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EDITORIAL

Original Articles

AIMS AND SCOPE:

The SAARC Journal of Tuberculosis, Lung Diseases and HIV/AIDS is the official journal of the STAC. The Journal's main aim is the continuing education of personnel and the dissemination of the most up-to-date information in the field of tuberculosis, lung diseases and HIV/AIDS. It is devoted to dissemination of knowledge concerning various aspects of tuberculosis, lung diseases and HIV/AIDS. All articles relevant to the practice of this Journal and quality health research are published. The Journal is an appropriate forum for the publication of articles concerning the social, economic, public health, epidemiology, diagnostics, genetics etc. in the area of tuberculosis, lung diseases and HIV/AIDS. The scientific manuscripts presenting the results of public health importance are encouraged. The novel case reports which adds to the existing knowledge and consistent with the scope of Journal will be considered for publication. The Journal accepts review/mini-review, case report, short communications, and letters to editors within the scope of the journal.

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Editorial

At the end of 2010, an estimated 34 million people (31.6 million–35.2 million) were living with HIV globally and although annual new HIV infections fell 21% between 1997 and 2010, according to UNAIDS 2011, still much efforts are needed to get zero new HIV infection and zero AIDS related deaths. Three decades after the emergence of the HIV epidemic, and despite the development of many efficacious individual, group and structural level interventions, it is clear that advances made in the prevention of HIV have not been sufficient to get ahead of the epidemics. Globally, 8.8 million (8.5 million–9.2 million) incident cases of TB occurred in 2010. As in other areas of public health and health service delivery, consensus surfaced that fundamental to this problem was insufficient use of scientific evidence in planning and delivering interventions. To address this gap, health programme planners and implementers were encouraged to adopt 'evidence-based methods' by bringing in evidence from the scientific literature and experts to inform their decision making. Gradually, researchers have been encouraged to engage in knowledge translation to ensure that the findings from their research is being made known to policy makers, planners and implementers to guide better decisions. While emphasizing the need to close the gulf between evidence and action, there is a growing sentiment that current concepts and approaches for doing so are inadequate, and new models are needed.

The common practice of conducting research by researchers tends to detach researchers from those involved in programme planning and implementation. So, it is a significant practice to engage jointly researchers, programme planners and implementers to develop program, focus on operational research, process evaluation, and outcome and to develop the knowledge base further, so as to determine what works best in different situations and why.

A newly devised approach, Program Science, may offer a structure that both expands the scope for knowledge development and provides an intersection between programme and science focused on resolving programme issues. Programme science can best be defined as the systematic application of theoretical and practical scientific knowledge to improve the design, implementation and evaluation of public health programmes. Program Science incorporates different spheres of practice including strategic planning and policy development, programme implementation and programme management with complementary spheres of knowledge, including epidemiology, transmission dynamics, policy analysis, intervention efficacy and effectiveness, surveillance, operations research and monitoring and evaluation.

Thus, Program Science brings programme and scientific research together to enhance the health impact of HIV/TB prevention, care and support programmes.

DO DOCTORS KNOW ENOUGH ON TUBERCULOSIS? A SURVEY AMONG GOVERNMENT MEDICAL OFFICERS IN THE CURATIVE HEALTH SECTOR

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ABSTRACT

Background: Tuberculosis (TB) is a global epidemic with over 2 billion people, equal to one-third of the world's population, are currently estimated to be infected. In Sri Lanka, despite an effective national programme for TB control and mass scale immunoprophylaxis, TB still remains a growing public health issue with over 9000 new cases being detected annually. Studies conducted in many high prevalent settings had clearly shown that the knowledge and practices among many general practitioners and family physicians do not conform to WHO guidelines or expectations. A survey among government medical officers in the curative health sector was carried out with the objective to assess the basic knowledge on tuberculosis (TB) among non-specialist medical officers (MOs).

Methodology: We assessed the basic knowledge on TB in five main fields, namely clinical pathology, diagnostic methods, treatment regimens, Direct Observed Treatment Short course (DOTS) and Bacillus of Calmette-Guerin (BCG) vaccination in 355 randomly selected MOs. According to the individual performance, marks were allocated and a grading of poor, average or good was offered for each component. An overall final rank was given to each subject according to the total marks achieved.

Results: Only 27% had good (marks >66%) overall knowledge. 67% had average, while 6% had poor (marks <33%) overall knowledge. Majority had good understanding on clinical pathology and DOTS, but the knowledge was most inadequate with regard to BCG and treatment regimens. Post-intern MOs had a lower understanding on anti-TB medications and different regimens, when compared to intern MOs ($x2=8.8047, p=0.012$), and their knowledge did not improve with service experience. MOs attached to out-patient departments and surgical units seem to be in need of extra attention.

Conclusion: The knowledge on basic aspects of TB is not satisfactory among most of the MOs. Applicability of targeted awareness and continuation of medical education programmes, assessment schemes, appraisal systems and antibiotic regulation and prescription policies for the local setup should be explored.

Keywords: Tuberculosis, Sri Lanka, TB, Knowledge, Medical officers

INTRODUCTION

Tuberculosis (TB) is a global epidemic with over 2 billion people, equal to one-third of the world's population, are currently estimated to be infected, with 9.4 million new TB cases identified worldwide and 1.8 million deaths annually. In 2008 the South-East Asia Region accounted for 34% of incident cases globally.[1] In Sri Lanka, despite an effective national programme for TB control and mass scale immunoprophylaxis, TB still remains a growing public health issue with over 9000 new cases being detected annually.[2]

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Despite the enormity of the issue and constant effort of World Health Organization (WHO), understanding and awareness on many aspects of TB, among the general public as well as many health care professionals, remains hesitant. Surveys carried out worldwide assessing not only the knowledge, but also attitude and practices of health care workers, have identified calamitous deficiencies warranting a well coordinated global effort in order to achieve WHO stop TB partnership targets.[3,4,5]

Studies conducted in many high prevalent settings had clearly shown that the knowledge and practices among many general practitioners and family physicians do not conform to WHO guidelines or expectations.[6,7] This, among many others, had been identified as an important iatrogenic factor which had lead to the identification of multi-drug resistant cases from over 100 countries and extensive drug resistant cases form 57 countries to date.[8]

Considering the rising incidence of resistant TB cases in Asian continent, Sri Lanka should be ever vigilant to keep the disease under control and to maintain its very low incidence of resistant cases. Further to a dedicated national programme for tuberculosis control (NPTC) and the supervision of every TB patient by a respiratory physician or qualified personnel, it is equally important for all of the health care personnel to be conversant with many basic aspects of tuberculosis. Intention of this limited survey was to assess the basic knowledge regarding few major aspects of tuberculosis among a group of Sri Lankan medical officers in the curative sector. The objectives of the survey were to evaluate the knowledge on tuberculosis among a representative sample of medical officers in the curative sector, to identify any categories of medical officers requiring special awareness programmes and to provide background information for the NPTC to plan out future training campaigns.

METHODOLOGY

Design and setting

We carried out a questionnaire based survey in two districts of the country; namely in Kandy and Kegalle. The study was conducted in General Hospitals Kandy and Kegalle and randomly selected secondary health care centres consisting of Base Hospitals Mawanalla, Karawanalla, Warakapola and District Hospital Rambukkana.

Recruitment of participants to the survey

A total of 355 medical officers were selected to participate in the survey. 200 were from Kandy district while 155 were from Kegalle district, proportionate to the employee number in each hospital concerned. The sample was selected using stratified randomization methods to represent different units in each hospital and to represent different categories of medical officers.

Data acquisition

A self administered questionnaire with ten multiple response questions was used as the data collecting tool (annexure 1). This was pretested at a pilot study conducted in early 2007 at Teaching Hospital, Kegalle.

Data collection was carried out in January to March 2007 in Kandy district and January to April 2009 in Kegalle district. We took special precautions to avoid possible re-recruitment of participants, since the study was carried out in two steps. Each selected participant was handed over the questionnaire after explaining the purpose of the study and advised to respond in the presence of the investigator. Average time taken by the participants was around fifteen minutes. Each answer sheet was checked twice and marks allocated by two independent investigators.

Assessment of knowledge

In this survey the investigators concentrated on the basic knowledge in five main fields about tuberculosis, namely clinical pathology, diagnostic methods, treatment regimens, direct observed treatment short course (DOTS) and Bacillus of Calmette-Guerin (BCG) vaccination. The questionnaire was designed to cover the above aspects.

Table 1. Allocation of marks and grading for each individual component

Topic & Question numbers	Total marks	Poor	Average	Good
Clinical Pathology (Q1,2,3)	3x3 = 9	≤3	4-6	7-9
Method of Diagnosis (Q4,10)	3x2 = 6	≤2	3-4	5-6
Treatments (Q6,7,8)	3x3 = 9	≤3	4-6	7-9
DOTS (Q9)	3x1 = 3	1	2	3
BCG (Q5)	3x1 = 3	1	2	3

According to the individual performance, marks were allocated and a grading of poor, average or good was offered for each component (Table 1). The overall final ranking was given according to the total marks achieved in all components (Table 2).

Table 2. Final ranking of knowledge according to total marks obtained

Total marks (out of 30)	Final ranking
≤10	Poor
11-20	Average
21-30	Good

Data analysis

Data were entered in to an MS Excel 2003 data sheet and analyzed using MS Excel and SPSS 12.0 statistical software. Chi square test was used where appropriate in calculating statistical significance at a 5% significance level at relevant degrees of freedom.

Ethical considerations and confidentiality

All participants to this survey are voluntary medical officers. The informed consent was taken from each participant after explaining the objectives of the survey by the investigators. All answered questionnaires were kept securely with access only to study group personnel. Name and other specific particulars of the participants, which will lead in to their identification, were not included in the survey.

RESULTS

We recruited a representative group of 286 post-intern MOs and 69 intern MOs. The overall knowledge in 67% was average, while 27% had good overall knowledge and 6% were found to have poor overall knowledge. When the responses for individual components were considered, the knowledge was most inadequate regarding BCG vaccination and treatment regimens, while majority had good understanding on clinical pathology and DOT strategy (Figure 1).

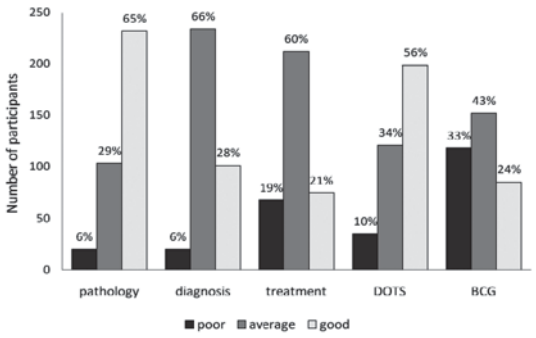

Figure 1. Comparison of the grades of knowledge achieved by th e participants for the different components

61% of MOs were having the misconception that BCG vaccination would prevent children from acquiring pulmonary TB, while 37% had poor understanding on the need for BCG revaccination in childhood. Many were not familiar with the different treatment regimens and their indications, and 24% were not aware on the use of levofloxacin and clarythromycin as second line anti-tubercular therapy. 10% were not confident regarding the diagnostic value of Mantoux test.

We further compared the knowledge among different categories of MOs. Intern MOs had better overall understanding than post intern MOs (Figure 2 χ^2=8.8047, p=0.012). This difference was mainly observed in relation to anti-TB medication and treatment regimens.

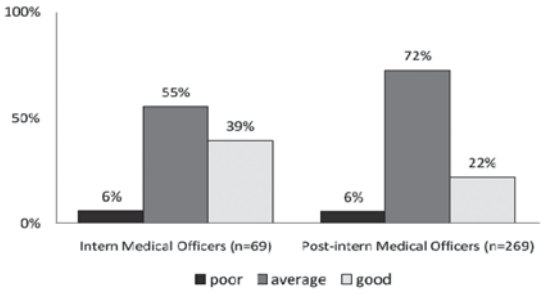

Figure 2. Comparison of knowledge (final overall ranking) between intern and post-intern medical officers

The specialties or units, to which MOs were attached, were categorized in to four. The medical group consisted of general medicine, general paediatrics and allied specialties, while the surgical group consisted of general surgery, allied specialties and gynaecology and obstetrics. Out patient department (OPD) was considered a separate group while anaesthesia, intensive care medicine and radiology units were considered together. Among them we noted that OPD and surgical categories had the highest number of poor grades, 8.6% and 7.5% respectively, even though the differences were not statistically significant (Figure 3 χ^2=9.7121, p=0.137).

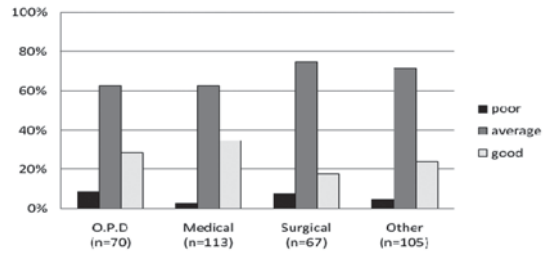

Figure 3. Comparison of knowledge (final overall ranking) among medical officers attached to different specialties / units

There was no significant difference among the ranks achieved by MOs with increasing service experience (Table 03; χ^2=2.3048, p=0.889).

Table 3. Final ranking of knowledge among medical officers with different service experience (x2=2.3048, p=0.889)

Service experience	Total (n)	Poor	Average	Good
Below 3 years	171	5%	68%	26%
3 to 6 years	78	6%	67%	27%
6 to 9 years	53	2%	68%	30%
Over 9 years	52	8%	63%	29%

DISCUSSION

Results showed that around three-fourths of the group were unable to achieve a minimum of 66% of the answers correct. This implies that the average knowledge on basic aspects of the disease is not satisfactory among most of the MOs. Since many post-intern MOs serve as primary physicians in the private sector of the country, it is important for these first contact medical persons to have a sound working knowledge on this common and growing health issue.

