Objectives of the study were to assess the baseline clinico-social and immunological profile of HIV positive children before the start of Antiretroviral Therapy, to study clinico-social and morbidity profile of HIV positive children at the time of their enrolment in ART centre and to study the association between immunodeficiency and clinical staging of CLHIV.

Methodology: The present study, conducted between December 2012 and March 2013, is a retrospective case review of 83 antiretroviral naïve Children living with HIV aged 8 months to 13 years and attending paediatric ART clinic of a tertiary care hospital of Delhi. After the infection was established on serological grounds, information on socio-demographic, clinical and immunological profile was studied.

Results: Majority (62.7%) of CLHIV were boys. Both parents of CLHIV were found to be positive in 63.9% while mother was found to be positive in 69.9%. The most common route of HIV infection was mother-to-child transmission (69.9%), followed by transmission though blood/blood products (12.0%). Every three out of four children (71.1%) were in WHO clinical stage III or IV. Three out of four (74.7%) children presented with fever, one in two with cough (56.6%). Diarrhoea (56.6%), pneumonia (41.0%), popular pruritic eruptions (18.1%), candidiasis (16.9%) and tuberculosis (14.5%) were the most common opportunistic infections in these children. The most common signs present were hepatomegaly (81.9%), anaemia (78.3%) and lymphadenopathy (72.3%).

Conclusion: Mother to child transmission is the most common route of transmission in CLHIV. At enrolment more than half of the children were in clinical stage III&IV. Fever, cough, diarrhoea, weight loss, rashes were common morbidities of the children. Majority of the children had hepatomegaly, anaemia and lymphadenopathy.

Key Words: AIDS;HIV; Opportunistic Infections; Immunodeficiency

INTRODUCTION

Globally, an estimated 35.3 (32.2–38.8) million people were living with HIV (PLHIV) in 2012, among which 3.2 million were children with a prevalence of 0.8%.[1] It is now estimated that half of all new episodes of HIV transmission in children occur during the breastfeeding period, when the majority of HIV positive lactating women may not be receiving the prophylaxis necessary for prevention of mother to child transmission (PMTCT) of HIV. India has the third largest number of PLHIV and their estimated number in 2011 was 2.09million. Children less than 15 years of age accounted for 7% (0.145 million) of all HIV infections.[2] The proportional contribution of the number of children living with HIV (CLHIV) out of the total PLHIV population was estimated to be 6.3% in 2007 and7% in 2011.[3]

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Dysfunction of immune system and resultant illnesses is more rapid in HIV infected children as compared to adults. HIV affects virtually all the systems of the body and presents with varied clinical manifestations. Children with AIDS present with disease patterns that are different in nature, severity and/or frequency as compared to immune-competent children. The clinical presentation varies with the degree of immune-suppression, ranging from asymptomatic infection to AIDS characterized by severe immuno-suppression and recurrent severe opportunistic infections.

This study was undertaken to assess the clinical profile of children living with HIV at the time of their enrolment in an ART centre in Delhi. The study also attempts to understand association between clinical staging and immunological profile (CD4 count/percentage) in HIV infected children. The findings of this study may help policy makers to plan better health care of CLHIV in resource constrained country like India.

METHODOLOGY

The present study was conducted between December 2012 and March2013 in ART clinic of Kalawati Saran Children Hospital(KSCH), a tertiary care hospital for children and the only pediatric centre of excellence for HIV in Delhi, since 2011. The study involved a retrospective case review of 83 antiretroviral naïve CLHIV between 8 months to 13 years of age attending the pediatric ART clinic of this hospital and resident of Delhi. After the HIV infection was established on serological grounds, information on demographic characteristics, clinical manifestation and immunological profile of the CLHIV was extracted from our data base using a standardized questionnaire. Clinical and immunological stage[4] were based on the WHO norms. WHO clinical stage 1 and clinical stage II were termed as early disease, while clinical stage III and IV as advanced disease. CD4 percentage was used to classify the immunological status of children below 5 years of age, while CD4 count was used in children aged 5 years or older.[4] Mode of HIV transmission was determined by establishing mother's HIV status, history of transfusion of blood or blood product and probable unsafe injection given to the children.

The diagnosis of tuberculosis was based on the WHO guidelines for National TB program forchildren[5]; cases were either smear positive or smear negative with clinical/radiological features were diagnosed to be TB positive. Other baseline investigations that were obtained were complete blood count, CD4 count, CD4 percentage, HBsAg and HCV assays. Anemia was defined using the WHO criteria: 6-59 months, <11g/dl; 5-11years, <11.5g/dl; 12-14years, <12g/dl.[6]

Data was collected after obtaining approval from the institutional protocol and ethical committee. All consecutive children attending the centre during the study period and residents of Delhi were eligible for inclusion in the study if their caregivers gave consent and study subjects who were more than 7 years gave assent. Collected data was transformed into variables, coded, entered and analyzed using SPSS version 12. All observations were in terms of mean, median, standard deviation, percentages and proportions. Tests of significance like chi square, t-test were applied for comparisons wherever required. P value less than 0.05 was considered statistically significant at 95% confidence level.

