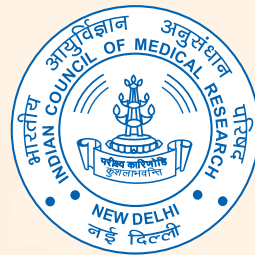


Disease Specific Documents for XII Plan

Leprosy



INDIAN COUNCIL OF MEDICAL RESEARCH

Disease Specific Documents for XII Plan

Leprosy

High Power Committee to Evaluate the Performance of ICMR, 2012-13



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1. Current situation of Disease with contribution of ICMR

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*. It is among the world's oldest and most dreaded diseases and it has been synonymous with stigma and discrimination due to the hideous deformities it produced, mystery around its etiology & transmission and lack of any effective remedy till recently. It mainly affects the skin and peripheral nerves, but has a wide range of clinical manifestations. The disease is characterized by long incubation period (generally 5-7 years) and is classified as paucibacillary (PB) and multibacillary (MB) types, depending on the bacillary load.

The Government of India started the National Leprosy Control Program (NLCP) in 1955, based upon dapsone domiciliary treatment through the vertical units, by implementation of survey, education and treatment (SET) activities. The Multi Drug Treatment came into wide use from 1982, following the recommendations by the WHO Study Group. The National Leprosy Eradication Programme (NLEP) was launched in 1983 in India with the support from WHO, ILEP, SMHF & Nippon Foundation, Tokyo, Novartis, DANLEP and the World Bank. Since 2005 NLEP is being continued with Government of India funds. In the last two decades, the reported global prevalence of active leprosy infection has dropped by almost 90 per cent by the combined efforts of the World Health Organization (WHO), local governments, health professionals, and non-governmental organizations (NGOs), however, a parallel drop in the incidence or new case detection rate (NCDR) has not occurred. From 1994 through 2011, more than 100,000 new cases are being detected annually globally, of whom maximum case load is from India. MDT has been the main weapon against leprosy since its inception in 1981 and by 2005, the prevalence in India was less than 1/10000. This was a landmark achievement in the history of leprosy in India. By the end of 2010, the prevalence had come down to 0.69/10000. In this context, it must be pointed out that cases of leprosy are not uniformly distributed but tend to cluster in certain localities, villages or taluks. Hence, while the country as a whole has eliminated leprosy, two States, Bihar and Chattisgarh are yet to achieve elimination (with a prevalence rate of 1.12 and 1.94, respectively).

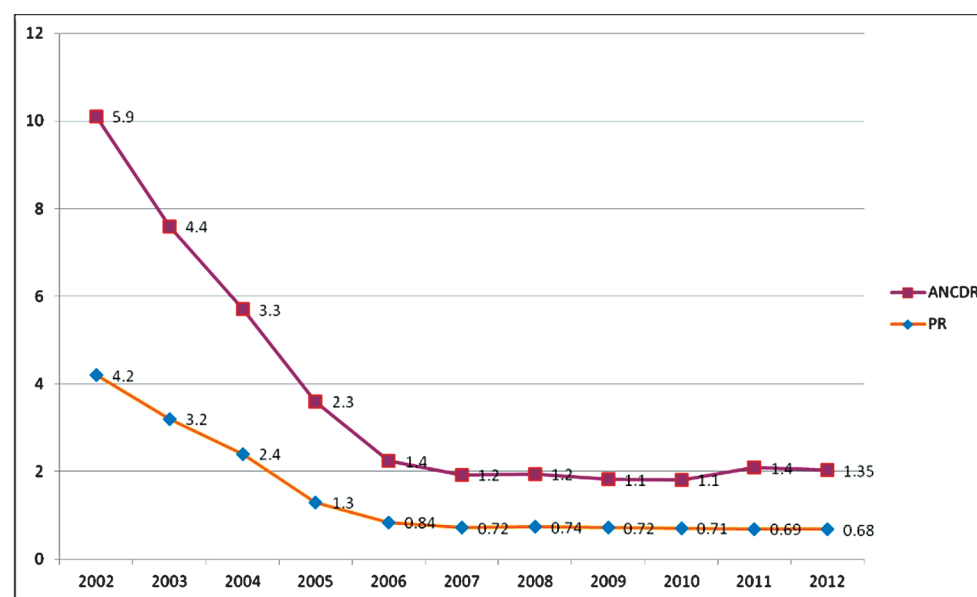


Fig. 1: Trends of leprosy prevalence (PR) and Annual new case detection rate (ANCDR) in India in last two decades.