The observations that the interns having better understanding on treatment aspects and that the knowledge not improving with length of service, raise the issues of lack of continuing education after graduation and lack of continuous assessment programmes in general. Recently NPTC has started a tuberculosis awareness programme for all pre-intern medical graduates, which will somewhat help to bridge this gap, but further campaigns targeting post-interns with special attention to OPDs and surgical units would be invaluable. Furthermore undergraduate medical curricula could be revised in order to minimize these deficiencies.

Poor understanding on anti-TB medications and different treatment regimens was highlighted in this survey. This necessitates the proposal of certain administrative legislations. Commencing and continuing ATT is currently under specialist supervision and we would be well advised to strictly adhere to that practice, as lessons should be learnt from the practices of other countries with high prevalence of resistant TB.[9,10,11] Antibiotic misuse is a major worldwide concern affecting TB control. Since irrational prescription and over the counter use of antibiotics is not uncommon in the country, we recommend for establishment of national antibiotic regulation and prescription policies. Use of important second line anti-tuberculosis antibiotics in general practice should be restricted to specialist authorization.

This study did not explore doctors' attitudes towards the disease, which would be interesting to look into since TB still carries a significant social stigma in

the local community. Another limitation was that the study was carried out in two phases due to practical inconveniences, which necessitated special precautions to avoid re-engaging same participant in both phases due to possible transfer among work stations. Nevertheless, this is not expected to interfere with the results as we do not observe a noteworthy change in the local education system or the hospital practices with regard to TB, during the past few years.

As the country has many primary care general practitioners (GPs) not attached to Government hospitals, it would be prudent to carry out a similar survey among them to identify any similar deficiencies. Awareness programmes may be organized jointly by NPTC and GP associations. Applicability of Continuation of Medical Education (CME) appraisal systems to both Government and non-Government health sectors in the country, followed by a well structured assessment programme should be explored[12]. Concurrently, hospitals should be provided with necessary facilities such as libraries, internet access, etc.

CONCLUSION

The knowledge on basic aspects of TB is not satisfactory among most of the MOs. We propose to conduct more targeted awareness programmes and to provide with necessary material and facilities to upgrade knowledge. Applicability of Continuation of Medical Education appraisal systems to the country with well structured assessment programmes should be explored. We recommend for antibiotic regulation and prescription policies and specialist supervision of all TB cases.

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DRUG SUSCEPTIBILITY PROFILE OF M.TUBERCULOSIS AMONG CATEGORY – II FAILURE PATIENTS UNDER RNTCP DRUG SUSCEPTIBILITY OF *M. TUBERCULOSIS*

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ABSTRACT

Introduction: The aim of this study was to evaluate level of drug resistance in Directly Observed Treatment, short course (DOTS) CAT-II failure Pulmonary tuberculosis patients admitted for retreatment according to Indian Revised National Tuberculosis Control Programme (RNTCP).

Methodology: From January 2006 to December 2008 sputum samples were collected from all patients of DOTS CAT-II failure and transported to laboratory for *Mycobacterium tuberculosis* culture and drug susceptibility testing (DST). Category II failure pulmonary TB includes those patients who remained sputum positive after 5 months of CAT- II TB treatment. AFB culture was done on Lowan stein Jenson slopes (Solid culture), at Choithram hospital and research center which is RNTCP accredited laboratory.

Results: DST results were available for 148 sputum smear positive DOTS CAT-II failure patients. Mean age of the patients were 33.96 years (range 15-65 years), male to female ratio was 1.79:1. Of the 148 patients, 50(33.78%) had Multidrug-resistant tuberculosis (MDR-TB) and 11(7.43%) had extensively drug-resistant tuberculosis XDR-TB. Out of 148 patients, 80(54.05%) had treatment after default and 68(45.94%) had treatment failure. The prevalence of MDR-TB and XDR-TB among category-II failure pulmonary tuberculosis patients was 33.7 and 7.43 per cent.

Conclusion: The prevalence of MDR-TB strains was dramatically high among patients with pulmonary tuberculosis who failed category II therapy. Capacity of drug sensitivity testing is essential for continuous monitoring of drug resistance trends, in order to assess the efficacy of current programme and epidemiological surveillance for planning.

Key words: Pulmonary tuberculosis, Drug susceptibility testing, Central India

INTRODUCTION

The Directly observed treatment, short course (DOTS) strategy is endorsed by the World Health Organization (WHO) as a current standard for tuberculosis treatment has been adopted for use by TB control programme in 148 countries.[1] The efficacy of DOTS in the treatment and control of TB is widely recognized.[2-5]. However, treatment failures occur that are most often due to limited resources, inadequate re-treatment regimens and incomplete treatment and increasing the risk of multi-drug resistant tuberculosis (MDR-TB). Since 1998 their has been a rapid expansion of the DOTS – based Revised National Tuberculosis Control Programme (RNTCP) in India. With such a large DOTS based

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programme in place, there is a need to assess the drug susceptibility profile among previously treated patients among programme condition. Drug resistance surveillance is considered useful tool to assess the effective functioning of tuberculosis (TB) control programmes.[6] Levels of drug resistance and its trends vary from place to place and serve as an epidemiological indicator to assess the extend of resistant bacterial transmission in the community.[7] Drug resistance levels among patients treated under TB control programmes are not available in many settings.

In poorer regions, DST is not routinely available. Empiric treatment regimens (ETRs) are generally customized to an individual's treatment and contact history but used in the absence of, or while awaiting, DST results.

We conducted this prospective analysis to evaluate level of drug resistance in DOTS CAT-II failure patients.

METHODOLOGY

A total 148 randomly selected patients having clinical and or radiological features of tuberculosis attending out patients department of MGM Medical College & MY Hospital, Indore were enrolled in this study during January 2006 to December, 2008. Approval for the study was obtained from the institutional ethics committee. All patients gave informed consent to participate in the study.

MY Hospital, the tertiary referral hospital for Indore District covering about 5,000,000 population Choithram hospital was the only laboratory performing Mycobacterial cultures in that period.

Subjects attending to hospital with the history of received DOTS CAT- II and complaint of cough with expectoration were screened for sputum smear microscopy using the Ziehl- Neelsen method. All participants had active pulmonary TB disease, and had failed previous DOTS CAT-II treatment.

Sputum samples were collected from all patients of DOTS CAT-II failure. Patients were provided with sterile bottles to collect sputum samples for culture and sensitivity test.

Sputum were collected and processed for culture by digestion, decontamination and concentration following modified Petroff's method and were inoculated on to two slopes of Lowenstein- Jensen (L-J) media for six weeks.

Sputum samples were process for culture of M. tuberculosis on Lowenstein - Jensen (LJ) slopes containing 0.5% pyruvate, glycerol and PNB (p-nitrobenzoic acid 500 mg/l) incubated at 370C for upto 6 weeks at Choithram hospital and research center which is RNTCP accredited laboratory. Culture positive for *M. tuberculosis*, with more than 10 colonies grown after 6 weeks of incubation were subjected to sensitivity testing by the simplified proportion method for Isoniazid (H), Rifampicin (R), Pyrazinamide (PZA), Ethambutol (E), Streptomycin (S), Para amino salicylic acid (PAS), Ethionamide (Et), Ciprofloxacin (C), and Kanamycin (K).

Drug susceptibility testing for Pyrazinamide was performed after making media acidic. Data was handled and analyzed in MS Office, Excel.

Inclusion criteria

DOTS CAT-II failure (Category II failure pulmonary TB includes those patients who remained sputum positive after 5 months of CAT - II TB treatment.)

Exclusion criteria

The following patients were excluded from this study: (i) presence of secondary immunodeficiency states like HIV, organ transplantation, diabetes mellitus, malignancy, (ii) hepatitis B infection; (iii) seriously ill and moribund patients with very low lung reserve (iv) abnormal renal function.

Information on associated factors for Drug resistance, such as age, sex, history of alcoholism, smoking, occupation, family history of contact with PTB, personal history of contact with PTB and prior antitubercular treatment received (Table 1).

RESULTS

Table 1. Showing associated factor for drug resistance (n=148)

Risk factor	No. of patients	Percentage
History of Smoking	15	10.13%
History of alcoholism	9	6.08%
Both smoking & alcoholism	40	27.02%
Family history of PTB	54	36.48%
History of contact with known PTB person	41	27.70%
Laborers	89	60.13%

A final total of 148 cases were included in the study, male 95 (64.18%) and female 53 (35.81%). Male to female ratio was 1.79:1. Mean age of the patients were 33.96 years (range 15-65 years).

Of 148 patients, 50 (33.78%) patients was resistant to at least Isoniazid and Rifampicin (MDR-TB) and 11 (7.43%) were resistant to Kanamycin and Ciprofloxacin along with Isoniazid and Rifampicin (XDR-TB).

The most common single drug resistance was of Isoniazid which was present in 11 (7.43%) patients followed by Streptomycin and Pyrazinamide. In 53 (35.81%) patients, resistance to more than 4 drugs were present (Table 2).

Table 2. Drug susceptibility profile among CAT-II failure patients (n=148)

Resistant to	No	Percentage
H	11	7.43%
R	3	2.02%
S	4	2.70%
Z	4	2.70%
SH	2	1.35%
SHE	1	0.67%
SHEZ	5	3.37%
RS	3	2.02%
RSE	1	0.67%
HR	50	33.78%
HRKC	11	7.43%
More than 4 Drugs	53	35.81%

When we analyzed associated factors in DOTS CAT-II failure patients, family history of contact with PTB was present in 54(36.48%), history of contact with PTB in 41(27.70%).Of the 148 patients, 64(43.24%) were addicted to either alcohol, smoking or both.

Out of 148 patients, 80 (54.05%) had treatment after default and 68 (45.94%) had treatment failure.

DISCUSSION

The main finding of the above study among 148 patients of DOT CAT- II failure 50 (33.78%) patients were MDR, and 11 (7.43%) patients were XDR-TB. 22 (14.86%) were resistant to at least Isoniazid, Rifampicin, Pyrazinamide and Streptomycin and 53 (35.81%) were resistant to more than 4 drugs. Patients with resistant to first line drugs were 95 (64.18%). The prevalence of MDR-TB among patients of DOT CAT - II failure was dramatically higher overall then among the previously detected patients in the WHO (20% MDR-TB, 2% XDR-TB) study. The National Tuberculosis Institute (NTI) Bangalore, reported that 77% of 271 patients treated with Cat - I regimen and organism susceptible to SM, INH and Rifampicin and 2.2 % had HR resistance.[8] Among 226 patients treated with the Cat-II regimen, 59% had drug-susceptible organism, 27.4% had INH-resistant and 12.8% had HR-resistance.[9] Whereas study from South India reported resistant to both INH and RMP was 1.7% among newly diagnosed patient and 12% in previously treated patients. The reminder had single drug resistance.[10] Malhotra B, Gupta PR et al have reported higher prevalence of MDR-TB.[11-12]

A high prevalence of MDR – TB in CAT- II failure is not unique to India and has been documented in Vietnam[13], Thailand[14], Rwanda[15] in low to moderate risk groups.

Routine surveillance of drug resistant profiles found in specific populations of previously treated patients provides information that is useful for adapting strategies for effective treatment within National tuberculosis programmes (NTP's) and is

essential for the care of persons with chronic active tuberculosis disease.

In the present study there was a large proportion of MDR –TB patients. If this findings is correct, it would mean that a high degree of resistant strains is already circulating in the community. In recent years, the WHO launched DOTS Plus initiate to procure second-line drugs at a lower cost that will facilitate the treatment of the many patients who currently require it. Establishment of programmes for the treatment of MDR-TB patients, when back by secure source of funds and technical assistance could strengthen an NTP. Several studies have estimated the prevalence of patients with resistant bacilli in specific locations and in recent years the IUATLD and the WHO have conducted large surveys to ascertain the importance of resistant TB throughout the world, utilizing a systemic approach and common laboratory techniques.[6-16]

CONCLUSION

The prevalence of MDR-TB strains was dramatically high among patients with pulmonary tuberculosis who failed category II therapy. Capacity of drug sensitivity testing is essential for continuous monitoring of drug resistance trends, in order to assess the efficacy of current programme and epidemiological surveillance for planning.

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A PROFILE OF PATIENTS REGISTERED AT ART CENTRE AT SURAT MUNICIPAL INSTITUTE OF MEDICAL EDUCATION & RESEARCH IN SURAT CITY, GUJARAT, INDIA

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ABSTRACT

Introduction: An estimated 2.4 million Indians are currently living with HIV. In India, the antiretroviral treatment program started with a free ART treatment in 2003. This study was conducted in order to understand the clinico-epidemiological profile of patients attending ART centre and the effectiveness of the therapy.

Methodology: A cross sectional study was conducted at an ART center of Surat Municipal Institute of Medical Education and Research (SMIMER), in Surat city of Gujarat State. The data of 2357 PLHAs (People Living with HIV/ AIDS) registered at ART centre in one year time duration starting from 21st January 2010 was included in the study.

Results: Among the total 2357 subjects, 1483 (63%) were males. In our study 42.3% of patients were between 31 to 40 years of age. Among the employed subjects, 32.9% of patients were laborer. Voluntary Counseling and Testing Centre (VCTC) was most common entry point of patient with 50% followed by private practitioner with 23% and NGOs with 6.2%. Other entry points are self referred, RNTCP etc. Tuberculosis and diarrhoea were the most common opportunistic infections. There was significant improvement in CD4 count, bodyweight and functional status of the subjects after receiving the ART for an average duration of 6 months.

