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SAARC Member States are implementing effective National TB Control Programmes focusing on DOTS strategy. DOTS Strategy is being implemented by all the SAARC Member States. All the SAARC Member States have adopted the Stop TB Strategy, “Address TB/HIV, MDR-TB, and the other challenges” being a major component. However, MDR-TB and the TB/HIV co-infection are the two areas which can very easily negate the gains made by the National TB Control Programmes.

WHO estimates that worldwide 500,000 cases of multi-drug resistant tuberculosis (MDR-TB) emerge every year, including 50000 XDR-TB (Extensively Drug-Resistant TB). XDR-TB is defined as Mycobacterium tuberculosis isolates resistant to at least Isoniazid, Rifampicin, any Fluoroquinolone and at least one of the three Injectable drugs (Amikacin, Kanamycin or Capreomycin). In the SAARC Region, about 125,000 cases of MDR-TB emerge every year, meaning thereby that the SAARC Region bears about 25% of the total burden of TB worldwide. The emergence of MDR-TB is a man made phenomenon. Inappropriate treatment regimen, inadequate treatment adherence, unregulated private sector, weak National TB Programmes, affordability issues, irrational use of 2nd Line Anti TB drugs, weak laboratory infrastructure and free availability of Anti TB drugs in the open market have been the causes of emergence of MDR-TB in the past. Inadequate response from the private sector for completion of treatment course for TB Patients, weakly implemented National Programme, weak laboratory infrastructure, irrational use of 2nd Line Anti TB drugs and inadequate capacity of the National Programmes to manage MDR-TB patients are the causes of further emergence of MDR-TB.

All the SAARC Member States have initiated Management of MDR-TB patients with a functional Culture & DST Laboratory or plans to have it. Expansion of the network of quality assured laboratories is an enormous challenge which all the Member States are facing. Management of the MDR-TB patients with 18 to 24 months or more of expensive and potentially toxic 2nd line Anti TB drugs following DOT principle is also a big challenge.

First and foremost requirement for management of MDR-TB management is development of quality assured infrastructure for diagnosis of MDR-TB. Establishment of the infrastructure for diagnosis, engagement and retention of trained human resources, procurement and ensuring continuous supply of 2nd line Anti TB drugs, adherence to treatment regimen of 18 months to 24 months or more duration, direct observation of treatment for such a long duration, comparatively much more expensive drugs and mobilization of resources for providing expensive drugs to patients free of cost, intake of comparatively more toxic drugs and management of their side effects, tailoring the regimen for special situations and management of co-morbidities are some of the challenges that the National TB Control Programmes have to face while rolling out and scaling up services for management of MDR-TB Patients.

SAARC Tuberculosis and HIV/AIDS Centre (STAC) has established a Regional TB Reference Laboratory which already has a networking with ten National level TB laboratories of the SAARC Member States. SAARC Regional TB Reference Laboratory is already supporting these laboratories for quality assurance of sputum microscopy through Proficiency Testing. SAARC Regional TB Reference Laboratory is also conducting Culture & DST jointly with the National TB Laboratory of Nepal. The Culture & DST conducted in this Laboratory has been accredited by the Gauting Laboratory, Germany which is a member of the Supranational TB Laboratory Network established by WHO. Currently, there is only one Supranational TB Laboratory in the SAARC Region, i.e. Tuberculosis Research Centre Laboratory, Chennai, India which is overburdened for fulfilling the responsibility of a Supranational TB Laboratory. STAC is scaling up its infrastructure and human resources requirements and has planned to take up the responsibility through supporting quality assurance of Culture & DST procedures in the SAARC countries National level laboratories in addition to sputum microscopy, as second Supranational TB Reference Laboratory for the SAARC region.
CHALLENGES IN HIV/AIDS PREVENTION, CARE AND TREATMENT PROGRAMME IN INDIA
Policies and Priorities under National AIDS Control Programme Phase-III (2007-12)

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ABSTRACT

This paper explores the pertinent challenges of Government’s HIV/AIDS prevention, care and treatment program in India. Study is mainly based on observations made during field visits, discussion during review meetings at various levels and during training programmes of various functionaries from April 2007-November 2009. The paper also takes into account the observations made on the conclusion of the Mid-term Review conducted by the Government of India in during July-December 2009 after completion of first 2.5 years of the 5-year plan of NACP-III. Additionally, interactions with key program managers involved in implementation and management of HIV/AIDS Program at the state, district and facility levels. Though numerous efforts have been made and continued by the Government and partners, HIV prevention, care and treatment services have not been able to reach to the most-at-risk population, specifically in the rural population. Socio-cultural and managerial issues are the key challenges reported by the most of the key implementers. There is an urgent need to address and strengthen the whole spectrum of health systems through a collaborative approach to achieve the millennium development goals of universal access to prevention, care and treatment services in India.

INTRODUCTION

Human Immunodeficiency Virus (HIV) has been a growing challenge worldwide from the last two decades.¹ A total of 33 million people are estimated to be living with HIV across the globe, 2.7 million people became infected with the virus and 2 million have lost their life due to AIDS. Every day, more than 6800 people become infected with HIV and more than 5700 die, mostly because they have no access to HIV prevention, care and treatment services.² The United Nations included HIV in its sixth millennium development goals which stated in combating and reversing the spread of HIV/AIDS by 2015 as well as to achieve universal access by 2010.³⁴ India, being committed to the Millennium Development goals, phase-III of National AIDS Control Program (NACP-III) was launched with the ultimate goal to reverse the HIV epidemic through various efforts for providing HIV prevention, care, support, and treatment services.⁵

India’s epidemic is concentrated in 195 districts, most of them in six states-where HIV prevalence was more than one percent⁶. Evidence suggests that HIV epidemic has now stabilized in the country and some states like Tamil Nadu have witnessed decline in the prevalence.⁷⁸ The epidemic is still concentrated in high risk groups and vulnerable population. However, information that the epidemic is spreading in new pockets in northern low prevalence states and in the rural areas, is a matter of concern. Prevention of HIV/AIDS through public awareness programmes, change in behaviour, use of condoms, blood safety and prevention of mother to child transmission are important interventions to prevent HIV infections. At the same time those who have got the infection and AIDS need to be provided care, support and treatment without any stigma and discrimination.

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The Government of India, with support from multi-lateral, bilateral and other partners, is implementing various activities in the field of HIV/AIDS. Despite the progress that has been made, many issues and challenges remain unidentified and not addressed at program management, implementation and service delivery levels in India. The number of new HIV infections continues to increase despite current efforts made to control the epidemic. There is an urgent need for systematic review to ensure effective response to achieve the ultimate goal of NACP-III. Therefore, an attempt has been made to primarily explore the pertinent challenges in HIV/AIDS prevention, care and treatment program in India to help the policy makers, program managers and health care providers to tailor, implement and manage the program in a better manner.

**METHODS**

The present study is mainly based on observations made during field visits, discussion during review meetings at various levels and during training programmes of various functionaries from April 2007-November 2009. The paper also takes into account the observations made on the conclusion of the Mid-term Review conducted by the Government of India in during July-December 2009 after completion of first 2.5 years of the 5-year plan of NACP-III. Additionally, interactions with key program managers involved in implementation and management of HIV/AIDS Program at the state, district and facility levels.

**RESULTS AND DISCUSSION**

Collectively, specific management issues and challenges in the implementation of prevention and treatment programs on HIV/AIDS were identified. These issues were organized into nine thematic categories including capacity building, monitoring and evaluation, finance and others.

1. **Strategic Planning and Management:**

The National AIDS Control program is structured and well designed. Many officers pointed out that the programs have been devised as a national response to HIV/AIDS under one uniform action framework. There are operational guidelines for SACS, DAPCU and all facilities to maintain quality and standards of the services uniformly across the country and monitor the programs consistently to assess progress against the national goals. These guidelines work as referral document for implementing units and service delivery systems.

Most of the officers agreed that strategic and programme interventions are evidence-based and local priorities are taken into consideration. Some states seemed to be satisfied with the current set up and admitted that there is considerable flexibility and scope for innovation in the program whereas others were of the opinion that program should be more flexible. Local needs and evidence should be taken into consideration while planning.

The state level officers also admitted that setting up of DAPCU has made program management and implementation easier, which has helped in better monitoring, linkages within the program and with general health systems at the district level. Officers of many SACS were of the opinion that all districts should have DAPCU irrespective of categorization of district based HIV Sentinel Surveillance alone. Districts not having DAPCU should have some alternate arrangement for monitoring at district level. DAPCU should be set up in a need-based and state specific manner depending upon the size the district, extent of the problem and estimated number of high-risk population.

Majority of the officers suggested that strong leadership is important for the program. There was consensus that the program suffered a lot due to frequent changes of Project Directors. In metros like Delhi, a peculiar problem is of four types of health institutions owned/ run by: (i) Delhi Government (ii) Municipal Corporation of Delhi (iii) New Delhi Municipal Corporation (iv) Central Government. It is very difficult to coordinate with different kind of health institutions as they are implemented by different agencies and have different level of hierarchy. The dissemination of information also becomes tough in such circumstances.

It was found that in states like Karnataka, DAPCU were well placed and functioning for the last two years. DAPCU officers were aware of their roles and responsibilities and prepare District annual action plan as per the local context. The major focus was to establish linkages within the program and
coordination with other national health programs. As far as implementation of the program is concerned, there are not many problems as HIV program in high prevalence states like Tamil Nadu, Karnataka and Maharashtra where the program received top priority. The interventions started early in these states and have now taken a definite shape. All high prevalence districts in these states have all the facilities: Targeted Interventions, Integrated Counseling and Testing Centres (ICTC), and Treatment and Care facilities (ART Centres and Community Care Centres), and therefore linkages and referral system within the programs are easier.

At facility level, the nodal officers of the ART Centre shared that Treatment program is quite systematic. Nodal officers at ART Centres reported that more clear directions are required regarding finances whereas nodal officer from ICTC pointed out that there is too much of compartmentalization of the program at the District level. The different facilities run independently and therefore implementation of the program as envisioned is not possible. However, majority of the health care providers opined that the systems have become better.

2. Human Resources:

In spite of two and a half years implementation of NACP-III, many states have vacancies at state and facility levels. It was indicated by most of the officials that human resource planning has been done in a very realistic manner by grouping states into different categories on the basis of disease burden. Some of the officials from SACS suggested that staffing in each component of the program at state level should be need-based and related to number of facilities being implemented in each state. It was also found that that the reporting by the Technical Support Units and Consultants appointed at SACS needs to be more streamlined. It was indicated that it is difficult to get good staff in the prescribed salary range. It was also observed that attrition rate amongst the contractual staff is high. All the contractual staff at state, district and facility levels revealed that there is sense of job insecurity. Moreover the salary package particularly at state and service delivery level is neither very attractive nor has any additional allowances and devoid of any promotional avenues. Similar opinion was also shared by the regular staff. Many senior officers both at central and state level suggested that the some authority or financial power may also be given to the divisional heads to avoid delays in taking routine decisions.

