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Editorial

Can Multidrug Resistance in Tuberculosis be Curbed?

Tuberculosis (TB) is an ancient disease, perhaps 3 million years old. The term Tuberculosis was coined way back in 1834 by Johann Schonlein. It was a dreaded disease as evident from its earlier names like 'phthisis' in ancient Greece, 'tabes' in ancient Rome, 'schachepheth' in ancient Hebrew, the 'white plague', 'white death', 'consumption', 'lung fever', 'galloping fever', 'graveyard cough' and 'Captain of men of death'. TB of the neck and lymph nodes was called scofula, believed to be different from pulmonary TB.

Currently, on the basis of treatment, TB is categorized into drug-susceptible, drug-resistant, multidrug resistant (MDR) and extensively drug-resistant (XDR). MDR-TB is disease that does not respond to at least isoniazid and rifampicin, the two most powerful anti-TB drugs. XDR-TB is a form of MDR-TB with additional resistance to more anti-TB drugs that therefore responds to even fewer available medicines. XDR-TB is accurately defined as in vitro resistance to first-line anti-TB drugs isoniazid and rifampicin plus any second-line oral drugs: fluoroquinolone and at least one of the second-line injectable drugs: amikacin, capreomycin or kanamycin. A label of Latent TB is given when a person has the TB bacteria within his/her body in very minute quantities that are kept under control by the body's immune system and do not cause any symptoms and are not infectious. It is estimated that 40-50 % of the Indian population has Latent TB.

Robert Koch isolated the tubercle bacillus, presented his finding to the Society of Physiology in Berlin on 24 March 1882 and also contributed to the elucidation of the infectious etiology of TB (1). A century later, March 24 was designated by the World Health Organization (WHO) as the World TB Day. In 1905, Koch won the Nobel Prize for Medicine and Physiology (2). In subsequent decades, the Pirquet and Mantoux tuberculin skin tests, Albert Calmette and Camille Guérin BCG vaccine, Selman Waksman streptomycin and other anti-TB drugs were developed (2). Dr. Koch's discovery was the most important step taken towards the control and elimination of this deadly disease. Having the capacity to spread rapidly being an airborne infectious disease and developing resistance to the commonly used medications, it is high time to identify the weak areas that need attention to curb the disease.

TB continues to be a major public health problem. A combined strategy, based on improving drug treatment, diagnosis and prevention modalities, is necessary in order to eradicate *Mycobacterium tuberculosis* by the year 2030, as committed by the WHO (3). Elimination as defined by the WHO is less than one case of TB for a population of a million people.

India has the highest number of deaths from TB in the world since the bacterial infection kills one person every minute (4). The latest report from WHO states that 33 % of deaths worldwide related to TB excluding HIV occurred in India in 2017 (5). As per the Global TB report 2017, the estimated incidence of TB cases in India was approximately 28,00,000 accounting for about a quarter of the world's TB cases (5). MDR-TB is on the rise across India, with an incidence of 1,47,000 cases (5). The two main

reasons why MDR-TB continues to emerge and spread are mismanagement of TB treatment and person-to-person transmission.

The reason TB found easy access to spread in India was the highly populous areas living in closed crowded locations with poor immunity added to the lack of awareness and personal hygiene. The disease is more common in certain states of India. Earlier, the poor were affected more and did not mind getting admitted in TB sanatoriums that was beneficial to them as they were properly looked after, got nutritious food and timely medications. Slowly the disease has now affected even the urban population as the rural people have migrated to towns and live in close contacts with the urban population as house care takers or workers in restaurants, factories, government offices or public and private funded hospitals. Another angle yet to be explored is the presence of TB in animals like cows, cats and dogs (6). There are innumerable stray animals in India who if infected can also contribute to spread of the disease, albeit of other etiologic species of Mycobateria.

The liberal availability of over-the-counter (OTC) drugs like Ciprofloxacin and liberal use of injectable Amikacin which are also second-line anti-TB drugs has led to development of resistance. Poor resources and lack of awareness of the communicable nature have led to spread of the disease. The patients, in order to save time and avoid travel, seek intermittent help from private practitioners and thus are not in close supervision or monitoring for compliance and response. Other reasons to seek private treatment include lack of awareness of disease symptoms, poor knowledge of free services available through the national public program, desire for confidentiality and personalized care. Lack of knowledge is a big factor that contributes to MDR-TB. The patients are non-compliant; after few weeks of treatment, they feel that have got well and leave the prescribed course of medicines.

In India, there are no guidelines to protect the other patients, staff and visitors once an infected case is admitted in Hospital till date. When I was trained as PG, each year two residents out of 4 in the unit used to get infected with the disease despite occasional use of the mask. However, in those days we used to routinely wash the loaded under-water seal chest tube drain bags and fill it with clean saline for reuse. Fortunately, the public hospitals have now been provided with sufficient funds for the chest bags. Patient should not spit on the ground as the infected sputum gets mixed with the dust particles and spreads the infection. Those infected should ideally either run their sputum into a drain, bury their collected sputum or burn it to prevent spread of infection. However, such instructions are not passed to majority of the patients by the doctors, who themselves might be misinformed or lack desire to inform. During General Surgery training, I was fortunate to work under a cardiothoracic surgeon and assisted hundreds of cases of chest TB surgeries including pneumonectomy, lobectomy, thoracoplasty and pericardectomy (7). It used to be a heroic surgery and thanks to the discovery of highly efficacious anti-TB drugs and designing of evidence-based anti-TB regimens, such an expertise is limited as the surgery for pulmonary TB is no longer in vogue. Currently, video-assisted thoracic surgeries or decortications are done with occasional lobectomy for very sick patients. The surgical expertise is also fading away with minimal invasive techniques that have the disadvantages of lack of palpation of the unhealthy lobe leading to incomplete resection.

The referral system also delays timely treatment. The super specialists forget the training they got during undergraduation or postgraduation and prefer to refer the patients to their colleagues for requesting investigations and writing the doses of the anti-TB drugs. Initiation of different protocols of anti-TB therapy in various hospitals may also lead to conversion of TB cases to MDR-TB or XDR-TB.

The large scale implementation of the Indian government's Revised National TB Control Program (RNTCP/ RNTCP I) was started in 1997. The entire nation was covered by the RNTCP. In March 2006, it was re-designated as RNTCP II that was designed to consolidate the gains achieved in RNTCP I, and to initiate services to address TB/HIV, MDR-TB and to extend RNTCP to the Private Sector. The RNTCP is responsible for carrying out the Government of India Five-year TB National Strategic Plan (NSP). Under the RNTCP both the diagnosis and treatment of TB were free. There is, theoretically, no waiting period for patients seeking treatment and TB drugs.

Contrary to WHO target of eliminating TB by 2030, the Government of India is calling for the elimination of TB by 2025, and there is a new NSP 2017-2025 with emphasis on reaching patients seeking care from private providers, building on the work already done with the new RNTCP guidelines. The RNTCP will also be helping private practitioners and hospitals to provide quality care and treatment, rather than encouraging the private providers to send their patients to get care from the RNTCP. The NSP plans to provide incentives to private providers for following the standard protocols for diagnosis and treatment as well as for notifying the government of cases. Also patients referred to the government will receive a cash transfer to compensate them for the direct and indirect costs of undergoing treatment and as an incentive to complete the course of treatment.

New WHO recommendations aim to speed-up detection and improve treatment outcomes for MDR-TB through use of a novel rapid diagnostic test and a shorter, cheaper treatment regimen. New technologies like whole genome sequencing help public health professionals see the patterns of TB transmission. The Genexpert test is a molecular test for TB (MTB) which diagnose TB by detecting the presence of TB bacteria DNA, as well as testing for resistance to the drug rifampicin. In December 2010, WHO endorsed the Genexpert technology and released a recommendation and guidance for countries to incorporate the new test into their anti-TB programs (8). In India, this test is known as the Cartridge Based- Nucleic Acid Amplification (CB-NAAT) Test. It uses a sputum sample and can give a result in less than 2 hours. It can also detect the genetic mutations associated with resistance to rifampicin.

In 2017 the Xpert Ultra assay was launched and found to be non-inferior to the standard Xpert MTB assay for the diagnosis of TB and the detection of rifampicin resistance. A new device called the GeneXpert Omni is currently under development. It is intended for point-of- care (POC) testing for TB and rifampicin resistance, using the same cartridges as those used in the current Genexpert machine.

Two new medicines have been introduced in the government-run hospitals with restricted, but available to those who require them most: bedaquiline and delamanid recommended by WHO for patients with XDR-TB who have documented evidence of resistance to any fluoroquinolones. Patients with TB are tested for HIV and patients with HIV are tested for TB. Drug treatment is moving from intermittent therapy to daily fixed-dose combinations (FDCs). Among repurposed drugs for the treatment of MDR-TB are clofazimine, linezolid, meropenem/clavulanate and ertapenem. Other regimens include high dose isoniazid (16-18mg/kg), pretomanid, moxifloxacin and pyrazinamide, addition of ethambutol if not added earlier, Aminoglycosides (kanamycin, and amikacin) and Polypeptides antibiotics (capreomycin and viomycin).

To conclude, to curb and prevent MDR-TB and XDR-TB, we need to identify contacts who could have contracted TB, promote awareness, improve environmental hygiene, encourage rapid diagnosis and appropriate treatment, assess response to treatment, assure compliance, identify and screen

immunodeficient people like those infected with HIV for presence of TB. Social and public health interventions need to supplement the efforts of the government to reach the desired goal of TB eradication.

Shilpa Sharma
Associate Professor, Department of Pediatric Surgery,
All India Institute of Medical Sciences, New Delhi.
New Delhi-110029. Email: drshilpas@gmail.com

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Mini Review

Glucose - Responsive Smart Insulin

Aishwarya Krishnamurthy, Ravinder Goswami
Department of Endocrinology and Metabolism
All India Institute of Medical Sciences, New Delhi.

ABSTRACT

The discovery of insulin in 1920s revolutionized the management of Type 1 Diabetes Mellitus. The evolution of insulin over the last nine decades has seen three phases, namely discovery and understanding of the molecule, advances in synthesis of the molecule and advancements in the optimization of insulin structure and delivery. These advances aim to minimize hypoglycemia while simultaneously improving anti-hyperglycemic efficacy. Glucose-responsive insulin (GRI) systems were first conceptualized in 1979. These are novel insulin formulations that provide anti hyperglycemic activity appropriate to circulating glucose levels but with a mechanism to avoid hypoglycemia, that often accompanies stringent glycemic control. GRIs are of three major types- algorithm-based mechanical, polymer-based, molecular GRI analog systems. They differ in the mechanisms of achieving glucose responsiveness. Algorithm-based systems are closed loop insulin pumps that adjust insulin delivery commensurate with blood glucose levels on the basis of predetermined algorithms, that terminate or increase insulin delivery according to blood glucose trends. The second category of GRI includes glucose-responsive polymer-based matrices that house insulin, releasing insulin as needed based on ambient glucose levels. The third approach is to incorporate glucose sensitive motifs in the insulin molecule itself that would decrease or increase insulin availability based on blood glucose levels. The mechanisms behind these novel approaches to insulin delivery and action will be the focus of this review article.

Introduction

The prognosis for patients with youth-onset diabetes mellitus was poor till the discovery of insulin in the early 1920s. This was preceded by several other complementary discoveries. Paul Langerhans identified islet cells in the pancreas while pursuing doctoral studies in 1869 (1). Twenty years later, in 1889, German researchers Oskar Minkowski and Joseph von Mering observed that removal of pancreas in dogs led to them developing symptoms suggestive of diabetes mellitus (2). In 1921, Frederick Grant Banting, an orthopedic surgeon and Charles Herbert Best, a medical student were able to extract insulin from dog's pancreas. They received the Nobel Prize for Medicine /Physiology in 1923 (3) for the discovery.

Mayer *et al* have described the chemical aspects of insulin's history in three distinct phases (4). Initial phase of 30 years culminated in protein sequencing of the insulin molecule by Sanger *et al* in 1954 (5). In the second phase, developments were related to advances in insulin synthesis in the laboratory by chemical synthesis, semi-synthesis and rDNA technology in 1970s (6). This provided unlimited amounts of pure insulin as an alternative to animal insulin. The third phase of insulin development

*Correspondence***:** Dr. Aishwarya Krishnamurthy, Department of Endocrinology and Metabolism, All India Institute of Medical Sciences, New Delhi-110029. Email: aishwaryakr1410@gmail.com.

pertains to optimization of insulin structure and delivery to minimize its current therapeutic limitations while simultaneously improving anti-hyperglycemic efficacy. Efforts in this direction include production of ultra-rapid and longer acting basal insulin analogues, single chain insulin analogues, hepato-selective analogues, insulin receptor isoform selective analogues, oral and pulmonary insulin delivery (7, 8).

Glucose-responsive insulin systems are another innovative approach that is being developed to reduce the hypoglycemic potential of insulin while retaining anti-hyperglycemic efficacy.

Glucose - Responsive Insulin Systems

The concept of glucose-responsive insulin (GRI) was first proposed in 1979 (8). GRI represent new insulin formulations that provide insulin activity appropriate to the circulating glucose levels so as to have the good control of hyperglycemia but no serious hypoglycemia. There are three categories of insulin/delivery systems fitting the theme of glucose regulated delivery :

(1) Algorithm-based mechanical GRI systems - closed loop continuous glucose monitors (CGMs) coupled insulin pumps (10).

(2) Polymer-based systems- in which insulin is housed in a glucose-responsive polymeric matrix-based vesicle or hydrogel insulin (11).

(3) Molecular GRI analog systems- which incorporate glucose-responsive motifs in the insulin molecule by way of altering bioavailability or activity (12).

Algorithm-based Mechanical GRI Systems

Closed loop pumps include CGMs, an insulin pump capable of receiving data from the CGM and a computer encoded algorithm which predicts dose of insulin. The limitations, of these systems, are delay in onset of action of insulin after subcutaneous injection, imprecision in estimation of algorithm-based insulin dose due to lag time between plasma and interstitial glucose. Besides, insulin action might persist once a bolus dose is delivered. Therefore, the current algorithms need fine tuning to provide insulin dosages appropriate for prevailing blood glucose in relation to the interstitial blood glucose (13-16). Modern pumps can provide glucagon hormone to counter hypoglycemia. These pumps are equipped with dual hormone algorithms that can predict hypoglycemia and stop insulin supply. These pumps reduce glycemic excursions and hypoglycemias better than conventional closed loop pumps (17, 18).

Intraperitoneal insulin delivery with an implantable insulin pump has the added advantage of providing first-pass metabolism similar to native insulin secretion (19, 20). Currently, these devices have not received regulatory approval because of susceptibility to catheter occlusion possibly due to pro-inflammatory amyloid formation from the peritoneal side and fibrillation/aggregation of insulin molecule within the pump.

Polymer-based Systems

In this system, glucose sensing mechanisms are coupled to a polymer-based matrix. Insulin is sequestered within this polymer matrix. The usual material for construction of the matrix are poly-N-vinyl-pyrrolidone, polyethyleneglycol (PEG), succinyl- amidophenyl-glucopyranoside (21-23) and modified peptides or lipids (24, 25). This matrix is injectable and is designed to function as a “smart” subcutaneous insulin depot. Structural changes occur in this matrix with increasing ambient glucose levels leading to increased permeability of encapsulated insulin or detachment of insulin from its structural attachment to the polymer matrix. The insulin release is similarly decreased with a fall in ambient glucose (Fig. 1). Molecular

glucose sensing/responsive motifs are incorporated into these polymer scaffolds that serve as on-off switches for effecting the structural change following alteration in glucose level. These glucose-responsive motifs are of three kinds.