RESULTS

Eighty three antiretroviral naïve children were enrolled in the study. The mean and median ages of the children at the time of HIV diagnosis were 5.4± 2.9yearsand 4.9 years (range 8 months to 13 years) respectively. There were 52 (62.7%) boys. Father was the head of the family in the majority of the subjects (68.7%), while one child was living in an NGO. Majority (74.7%) belonged to upper lower Socio-economic class according to Kuppuswami scale CPI 2013.[7] Twenty seven children (32.5%) were orphaned, of whom 16 (59.3%) and 11 (40.7%) were single and double orphans respectively. Both parents were found to be positive in 63.9% of the CLHIV while mother was found to be positive in 69.9% and father was positive in 64.5% of the children. In 13 (15.7%) CLHIV, siblings were also found to be positive. Mother was the primary caretaker in almost two-third of the children. Socio-demographic characteristics of CLHIV are shown in table 1.

Table1. Socio-Demographic Characteristics of The HIV Infected Children (N=83)			
Socio-demographic characteristics		**Number**	**%**
Age	Less than 5 years	42	50.6
	Greater than 5 years	41	49.4
Gender	Male	52	62.7
	Female	31	37.3
HIV status of parents	Mother positive	58	69.9
	Father positive	53	63.9
	Both parents positive	53	63.9
	One parent positive	5	6.0
	None positive	19	22.9
	Siblings positive	13	15.7
Orphaned	Yes	27	32.5
	No	56	67.5
Primary care taker	Mother	63	75.9
	Father	10	12.0
	Maternal relatives	6	7.2
	Paternal relatives	2	2.4
	Siblings	2	2.4

The most common route of HIV infection was mother-to-child (69.9%). Blood transfusion and probable unsafe injections contributed 12.0% and 6.0% respectively. 71.1% of the children were in advanced (WHO clinical stage III and IV) stage and were in moderate to severe immuno-compromised stage (table 2). The most common symptoms seen in these children were fever (74.7%), cough (56.6%)and weight loss/failure to gain weight (34.9%). Diarrhoea (56.6%), pneumonia (41.0%), popular pruritic eruptions (18.1), candidiasis (16.9%) and tuberculosis (14.5%) were the most common opportunistic infections seen in these children. Hepatitis B and C co-infections were seen in 6.0% and 2.4% of the children respectively.

Table 2. Mode of HIV Transmission, Clinical Staging and Immunological Staging In CLHIV (N=83)			
Mode of HIV transmission		**Number**	**%**
Mother to child		58	69.9
Blood /Blood products transfusion		10	12.0
Probable unsafe injection		5	6.0
Unknown		10	12.0
Clinical Staging			
Early disease	WHO Clinical Stage I	3	3.6
	WHO Clinical Stage II	21	25.3
Advanced disease	WHO Clinical Stage III	33	39.8
	WHO Clinical Stage IV	26	31.3
Immunological Staging			
Not Immunodeficient		7	8.4
Mild Immunodeficient		17	20.5
Moderate Immunodeficient		17	20.5
Severe Immunodeficient		42	50.6

Four out of five children were having hepatomegaly while anaemia was seen in three out of four children. Lymphadenopathy was seen in 72.3% of the children, mainly in cervical and axillary regions accounting for 57.8% and 43.4% of cases. Clinical features of CLHIV are shown in table 3. Mean CD4 % in children less than 5 years (n=42) was 14.9 ± 6.7 while mean CD4 count in children aged 5 years and above (n=41) was 332.9 ± 224.6 cells/mm.[3] (table 4).

DISCUSSION

In the present study, approximately two-thirds (62.7%) of the study subjects were boys. Proportion of HIV positive boys in other studies in India was also more than 50% (63.4% -76%).[8,9,10,11] According to National AIDS Control Organisation (NACO), mother to child transmission(MTCT) is the primary route of transmission for HIV among children. It is estimated that without any intervention, the risk of transmission of HIV from infected mother to her child is between, 20% to 45%.[12]

In majority (69.9%) of the study subjects, mothers were HIV positive, which is similar to findings in an earlier study conducted in New Delhi.[9] This clearly shows that MTCT is the most common route of transmission. Hence preventive strategies need to be strengthened for preventing MTCT. Both parents were found to be HIV positive in nearly three fourth of the study subjects. Similar finding (74%) was seen in a study conducted in Surat[13], India while inChennai[14], a relatively lower proportion (38%) of HIV infected parents was observed indicating that mode of transmission may vary from place to place. Another study conducted by Okomo U, et al in West Africa[15], parents of 10.8% of children were HIV positive.