Conclusion: The economically productive & sexually active people and those with lesser education are at higher risk of becoming sero-positive. The subjects showed significant improvement after receiving ART with respect to the CD4 count and average body weight.

Key words: *ART, Sero-positives, Opportunistic infections, VCTC*

INTRODUCTION

The first case of HIV/AIDS in India was identified in Chennai in 1986.[1] And 24 years later, around 2.4 million Indians are HIV positive.[2] According to the National AIDS Control Organization HIV prevalence rate in India is 0.34% (0.25%-0.43%).[3] The prevalence rate of HIV in Gujarat is 0.25%.[4] As HIV treatment programs are implemented across the world, increasing numbers of HIV-infected persons are being treated with antiretroviral therapy (ART). In India, the antiretroviral treatment program started with a free ART treatment in 2003. Although data gathered by National AIDS Control Organization (NACO) in 2007 has revealed that HIV prevalence has stabilized, at least in Tamil Nadu,

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Andhra Pradesh, Karnataka and Maharashtra, it is increasing in at-risk populations in other states. As a result, overall HIV prevalence has continued to rise.[5] Despite the fact that ART is provided free by the government, there are a large number of sero-positive people who do not come forward to receive treatment. Non-adherence is another aspect even when they come for ART. These problems affect the overall success of the programme.[6] There is a need to study the profile of patients who come to ART centres and their clinical and socio demographic profile. Therefore, the study was conducted in order to understand the clinico-epidemiological profile of patients attending ART centres and the effectiveness of the therapy. This information can be utilized to enhance the utility and adherence to therapy among attendees.

METHODOLOGY

A cross sectional study was conducted at an ART center of Surat Municipal Institute of Medical Education and Research (SMIMER), a tertiary care hospital and one of the teaching hospitals in Surat city of Gujarat State. Surat is a rapidly developing area, resulted in a high number of migrating and transit populations contributing to the HIV/AIDS disease burden in the area. The SMIMER hospital caters to patients from neighboring districts also. The data of 2357 PLHAs (People Living with HIV/AIDS) registered at ART centre in one year time duration starting from 21st January 2010 was included in the study. Permission was obtained from the Gujarat State AIDS Control Society (GSACS) and the in charge Medical Officer of the ART centre; following which the data was collected using the records of the PLHAs. The demographic information of the subjects was collected along with the clinical profile, place of referral for ART and the treatment details. The clinical staging was done according to the WHO clinical staging of HIV/AIDS (2005) Stage I: HIV infection is asymptomatic and not categorized as AIDS Stage II: includes minor mucocutaneous manifestations and recurrent upper respiratory tract infections and unexplained weight loss (<10% of presumed or measured body weight) Stage III: includes unexplained chronic diarrhea for longer than a month, severe bacterial infections and pulmonary tuberculosis and unexplained weight loss (>10% of presumed or measured body weight) Stage IV: includes toxoplasmosis of the brain, candidiasis of the esophagus, trachea, bronchi or lungs and Kaposi's sarcoma; these diseases are indicators of AIDS.

The functional status of the subjects was done as working, ambulatory and bed ridden.7 The records with incomplete information were excluded from the study. Data was entered and analyzed using Epi-info statistical software and the results presented as proportions in the form of tables. For statistical analysis, paired t-test test was used and $p < 0.05$ was considered significant.

RESULTS

Socio-demographic profile of sero-positives

Our study included 2357 sero-positive patients who had received ART. Table 1 shows the demographic profile of the study population. Among the subjects, 1483 (63%) were males. In our study 42.3% of patients were between 31 to 40 years of age. Interesting to note that, out of total 862 female patients, maximum 344 (40%) were from age group of 20 to 30 years. Where as, maximum number of male patients that is 688 (46.4%) were from age group of 30 to 40 years.

Table 1. Socio-demographic characteristics of the study population (n=2357)

Characteristic		Males (%)	Females (%)	Transgender (%)	Total (%)
Age group (years) n=2354 [Data of 3 patients not available]	≤10	36(2.4)	25(2.9)	0	61(2.6)
	11-20	25(1.7)	36(4.2)	0	61(2.6)
	21-30	342(23.1)	344(40.0)	5(55.6)	691(29.4)
	31-40	688(46.4)	304(35.3)	4(44.4)	996(42.3)
	40-50	296(19.9)	118(13.7)	0	414(17.6)
	>50	96(6.5)	35(4.1)	0	131(5.5)

Table 1. Continuous

Marital status n=2354 [Data of 3 patients not available]	Unmarried	171(11.5)	36(4.2)	5(55.6)	212(9.0)
	Married	1150(77.6)	575(66.7)	2(22.2)	1727(73.4)
	Divorced	38(2.6)	21(2.4)	0	59(2.5)
	Widow/ Widower	86(5.8)	210(24.4)	0	296(12.6)
	Separated	36(2.4)	20(2.3)	2(22.2)	58(2.5)
	Live-in Relationship	1(0.1)	1(0.1)	0	2(0.1)
Educational Status(n=2350) [Data of 7 patients not available]	Illiterate	273(18.4)	307(35.7)	2(22.2)	582(24.8)
	Primary School	666(45.0)	301(35.0)	4(44.4)	971(41.3)
	Secondary School	497(33.6)	226(26.2)	3(33.3)	726(30.9)
	Collage & above	44(3.0)	21(3.1)	0	71(3.0)
Employment Status n=2357	Unemployed	130(8.8)	75(8.7)	0	205(8.7)
	Student	31(2.1)	17(2.0)	0	48(2.0)
	Service	539(36.3)	21(3.3)	1(11.1)	569(24.2)
	Self-employed	155(10.5)	12(1.4)	3(33.3)	170(7.2)
	Labourer	626(42.2)	144(16.7)	5(55.6)	775(32.9)
	Housewife	2(0.1)	588(68.1)	0	590(25.0)

Around 9% of patients were unmarried. Overall, 86.3% of the subjects were from Choriyasi taluka of Surat District. Among the total 862 female attendees 588 (68.1%) were housewife. Among the employed subjects, 32.9% of patients were labourer. Voluntary Counseling and Testing Centre (VCTC) was most common entry point of patient with 50% followed by private practitioner with 23% and NGOs with 6.2%. (Table 2).

Table 2. Entry point of PLHAs (n= 2347)*

Entry Point	Males (%)	Females (%)	Transgender (%)	Total (%)
VCTC	712(48.4)	452(52.8)	5(55.6)	1169(50.0)
TB/RNTCP	44(3.0)	10(1.2)	0	54(2.3)
PPTCT	13(0.9)	47(5.5)	0	60(2.6)
Private	363(24.7)	174(20.3)	1(11.1)	538(23.0)
NGO	110(7.5)	35(4.1)	1(11.1)	146(6.2)
Self referred	66(7.7)	50(3.4)	2(22.2)	118(5.0)
Other	164(11.1)	88(10.3)	0	262(11.2)

* Data of 10 patients are not available

Clinical profile of study subjects

The functional status of sero-positive attendees revealed (Table 3) that 89.9% of the study subjects were in "working", 8.3% were in "ambulatory" stage and 1.8% were bed-ridden. As per the WHO staging, at the time of registration, 60.6% of subjects were in stage 1 and 18.9% were in Stage 2 and 14.70% were from stage 3 (Table 4). Among total attendees 8.10% had Tuberculosis.

Table 3. Functionality status of subjects (n=2336)*

Characteristic	Male (%)	Female (%)	Transgender (%)	Total (%)
Working	1301(88.4)	792(92.6)	8(88.9)	2101(89.9)
Ambulatory	139(9.4)	55(6.4)	0	194(8.3)
Bedridden	32(2.2)	8(0.9)	1(11.1)	41(1.8)

* Data of 21 patients are not available

Table 4. WHO clinical staging of HIV/AIDS (During first visit) (n=2327)*

WHO Stage	Male (%)	Female (%)	Transgender (%)	Total (%)
Stage 1	547(64.4)	858(58.4)	6(66.7)	1411(60.6)
Stage 2	176(20.7)	263(17.9)	1(11.1)	440(18.9)
Stage 3	98(11.5)	244(16.6)	1(11.1)	343(14.7)
Stage 4	29(3.4)	103(7.0)	1(11.1)	133(5.7)

* Data of 20 patients are not available

Health status of the sero-positives before and after ART

Table 5 shows the comparison of the CD4 count and weight before and after receiving the ART of 1346 PLHAs. The mean CD4 count and the body weight of the subjects increased after receiving ART. There was a statistically significant difference in both CD4 count and weight; before and after receiving the ART on an average for 6 months. (Paired t- test and <0.05 – level of statistical significance).

Table 5. Comparison of Health status of the subjects before and after receiving ART (n=1346)*

Characteristic	Before ART (n=1346)	After ART (n=1346)	Difference of Mean	p value
CD4 count Mean (95% CI)	156.24 (151.41 - 161.07)	328.91 (322.54 - 335.28)	172.67	<0.0001#
Weight (kg.) Mean (95% CI)	43.81 (43.19 - 44.43)	51.42 (50.80 - 52.04)	7.61	<0.0001#

* Out of total 2357 PLWHA registered, 1346 were put on ART

Paired t-test and <0.05 – level of statistical significance

DISCUSSION

The present study revealed that males constituted 63% of the total subjects which was almost equal to National figures, i.e. 61%.[5] Result of other study done at Manglore shows 64.4% of patients were male, which is consistent with results of our study.[8] This is similar to the findings in a study conducted in the Udupi District by Kumar A et al.[9] Similar observation was made by Sarna A et al6 and Cauldbeck et al in Banglore[10], where majority of the attendees were male(84%).

Majority of the subjects belonged to the age group 31-40years (42.3%). Our study results were consistent with that of study by Cauldbeck et al which had 50% of the subjects in 30 to 40 years age group and the overall mean age of the subjects 39.9 years.10 The distribution according to marital status showed that unmarried subjects constituted only 9%. In comparison with study done at Manglore which had 58.45% married patient registered, our study shows higher number of them registered at the ART centre.8 This indicates more number of married patients taking ART at the centre and important role of PPTCT in the institution.

The distribution according to educational status showed that the sero-positivity was higher among the subjects with lesser education. These findings

are similar to the study conducted by Jayaram S et al[11] and to that of the study conducted by Safren SA et al (17%)[12] though the number of subjects with primary education was lesser than those with secondary education and above. But Cauldbeck et al observed no trends for education level with respect to the seropositivity.[10]

In our study, maximum (32.9%) patients were labourer, reveals more prevalence of HIV in this group of people. Low education status, migration and less awareness regarding safe sex can be the reason for high prevalence among this group of people.

Among the total 862 female attendees 588 (68.1%) were housewife. This result is higher than the study conducted by Kumar A et al (44.5%).[9]

The reason for more number of attendees those were housewife can be gaining of infection by unsafe sex from their HIV positive husband or any other sexual partner. This point-out the unsafe sexual practices among married couple.

In our study, 86.3% of the subjects were from Choriyasi taluka of Surat District. In the present study VCTC centre was most common entry point of patient with 50% followed by private practitioner with 23% and NGOs with 6%. Other entry points like PPTCT, TB, In and Out patients, PLHA network, MSM network and STI clinic were contributing less than 5%. Patients self-referral rate was around 5%.

Study at Manglore shows that 84.1% have availed the services through VCTC and 9.4% referred from NGO.[8] This point out lower rate of referring from VCTC at Surat. And there is a need to focus to lower the loss of patient from VCTC.

Tuberculosis and diarrhoea were the most common opportunistic infections. These findings were similar to the study conducted by Sharma SK et al (71%) in North India[13] and Kumarasamy N et al in South India.[14] In our study, the health status of seropositives revealed improvement in the CD4 count, average body weight and functional status of the subjects after receiving ART indicating efficacy of ART (Table 5). Pant Pai N et al in San Francisco also observed an improvement in CD4 cell counts after continuous ART therapy for HIV/AIDS.[15]

CONCLUSION

More than half of the subjects were in economically productive age group and majority of patients who sought ART were males. People from outside the district under study also sought ART from the study area. TB and diarrhoea were the most common opportunistic infections. The subjects showed significant improvement in CD4 count and average body weight after receiving ART.

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MOLECULAR CHARACTERIZATION AND COMPARISON OF MULTI DRUG RESISTANT STRAINS OF MYCOBACTERIUM TUBERCULOSIS BY PHENOTYPIC AND GENOTYPIC METHOD

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ABSTRACT

Introduction: Drug resistant tuberculosis is a significant threat to tuberculosis control because only a few effective drugs are available against *M. tuberculosis*. The aims of this study were to compare multi drug resistant (MDR) strains of tuberculosis by phenotypic and genotypic method and determine type, location and frequency of *rpoB* and *KatG* gene mutations.

Methodology: Anti-tuberculosis drugs susceptibility test of *M. tuberculosis* grown on Lowenstein Jensen medium was performed by proportion method. MDR cases were analyzed for mutation of rpoB and KatG genes. The regions of these genes were amplified by polymerase chain reaction (PCR) and sequenced.

Results: Two different mutations were identified in rifampicin resistant strains. The most common point mutations were in codons TCG 531→TTG (85%) and GAC 516→TTC (15%) of the *rpoB* gene. Two different mutations in *KatG* gene were detected. The most common *KatG* point mutations were AGC 315 ACC (Ser→Thr) (85%) and CGG 463 CTG (Arg→Leu) (10%). In this study DNA sequencing analysis did not find mutation on *KatG* gene of one of the strain tested. Male and female were equally affected by MDR tuberculosis and majorities (35%) of them were found in 21-30 years age group.

Conclusion: The present investigation agrees that genetic mutation is responsible for change in phenotypic characteristics of M. tuberculosis.