DAPCU officers highlighted the need of authority to district level officers. Due to contractual position of district program managers, they are not in position to take any decisions. It was highlighted by majority of the respondents, that regular staff does not follow the instructions of the contractual officers.

Staffing at the facility level is based on volume of case load. Staffing is adequate but the attrition rate is very high. The staff is appointed on contractual basis and there is a sense of job insecurity. Most of the staff from various facilities included in the study has complained of the lower salary status and no other benefits. It was also found that there are no growth avenues particularly for contractual staff. Higher level SACS officials were of the opinion that program implementation suffers a lot due to high staff turn over of contractual staff. It was suggested that salaries of the contractual staff should be at par with other staff with same qualification in general Health system in the concerned state. Many health care providers at facility level informed that there were frequent delays in payment of the salary.

3. Capacity building:

Most of the personnel agreed that the training programs are very well designed. The staff who have worked with other health programs earlier or have regular interaction with other national programs expressed that training component of NACP III is stronger and intensive. There are both induction as well as in-service/ refresher training for most of the health care providers. But at the same time it was observed that there are no formal trainings for staff at SACS. Non-availability of any training modules for them makes it difficult for them to understand the program and their job responsibilities.

DAPCU offices were of opinion that some of them were provided induction training but few of them have no clarity of their responsibilities. It was found that all the district supervisors were given induction training. The DAPCU staff was of the opinion that more intensive trainings were required especially in quality
data management and Computerized Management Information System (CMIS). The DAPCU officers suggested need for refresher training to update their knowledge on the national program and strategic planning.

Majority of the staff expressed their satisfaction on the ongoing training program. Most of the staff had undergone training. There was a very good response on the team trainings. The Medical officers from ART suggested that revision and updation in the curriculum was required as new initiatives such as 2nd Line ART and alternate first line are being rolled out. Some of the nodal officers and Medical Officers from ART also suggested that there were separate training from time to time for various components such as Pediatrics, 2nd line ART, alternate first-line ART, M&E, HIV-TB etc. To attend training repeatedly causes lot of inconvenience to the staff as well as to the patients. They suggested that there should be planned refresher trainings with standard and consolidated modules on all new updates. The staff nurses at LAC expressed they are given team training by Nodal Officers but it would be better if a more thorough training could be given to them on ART. The TI NGO in Delhi indicated that there were too many training programs which need to be streamlined.

4. Coordination and Linkages within the program:

Better Coordination and linkages are required between various services notably by Targeted Interventions for (TI) High Risk and vulnerable population. Under NACP-III, all TIs are attached to nearest ICTC. The linkages and referral from targeted interventions to testing and counseling services are not up to expected levels as all clients from TIs do not come to ICTC. Some TI NGOs mentioned difficulty to take High risk groups to ICTC due to timing problems. The problem is more severe with IDUs as most of the times these people are intoxicated and tend to run away. Funds are not sufficient enough to bear travel cost to take such people to ICTC. The FSW TI NGOs shared that the day timings of the ICTC are not suitable as most of the FSW tend to sleep during day hours. The SACS Officers in Delhi informed that Mobile ICTCs are being encouraged for testing and reporting on the same day.

PPTCT coverage and follow up is low. The PPTCT health care providers shared that the major reason for this low number of institutional deliveries. Most of the women go to their maternal houses for delivery.

The linkages are improving between testing and treatment services. It was informed that there has been a continuous rise in the number of HIV positives accessing ART services. It was shared by some of the district level staff and ICTC staff that in some of the ART Centers, CD4 test is done once a week for limited time. Patients have to go at least twice, once for registration and the next time for CD4 testing. This leads to additional costs to the patients. Also the patients from HRG find it difficult to access ART services due to problems of timings.

5. Coordination and Linkages with other Health Systems:

Most of the SACS officers were of opinion that coordination with Health Systems was difficult because of multi-structured Health systems. However it was observed that there was good integration between NRHM and NACP-III in those states where the coordination between Project Director, SACS and Mission Director, NRHM. In such settings linkages between various health programs and systems became easier.

Many of the senior level officers at SACS indicated that in general, there is good cooperation from Nodal officers but since these officers are Head of their Departments, it is very difficult for them to supervise day-to-day activities of the facility. It was suggested that nodal officer should not be selected by virtue of being Head of the Department. Alternatively, Head of Department should depute one of the officers from the department to monitor day-to-day working of the facility.

All program officers and health care providers unanimously accepted that linkages with RNTCP are good. District Supervisors attend District TB Officer meetings. The cross referrals from HIV into TB and from TB into HIV are quite satisfactory. The linkages at program as well as facility level are relatively good. The ICTC Health care providers informed that with the implementation of Provider-Initiated HIV Testing
and Counseling of Tuberculosis patients under the TB Control Program (PITC) has improved the referral to HIV services, detection of new cases and initiation of ART. It was also informed that all patients at ART Centers are screened for TB and if found positive are referred to RNTCP.

The DAPCU staff shared that in hospital settings more referral clients were coming to ICTC from other departments. This indicated that the health care providers are sensitized on the need of HIV screening in patients with risk behavior. Majority of the officers from DAPCU emphasized the need for integration of NACP III Services with NRHM & General Health Services at gross root level.

6. Procurement and Supply Chain Management:

During the study it was found that ART drugs, CD4 kits and HIV test kit (Antigen 1, Elisa) are provided centrally. PEP drugs, HIV Kits Antigen 2 & 3, Nevirapine are to be procured by states and supplied to facilities. States suggested that there were problems in procurement at state level due to the procedures as well as lack of capacities.

Some of the SACS staff indicated that procurement of all kits should be done by NACO. This will help to manage the supply chain and also procure goods at competitive rates due to large volume. Rate contract will perhaps be better option for procurement of drugs whereby NACO can fix up the rates and SACS can procure them as per requirement. This will improve the supply chain management and reduce storage problems. It was also suggested by some officers that procedures like e-tendering (where open bids are available) can also be adopted. It will make procurement process more transparent. It was observed during the study that procurement is weak in several states. On discussion, it was informed that though there were no major issues with global fund procurement but some there were definite delays in procurement under the World Bank funded procurement.

Most of the DAPCU reported shortage of space for storing kits. Majority of the DAPCU officers depicted that decentralization of supply chain management at DAPCU Level is required. This is particularly true for large states with large number of ICTCs in each district. The transportation causes a lot of inconvenience and maintenance of cold chain is also difficult. It was suggested that Warehouse System is required at the district level for storage of kits and other consumables.

It was found that supplies of ART drugs are regular and systematic. The process is centrally controlled by NACO. Relocation of Drugs is done as per requirement. There were problems regarding the supplies of OI drugs. ART Centres staff told that recently some funds were released to procure OI drugs. In the ICTCs, there were problems of erratic supplies of kits, drugs and consumables. The TI and CCC NGOs are given grants to buy drugs and consumables.

7. Monitoring and Evaluation:

NACP III is based on three ones principle and has a national Monitoring and Evaluation (M&E) plan. It aims at developing a consolidated Strategic Information Management System (SIMS) at national and state levels to focus on strategic planning, monitoring, evaluation, surveillance and research. It is aimed to provide effective tracking response to HIV epidemic. This will be a web-based system networking all facilities developed/supported under NACP-III.

The program officers implementing various programs pointed that M&E systems are well placed at SACS and M&E officers compile, upload and analyze the data. The M&E officer of few states also revealed that the reporting from some units was low, for example: blood bank, TI, CCCs. Some officers also raised concerns about the quality of data due to inadequate training of the staff at primary data collection units. Many program officers at SACS also informed lack of routine feedback from NACO to SACS and from SACS to primary data collection units. At DAPCU level, it was found that M&E officers were available in many states but they need intensive training in data management, analysis and report generation so that the data can be used to focus program intervention. It was observed that ART Centers are computerized and have PLHA Software. Soft copy of report is directly sent to NACO with a copy to SACS. The staff at ART Centre complained of too much of documentation and recording. The Medical officer at one of the centers said that it was very difficult to maintain all record and reports with such high load of patients.
ICTC are under the process of computerization. All reports (physical copy / soft copy) are first received at SACS and then checked and compiled and sent to NACO. Postal delay in getting information is also a problem. Reporting from facilities is very poor. Constant reminders are required. Blood banks do not feel accountable to SACS. Reports are sent physically and then collated at SACS.

The DAPCU officers reported lack of coordination from NGO led TIs and CCCs. The CCC staff in some of the states pointed that they have to maintain two kinds of records, one prescribed by NACO and the other suggested by implementing agencies. The reporting was not very streamlined in case of TI NGOs also. One of the TI staff reported that the reporting formats were frequently changed and it is difficult to keep pace with them.

8. Supervision of the Program:

Senior Officials at SACS mentioned that regular review meetings are conducted at the Central level for all components to assess the performance of each state. These meetings also serve as forums where states can share their problems and seek guidance from NACO. It was also told that there are continuous supervisory visits of the senior officers from Centre to the SACS and facilities to look into the gaps in implementation. Majority of the officers said that the visits help to maintain the quality of services and help to sort out issues related to other health departments.

It was also found during the study that regular staff meetings are conducted at SACS level headed by the Project Director. The SACS also conducts component wise meetings within the state to disseminate information, review the progress, get an update on implementation and plan future strategies.

There are monthly reviews of DAPCUs by SACS. All District Supervisors are given quarterly plan. The District supervisors also shared that there were quarterly review of all ICTC Counselors of the state. In addition, the district supervisors conducted monthly meetings of counselors. The District Supervisors also brought forth the issue of daily reporting to SACS and NACO but they find it difficult due to lack of Computer/Laptop. The district supervisors are also given motor bikes to be in continuous touch with facilities.

The Health care providers at ART Centers and CCC revealed that Regional Coordinators appointed by NACO provide constant mentoring and supervision. For Targeted intervention program, TSU provided guidance to the NGOs. ICTC staff shared that they got guidance from the District Supervisors. The district level monthly meeting of the ICTC also provided forum to discuss and sort out local issues. In addition, there were quarterly meeting of the ICTC at SACS which helped ICTC to plan their action. In the LACs it was found that regular supervision was missing.

9. Finance:

It was revealed by SACS that the entire program is centrally funded. The funds are allocated to states based on their annual action plan. The finance officers at SACS pointed that there was flexibility to re-appropriation of funds in a given component under various heads. The program officers revealed that there is provision of even submitting the new proposals or change in the program strategies even in the midst of the year provided there is sufficient justification. Majority of the officers pointed that fund flow from centre to SACS is fine. Approval has to be taken for already approved activities. Fund utilization at the facility level is disorganized. Funds from SACS are released to the accounts of the Medical Superintendent/Head of the institution. Any flow of funds to the facility level is done through Finance department of the institution. It is very difficult for Nodal Officers get the funds mobilized under such cases. Many times, the nodal officers are not aware of the availability of the funds and financial reporting from the institutions is very chaotic. Most of the DAPCUs did not mention any problem regarding fund flow.