Glucose-binding proteins

Concanavalin A (Con A), a lectin was used by Brownlee and Cerami (25) in their initial model of glucose-responsive insulin. Glucose responsiveness in this system can be achieved through two mechanisms. First is through immobilization of a sugar-modified insulin on Con A, which is released from the injected subcutaneous deposit upon displacement by ambient glucose. The second is by incorporating Con A lectin with its tetrameric structure and four sugar-binding sites as a cross-linker in a polymer backbone. Competitive binding of glucose with Con A will disrupt the structural integrity of the polymer and release the insulin contained within it (Fig. 2). The clinical utility of Con A is limited by its non- physiological glucose affinity (competition for glucose binding at ambient glucose levels that are higher than typical diabetic range), immunogenicity and mitogenic potential (26-28).

Glucose oxidase

This enzyme catalyses glucose oxidation. This reaction results in the formation of gluconic acid, H_2O_2 and drop in pH. The resulting change in pH leads to structural changes in polymers that are built to respond to pH by virtue of their acidic/basic functional groups. A polymer with predominantly acidic group would shrink and one with basic groups would swell. Some polymers are built to degrade in an acidic environment thus releasing the contained insulin. (Fig. 1, 3). Glucose oxidase (GoD) based polymersomes incorporated on cross-linked hyaluronic acid microneedle arrays are being developed for painless transcutaneous delivery (29). Experimental data suggests these glucose sensing system can lower blood glucose within one hour followed by maintenance up to five hours without any hypoglycemia in mice pre- treated with insulin.

Phenylboronic acid

Glucose responsiveness property of Phenylboronic acid (PBA) is achieved by its formation of reversible esters with cis- diol molecules. The ambient glucose acts as a competitive inhibitor of diol molecules bound to PBA. In PBA based system, diols are integrated with insulin and immobilized on a PBA based polymer scaffold. With hyperglycemia, diol-insulin ester bond with PBA is dissociated and insulin is released in proportion to glucose levels. PBA has also been used, like Con A lectin as a component maintaining the structural integrity of the polymer backbone, competitive binding to glucose would cause degradation of a polymer leading to insulin secretion (Fig. 4). The advantage of PBA over Con A is due to its affinity for glucose which is in the physiological range (11). However, it has a tendency for spontaneous degradation (11) and the interaction, which is mediated by the diol group, is not specific to glucose, thereby limiting its clinical translational value.

Currently, polymer based GRI systems have several limitations. These challenges include limited particle stability, non-physiological range of action and too slow or too rapid response. Strategies being considered to overcome these hurdles include: use of multiple glucose sensing mechanisms like GoD and PBA in polymer matrix, variations in particle size, permeability of surface area and use of multilayered polymer matrices. Another interesting innovation is the integration of glucose-responsive system with externally positioned β-cell capsules. Ye *et al* (30) fashioned beta cells embedded in an alginate based microgel capsule, fitted with a microneedle array made of hyaluronic acid. The principle is that the interstitial glucose permeates through the microneedle patch and reaches the β-cells stimulating insulin release.

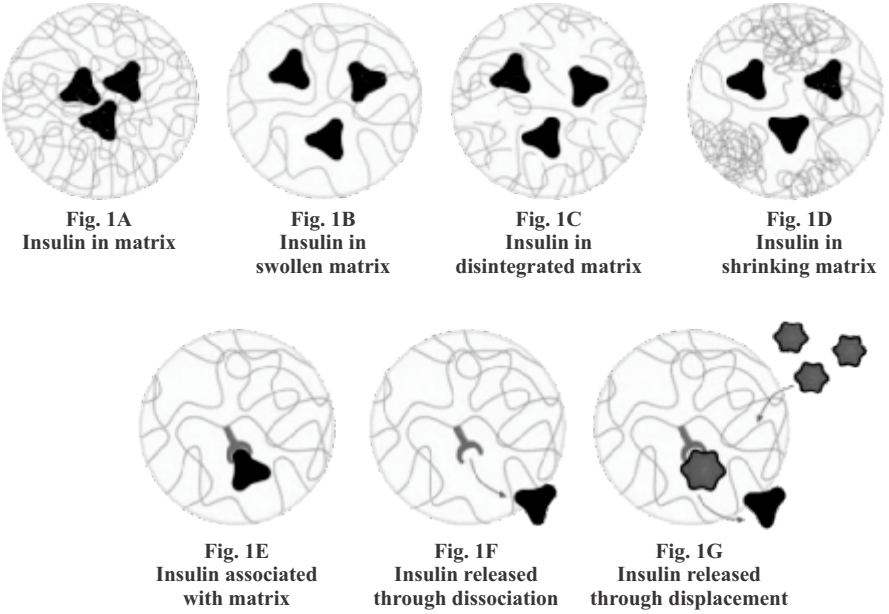

Fig. 1 (A-G) : Different types of changes in injectable polymer matrices leading to release of encapsulated insulin.

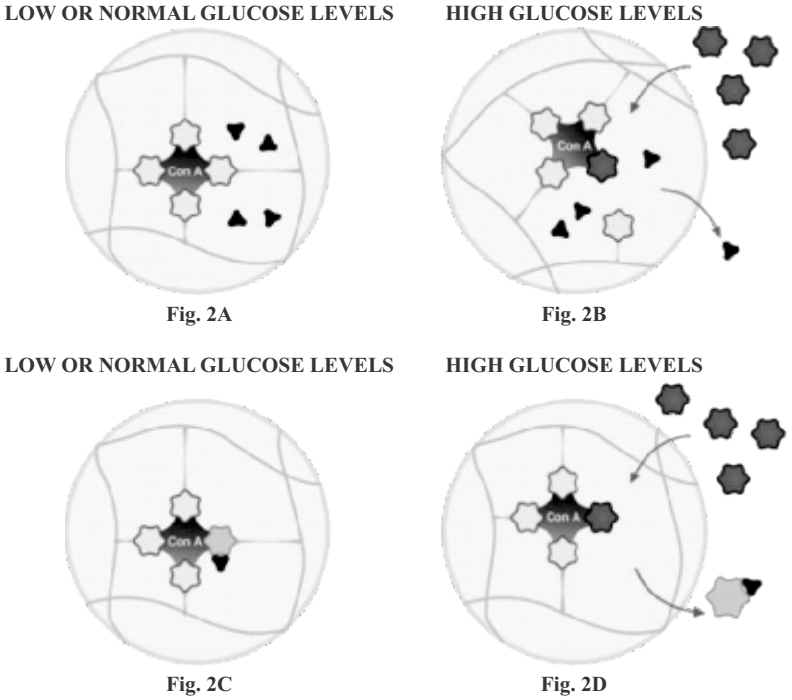

Fig. 2 (A&B) : Concanavalin A forms an important structural component of the matrix that entraps insulin molecules. When it interacts with the ambient glucose molecules the interaction is interrupted leading to disintegration of the matrix and release of insulin from the polymer matrix.

Fig. 2 (C&D) : Concanavalin A forms an integral part of the polymer matrix along with glucose conjugated insulin molecule. Ambient glucose displaces the insulin glucose conjugate by competitive binding to concanavalin A, thereby effecting the release of insulin from the polymer.

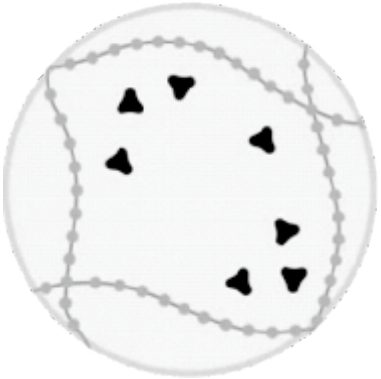

Fig. 3A

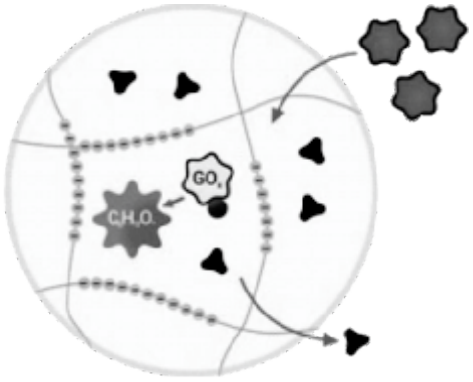

Fig. 3B

Fig. 3 (A): shows a vesicle (polymer matrix) with the encapsulated insulin.
Fig. 3 (B): shows production of gluconic acid by interaction of glucose oxidase enzyme contained within the vesicle with the ambient glucose molecule (resulting in an acidic environment). The vesicle being acidic shrinks in an acidic environment and the pores increase in size resulting in escape of insulin.

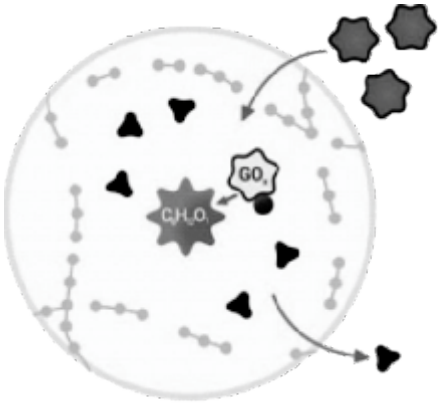

Fig. 3C

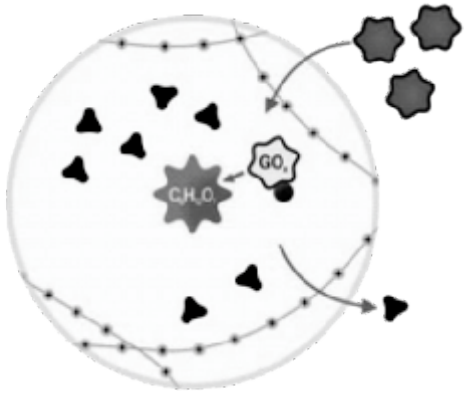

Fig. 3D

Fig. 3 (C): shows the same reaction resulting in production of gluconic acid from glucose oxidase contained within the vesicle and ambient glucose (pink) and the disintegration of the vesicle matrix with release of insulin in an acidic environment.
Fig. 3 (D): shows the same reaction with a basic vesicle matrix demonstrating swelling and release of contained insulin in response to an acidic environment due to production of gluconic acid by glucose oxidase enzyme acting on ambient glucose.

However, it was found that the diffusion of glucose through the microneedle patch is inadequate, resulting in an insignificant insulin release. To overcome this, the microneedles, are embedded with α-amylose as well as self-assembling polymers containing GoD, α-amylase, and glucoamylase enzymes. The GoD in these microneedles converts interstitial glucose into gluconic acid leading to pH mediated disintegration of the polymers on the microneedle arrays. Release of α-amylase from within the polymers hydrolyses α-amylose embedded on the microneedle into disaccharides and tri-saccharides. Glucoamylase converts

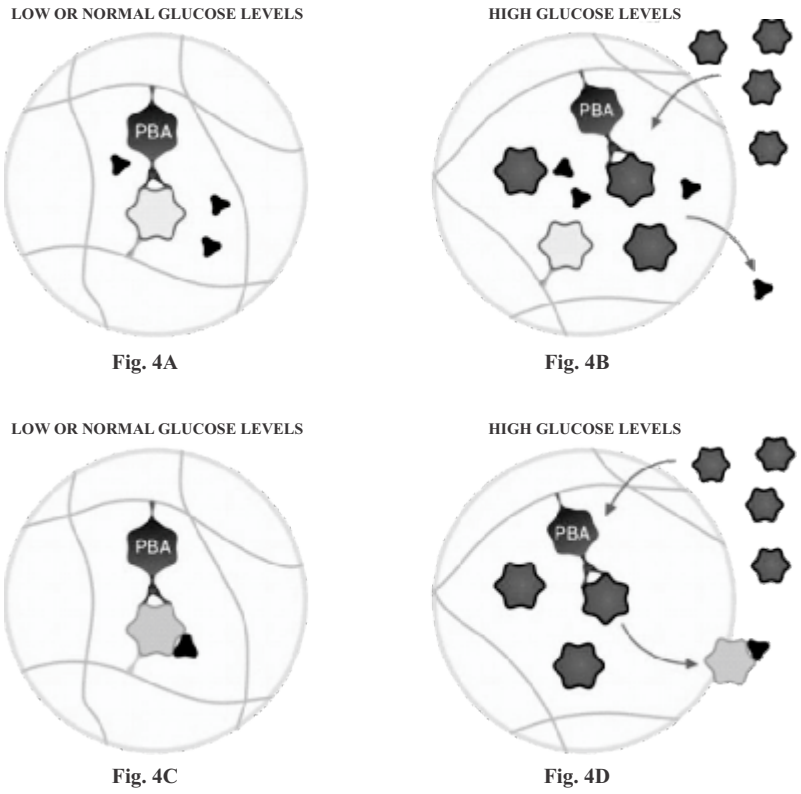

Fig. 4A Fig. 4B

Fig. 4C Fig. 4D

Fig. 4 (A&B): Phenylboronic acid (PBA) is an integral part of the polymer structure that houses insulin. The interaction in the polymer is mediated through another monosaccharide unit which gets competitively displaced by ambient glucose causing disruption of the structural attachments of PBA and disintegration of the matrix with release of insulin.
Fig. 4 (C&D): PBA and glucose conjugated insulin form an important component of the polymer structure. The ambient glucose displaces the insulin glucose conjugate from the PBA molecule by competitive inhibition thereby freeing the glucose conjugated insulin from the matrix.

these saccharides to glucose. Thus effectively the glucose concentration in the vicinity of the microneedles/beta cell capsule structure increases, providing stimulus to beta cells for insulin secretion. This strategy of amplifying glucose signal overcomes the disadvantage of beta cell capsules, i.e. poor response at physiological glucose levels (Fig. 5). A single patch of this type of GRS was effective in Type 1 diabetic mice for up to 10 hours (30).

In brief, all the three technologies discussed above were based on sequestering insulin in a glucose-responsive matrix followed by appropriate release of insulin as per the ambient glucose.

Molecular GRI Analog Systems

Molecular GRI are different class of system wherein the glucose responsiveness is inbuilt in the insulin molecule itself. The strategy involves altering the structure of insulin to modulate its pharmacokinetics.

GoD based approach uses GoD-insulin compound wherein a cysteine based linkage is disrupted by the enzymatic glucose oxidation to affect insulin release. GoD-based acidification at local injection site can also increase the bioavailability of glargine due to its enhanced solubility at acidic pH (31). Both the approaches have not resulted in any clinically significant

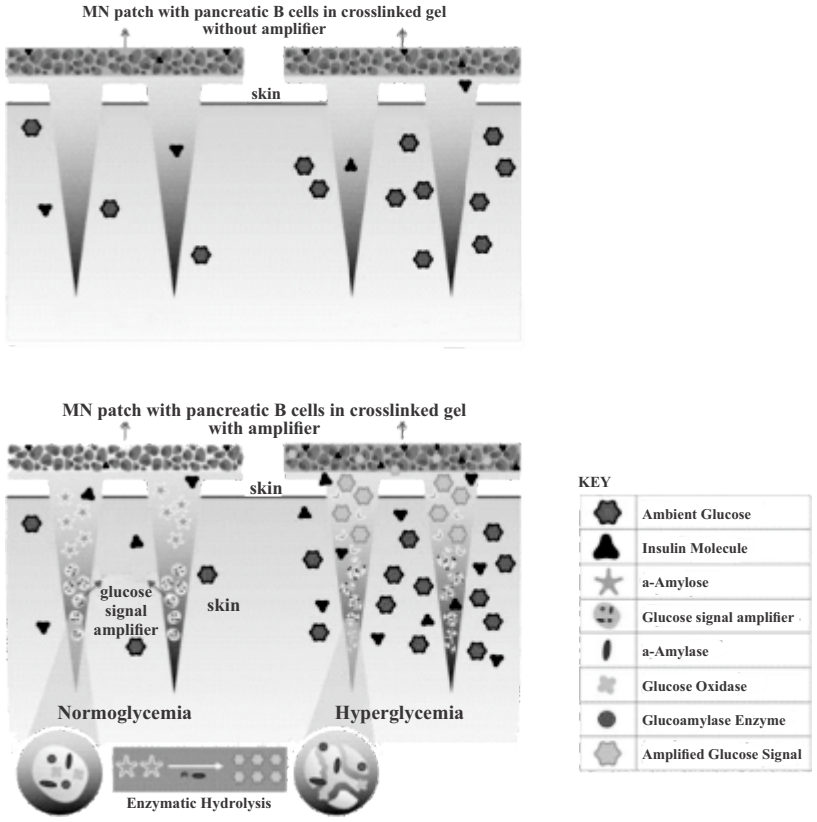

Fig. 5: β cell capsule with an α-amylase and polymers (encapsulating glucose oxidase, α-amylase, glucoamylase enzyme) embedded micro needle patch. This system helps to amplify the glucose concentration in the vicinity of the β cells so as to elicit a more robust insulin secretory response.