Key words: *Mycobacterium tuberculosis*, Multi drug resistant, *KatG, rpoB*

INTRODUCTION

The complete genome of *M. tuberculosis* strain has been mapped as a length of about 4. 4Mb, However, each gene of *M. tuberculosis* may have separate function including the specific complex formation with important drugs being used as tuberculosis treatment regime. Unusual genetic alteration of bacterial genes leads to the development of drug resistance.

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The *KatG* encodes catalase-peroxidase which is necessary to activate INH to a toxic substance in the bacterial cell.[1] This toxic substance subsequently affects intracellular targets such as mycolic acid biosynthesis which eventually results in loss of cellular integrity and the bacteria die.[2] Middlebrook et al. initially demonstrated that a loss of catalase activity can result in INH resistance.[3] The majority of INH-resistant clinical isolates become resistant by losing or altering *KatG* activity, nevertheless, only *KatG* mutations do not account for all observed INH resistance, but mutations in another putative INH

target called inhA also. The Ser315Thr substitution is estimated to occur in 30–60% of INH resistant isolates.[4,5,6] The *KatG* (CGG463CTG) (Arg-Leu) amino acid substitutions is the most common polymorphism found in the *KatG* gene and is not associated with INH resistance.

Rifampicin (RIF) binds to the β-subunit of DNA-dependent RNA polymerase hindering transcription and thereby killing the organism. Extensive studies on the *rpoB* gene in RIF resistant isolates of *M. tuberculosis* identified a variety of mutations and short deletions in the gene. A total of 69 single nucleotide changes; 3 insertions, 16 deletions and 38 multiple nucleotide changes have been reported.[7] More than 95% of all missense mutations are located in a 81bp core region (Rifampicin resistance determining region) of the *rpoB* gene between codons 507–533 with the most common changes in codons Ser531Leu, His526Tyr and Asp516Val. These changes occur in more than 70% of RIF resistant isolates.[4, 8,7]

Drug susceptibility testing (DST) of *M. tuberculosis* in clinical specimens is time-consuming. INH and RIF are crucial elements of the standard treatment regimen of tuberculosis, and resistance to these drugs requires extension of therapy.[9] Proportion method is a widely used method, especially in resource-limited settings (RLS). It uses solid media, Lowenstein-Jensen to determine the proportion of resistant mutants to a given drug. Its turnaround time (TAT) is between 4-6 weeks.[10, 11] The vast majority of RIF resistance is caused by mutations located in the 81-bp region of the *rpoB* gene.[12] INH resistances are more complex, as the mutations conferring resistance are located in several genes and loci. INH resistance has been associated mainly with mutations in *KatG, inhA, ahpC,* and *kas.A.*[13-16] Sequencing of PCR-amplified products of *rpoB* and *KatG* has become the most widely used genotypic method for detecting drug resistance in *M. tuberculosis*; it is accurate and reliable and it has become the reference standard for mutation detection.[17] DNA sequencing has been widely used for characterizing mutations in the *rpoB* gene in RIF-resistant strains and to detect mutations responsible for resistance to other anti-tuberculosis drugs.[12, 18, 19]

Aims of this study were to compare multi drug resistant cases of tuberculosis by phenotypic and genotypic method and determine types, location and frequency of mutation on *rpoB* and *KatG*.

METHODOLOGY

This study was carried out at the GENTUP Kathmandu and HNB Garhwal University during January 2008 to December 2008. Ethical approval was taken. Data on the MDR-TB patients were collected and recorded on a standardized form. The data were collected on age, gender and type of disease (new or old). This study included 9 primary and 11 acquired drug resistant cases. The research objectives and methods were explained to the patients and verbal consent obtained from them before the sputum samples were collected. The cases were selected using random sampling technique. Drug susceptibility test was performed on *M. tuberculosis* isolates by proportion method as standard protocol.20 *M. tuberculosis* strains were tested against four antibiotics used in DOTS program of Nepal such as isoniazid, rifampicin, ethambutol and streptomycin.

Twenty multi drug resistant strains of *M. tuberculosis* were screened for mutations of *rpoB* and *KatG* gene associated with resistance to rifampicin and isoniazid respectively by PCR-DNA sequencing method. Spin column method was used for extracting *Mycobacterium tuberculosis* DNA following the manufacturer instructions.[21] The estimation of the extracted DNA from the samples was carried out using spectrophotometry method.22 A 210-bp and 750-bp segment of the *katG* gene and 411-bp fragments of the *rpoB* gene, containing the sequence of the 157-bp fragment were amplified by standard polymerase chain reaction (PCR).[23]

PCR amplified products were sequenced directly on an Applied Bio-systems ABI Prism 3100-Avant automated DNA sequencer. Sequencing was done with big dye terminator cycle sequencing kit from ABI, following the manufacturer's instruction.

RESULTS

Age wise distribution of MDR cases is shown in figure 1. Majority, 7 (35%) of the MDR cases of tuberculosis were found in 21-30 years age group. Similarly gender wise distribution of MDR cases of tuberculosis were found equal in both the gender. Among twenty strains of MDR *M. tuberculosis* 15% and 40% were sensitive to streptomycin and ethambutol respectively. PCR-DNA sequencing results of MDR strains found 100% and 95% mutation in *rpoB* and *KatG* respectively and they correlated well with the phenotypic method (Table 1).

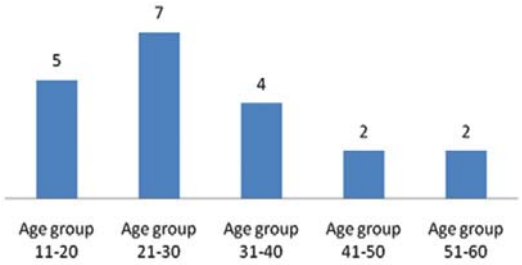

Figure 1. Age wise distribution of MDR strains

Table 1. DNA sequencing results of MDR strains of *Mycobacterium tuberculosis*

S. No	Age	Sex	*rpoB* gene	*KatG* gene	Case
1	60	M	TCG 531 TTG	S 315 T	New
2	42	F	TCG531 TTG	S 315 T	Old
3	25	F	TCG531 TTG	G 463 T	Old
4	24	F	TCG531 TTG	S 315T	New
5	15	F	GAC516 TTC	S 315T	Old
6	40	M	TCG531 TTG	S 315T	Old
7	16	M	TCG531 TTG	S 315 T	New
8	35	M	TCG531 TTG	S 315 T	New
9	17	F	TCG531 TTG	S 315 T	New
10	38	M	TCG531 TTG	S 315 T	Old
11	23	F	TCG531 TTG	S 315 T	New
12	16	F	GAC516 TTC	S 315T	New
13	22	M	TCG531 TTG	S 315 T	Old
14	30	M	TCG531 TTG	S 315 T	Old
15	56	M	TCG531 TTG	S 315 T	Old
16	25	M	GAC516-TTC		Old
17	17	F	TCG531-TTG	S 315 T	New
18	36	F	TCG531-TTG	S 315 T	New
19	35	M	TCG531-TTG	G 463 T	Old
20	45	F	TCG531-TTG	S 315 T	Old

DISCUSSION

The mycobacterium uses various mechanisms to evade killing by drugs, including mutations in genes that code for drug target proteins, a complex cell wall which blocks drug entry, and membrane proteins that act as drug effux pumps.[24, 25] Along with HIV/AIDS, MDR-TB is the most important threat to TB control. Countries with a high MDR-TB prevalence generally have a history of poor TB control. There are both preventive and restorative strategies to combat resistance –DOTS and DOTS-Plus. The major barrier to MDR-TB treatment is the high cost of second line drugs which are at least 300 times more expensive than first line drugs based on Green Light Committee (GLC) prices and between 1000-3000 times more expensive when market prices are used. Additional barriers include extensive laboratory requirements to conduct culture and drug susceptibility testing (DST), severe adverse events associated with second-line drugs and fear of development of resistance to second line drugs. The misuse of second line drugs could lead to the creation of TB strains resistant to all known anti-TB drugs.[26]

Twenty MDR cases of *M. tuberculosis* were included in this study. Two loci associated with drug resistance were selected for characterization viz., *rpoB* (RIF) and *KatG* (INH). Two different mutations were identified in rifampicin resistant *M. tuberculosis* strains. The most common point mutations were in codons 531 (85%) followed by 516 (15%) of the *ropB* respectively. Similar study conducted by Sajduda et al. (2004)27 in Poland showed that nineteen different mutations were identified in 64 rifampicin resistant strains, and five new alleles were described. The most common point mutations were in codons 531 (41%), 516 (16%), and 526 (9%) of the *rpoB* gene. These findings are in agreement with those reported by Spindola de Miranda *et al.* (2001)[28] the later showed that among rifampicin resistant strains a double point mutation which had not been reported before was detected in one strain from France. The mutations were found in codons 531 (31.2%), 526, 513 and 533 (18.7% each). In Brazilian strains the most common mutations were in codons 531 (72.2%), 526 (11.1%) and 513 (5.5%). The heterogeneity

found in French strains may be related to the fact that most of those strains were from African or Asian patients.

PCR-DNA sequencing of isoniazid resistant *M. tuberculosis* strains identified two different types of mutations in *KatG*. The most common point mutations were in codons 315 (85%), AGC→ACC (Ser→Thr) followed by 463 (10%) CGG→CTG (Arg→Leu) of the *KatG*. Mutation were not found in 1 (5%) in KatG of the isolate. Similar study conducted in Bostanabad et al. (2008)[29], showed that most mutations were in *KatG* gene codons 315, 316 and 309. Four types of mutations were identified in codon 315: AGC→ACC (85%), AGC→AGG (2.3%), AGC→AAC (4.7%), AGC→GGC (2.3%). The highest frequency of mutations sharing between primary and secondary infection was found in codon 315. Another similar study conducted by Sajduda et al. (2004)[27] in Poland showed that six different mutations in the *KatG* gene of 83 resistant strains were detected. Fifty-seven (69%) isolates exhibited nucleotide substitutions at codon 315. The majority of hot mutations in *katG* gene of *M. tuberculosis* have been reported in codon 315 (Ser→Thr) and less in other codons.[30,31,32] Most reports suggest that resistance of *M. tuberculosis* to isoniazid mostly corresponds to changes in codon 315.[31,33] Finding of this study were similar with 85% of all isolates showing mutation in codon 315.

In this study sequencing analysis did not find mutation on *KatG* gene of one (5%) of the strain tested, although that strain was resistant to isoniazid as determined by the proportion method. Other study revealed that a mutation associated with isoniazid resistance can also be located outside the *KatG* gene such as *inhA* gene, *kasA* gene, *ndh* gene and *ahpC* gene.[27,34] Although this does not occur so frequently. In this case mutation may present to other genes. Other possibilities are that in this resistant strain other mechanism of resistance may be involved.

CONCLUSION

PCR-DNA sequencing shows MDR strains of *M. tuberculosis* isolates has mutation on *rpoB* gene and 95% strains had mutation on *KatG* gene. The most common point mutations were found in codons 531 (85%) of the *ropB* gene and codons 315 (85%) of the KatG gene. This study showed that genetic changes in the *rpoB* and *KatG* genes were more consistently associated with resistance phenotype.

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NATIONAL EXTERNAL QUALITY ASSURANCE SCHEME FOR HIV TESTING USING DRIED BLOOD SPOT: A FEASIBILITY STUDY

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ABSTRACT

Introduction: Dried Blood Spot (DBS) for National External Quality Assurance Scheme (NEQAS) is becoming popular in resource limited and geographically constrained settings. The feasibility of using DBS for EQAS for HIV testing in Nepal is studied.

Methodology: National Public Health Laboratory (NPHL) and five Voluntary Counseling and Testing Centers (VCT) located at the Mid-Western and Central region of Nepal were chosen as the organizing and participating laboratories, respectively. HIV tests were performed on 35 samples using rapid test kits following the National algorithm. Two sets of samples were blotted on a DBS card, dried, packed, stored and posted to NPHL using normal postal delivery. The eluted DBS were tested by rapid test and ELISA and results were compared.

Results: All DBS cards were received in a good condition. The rapid test result obtained at VCT and NPHL was 100% concordant. By ELISA, 32 (91.4%) and 3 (8.6%) samples were concordant and discordant, respectively. The sensitivity and specificity of the rapid test were 88.9% and 94.0%, respectively. The DBS was stable with mean transportation time of 2.25 days and storage time of one month.

Conclusion: DBS is an easy, simple, cost effective and a dynamic platform for blood collection in a country like Nepal and can be considered for implementation for NEQAS for HIV testing.

Key words: Dried Blood Spot, External Quality Assurance Scheme, HIV Testing, Nepal

INTRODUCTION

The diagnosis of HIV relies on accuracy of HIV testing laboratory services in the country and accuracy of the result depend upon pre-analytic, analytic and post-analytic steps. Therefore to ensure accurate test results, it is now accepted that quality assurance, quality control and quality assessment constitutes an integral part of all HIV diagnostic testing available globally. This is only possible through development of External Quality Assurance Scheme (EQAS) for HIV/AIDS testing. There exist three types of EQAS; proficiency testing, on site validation and retesting. Retesting as the means of EQAS is becoming popular these days in resource limited settings and geographically diverse countries where the whole blood can be collected in filter paper, dried at room temperature and then mailed through regular post to the organizing laboratory.[1]

The use of whole blood in a filter paper for screening phenylketonuria in infants date back to

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1973[2] and this technique was first evaluated for HIV-1 sero-prevalence study in pregnant women in 1988.[3] Preparing of dried blood spot (DBS) is simple, doesn't need advance expertise, is cost effective and remains stable at room temperature for a month and for 2 years at -20°C.1,[4] The DNA as well as proteins remain stable in the filter paper and is appropriate for testing HIV antibodies along with genome detection for diagnosis and viral load monitoring.[5,6,7] The eluted whole blood from a DBS can be tested using ELISA and western blot and the use of rapid test kits (Unigold, Determine and Oraquick) has also been validated.[8] The DBS could be an EQA material for HIV testing in resource limited country like Nepal. Hence, we have studied the feasibility of DBS as a material for HIV EQAS in Nepal.