CONCLUSION

The current study provides an opportunity to assess the HIV response to understand what must be done to ensure that India is on course to achieve the goal of universal access to prevention and treatment.

A systematic and comprehensive approach based on scientific and programmatic evidence is the key for successful implementation of Public Health Programs. The NACP-III is guided by the three-ones principle and this acts as a unifying factor for the national response.
and helps to set standards of quality for HIV/AIDS prevention and treatment programs.

Strategic and program interventions are evidence-based and result-oriented with scope for innovations and flexibility. Priority is accorded to specific local contexts and interventions planned accordingly. Study findings conclude that program is well-structured and well-tailored considering the national as well as local needs, ensuring full implementation of evidence-informed policies and programs. Being a large country with wide variations in social, geo-physical and health service patterns across the States and regions, it was necessary to prepare a plan that is technically sound and uniform but allows flexibility and adaptability at the local level.

A number of challenges and gaps have been identified, namely: low reporting by primary data generation units; lack of skills to appropriately use information generated through CMIS; poor quality of data due to inadequate training of the primary data collection units; lack of routine feedback from NACO to SACS and from SACS to primary data collection units. The convergence of decentralized delivery system with other health system is helpful in long term sustainability of the program. The main challenge lies in increasing the availability of prevention and treatment in resource-limited countries. The expansion of HIV prevention and treatment services is currently hindered by weak infrastructure, limited human and financial resources, and poor integration of HIV-specific interventions within broader reproductive and child health services. Technical staff appointed for program implementation and service delivery is contractual. The attrition rate of contractual staff is very high due to job insecurity, lack of benefits and incentives and lack of growth options. High turnover of staff and frequent transfer of regular staff especially Project Directors is also a constraint in some States. There is need to strengthen monitoring and supervision and training of program managers at SACS and DAPCU to take informed decisions.

Our study is based on observations of a few program sites, DAPCU & SACS Offices as representativeness of sites was not the goal of the study. However, recently held mid-term review has revealed similar problems in many States. It would be important to examine these issues at national and State levels and take corrective actions to achieve the goal of halting and later reversing the epidemic of HIV/AIDS in India.

REFERENCES

AWARENESS OF MOTHER TO CHILD HIV TRANSMISSION AMONG WOMEN ATTENDING ANTENATAL CLINIC, SMIMER, SURAT

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Surat Municipal Institute of Medical Education and Research (SMIMER), Surat, India

ABSTRACT

The study was conducted among 946 women attending antenatal clinic in SMIMER teaching hospital during 2008 to know their awareness regarding various aspects of mother-to-child transmission (MTCT) of HIV and it was found that though the general awareness of HIV is high, awareness of MTCT and its prevention is low which need to be improved through focused IEC campaign.

Background: More than 90% of HIV infections in children aged <15 years are due to mother-to-child transmission (MTCT). The MTCT may reverse the gains of the child survival strategy in the country. Awareness of HIV and MTCT among antenatal women plays crucial role in preventing MTCT.

Objective: To evaluate the awareness and knowledge of HIV/AIDS, MTCT of HIV and the methods to prevent MTCT of HIV.

Methods: This is a descriptive cross sectional study carried out among antenatal women at the SMIMER Teaching Hospital during 2008.

Results: Out of 946 women interviewed, 78% had heard of HIV/AIDS and main sources of information were television (48.8%) and radio (37.8%). Ninety-one percent of women were aware of MTCT of HIV. Trans-placental route (41%) was the commonly identified route of transmission. Awareness of HIV/AIDS, HIV co-existence with pregnancy and Mother to child transmission was significantly higher among women with at least secondary education (P<0.05). The level of knowledge and perceptions of MTCT of HIV is inadequate.

Conclusion: There is an urgent need to scale up HIV awareness programme focusing on mother-to-child transmission in the region.

Keywords: Awareness, education, mother-to-child transmission, prevention, HIV

INTRODUCTION

India accounts for about 7% of all HIV/AIDS cases in the world.¹ Globally, women constitute 48% of adults infected with HIV; in India, they constitute 39%. The prevalence is highest among productive young people between the ages of 20 and 29 years, with 60% of new infections occurring in the 15-25 years age group.²³ Heterosexual transmissions account for more than 80% of all infections.⁴ The pandemic is having a serious effect on the reproductive health of women in India.

There is an increase in the number of children infected with HIV in recent years as the number of HIV-positive women has increased.¹ More than 90% of HIV infections in children aged less than 15 years are due to mother-to-child transmission of HIV.⁵ In many developed countries, testing, antiretroviral therapy and infant-feeding modifications have been effectively used to eliminate mother-to-child transmission of
In countries like India, HIV continues to be a problem due to lack of information, testing services and antiretroviral therapy. The high prevalence of mother-to-child transmission has reversed the gains of the child survival strategy in the country. There is an urgent need to combat this menace.

The "Prevention of Mother-to-Child Transmission of HIV" service was started in October 2003 at SMIMER hospital, Surat. Following the designation of this center as a service center for the program of prevention of mother-to-child transmission, a counselor appointed to run the centre. The current study was undertaken to assess the knowledge and awareness about HIV/AIDS and mother-to-child transmission among women attending the antenatal OPD. The information obtained would influence the counseling and education of patients and the community about HIV/AIDS.

MATERIAL AND METHODS

The study was carried out at the antenatal outpatient department of SMIMER Teaching Hospital, Surat, from 1st January 2008 to 30th December 2008. Women included in the study were in first contact with HIV facilities, which was at our center, before any form of counseling. The instrument used was a questionnaire designed to assess awareness of the women about HIV/AIDS, evaluate their knowledge of possible routes of transmission, particularly mother-to-child transmission, and measures to prevent vertical transmission from mother to child. It included socio-demographic information such as age, marital status and level of education. Questions were asked on whether they were aware of HIV/AIDS, the routes of transmission of HIV, the possibility of HIV coexisting with pregnancy and transmission to the infants, the timing of transmission from mother to child, measures to prevent mother-to-child transmission; and whether they felt that a healthy person could be infected with HIV. The questionnaire was then pre-tested for comprehensibility, appropriateness of language, sensitivity of questions and average duration of administration. The interviewers were trained in interviewing techniques, were aware of the eligibility criteria of respondents and were capable of providing a detailed explanation of each question in the local language. Ethical approval was obtained from the institutional ethical committee of the SMIMER Teaching Hospital, Surat.

Sample size

Sample size was calculated from the expression n = 4pq/L², where p is expected frequency of factor under study and q is ‘1-p’. The confidence level was specified as 95%, and the tolerable error margin (L) was 5%. Specifications for p were determined based on the study objectives. The largest sample size that satisfied all objectives was used and a sample of 788 respondents was needed (p= 67, which was the prevalence of awareness among urban women of transmission of HIV from mother to her infants in NFHS 3. The sample size was adjusted to compensate for a non-response rate of 10%. The final, minimum sample size was 867.

Data analysis

Data was analyzed using Epi-info for the descriptive aspects of analysis, and frequency distributions were generated for all categorical variables. Means and standard deviations were determined for quantitative variables. The Chi-square test was applied for comparisons of proportions and for evaluating association of categorical variables. Statistical significance was said to be achieved when the P value was < 0.05.

RESULT

A total of 946 women attended the antenatal clinic and given consent were interviewed during the study period. The age range of the respondents was 17-42 years, with a mean age of 27.2 (SD 4.71) years. Nine hundred thirty one respondents were married, and 516 (54.5%) had at least secondary level of education (Table 1).

| Table 1: Socio-demographic characteristics of study population |
|-----------------|-----------------|-----------------|
| Age (years)     | Number (n=946)  | Percentage |
| 15-19           | 23              | 2.4           |
| 20-24           | 290             | 30.7          |
| 25-29           | 312             | 33.0          |
| 30-34           | 275             | 29.1          |
| >35             | 46              | 4.9           |
| Marital Status  |                 |               |
| Married         | 931             | 98.4          |
Table 2 shows the awareness and knowledge of HIV/AIDS among the respondents. 78% respondents interviewed were aware of HIV/AIDS, and among them 55% had been aware of the disease for more than 5 years. The main sources of information included television (48.8%), radio (37.8%), public awareness campaign (35.3%) and health workers (35.4%). All women who have heard of HIV/AIDS identified sexual intercourse as a route of transmission of HIV. Sharing of sharps (needles, razors) and blood transfusion were identified as additional routes of transmission by 17% and 48% of the respondents, respectively. Higher proportion (62%) of the respondents think that HIV infected person cannot be look healthy.

<table>
<thead>
<tr>
<th>Table 2: Awareness and knowledge of HIV/AIDS</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heard of HIV/AIDS (n=946)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>736</td>
<td>77.8</td>
</tr>
<tr>
<td>No</td>
<td>210</td>
<td>22.2</td>
</tr>
<tr>
<td>Duration of awareness (n=736)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5 years</td>
<td>332</td>
<td>45.1</td>
</tr>
<tr>
<td>&gt;5 years</td>
<td>404</td>
<td>54.9</td>
</tr>
<tr>
<td>Source of information (n=736)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Television</td>
<td>359</td>
<td>48.8</td>
</tr>
<tr>
<td>Radio</td>
<td>278</td>
<td>37.8</td>
</tr>
<tr>
<td>Public campaign / rally</td>
<td>260</td>
<td>35.3</td>
</tr>
<tr>
<td>Health workers</td>
<td>251</td>
<td>34.1</td>
</tr>
<tr>
<td>Newspaper</td>
<td>233</td>
<td>31.7</td>
</tr>
<tr>
<td>Friend/relative/neighbor</td>
<td>179</td>
<td>24.3</td>
</tr>
<tr>
<td>Route of transmission (n=736)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sexual intercourse</td>
<td>736</td>
<td>100.0</td>
</tr>
<tr>
<td>sharing/reusing needle/syringe</td>
<td>128</td>
<td>17.4</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>350</td>
<td>47.6</td>
</tr>
<tr>
<td>Infected person can be healthy (n=736)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>78</td>
<td>10.6</td>
</tr>
<tr>
<td>No</td>
<td>457</td>
<td>62.1</td>
</tr>
<tr>
<td>Don’t know</td>
<td>201</td>
<td>27.3</td>
</tr>
</tbody>
</table>

Trans-placental route as a mode of mother-to-child transmission of HIV was known by 60% of the respondents. A significantly lower proportion of the respondents identified vaginal delivery and breastfeeding as routes of HIV transmission (40% and 53%, respectively; P < 0.05).