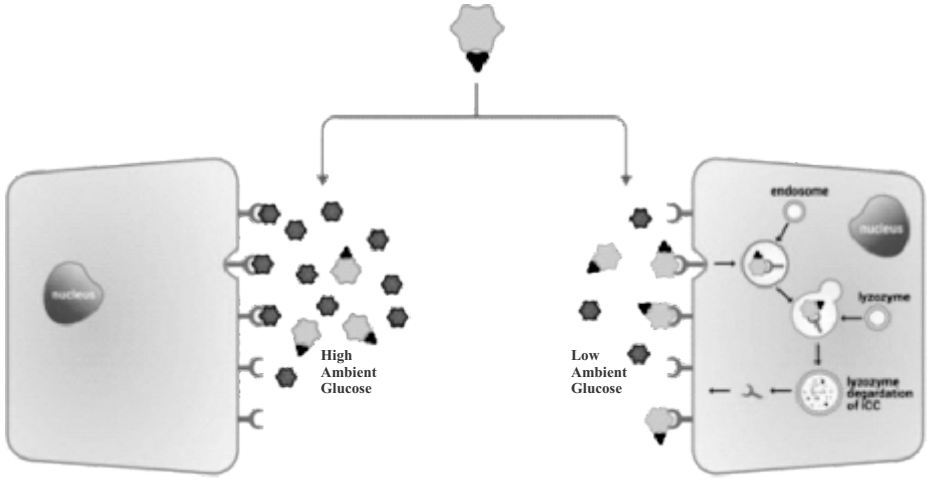

Fig. 6(A) Fig. 6(B)

Fig. 6: Insulin carbohydrate conjugates (ICC) compete with ambient glucose for binding to the mannose receptors (MR) on hepatocyte cell surface. At low ambient glucose levels (Fig. 6B), more molecules of ICC bind to the MR and are subjected to lysosomal degradation, thereby clearing excess insulin and preventing hypoglycemia. At high ambient glucose levels (Fig. 6A) there is little attachment to the MR and therefore minimal degradation.

effects due to adverse effects. The important drawback is the low Km of GoD for glucose leads to excessive insulin release at even low ambient glucose levels and results in hypoglycemia. Besides, tissue damage can occur at the local injection sites due to liberation of H_2O_2 (32). The *in-vitro* efficacy of GoD-based glargine, in reality, did not translate to improved glycemic control in pilot animal studies (33).

PBA based approaches to generate molecular GRI analog have been attempted. Insulin conjugated to a diol or sugar molecule is anchored to PBA molecule through a reversible ester bond. The interstitial glucose, competitively binds to PBA molecule thus freeing the diol labelled insulin for action. A second approach is through addition of a PBA tag to the insulin analogue detemir. Detemir has long half-life consequent to its myristic acid chain mediated binding to albumin and slow release from albumin. This release remains unrelated to the glucose concentration in blood. Addition of a PBA molecule to detemir in such a way that the interaction of detemir with albumin becomes glucose-responsive has been attempted. However, both the above approaches were unable to achieve the desired result in-vitro (34).

Zion and Lancaster proposed another novel alternative strategy for a GRI, based on endogenous lectin–based clearance (35). In this approach, addition of saccharides to the native insulin molecule results in an analog that can bind to insulin receptor as well as the mannose binding receptor. The mannose receptor (MR) normally binds and transports proteins and pathogens tagged for intracellular destruction and degradation through lysosomes without eliciting any immune response. Glucose is a competitive inhibitor of MR binding. At high ambient glucose level, insulin binding to MR and destruction is decreased. As a result more insulin is available for normal action through insulin receptor. In hypoglycemia there is a reverse sequence of events, i.e. higher fraction of circulating modified insulin is destroyed via uninhibited binding to MR (Fig. 6). Kaarsholm *et al* (36) were able to demonstrate *in vitro* glucose responsiveness of this approach, with the molecule MK 2640. However, the insulin analogue though safe and well tolerated, had poor *in vitro* potency for clinical use. Further modifications in this approach are underway, to address the potency of these formulations.

Conclusion

The journey of insulin over the last century from the initial crude alcoholic extracts of canine pancreatic tissue to a sophisticated molecule is inspiring. Recombinant DNA technology has resulted in ease of availability of human insulin molecules. Modification of insulin amino-acid sequence and addition of side chain has given insulin of different duration of action to facilitate better glycemic control. Currently efforts are on to make smart insulin molecules which are released in a controlled manner as per the ambient glucose in order to prevent hypoglycemia while maintaining normal glycemic condition for prolonged duration. On the other hand parallel efforts are on to design smart insulin, which is degraded in proportion to the ambient glucose so as to reach the same objective, i.e. good glycemic control without any risk of hypoglycemia.

Declaration

The authors have nothing to declare and there is no conflict of interest.

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HCV Seroreactivity and Detection of HCV RNA in Hepatitis Cases

Mahantesh B Nagamoti [1], *Chidanand S Patil* [2], *Jyoti M Nagamoti* [3]
Department of Microbiology, J.N. Medical College, KLE University Belagavi [1, 3]
USM-KLE Medical School Belagavi[2].

ABSTRACT

Background: Hepatitis C virus (HCV) known to be associated with wide variety of liver pathology. It is less studied in India as compared to western region.

Methods: Suspected patients sera screened for HCV by ELISA and confirmed with reverse transcription polymerase chain reaction (RT-PCR) along with routine investigations and liver profile. All HCV positive patients were undergone liver biopsy.

Results: All 24 HCV ELISA reactive and two ELISA indeterminate sera are confirmed by RT- PCR. The liver biopsy of these patients showed normal picture (19.2%), Acute hepatitis (11.5%), Chronic hepatitis (23.7%), Cirrhosis (34.72%), Hepato-cellular carcinoma (HCC) (15.38%). ALT levels were not significant.

Conclusion: All the suspected HCV cases need to be confirmed for HCV by RT-PCR.

Keywords: Hepatitis C virus (HCV), HCV RT-PCR, ALT levels, liver pathology.

Introduction

Hepatitis C virus (HCV) is a RNA virus, belongs to *Hepacalci* virus genus, transmitted parenterally leading to acute to chronic hepatitis and rarely may cause hepato-cellular carcinoma (1, 2). It is slowly progressive infection, affecting about 170 million people worldwide (3). There is significant geographic variation of prevalence of HCV across various regions of India (4). Approximately, 12-18 million people are infected with HCV in India having prevalence around 1 to 1.5% (5).

Due to rapidity and easiness, ELISA and rapid tests are most commonly used to detect HCV reactivity. Molecular detection methods are effective to detect the infection and also help to record changes in the period of therapy and to monitor the period of treatment. Even though, these techniques are more sensitive, rapid but require sophistication and have some limitations (6-8).

There is paucity in the epidemiology and clinical picture of HCV in India. There are very few reports from North Karnataka region. In this connection, we have screened the suspected hepatitis patients for HCV, using ELISA along with their liver pathology and further confirmed with reverse transcription polymerase chain reaction (RT-PCR) for HCV.

Materials and Methods

***Subjects*:** A total of 612 sera from suspected

Correspondence: Dr. Mahantesh B. Nagamoti, Professor of Microbiology, J.N. Medical College, KLE University, Belagavi, Karnataka. Email: drmbnagamoti@gmail.com.

cases of hepatitis were collected from the patients were are attending KLE Society's DR Prabhakar Kore Hospital & MRC Belagavi, (Karnataka) India, a teaching Hospital attached to the J N Medical College KLE University Belagavi, for testing of HCV, after taking approval by Institutional Ethical Committee. A detailed history and risk factors were noted and detailed investigations were carried out including liver profile (biochemistry/ biopsy).

Serology **:** All the sera were tested for HCV by ELISA using commercial kits (X-cyton India, Ranbaxy Diagnostics, England and Innogenetics Belgium) according to manufacturer's instructions. All HCV reactive and indeterminate sera were further confirmed by repeat ELISA.

RT-PCR **:** RT-PCR technique was used to detect HCV RNA, employing the primers from a highly conserved 5' Non Coding Region (5'NCR) (Table 1). Pre and post amplifications steps were performed in different sections, situated on two different floors of the Hepatitis Division of NIV, Pune. Positive and negative controls were included in every PCR experiment.

a. RNA Extraction: RNA was extracted from the patient's plasma samples, using Trizol L.S. reagent (Invitrogen). The extracted RNA was stored at 20°C until the PCR reaction was put up.

b. Reverse Transcription-PCR: Nested PCR was performed using 50 µL of the extracted RNA as template. Briefly, RT-PCR was carried out in 100 µL of reaction mixture containing 25 mM dNTPs, 50 ng/mL BSA, 10X PCR buffer, 20 pmol outer primers JENS1 and JENS 2, 0.5 mL of AMV reverse transcriptase (Promega) and 0.5 U of Taq DNA polymerase (Promega). The above mixture was incubated at 42°C for 1 hour. Amplification was performed for 35 cycles in an automated Thermocycler (Perkin Elmer Cetus), with denaturation at 94°C for 1 minute. Primer Annealing at 55°C for 1 min and amplification at 72°C for 1 min. Final extension was at 72°C for 2 min.

Five µL of the amplified product of the first PCR cycle was used for the second round of PCR (35 cycles) with inner primers. For 50 µL reaction master mix was prepared using Primer (Jens 3 and Jens 4), Taq polymerase, dNTPs and DW. Amplification was performed for 35 cycles in an automated Thermocycler (Perkin Elmer Cetus), with denaturation at 94°C for 1 minute. Primer Annealing at 55°C for 1 min and amplification at 72°C for 1 min. Final extension was at 72°C for 3 min.

c. Detection of the RT-PCR product : A 250 bp product was detected in an ethidium bromide stained 2% Agarose gel and compared with a molecular weight marker 100 bp ladder. Gel photograph was taken using Gene snap from Synergie US gel documentation system (Fig. 1).

Results

Of the 612 suspected cases of hepatitis, 24 (3.92%) of the sera turns out to be HCV reactive by ELISA and two were indeterminate

Table 1: Primer list

Primer	Sequence
Jens 1	5[1] ACT GTC TTC ACG CAG AAA GCG TCT AGC CAT '3'
Jens 2	5[1] CGA GAC CTC CCG GGG CAC TCG CAA GCA CCC '3'
Jens 3	5' ACG CAG AAA GCG TCT AGC CA T GGC GTT AGT 3'
Jens 4	5' TCC CGG GGC ACT CGC AAG CAC CCT ATC AGG 3'

(0.32%). All HCV-ELISA reactive 24 cases and two indeterminate (0.32%) were confirmed by RT-PCR (4.24%). Of them 16 were males (66.66%) and 10 (41.67%) were female patients. The youngest was 20 year old and the oldest was 55 years. The mean age for the male patients was 38.44 years with a standard deviation of 9.99 and that for female patients was 36.63 years with a standard deviation 9.3. There was no significant variation between the mean ages of male and female patients ($t = 0.29$, $df = 22$, $p = 0.78$).

The ALT levels (Mean±SD) in the HCV infected patients were 73.87±50.73 (were statistically significant 'p' < 0.001). The ALT levels in all our patients with cirrhosis, acute hepatitis and in those with normal liver cell morphology was similar (58.5 IU). On the other hand, highest ALT levels were observed in patients with chronic hepatitis (114.17± 87.69). Moderately high ALT levels were found in patients with hepatocellular carcinoma.

The analysis of liver biopsy of these 26 patients were as follows; Normal-05 (19.2%), Acute hepatitis - 03 (11.5%), Chronic hepatitis - 06 (23.7%), Cirrhosis - 08 (34.72%), Hepato-cellular carcinoma (HCC) - 04 (15.38%).

Discussion

HCV infection known to be associated with wide variety of changes in the liver (9). HCV infection in our case also proved that it is associated with multiple histological changes including acute to chronic hepatitis and cirrhotic changes with HCC. Majority of the western studies proved the presence of HCV infection in cirrhotics in 65% of the cases and in India it is less (10-24%) (10). The association is slightly higher in our study as compare to Indian studies. It may be due to the fact that, the prevalence of HCV in chronic liver disease varies according to the background endemicity of HCV in the population (11).

We have observed, HCV viraemia in the presence of specific antibodies was observed in 24/62 (4.24%) and absence of specific antibodies was observed in two of our patients. The same has been observed by Marin *et al* (12). HCV RNA detection may be said to provide early diagnosis of HCV infection. The reason of HCV being negative by ELISA may be because of genetic heterogeneity or a weakened host immune response or due to molecular techniques, which are more sensitive than ELISA (9, 13-15).

It is a well known fact that, ALT has also been widely investigated as a surrogate marker for liver pathology and HCV infection (15). ALT is known to fluctuate in chronic HCV infection, and recent studies suggest that elevated levels do not correlate with viraemia (15). Herewith we also found that ALT levels are variable in various stages of the diseases. This finding suggests that, the serum ALT levels are not the reliable predictors of HCV - induced liver pathology.

We have observed a significant correlation between HCV reactivity with all types of histological lesions of the liver. Further this association may be well co-related, if we have titrated the HCV RNA using real time PCR. Herewith we conclude that, HCV RNA detection can be applied in all suspected hepatitis cases especially in early infective cases and ALT levels

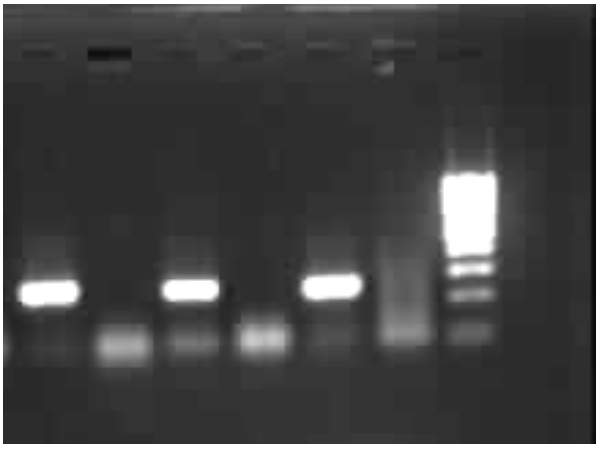

Fig. 1: HCV RNA - RT PCR results (lane 1, 3, 5 HCV positive sera, Lane 2,4 & 6 were negative sera and lane 7 Mol wt marker 100 bp ladder (left to right).

are insignificant in HCV liver pathology.

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Review Article

Atopic Dermatitis: Drug Delivery (Management) and Approaches (Strategies) in Perspective

Virendra N. Sehgal
Dermato-Venereology (Skin/VD) Center, Sehgal Nursing Home,
Panchwati, Delhi.