METHODOLOGY

This study was conducted between October to December 2009. National Public Health laboratory (NPHL), Kathmandu was chosen as the organizing laboratory and 5 VCT centers from Central Development Region (Bharatpur Hospital (BH), Family Planning Association of Nepal (FPAN) and Mid-Western Region (Western regional Hospital (WRH), Nepal Red Cross Society (NRCS) and Walling Primary Health Care Center (WPHC)) were chosen as participating laboratories. These sites are run by government and non-government organizations and are located at different geographical locations. BH, FPAN, WHR, NRCS and Walling VCT center are at 155, 157, 200, 200 and 270 kilometers from NPHL, respectively.

Sample collection, testing, storage and transportation from the participating laboratories

With all aseptic precautions and fulfilling the norms of VCT centers, whole blood from the clients visiting the VCT centers was collected in two vials, with or without Ethylenediamine tetraacetic acid (EDTA). Thirty five paired samples (for onsite HIV testing and for preparation of DBS cards) were evaluated for this study. The serum was separated from non-EDTA vial for onsite HIV test using HIV rapid test kits following the National serial algorithm which is based on WHO/UNAIDS (Determine; test 1, Unigold; test 2 and SD Bioloine; tie-breaker test).[1] The samples were spotted in a DBS card from an EDTA whole blood following WHO guidelines.[1] Two DBS cards for each sample were prepared. The DBS card was packed in a small zip-lock bag with some desiccants and was left at room temperature. Ten small zip-lock bags containing DBS cards were finally kept in bigger zip-lock bag along with the sheet mentioning the test result. One set was transported to NPHL immediately and the other was stored at room temperature and posted using normal postal service by the end of the month. The NPHL and Nepal government's criteria for transportation of bio-hazardous materials were met. Pre-analytical, analytical and post-analytical skills of the laboratory staff and space and equipment used in the laboratories were assessed through pre-evaluated questionnaire and checklist.

At the participating laboratory, NPHL

One staff was assigned for receiving, registering and storage of DBS card. The DBS card that was received at NPHL was checked for validity (condition of the DBS card, quality of spots, packing, any deterioration of desiccants and code number) and DBS cards with good quality were registered. Different code number for each DBS card was reassigned and was kept confidential. The cards were left at room temperature if the card was tested within 1 month of the preparation or was stored at -20C, if it was tested after 1 month. The DBS spots from the DBS cards were eluted as per the manufacturer's guidelines (Sigma-Eldrich, Germany). The eluent was tested using rapid test following the same algorithm followed at participating laboratories and was also tested using ELISA (HUMAN, Australia). Different person was assigned to test samples with different assays. Third person was assigned to manage the data. The results were compared between rapid test and ELISA and were also compared with the results that were obtained at the participating laboratory. The result was recorded in Excel spread sheet and was analyzed using Statistical Package for Social Science (SPSS) 15.0 for windows.

RESULTS

Thirty five DBS cards were received at NPHL on 2, 1, 3, 3 days (mean, 2.25 days) (BH, FPAN, WHR and NRCS, respectively) using the normal postal delivery. Samples were not received from WPHC. These 35 posted DBS samples and those transported immediately to NPHL were of good quality and were finally processed. Concordant result was obtained between the two samples received immediately and by postal delivery using serial testing algorithm by rapid HIV test kits (Determine; test 1, Unigold; test 2, SD Bioline, tie-breaker test).

However, when these DBS eluted samples were tested with ELISA some discrepancies were seen. Reactive samples with rapid test were 17 (48.6%) while 18 (51.4%) were reactive with ELISA. Similarly, non-reactive samples with rapid test were 18 (51.4%) and with ELISA were 17 (48.6%). Thirty two samples were concordant between two assays and 3 samples were discordant (Table 1). Altogether, 32 (91.4%) samples were concordant between two assays and 3 (8.6%) samples were discordant.

Table 1. Number of DBS samples tested by Rapid test and ELISA

Sample	Result	Assays			
		*RT		ELISA	
		No.	%	No.	%
DBS	Reactive	17	48.6	18	51.4
	Nonreactive	18	51.4	17	48.6

*RT, Rapid Test

Table 2. Comparison of Rapid test results in different VCT

VCT sites	No. of specimen	ELISA positive	Determine HIV-1/2 positive	ELISA negative	Determine HIV-1/2 negative	FP	FN
BH	24	16	15	8	7	1	1
FPAN	2	0	0	2	2	0	0
WRH	5	1	1	4	4	0	0
NRCS	4	1	0	3	4		1

FP, False positive; FN, False negative

Results obtained at FPAN and WRH were concordant with the results obtained at NPHL using both assays while results obtained at BH showed one false positive and one false negative result (Table 2). Similarly, one false negative result was obtained in sample obtained from NRCS.

Sensitivity of rapid test (Determine HIV-1/2) was determined using the reference assays, ELISA. Sensitivity was 88.9% and specificity was 94%. The positive and negative predictive values were 94% and 88.9%, respectively. Personal and infrastructural need of a laboratory and analytical skills of each staff was assessed using the pre-evaluated questionnaire and checklist. All the laboratories had enough well maintained space for performing rapid test and preparing a DBS card. Staff were adequately assigned for HIV testing and only some staff had formal training on rapid HIV testing. The lines of supervision were clear to the staff and supervisor checked the result before it was dispatched. Most of the equipments were present in all laboratories except stop watches and incinerator in some laboratories. The laboratory waste had been managed according to the bio-safety rules. The micropipettes that had been used for testing were not calibrated in all laboratories. Although the staff were aware of DBS card, none of the staff had hands on practice with it.

DISCUSSION

The feasibility of DBS as a method of sample collection for NEQAS for HIV testing was studied. The DBS cards were transported using normal postal service and were found to be stable. Similar stability of a DBS card has been reported. We tested the DBS eluted sample using rapid test kit and ELISA. The rapid HIV test result showed 100 % concordance using the serum and eluent from the DBS card. Similar results were obtained by Center for Disease control (CDC), Atlanta when using the rapid test kits on the DBS eluent but variation were seen among the test kits used.[8] In this study, some discrepancies were noted when the DBS eluent was tested by ELISA. This might reflect some variations in the HIV-1 strains in Nepal and warrants for HIV-1 strain characterization in different risk

groups and also for local manufacturing of a rapid test kit coated with antigens prevalent in Nepal. At the current situation where locally manufactured tests kits are not available, imported rapid test kits should be validated in the country before being used. The DBS eluent was assayed by ELISA kit, HUMAN, Australia. It would have been clear, if DBS eluent was also assayed by different ELISA kits as discrepancies has also been reported between different ELISA kits.[9]

The sensitivity and specificity of a Determine HIV-1/2 rapid test were 88.9% and 94%, respectively and the positive and negative predictive values were 94% and 88.9%, respectively using ELISA as a reference method. Testing of DBS eluent using Determine HIV-1/2 rapid test gave sensitivity of 83-92 % and specificity of 80-89 % .[8] Although, WHO recommends test kits with >95% sensitivity and specificity in HIV diagnostics, it is necessary to use a test kit having higher sensitivity and specificity. The sensitivity and specificity of HIV rapid test also depends on the HIV strains circulating in this region. The staff and equipments were sufficient to carry on the HIV NEQAS using a DBS card.

EQAS for HIV testing using a DBS sample is easy to collect and cost effective for resource limited settings. On top of this, DBS material could also be used for HIV-1 RNA and DNA PCR for diagnosis in infancy, HIV genotyping, HIV viral load monitoring and phenylketonuria and Palmodium falciparium diagnosis.2,10-12 HIV EQAS can also be performed with proficiency testing and onsite validation. Due to the laboratory infrastructural and geographical constraints, National EQAS using the proficiency panel (serum) is cumbersome as the specimen would take longer time to reach the corners of the country and is also almost impossible to maintain the cold chain and the integrity of the specimen during transportation.[1] On site validation and correction of errors would be an idle choice but requires a team to travel to the participating laboratories regularly. This can become a hurdle when number of HIV tests/day is minimum and team would have to wait till the specimen arrives in the laboratory for on-site testing. The VCT centers studied were located around 270 kilometers (kms) and mean duration of postal delivery to NPHL was 2.25 days. Most of the VCT centers are located in major cities and these are located around 500-600 kms from NPHL and postal delivery will not take longer than 7-10 days. DBS is stable for one month at room temperature and can be safely delivered to NPHL from any VCT centers in the country. Further study is warranted to support this notion. Consideration of this method needs evaluation with larger sample size in wide geographical area. We could not include larger sample size representing laboratories located at different geographical areas as this study was a time bound study.

CONCLUSIONS

DBS is a simple and cost effective method for sample collection in a resource limited and geographic constrained country like Nepal. The DBS cards were easily posted, reached the laboratory with a mean duration of 2.25 days, sample was stable and the results obtained in all participating and organizing laboratories were concordant for most of the samples. Hence, DBS as a method of sample collection for HIV EQAS will offer a dynamic platform in Nepal. The spectrum of its use can also be extended for collection of samples for HIV surveillance, screening HIV in newborn, and study of HIV drug resistance.

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A COMPARISON OF THREE VERSUS TWO SPUTUM SMEAR MICROSCOPY IN A GOVERNMENT MEDICAL COLLEGE, PATIALA, INDIA

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ABSTRACT

Introduction: Under the Revised National Tuberculosis Control Programme of India, three sputum samples are examined and 2 samples positivity criteria are used for labeling the patient as sputum positive pulmonary tuberculosis. Recent studies advocate use of two samples (one spot & one morning) for diagnosis of Tuberculosis. The objective was to compare three versus two sputum smears and to study the relevance of third sputum sample for microscopy in the current practice under Revised National Tuberculosis and Control Programme.

Methodology: A study of the laboratory register of the designated microscopic centre for the calendar year 2008 was undertaken. In all 9028 suspects were examined. An analysis of contribution of various sputum samples, S1 (first spot sample), M (early morning) & S2 (second spot) towards diagnosis of Pulmonary Tuberculosis was undertaken.

Results: Sputum smear examination results of all the patients examined during 2008 were analyzed. Twelve hundred and eighty eight patients (99.3%) were labeled as smear positive tuberculosis when three sputum samples positivity criteria was considered. By applying two samples and any smear positivity criteria 1296 (99.9%) patients were labeled as sputum smear positive. Among 1296 smears, S1 was positive in 1088 (83.8%) and M in 1293 (99.6%) patients. Early morning sample positivity yield was found higher.

Conclusion: Considering 2 samples for examination with at least one morning specimen and one sample positivity criteria, the work load on laboratory can be reduced by 1/3rd without affecting case detection rate.

Key words: Revised National Tuberculosis Control Programme, Three sputum versus two sputum smears

INTRODUCTION

In a suspected case of pulmonary tuberculosis under the Revised National Tuberculosis Control Programme of India, 3 sputum smear examination are undertaken for diagnosing a case of pulmonary tuberculosis. However recent studies have reported that 2 sputum smear examination is enough for diagnosing pulmonary tuberculosis.[1] International Standard of TB Care also advises 2 or 3 sputum smear examination for diagnosing pulmonary tuberculosis. Under the present strategy every patient has to visit the Designated Microscopy Centre for 2 days in order to get his sputum examination done. It is observed that many times some patients do not turn up for giving

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their early morning and second spot samples due to various reasons. The only sample submitted by some of these patients is found to be smear positive. This leads to increased work load for the programme staff, who have to trace these patients. So in order to see the contribution of first, second and third sputum sample for the diagnosis of pulmonary tuberculosis especially the 2nd spot sample, a retrospective study was undertaken by the Tuberculosis and Chest Department/ Hospital Government Medical College, Patiala, India.

METHODOLOGY

Analysis of Tuberculosis laboratory register of Designated Microscopy Centre of Tuberculosis and Chest Department/Hospital Government Medical College, Patiala for calendar year 2008 was undertaken. In all 9028 suspects had undergone sputum smear examination which include those persons, who were symptomatic (cough> 3 weeks) and others are having cough <3 weeks but symptoms associated with comorbities(Diabetes Mellitus, Bronchial Asthma, Chronic Obstructive Pulmonary Disease and HIV). Result of sputum smear of first spot (S1), morning (M), and second spot (S2) smear was recorded and analyzed.

RESULTS

In 9028 patients examined, 3 sputum smears were From above table, in all 9028 positive in 1059 (82%) patients (Table 1). 1288 (99.3%) patients were found to be sputum smear positive when 2 sputum smear positivity was considered out of three samples. When 2 samples and 2 sputum smears positivity criteria was adopted, 1085 (83.7%) patients were positive. Similarly, when two samples and one sputum positivity criteria was considered 1296 (99.9%) patients were positive. Yield of early morning sample was higher than first spot specimen. If two samples and 1 smear positivity criteria is adopted instead of 3 samples and 2 positivity criteria as is being presently used, number of patients diagnosed as sputum positive will be 1296 and 1288 respectively. By applying any sample positivity criteria and ignoring the second spot sample, only 1 patient was not fulfilling the criteria of sputum positivity. While applying 2 sample criteria, out of total 1297 the morning sample was positive in 1287 (99.2%) and negative in 1 (0.07%) patients. This signifies the importance of early morning sample for the diagnosis of pulmonary tuberculosis.