Cesarean section was believed to be a route of transmission by 188 (41%) respondents. This proportion was significantly higher than the 39% of the respondents that identified vaginal delivery as a route of mother-to-child transmission of HIV (P < 0.05). Ninety-five (21%) women could not identify any route of mother-to-child transmission. The use of antiretroviral drugs in pregnancy and avoidance of breastfeeding were identified as methods of reducing mother-to-child transmission of HIV by only 17%

Table 3 shows that majority (91%) of the respondents were aware that HIV could coexist with pregnancy, but only 62% were aware of mother-to-child transmission of HIV. The observed difference between their two proportions was statistically significant (P < 0.05).

<table>
<thead>
<tr>
<th>Table 3: Knowledge on mother to child transmission</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV co-exist with pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>673</td>
<td>91.4</td>
</tr>
<tr>
<td>No</td>
<td>41</td>
<td>5.6</td>
</tr>
<tr>
<td>Don’t know</td>
<td>22</td>
<td>3.0</td>
</tr>
<tr>
<td>Mother to child transmission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>453</td>
<td>61.5</td>
</tr>
<tr>
<td>No</td>
<td>117</td>
<td>15.9</td>
</tr>
<tr>
<td>Don’t know</td>
<td>166</td>
<td>22.6</td>
</tr>
<tr>
<td>Route of transmission to child</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placenta</td>
<td>271</td>
<td>59.8</td>
</tr>
<tr>
<td>Vaginal Delivery</td>
<td>180</td>
<td>39.7</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>188</td>
<td>41.5</td>
</tr>
<tr>
<td>Breast feeding</td>
<td>240</td>
<td>53.0</td>
</tr>
<tr>
<td>Don’t know</td>
<td>95</td>
<td>21.0</td>
</tr>
<tr>
<td>Method of Preventing MTCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ART in Pregnancy</td>
<td>77</td>
<td>17.0</td>
</tr>
<tr>
<td>ART during labor</td>
<td>50</td>
<td>11.0</td>
</tr>
<tr>
<td>Delivery by caesarean</td>
<td>28</td>
<td>6.2</td>
</tr>
<tr>
<td>Special medication to newborn</td>
<td>36</td>
<td>7.9</td>
</tr>
<tr>
<td>no breast feeding</td>
<td>110</td>
<td>24.3</td>
</tr>
<tr>
<td>Don’t know</td>
<td>265</td>
<td>58.5</td>
</tr>
</tbody>
</table>
and 24% of the respondents, respectively. Delivery by cesarean section was identified as a method of prevention of mother-to-child transmission by only 28 (6%) respondents. A significantly higher proportion of respondents (58%, P < 0.05) did not know any method to prevent mother-to-child transmission of HIV.

Table 4 shows the knowledge about mother-to-child transmission of HIV among women with no or primary education as compared with women who had at least secondary education. Awareness of HIV/AIDS, HIV co-existence with pregnancy and Mother to child transmission was higher among women with at least secondary education. The observed differences were statistically significant (P<0.05).

<table>
<thead>
<tr>
<th>Heard of HIV/AIDS (n=946)</th>
<th>No or Primary education</th>
<th>At least Secondary education</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>317 (73.7)</td>
<td>419 (81.2)</td>
<td>0.005</td>
</tr>
<tr>
<td>No</td>
<td>113 (26.3)</td>
<td>97 (18.8)</td>
<td></td>
</tr>
<tr>
<td>HIV co-exist with pregnancy (n=736)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>246 (88.8)</td>
<td>427 (93.0)</td>
<td>0.047</td>
</tr>
<tr>
<td>No</td>
<td>11 (4.0)</td>
<td>30 (6.5)</td>
<td></td>
</tr>
<tr>
<td>Don't know</td>
<td>20 (7.2)</td>
<td>2 (0.4)</td>
<td></td>
</tr>
<tr>
<td>HIV transmit from Mother to her child (n=736)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>53 (19.1)</td>
<td>400 (87.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No</td>
<td>68 (24.5)</td>
<td>49 (10.7)</td>
<td></td>
</tr>
<tr>
<td>Don't know</td>
<td>156 (56.3)</td>
<td>10 (2.2)</td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

More than three forth of women in this study were aware of HIV/AIDS, and the majority also demonstrated knowledge of mode of transmission and the course of the disease. This is commendable and may be attributed to many factors, including the high level of education of the respondents. Such high levels of awareness have been reported in Delhi and Pune in India and in other parts of the world. The high levels of awareness and knowledge of HIV/AIDS reported in most parts of India may be the contributory factor for steady HIV prevalence among pregnant women, as shown by sentinel surveys. The main media of information on HIV/AIDS among women in this study were television, radio, health worker and public rallies. Mass media communications have been quite successful in increasing knowledge about HIV/AIDS and have been the first source of knowledge about HIV/AIDS for many.

Sexual intercourse was identified as a route of transmission by all the respondents who were aware of HIV/AIDS. This agrees with a worldwide trend in which sexual intercourse is the route of transmission mostly known to respondents. Blood transfusion and the sharing of razors and other sharp objects were identified by 48% and 17% of respondents, respectively, in this study as routes of transmission. It reflects lower level of awareness of HIV transmission by these routes.

Ninety-one of the respondents were aware that HIV infection could coexist with pregnancy, while a significantly lower proportion (61%) of respondents were aware of mother-to-child transmission of HIV. The study also reveals that illiterate or literate up to primary level respondents had poor in their awareness of mother-to-child transmission of HIV. Furthermore, specific knowledge of routes of transmission or measures available to prevent transmission was low. Similar low levels of knowledge were reported in other parts of India and Nigeria.

More than half of the women in this study did not know of any method of preventing mother-to-child transmission of HIV. Avoiding breastfeeding was identified by 24% of the respondents as a means of preventing transmission from mother to child, while only 6.2% of the respondents mentioned cesarean section as a method of preventing mother-to-child transmission of HIV. It is known that breastfeeding contributes 30-40% of vertical transmissions. This is a noteworthy and contemporary issue, since in India and other developing countries, breastfeeding is the cultural norm and exclusive breastfeeding is advocated because of high infant mortality and morbidity from diarrheal diseases and malnutrition. Women are likely to opt for breastfeeding to avoid being stigmatized as HIV/AIDS victims. However, there is a need for government and nongovernmental policies that will ensure a sustainable and effective breast milk substitute supply and its utilization by infants of HIV-positive women, just as antiretroviral drugs are currently being made available to these women.
This study provides information about knowledge of mother-to-child transmission of HIV and its prevention among women targeted for intervention in a high-prevalent urban area. The women in this study were interviewed at their first visit to the antenatal clinic. Some of the women could have had some information prior to presentation to the clinic. This study therefore has a reflection on the larger community.

In our locality, the opinion of the male partners strongly influences the adoption of health policies and programs by women.

For community education, a public media campaign should dwell more on the aspect of prevention of mother-to-child transmission. There is also the need for increased collaboration with HIV/AIDS prevention programs. Finally, a more comprehensive evaluation of knowledge and attitudes of both men and women in the community about HIV/AIDS and mother-to-child transmission will provide added information for establishing community intervention programs.

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IDENTIFICATION OF SOCIO-DEMOGRAPHIC FACTORS AND THE CAUSES OF POOR ADHERENCES OF URBAN DOTS IN BIRATNAGAR

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ABSTRACT

Background: TB is a major public health problem. Though investigation and chemotherapy is free of cost we are still facing poor compliance and severe degree of disease among the poorly treated ones. We have tried to explore into reasons behind it.

Methodology: It was a retrospective cross-sectional study done at Biratnagar Sub-metro Politian City.

Result: People accept DOTS. They visit as early as they are referred but are diagnosed lately. They have multiple reasons to quit the treatment before completion.

Conclusion: We found that early diagnosis and proper counselling about the need of prolonged regime of treatment and its side effects are important factors in preventing transmission and poor compliance.

Keywords: TB, DOTS, HIV, BPKIHS, cases, defaulters

INTRODUCTION

TB is a major public health problem. It is neither the stuff between patient and doctor nor controlled with provision of drugs made fifty years back and diagnostic modality developed hundred years ago. In 2006 there were estimated 1.5 million deaths among TB in HIV-negative people and 0.2 million among HIV positives. DOTS remain the only hope to control this public health problem amongst the third world country, but poor implementation of DOTS could be a double-edged-sword. DOTS therapy consists of two months of health worker observed treatment, followed by six months of treatment collected weekly from health facilities (2HRZE/6HE). Resistances to Mycobacterium tuberculosis is being man made phenomena rather than bacterial virulence. DOTS is considered as the best strategy to provision and monitoring of chemotherapy but is not free of limitations. It does not address multi-drug resistant TB and TB/HIV co-epidemic. International efforts are towards these burning issues whereas proper implementation of DOTS at root level is being placed in a shadow. Though people react differently to TB all of them are afraid of it. Despite implementation of DOTS for thirteen years, TB still remains as difficult-to-control. The growing burden of drug resistance and co-epidemic with HIV has made TB more complicated than it was ever thought. Adequate amount of drugs could not solve the problem as expected. Many pitfalls will never be solved by DOTS alone. Keeping this in view we had conducted a study with the following objectives to identify the socio demographic factors, health seeking behaviour and health services provision and the causes of poor adherence among cases in Biratnagar DOTS Clinic.

In South East Asian Region the estimated incidence of TB is 1.4 million cases every year. Most cases occur in the age group of 15-54 years, with males being...
disproportionately affected. The male/female ratio among newly detected cases is 2:1. The TB epidemic in the region is in constant flux due to variations in the quality and coverage of various TB control interventions, population demographics, urbanization, changes in socioeconomic standards and, more recently, the growing HIV epidemic.5

About 45% of population in Nepal is infected with TB out of which 60% are adults.6 TB affects 40,000 people annually of which 50% will spread disease to others.6 DOTS has been successfully implemented throughout the country since April, 2001. It is recommended as the “National Standard Care” in many countries for the treatment of tuberculosis but non-compliance to treatment is giving the patient a “chance to fail” especially when failure can be so devastating not only to the patient, but also to the community. The devastating consequences of failure are relapse, treatment failure, RAD, MDR-TB and XDR-TB.

By achieving global target of diagnosing 70% of new cases and treating 85% of them, we will be able to prevent 6000 deaths and reduce the transmission rate yearly. Currently, with 874 centers and 3117 sub centers, DOTS is being implemented throughout the country. Inspite of this, about 10% of cases present themselves as non adherent. By keeping patient alive and failing to cure them, poor treatment augments the spread of TB and proportion of drug resistant cases. Non adherence is a multi factorial phenomenon. Adherence is the series of responsibility among patients, health care workers and society. Drug resistant TB is unavoidable in near future unless major steps are taken immediately. Four national surveys of anti-tuberculosis drug resistance have been undertaken in Nepal. The first carried out in 1996 showed a prevalence of 1.1% of multidrug resistance in patients never previously treated for tuberculosis. This prevalence was 1.0% in 1999, 1.3% in 2001 and 2.9% in 2006.7

As we keep on solving these problems we will be facing new problems. Hence, exploring our knowledge beyond microbiology, pharmacology and internal medicine is important.