ABSTRACT

Age-related cutaneous manifestations are definitive pointer to the diagnosis of atopic dermatitis, the confirmation of which is solicited by 3 major and 3 minor criteria. Its unpredictable course is punctuated by exacerbations and remissions. Several treatment options, namely: 1st, 2nd and 3rd line are in vogue ever since. The 1st line envisages general measures, 2nd encompasses topical applications, while the 3rd take into account drug therapy comprising, systemic Corticosteroids, Cyclosporin, Azathioprine, Thymopentin, Interferon–therapy, Topical Calcineurin inhibitors: Tacrolimus and Pimecrolimus. The mode of action, their dosages and adverse drug reaction (ADR), in particular, have been focused in this paper with special attention to refresh their drug delivery (management) approaches (strategies) in perspective. An endeavor to focus attention to emerging etio-pathogenesis, and its application in the contemporary context has also been made.

Keywords: Atopic dermatitis, Immunoglobulins E, Cyclosporin, Azathioprine, Thymopentin, Interferon-therapy.

Introduction

Atopic dermatitis (AD) is a non-contagious, intensely pruritic, inflammatory, chronic skin disorder having a course of exacerbations and remissions, occurring in infancy and childhood running in families with a history of atopy. It is frequently associated with an elevated immunoglobulins E (IgE) levels in serum. Disease has an intricate immunological basis influenced by genetic/ familial predisposition, and certain environmental, life style and dietary factors. Recent trends suggest a continuous rise in the prevalence of atopic dermatitis in developed nations and in countries undergoing rapid urbanization and industrialization. The clinical phenotype that characterizes AD as the product of complex interactions among susceptibility genes, the host's environment, defects in skin barrier function, and systemic and local immunologic responses (1-4). IgE discovered in 1966 by the Japanese scientist couple Teruka and Kimishige Ishizaka (5), plays an important role in allergy, and is especially associated with type 1 hypersensitivity.

Serum IgE levels in a normal, non-atopic individual are only 0.05 percent of the IgG concentration, the isotype responsible for most of the classical adaptive immune response (6). Although IgE is typically the least abundant

Correspondence : Dir. Prof. (Dr.) Virendra N Sehgal, Dermato Venerology (Skin/VD) Centre, Sehgal Nursing Home, A/6 Panchwati, Delhi-110 033 (India). Ph: 011-27675363, 9810182241. Email: drsehgal@ndf.vsnl.net.in.

isotype, it is capable of triggering the most powerful immune reactions. Increased serum levels of IgE have subsequently been reported in patients with asthma, hay fever, atopic dermatitis (7-9) and also in patients infested with intestinal parasites (7). In several studies the concentrations of other immunoglobulin classes have been investigated in atopic subjects. Varelzidis *et al* (10) found a significant rise in the level of IgG in a study of adults and children with atopic eczema. High serum levels of IgA in atopic subjects have been described by Ortiz (11). Drug delivery approaches (12), in particular, are adopted for largely it takes into account formulations, technologies, and systems for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effect.

Drug Delivery (Management) Approaches (Strategies)

Due to variations in presentation in different age groups and severity of the disorder, the therapy has to be individualized. The treatment of AD envisages reducing symptoms, preventing exacerbations and recurrences, and minimizing side-effects from medications. The use of emollients, wet dressings, topical corticosteroids, antibiotics for infections, antihistamines, stress management, counseling and avoidance of allergens or triggers (1) are its main stay, the brief of treatment modalities (13) are recounted below:

A. First (1st) Line Therapy

a. General advice - decreasing scratching, improving family interaction, correcting sleep disturbances, and avoidance of trigger factors.

b. Reduction of trigger factors
   i. Curtail use of Soap and detergent
   ii. Avoid contact with wool
   iii. A central or room humidifier may help in obviating xerosis
   iv. Eliminate air borne allergens
   v. Avoid house pets
   vi. Alleviate patient and/or family stress

c. Topical therapy
   I. Bathing followed by emollients, moisturizing creams and emollients are useful and important treatment adjuncts for the daily skin care of patients with dry and inflamed skin
   ii. Initial administration of mild/mid potent topical steroids
   iii. Maintenance by ichthammol /coal tar

d. Systemic therapy
   i. Oral antihistaminic therapy comprising hydroxyzin hydrochloride / dihydrochloride at bed time, and antibiotics when impetigo develops.

B. Second (2nd) Line Therapy

a. Intensive topical potent corticosteroids for short periods
b. Wet-wrap techniques: affected parts covered with emollient followed by a wet inner and dry outer dressing used over night
c. Contact allergy to medicaments is possible, change to different preparations or patch test to topical agents.
d. Phototherapy, a useful treatment option in moderate to severe, resistant disease which frequently needs systemic immunosuppressive.

PUVA is recognized to be beneficial in the management of adult AD, and children over 12 years of age. Narrowband UVB (14) is preferred in pediatric age group. Limitations are visits to treatment center and risk of premature skin aging and cutaneous malignancies.

C. Third (3rd) Line Therapy Drugs

Systemic corticosteroid

Acute flare-up, in patients of AD may benefit from a short course of systemic therapy with corticosteroids (15, 16), but long-term use in adults and any use in children should be avoided.

Cyclosporin

Cyclosporin (CSA) is an immuno-suppressant drug widely used in organ transplantation to prevent rejection (17, 18). It reduces the activity of the immune system by interfering with the activity and growth of T cells (19). T-cell receptor activation causes release of intracellular calcium that in turn binds to calmodulin, and activates calcineurin. The calcineurin complex then dephosphorylates the nuclear factor of activated T cells (NFATc), which migrate into the nucleus and make a complex that is a transcription factor for inflammatory cytokines, (e.g., IL-2). Cyclosporin binds to cyclophilin (intracytoplasmic proteins–immunophylin), which blocks the dephosphorylation of NFAT, resulting in a decrease of T-helper cells (CD4), and cytotoxic (CD8) in the epidermis. Cyclosporin A (CyA) inhibits calcineurin-dependent pathways, resulting in reduced levels of pro-inflammatory cytokines, such as IL-2 and IFN-g. CyA is effective in treatment for both adult and childhood AD (20). It is an isolate from Tolypocladium inflatum, the fungus found in a soil samples (21). It is a cyclic non-ribosomal peptide of 11 amino acids, and contains a single D-amino acid, rarely encountered in nature (22).

Dosage schedule

Unresponsive atopic dermatitis to topical therapy may require high-dose comprising 5 mg/kg/day divided into two doses of CSA as an acute management strategy for a period of 6 weeks, followed by randomization to receive maintenance treatment with either CSA 3 mg/kg/day or enteric-coated mycophenolate sodium (1440 mg/day) for 30 weeks, followed by a 12-week follow-up period (23, 24). However, for long term treatment lowest effective dose is recommended (25).

Adverse drug reactions (ADRs) of CSA are (26)

- Enlargement of the gums
- Convulsions
- Peptic ulcers
- Pancreatitis
- Fever,
- Vomiting
- Diarrhea
- Confusion
- Hypercholesterolemia,
- Dyspnea
- Numbness and tingling particularly of the lips
- Pruritus
- High blood pressure, potassium retention possibly leading to hyperkalemia
- Kidney and liver dysfunction (nephrotoxicity) and hepatotoxicity

Azathioprine

Azathioprine (AZA) Imuran, is an immuno supressive drug used in organ transplantation, and autoimmune diseases including atopic dermatitis (27-29). It belongs to the chemical class of purine analogues (29).

Dosage schedule

Usual pediatric dose for atopic dermatitis in patients greater than 17 years: 2.5 mg/kg orally once a day, in the morning, for 3 months.

Adverse drug reactions (ADRs)

It has several side-effects including myelo suppression, hepatotoxicity, and susceptibility for infection (30).

Thymopentin (31-38)

Thymopentin (31) an immuno-stimulant (32), is a thymic polypeptide (33), which interacts with T cells (34, 35), causing T – helper cell activation resulting in enhanced interleukin-2 production with subsequent proliferation of cytotoxic T lymphocytes and natural killer cells which are capable of producing immune interferon (36), thus helps to improve

immunological condition(s) including atopic dermatitis (37).

Dosage schedule

Thymopentin therapy reduces the clinical severity of atopic dermatitis at a dose of 1 mg/kg, three times per week for 8 weeks (38, 39).

Adverse drug reactions (ADRs)

No significant adverse drug reactions are noticed, except for an increase in the T-helper/ T-suppressor ratio.

Interferon –therapy (40-48)

Interferons (IFNs) (40) are a group of signaling proteins (41), the cytokines, molecules used for communication between cells to trigger the protective defenses of the immune system that help eradicate pathogens such as viruses, bacteria, and parasites. Interferons are named for their ability to interfere with viral replication by protecting cells from virus infections (42). Their other functions are to activate immune cells, such as natural killer cells and macrophages; they increase host defenses by up-regulating antigen presentation by virtue of increasing the expression of major histocompatibility complex (MHC) antigens. Certain symptoms of infections; fever, muscle pain and flu-like symptoms, are also caused by the production of IFNs and other cytokines. Currently, AD is classified into Extrinsic atopic dermatitis (eAD) (43), IgE associated allergic atopic dermatitis, and Intrinsic atopic dermatitis (iAD), non-allergic atopic eczema/dermatitis syndrome (AEDS) (44). eAD has elevated Th2- and decreased Thl-expressing cells in the peripheral blood, with elevated interleukin (IL-4 and IL-13) expression, increased, IgE levels and decreased IFN-gamma production. Accordingly, it is imperative to highlight the role of recombinant interferon-gamma therapy in severe atopic dermatitis (45-47).

Dosage schedule

50 micrograms/m2 rIFN-gamma (n = 40) or placebo (n = 43) by daily subcutaneous injection for 12 weeks.

Adverse drug reactions (48, 49)

· Early: Flu-like syndrome : Fever, chills, generalized aches and pains, headache, poor appetite.
· Fatigue, drowsiness.
· Low blood counts

Topical immunosuppressant (50-64)

Calcineurin inhibitors

- **Tacrolimus** (50)

Tacrolimus (FK506), a macrolide lactone produced by soil fungus Streptomyces tsukubaensis. It was originally used intravenously or orally for prevention of organ rejection after transplant. Tacrolimus, especially through the topical route of administration, gained entry into therapy for inflammatory dermatoses (51), such as atopic dermatitis, without significant risk of toxicity (47). The use of tacrolimus (53, 54) (Prograf) and pimecrolimus (55) (Elidel) ointments has been licensed in the UK since 2001 subject to National Institute for Health and Clinical Excellence guidance. Tacrolimus ointment is restricted to use in adults (0.1%) and children over 2 years of age (0.03%) with moderate to severe atopic dermatitis not controlled by topical corticosteroids. Pimecrolimus cream is for use in corticosteroid resistant facial dermatitis. They are perceived as second-line agents, but conditions of use vary.

Many studies have demonstrated their efficacy, and their attraction is the absence of the cutaneous side-effects, atrophy of skin, striae, telangiectasia and bruising that may be seen with prolonged or inappropriate corticosteroid use (56). The US Food and Drug Administration

(FDA), however, has warned of their injudicious use due to theoretic side-effects (57) related to immuno-suppressant, including cutaneous or internal malignancies children.

A pilot study (58) was initiated to evaluate the efficacy of tacrolimus 0.1% ointment in the treatment of atopic hand eczema (AHE). The study was an open-label non-comparative using tacrolimus 0.1% ointment in 10 patients with AHE. Inclusion criteria included patients with hand eczema, known history of atopy, AD, hay fever and/or asthma. Patients had to stop topical application of steroids and systemic use of steroids or antihistamines for 4 weeks. Patients applied tacrolimus 0.1% ointment twice daily for 4 weeks. Evaluation was performed before treatment, after 4 weeks of treatment, and after a follow-up period of 4 weeks. During follow-up, the patients used emollients. Treatment efficacy was established at each visit based on the following parameters: itch and/or burning sensation, dryness, erythema, lichenification, erosions and fissures. Of the 10 patients, four had marked or complete improvement at the end of treatment, four other cases had partial improvement, while in one patient the treatment failed. One patient left the study due to side-effects.

· **Pimecrolimus** (59)

Pimecrolimus is an ascomycin macrolactam derivative, which has a potential to bind to macrophilin-12 (FKBP-12) *in vitro* and inhibits calcineurin. Thus it inhibits T-cell activation by inhibiting the synthesis, and release of cytokines from T-cells. It also prevents the release of inflammatory cytokines and mediators from mast cells (60-64).

Azathioprine and Betamethasone *versus* Betamethasone Therapy (65)

Efficacy of combine topical emollients containing azathioprine (AZT) and betamethasone (BM), and betamethasone alone was used in two groups of moderate-to-severe atopic dermatitis, twice a day for a period of 8 weeks. The recurrence, and presence of side-effects were evaluated, the former was found to be superior in contrast to betamethasone, suggesting usefulness of AZT in the future dispensation (65).

Topical Therapy, Evolving Scenario (66-76)

Although, several treatment options are in vogue, its treatment continues to loom large, because its etio-pathogenesis is largely seem to be enigmatic (66, 67) nevertheless, the endeavors to unfold it are relentlessly continuing. The advent of stratum corneum (66, 68), the impeccable skin barrier has taken the central stage. Accordingly, its micro-anatomy (structure) and physiology (function) has added refreshing dimensions. The alterations in its patho-physiology have been a definitive step forward, enshrining the role of flaggrin (69) and serine proteases (70) in particular. The changing pattern in the former may impede its physical strength, hydration status, skin pH, and buffering capacity amongst other physio-chemical properties. Filaggrin (71, 72), the epidermal barrier protein is, therefore, a major pre-disposing factor in the pathogenesis of AD. In addition, up-regulation of serine protease activity may cause adverse structural changes due to degradation of certain proteins, the part component of epidermal structure and its functions, thus interfering in the formation of the stratum corneum intercellular lipid membrane, regulating epidermal water flux and gradient, resulting in induction of TH2 (Subset Type pattern) of inflammation. Simultaneous, immune system (73) and its dysfunction through IgE mediated sequence of events capped by genetic (74) undertones may initiate and/or perpetuate the clinical expression of AD. Hence, the preceding rumblings responsible for the clinical connotation of AD, may bring to the fore

newer modalities to counter the intricate often difficult to treat condition. Thus, atopic dermatitis treatment envisages (75).

- Eliminating inflammation and infection
- Hydrating the skin
- Controlling pruritus, and
- Avoiding exacerbative factors

Addition of moisturizer to a low-potency corticosteroid lotion in separate regimens is effective in treating the signs and symptoms of mild-to-moderate atopic dermatitis (75, 76).

Bathing and Emollients (77-80)

Foaming detergents and soaps should be avoided. A soap substitute should be used for cleansing (77-78). A regular use of emollient(s) may even protect against inflammation provoked by irritants, thus increasing the benefit obtained from topical corticosteroid therapy (79). Indeed, ceramide-rich emollients may lead to improvements in childhood atopic dermatitis through a specific barrier repair mechanism (80).

Topical Suppression of Inflammation (81)

Topical steroids are the predominant treatment for the inflammation of atopic dermatitis, and are very safe. The strength and mode of application of the topical steroids may depend on the severity of the dermatitis, the site(s) and the age of the patient. Less potent topical steroids should be used on the eyelid, face, axillae, groins and inner thighs. Less potent topical steroids are used in less than 1 year old children. Systemic absorption may occur, even with 1% hydrocortisone ointment. It is therefore recommended to apply the ointment once daily in the evening, morning application of emollients, may be effective. There appear to be no differences in efficacy or side-effects between pulsed potent corticosteroid creams and the continuous use of mild topical corticosteroids in patients with mild to moderate disease (81).