The Union Territory of Dadra and Nagar Haveli also reports high prevalence of leprosy. There are a few pockets of higher leprosy prevalence in Agra, Firozabad and Kanpur districts as per epidemiological studies being conducted by NJIL&OMD. Of the total of 640 districts in the country, 110 districts still have prevalence rates between 1 and 2/10000, while in 530 districts, elimination has been achieved.

The decline in prevalence rate not accompanied with a decline in the incidence rates indicates continued transmission. The incident cases could also be derived from the infected pool since the incubation period is long and variable. Despite the discovery of *Mycobacterium leprae* more than a century ago and world wide research since then, several epidemiological features of leprosy are still poorly understood. Among the classical epidemiological triad, the agent *M. leprae* is probably best described, but its entry and exit from the host, its passage into the Schwann cells of peripheral nerves, the incubation period from entry to manifestation of the disease, are at best guesstimates. The distribution of the disease among the males and females is poorly understood with a higher prevalence observed in males. This could also be possible with differential treatment seeking behavior among the males and females. The relationship of the disease with its other determinants such as poverty, nutrition also lacks clear demonstration.

Thus, there is need for research on tools for early diagnosis, short and effective treatment, and prevention of deformities/disabilities to curb transmission and reduce the incidence of leprosy. Evaluating the role of immunotherapy and immunoprophylaxis will also lead us to better understanding of their mode of action. Further molecular analysis of *Mycobacterium leprae* genome may provide the requisite basis for better diagnosis and treatment of leprosy in India. The current reality is that there is a need to sustain and provide quality leprosy services to all persons through general health services, including good referral system. Efforts need to be made to reduce deformity through early detection, self-care, physiotherapy, reconstructive surgery and developing comprehensive surveillance systems. ICMR through its premier leprosy Institute at Agra and its Field unit/centre, Model Rural Health Research Unit (MRHRU), Ghatampur has contributed to leprosy control and eradication through research and treatment.

Ghatampur Unit

NJIL&OMD, Agra has an old association with Ghatampur area. During Japanese period (1966-1976), Ghatampur unit was a field satellite treatment clinic of original India Centre of JALMA. The doctors and paramedical staff used to visit Ghatampur periodically from Agra. After JALMA was taken over by ICMR in 1976, the focus shifted to research-cum-hospital activities at Agra. The link with Ghatampur was entirely lost for nearly 23 years till 1999 when the scientists of the Institute under the able supervision of Dr. Kiran Katoch, former Director, NJIL&OMD were assigned the job of independent validation of third survey of Mw vaccine (now renamed as *Mycobacterium indicus pranii*- MIP) trials. After assessing the initial logistics, local field office of the project was shifted from Kanpur to Ghatampur. Besides the main unit, a satellite centre has been set up at Banda which is addressing the issues of epidemiology of tuberculosis specially drug resistance.

Keeping in view of the objectives of Rural Health Mission of Prime Minister, ICMR provided all support to field unit at Ghatampur and made this into a Model Rural Health Research Unit (MRHRU). Mw vaccine was administered in leprosy patients and their contacts in a double blind

manner between 1986-1989 in Ghatampur area. The third resurvey was conducted with NIMS and JALMA (ICMR) and the effect was monitored in the population against leprosy. Subsequently, after re-establishing the links with the local population and observing the beneficial effects of vaccine in leprosy, the effect of this vaccine is being evaluated against pulmonary tuberculosis also(both Cat I and Cat II patients). Since 1999, the Institute has established a vibrant field programme with renewed link with the public of Ghatampur. This programme has been expanded as the area is highly endemic for leprosy, tuberculosis and also filariasis whose endemicity has been linked with leprosy prevalence in Africa. MRHRU has emerged as excellent example of partnership between state and centre as well to demonstrate as what a collective support by different international (WHO), national-(DBT, ICMR and Govt. of India) and state (Govt. of UP) agencies can achieve to generate knowledge to improve the health of the people of rural India.