By applying 3/2/1 samples and 2/1 smear positivity criteria, number of patients which could be diagnosed smear positive pulmonary TB is given in table 2. Out of 9028 suspect patients single spot sample was obtained from 1360 (15.06%) of patients of which 63 (4.6%) were found to be sputum positive (Table 1). If only one sample criteria was used then out of 9028 patients 1151 (12.7%) patients were found to be sputum positive (Table 1 and Table 2).

Table 1. Results of various sputum samples examination

No. of Positive Samples	Result of various sputum samples	No. of Patient Positive	%
All 3 Samples positive	S1(+),M(+),S2(+)	1059	82%
Any 2 Samples Positive	S1(+), M(+), S 2(-) S1(-), M(+), S 2(+) S1(+),M(-),S2(+)	26 202 1	1.15% 15.3% 0.07%
Any 1 Sample Positive	S1(+),M(-),S2(-) S1(-),M(+),S2(-) S1(-),M(-),S2(+)	2 6 1	0.15% 0.23% 0.07%
Only 1st Spot sample given in 1360	S1(+)	63	4.6%

S1 (+) = First Spot Positive M (+) =Morning Positive S2 (+) =Second Spot Positive
S1 (-) = First Spot Negative M (-) =Morning Negative S2 (-) = Second Spot Negative

Table 2. 3/2/1 sputum smears versus 2/1 Smear Positivity Criteria

No of Sputum Sample	Positivity criteria	Patients Diagnosed as Sputum Positive
3	2	1288(99.3%)
2	2	1085(83.7%)
3	1	1297(100%)
2	1	1296(99.9%)
1	1	1151(88.7%)

DISCUSSION

The present study is comparable with a study by Santha et al (2000) reporting third sputum smear positivity in only 3 (1.4%) out of 1715 patients.[3] A study has also reported third sputum sample positivity in only 8 (< 1 %) out of 7927 patients and is comparable with the present study. The present study can also be compared with others who have reported third sputum sample positivity in 1 (< 1 %) out of 2560 patients.[4,5] This study is also comparable with a study by S. Rao et al, 2009 reporting third sputum smear positivity in only 12 (2%) out of 546 patients.[2] If we reduce the no. of samples from 3 to 2, then it will reduce the work load by 1/3 without much effect on case detection rate.[1,6,7] If we use 1 sample and 1 positivity criteria, the number of sputum positive patients will decrease by 11.3%. So it is important to take 1 spot and 1 morning sample for diagnosing the sputum positive pulmonary tuberculosis.[2] However in order to have single day disposal of the patient the information, education and communication (IEC) activities under RNTCP need to be accelerated so that masses are educated enough to bring early morning sample on day 1 itself.[8] This will lead to saving for 1 day wages and cost of transport of the patient and the attendants. Thus by applying 2 samples and any one smear positivity criteria number of cases will be almost equal, but the workload will be decreased by 1/3rd.[4] Since early morning sample yield is higher than spot specimen, so out of two samples one should be early morning.

CONCLUSIONS

By applying 2 sputum smear criteria and 1 sputum smear positivity criteria; workload can be reduced by one third while maintaining the sensitivity of diagnosing smear positive patients. External Quality Assurance programme should be stringently applied while adopting 2 sample and 1 positivity criteria to monitor quality of sputum microscopy.

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OCULAR MANIFESTATIONS IN HIV POSITIVE PATIENTS ATTENDING KHYBER TEACHING HOSPITAL PESHAWAR

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ABSTRACT

Introduction: Human immunodeficiency virus (HIV) infection is a global health problem. Around 90% of infected persons live in developing countries, particularly those in sub-Saharan Africa and Southeast Asia. Ocular manifestations occur in approximately 70% of these patients. The objective of this study was to document ocular manifestations in HIV positive patients attending Khyber Teaching Hospital Peshawar, Pakistan.

Methodology: It was a descriptive case series. The study was conducted at Khyber Teaching Hospital Peshawar from January to December 2007. A total of 14 patients were examined. These patients underwent complete ocular examination including assessment of visual acuity, pupillary reaction, ocular motility, ocular adnexa, anterior segment and posterior segment. CD4 count was done in all the patients.

Results: Out of the 14 patients examined 6 (42.9%) had ocular manifestations, all of whom were male. The ocular manifestations included herpes simplex keratitis, herpes zoster ophthalmicus with neurotrophic keratitis, iridocyclitis, HIV retinopathy, retinal vasculitis and cytomegalovirus retinitis in one patient each. Amongst those with ocular manifestations, 5 patients (83.3%) had CD4 cell count of 100/mm^3 or less and 1 patient (16.7%) had CD4 count between 101 and 200/mm^3; and the mode of transmission was homosexual contact in 5 patients (83.3%) and vertical transmission in 1 patient (16.7%).

Conclusion: Ocular manifestations occur in a considerable number of HIV positive patients particularly in those with CD4 cell count less than 100/mm^3. Therefore, all HIV positive patients should be screened for ocular manifestations.

Key words: HIV, AIDS, CMV retinitis, HAART, CD4 count.

INTRODUCTION

Human immunodeficiency virus (HIV) is a retrovirus that causes a wide range of diseases,[1,2,3,] like an acute mononucleosis-like syndrome, an asymptomatic carrier state, persistent generalized lymphadenopathy, AIDS-related complex, and AIDS (acquired immune deficiency syndrome). AIDS is characterized by a gradual decrease in circulating $CD4^+$ T cell count which is responsible for the various opportunistic infections and neoplasms.[4] According to the Joint United Nations Program on HIV/AIDS (UNAIDS), there were approximately 33 million people living with HIV worldwide in 2007. In 2007, 2.7 million people contracted the virus and 2 million died from AIDS.[5] Around 90% of HIV infected persons live in developing countries, particularly

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those in sub-Saharan Africa and Southeast Asia.[6,7] According to UNAIDS, approximately 96000 people had HIV infection in Pakistan at the end of 2007 and the estimated adult prevalence was approximately 0.1%.[8]

Transmission of HIV is predominantly by sexual contact, by parenteral (intravenous drug use) or mucous membrane exposure to contaminated blood or blood products and perinatally.[4] Early diagnosis and treatment of HIV/AIDS is critical in prolonging life expectancy and reducing opportunistic infections. Highly active antiretroviral therapy (HAART) is a combination of antiretroviral drugs which make them more potent and probably reduce or postpone the occurrence of drug resistance.[9] Ocular manifestations occur in approximately 70% of HIV infected persons.[4] Ocular lesions associated with AIDS can be categorized into the following groups:[9]

- HIV retinopathy
- Opportunistic infections (caused by bacteria, viruses, fungi and protozoa)
- Neuro-ophthalmic lesions
- Unusual neoplasms like Kaposi sarcoma

CD4 count can be used to predict the onset of certain ocular manifestations of AIDS. CD4 count less than 500/mm^3 is associated with Kaposi sarcoma, lymphoma and tuberculosis; CD4 count less than 250/mm^3 is associated with pneumocystosis and toxoplasmosis; and CD4 count less than 100/mm^3 is associated with retinal or conjunvtival microvasculopathy, cytomegalovirus (CMV) retinitis, varicella zoster virus (VZV) retinitis, mycobacterium avium complex infection, cryptococcosis and microsporidiosis.[10]

To the best of our knowledge, no work has been done regarding ocular manifestation in HIV positive patients in Pakistan. The purpose of our study was to document ocular manifestations in HIV positive patients attending Khyber Teaching Hospital Peshawar. Based on the results of our study, we plan to develop a large screening program for HIV positive patients in Khyber Pukhtoon Khwa (KPK), Pakistan.

METHODOLOGY

It was a descriptive case series. The study was conducted at the Department of Ophthalmology, Eye 'B' Unit, Khyber Teaching Hospital Peshawar Pakistan, which is a tertiary care hospital. Duration of study was 1 year i.e. from January to December 2007. Our study population included HIV positive patients of Khyber Pukhtoon Khwa (KPK) Pakistan. A total of 14 HIV positive patients including 10 male and 4 female were included in the study. They were all diagnosed by the physician. Diagnosis was based on detection of anti-HIV antibodies in the serum by both enzyme-linked immunosorbent assay (ELISA) and western blot tests. These patients were referred for screening for ocular manifestations of HIV infection. Informed consent was taken from all the patients. Detailed history was taken including questions about the mode of transmission, time since the diagnosis of disease and any systemic morbidity. Complete ocular examination was done including best corrected visual acuity using Snellen visual acuity chart, pupillary reaction with torch, ocular motility, examination of the ocular adnexa with torch and slit-lamp (Takagi SM-70, Japan), examination of anterior segment and vitreous with slit-lamp and fundus examination with indirect ophthalmoscope (Neitz, Japan) and 78/90D lens (Volk, USA) after pupillary dilatation with 1.0% tropicamide and 10% phenylephrine eye drops. CD4 count was done in all the patients. All the relevant data was recorded on a proforma. The study was approved by the ethical review board of the hospital.

RESULTS

Total of 14 patients were examined including 10 males (71.4%) and 4 females (28.6%). Patient's age ranged from 2 to 50 years with a mean of 32 years. The time period between the diagnosis of disease and ocular examination was ranging from 4 months to 10 years. Mode of transmission was homosexuality in 8 (57.1%), heterosexuality in 4 (28.6%), blood-borne in 1 (7.1%) and vertical in 1 patient (7.1%). The CD4 count of the patients was ranging from 5 cells/mm^3 to 427cells/mm^3. The CD4 count was 100 or less in 6 patients (42.9%),

101-200 in 4 patients (28.6%), 201-300 in 1 patient (7.1%), 301-400 in 1 patient (7.1%) and 401-500 in 2 patients (14.3%).

Ocular manifestations were present in 6 patients (42.9%) all of whom were male. Mean age of patients with ocular manifestations (29.5 years) was slightly lower than those with no ocular manifestations (34 years). The time period between the diagnosis of disease and ocular examination in patients with ocular manifestations, was ranging from 7 months to 6 years. In patients with ocular manifestations, the CD4 count was ranging from 5cells/mm^3 to 130cells/mm^3. 5 out of those 6 patients (83.3%) had CD4 count of 100cells/mm^3 or less and 1 patient (16.7%) had CD4 count between 101 and 200cells/mm^3 and the mode of transmission was homosexuality in 5 patients (83.3%) and 1 patient (16.7%) had vertical transmission of disease from his mother.

Ocular manifestations were present in 6 out of the 10 male patients (60%); none of the female had ocular manifestations. Ocular manifestations seen in our patients included herpes simplex keratitis, herpes zoster ophthalmicus (HZO) with neurotrophic keratitis, iridocyclitis, HIV retinopathy, retinal vasculitis and cytomegalovirus (CMV) retinitis in 1 patient each (Table 1).

Table 1. Summary of clinical features of all patients

Age	Gender	Mode of transmission	Duration of disease	CD4 count	Ocular Manifestations
50 years	Male	Homosexual	07 months	34	HSV keratitis
30 years	Female	Heterosexual	11 months	436	None
35 years	Female	Heterosexual	10 months	129	None
40 years	Male	Homosexual	02 years	05	CMV retinitis
27 years	Male	Homosexual	01 year	134	
47 years	Male	Homosexual	06 years	83	HZO with neurotrophic keratitis
35 years	Male	Homosexual	02 years	210	None
32 years	Male	Blood borne	10 years	326	None
50 years	Male	Homosexual	04 years	427	None
30 years	Female	Heterosexual	07 months	125	None
20 years	Male	Homosexual	06 years	43	Retinal vasculitis
33 years	Female	Heterosexual	01 year	61	None
18 years	Male	Homosexual	04 months	130	HIV retinopathy
02 years	Male	Vertical	02 years	70	Iridocyclitis

HSV keratitis - Herpes simplex virus keratitis;
CMV retinitis - Cytomegalovirus retinitis;
HZO - Herpes zoster ophthalmicus;
HIV - Human immunodeficiency virus.

DISCUSSION

The lifetime risk of developing ocular feature in a HIV positive patient varies from 52 to 100%.[11] The frequency of ocular manifestations reported from a study done in Ethiopia was 60%.[12] In another study conducted in Japan 80% of the AIDS patients had ocular manifestations.[13] In our study 42.9% patients had ocular manifestations. All these patients were male; none of the female patients had ocular manifestation. The prevalence of ocular manifestations is higher in patients who have CD4 count of 100cells/mm^3 or less.[14] In our study 5 out of the 6 patients with ocular manifestations had CD4 count less than 100 and 1 patient had CD4 count between 101 and 200. The mode of transmission was homosexual contact in 5 out of these 6 patients who had ocular manifestations and vertical transmission in 1 patient. In literature no association has been found between the mode of transmission and the presence of ocular manifestations of HIV.

HIV retinopathy is the most common ocular feature of AIDS.[15] It affects almost 50-70% of the patients and is characterized

by cotton-wool spots, intraretinal hemorrhages and retinal microaneurysms.[16,17] It is usually asymptomatic.[18] In our study HIV retinopathy was seen in 1 patient. The patient had bilateral cotton wool spots and microaneurysms. The patient was asymptomatic and had normal visual acuity in both eyes.

In our study 1 patient had cytomegalovirus (CMV) retinitis. CMV retinitis is the most common opportunistic ocular infection in AIDS patients and can occur in approximately 40-50% of untreated patients.[15] It occurs almost exclusively in patients with CD4 count below 50cells/mm^3 and may be unilateral initially but upto 52% eventually develop bilateral disease.[19] Diagnosis of CMV retinitis was made on the basis of clinical features. The patient had CD4 count of 5cells/mm^3 and had marked systemic debility and other opportunistic infections.