At the time of the study, both parents were alive in almost 65.6% of the study subjects while approximately 13.3% children had lost both parents. These findings were almost similar to another

Table 3. Clinical Features Of CLHIV Before Art Initiation (N=83)*					
Clinical features	**N (%)**	**Immunodeficiency**		**CI=95%**	
		No to Mild (n=24)	**Moderate to Severe (n=59)**	**p -value**	**OR**
Symptoms					
Fever	62 (74.7)	16	46	0.2	0.5 (0.2-1.6)
Cough	47 (56.6)	11	36	0.2	0.5 (0.2-1.4)
Weight loss/failure to gain weight	29 (34.9)	8	21	1.0	0.9 (0.3-2.5)
Rashes	26 (31.3)	9	17	0.4	1.5 (0.5-4.0)
Ear discharge	25 (30.1)	6	19	0.6	0.7 (0.2-2.1)
Opportunistic infections					
Diarrhea	47 (56.6)	8	39	0.0	0.3 (0.1-0.7)
Pneumonia	34 (41.0)	7	27	0.2	0.5 (0.2-1.4)
Popular pruritic eruptions	15 (18.1)	7	8	0.1	2.6 (0.8-8.3)
Candidacies	14 (16.9)	2	12	0.3	0.4 (0.1-1.7)
Tuberculosis	12 (14.5)	3	9	1.0	0.8 (0.2-3.3)
Other signs					
Hepatomegaly	68 (81.9)	17	51	0.1	0.4 (0.1-1.2)
Anemia	65 (78.3)	12	53	0.0	0.1 (0.0-0.3)
Lymphadenopathy	60 (72.3)	16	44	0.6	0.7 (0.2-1.9)
Splenomegaly	48 (57.8)	13	35	0.8	0.8 (0.3-2.1)
Hepatosplenomagaly	48 (57.8)	13	35	0.8	0.8 (0.3-2.1)
Failure to thrive	10 (12.0)	2	8	0.7	0.6 (0.1-2.9)
Developmental delay	8 (9.6)	2	6	1.0	0.8 (0.2-4.3)

*multiple response

Table 4. clinical staging and opportunistic infections with respect to immunodeficiency in CLHIV (n=83)						
WHO clinical stage	Number (%)	CD4		WHO classification of Immunodeficiency		p-value
		Mean	SD	Not significant to Mild	Moderate to Severe	
In children less than 5 years (n=42)*						
CD4 Percentage						
Early	13 (31.0)	18.9	7.4	7	6	0.00
Advanced	29 (69.0)	13.1	5.7	3	26	
In children greater than 5 years (n=41)**						
CD4 Count						
Early	11 (26.8)	586.5	232.7	10	1	0.00
Advanced	30 (73.2)	239.9	132.7	4	26	
Opportunistic Infections (N=83)***						
CD4 Percentage						
0-2	38(45.8)	16.2	7.4	6	32	0.04
≥ 3	45 (54.2)	12.2	6.0	1	44	

* there was statistically significant association seen between clinical stage and immunodeficiency (CI=95;p=0.00); also there was statistically significant association between clinical staging and mean CD4 % (p=0.02)

** there was statistically significant association seen between clinical stage and immunodeficiency (CI=95;p=0.00); also there was statistical significant association between clinical staging and mean CD4 count (p=0.00)

*** there was significant statistical association seen between immunodeficiency and number of opportunistic infections (p=0.04); also there was statistical significant association between the number of opportunistic infections and mean CD4 % (CI=95;p=0.00)

study in Delhi[9] where both parents were alive in 58% instances and had expired in case of 8% of children. However in a study conducted by Patel et al[13] both parents were alive in 40.8% of the cases, whereas one in four children had lost both parents. In another study by Pol, et al[10] (Karnataka, 2007) 42% of children had single parent and 12.67% had lost both parents. Such differences can be attributed to availability, accessibility and utilization of ART services in differential study settings. The increased life expectancy of PLHIV with ART could also be one of the reasons for variation in these findings.