To summarize the achievements by MRHRU, Ghatampur and the important findings of the studies in leprosy from MRHRU are:

- Mw vaccine shows protection against household contacts of leprosy and this effect is reduced over 8-10 year period.
- In addition, Mw vaccine has protection against pulmonary tuberculosis.
- Collaborative studies have indicated the role of certain genes in susceptibility towards leprosy.
- The epidemiological studies in leprosy at Ghatampur have provided new information on prevalence of leprosy in endemic pockets which appear to be due to backlog; subsequently there is change in clinical and bacteriological profile of disease. Original information about genotypes of leprosy bacillus was obtained and published; presence of live *M.leprae* in the environment has been established and the effect of various interventions are being evaluated. The entire information has public health importance and has been taken note by the Programme managers.
- Uniform MDT in leprosy (Addition of clofazimine to MDT of monthly once Rifampicin and daily DDS): This concept was originally thought by the Group for Paucibacillary leprosy and has been adopted by WHO as UMDT for wider application.

In view of aforesaid background, research studies related to leprosy and carried out at NJIL&OMD as well as MRHRU (Ghatampur) can be categorized into:

- Basic Research (on leprosy pathogenesis)
- Clinical Research (diagnostics, and drug trials)
- Epidemiological/Operational research (transmission dynamics).
- Translational Research (transfer of research outcome for use by national programme/industry/policy makers)

2. MAJOR ACHIEVEMENTS WITH LEADS EMERGED DURING XI PLAN

Basic Research

- Mechanism of endothelial cell infection and their role in transmitting the disease in peripheral nerves in leprosy has been studied.
- A study on the circulating immune-complexed anti cytokine antibodies in leprosy patients and their role in reactions showed significant difference with some immune complexed anti cytokine antibodies in Borderline Tuberculoid patients during reactions; The studies need be pursued with appropriate sample size and follow up in BT patients with and without reactions.
- To develop tools for early and definitive diagnosis of leprosy, *in situ* PCR studies were conducted in biopsy specimens as well as skin smears targeting 18kDa, 36kDa and 65kDa . The established techniques can be used routinely in clinical specimens, smears, experimental animal specimens for establishing the diagnosis/pathogenic interaction between *M.leprae* and host tissues .The technique can be used for demonstrating the presence of *M. leprae* /products. (Taken over for studies in next plan period; collaborating with NIOP on the use of technology).
- Addition of MIP (Mw) with standard MDT in BT/ BB and BL cases of leprosy led to faster bacterial clearance, achievement of smear negativity and reduction of reactions and neuritis in these group of patients.

Clinical Research

- Nerve decompression and re-constructive surgery for repair/enablement of leprosy patients Decompression of peripheral nerves has been beneficial for preventing deformities in leprosy.
- Neurophysiological and nerve conduction studies to understand nerve damage in leprosy have shown that increase in latent period of conduction in peripheral nerve occurs before derangement of nerve conduction velocity in the nerve, and can be used as a marker to predict impending nerve damage.

Epidemiological/Operational Research

- Screening for drug resistant *M .leprae* in relapse cases of leprosy using mouse foot pad; Next study initiated using both mouse foot pad and molecular methods.
- Epidemiological studies in leprosy in Agra and Firozabad districts with the objective to assess trends of leprosy incidence in Agra and Firozabad, have shown a higher period prevalence during house to house survey in Agra district .