Herpes zoster ophthalmicus (HZO) occurs in 5-15% of HIV positive patients, resulting from reactivation of previously established primary varicella-zoster virus (VZV) infection. It is characterized by painful, vesiculobullous skin rash over the distribution of the ophthalmic division of trigeminal nerve. VZV may also cause keratitis, iridocyclitis and retinitis (acute retinal necrosis and progressive outer retinal necrosis).[10] In our study 1 patient had HZO. He had vesicular skin rash in the distribution of ophthalmic division of trigeminal nerve and had neurotrophic keratitis due to which his visual acuity was decreased to 6/36 in the affected eye.

In our study 1 patient had dendritic corneal ulcer with decreased corneal sensation. A clinical diagnosis of herpes simplex virus (HSV) epithelial keratitis was made. The patient had CD4 count of 34cells/mm^3 and had visual acuity of 6/9 in the affected eye. In one study, the prevalence of HSV keratitis was higher in HIV positive patients as compared to the general population, and approximately 67% of HSV infected patients developed epithelial keratitis.[10] According to another author, except for the recurrent rate, the incidence and clinical course of HSV keratitis was similar among patients positive and negative for HIV.[20]

Iridocyclitis was seen in 1 patient in our study. It was unilateral and non-granulomatous. Iridocyclitis, in HIV positive patients, may be associated with retinal or choroidal infection with Cytomegalovirus, Herpes simplex virus, Varicella zoster virus, Candida, Cryptococcus, Toxoplasma gondii, Treponema pallidum and Mycobacteria;[21] and some medications, such as rifabutin[22] and cidofovir.[23] Serological investigations for most of these organisms were not available in our hospital. Only the few available investigations (Anti-toxoplasma IgG and IgM antibodies by ELISA, Venereal Disease Research Laboratory, Montoux test and Chest X-ray) were done but they were negative and therefore, no cause could be found for iridocyclitis.

In one study vasculitis involving peripheral retinal vessels was observed in 31% of the HIV positive patients.[24] In our study retinal vasculitis was seen in one patient. It was unilateral, involving the peripheral retina and the patient had visual acuity of 6/12. The patient was assessed clinically and relevant investigations were done to exclude other causes of retinal vasculitis. Chest X-ray, serum angiotensin converting enzyme (ACE), montoux test, computed tomography (CT) thorax, magnetic resonance imaging (MRI) brain, antinuclear antibody (ANA) and anti-neutrophil cytoplasmic antibodies (ANCA) were done, but all of them were normal.

There are a number of drawbacks in our study. Our sample size is very small. The reason being that we started our study in collaboration with a non-governmental organization (NGO) which was working for HIV positive patients in Khyber Pukhtoonkhwa (KPK) Pakistan, but due to the political situation and due to security concerns that NGO became completely non-functional and we could not continue our project. Our study was a cross-sectional study. Most of the patients developed marked systemic debility that's why follow up was not possible in those patients. Further studies, with larger sample size and long follow up, need to be done to know precisely about the disease pattern in our population.

Since we belong to a developing country and even in our tertiary care hospitals many modern facilities

are lacking. At the time of conducting this study, a functional fundus camera was not available in our hospital and since it is very inconvenient for the ill patients to go to other centres, we could not take fundus photographs of the patients and could not do Fundus Fluorescein Angiography (FFA).

CONCLUSION

Ocular involvement occurs in a considerable number of HIV positive patients particularly in those patients with CD4 cell count less than 100/ mm^3. All HIV positive patients should be screened by an ophthalmologist in order to identify the ocular manifestations at early stage and manage them properly.

To the best of our knowledge, no work has been done regarding ocular manifestation in HIV positive patients in Pakistan. Therefore further research needs to be done to know about the disease pattern in Pakistani population.

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AWARENESS REGARDING HIV/AIDS AMONG COLLEGE STUDENTS IN KHYBER PAKHTUNKHWA

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ABSTRACT

Introduction: HIV is still a major global health problem. In 2009, globally there were an estimated 2.6 million (2.3 million–2.8 million) incident cases of HIV. According to UNAIDS in 2009 a total of 98,000 (79,000 – 120,000) people are living with HIV in Pakistan. Pakistan was classified as a low-prevalence country with many risk factors that could lead to the rapid development of an epidemic. In 2004, a concentrated outbreak of HIV was found among Injecting Drug Users (IDUs) in Karachi, where over 20 percent of those tested were found to be infected. Prevention efforts are beginning to bear fruit, with indications of behaviour change and declines in prevalence rates in a number of high-burden countries. The study aimed to determine the level of awareness among college students regarding HIV/AIDS in Khyber Pakhtunkhwa.

Methodology: This cross sectional study was conducted in the two colleges of Peshawar. A total of sixty students were enrolled in the study thirty from each college through random sampling. The students were from second year class. One of the colleges was from Public Sector, Government College Peshawar and the other was from private sector Peshawar Model College (PMC).

Results: It was interesting to note that 60% of the students of Government (Govt.) college and 70% of the students of PMC knew that a person having a healthy look might have been infected with HIV. Seventy percent of the students of Govt. college and 60% of the students of PMC reported that there is no curative treatment for HIV/AIDS. Major source of information regarding HIV/AIDS was through TV and friends. Condoms were regarded as main protection from HIV/AIDS as 35% of the students of Govt. College and 40% of the students of PMC reported as a method of prevention, Other preventive methods were also reported by the students.

Conclusion: The study depicted that there remain gaps in certain areas of awareness of the students which needs initiation of HIV/AIDS awareness programmes among college students.

Key words: HIV/AIDS, IDUs, Peshawar

INTRODUCTION

HIV is still a major global health problem. Since the first cases were recorded in 1981, acquired immunodeficiency syndrome (AIDS) and its causative agent, the human immunodeficiency virus (HIV), have taken an enormous toll around the world. In 2009, globally there were an estimated 2.6 million (2.3 million–2.8 million) incident cases of HIV. The decline in mortality due to HIV is about 25% and reflects the increased availability of antiretroviral therapy, as well as care and support, to people living with HIV, particularly in middle-

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and low-income countries; it is also a result of decreasing incidence starting in the late 1990s.[1]

According to UNAIDS in 2009, a total of 98,000 (79,000 – 120,000) people were living with HIV in Pakistan. The mortality due to AIDS is placed at 5,800 (4,500 – 7,400).[2] Pakistan was classified as a low-prevalence country with many risk factors that could lead to the rapid development of an epidemic.

Knowledge about HIV is the first step to avoid its transmission. Yet less than one third of young men and only a fifth of young women in developing countries know basic facts about the virus. Although condom use has gained acceptance in some countries, global use remains low, especially among young adults in developing countries.[3]

Worldwide efforts to address HIV/AIDS have advanced in recent years. Prevention efforts are beginning to bear fruit, with indications of behaviour change and declines in prevalence rates in a number of high-burden countries. Many countries—supported by the WHO/Joint United Nations Programme on HIV/AIDS (UNAIDS) '3 by 5' Initiative and the efforts of many other partners—have also made significant progress in expanding access to antiretroviral therapy. Still much more remains to be done if the goal of universal access is to be achieved. Global coverage of many of the key health sector interventions against HIV/AIDS remains low, and growth in the numbers of new infections and people in need of treatment continues to outpace the capacity of health services to respond. Global financial resources also fall short of what will be needed to achieve universal access, and the sustained political commitment needed to tackle AIDS over the long term is still lacking in some countries.[4]

Lack of awareness and fear of HIV/AIDS create key apprehensions among students and general public and may generate a barrier to successful educational endeavors regarding the disease. This might lead to a range of unfavorable outcomes such as seeking advice or reluctance to treat AIDS patients.[5] Concerns about AIDS panic have among other things been linked to lack of appropriate knowledge about HIV and its transmission routes.[6]

The study was conducted with the aim to determine the level of awareness among college students regarding HIV/AIDS in Khyber Pakhtunkhwa.

METHODOLOGY

This cross sectional study was carried out from January – March 2010 among two colleges of Peshawar, Khyber Pakhtunkhwa, namely; Peshawar Model College, Peshawar and Government college, Peshawar.

The study population consisted of students of second year class of these two colleges. Sixty students were enrolled in this study; thirty from each college.

Sampling strategy

From each college 30 students were randomly selected from the total of 60 students from second year class. Each of the 30 students was explained about the purpose of the study. Informed consent was taken from all the participants. One of the colleges was of public sector (Govt. college Peshawar) while the other belonged to private sector (Peshawar Model college, Peshawar). The questionnaires were filled by experienced data collectors from the students.

Those students who gave consent to take part in the study were included while those who refused to give consent were excluded from the study.

Data was entered and analyzed in Statistical Package for Social Sciences (SPSS 14). [7]

Mean and standard deviations were calculated for continuous variables while frequencies and percentages were calculated for categorical variables.

RESULTS

The response rate of the sixty students enrolled was 100%. Overall mean age of the students was approximately twenty years with students from Govt. college (18 yr) younger than that of PMC (20 yr).

It was observed that p-value was not significant regarding the responses of the students of the two colleges, hence it was concluded that the responses for the awareness regarding HIV/AIDS were similar for the students of the two colleges.

Table 1 describes the knowledge regarding HIV/AIDS among college students in Peshawar. It is interesting to note that 60% of the students of Govt. College and 70% of the students of PMC knew that a person having a healthy look might be having HIV in his body. But on the contrary 80% of the students of Govt. college and 78% of the students of PMC said that they were not at risk of contracting HIV. This might be due to the reason that health education regarding HIV/AIDS was not imparted in educational institutions and needs revision of their curricula.

Table 1. Knowledge regarding HIV/AIDS among college students in Peshawar

Variable	Govt. College	PMC	Total
Healthy looking man may have HIV***			
Yes	18 (60%)	21 (70%)	39 (65%)
No	09 (30%)	06 (20%)	15 (25%)
Don't know	03 (10%)	03 (10%)	06 (10%)
Risk of contracting HIV by the respondents			
Yes	04 (15%)	03 (10%)	07 (12%)
No	24 (80%)	23 (78%)	47 (78%)
Don't know	02 (05%)	04 (12%)	06 (10%)
HIV is not transmitted by			
Hand Shaking	10 (35%)	11 (25%)	21(35%)
Eating in same utensils	06 (20%)	04 (15%)	10 (17%)
Studying together in college	14 (45%)	15 (50%)	29 (48%)
Preventive measures from HIV			
Safe blood transfusion	09 (30%)	08 (25%)	17 (28%)
Using condoms	10 (35%)	12 (40%)	22 (37%)
Sterilized syringes	03 (10%)	03 (11%)	06 (10%)
New razors/blades	08 (25%)	07 (24%)	15 (25%)
Is treatment for HIV present			
Yes	09 (30%)	12 (40%)	21(35%)
No	21 (70%)	18 (60%)	39 (65%)

One of the main findings was that majority of the students believe that HIV was transmitted through unsafe sexual activities while knowledge regarding other routes of transmission is lacking. Condoms were regarded as main protection from HIV/AIDS as 35% of the students of Govt. College and 40% of the students of PMC reported it as a method of prevention, Other preventive methods were also reported by the students. It is also interesting that 70% of the students of Govt. College and 60% of the students of PMC reported that there is no treatment for HIV/AIDS.

Figure 1 shows the different routes of HIV transmission responded by the students. It is evident from the graph that unsafe sex is accounted as the major route of transmission. And approximately 60% of the Govt. College and 50% of the PMC responded that unsafe sex is major route of transmission while contaminated blood transfusion accounted for 10% (Govt. College) and 05% (PMC) as route of transmission for HIV.

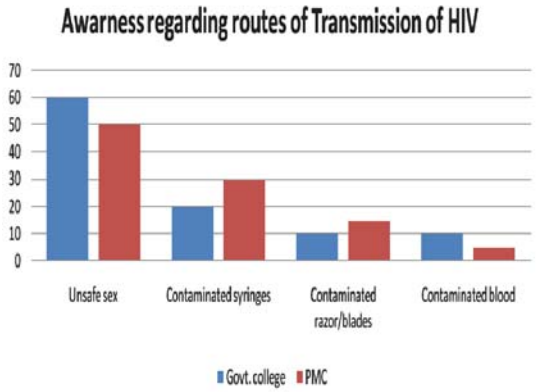

Figure 1. Routes of HIV transmission

Sources of information regarding HIV infection are depicted in figure 2. Television for the Govt. College (35%) & PMC (45%) and friends for the Govt. College (45%) & PMC (40%) were the most frequently reported source of information regarding HIV. While radio for both Govt. College & PMC (5%) was the less frequently reported source of information regarding HIV infections.

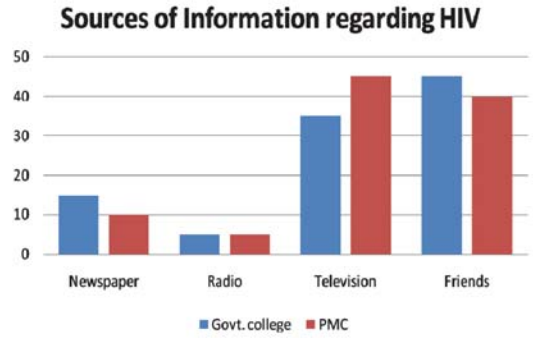

Figure 2. Sources of information regarding HIV infection

DISCUSSION

This study compared the awareness level regarding HIV among two groups of college students in Peshawar. The study enrolled college students with the assumption that a younger age group of population was targeted and who were thus prone to risky behavior.

The study showed that almost all of the students of the two colleges have heard of HIV/AIDS. Similar observation was made in other studies conducted in Africa.[8]

Friends and television were the most frequently reported sources of HIV information among both public and private college students. TV was considered to be a major source of information regarding HIV/AIDS.