Loss of parents means not only loss of social security for these children but also adverse upbringing of children emotionally and financially. Nearly a third of (29.1%) of children had lost their fathers, affecting the family economically which may have influenced medical treatment and regular follow up. These observations emphasize the family dimensions of the HIV infections. The increasing number of children orphaned due to HIV/AIDS is an emerging problem in many developing countries.[9,10,13,15]

As the most common route of transmission was vertical (MTCT), the gender distribution of CLHIV should have been almost equal (at least in the same proportion as the sex ratio at birth). Higher proportion of boys (62.7%) could be due to low level of care and lower utilization of services for the infected girls.HIV infection in children is primarily restricted to perinatal transmission or is transfusion acquired. The present study has shown similar results, where MTCT was the most common route of transmission seen in 69.9% of the study subjects. Similar observations were made in other studies also.[25]

In spite of the fact that mandatory screening of donated blood for HIV antibodies has been in force since 1993, it is seen that 12% of the study subjects had acquired HIV through transfusion of blood and blood products. However other studies,[11,16,17] observed a higher percentage of children who had acquired HIV infection through blood/blood products (19.3% - 39%). Presuming adequate screening for anti-HIV antibodies, transmission of HIV may still be possible during the window period. This calls for more prudent usage of blood or blood products. Feasibility of antigen assays or PCR assays to screen blood products for checking acquisition of transfusion mediated HIV also needs to be explored. Stringent quality control practices need to be instituted in HIV testing laboratories.

In the present study, it was seen that nearly three-fourth (71.1%) of the study subjects were categorized as having moderate to severe disease (WHO clinical stage III & IV). Similar observations were made by number of researchers[13,18,19,20,21] who reported a significant proportion of children (started on HAART) to be suffering from moderate or severe form of disease (31% - 76%). 11 out of 83 (13.3%) children were ambulatory, one was bedridden, while the rest of the study subjects (85.5%) were in working functional status. The signs, symptoms and opportunistic infections were almost similar to those reported earlier from developed and developing countries, including India[11,17,22,23,24], although small variations can be attributed to the wide spectrum of disease all over the world. Management of these infections should be ensured in the ART clinics.

One of the important observations in this study was that CD4percentage and CD4 count declined with deterioration in the WHO clinical stages of HIV infection (table 3). Similar findings were also observed in a few other studies.[25,26] This observation indicates that CD4 values are reliable marker of clinical status. With deteriorating immunity, there is worsening of clinical staging and increase in opportunistic infections. Children with lower CD4 values had more opportunistic infections as compared to children with better values of CD4 count who had no or less opportunistic infections and these findings have been reported in other studies[22,25,26] CD4 estimation has been studied as a marker of deterioration of HIV status and it is also a measure of relative risk of developing opportunistic infections in HIV positive children. Therefore CD4 values can be considered as a reliable marker of HIV progression. However there is a need of further studies on CD4 estimation in relation to antiretroviral therapy.

Limitations: Since it was a cross-sectional study, the response of HAART on immunodeficiency and morbidity cannot be assessed. Also viral load as a predictor of illness have not been studied. Moreover the results of this study in limited setting in Delhi cannot be generalized. However, association

of immunological stage with clinical stage and opportunistic infections warrants further longitudinal studies in larger cohort.

Recommendations: As MCTC is a major route of acquiring HIV infection in children, emphasis must therefore be laid on PMTCT guidelines, investigations and treatment in pregnant mothers to prevent or reduce risk of HIV transmission to their children. There should also be more judicious usage of blood or blood products as even if we presume adequate screening for HIV infection, transmission may still be possible during the window period. Use of PCR assays or antigen assays for screening blood products before transfusion also needs to be considered. There should also be strict quality control practices in HIV testing laboratories and blood banks to prevent blood transfusion of HIV. Clinical manifestations and opportunistic infections of HIV infection are variable and mimic a number of other ailments. A high index of suspicion and appropriate investigation may help in early diagnosis. Training of paediatricians should also be organized on this subject for early diagnosis and management of HIV, related illnesses and opportunistic infections. Those children on treatment should be regularly followed up for drug adherence and treatment outcomes.

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Short Communications

WHAT EMPOWERED COMMUNITY CAN DO FOR TB CARE? EXPERIENCE FROM INDIA

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ABSTRACT

The community engagement strategies of Tuberculosis programmes prioritized on increasing case finding. There are limited evidences on - what an empowered community can do for Tuberculosis care? An innovative community empowerment model was developed and implemented in districts of India as "District Tuberculosis Forum" (DTF) under Project Axshya to engage and empower community. The paper here describes the contributions made by empowered forum members. We collected quantitative data from 77 districts for activities conducted during June 2013 to July 2014. The analysis is focused on activities conducted within patient centric, community and programme centric approaches. Empowered community members sensitized over 9000 TB patients on their rights and responsibilities and generated resources to support nearly 700 patients. The model is promising with key stakeholders at district level coming forward to get involved in activities supporting Tuberculosis prevention and care with an aim to END –TB.