- Phase II of epidemiological studies on leprosy at Ghatampur, MRHRU to (i) Re-survey of the population for new case detection and profile of the leprosy cases to determine the trends as well as treatment outcomes and (ii) to study the transmission of leprosy by molecular methods: Supervised treatment of leprosy brought down the incidence and prevalence of leprosy in the area (614 new cases were detected as against 3152 in the 1st phase survey). In addition no deformities were detected in new cases. Utilization and re-training of the existing health services helped in early detection and treatment completion of cases in the area. Molecular tools were established to understand the transmission dynamics of the disease.

Translational Research

- **Early Mobilization of claw hand can reduce the post operative duration of stay in the hospital and restore functional capacity of hand.**
 - **Follow up of patients on different drug regimens of leprosy:** The outcome of patients treated with different treatment regimens at MRHRU, Ghatampur show that the addition of clofazimine to paucibacillary leprosy patients was well accepted and relapses as well as reactions were reduced as compared to the standard regimen and that the addition of supervised doses of minocycline and ofloxacin in treatment of MB cases led to lesser relapses in MB patients. Addition of clofazimine to paucibacillary MDT was adopted by WHO as UMDT for all types of leprosy patients. Findings of the trials at Ghatampur field unit area have shown that addition of supervised doses of minocycline and ofloxacin in WHO-MDT of MB cases as a treatment regimen can be potentially useful .
 - **Novel proteins of *M.leprae* as potential therapeutic target:** New potential drug target genes were identified. These gene targets could have broader application for drug development for tuberculosis and other mycobacterial diseases; Indian patent application number has been allotted after granting of the patent, this technology will be transferred to the relevant industry through Government of India.
 - Expression of virulence factors of *M.leprae* in host during infection by functional genomics approaches; Understanding the virulence of *M. leprae*. A DNA Chip for the identification of differential expression of associated genes of *Mycobacterium leprae* during the disease spectrum in the human host has been developed and can be used as potential future drug target/ vaccine production for mycobacterial diseases by using comparative and structural genomic approaches. Indian patent application number has been allotted. After granting of patent, this technology will be transferred to the relevant industry through the Government of India.
 - **JALMA Flap :** To fill up hollowness of 1st webspace due to ulnar nerve paralysis in leprosy; A viable Adipocutaneous flap is used from upper fore arm and shown good results on follow up studies during 11th Plan period.
- 3. RESEARCH PAPERS PUBLISHED: 29 in leprosy (during 2008-2012) of total 130 publications (including TB/HIV research and miscellaneous)**

4. TECHNOLOGIES TRANSFERRED TO INDUSTRY

NJIL & OMD played a major role in Mw ((MIP)vaccine trials .

5. TECHNOLOGIES READY FOR TRANSFER:

In situ PCR for early diagnosis of leprosy

6. TECHNOLOGIES TRANSFERRED TO HEALTH AGENCIES : Nil

7. LISTS OF PATENTS FILED : Four Patent Applications have been filed

- (i) 'Novel Drug Efflux Proteins of *M.tuberculosis* as Potential Therapeutic Targets' (2071/DEL/2007)
- (ii) 'Probes and primers for identification of mycobacterial proteins useful as potential drug targets (Indian patent Appl No.884/DEL/2007)'.
(iii) Expression of virulence factors of *M.leprae* in host during infection by functional genomic approaches (Indian Patent Application No. 2012/DEL/2007)
- (iv) Primers and methods for identification of pathogenic mycobacteria (Granted No 2418/DEL/2006)

8. MANPOWER TRAINED

The Following Manpower was trained during XIth Plan

- | | |
|----------------------------|-------|
| 1. Public Health Personnel | : 450 |
| 2. Scientists | : 40 |
| 3. Students/ Teachers | : 560 |

9. NEW HUMAN RESOURCE GENERATED (2008-2012)

- Ph D degree awarded : 21
- Pursuing for Ph D degree : 19
- M.Sc. Dissertation : 350
- M.D. : 5

Table I. Status of Completed Research Studies undertaken during XI Plan and some carried over to XIIth plan in Leprosy