Antipruritic Agents (82-84)

1. Antihistamines (82-84)

H1-receptor antagonists (82) are used predominantly for their sedative effect. Agents such as promethazine or trimeprazine given 1 h before bedtime can be useful when there is severe nocturnal itching. However, they can cause drowsiness and lack of concentration the next morning. In infants, these preparations may occasionally cause paradoxical excitation. They are best used in short courses, for example 10–14 days, as tachyphylaxis can occur with prolonged use (83). Most studies (84) concluded that non-sedating antihistamines are of little value for the pruritus of atopic dermatitis

2. Antibiotics (85-87)

Systemic antibiotic treatment is indicated for widespread bacterial secondary infection, primarily *S. aureus*. First- or second-generation cephalosporins or semi-synthetic penicillins administered for 7 to 10 days are usually effective. Clindamycin or oral fusidic acid (85) are possible alternatives in cases of penicillin or cephalosporin allergy. Besides, heavy colonization of klebsiella pneumonia (86) may also be recovered, requiring administration of metronidazole specific for anaerobic gram negative bacteria (87).

3. Leukotriene Antagonists (88)

Leukotriene antagonists (montelukast and zafirlukast) are useful for the treatment of asthma and allergic rhinitis. In AD therapy they are not fully elucidated. Zafirlukast (Accolate) is approved in AD and asthma for adolescents, and adult. In chronic AD montelukast (Singulair) achieved little success. It is administered in the doses of 5mg daily for 4 weeks in a clinical double-blind study (88) of moderate to severe AD in young patients between 6 to 16 years showed a significant decrease in disclosed severity, but in another study with severe AD and different doses of 5 mg, 10 mg, 20 mg, a partial

improvement comprising relief of pruritus; and erythema, in a few patients. AD patients *per se* failed to show any benefit from leukotriene receptor antagonist therapy.

Phototherapy

Phototherapy options namely:

- broad-band UVB (280 to 320 nm)
- narrowband UVB (311 to 313 nm),
- UVA (320 to 400 nm), UVA1 (340 to 400 nm)
- PUVA, and PUVA bath
- Combinations of UVB, TCs and UVB with UVA as well as UVA1

Medium- and high dose therapy are useful in AD. In the pediatric population, UV therapy should be restricted to children older than 12 (89)

Bioengineered Immuno-modulators (90)

Most of the new approaches aim at inhibiting components of the allergic inflammatory response, including cytokine modulation, the tumor necrosis factor [TNF] inhibitors, blockade of inflammatory cell recruitment, the chemokine receptor antagonists, cutaneous lymphocyte antigen inhibitors, and inhibition of T-cell activation (alefacept and efalizumab). Currently, bioengineered immune- modulators are in a clinical trial phase for AD treatment (90).

IgE-blocking Antibody (91)

IgE-blocking antibody omalizumab is a recombinant human monoclonal antibody that targets specific antihuman IgE drugs, which binds free serum IgE and avoids binding to Fc3RI receptors as well as Fc3RII [CD23] receptors on mast cells, basophiles, and antigen-presenting cell surfaces, which stops release of pro-inflammatory mediators.

Omalizumab is for use in adults and children older than 12 years with asthma and AD for 3 months; subcutaneous injections of 0.015 mg/kg IgE and 0.03 mg/kg each 2 weeks or 4 weeks. The role of omalizumab in dermatology and for AD is probably best directed towards patients who have high levels of IgE, and in whom the IgE is an etiologic factor for their disease (91).

Probiotics (92, 93)

They have also been tried in AD. Probiotics are cultures of potentially beneficial bacteria that positively affect hosts with adverse reactions to certain foods, as may be the case in AD. Many interventional studies have reported variable outcomes with manipulation of diet and environment in pregnant women, the primary prevention, and children with established AD, the secondary prevention. However, more work is required to determine the effect of such measures on the long-term outlook of patients with AD. Early treatment with microbial probiotics may be beneficial by boosting Th1 immune responses in AD (92, 93).

It is difficult to predict the outcome of the disease. It is found to be more persistent and prevalent in young children, the episodes of remission become more frequent and of longer duration in an aging child. A spontaneous remission after the age of 5 may be observed in 40 to 60 percent of patients who develop the disease in infancy. A poor prognosis is seen in individuals who have widespread disease in childhood, associated rhinitis/asthma, a positive family history, and/or very high serum IgE levels (1).

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Epidemiological Correlates and Treatment Outcomes among Patients with MDR Tuberculosis in Northern India

Anjana Singh[1], Ved Prakash[2], Ravi Kant[3]
All India Institute of Medical Sciences, Rishikesh[1]
King George's Medical University, Lucknow[2, 3].

ABSTRACT

Introduction: Multi drug resistant-TB (MDR-TB) threatens global TB control and is a major public health concern in several countries. The present study was undertaken to detect the epidemiological correlates and treatment outcomes among patients with MDR-TB previously or currently admitted in Department of Respiratory Medicine and Pulmonary and Critical Medicine, KGMU, Lucknow.

Material & Methods: This retrospective study included 2370 TB patients admitted in the Department of Respiratory Medicine and Pulmonary and Critical Medicine, KGMU, Lucknow between years 2012 to 2015. Treatment outcomes were observed. SPSS software was used for data analysis.

Results: The total number of MDR-TB cases enrolled were 2370. There were 772 (32.6%) males (95% CI: 30.7 % -34.5%) and 1598 (67.4%) females (95% CI: 65.5% -69.3%) registered for MDR-TB treatment. The treatment outcomes were as follows: majority (77.1%) were under treatment, 279 (11.8%) patients were declared cured, 10 (0.4%) were failure cases, while 64 (2.7%) were defaulters, 149 (6.3%) had died and 41(1.7%) were transferred out.

Conclusion: Emergence of MDR-TB has the potential to be a serious public health problem in Northern India and this necessitates strengthening of TB control and improved continuous monitoring of therapy.

Keywords : MDR-TB, treatment outcome.

Introduction

Tuberculosis (TB) is a chronic specific bacterial infection caused by *Mycobacterium tuberculosis*. It primarily affects the lungs (pulmonary tuberculosis) but, in a minority of cases other organs may also be involved (extra-pulmonary tuberculosis). India has the largest burden of human tuberculosis in the world, the annual reported cases being 2.2 million. In 2015, there were an estimated 10.4 million new cases of tuberculosis and 1.8 million deaths due to tuberculosis reported globally (1). Of these, there were an estimated 2.8 million new cases in India, and 0.48 million people died in India due to TB (1). The actual data may not be accurate due to lack of proper identification and reporting (2). Over a million of patients are missing, and government has no data about these missing patients (3). About 80% of healthcare setup in India is in private sector and as the experience showed, this sector may not report its cases. However, after the legal binding for notifying the TB patient to the government health

Correspondence: Dr. Ved Prakash, Associate Professor, Dept. of Pulmonary and Critical Care, King George's Medical University, Lucknow-226003.

authorities and penalty clause as mandated by the government of India recently may improve the situation for the better reporting. Thus, until now there is a gross underestimate of the disease due to poor surveillance (2).

Worldwide, anti-tubercular drug resistance is a major public health problem and become hurdle in the TB control programme. Anti-tubercular drug resistance can be with only one drug (mono-resistance) or more than one drugs (poly-resistance). It is called multi-drug resistance-TB (MDR-TB), when mycobacteria is at least resistance to both Rifampicin and Isoniazid. MDR-TB can be primary when infection occurs *de novo* with the resistant strain or secondary when it is a result of improper drug therapy resulting in to development of resistance to drugs during the course of anti-TB drug therapy. There is scarcity of data on MDR-TB, especially from developing countries. With this background the present study was undertaken to find out the demographic features and outcome of MDR patients enrolled at a tertiary care centre of District Lucknow.

Material and Methods

This retrospective hospital record based study was conducted in the Department of Respiratory Medicine and Pulmonary and Critical Medicine, KGMU, Lucknow for a period of 4 years from 2012 to 2015. The study protocol was cleared for ethics by research institutional review board. Information on characteristics like age and gender was collected from the patients enrolled under DOTS plus regimen. Treatment outcome were observed.

An MDR-TB case was defined as one whose sputum was culture-positive for *Mycobacterium tuberculosis* and resistant *in vitro* to both isoniazid and rifampicin, with or without resistance to other anti-tubercular drugs, on the basis of DST results from an RNTCP certified culture and DST laboratory.

Treatment outcomes were defined as follows:

- **Cured**: was defined as someone who completed treatment without evidence of treatment failure and who had three or more consecutive negative cultures taken at least 30 days apart, after the completion of the intensive phase.

- **Treatment completed**: was defined as a patient who had completed treatment but did not meet the definition for cured due to lack of bacteriological results.

- **Treatment failure:** was defined as treatment terminated or a need for permanent regimen change of at least two anti-TB drugs due to an adverse drug reaction, or lack of culture conversion by the end of the intensive phase, or bacteriological reversion in the continuation phase after conversion to negative after intensive phase, or evidence of acquired additional resistance to fluoroquinolones or second-line injectable drugs.

- **Default**: was defined as a patient whose treatment was interrupted for two consecutive months or more.

- **Death:** was defined as those who died for any reason during the course of anti-tuberculor treatment.

Statistical Analysis

Data entry was made in MS Office Excel software in codes and analysis was done by SPSS software. Descriptive statistical analysis, which included frequency and percentages was used to characterize the data. Association with the factors was tested for significance using Chi-square test and $p < 0.05$ was considered statistically significant. Graphs were made to visualize various findings.

Results

The total number of MDR-TB cases enrolled from 2012 to 2015 was 2370. The number of enrolled cases increased from 61 in the year 2012 to 1027 in 2015 (Fig. 1). There were 772 (32.6%) males (95% CI: 30.7 % - 34.5%) and 1598 (67.4%) females (95% CI: 65.5% -69.3%) registered for anti-TB treatment (Fig. 2). The majority of MDR-TB cases were aged between 21 - 30 years (37.7%) followed by those aged less than 20 years (22.5%) and those between 31-40 years (20.9%) (Table 1).

Treatment Outcome

Out of total 2370 patients, majority (77.1%) were under treatment; while 279 patients (11.8%) were declared cured, 10 (0.4%) were failure cases, while 64 (2.7%) were defaulters, 149 (6.3%) had died and 41(1.7%) were transferred out (Table 2). Nearly 57.4% and 53.4% cases were cured in 2012 and 2013, respectively while failure rate was 3.3% in 2012 and 1.8% in 2013 (Fig. 3). About 1.1% of patients died and 98.6% patients were continuing treatment in 2015 (Table 3).

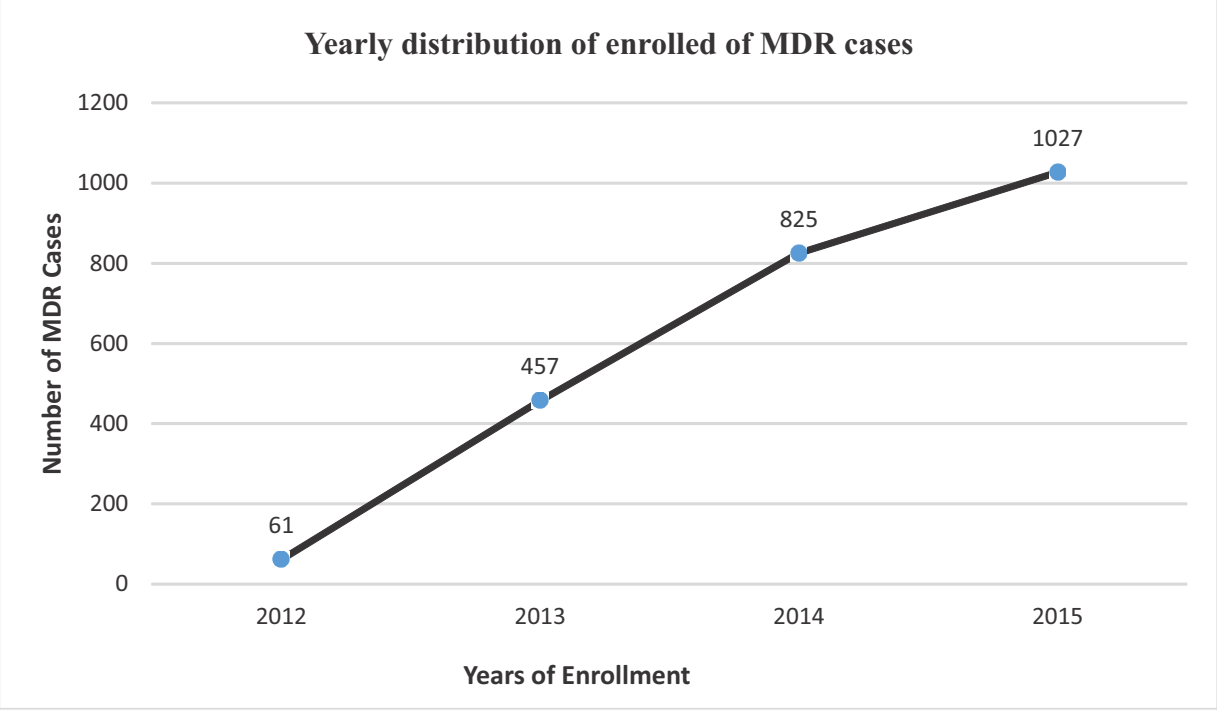

Fig. 1: Yearly distribution of enrolled cases.

Table 1: Age distribution of MDR- TB Cases

Age Group (Years)	N (%)	95% CI	
		Low	Upper
≤ 20	533 (22.5)	20.8	24.2
21 - 30	894 (37.7)	35.8	39.7
31 - 40	496 (20.9)	19.3	22.6
41 - 50	258 (10.9)	9.6	12.1
51 - 60	135 (5.7)	4.8	6.6
≥ 60	54 (2.3)	1.7	2.9

Table 2: Distribution of treatment outcome

Treatment Outcome	N (%)	95% CI	
		Low	Upper
Cured	279 (11.8)	10.5	13.1
Failure	10 (0.4)	0.2	0.7
Default	64 (2.7)	2.0	3.4
Died	149 (6.3)	5.3	7.3
Treatment Continue	1827 (77.1)	75.4	78.8
Transfer out	41 (1.7)	1.2	2.3
Total	2370 (100)		

Table 3: Year wise distribution of treatment outcome

Treatment Outcome	2012 N(%)	2013 N(%)	2014 N(%)	2015 N(%)	Chi sq	p-value
Cured	35 (57.4)	244 (53.4)	0	0	2085	<0.0001
Failure	2 (3.3)	8 (1.8)	0	0		
Default	6 (9.8)	55 (12)	2 (0.2)	1 (0.1)		
Died	13 (21.3)	108 (23.6)	17 (2.1)	11 (1.1)		
Treatment Continuing	3 (4.9)	14 (3.1)	797 (96.6)	1013 (98.6)		
Transfer out	2 (3.3)	28 (6.1)	9 (1.1)	2 (0.2)		
Total	**61**	**457**	**825**	**1027**		

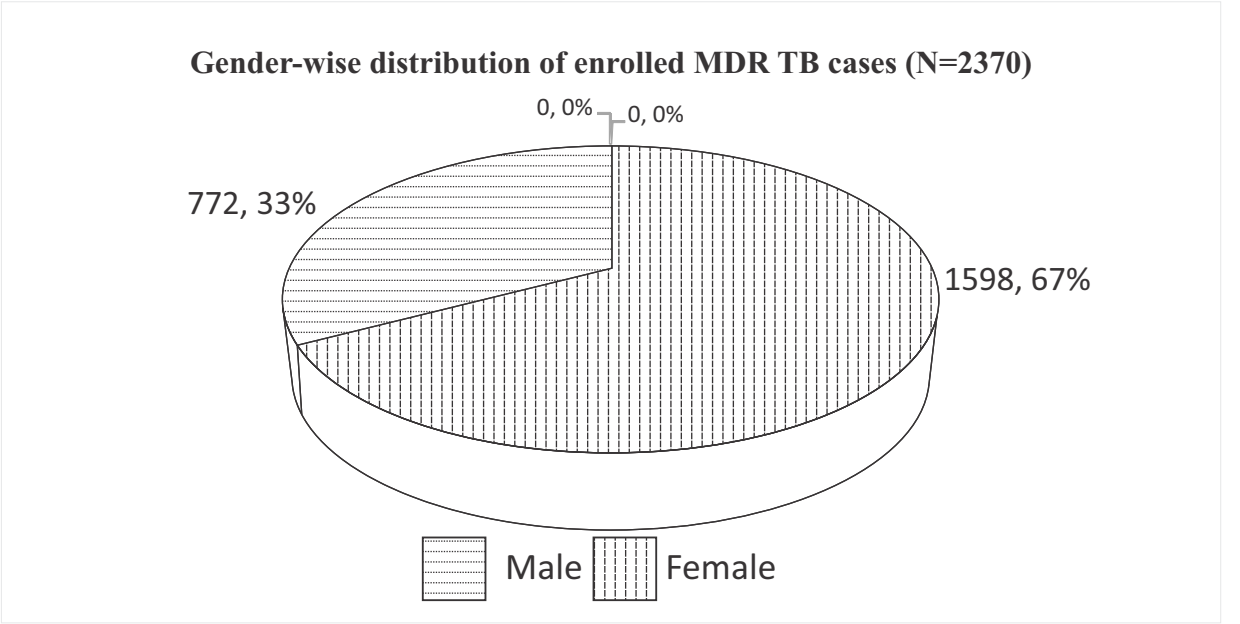

Fig. 2: Gender-wise distribution of MDR-TB cases.