Key Words: Community Empowerment, Patient Centric approach, District Tuberculosis Forum, Project Axshya

INTRODUCTION

Community is the central focus of programmatic intervention and key strategy to increase universal access to healthcare. Globally, the programmatic approaches are suggestive of having community participation or per-se community engagement aimed at outreach activities.[1] In Tuberculosis programme, community engagement strategies prioritized mainly on increasing case finding, disseminate information about Tuberculosis and engage volunteers as DOTS providers.

The World Health Organization (WHO), END-TB strategy and programme guidelines of India, also emphasize on need to engage and empower communities for TB care.[2,3] Various modalities are outlined for engaging and empowering, however, limited is known on what empowered communities could do. An empowerment model was designed to identify, sensitize, engage and empower key stakeholders at district level by constituting a forum christened as "District Tuberculosis Forum" (DTF). This forum is constituted with support from Project Axshya (A Global Fund TB grant to India).[4] The paper here describe the contributions made by the empowered DTF members in India.

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METHODOLOGY

District level project staff, encouraged identified key-stakeholders to be part of DTF and sensitized members on "patient -charter" of WHO to interact with patients on one-to-one basis ("patient centric approach") and to empower them about patients' rights and responsibilities.[5] Patient empowerment is one of the core strategy of this model with an aim to have a positive treatment outcome.[6]

Community level participation of forum members ("community centric approach" to empower communities) is expected in project activities like local community meetings, where members inform about TB care and address issues related to "stigma" and or mobilize resources to support needy patients.[7]

This loop is completed by integrating empowered community to support the programme ("programme centric approach"). Forum members document observations related about programme that are limited to; visiting houses of TB patients who are recently diagnosed or irregular on treatment provide them with counselling support and social support. Forum members also visit the healthcare facility where TB services are provided, to conduct social or community monitoring and address any issue that cause hinder in providing the services.

In this paper we have used quantitative data for activities conducted by DTF members during June 2013 to July 2014that were available from 77 out of 96 districts under the preview of implementation partner Catholic Health Association of India (CHAI).[4] The analysis of activities conducted were grouped under "patient centric approach", "community centric approach" and "programme centric approach" within the framework of Advocacy, Communication and Social mobilization (figure 1). Ethical approval was not sought as the data used do not have any patient identifiers or other individual identifiers and the study did an analysis of record review of available or reported data from the project.

RESULTS

About Forum members: Out of 77 DTFs reports analyzed, 58(75%) forums hadrepresentation of TB patients. Representation from local leaders and print media was seen in 55% of forums. Majority of DTF (in 72 districts) had representation from civil society (Table 1).

Patient Centric Approach: A total of 9,732 TB patients were sensitized on "patient-charter". During this sensitization process members addressed the issues raised by TB patients and communicated to programme officers. Apart from sensitization, forum members from 49 districts had conducted home visits to meet 480 TB patients to counsel family members and patients. In addition, provided nutritional support to 663 TB patients (reported from 75% of forum) and 100 Multi-Drug-Resistant (MDR) TB patients. This activity was supported through self-contribution by members in 64% of districts.

Community Centric Approach: Community centric approach showed participation of members (in 90% of DTFs) in community awareness programmes organized by the project. Members discussed about Tuberculosis transmission, prevention, treatment and care (in 65% of DTFs) in these meetings.

Empowered community members advocated for Nutritional support to patients (in 75% of77 DTFs) with district level administrative authorities. These efforts led to policy revisions and one such example of policy revision is documented from the state of Kerala, India - to support a million plus poor TB patients. (*Box 1*).

Programme Centric Approach: The advocacy efforts of members focused on ensuring examination and exemption of User Fees, for presumptive TB referrals/sputa from community (in all 77districts). Other advocacy efforts reported were – behaviour change among laboratory technicians (in 6 districts), recruitment of doctors (in two districts) and new diagnostic centers at government facilities (in four districts). Members also reviewed about availability of medicines at government facilities and with TB patients during their community visits (in 14 districts); including retrieval of lost to follow-up TB patients to programme (in 8 districts) (Table 1).