**METHODOLOGY**

It was a cross-sectional study carried out for 15 days from 01/04/006 to 15/04/006. The study was carried out in Biratnagar, where DOTS is run by a non-governmental organization, National Anti tuberculosis Association (NATA). The site and duration was chosen according to our convenience. Pre tested questionnaires containing close ended questions were used to collect information from TB cases and defaulters.

The questionnaire was prepared in English and translated in Nepali. All interviews were carried out after receiving the informed consent. TB cases were interviewed at DOTS centres and sub centres when they came for chemotherapy. Defaulters were traced out with the help of local personnel and were interviewed at their residences. Fund and logistics support were provided by B.P. Koirala Institute of Health Sciences (BPKIHS). The study was carried out under the guidance of School of Public Health & Community Medicine, BPKIHS. Among the 176 cases, only 113 cases were interviewed due to various reasons like refusal of consent, absence at the time of visit, inadequate number of interviewer with respect to the number of sub centres etc. Among the 16 defaulters, 14 were traced out of which 2 denied the interview. The limitations of the study were recall bias of the cases, short duration of study and need of translator among the cases whose local language were other than Nepali.

**RESULTS**

<table>
<thead>
<tr>
<th>Table 1: Demographic factors among the interviewed patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Factors</strong></td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>1. Category</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>2. Age(years)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>3. Sex</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>4. Religion</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>5. Marital status</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>

(Numbers in the parenthesis indicates percentage)
Male outnumbered female in both cases and defaulters’ category (Table 1). Majority of the cases were Hindus (83%) but most of the defaulters were Muslims (58.33%). The proportion of divorce was higher among defaulters (25%) than that among cases (4.46%).

Table 2: Socio cultural factors among the interviewed patients

<table>
<thead>
<tr>
<th>Factors</th>
<th>Subclass</th>
<th>Cases (%)</th>
<th>Defaulter (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.Family size</td>
<td>&lt;4</td>
<td>15(13.27)</td>
<td>0(0)</td>
</tr>
<tr>
<td></td>
<td>4-6</td>
<td>65(57.52)</td>
<td>59(41.66)</td>
</tr>
<tr>
<td></td>
<td>&gt;6</td>
<td>33(30.21)</td>
<td>7(58.34)</td>
</tr>
<tr>
<td>2.Over crowding</td>
<td>Yes</td>
<td>89(78.76)</td>
<td>9(75)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>24(21.24)</td>
<td>3(25)</td>
</tr>
<tr>
<td>3.Literacy</td>
<td>Literate</td>
<td>65(57.52)</td>
<td>2(16.66)</td>
</tr>
<tr>
<td></td>
<td>Illiterate</td>
<td>48(42.48)</td>
<td>8(66.66)</td>
</tr>
<tr>
<td>4.Earning</td>
<td>Daily wages</td>
<td>51(45.13)</td>
<td>8(66.67)</td>
</tr>
<tr>
<td></td>
<td>Monthly</td>
<td>29(25.68)</td>
<td>0(0)</td>
</tr>
<tr>
<td></td>
<td>salary</td>
<td>20(17.69)</td>
<td>1(8.33)</td>
</tr>
<tr>
<td></td>
<td>Self</td>
<td>13(11.50)</td>
<td>3(25)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.Substance abuse</td>
<td>Smoking</td>
<td>51(45.13)</td>
<td>9(75)</td>
</tr>
<tr>
<td></td>
<td>Alcohol</td>
<td>38(33.62)</td>
<td>5(41.66)</td>
</tr>
<tr>
<td></td>
<td>Drugs</td>
<td>04(3.53)</td>
<td>1(8.33)</td>
</tr>
<tr>
<td>6.Travel time to DOTS Clinic (in minutes)</td>
<td>&lt;15</td>
<td>50(44.24)</td>
<td>0(0)</td>
</tr>
<tr>
<td></td>
<td>15-30</td>
<td>52(46.01)</td>
<td>2(16.67)</td>
</tr>
<tr>
<td></td>
<td>&gt;30</td>
<td>11(9.73)</td>
<td>10(83.33)</td>
</tr>
</tbody>
</table>

(Numbers in the parenthesis indicates percentage)

Overcrowding was experienced by 78.76% of the cases and 75% of the defaulters (Table 2). Literacy rate among cases was found to be 57.52% but the literacy rate among defaulters was only 16.66%. Tobacco was the main substance used by both cases (45.13%) and defaulters (75%).

Table 3: Health related behaviour among the interviewed patients

<table>
<thead>
<tr>
<th>Factors</th>
<th>Subclass</th>
<th>Cases (%)</th>
<th>Defaulter (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.BCG vaccination</td>
<td>Yes</td>
<td>88(77.87)</td>
<td>11(91.67)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>25(22.13)</td>
<td>01(8.37)</td>
</tr>
<tr>
<td>2.Gap between symptoms and seeking help (in days)</td>
<td>&lt;15</td>
<td>46(40.70)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>16-30</td>
<td>41(36.28)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>31-45</td>
<td>03(2.65)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>19(16.81)</td>
<td>NA</td>
</tr>
<tr>
<td>3.Gap between seeking help and diagnosis (in days)</td>
<td>&lt;15</td>
<td>13(15.92)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>16-30</td>
<td>20(17.69)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>31-45</td>
<td>12(10.61)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>21(18.58)</td>
<td>NA</td>
</tr>
<tr>
<td>4.Symptomatic Relief (in weeks)</td>
<td>&lt;4</td>
<td>55(48.67)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>4-6</td>
<td>27(23.89)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>&gt;6</td>
<td>31(27.43)</td>
<td>NA</td>
</tr>
<tr>
<td>5.Faith on DOTS Treatment</td>
<td>yes</td>
<td>98(86.72)</td>
<td>2(16.67)</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>08(7.07)</td>
<td>8(66.66)</td>
</tr>
<tr>
<td></td>
<td>no comment</td>
<td>07(6.19)</td>
<td>2(16.66)</td>
</tr>
</tbody>
</table>

(Numbers in the parenthesis indicates percentage)

Table 3 shows that most of the cases seek medical help within 30 days but our medical and laboratory facilities diagnose them only after 30 days. Most of the Cases have faith on the DOTS (86.72%) but most of the defaulters do not (66.66%).

REASONS TO QUIT DOTS (IN %)

- none to escort
- long travel time
- DOTS staffs are less friendly
- need to return my job
- treatment of prolong duration

Fig. 1 Reasons for quitting DOTS among the traced drop out cases:
Above bar diagram shows that reasons for defaulting were multiple. The most common reported reason for defaulting was long duration of treatment (83.33%).

**DISCUSSION**

Like national demographic distribution, majority of cases belonged to CAT I (59.29%) and working age group (85.84%). TB is a disease of productive age group and majority of cases are infectious. About seventy eight percent were vaccinated. Different risk factors like overcrowding (78.76%), smoking (48.13%), and alcohol intake (33.62%) were present in the study subjects. About fifty eight percent of cases were found literate contradicting with the national figure. It could be due to health seeking behaviour among literates. This study showed that 83.33% of the cases were adherent to urban DOTS launched by an NGO. Similar results were found in Uganda with 81% adherence to community based DOTS and 48% to non community based DOTS by Byaranhanga et al. P.G.Gopi et al found nongovernmental DOTS centre as a risk factor for poor adherence which contradict with our study finding. Study in Kathmandu valley found a need of linking organisation between public and private sectors in treatment of TB patient. NATA might have played bridging role, which could be the reason for good adherence. The associated factors among the defaulters were 58.33% male sex, 58.33% Muslim religion, 75% married, 83.34% illiterate and 66.67% daily wage workers. A study carried in 1997 in Islamabad found poor association between adherence with widow and divorcee.

The study conducted by P. Mishra et al in a Western hilly district of Nepal concluded that the risk of non-adherence to TB treatment was significantly associated with unemployment (OR= 9.2), low occupational status (OR= 4.4), low annual income (OR= 5.4), and cost of travel to the TB treatment facility (OR =3.0). Regarding accessibility, 33.33% of defaulters felt long travel time, 16.67% have no one to escort and 58.33% have to return to work place away from DOTS centre. Regarding knowledge, 25% never knew the duration of treatment, 58.33% thought microscopy negative means cure, 66.67% felt being cured after symptomatic relief. About forty two percent do not like chemotherapy for half a year due to side effects and same percent found DOTS staff being less friendly. P.G.Gopi et al found similar facts; among the non adherent, 67% have inadequate knowledge about treatment, 57% have difficulty in accessing health facility and 38% found DOTS staff less friendly. A study carried by T.S.Bam et al found that 61% non adherent had insufficient knowledge about the need of taking drugs for more months after feeling better and 59% with long travel time. A study by Garner et al among prisoners found that 60% of defaulters blamed the side effects of the drugs.

The other very important finding of our study is lag period of about two months between health seeking behaviour and diagnostic modality. Following is the graph plotted from the factor number 2 and 3 from table 3 with x axis as duration in days and y axis as numbers of cases. In the graph health seeking behaviour is right skewed whereas the diagnosis is left skewed.

![Graph showing relation between health seeking nature and diagnosis](image-url)
Health seeking nature before 30 days of symptoms covers more than 75% of areas whereas more than 75% of diagnosis falls after 30 days. There can be many explanations to it. As we know diagnosis is a multistep procedure involving sample collection, processing, reporting and report collection. So delay in any above steps could lead to delayed diagnosis. The delay could be due to late referral from local practitioner or improper sample collection technique like three samples collected on third day, sample contaminated with saliva, postponing sample collection to office day if the third day is a public holiday etc. We neither have any screening test nor have any diagnostic test that can diagnose all types of TB. We hardly culture specimen of the highly suspected stain negative patients; even if cultured the reporting is after six weeks. Our reporting system is not equipped with good technology and technicians. We are not being able to afford recent diagnostic methods. There is a chance of delayed report collection because most of the patients rely on daily earning and so they hardly want to lose a day for report collection. Whatever the reason there is a great impact on transmission of disease.

More than 75% TB cases will be spreading bacteria for two and half months since the first day of symptomatic presentation, provided the DOTS is started on them. Hence early referral or rapid diagnostic approach or both are very necessary to prevent this transmission.

**CONCLUSION**

DOTS was well accepted by TB patients in Biratnagar. Despite early help seeking nature they are received at the desk of DOTS lately. Lack of knowledge about the duration and the side effects had made the cases to quit the course before schedule.