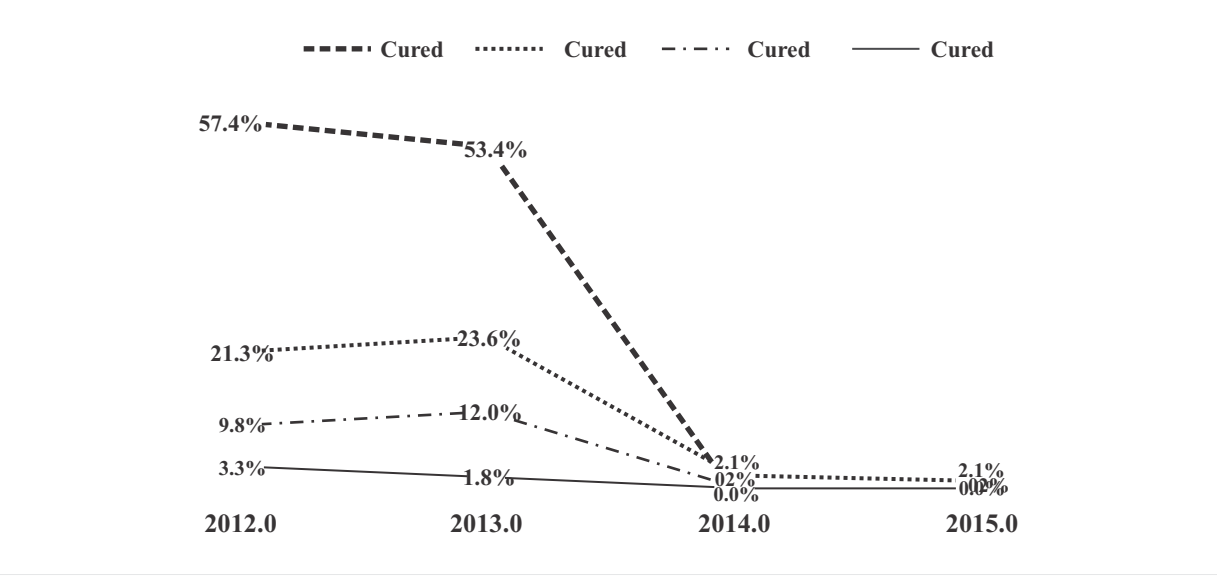

Fig. 3: Year-wise distribution of treatment outcomes.

Discussion

As per the WHO Global Tuberculosis Report 2018, a total 558,000 (range 483,000 – 639,000) new cases of MDR-TB estimated in 2017 of which 161,000 new cases were detected and reported. A 139,114 cases (87%) were started on second line ATT in 2017, up from 129,689 in 2016 (1). The estimated MDR-TB cases in India was 84,000 in 2016 (4). The result of present study shows that the number of MDR-TB cases increased from 61 in 2012 to 1027 in 2015. This is again in agreement to the increasing burden of MDR-TB. The greatest burden of TB incidence and mortality in India is in adults aged 15-60 years, which include the most productive members of the society (5). This is similar to the present study results, where TB incidence was higher among those aged up to 50 years. Nearly 57.4% and 53.4% cases were cured in 2012 and 2013, respectively. These results are consistent with reported global (52%) MDR-TB treatment success rates (1). The overall rate of treatment success was 57% (95% CI: 52%–61%) as reported from a study conducted in China (6). Results from a tertiary referral private hospital in Mumbai with sound mycobacterial laboratory back-up showed 68% success rates of treatment of a prospective cohort of MDR-TB patients (7). The highest estimated incidence and lower treatment success rates of TB are restricted to countries with low human developmental index (HDI). Life expectancy, education level, gross national income (three essential indicators taken in to accounting the HDI, and urbanisation are significantly associated factors with both TB incidence and treatment success rates (8). As envisaged in sustainable development goals (SDGs), shorter and new MDR-TB regimen needs to be adopted to fulfill the goal of end TB strategy by 2030 (9). In the present study, out of total 2370 patients, majority (77.1%) were under treatment, while 279 patients (11.8%) were cured, 10 (0.4%) were failure cases while 64 (2.7%) were defaulters, 149 (6.3%) had died and 41(1.7%) were transferred out. This is comparable to MDR-TB treatment outcome of another study by Ibrahim *et al* (10) as follows: cured cases were 300 (52%), defaulted patients were 54 (9.4%), treatment failure was 17 cases (2.9%), 52 cases (9%) completed their treatments, 96 cases (16.6%) were still under treatment and 57 cases (9.9%) died.Adherence to treatment can be attained by strong health education to the patient and their family members prior to start treatment and at different periodic intervals and involvement of community and family in

providing treatment (11). Primary transmission of MDR-TB can be prevented by improving the social support, living standards, and medical security of each patient (12). Ending TB will not be possible without research. 'India TB Research Consortium (ITRC)' initiative 2016 by the Indian Council of Medical Research (ICMR), is a key step to bring together all major stakeholders to enhance TB research and develop new tools for TB (13).

Long-term follow-up is important for understanding the overall impact of TB on patient's physical, functional, and socioeconomic well-being. Focus beyond bacteriological cure of MDR-TB patients is needed. Medical management and social support for post-treatment sequelae of MDR-TB should be incorporated in the national programs (14).

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Multiple Myeloma: Front Line Therapy and Autologous Stem Cell Transplantation

Lalit Kumar
Department of Medical Oncology
Institute Rotary Cancer Hospital, All India Institute of Medical Sciences, New Delhi.

ABSTRACT

Prognosis of multiple myeloma (MM) has improved during the past two decades. This has been attributed to the better understanding of the biology of disease leading to introduction of two new classes of molecules, namely immune-modulators (e.g. thalidomide, lenalidomide), and proteasome inhibitors (e.g. bortezomib), use of high dose chemotherapy and autologous stem cell transplantation (ASCT) and better supportive care. Current management of myeloma for young patients (≤65 years) includes initial induction therapy followed by consolidation with ASCT followed by maintenance therapy with low dose thalidomide or lenalidomide or bortezomib for 1-2 years.

The choice of initial therapy for patients of MM is based upon their eligibility for ASCT which in turn is based on their age and major co-morbid conditions pertaining to cardiac and renal systems. Patients who are ≤65 years of age (or 65 to 70 years) with no major co-morbid conditions are considered potential candidates for ASCT. Four cycles of induction therapy are administered; a combination of 3 drugs (bortezomib, thalidomide, and dexamethasone (BTD) or bortezomib, lenalidomide, and dexamethasone (BLD) or bortezomib, cyclophosphamide and dexamethasone (BCD) is associated with higher complete response (CR) (approx. 30-40%) and very good partial response (VGPR) and better progression free survival (PFS). Further consolidation with ASCT results in CR rates of 50%–70%; patients who achieve CR, have improved event-free and overall survival. Our initial experience with 225 ASCT supports these observations.

It is now possible to individualize therapy in a given patient. For example, for patients with renal failure (present in 20-30% of patients at diagnosis) —bortezomib, dexamethasone and/or doxorubicin combination could be an option; for patients with pre-existing peripheral neuropathy—lenalidomide and dexamethasone is preferred; for patients at high risk of venous thrombo-embolism bortezomib-based regimens can be used safely. Treatment with bortezomib or bortezomib + lenalidomide for patients with poor cytogenetics (chromosome deletion t(4;14), t(14;16), 17p–) appears to result in an outcome similar to that in patients without these abnormalities.

In conclusion, from being incurable, myeloma is now a chronic illness. Along with earlier diagnosis, improved treatment and better management of complications have resulted in longer disease control and survival with a better quality of life. Novel agents have provided an opportunity to tailor therapy in an individual patient. Further research is needed to improve outcome for patients who fail to achieve complete response, those with ISS stage III, and extra-medullary disease. Availability of oral proteasome inhibitors and monoclonal antibodies (e.g. IL-6 receptor) are likely to expand choice of agents for maintenance therapy in future.

Correspondence: Dr. Lalit Kumar, Department of Medical Oncology, Institute Rotary Cancer Hospital, All India Institute of Medical Sciences, New Delhi-110029. Email: lalitaiims@yahoo.com.

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Introduction

Multiple myeloma (MM)- a disease of malignant plasma cells accounts for 1% of all malignant disorders and 10-15% of haematological malignancies. While incidence of myeloma is lower in Asia and in India compared to West, there is evidence that in metropolitan cities, incidence of MM is gradually rising (1). Some of key differences seen in presentation in India compared to western population include- younger age at presentation (median 55-60 years compared to 65 years), delay in diagnosis, lower proportion of asymptomatic patients (1-2% Vs 10-20%), higher proportion of patients with anaemia (Hb <10g/dL) , ISS stage III (30 to 50%), renal failure (eGFR <40 ml/mt in 25%) and higher proportion of patients with extra-medullary disease (10-20%) (2). Limited data suggest that proportion of high risk cytogenetics [17p del, t(4;14), t(14;16)] is similar (10-15%) (unpublished data).

Survival of MM patients has improved significantly during the past 2 decades. This has been attributed to novel agents based induction, autologous stem cell transplantation (ASCT) in eligible patients, and use of maintenance therapy (3). Prior to year 2000, initial therapy for myeloma patients included cytotoxic chemotherapy – melphalan and prednisolone or VAD (vincristine, adriamycin, and dexamethasone) as continuous infusion. Treatment was associated with low complete response rates (5-15%), and short progression free and overall survival (2.5 to 3.5 years). Introduction of immunomodulators (thalidomide, lenalidomide), proteasome inhibitors (bortezomib) and dexamethasone confirmed higher response rates, and improved progression free and overall survival (PFS/OS). Currently, these are the back bone of myeloma treatment.

Treatment

Current management of myeloma is based on the initial assessment for transplant eligibility. Patients who are ≤65 to 70 years of age, in good ECOG performance status and without significant co-morbidities are considered transplant eligible. Such patients receive 3 to 6 cycles of induction therapy followed by ASCT followed by maintenance therapy. Goal of induction therapy is to reduce plasma cells burden and improve depth of response. Patients who are 'transplant ineligible' or elderly are advised induction therapy (9-12 cycles) followed by maintenance therapy. In addition all patients should receive supportive care in the form of bisphosphonates, initially 3 monthly for 1-2 years then at longer intervals (4).

Induction Therapy

Initial studies have used 2 drug-based induction in the form of thalidomide plus dexamethasone, lenalidomide plus dexamethasone or bortezomib plus dexamethasone. In last five years -3 drug combination – one immunomodulator (thalidomide or lenalidomide), one proteasome inhibitor (bortezomib) and dexamethasone are being used for induction. Three drug combinations are associated with higher response rate (complete and very good partial response), and better PFS and OS. Commonly used combinations include- bortezomib, thalidomide plus dexamethasone (VTD), bortezomib, lenalidomide plus dexamethasone (VRd), or bortezomib, cyclophosphamide plus dexamethasone(VCd) or bortezomib, liposomal doxorubicin plus dexamethasone (PAd). There is no head to head comparison between these combinations. A number of randomized trials (5-10) have confirmed high response rates (Table 1).

AIIMS Experience: In a randomized study (11), we compared lenalidomide–dexamethasone (n=97) versus thalidomide-dexamethsone (n=96). Response rate was 72.2% versus 68.7%, p=0.34. At a median follow-up of 70 months, median overall survival was not reached in len-dexa arm versus 63 months in thal-dexa arm (p=0.50). Subsequently, in

Table 1: Novel agents based induction therapy prior to transplant
(Adapted from ref 5 : Moreau *et al*, Blood 2015)

Study (Ref)	Treatment scheme	No of Pts	Response rate (%)		Post transplant CR+ VGPR (%)	Long term outcome
			ORR	CR+VGPR		
IFM (5)	VADx4±DCEP x2-ASCT VDx4±DCEP x2-ASCT	242 240	63 78	1+15 6+38	9+37 16+54	PFS 30 months PFS 36 months
GIMEMA (6)	VTDx3-ASCT-VTD x2-TDx3-ASCT-TDx2-Dexa maintenance	236 238	93 79	≥62 VGPR ≥28 VGPR	≥82VGPR ≥64VGPR	3Yr PFS:68, OS:86mo 3 Yr PFS:56,OS:84mo
PETHEMA (7)	TDx6-ASCT-IFNm/Tm/VTmx 3Y VTDx6-ASCT-IFNm/Tm/VTmx 3Y VBMCP/VBADx4-Vx2-ASCT-IFNm/Tm/VTmx3Y	127 130 129	62 85 75	≥29VGPR ≥60VGPR ≥36VGPR	CR: 40 CR:57 CR:48	PFS 28 mo, OS 65% @4 Yr PFS:56 mo, OS 74% PFS:35 mo, OS:70%
IFM (8)	VDx4-ASCT VTDx4-ASCT	99 100	81 88	≥36VGPR 49≥VGPR	58≥VGPR 74≥VGPR	PFS : 30 mo PFS:26 mo
HOVON-65 (9)	VADx3 -CAD-ASCT-Tmx2Y PADx3 -CAD-ASCT-Vmx2Y	414 413	54 78	14≥VGPR 42≥VGPR	36≥VGPR 62≥VGPR	PFS:28mo,OS 55% @5 Yr PFS:35 mo, OS 61% @5 Yr

Abbreviations: CAD: cyclophosphamide-doxorubicin-dexamethasone; DCEP: dexamethasone-cyclophosphamide-etoposide-cisplatin; Dm: dexamethasone maintenance; GIMEMA: Gruppo Italiano Malattie Ematologiche dell'Adulto; HOVON: Dutch-Belgian Hemato-Oncology Group; IFM: Intergroupe Francophone du Myélome; IFNm: interferon maintenance; NR: not reported; ORR: overall response rate; PAD: bortezomib-doxorubicin-dexamethasone; PETHEMA/GEM: Programa para el Estudio y la Terapéutica de las Hemopatías Malignas/Grupo Español de Mieloma; PR: partial response; TD: thalidomide-dexamethasone; Tm: thalidomide maintenance; V: bortezomib; VBAD: vincristine-BCNU-doxorubicin-dexamethasone; VBMCP: vincristine-BCNU-melphalan-cyclophosphamide-prednisone; Vm: bortezomib maintenance; VTD: bortezomib-thalidomide-dexamethasone; VTm: bortezomib-thalidomide maintenance.

another study (12) a combination of bortezomib-lenalidomide and dexamethasone (VRd) was compared to lenalidomide plus dexamethasone (Rd). Overall response rates (sCR+CR+VGPR +PR) was 78.4% vs 73.9% in VRD and Rd arms, respectively, p=0.6; sCR + CR 21 (28.4%) and 21 (30.4%), respectively, p=0.86. At a median follow-up 17.1 months (range 1 to 33), median OS is 30.2 months (95% CI 28.2 to 32.2) and 28.6 months (95% CI 26 to 31.3) in VRD and Rd arms, respectively, p=0.3. Median PFS was 27.8 months (95% CI 25.4 to 30.2) and 28 months (95% CI 24.6 to 31.4), respectively, p=0.3. Estimated one-year OS is 88% vs 85% in arms A and B, and PFS 83% vs 72%, respectively (12).