The sources of HIV/AIDS information reported in this study are similar to those identified previously among students/dental health care workers from USA as well as in students of medical subjects from Iran and Pakistan.[9,10] Similar findings regarding media as a main source of information for HIV/AIDS was found in a study conducted in Sudan and Ethiopia.[11,12]

In our study majority of the students considered unsafe sex as the major route of transmission while contaminated instruments is accounted as low risk route for transmission. In a study from United Kingdom, dentists demonstrated good knowledge regarding oral lesions associated with HIV and AIDS, but were less familiar with HIV and AIDS transmission routes.[13]

Generally, the respondents seem to have favorable awareness level on prevention of the disease. Prevention strategies are well known in developed countries, however, recent epidemiological and behavioral studies in Europe and North America have suggested that a substantial minority of young people continue to engage in high-risk practices and that despite HIV/AIDS knowledge; young people underestimate their own risk of becoming infected with HIV.

This study depicts that knowledge regarding transmission of HIV through hand shaking and eating in utensils of HIV patients is low and is similar to the results of a study conducted in India by G.S. Basavayya in his study he observed that 20% & 15% girls had false notion that HIV spread by kissing, playing together and through mosquito bites.[14]

A very significant finding of this study was that all the students who participated in this study had a good level of awareness regarding HIV/AIDS and studies conducted in India had also reported high level of knowledge among participants regarding HIV/AIDS.[15]

CONCLUSION

The study depicted that there remain gaps in the certain areas of awareness of the students which needs initiation of HIV/AIDS awareness programmes in the first year of their studies. Health education regarding HIV/AIDS transmission and prevention should be included in the curricula of education. Hence, appropriate health education should be given in a way to bring behavioral change targeting at individual risk behavior.

However, it is suggested to conduct a large scale study conducting different strata of the younger population before considering implementation of the above mentioned recommendation,

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DISTRIBUTION OF ABO AND RH BLOOD GROUPS IN HIV SEROPOSITIVES AT AN INTEGRATED COUNSELING AND TESTING CENTRE IN KARNATAKA, INDIA

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ABSTRACT

Introduction: ABO blood group system was the first human blood group system to be discovered. Subsequent to the discovery of blood groups by Landsteiner and advancement in its study many workers tried to find out associations between blood groups and the incidence of various diseases. The objective of the study was to create a blood group database which would probably help in transfusion services and find out the distribution of blood groups in the seropositive population.

Methodology: Blood groups were ascertained for 1809 patients who were HIV seropositive enrolled at ICTC in a tertiary care teaching hospital in Karnataka, India from April 2004 to January 2010 using the simple tile method. The results were compiles and statistically analyzed.

Results: Blood grouping was done for 1809 patients, out of which 1749 (96.68%) were adults and 60(3.32%) were paediatric patients. O Rhesus positive was the most prevalent blood group in both adult (40.13%) and paediatric (43.33%) seropositives. B Rhesus positive was next commonest group in adults (26.12%) and A Rhesus positive in paediatric(30.0%). AB Rhesus negative (Adults-0.34% and Paediatric-0%) blood group was the least prevalent in the study population.

Conclusion: It is important to create blood group database for the Indian seropositives population, to know any probable association between blood group and HIV infection. This study is an attempt to create a blood group database in a modestly large seropositive which would play a vital role in transfusion services and future research . Larger nation-wide studies would be required to substantiate any association between blood groups and HIV infection.

Key words: HIV seropositive, ABO, Rhesus blood groups.

INTRODUCTION

ABO blood group system was the first human blood group system to be discovered.[1.] Subsequent to the discovery of blood groups by Landsteiner and advancement in its study many workers tried to find out associations between blood groups and the incidence of various diseases. For example, strong associations have been described between *O* blood group and peptic ulcer.[2] A blood group and gastric carcinoma.[3] AB blood group and carcinoma cervix[4], and so on. In the recent times, according to research done by Swedish scientists, the risk of being infected by Human Immunodeficiency Virus (HIV) may be determined by the presence of the carbohydrate based blood group moiety P^k. Individuals with high P^k levels exhibited a

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greater natural resistance to HIV infection.[5] World literature has few articles describing associations between HIV infection and blood groups .With this background, an attempt was made in this study to create a blood group database in HIV seropositives both adult and paediatric to ascertain the distribution of various ABO/Rh blood groups in the study population. The database so created would probably be of help in blood transfusion services particularly in this locality.

METHODOLOGY

This is a cross sectional study in which blood groups were determined for 1809 HIV seropositives(1749 adults and 60 paediatric) at blood bank of a tertiary care hospital in Karnataka, India, during the period April 2004 to January 2010. Informed consent had been taken from the patients to perform the test. The ABO and Rhesus(Rh) blood grouping was done by a simple tile method.[6] A drop of blood from each subject was placed on a clean white tile in three places. A drop of each of the antisera, anti A, and anti B and anti D was added and mixed with each blood sample with the aid of glass rods. Blood groups were determined on the basis of agglutination.[7]

The HIV seropositive status was confirmed by standard ELISA/RAPID tests according to National AIDS Control Organization (NACO) guidelines at the Integrated Counseling and Testing Centre (ICTC). According to the earlier guidelines, HIV testing was confirmed by ELISA. Revised guidelines follow strategy 3 i. e. using 3 rapid tests for confirmation of positive status.[8] Baseline CD4 cell counts were done for all the participants of the study at the Department of Microbiology. The blood group of the control population was obtained from the records of 1168 consecutive personnel maintained at the blood bank of the hospital. Control group was included in this study in order to create a database and also compare the distribution of blood groups among seropositives against the general population so as to determine any significant correlation if present. Age, gender, blood group and CD4 data were compiled and subjected to relevant statistical analysis using SPSS Version 16.

RESULTS

Blood grouping was done for 1749 adult HIV seropositives, 60 paediatric HIV seropositives between the age group of 18 months and 70 years. The adult seropositive group consisted of 1030(58.9%) males and 719 (41.1%) females. The paediatric seropositive group consisted of 25 (41.7%) males and 35 (58.3%) females. The control group comprised 1130 adult and 38 paediatric individuals. The adult controls comprised 662(58.6%) males and 468 (41.4%) females. The paediatric age group consisted of 16(42.1%) males and 22(57.9%) females

The adults seropositive group had 1649(94.28%) *Rhesus* positive blood group while 100(5.72%) were *Rhesus* negative. In the paediatric seropositive group 59(98.53%) were *Rhesus* positive and one (1.66%) was *Rhesus* negative. In the adult control group 1067(94.33%) were *Rhesus* positive and 64(5.67%) were *Rhesus* negative while in the paediatric control group 36(97.36%) were Rhesus positive while 1 (2.63%) were Rhesus negative as shown in Table1.

Table 1. Rhesus Positive and Rhesus Negative

Study Population	Rhesus Positive		Rhesus Negative	
	Number	%	Number	%
Adult Seropositive	1649	94.28	100	5.72
Paediatric Seropositive	59	98.53	1	1.66
Adult Control	1066	94.33	64	5.67
Paediatric Control	37	97.36	1	2.63

Figure 1 shows that O Rh positive blood group was the commonest in 702(40.13%) adult seropositives and 26(43.33%) in paediatric seropositives as well as 407(34.84%) in the control group. In the adult seropositives population B Rh positive blood group was the next commonest in 457(26.12%) followed by A Rh positive blood group in 395(22.58%).The control group also showed a similar trend with 341(29.19%) individuals having B Rh positive blood group and 279(23.88%) having A Rh positive blood group. The paediatric seropositive population

showed a variation with more number of females than males and A Rh positive blood group being the next commonest in 17(28.33%) followed by B Rh positive blood group in 14(23.33%). Figure 2, 3 and 4 show the gender-wise distribution of blood groups in adult, paediatric seropositive and the control population respectively. In all the three groups AB Rh negative was the least commonly observed blood group.

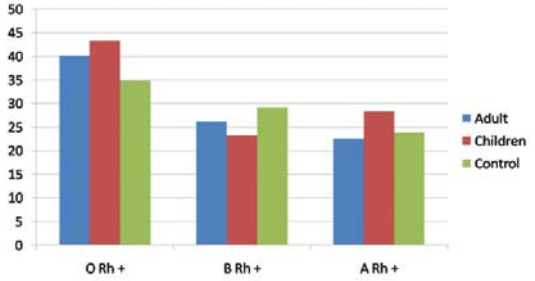

Figure 1. Bar graph showing the percentage (Y axis) of adult, paediatric and control subjects positive for O Rh+, B Rh+ and A Rh+ blood groups

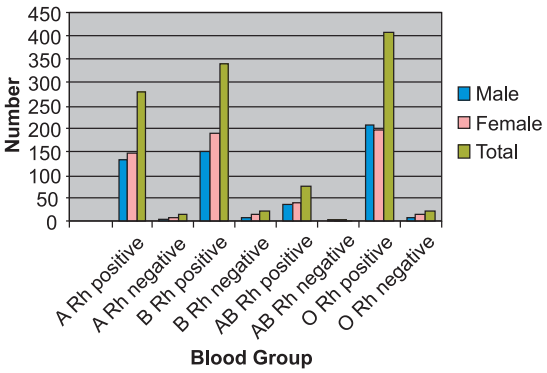

Figure 2. Gender-wise Distribution of Blood Groups in Adult Seropositives

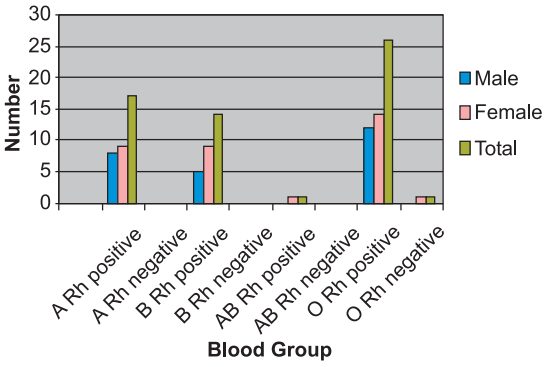

Figure 3. Gender-wise Distribution of Blood Groups in Paediatric Seropositives

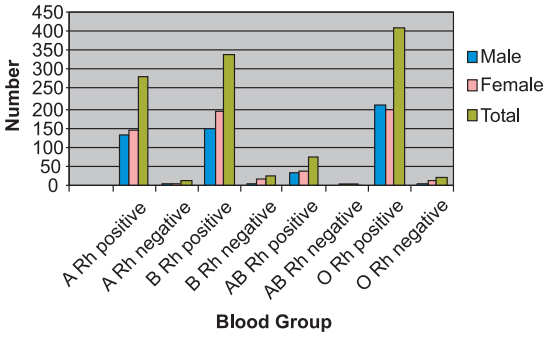

Figure 4. Blood Group Distribution in Control Population

The mean baseline CD4 cell count of the study population was 121.77 cells/µl.

DISCUSSION

Few studies from the world have researched probable association between blood group and HIV infection. Very few large scale studies have been conducted in India to know the distribution of blood groups of HIV seropositive population. In this study it was observed that O Rh positive was the commonest among the adult 702(40.13%) and paediatric 26(43.33%) seropositive population as well as the control 407(34.84%) population. An Indian study which determined the distribution of blood groups in 104 HIV seropositives observed *O* blood group in 40(38.5%) individuals which was most common, *A* blood group was seen in 28(26.9%), 26(25%) had *B* blood group and 10(9.6%) had *AB* blood group.[9] The current study showed 457(26.12%) with B Rh positive blood group in adult and 17(28.33%) with A Rh positive blood group in paediatric study population, which were the next most prevalent blood groups. In addition, there were more female seropositives than males in the paediatric study group. In a study from Nigeria, HIV status and blood groups determination (*Rhesus* and *ABO* groups) in 3691 pregnant women attending antenatal clinic and 1199 non-pregnant women visiting the same institution between 1999 to 2002 were studied. Overall, the prevalence of blood group O Rh positive was higher than in the general population with highest rate of 62.9% in HIV positive pregnant women followed by 58.4% in HIV negative pregnant women and 58.0% in

non-pregnant women. No significant difference was observed in blood groups for the three categories of subjects studied showing that no particular blood group type could be linked to the occurrence of HIV infection.[10] In another Nigerian study which determined *ABO* blood groups for 216 seropositives, 42.1% were O group, 11.1% *A* group, 9.4% *B* group and 8.7% were *AB* group and 5 out of 7(71.4%) infected subjects with HIV-2 only, belonged to blood group AB.[11] The present study also had one HIV-2 infected individual who was found to be B Rh positive.

ABO histo-blood group antigens have been postulated to modify pathogen spread through the action of natural antibodies and complement. Incorporation of *ABO* antigens by HIV-1 may affect transmission of virus between individuals of discordant blood groups by interaction with host natural antibody and complement. While much research has been devoted to searching for genetic factors that confer resistance to HIV-1 transmission, the most notable being the MHC and CCR5 loci, little is known about the role that some of the most well-studied blood group polymorphisms may play in viral transmission.[12] Swedish researchers investigated cell surface–expressed P^k in HIV infection. They concluded that P^k expression strongly influences susceptibility to HIV-1 infection, which implicates P^k as a new endogenous cell-surface factor that may provide protection against HIV-1 infection. Individuals with high P^k levels exhibited a greater natural resistance to HIV infection.[5]

It is important to create blood group database for the Indian seropositives population, to know about blood group distributions and any probable association between blood group and HIV infection. This study is an attempt to create a blood group database in a modestly large seropositive population which would play a vital role in blood transfusion services and future research protocols. Larger nation-wide studies would be required to substantiate any association between blood groups and HIV infection.

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