Sr. No.	Thematic area and Title of the Study	Objectives	Completed with outcome of the study	If off-shoot, Refer to XII th plan study	Institution
Basic Research					
1	Mechanism of endothelial cell infection and their role in transmitting the disease in peripheral nerves in leprosy	To understand the mechanism of propagation of <i>M. leprae</i> in nerve	Using electron microscopy, & scanning microscopy showed that <i>M. leprae</i> persist in these cells which also provide nutrition to the nerve; bursting of these cells lead to bacteremia which causes fresh "seeding" of bacteria in new areas.	More basic research on its mechanisms of progression of disease in the peripheral nerve required.	NJIL & OMD, Agra
2	Seroassays for leprosy using newer antigens/ techniques for early diagnosis of leprosy	To develop a test using a combination of antigens and antibodies for development of sero-based test for early and definitive diagnosis of leprosy	At present cannot differentiate between sub clinical infection and disease	Completed	NJIL & OMD, Agra
3	A study on circulating immune-complexed anti cytokine antibodies in leprosy patients	To study the circulating immune-complexed anti cytokine antibodies in leprosy patients and their role in reactions	More immune complexed anti cytokine antibodies observed in Borderline tuberculoid patients during reactions	Proposed to be studied with appropriate sample size and follow up in BT patients with and without reactions	NJIL & OMD, Agra
4	Study on IL-18 production, TLR 2 expression and level of auto antibodies in leprosy patients with Type 1 and 2 reactions	1. Study on IL-18 production in patients with reactions in leprosy 2. TLR 2 expression and level of auto antibodies in leprosy patients with Type 1 and 2 reactions	Higher levels of circulating IL-18 observed in patients with reactions. Measuring it in different time points in disease may lead to discovery of biomarker for reactions	Completed	NJIL & OMD, Agra

5	Addition of MIP(Mw) to standard chemotherapy in BT/ BB and BL cases of leprosy	To study the mechanism of immunomodulation in Borderline patients	Addition of MIP with standard MDT in these patients lead to faster bacterial clearance, achievement of smear negativity and reduction of reactions and neuritis in these group of patients.	This study is Completed in 2008	NJIL & OMD, Agra
Clinical Research					
1	Study of hormonal profile in female leprosy patients	Harmonal changes in women <i>vis- a- vis</i> its relationship to reactions in leprosy	Hormonal changes at, puberty, pregnancy, lactation are known to trigger/perpetuate reactions in the reproductive age group. However, in the present study these changes in menopause also may be responsible for increase in reactions & relapses in this group.	Completed	NJIL & OMD, Agra
2	Neurophysiological and nerve conduction studies to understand nerve damage in leprosy	Neurophysiological and nerve conduction studies to understand nerve damage in leprosy	Increase in latent period of conduction in peripheral nerve occurs before derangement of nerve conduction velocity in the nerve, and can be used as a marker to predict impending nerve damage.	Follow up of patients continuing	NJIL & OMD, Agra
3	<i>In situ</i> RT-PCR and use of 16S mRNA as viability marker in leprosy	Demonstration of 16S RNA as a viability marker in tissues of leprosy patients during reactions by <i>in situ</i> real time PCR	i)Viable bacilli persist after completion of FDT ii) They also are present in reactional lesions and may be the initiating/ perpetuating the reaction.	Completed. Technology ready to be used in other studies.	NJIL & OMD, Agra