Table 2 :High dose chemotherapy and autologous stem cell transplantation with novel agents based induction therapy: Randomized trials
(adapted from ref. 17 Dhakal *et al*, 2018)

Author (Ref)	No of Pts	Induction	Conditioning Vs standard therapy	Maintenance	Follow-up (in months)	Comments
Palumbo *et al*, 2014 (13)	273	Rd	Mel 200 mg x2 Vs MPR	Len Vs observation until progression	51.2	Median PFS 43 mon Vs 22.4 mon, p<0.001 OS @4 Yr 81.6% vs 65.3%,p<0.02
Gay *et al*, 2015 (14)	256	Rd	Mel 200 x2 Vs CRd	Len +P vs Len until progression	52	median PFS 43.3 mo vs 28.6 mo,p<0.0001
Attal *et al*, 2015 (15)	700	RVd	Mel 200x1 Vs RVd x 8 cycles	Len for one year	44	Median PFS 50mo vs 36 mo,p<0.001 OS @ 4 Yr 81% vs 82%,p=ns
Cavo *et al,* 2016 (16)	1192	CyBord	Mel200x 1 or 2 VsVMPx4 cycles	Len until progression	26	VGPR 84% vs 74%,p<0.0001 PFS better with HDCT, p<0.01

Rd: lenalidomide-dexamethsone; RVd: bortezomib, lenalidomide and dexamethsone; Len: lenalidomide; MPR: melphalan, lenalidomide- prednisolone, CRd: cyclophosphamide; lenalidomide: pdexamethasone; Mel: melphalan; VMP: bortezomib, melphalan, prednisolone; PFS: progression free survival; mo: months; HDCT: high dose chemotherapy.

Autologous Stem Cell Transplantation (ASCT)

Post induction therapy, transplant eligible patients undergo ASCT. A number of randomized studies have confirmed superiority of ASCT over conventional cytotoxic chemotherapy in these studies conducted before the year 2000. These studies confirmed superiority of high dose chemotherapy and stem cell transplant over conventional chemotherapy. With availability of novel agents from year 2000 onwards, four randomised studies (13-16) have been reported. Data from these studies have been summarized (17) in Table 2. High dose chemotherapy (HDCT) was associated with superior CR rates and improved PFS, confirming that even in novel agents era- HDCT followed by ASCT is the standard of care for transplant eligible myeloma patients.

Procedure

Prior to transplant all patients were evaluated for their fitness for transplant- for organ function, performance status and disease status. For peripheral blood stem cell mobilization patients receive inj G-CSF 10 mcg/day in 2 divided doses for 5 days followed by aphaeresis. Target is to collect 2-2.5x10(6) CD34+ cells. About 10-20% patients may have poor mobilization. These can be identified by

Table 3 : Maintenance therapy following ASCT : Phase 3 trials
(Adapted from ref. 5 Moreau *et al*, Blood 2015)

Study (Ref)	**No of Pts**	**Initial Dose**	**Response Vs comparator**	**Median FU in months**	**EFS or PFS Vs Comparator**	**OS Vs comparator**
Thalidomide						
Attal *et al* (18)	597	400 mg	CR+VGPR 67% Vs 55%	30mon	3 Yr EFS 52% Vs 36%	87% Vs 77% @ 4 Yr
Barlogie *et al* (19)	668	400mg	CR: 64% Vs 43%	72mon	Median EFS 6.0 vs 4.1 Yr	57% Vs 44% @ 8 Yr
Spencer *et al* (20)	269	200mg	CR+VGPR 63 % Vs 40%	36mon	PFS 42% Vs 23% @ 3Yr	86% Vs 75% @3 Yr
Lokhorst *et al* (21)	556	50mg	CR:31% Vs 23%	52mon	Median PFS 34 Vs 25 mon	Median OS 73 Vs 60 mon
Morgan *et al* (22)	492	50mg	NR	38 mon	Median PFS 30 Vs 23 mon	75% Vs 80% @ 3yr
Steward *et al* (23)	332	200 mg	NR	4.1 Yr	PFS : 32% Vs 14% @ 4 Yr	68% Vs 60% @ 4 Yr
Lenalidomide						
Attal *et al* (24)	614	10 mg	CR+VGPR: 84% Vs 76%	45 mon	Median PFS: 41 Vs 23 mon	73% Vs 75% @ 4 Yr
Mc Carthy *et al* (25)	460	10mg	NR	34 mon	Median TTP: 46 Vs 27 mon	88% Vs 80% @ 3 Yr
Bortezomib						
Sonneveld *et al* (10)	827	1.3mg/m2	CR+VGPR:76% Vs 56%	41 mon	Median PFS:35 Vs 28 mon	61% Vs 55% @ 5 Yr
Rosinol (26)	266	1.3mg/m2	NR	24 mon	2 Yr PFS 78% Vs 63% Vs 49%	NR

CR: complete response; VGPR: very good partial response; EFS: event free survival; NR: not reported; PFS: progression free survival; OS: overall survival; TTP: time to progression.

Table 4 : Induction therapy for transplant ineligible patients : Phase 3 studies and meta analysis results
(Adapted from ref. 5 Moreau *et al*, Blood 2015)

Study (Ref)	Scheme	No of pts	Median FU	Best response	PFS in Months	OS in months
MPT meta analysis (27)	MPTx8 vs 12 vs until relapse	1685	Not available	VGPR25%	20.3	39.3 mo
MPT First trial (28)	MPTx12 cycles	547	37 mon	CR9.3%	21.2	51.4% @ 4 Yr
CTD (29)	CTD -9 cycles	426	44	CR13.1%	13	33.2
VMP (30) VISTA trial	VMP -9 cycles	344	60.1	CR30%	21.7	56.4
MPR-R (31)	MPRx9 cycles followed by R until progression/relapse	152	30	CR9.9%	31	59%@4 Yr
VMPT-VT(32-33)	VMPTx9 followed by VTx 2 yrs or until progression/relapse	254	54	CR38%	35.3	61%@5 Yr
VMP/VTP -VT(34-35)	VMP or VTPx6 f/b VT up to 3 Yrs	91	46	CR46%	39	69%@5Yr
Rd continuous (36)	RD until disease progression	535	37	CR15.1%	25.5	59.4% @4 Yr

Abbreviations: CAD: cyclophosphamide-doxorubicin-dexamethasone; DCEP: dexamethasone-cyclophosphamide-etoposide-cisplatin; Dm: dexamethasone maintenance; GIMEMA: Gruppo Italiano Malattie Ematologiche dell'Adulto; HOVON: Dutch-Belgian Hemato-Oncology Group; IFM: Intergroupe Francophone du Myélome; IFNm: interferon maintenance; NR: not reported; ORR: overall response rate; PAD: bortezomib-doxorubicin-dexamethasone; PETHEMA/GEM: Programa para el Estudio y la Terapéutica de las Hemopatías Malignas/Grupo Español de Mieloma; PR: partial response; TD: thalidomide-dexamethasone; Tm: thalidomide maintenance; V: bortezomib; VBAD: vincristine-BCNU-doxorubicin-dexamethasone; VBMCP: vincristine-BCNU-melphalan-cyclophosphamide-prednisone; Vm: bortezomib maintenance; VTD: bortezomib-thalidomide-dexamethasone; VTm: bortezomib-thalidomide maintenance.

doing peripheral blood CD34 counts on day 4 of G-CSF. Patients with CD34 + cells <20/cmm are likely to have poor mobilization. Options for such patients include- chemo-mobilization using cyclophosphamide 2-4 g/m^2 or Plerixafor, a CXCR4 –chemokine nhibitor. Patients with prior melphalan or radiation are poor mobilizers and therefore these should be avoided during induction in transplant eligible patients. Once adequate number of stem cells are harvested these can be cryopreserved at -80 degree Cels or in liquid nitrogen for long term storage. At our centre, we collect stem cells electively, keep at 4 degree Cel. This is followed by high dose chemotherapy with melphalan 200 mg/m^2 IV followed by stem cell infusion 24 hours later. This practice of keeping stem cells at 4 degree is cost effective and stem cells are viable (>90%) up to 96 hours. Twenty four hours after stem cells, patients are started on G-CSF 5 mcg/kg once daily until engraftment. Once stable, patients are then discharged and followed-up in the out patients department with reassessment for response on day 100. Our current policy is to give 2 more cycles of VRd regimen as consolidation from day 100 (±7 days) onwards followed by maintenance therapy using lenalidomide 10 mg daily for 21 days every 28 days for 2 years. Patients intolerant to lenalidomide receive inj bortezomib 2 mg subcutaneously every 2 weeks. In earlier period we have used low dose thalidomide (50 mg daily).

Post transplant consolidation

A number of studies have suggested that 2-3 cycles of consolidation using VRd or VTd may further improve CR rate and more patients have 'nil' minimal residual disease. In an ongoing prospective study at our centre among 58 patients CR rate improved from 81.3% (post ASCT at day 100) to 89.8 % post consolidation, 32 of 58 were MRD negative at day +100, 26 were MRD +ve, of these 16 (61.5%) became MRD negative post-consolidation as assessed by 8 colour flow cytometry) (unpublished data).

Maintenance therapy

Initial studies have used thalidomide 100 to 200 mg per day; among six randomized studies (18-23) 3 had shown improved PFS and OS. Neuropathy was the main toxicity. Subsequent studies have used lenalidomide 5-10 mg daily 15-21 days every 28 days. These studies have shown improved PFS. Second malignancy has been reported in 4-6% of patients. Inj Bortezomib 2 mg every two weeks has been used in studies from Europe. Currently, Lenalidomide 10 mg daily for 21 days out of 28 days for two years is recommended (Table 3).

Induction therapy for transplant ineligible or elderly patients

Initial studies confirmed superiority of melphalan- thalidomide and prednisolone (MPT) combination over MP alone as regards to response rate, PFS and OS (Table 4). VISTA trial compared VMP (bortezomib, melphalan and prednisolone) with MP; VMP was superior in terms of response rate, CR rate, median time to progression (24.4 months vs. 16.6 months) and OS. In a recent update at 60 months, these results still hold; median OS 56 months versus 43 months (32-33). Recent studies have used a combination of MP-lenalidomide (MPR) (31). Another phase 3 study has compared lenalidomide plus dexamethasone (Ld) as continuous therapy or 18 cycles to MPT. At a median follow-up of 37 months; continuous Ld was better compared to Ld 18 cycles and MPT regimen (36). For patients who are frail or have significant co-morbidities- two drug combinations is a reasonable choice.

Conclusions

Survival of patients with myeloma has improved significantly in the past two decades. Compared to median survival of 3-to 3.5 years prior to year 2000, presently median survival is 5.5 to 6 years. For patients undergoing ASCT median survival is 8 to 9 years. Recently a number of newer agents have been added with

significant activity and have been approved for the treatment of relapse. These include- carfilzomib, ixazomib, pomalidomide. In addition, two monoclonal antibodies- daratumab -anti CD38 and elotuzumab (anti SLAM F7) have been moved to front line. These are now being compared in combination with bortezomib, carfilzomib, or lenalidomide based combinations. Other strategies currently being explored include- vaccination against MM antigens, along with immunomodulatory agents such as IMiDs or the anti-PD-1 antibody and CAR-T cell therapy. It is hoped that these strategies would lead to further improvement in response and long-term control of the disease in near future.

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New Perspective of Lymphatic Filariasis-Towards Elimination

Shantanu Kumar Kar, Jagadish Hansa
IMS & SUM Hospital, Siksha 'O' Anusandhan University,
Bhubaneswar, Odisha.

ABSTRACT

Lymphatic filariasis (LF) is an important public health problem globally as well as in India. LF causes both acute and chronic morbidity with significant impediment to socio-economic development. The aim of this review was to analyze the current scenario in filariasis research. This debilitating disease which carried a serious social stigma and once thought to be difficult to treat, control, and eradicate, now could achieve significant success in its treatment and elimination globally by 2020, including from India. Achievement of success so far could be possible through series of evolution in understanding pathology of the disease, pathogenesis of the etiologic agent parasite, diagnostic tools, therapeutic and preventive approaches through new knowledge, techniques and development of investigative tools.

Keywords: Lymphatic filariasis, *W.bancrofti,* lymphedema.

Introduction

Tropical and vector-borne diseases are a challenge to the community as they impose a relatively high degree of social impact on the financial and economic resources and disturb the affected patients psychologically. Lymphatic filariasis (LF) is a tropical disease which is prevalent in the developing countries and least developed countries. The aim of this review was to analyze the current scenario in filariasis research. The focus is to depict the contribution of India and its impact on the global filariasis research.

Global Scenario

LF caused by three parasitic species of nemotode parasites like *Wuchereria bancrofti* (*W.bancrofti*), *Brugia malayi* and *Brugia timori*, characterized by chronic morbidity in the form of hydrocele, lymphedema and elephantiasis, and is considered a major public health problem that belongs to group of neglected tropical diseases (NTDS) globally (1).

While 1.34 billion people are at risk of this mosquito-borne disease globally, large majority (65%) are shared by south East Asia (2). Around estimated 36 million are chronically disabled that constitutes a leading cause of physical disability globally. Almost 19 million are affected by scrotal hydrocele in men, and some 17 million by lymphedema (3). The lymphedema mainly affects lower limbs and also arms, breasts and scrotum. The other LF infected persons are at risk of developing lymphedema or hydrocele.

LF is targeted for elimination by the world health assembly (World Health Assembly Resolution WHA 50.29: Elimination of LF as a

Correspondence: Dr. Shantanu Kumar Kar, Directorate of Medical Research IMS & SUM Hospital, Siksha 'O' Anusandhan University, Bhubaneswar-751003, Odisha. Email: shantanukar123@gmail.com.

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public health problem. Fifth World Health Assembly, 5-14 May, 1997, Resolution and Decisions). The Global Programme to Eliminate Lymphatic Filariasis (GPELF) consists of two "Pillars", stopping new infections by 2020 and managing morbidity and preventing disability for persons already infected (4). Out of 73 endemic countries outlined above, Mass Drug Administration (MDA), has been already started in 62 by WHO in 2014 (5) and 45 countries are in process aiming to achieve elimination targets by WHO by 2020 (6). Significant achievement has been done in the first pillar of interruption of transmission thereby preventing new infection only 24 of 73 countries have addressed the morbidity management and disability prevention process by 2014 (6). Out of 73 countries globally endemic for the disease are mostly situated in tropical countries but found commonly in Africa and India. The distribution of the disease shows that most countries are in Africa on either side of equator. India unfortunately has bulk of the disease and bears a disproportionate burden almost hay of the global burden.

Indian Scenario

LF is still considered a major public health problem in India. Around 256 districts of about 630 million populations are being targeted in India (NVBDCP: 2017 personal communication). India alone contributes to forty percent of world burden of LF (7). While *W. bancrofti* filarial parasite accounts for 95% of the disease burden, only 5% is contributed by *Brugia malayi.* Out of 1.3 billion populations in the country, only 630 million population are at risk, over 23 million people suffer from disease and 31 million are estimated to be carriers (8). The disease is considered to be tropical debilitating disease only next to malaria (9). The total disability adjusted life years lost is nearly 2.06 million resulting in wage loss of US 811 million (10). The current data (2017) estimates round 0.38 million hydrocele and 0.84 million lymphedema in India (NVBDCP: Personal communication). In India the major chronic manifestations are lymphedema, hydrocele, elephantiasis and chyluria Tropical pulmonary eosinophilia is considered as a form of occult filariasis caused by parasite(s). The disease has a great economic impact in the country. While 2 billion USD lost per year both direct cost (out of pocket payment for care and cost to health system) and indirect cost includes lost to productivity (around 30%) and missed earning both patient and employer (NVBDCP: Personal communication).