**RECOMMENDATION**

In the race of achieving the global target we might be putting less effort regarding proper implementation of DOTS. We may be adopting the new internationally accepted methods without modifying socio cultural factors and addressing the factors not touched by DOTS. Non adherent and defaulters go on increasing unless we understand this as a multi-fatorial phenomenon. Development of new drugs and rapid diagnostic methods will be important tools to control TB but the proper implementation of any programme at grass level is equally important. Early diagnosis and treatment are fundamental to tuberculosis (TB) control. Nevertheless, the effectiveness of TB management continues to be influenced by treatment adherence. The diversity of patients' attitudes towards the disease and the extreme variability of access to care, especially in resource-poor countries, are amongst the many factors of social context that profoundly affect the ability of control programmes to implement this policy effectively.

**ACKNOWLEDGEMENT**

We would like to thank Dr. Ankur Podar, and Dr. Ramesh Baniya for their kind help during the data collection as well as people who have directly or indirectly contributed in their own way during the whole process and finally, we are grateful to School of Public Health, BPKIHS, for providing this opportunity to come up with this article.

**REFERENCES**


MALE CIRCUMCISION SHOULD BE PROMOTED IN DEVELOPING COUNTRIES AS A MAJOR MEANS OF HIV PREVENTION

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INTRODUCTION

Male circumcision is a religious practice in some of the countries in Asia and Africa. In the Muslim community it is particularly a very important ceremony where the foreskin of the penis is cut to remove normally before the fifth birthday of the boys particularly in Indian Muslim community. There are some other ethnic groups that perform this practice across Africa. However in the countries such as USA, Canada, Australia and South Korea it is performed irrespective of the culture, religion and ethnicity.1

Male circumcision is deeply rooted in the culture and moral values of many communities in the developing world and hence remains a sensitive issue when it comes to link it with the issues of reproductive health and sexuality. The issue becomes more sensitive in the wake of STIs and HIV/AIDS. Linking the transmission of HIV and uncircumcised male has witnessed intense resistance and doubt and silence over the years. This is reflected well in less availability of public health interventions and information on this issue.2

Almost 20 years have passed when Cameron and colleagues presented a study that showed that there is 8 times more risk for contracting HIV -1 among uncircumcised males.3 In this article I discuss the promotion of male circumcision in developing countries as a major means of HIV prevention.

DISCUSSION

Table 1: Comparative figures of per cent of male circumcision and HIV seroprevalence in 8 different countries of Asia and Africa

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Country</th>
<th>Per cent of circumcised male</th>
<th>HIV Seroprevalence rate in low risk groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Zimbabwe</td>
<td>10.1 %</td>
<td>19.7 %</td>
</tr>
<tr>
<td>2.</td>
<td>Bostwana</td>
<td>11.0%</td>
<td>41.6 %</td>
</tr>
<tr>
<td>3.</td>
<td>Namibia</td>
<td>21%</td>
<td>16%</td>
</tr>
<tr>
<td>4.</td>
<td>Thailand</td>
<td>13%</td>
<td>1.1%</td>
</tr>
<tr>
<td>5.</td>
<td>Philippines</td>
<td>93%</td>
<td>0.3%</td>
</tr>
<tr>
<td>6.</td>
<td>Kenya</td>
<td>83.0%</td>
<td>10.5%</td>
</tr>
<tr>
<td>7.</td>
<td>Congo</td>
<td>90%</td>
<td>3.1%</td>
</tr>
<tr>
<td>8.</td>
<td>Cameroon</td>
<td>&gt;80%</td>
<td>6.9%</td>
</tr>
</tbody>
</table>

Let us have a look of the figures presented in table No.1. If we compare the figures by segregating the data into two categories of those countries where the per cent male circumcised is more than 80 per cent and less than 20 per cent and compare it with the prevalence rate of HIV seropositivity, we find a beneficial effect of male circumcision in terms of low HIV seropositivity.

Though there are different conditions prevailing in these countries with respect to health system, risk factors, socio-economic and cultural values and norms and interventions on HIV/AIDS even though the seropositivity differences are worth noticing. Daniel et al also argues on the similar lines in their study on male circumcision and HIV infection.1
There are several studies that explain uncircumcised males are more prone to contract HIV and other pathogen through the foreskin. Simonsen et al reported as early as in the year 1988 that foreskin contain higher density of primary target cells that facilitate sexual transmission of HIV than cervical, vaginal and rectal mucosa. Patterson et al in the year 2002 reported that under in vitro culture, human foreskin is highly susceptible to HIV -1. Hussian et al reported that in histopathological studies, epithelium of human foreskin provide main portal of entry for HIV-1 into the penis. The biological phenomenon reflects that there is a strong relationship between transmission of HIV -1 and presence of foreskin in the heterosexual activities suggesting that the removal of the foreskin of penis could be used as a public health strategy to prevent the transmission of HIV-1.

A study was conducted in India with 2298 HIV uninfected males attending clinics of sexually transmitted diseases. In this study it was found that the male circumcision was strongly protective against HIV -1. A similar trail was also conducted in Kenya with the young men in Kisumu for HIV prevention. In this trial also it was found that male circumcision significantly reduces the risk of acquisition of HIV. This study suggests that male circumcision services should be integrated with HIV prevention strategies.

A trail was conducted in peri-urban settings in South Africa with 3274 men aged between 18-24 years. This trail recruited the participants from the general population and so loss to follow up was less in this trail. This also ensures the generalisability of the results of this trail. The results of the trial suggest that there is 60 per cent less relative risk of HIV in circumcised men.

Seeing the benefits that male circumcision provides WHO and UNAIDS recommended that those men who want to get circumcised should be provided the procedure. These services should also be provided to those who do not wish to undergo HIV testing and irrespective of the HIV status of men.

The benefits of male circumcision for men with respect to reduced risk of HIV transmission from the sex partners are evident but, the protection for the women is not obvious. Maria Wawer and colleagues conducted a clinical trial in Uganda. This trail was stopped in between as no benefit was found in terms of risk reduction for women whose HIV infected partners were circumcised. The data of this study suggest that the risk for the women increased whose partners resumed the sexual activities before the wound healing. No HIV intervention based on single stand alone approach would be effective unless a comprehensive package of various HIV prevention strategies are combined together. Male circumcision provides one such window of opportunity in long term benefits for both male and females considering the fact that if less number of male are infected the transmission in heterosexual relationships from male to female would be less. The benefits of couple counseling, constant and consistent use of condoms and couple testing for HIV can not be undermined. The benefits of circumcision in reducing men’s risk of HIV are clear, and circumcision roll-out presents a unique opportunity to enhance HIV prevention services for both women and men.

Male circumcision does not provide 100 per cent protection against the transmission of HIV and the use of condoms remains an important aspect of HIV prevention efforts. Similarly there are issues involved with the consistent and correct use of condoms. In the population the condom use is found to be inconsistent. So combining the benefits of condom use along with male circumcision provides a benefit that is worth noticing.

### MALE CIRCUMCISION AS A PUBLIC HEALTH INTERVENTION ACCEPTABILITY

Circumcision is normally done for three main reasons. These are religious, ethnic and medical condition. The communities in Kenya, South Africa and Botswana have reported the acceptance of circumcision in the range of 51 % to 61 % among uncircumcised men. The biggest challenge in the intervention related to circumcision is that those communities that are at higher risk of HIV would be least accepting it as they are traditionally noncircumcising communities. There is also a stigma associated with circumcision in some communities and a systematic study needs to be carried out to understand the perception of these
communities. The fear of pain, bleeding, reduced sexual pleasure and access to health services are some of the concern of the people. Related to the acceptability is the false perception of the people that circumcision would protect them from contracting HIV. This poses a huge problem and may cause a sense of false security. A study conducted in South Africa revealed that 30 per cent of circumcised men believe that they can safely have sex with multiple partners.14

SAFETY

Circumcision is a surgical process however in traditional settings it is being done by non-licensed practitioners. It can lead to complications if performed by untrained persons in nonmedical settings. A study done in Turkey reported that when circumcision was done in non-medical settings, 85 per cent of male adults faced complications. On the other hand when it was performed by licensed professionals only 2 per cent faced some kind of complications.15 From the above study it can be concluded that circumcision if performed in a medical settings by a trained professional can be a safer intervention.

SOME OTHER CONSIDERATIONS

There are some other issues that needs to be taken into consideration and these are legal issues, cultural sensitivity, human rights issues, ethical consideration when designing the interventions of male circumcision as a mean of HIV prevention.16

CONCLUSION

There are convincing evidences across various regions that male circumcision has the potential to reduce the transmission of HIV and thus reduce the reproductive rate of HIV in a population. Male circumcision could eventually be used as one of the strategies in controlling the spread of HIV within the comprehensive HIV/AIDS control programme in a developing country. The issues such as false security, safety period, infection control, cultural issues, women’s safety issues, couple counseling for circumcision etc. should be carefully considered.

This strategy needs to be made affordable and safely available to the populations having high burden of the disease. The role of government, nongovernment organizations and funding agency become critically important in promoting this strategy.

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STUDY OF NOVEL POTASSIUM PERMANGANATE STAINING IN COMPARISON WITH CONVENTIONAL ZN STAINING FOR THE DIAGNOSIS OF PULMONARY TUBERCULOSIS

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ABSTRACT

An estimated 1.8 billion individuals are infected worldwide with the Tuberculosis and there is one death due to it every 15 minutes. It is essential that a fast, effective and inexpensive diagnostic test for TB is developed as early diagnosis stands central to the control of the disease. Traditional diagnosis of tuberculosis is still dependent on sputum microscopy by ZN stain that requires expertise and lacks sensitivity. In this study, we tried to evaluate the use of potassium permanganate staining which is easy, inexpensive and does not require much expertise for screening. Potassium permanganate oxidises Mycolic acid present in cell wall of Mycobacterium sp. which evolves an identifiable oxidation pattern. This study was undertaken to investigate this property of potassium permanganate as a typical laboratory test bridging the gap between sensitivity and time restraint required for an early diagnosis of tuberculosis. The sputum samples (early morning – spot) were collected from patients. From each sample two slides were prepared; one was stained with ZN stain and other with modified potassium permanganate staining. Sputum samples were simultaneously cultured on LJ media after decontamination and concentration. The pattern evolved on staining with potassium permanganate was observed under light microscope and assessed as a diagnostic tool. We found that the pattern formed on oxidation was in association with the AFB status of the sputum (p= 0.0001).

Keywords: Acid- Fast bacilli; Mycolic acid; Potassium permanganate oxidation.