Table 1. An overview of District TB Forum members

S. No.	Indicator	Number	Additional information
Composition of DTF members (Districts)			
A	Non-government organization (NGO) representation	72	
B	TB patient as key stakeholder	58	In 75% districts included TB patient as key stakeholder to share their experience of being a "TB patient"during community/patient meetings.
C	Social Worker	51	Multiple representation of key stakeholders were part of DTF. Each stakeholder assumed respective role within the model. Others, included, volunteers, community health workers, local business men, philanthropies etc
D	Local print media	42	
E	Local Politician	42	
F	Lawyer	32	
G	HIV infected community	35	
H	Private Doctors	10	
I	Others	53	
	Total	77	
Patient centric approach to empowerment (Patients)			
a	Patient sensitization on patient charter	9732	Patients were sensitized on "rights and responsibility" by one of the member – mainly the chairman of TB forum group with support from TB patient.
b	Home visits by DTF members	480	Counselling of patients on DOTS and their family members about TB prevention and care.
c	Nutritional support to patients on DOTS	663	Locally generated resources, through contributions from community members. DTF members also contributed to nutritional support.
d	Nutritional support to MDR-TB patients	100	Major contribution from private practitioners (doctors).
Community centric approach to empowerment (Districts)			
a	DTF members participation in community meetings	58 (75%)	Mainly meeting with PRI members, village functionaries to sensitize on TB prevention and care.
b	DTF members organising mid-media campaign	58 (75%)	Announcements in villages about "key messages" of TB prevention and care.
c	Advocacy for TB pension schemes	n.a	Million plus poor TB patients supported in the state of Kerala. Similar efforts are underway in other states.
Programme centric approach to empowerment (Districts)			
a	Facilitate services for TB patients	6	DTF members advocated for exemption of User Fees at public facilities
b	Ensuring availability of medicines to TB patients	14	DTF members reviewed availability of medicines at DOTS center and at Tuberculosis units.
c	Feedback on programme performance	19	Members provided feedback about the performance of programme to respective programme officers.

Box I. Pension schemes for tuberculosis patients: An advocacy effort from TB forum Members of Kerala

Kerala is one among first the states to introduce financial support scheme for TB patients in 1963. The utilization of scheme was abysmally low due to lack of information. Information about the scheme was known following revisions in 2010 to Rs 300 per month (USD 4.8) and subsequent revisions to Rs 525 per month in 2012 and to Rs 800 in 2013 (USD 13). Revisions in monetary terms did not follow revisions in inclusion criteria. Any family to avail the scheme need to be in the annual income cut-off of Rs 2400 i.e, USD 39. Project Axshya team sensitized DTF members about the issue and a channel of advocacy efforts were initiated.

The advocacy efforts led to acceptance of government for revisions in inclusion criteria. The government proposed decision for revisions in front of state elected representatives, to as "eligibility on annual income from Rs 2400 to Rs 100,000 (USD 1613) and increase in pension amount to Rs 1000 (USD 16). Both the proposals were accepted and about Rs 1,651,000 (USD 26,630) is earmarked by government of Kerala in the current financial year. The efforts of DTF supported nearly 300 patients from 26 Taluks (sub-divisions of the district). The resolution will support all poor TB patients of the state in years to come.

Source: Jinesh Lal, Joltin C, Gadala Srinivasa, Krithika, *Banuru Muralidhara Prasad*, Tomi Thomas, Pension schemes for tuberculosis patients: An advocacy effort from TB forum members of Kerala, India, 45th World Lung Conference, Barcelona Spain, 28th October to 1st November 2014.

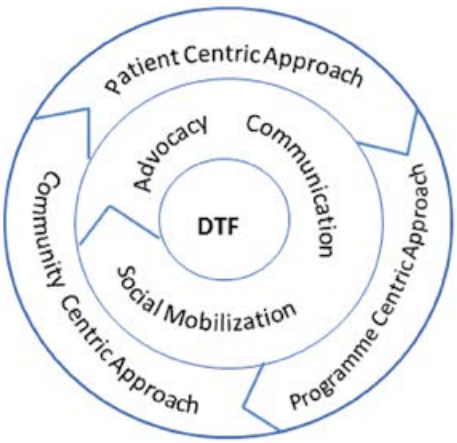

Figure 1. Diagrammatic representation of DTF Empowerment Model

DISCUSSION

The "District Tuberculosis Forum" –a community empowerment model is an innovative model of Project Axshya. Resources from the project supported sensitization of DTF members on "patient charter", organize quarterly meetings of members and supported home visits. This model has close resemblance to "TB patients' empowerment and involvement" described by Jean Macq in 2007 where empowered TB patient take control of own health by involving key stakeholders from community.[6]One of the key stakeholder in our study is TB patient who were involved to share their experience with community. Similar experience is documented from a rural district of Ethiopia where patient based support groups are created to support the programme.[8]

The study has demonstrated that by enhancing capacities of key stakeholders in community, a holistic community empowerment model could be developed for TB care. At the policy level the model could be envisaged as a social-capital that bridge community-system- programme to achieve Universal Healthcare Coverage for TB services in India and elsewhere.[9]In this study we included activities that could be documented and quantified; other qualitative discussions with community/ programme had limited documentation to be included.