Epidemiological/Operational Research					
1	Screening for drug resistant <i>M. leprae</i> using mouse foot pad in relapse cases of leprosy	Screening for drug resistant <i>M. leprae</i> in relapse cases of leprosy using mouse foot pad	Clinically diagnosed suspected relapse cases from 7 centres i.e. School of Tropical Medicine, Kolkatta; PGIMER, Chandigarh; SLTRI, Karigiri; LNJP and RML, N Delhi; Blue Peter, Hyderabad; & NJIL & OMD, Agra No drug resistant <i>M. leprae</i> could be detected using this method	Completed. Next study initiated using both mouse foot pad and molecular methods	NJIL & OMD, Agra
2	Epidemiological studies in leprosy in Agra and Firozabad districts	Assess trends of leprosy incidence in Agra and Firozabad	Higher period prevalence observed during house to house survey in Agra district which is as anticipated in active search	Completed in Agra. Firozabad expected to be completed by next year	NJIL & OMD, Agra
3	Phase II of epidemiological studies on leprosy at Ghatampur, MRHRU	(i) Re-survey of the population for new case detection and profile of the leprosy cases to determine the trends as well as treatment outcomes. (ii) To study the transmission of leprosy by molecular methods.	Supervised treatment of leprosy brought down the incidence and prevalence of leprosy in the area (614 new cases were detected as against 3152 in the 1 st phase survey). Also no deformities were detected in new cases. Utilization and re-training of the existing health services helped in early detection and treatment completion of cases in the area. Molecular tools were established to understand the transmission dynamics of the disease.	Completed Two new task force projects initiated to understand the transmission dynamics. Translational study initiated to follow-up patients on different regimens	NJIL & OMD, Agra

Translational Research					
1	Novel proteins of <i>M.leprae</i> as potential therapeutic target	Novel drug targets in leprosy	New potential drug target genes identified. These gene targets could have broader application for drug development for tuberculosis and other mycobacterial diseases.	Indian patent application number has been allotted after granting patent this technology will be transferred to the relevant industry through Govt of India.	NJIL & OMD, Agra
2	Expression of virulence factors of <i>M.leprae</i> in host during infection by functional genomics approaches	Understanding the virulence of <i>M. leprae</i>	A DNA Chip for the identification of differential expression of associated genes of <i>Mycobacterium leprae</i> during the disease spectrum in the human host developed. Can be used as potential future drug target/ vaccine production for mycobacterial diseases by using comparative and structural genomic approaches.	Indian patent application number has been allotted after granting patent. This technology will be transferred to the relevant industry through Govt of India.	NJIL & OMD, Agra
3	Treatment of Early Neuritis	To find out whether steroids would be useful in prevention and progression of deformity in subclinical nerve damage	Electrophysiologically subclinical nerve damage can be demonstrated	To be continued	Multicentric international study funded by Instt. of Tropical Medicine, The Netherlands.
4	JALMA Flap	To fill up hallowness of 1 st webspace due to ulnar nerve paralysis in leprosy	A viable Adipocutaneous flap is used from upper fore arm and shown good results	Continued	NJIL & OMD, Agra

5	Understanding the role of CCL2 and associated gene in leprosy susceptibility and in leprosy reactions	To investigate the gene polymorphism in regulatory region of mcp1/ccl2 and nos2 in leprosy cases and controls to reveal the association with susceptibility or resistance to leprosy, occurrence of leprosy reactions and to understand the functional role of these polymorphisms.	Initiated in 2010	To be continued	NJIL & OMD, Agra
6	Role of FOXP3+ regulatory T cells in polarized immunity among leprosy patients	To evaluate the role of regulatory T cells in determining the polarization of disease manifestations in leprosy patients, to delineate the hierarchic role of chemokine receptors on the T regulatory cells in their selective recruitment at the pathologic sites and elucidate their function	Initiated in 2010 and to be continued till end of 2013.	T Reg cells were higher in BL/LL patients in comparison to BT/TT patients and Healthy individuals.	NJIL & OMD, Agra
7	Study of Mechanism of regulation of T cells responses in Leprosy	Evaluation of Th1, Th2, and Th17 cell subsets across leprosy spectrum and healthy contacts and their role in outcome of the disease. Evaluation of Notch and various transcription factors in cytokine expression and in regulation of T cell responses in leprosy	Initiated in 2010	To be continued till 2013	NJIL & OMD, Agra