INTRODUCTION

Worldwide increase in tuberculosis is indicative of our failure to use modern medical advances to control an ancient enemy. Despite killing 2 million people every year, TB is arguably the most neglected disease. India ranks number 11 and constitute nearly 30% of the global TB burden. Co-infection of TB with HIV-AIDS and the emergence of multidrug-resistant tuberculosis (MDRTB) have brought tuberculosis back into the priority list of the health care providers globally. It is essential that a fast, effective and inexpensive diagnostic test for TB is developed as early diagnosis stands central to the control of the disease.

The diagnosis of TB traditionally depends on demonstration of tubercle bacilli in clinical specimen. Direct smear examination after ZN stain is a simple and rapid test, but it lacks sensitivity (detects >10^4 bacilli/ml). This continued dependence on more than hundred years old smear test which has a sensitivity of 50% and is compounded by its inability to diagnose drug resistant bacilli. Conventional culture methods using LJ medium, despite being more sensitive (detects 10-100 bacilli/ml) is time consuming (3 to 12 weeks), and the modified culture system, BACTEC, takes 7-12 days. Advances in the field of molecular biology have provided rapid diagnostic tools such as PCR based on nucleic acid amplification with reduced turnover time for the unavailability in peripheral centers, cost ineffectiveness and the requirement for specialist personnel has limited its use.
The unique cell wall structure of Mycobacterium tuberculosis accounts for its unusual low permeability and resistance towards common antibiotics. The main structural element consists of a cross-linked network of peptidoglycan in which some of the muramic acid residues are replaced with arabinogalactan, which in turn is acylated at its distal end with mycolic acids. The entire complex is essential for viability in M. tuberculosis and other Mycobacteria. 2

Potassium permanganate oxidises Mycolic acid present in cell wall of Mycobacterium sp. which evolves an identifiable oxidation pattern. This study was undertaken to investigate this property of potassium permanganate as a typical laboratory test bridging the gap between sensitivity and time restraint required for an early diagnosis of tuberculosis.

**METHODOLOGY**

This prospective study was done to evaluate the use of the above stated method as a diagnostic tool for tuberculosis. This short term research project was conducted in Department of Microbiology, Bowring & Lady Curzon Hospital, Bangalore Medical College and Research Institute, Bangalore over a period of three months (June – August 2009). We included patients attending RNTCP centers in Victoria and Bowring Hospitals, Bangalore with clinical presentation indicative of tuberculosis (like chronic cough > 3 weeks, haemoptysis, etc) who were not on ATT to take part in the study with an informed consent. The sputum sample was collected from the RNTCP centers, where the treating team routinely uses sputum samples for diagnostic purpose. On the basis of ZN staining results, 50 samples were collected, of which 25 were AFB positive and 25 were AFB negative according to RNTCP criteria.3 The ratio of AFB positive to AFB negative samples was deliberately kept 1:1 to have an ample opportunity to investigate AFB positive samples.

Processing of the samples: The selected samples were transported to the laboratory and processed on that very day itself. All the samples were handled carefully in a bio safety cabinet (Class II). Three parts were prepared from each specimen, one used for the ZN technique4, one for potassium permanganate stain; and the other for culture inoculation. All the samples were cultured on LJ media using standard precautions and were incubated for 8 weeks and the culture positives were identified using standard techniques.5

**Potassium permanganate (PP) staining technique:**

1) One ml of sputum sample was centrifuged at 2000 rpm for 5 minutes and the supernatant was discarded.

2) The pellet is washed once with 500 μl sterile water and again centrifuged at 2000 rpm for 5 minutes.

3) The pellet was suspended in 15 μl of sterile water. 10 μl of suspension is placed on a microscopic slide and heat fixed.

4) Then 10 μl saturated potassium permanganate solution was added and the slide is incubated at 56°C for 5 minutes until dehydrated.

5) The slide is washed by sterile water until excess of color of potassium permanganate is removed.

6) After drying the slide at room temperature, the slide is subjected for microscopic examination.

**Statistical analysis:** Statistical analysis was performed using the Statistical Package for Social Sciences (version 16.0). The groups were compared using chi-squared test. Calculation of p-value was done using Fischer’s exact test. Statistical significance was fixed at p<0.05.

**OBSERVATION AND RESULTS**

The microscopic examination of the smears stained with modified potassium permanganate technique revealed a distinguishable pattern between AFB positive and AFB negative specimen. The positive or negative were strongly supported by results obtained from ZN staining and culture growths. The observations were made under light microscope at a magnification of 10X, 40X and 100X oil immersion objectives.

The salient features of the pattern evolved under observed under oil immersion magnification have been mentioned below:-
AFB positive smears:
- Deep brown to orange irregular patches seen (of 2-3 mm diameter in size).
- Patches are generally broken into polygons of indefinable shapes and sizes (Honey comb pattern).
- Pattern rests against a translucent background.
- More prominent in the centre of the smears.

AFB negative smears:
- The above mentioned patches are absent.
- The smear shows a uniformly translucent background with occasional artifacts.

Out of 50 sputum samples, 25 were AFB positive and 25 AFB negative based on the results obtained from ZN staining technique and culture confirmed. The Potassium Permanganate staining successfully picked up all 25 AFB positive. All the other 25 AFB negative samples were also shown negative by this staining technique. The results show a definite association between the pattern formed and the AFB status of sputum sample.

**DISCUSSION**

This is a pioneering study demonstrating the recognition of the pattern formed on oxidation of mycolic acid using KMnO₄. In the present study, the results of AFB ZN smear and culture on LJ media were analysed in comparison with Potassium Permanganate stain from 50 cases of clinical suspicion of pulmonary tuberculosis. In the study, 25(50%) cases were culture positive. ZN stain was positive in 24(48%) cases, whereas Potassium permanganate stain was positive in 25(50%) culture positive cases. The possible reason of 1 case being negative by ZN stain is that the samples stained with potassium permanganate were centrifuged prior to it. This would increase the bacterial concentration in the sample and possibly increase the sensitivity of the test. Ellena M Peterson et al (6) have reported that concentration smears of AFB have increased the sensitivity to 91% as compared to 81% of direct smears.

The comparison of the results obtained on PP staining with the AFB status of sputum samples reveals a definite association between the two where potassium permanganate staining was positive in all the ZN positive smears. Since no data was available about a similar study, no comparisons can be made.

The major difficulty in staining tubercle bacilli arises because their surface is coated with an unsaponifiable waxy substance. For such bacilli, the success of conventional staining techniques depend on the ability of the dye to uniformly penetrate the cell wall through this waxy barrier, while leaving intact the acid fast character of the bacilli (in ZN staining technique, heating with conc. carbol fuschin). In this novel potassium permanganate staining technique, the whole cell wall of bacilli is oxidised using a saturated solution of potassium permanganate. In turn, mycolic acid, present in the cell wall, is oxidised to evolve ‘honey comb pattern’.

This test avoids the complexity involved in other methods and presents an easier method. The pattern evolved in AFB positive samples is distinguishable and could be successfully employed for diagnosis of tuberculosis. It is easier for the eye to detect brown honeycomb pattern rather than a red fuschin stained bacillus in of surrounding. The time required by PP staining before declaring smear as negative was 2 minutes compared to 8 minutes with ZN staining(7). It’s a fast test and is highly cost effective at the same time as the only reagent required is potassium permanganate. Moreover the expertise required in handling reagents is less and reading results is easier.

The possible application of this test lies in its potential to be used as a mass diagnostic modality as it is easier, cost effective, and takes the minimal time. This makes it aptly suited for the primary health centers in a developing country as ours. It could also be used to funnel down the patients being investigated for tuberculosis.

This study has many strength. The AFB positive samples suggested by ZN staining technique were confirmed by culturing them on LJ media. ZN staining lacks sensitivity, the sputum samples with a bacterial load of less than 10⁴ bacilli/ml are not detectable (1). Culturing the samples picks up even those which have a bacterial load of 10-100 bacilli/ml, much more sensitive than ZN staining alone. A sincere attempt
was made to standardise the method to reduce the possible error that may have crept in while the experiments.

Certain limitations and caveats should be kept in mind while interpreting the results of this study. Firstly, sensitivity and specificity of the method has not been evaluated. The sample size is too small as still it is an ongoing study.

CONCLUSION

The microscopic examination of the smears stained with modified potassium permanganate technique revealed a distinguishable pattern between AFB positive and AFB negative specimen. This implies a possible application of potassium permanganate staining as a method in diagnosis of tuberculosis.

FUTURE PROSPECTIVES OF THE STUDY

- The sensitivity and specificity of the method has to be estimated, and compared with the standard ZN staining technique being used by including more subjects.
- The mechanism of oxidation and the products hence formed needs to be looked into. Colour tests based on the detection of product formed could be the shape of the things to come in future.

REFERENCES

EVALUATION OF NITRATE REDUCTASE ASSAY (NRA) FOR RAPID DETECTION OF DRUG RESISTANT TUBERCULOSIS AT NATIONAL TUBERCULOSIS CENTRE, NEPAL

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2 SAARC TB and HIV/AIDS Centre, Thimi, Bhaktapur, Nepal.

ABSTRACT

Background: Treatment of drug-resistant tuberculosis is often based on drug susceptibility testing results. Thus simple, rapid and economic test is very important for diagnosis of drug resistant tuberculosis and such method aids in TB control effectively. One such method is a Nitrate Reductase Assay (NRA).

Objective: To evaluate feasibility and performance of Nitrate Reductase Assay in the screening of drug-resistant tuberculosis.


Methods: A prospective study comparing the sensitivity and specificity of the Nitrate Reductase Assay with the gold standard Lowenstein Jensen proportion method in determining drug susceptibility pattern to four primary anti-tubercular drugs i.e. isoniazid, rifampicin, streptomycin and ethambutal among clinical isolates.

Results: Among 121 specimens, the sensitivity and specificity of the Nitrate Reductase Assay for detection of Isoniazid resistance was 100% and 91%, for rifampicin was 100% and 98.95%, for streptomycin was 96% and 91.66% and for ethambutal was 100% and 98% respectively.

Conclusions: The Nitrate Reductase Assay is sensitive and specific enough for the detection of drug resistant tuberculosis. It is rapid, easy to use and inexpensive, making it suitable for developing countries. Its usefulness for national drug resistance surveys should be assessed.

Keywords: diagnosis, drug resistance, sensitivity, specificity, NRA

INTRODUCTION

Tuberculosis (TB) is a serious public health problem in many developing countries. The drug resistant tuberculosis (DR-TB) and particularly multi-drug resistant tuberculosis (MDR-TB), defined as resistance to at least the two major anti-tuberculous drugs isoniazid (INH) and rifampicin (RFP), has emerged in many countries in different regions of the world (WHO, 2009).