CONCLUSION

The study is a brief description of activities focused at patient-community-programme centric approach that an empowered community through an organized forum – like DTF could perform. The model is promising with key stakeholders at district level coming forward to get involved in activities supporting Tuberculosis prevention and care with an aim to END –TB.

Acknowledgements: We would like to acknowledge the contribution of District level Project staff of Catholic Health Association of India (CHAI) and members of District Tuberculosis Forum.

Author Contribution: BMP conceptualized the paper, collated the information and wrote the paper. GSR implemented the project and reviewed manuscript. JR, DS, KKR, KK, SD, BDG, co-ordinated for implementation, and collected data. SC and SM conceptualized DTF model, reviewed and approved the manuscript.

Conflict of Interest: None declared.

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Case Report

CONGENITAL TUBERCULOSIS IN AN INFANT – EPIDEMIOLOGICAL INVESTIGATION

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ABSTRACT

Congenital Tuberculosis was diagnosed in a 40-days-old premature infant. The infant had fever. A chest radiograph showed infiltrates which was thought to be bacterial infection. Gastric aspirate revealed acid-fast bacilli by Ziehl-Neelsen staining and fluorescent microscopy later confirmed to be *Mycobacterium tuberculosis* by Gene Xpert MTB/RIF test. Her 22 years old mother was later diagnosed as a case of tuberculosis with symptoms, signs and radiologic manifestation of mild pleural effusion with infiltration. Infant was treated with isoniazid, syrup rifampicin, pyrazinamide and pyridoxine and mother with RNTCP Cat I regimen.

Key-words: Gastric Aspirate; Tuberculosis; Ziehl-Neelsen; Congenital; Gene Xpert MTB/RIF Test

INTRODUCTION

Tuberculosis (TB) is a global public health problem, India and China together account for almost 40 percent of the world's TB.[1] Congenital tuberculosis (TB) is a rare entity with 300 cases reported so far[2] and only 11 cases from India.[3] Approximately 30 cases of congenital TB have been reported since the review in 1980 by Hageman, et al.[4] The transmission of tubercular bacilli to the foetus occurs by haematogenous spread through placenta, in-uterus aspiration and ingestion of infected amniotic fluid or secretions during delivery. In addition, post natal infection may occur from contact with a contagious mother or ingestion of infected breast milk from a mother with tuberculosis breast abscess.[5]

CASE REPORT

A 40 day old female infant, weighing 3000 gm, presented to us with a history of fever for 20 days, fast breathing for 2 days and refusal to feed for one day. The baby was delivered as full term by spontaneous vaginal delivery with birth weight of 2750gm. Physical examination revealed a febrile (102°F). Investigations revealed: Haemoglobin: 9.4 gm/dl, Platelet 86,000 with total WBC 850/ CU mm, 60% Neutrophils, 32% Lymphocytes, 4% Monocytes and 4% Eosinofils. Erythrocyte sedimentation rate was 6mm after the 1st hour. Cerebrospinal fluid examination was normal. Serum electrolytes, creatinine, urea and blood sugar were normal. Ultrasound Sonography Test was showing liver normal in size and shape Parenchymal echotexture was homogenous. Spleen was enlarged (5.6 cm), multiple small hypoxic well defined round to oval lesions were seen throughout splenic parenchyma (granuloma). Splenomegaly with splenic microabcess and lymphoma of spleen was seen. The chest radiograph showed parenchymal infiltration in both lung fields (figure-1).

Gastric aspirate showed Mycobacterium tuberculosis by Gene Xpert MTB/RIF test. Acid Fast Bacilli were seen by florescent microscopy and Zeihl-Nelson staining. The infant's family was screened for tuberculosis. Only her mother was found to be the source of infection. Her chest radiograph showed evidence of pleural effusion,

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which was confirmed by a diagnostic pleural fluid. The pleural fluid was pale yellow with a cobweb coagulum formation. The pleural fluid Adenosin deaminase was 38 IU/l. The cytochemical examination of pleural fluid showed total leukocyte count of 1500 cells/cu mm, 10% Polymorphs and 90% Lymphocytes, protein : 3.4 g/dl and sugar : 50.4 mg/dl. Left mild pleural effusion with basal lung vasodilatation was also seen. (figure 2)

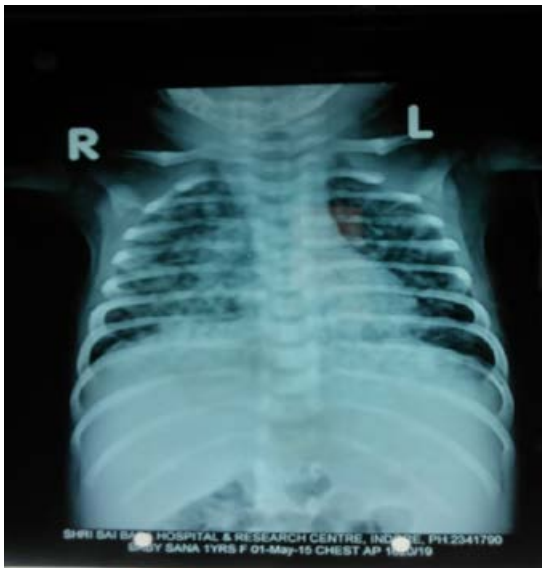

Figure 1. Chest X-Ray of infant showing parenchymal infiltration in both lung fields.