Table 2. Projects/studies in leprosy at NJIL & OMD in the 12th plan

Sr. No.	Title/subject	Objectives	Leads observed	Completed /taken over to next plan period	Institute
Basic Studies					
1	<i>In situ</i> PCR studies in biopsy specimens targeting 18kDa, 36kDa & 65kDa for early and definitive diagnosis of leprosy	To develop tools for early and definitive diagnosis of leprosy	Established the techniques. These can be used routinely in clinical specimens, experimental animal specimens for establishing the diagnosis/pathogenic interaction between parasite and host. tissues	Being used in all studies to demonstrate presence of <i>M. leprae</i> /products Taken over for studies in next plan period; collaborating with NIOP on the use of technology	NJIL & OMD, Agra
2	Study of predictors of therapeutic responses governing the regression and clearance of granulomas in response to chemotherapy with immunotherapy in borderline leprosy (ICMR Task Force project)	(a) Proteomic analysis to identify and characterize the proteins involved in granuloma clearance (b) To correlate these findings with histopathology and immunohistochemistry using markers of cellular phenotypes (CD4,8,68) and their mediators (IF-gamma, TNF-alpha, IL-12 & TGF-beta)	Double blinded study. Leads to be discovered after decoding	Carried over in 12 th Plan Period (2010-13)	NJIL & OMD, Agra
Clinical Research					
1	Nerve decompression and re-constructive surgery for repair / enablement of leprosy patients	Enablement of disabled leprosy patients	Decompression of peripheral nerves has been beneficial for preventing deformities in leprosy.	Patient care; Carried over in 12 th plan period	NJIL & OMD, Agra

Translational Research					
1	Early Mobilization of claw hand	Reduce the post operative duration of stay in the hospital and restore functional capacity of hand	Study undertaken in Odissa showed to be superior to the conventional method. It would help in cutting short the admission time in hospital	Multicentric evaluation of technique	NJIL & OMD, Agra One of the centres participating in the study
2	Follow up of patients on different drug regimens of leprosy	Outcome of patients treated with different treatment regimens at MRHRU, Ghatampur	Addition of CLF to paucibacillary leprosy patients well accepted and relapses as well as reactions were lesser as compared to the standard regimen. Addition of supervised doses of Minocycline and Ofloxacin in treatment of MB cases led to lesser relapses in MB patients.	Adopted by WHO as UMDT for all types of leprosy patients. Addition of supervised doses of Minocycline and Ofloxacin in treatment of MB cases accepted as a treatment regimen	NJIL & OMD, Agra

Table 3. Miscellaneous activities during the XIth plan period at NJIL & OMD and to be continued in the XIIth plan period.

Sr. No.	Title/Subject	Funding	Objectives/Outcome	Status
1	HIV testing and counseling	Intramural and some part by NACO	Accredited centre of NACO	Patient care will continue in XII th plan
2	Monitoring of ART of HIV positive patients treated at Medical College	Extramural	Programme support of NACO	Patient care will continue in XII th plan
3	Experimental animal National Guinea pig facility for intranasal tuberculosis infection	Initially was established by extra mural support now intramural	National facility with drug testing and vaccine testing animal facility with experiments done for over 30 groups till now	Will continue
4	National facility for mouse foot pad testing for leprosy	Intramural	One of the 2 facility remaining in the country for detection of drug resistance <i>M. leprae</i> by MFP studies	Will continue in XII th plan period
5	2 week summer training for M.Sc students	Intramural	Has provided biosafety and laboratory technique advances to more than 600 students	Will continue
6	4-6 months project dissertation courses for M.Sc students	Intramural	Conducted dissertations for more than 300 students	Will continue in XII th plan
7	Provides training and dissertations for MD and PhD students	Intramural	Human resource development to more than 50 students	Will continue in XII th plan
8	Training of Staff employed in various laboratories in Colleges/ Universities on new technologies	Intramural	Human Resource development	Will continue in XII th plan
9	Construction of new BSL3 diagnostic complex and animal experimental laboratory	Intramural	SFC accepted and funds allotted for construction of new lab for molecular characterization of TB strains, HIV work and animal experiments on TB	Construction will be completed in XII th plan and experimental work initiated.