In Nepal, tuberculosis is a major public health problem and recognized by the Government as a priority number one (P1) program. About 45% of the total population is infected with TB, out of which 60% are adult. Every year about 40,000 people develop TB, of whom 20,000 have infectious pulmonary disease. Introduction of treatment by DOTS has already reduced the number of death, however it is estimated 5000-7000 people still die per year from TB (NTC, 2008). A national prevalence of MDR-TB is 2.9% among new TB cases and 11.7% among previously treated TB cases (STAC, 2008).
Rapid and precise diagnosis of drug resistant tuberculosis is necessary for effective patient treatment and to prevent transmission of the disease. Isolation, identification and susceptibility testing are essential procedures that should be performed as quickly as possible, so that adequate treatment can be prescribed. The drug susceptibility test currently available is the conventional proportion method on Lowenstein-Jensen (LJ) medium, which takes 4-6 weeks to yield culture result plus another 4-6 weeks for drug susceptibility test results. More rapid tests using liquid media, such as the BACTEC 460TB radiometric method and the MGIT, require specific equipment and consumables, and are expensive. Likewise, the new rapid tests based on molecular tools are generally not easy to use and need specialized staff. Rapid diagnosis of MDR patients is nevertheless necessary to avoid the spread of MDR strains. For developing countries, like Nepal the test should be specific, simple and applicable that can rapidly detect MDR Mycobacterium tuberculosis strains.

One rapid drug susceptibility testing method that could potentially satisfy these criteria is a Nitrate Reductase Assay, a colorimetric assay also known as Griess method (Golyshevskaia et al. 1996). This assay was initially developed at the Central Tuberculosis Research Institute in Moscow, Russia, where it was called the Griess method, after J.P. Griess, who discovered the chemistry of the detection method used (Griess and Benerkungen, 1879). It is a low-cost DST method that can be employed in areas of limited resources and low technical capacity (Canetti et al., 1969). It is based on the ability of M. tuberculosis to reduce nitrate to nitrite, which is routinely used for biochemical identification of mycobacterial species. The presence of nitrite can easily be detected with specific reagents, which produce a color change (Kent and Kubica, 1985).

**METHODOLOGY**

Specimen processing: Sputum specimens were initially processed by modified petroff’s method and ZN acid-fast bacilli (AFB) staining of the sediment was done according to standard protocols (WHO, 1998).

**Conventional Lowenstein-Jensen method:** Processed specimen was cultured on two slopes of LJ-medium and drug susceptibility test of isolate was performed by 1% proportion method according to standard protocols (WHO, 1998).

**Nitrate Reductase Assay method :**

**Media preparation :** Complete LJ-media was prepared and then 1g/1000 ml NaN0₃ was added to LJ medium and completely dissolved by stirring. The drugs were then added to the modified medium to prepare the drug containing medium and the medium was a liquated and inspissated. Six tubes with modified LJ medium are needed for each specimen: one containing INH at critical concentration (0.2 μg/ml), one containing RFP at critical concentration (40 μg/ml), one containing SM at critical concentration (4 μg/ml), one containing EMB at critical concentration (2 μg/ml) and two control tubes without any drugs added.

**Procedure :** The test was performed only on specimens with an AFB smear positive result (1+and above). Each of the six tubes described above was inoculated with 0.2 ml of the processed sputum sediment. After 28 days of incubation at 37°C, 0.5 ml of freshly prepared Griess reagent was transferred into one of the growth control tubes, and development of color was observed. If the color intensity was sufficient, the same amount of Griess reagent was pipetted into each of the drug containing tube and other control tube. The color intensity in the drug-containing tube was then compared to the control tube.

**Interpretation of results :** The results were classified as negative if there were no color changes or a very pale pink color was observed. Positive results varied from pink to deep red or violet. The results were thus, interpreted as follows:

- **Resistant (R)** - An isolate was considered resistant to a certain drug if there was a positive color change in the drug tube in question and in the drug-free control tube.
- **Sensitive (S)** - An isolate was considered sensitive to a drug if there was no color change in the drug tube in question and positive color change in the drug-free control tube.

If no color changes or pale pink color were observed in the control tube, the test was considered to be invalid.
Preparation of Griess reagent: Shortly before use, one part of 50% (vol/vol) concentrated hydrochloric acid was mixed with two parts of 0.2% (wt/vol) sulfanilamide and two parts of 0.1% (wt/vol) n-1-naphthylenediamine dihydrochloride.

Preparation of inoculum: 1 ml sterile distilled water was added to the sediment obtained after processing the sputum specimen.

RESULTS

The test was completed on 121 specimens of 144 smear-positive (31 1+, 31 2+ and 82 3+) specimens. Of the remaining 23 specimen for which the test was not completed, 12 were culture-negative by both gold standard and nitrate reductase assay method and 11 were contaminated on culture by either one or both methods.

Comparison of the Nitrate Reductase Assay results with conventional DST was determined to be 100% and 91% for INH; 100% and 98.95% for RFP; 91.66% and 91% for SM; and 98% and 98% for EMB. Not a single isolate was falsely identified as being INH susceptible. Among 93 strains that were susceptible to INH by the conventional method, 85 (91.39%) were also found to be susceptible by the direct nitrate reductase assay method. Similarly, 26 of 26 (100%) isolates that were resistant to RFP by the conventional DST were also found to be resistant by the nitrate reductase assay method. Among 95 strains that were susceptible to EMB by the conventional method, 98 (98.9%) were found to be susceptible by the nitrate reductase assay method.

Comparison of the Nitrate Reductase Assay results with conventional DST was determined to be 100% and 91% for INH; 100% and 98.95% for RFP; 91.66% and 91% for SM; and 98% and 98% for EMB. Not a single isolate was falsely identified as being INH susceptible. Among 93 strains that were susceptible to INH by the conventional method, 85 (91.39%) were also found to be susceptible by the direct nitrate reductase assay method. Similarly, 26 of 26 (100%) isolates that were resistant to RFP by the conventional DST were also found to be resistant by the nitrate reductase assay method. Among 95 strains that were susceptible to EMB by the conventional method, 98 (98.9%) were found to be susceptible by the nitrate reductase assay method. Considering proportion method as standard, the sensitivity and specificity of direct NRA was determined to be 100% and 91% for INH; 100% and 98.95% for RFP; 91.66% and 91% for SM; and 98% and 98% for EMB.

### Table 1a: Drug Susceptibility Pattern of M. tuberculosis (n=121) determined by the proportion method and Nitrate Reductase Assay method

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Proportion Method</th>
<th>Nitrate Reductase Assay method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant</td>
<td>Sensitive</td>
</tr>
<tr>
<td>INH</td>
<td>28</td>
<td>93</td>
</tr>
<tr>
<td>RFP</td>
<td>26</td>
<td>95</td>
</tr>
<tr>
<td>SM</td>
<td>25</td>
<td>96</td>
</tr>
<tr>
<td>EMB</td>
<td>21</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 1b: Comparison of Nitrate Reductase Assay results with Conventional DST

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Conventional method</th>
<th>Direct Nitrate Reductase Assay method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant</td>
<td>Sensitive</td>
</tr>
<tr>
<td>INH</td>
<td>Resistant = 28</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Sensitive = 93</td>
<td>8</td>
</tr>
<tr>
<td>RFP</td>
<td>Resistant = 26</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Sensitive = 95</td>
<td>1</td>
</tr>
<tr>
<td>SM</td>
<td>Resistant = 25</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Sensitive = 96</td>
<td>8</td>
</tr>
<tr>
<td>EMB</td>
<td>Resistant = 21</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Sensitive=100</td>
<td>2</td>
</tr>
</tbody>
</table>
In this study, high sensitivity and specificity of the nitrate reductase assay method, relative to conventional methods were demonstrated in the identification of resistance of M. tuberculosis to four primary anti-tubercular drugs i.e. INH, RFP, SM and EMB. The sensitivity and specificity of the direct nitrate reductase assay for detection of isoniazid resistance was 100% and 91%, for rifampicin was 100% and 98.95%, for streptomycin was 96% and 91.66% and for ethambutal was 100% and 98% respectively.

In a similar study by Sloutsky et al. (2005) in Lima, Peru showed the sensitivity and specificity of the method for INH resistance was 99.1% and 100%, and for RMP resistance was 93.5% and 100% respectively. Study by Angeby et al. (2005) showed sensitivity and specificity of the direct NRA using the direct proportion method as reference for INH, RFP, SM, and EMB were 100 and 100%, 93 and 100%, 76 and 100% and 55 and 100% respectively. In another report by Angeby et al. (2002), sensitivities and specificities for drugs as determined by the NRA method compared to those determined by the BACTEC 460 method were 100% and 100% for rifampicin, 97% and 96% for isoniazid, 95% and 83% for streptomycin, and 75% and 98% for ethambutol, respectively. Other study of the performance of the indirect NRA method by Golyshhevskia et al. (1998) in Sweden, sensitivity and specificity for INH were 97% and 96% respectively, and 100% for RMP.

The ability to reduce nitrate is typical for M. tuberculosis, although some other mycobacterial species, like Mycobacterium kansasii, and most rapid growers share this characteristic (Kent and Kubica, 1985). Nitrate reductase-negative strains of M. tuberculosis are rare (1%) (Kent and Kubica, 1985) and would create no false results since the control would be negative and the test would therefore be invalid. No such strains were encountered in this study. Strains of Mycobacterium bovis do not reduce nitrate, for which reason the NRA technique is not applicable to DST of them. Another possible limitation is that nitrite might be further reduced to nitric oxide, which cannot be detected by the reagents used. When the nitrate reduction test is performed for the purpose of species identification, zinc powder is added to all negative tubes (Kent and Kubica, 1985). Zinc reduces nitrate rapidly, and a true negative test will directly turn red, while there will be no color change in a tube where reduction has passed beyond nitrite. Since the result was always confirmed, this step was omitted in this study. However, further studies will be needed to clarify the role of zinc powder in the NRA.

This study demonstrates the potential usefulness of the direct NRA as a rapid, susceptible and specific screening tool for MDR-TB. Even though more studies are needed to further assess the accuracy and applicability of the method, the direct nitrate reductase assay has the potential to become an
inexpensive alternative for DST where resources are scarce, especially for RFP, the most important anti-tuberculosis drugs. It might then be used either as a rapid screening tool alone or in combination with other methods. In addition to its rapidity, the direct NRA has further obvious benefits that would facilitate its institution in resource poor setting. Furthermore, the test uses only simple reagents that are inexpensive and easily obtained, does not require maintenance of any specialized equipment, and requires minimal laboratory space and staffing.

CONCLUSION

In this study, direct NRA test for culture and susceptibility testing was evaluated in comparison to gold standard proportion method. The method was found to be having high sensitivity and specificity. This study revealed that the direct nitrate reductase assay method has capability of accurate and rapid drug susceptibility testing for primary four anti-tuberculosis drugs viz, INH, RFP, SM, and EMB. More importantly, the test will provide patients and clinicians with the benefits of greater access to fast and accurate drug susceptibility testing result for these first-line drugs.

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