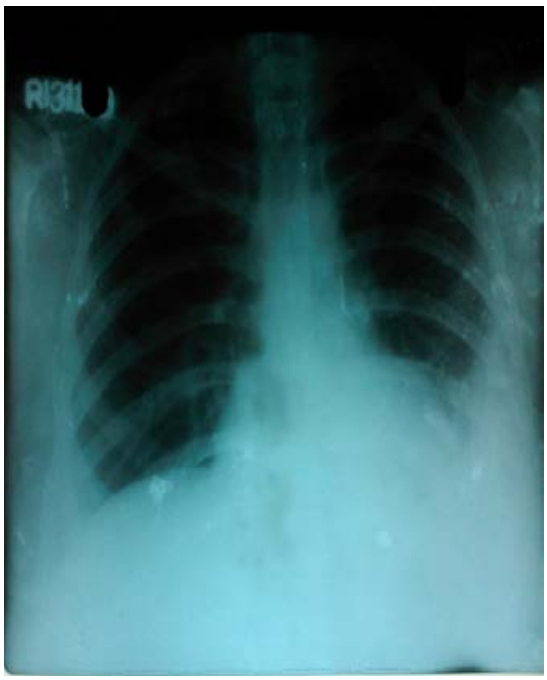

Figure 2. Chest X-Ray of her mother showing pleural effusion in lung fields

DISCUSSION

The diagnosis of congenital TB is often difficult. The clinical features of congenital TB have been described by Hudson (1956).[6] Cantwell et al [7] have proposed revised diagnostic criteria for the diagnosis of congenital TB. The infant must have proved tuberculous lesions and at least one of the following: (i) Lesions in the first week of life; (ii) A primary hepatic complex or caseating hepatic granulomas (iii) Tuberculous infection of the placenta or the maternal genital tract or (iv) Exclusion of the possibility of postnatal transmission by a thorough investigation of contacts, including the infant's hospital attendants and by adherence to existing recommendations for treating infants exposed to tuberculosis.[7] In our case only her mother was diagnosed with pulmonary tuberculosis after screening. Spleen of infant was with multiple hypoxic lesions throughout splenic parenchyma.

Although gastric aspirate cultures are said to be a poor diagnostic tool.[8] It has been associated with a high yield of positive cultures for *Mycobacterium tuberculosis* in most of the reported cases of congenital tuberculosis. In our patient, Gene Xpert MTB/RIF test of her gastric aspirate showed the presence of *Mycobacterium tuberculosis* and Rifampicin sensitive. Our patient has respiratory distress and hepatosplenomegaly. The milliary pattern in her chest radiograph was the clue that prompted us to get a chest radiograph of the mother. This led to further investigations that conclusively documented tuberculosis in order to start anti-tuberculosis therapy.

In infants who are suffering from pneumonia, it is advisable to send their gastric aspirate/lavage for Gene Xpert MTB/RIF test so that diagnosis of tuberculosis becomes as early as possible.

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The drug names should be provided in generic names, the use of generic name is not permitted.

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Binary names, consisting of generic name and a specific epithet (e.g. *Mycobacterium tuberculosis*) must be used for all organisms. A specific epithet must be preceded by a generic name, written out in full in its first appearance (eg. *Mycobacterium tuberculosis*) and can be abbreviated on subsequent uses (e.g. *M. tuberculosis*).

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This section provides an opportunity of correcting errors that occurred during the writing, typing, editing, or publication. These errors could be a misspelling, a dropped word or line, or mislabeling in a figure in a published article. Authors can submit errata using the online manuscript submission or via the email (See below).

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Manuscripts can be submitted online (www.saarctb.org) or through email to the Chief Editor, SAARC Journal of Tuberculosis, Lung Diseases and HIV/AIDS. Authors should ensure following documents to be sent if he/she wishes to send manuscript via email or online system. 1) Cover letter 2) Authorship form 3) Declaration form 4) Manuscript (Title page, Abstract, Body of article, References) and 5) Letter of Ethical Approval or A statement of clearance of the study protocol and the study by the Ethical Committee/Board mentioned in Methodology.

5. Publication charge

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