Out of all countries in globe, India has the largest Mass Drug Administration (MDA) programme. The country targets elimination of LF by 2020. Lymphatic disease also imposes a great social burden. The disease carries a significant social stigma interfering marriage, prospects, sexual disability and decreased prospect of getting manual jobs. Frequent episodes of Acute Dermato Lymphangio Adentitis (ADLA) in cases with lymhedema and elephantiasis not only lead to painful deviating condition but also loss of wages lifelong that affect the family as well as the community. The disease is well known in ancient times, as evidenced by the script of a hydromel case in famous Konark temples of Odisha built in around 1000AD.

Historical Perspective

LF is an ancient disease and its grotesque lymphedematous limb and massive hydroceles were well known since long. India has a long history in taking efforts to control the disease through National Filaria Control Programme (NFCP). Odisha has first witnessed the LF control measures during 1950s. Several control measures were taken in many parts of the country subsequently using drug Diethylcarbamazine (DEC) and use of anti larval, anti adult entomocogical measures tools in last 4 to 5 decades. In 1980's lymphatic filarial control programme was primarily targeting towards vector control and medical impact at both clinical and immunological level. There was no concept on elimination as a public health measure. Several surgical techniques were used

to reduce limb size and drugs to address filarial sepsis caused due to infection. Until recently when paradigm shift of approach to eliminate the filarial infection and the disease occurred with advent of newer knowledge and techniques.

Clinical Disease and Infection

Both acute and chronic clinical manifestations of LF are well known since long, but the understanding about its pathogenesis, clinical progression, tools for diagnosis therapeutic strategy and approach for its elimination was not known until recently. The understanding of these filaria parasites in its biological and genomic aspect has significantly improved recently paving way for use of newer diagnostic tool to detect infection in convenient way accurately. Elegant studies now could demonstrate the location of adult parasite in human, using new tool like Ultrasonography, and Lymphoscintigraphy. The understanding as to how the parasite evades the immune attack including the role of sheath in such maneuvers.

The genetic makeup of these parasites could be revealed. The sheath of *W.bancrofti* demonstrates albumin and immunoglobulin in its surface (11). The clinical spectrum of the disease is classified in to 3 groups; microfilaria carrier, acute and chronic filarial disease. The larval form is called microfilarae that circulates in peripheral blood is considered innocuous; the adult form of parasite that remains hidden in lymphatics or in lymphnodes is responsible to initiate pathology. Atypical clinical features like lymphatic nodule due to the adult parasite seen in same human host. Lymphatic nodules were demonstrated as early as in 1980 (12, 13).

Recurrent adenolymphangitis (ADL) is considered hall mark of the disease and constitutes important risk factor for its progression to lymphedema stage (14, 15). Repeated acute episodes of ADL affects regional lymphatics followed by peripheral lymphatic sclerosis eventually towards occlusion was considered hallmark of the pathogenesis of filarial lymphedema (16). During course of the carrier stage the occurrence of adenolymphangitis associated with filarial fever is observed possibly due to host humoral response (17). These adenolymphangitis ADL episodes had a great economic impact on patients (18). Subsequently, Dreyer *et al* demonstrated Acute Dermatol-lymphangio Adenitis (ADLA) which is one of the two forms lymphangitis caused by secondary bacterial infections particularly among lymphedema patients (19) and possibly leads to progression of lymphedema to elephantiasis. Further, it was postulated that impaired lymph drainage and lack of elimination of penetrating bacteria are responsible factors for progression of lymphedema and attacks of ADLA. The broken skin of affected limb and lymph stasis encourages the bacterial growth sustaining the infection causing inflammation. The commonest organism isolated in recurrent ADLA cases is mostly streptococci that respond to penicillin and other common antibiotics.

Recent understanding on the pathogenesis of disease and its progression emerge from discoveries in identification of Wolbachia which are endosymbiotic bacteria present in these cases. These bacteria not only influence development and metabolism of parasite but also responsible for many aspect of pathogenesis of disease. The pro-inflammatory effects of cytokines released by bacteria are largely leads to post treatment reaction such as fever and more importantly for development of chronic pathology. This observations lead to another pathway of treatment of filarial infection by using tetracycline for Wolbachia. With tetracycline treatment in microfilaraemic subjects, the microfilaria (mf) cleared rapidly as opposed to those who did not receive tetracycline.

Recent Advances in Pathology

Few studies have brought more clarity to get better understanding of its pathogenesis and pathology. It is now well documented that in filarial endemic areas most of the children are infected early in life while clinical manifestation

occur during late adolescence stage or in adult. We also now recognize that lymphatic pathology in adults results from variety of factors that act on damaged lymphatics. Our recent study (20) on *W.bancrofti* infected asymptomatic young endemic children between five to eighteen years documented occurrence of subclinical lymphatic pathology shown by lymphoscintigraphy of both lower limbs.

Majority (>70%) *W.bancrofti* infected but asymptomatic children had demonstrable suclinical lymphatic pathology in their lower limbs with lymph flow observation features. All these children were treated with single dose DEC Plus Albendazole (dose as per National Programme) either annually or biannually after randomizing the group. Results clearly indicated either reversal or improvement of clinical pathology following use of single dose treatment (DEC+Albendazole) in 2 years in more than 80% children.

Post treatment reversibility and change in foot circumference was also documented in symptomatic children with lymphedema. This finding has important implication to act as a strong advocacy tool for LF programme. Finally, above study has shown that MDA is not only for interruption of transmission, but also serve as an effective tool for disability prevention and reduction of morbidity like early lymphedema in children.

Clinical Features: Cause of Disability

Once the filarial infection is established, the clinical course of the disease passes through various stages and time period may progress to end-up with chronic stage that causes serious disability. Although the ADL episodes accompanying mild edema distally in limb resolve in 3-7 days, these often tend to recur, where edema becomes persistent and non pitting with stasis of lymphatic fluid at interstitial tissue spaces in edematous site in limb. With recurrence of adenolymphagitis, edema progress to full form of irreversible lymphedema due to gross changes in lymphatic architecture, lymph dysfunction and lymph stasis. The affected area usually in extremities or sometime in scrotum becomes potential sites of inflammation in presence of precipitating factor.

Once lymphatic drainage to the limb or scrotum in male get obstructed in late stage of lymphedema, the affected limb or scrotum favor entry of bacteria, causing acute inflammation of the affected skin, draining lymph vessels and regional lymphnode, called ADLA. In unhygienic situation, the organism gain entry through inter digital spaces site of injury, eczema or cracks in the feet. Higher grades of lymphedema sometimes affected by fungal infection aggravated in rainy season, when feet are soaked with water. Repeated attacks of ADLA further damage the lymph drainage and lymphedema progress to elephantiasis, during attack of ADLA, the limb/scrotum becomes extremely painful, warm, red, swollen, and tender with systematic reaction like higher fever.

A paradigm shift is reflected in our knowledge as to how the attack of ADL or ADLA occurs in Filariasis. While the early asymptomatic mf carrier stage, exhibits ADL due to periodic release of toxic material from parasite. Late stages of lymphedema clearly established the role of secondary bacterial infection that invades the already damaged lymphatics. Above, observation paved way to the concept of current management of ADLA associated with LF.

Advancements in the Diagnosis of LF

Newer diagnosis have not only made the process convenient during field collection and processing of blood specimens in daytime, but also yielded most accurate assessment of detection of filarial infection by antigen detection test (ICT) and mapping of the disease to identify endemic areas. The OG4C3 antigen test assay allowed testing of large numbers of specimens in laboratory. To detect brugia infection, brugia rapid assay is used based on the detection of antibody. Use of color droppler ultrasonography, the adult parasite can be

detected in non-invasive manner from hidden locations, usually in axilla, inguinal region or afferent lymph channels to draining lymphnode. The array of lymphatic architecture and lymph flow pattern can be studied through lymphoscintigraphy that made a paradigm shift to our knowledge.

Newer Pharmacotherapeutics for LF Elimination

Dramatic change in concept has been witnessed in last decade that added knowledge on therapeutics from 12 days individual treatment to a single dose MDA to achieve elimination of LF. This has paved the way to formulate a global program for elimination of Filariasis. DEC has been the drug of choice and was given for 12 days for treatment of Filariasis to suppress mf for long period in a slow but sustained manner. Studies clearly demonstrated that single dose of DEC can achieve mf suppression equally as that of 12 days regimen. The observation of effect of 6mg/kg dose of DEC given in single dose followed the study that evaluated invermectin when administered in single dose and compared with DEC as a powerful microfilariacide (21). This useful observation had paved way for genesis of MDA strategy.

A community based trial undertaken to evaluate effectiveness and side reaction score of alternative doses of DEC used as MDA with low doses 100 mg, 200 mg or 300 mg dose given uniformly in all age groups in 3 different matched population groups. The result revealed that low dose of DEC (100 mg) is comparable to enhanced doses of DEC in of mf suppression with significantly less side reaction. It can be believed that this finding will have important impact on improving compliance, ease of drug delivery and in decreasing drug requirement if low dose DEC (100 mg) is used to all age groups above 2 years. Currently programme uses 300 mg of DEC Plus Albendazole 400 mg given annually. Further the study addressing doubling the dose of Albendazole to 800 mg given bianually has significantly prolonged mf suppression along with reduction of mf density in 2 year study (22). Beside this regimen has exhibited clearance of the adult parasite as well as antigenemia.

The recent findings on trials with Doxycycline given in doses of 200 mg daily for 6 weeks has shown promise as powerful filariasis effect with its anti-inflammatory action which are independent of anti-filarial activity and can reduce lymphedema. Some countries have also attempted to use DEC fortified salt. Summarizing these results it is now clear that there are several options open for use as drug regimen for MDA to achieve better compliance, effective mf and adult worm clearance and the possibility of reducing the number of rounds the MDA programme has to continue to achieve desired result.

Management of ADLA

With better understanding of pathogenesis of acute attack of ADLA, it is now possible to formulate simple strategies for management of simple acute attack (19). ADLA can be easily treated and further attacks are prevented. Bed rest and paracetamol are enough in mild cases for treatment. Local precipitating factors to be assessed and treated appropriately like injury and associated fungal or bacterial infection, Moderate to severe ADLA may require oral or parenteral antibiotic along with analgesics or antipyretics drug. Community awareness about ADLA and its prevention on has been initiated in many endemic areas to curtail the episode and improve quality of life.

Management of Lymphedema

With new knowledge on pathogenesis of LF, the management of filarial lymphedema now relies on limb washing, foot care, physiotherapy and precautions to prevent acute attack.

Current Programme for Elimination of LF

New insights on the disease, the parasite diagnostic tools and available chemotherapy

options have been used to initiate Global programme for elimination of LF, in year 2000. The programme was launched first to eliminate LF as a public health problem by 2020. Besides the two pillars of GPELF the vector control and integrated vector management play supplementary role are already illustrated. MDA strategy is the basis of interruption of transmission is based on the earlier studies that can suppress mf for long period, thereby annual single dose of MDA given in 5 to 6 rounds can reduce mf level to a point where no new infection can occur. The addition of Albendazole to either DEC or Ivermectin enhances the effect of long term mf suppression that forms the basis for MDA.

For future, based on recent ongoing clinical trials under taken in the globe including India indicates that triple drug therapy, i.e. annual single dose of MDA consisting of DEC, Albendazole and Ivermectin can not only achieve prolonged mf suppression but also sustain the effect for long period, with clearance of adult parasite, Thus this provides opportunity of reducing the number of rounds to achieve interruption of transmission. This regimen can be applied to areas where either MDA is not achieved yet or areas where it is not initiated.

These new chemotherapy trials can facilitate effective MDA application since these can be distributed by community health volunteers and can be given once a year. The other advantage is that these drugs are already in clinical practice and now management of side effects can be easily carried out.

The filarial elimination programme has several potential benefits apart from addressing filarial elimination. Since these drugs are very effective in treatment of soil transmitted helminthes, the benefits like decrease in intestinal helminthes burden and consequent decrease in anemia and increase in nutrition & growth can be achieved. Additional benefits of treatment of scabies by Ivermectin (21) together that constitute what is called –benefits beyond filariasis effect.

The Indian programme to eliminate LF is considered as largest such programme in the world. It requires the massive distribution of more than one billion DEC and Alb dosage every year. Nearly 2.5 million workers are required every year for their distribution. Thus it is often said the success of global programme will be more pronounced as per success of Indian programme.

Current Global LFE Programme

Global programme for elimination of lymphatic filariasis (GPELF) was launched in year 2000. MDA scaled up dramatically globally after launch of GPELF and this considered is one of the most rapidly expanding global health programme in history of public health. During 1st 10 years after it is launched, MDA increased from 3 million in 12 countries in year 2000 to 466 million 53 countries in 2010. Between 2000 to 2014 cumulative total of 5.62 million treatments were delivered to over 1 billion people at least once. Out of 73 endemic countries, all implementation units (IU) of 23 countries could not start MDA, but 21 had achieved 100% coverage, and 18 states transitioned to post MDA surveillance and no longer require MDA, Effective water, sanitation and hygiene (WASH) campaigns, environmental sanitation and house construction significantly eliminated filariasis in china and Korea. Alternative treatment strategies and intervention approaches like MDA at high coverage (100% coverage) twice yearly treatment, different combination of drug (like Annual single dose combination MDA regimen (DEC+Albenazole+Ivermectin) and supplementary vector control measure could accelerate the interruption of transmission.

WHO predicted that MDA with DEC+Albendazole will require coverage of 70% or more in transmission zones to achieve LFE by 2020? Addition of Ivermectin to existing MDA regimen may improve mf clearance and provide a long lasting effect and require less rounds of MDA. Yet another new therapeutic option has been shown that 6 week course of

Doxycycline 200 mg daily dose for six weeks may help in reverting or halting the early stage lymphedema, regardless of the presence of an active infection.

While the first part of GPELF goal (MDA) for interruption transmission is mostly realized, through sequential mapping, MDA, post MDA surveillance and verification in many endemic areas, efforts are on the way to analyze country situation on MMDP, developing plan and providing access to a minimum package of care to affected cases with chronic morbidities like lymphedema and hydrocele cases with 100% geographical coverage. Each endemic country has to pass the Transmission assessment surveys (TAS) undertaken by WHO in 3 phases in 4 years after stoppage of MDA for verification and certification by WHO Each country can identify the area where LFE can be integrated to prevention of other conditions of NTDS soil transmitted helminthes, leprosy, diabetes foot care and malaria to make it more robust & economical.

Elimination of LF as a public health problem: as desired is defined as reduction in measurable prevalence of infection in endemic areas below a target threshold (<1% mf & Ag) at which further transmission is considered unlikely. TAS in 3 stages will assess above to ensure sustainability in 100% coverage implementation.

Now What does the Future Hold for LF?

Whether the global programme will be finally completely successful? We can envisage world free of LF, and its morbidities however several challenges have to be overcome and opportunity utilized to achieve ultimate goal. All over the tropical world several NTDS occur in the same endemic area, e.g. in India there are areas with at least 3 NTDS are present. LF, STH and Visceral Leishmaniasis (VL) in parts of India or more diseases like Hansen's disease may co-exist in the same region. Treating the diseases where they co-exist remains a challenge in terms of chemotherapeutic approaches, drug delivery and monitoring and evaluation. However, source of the beyond filariasis effect of the drug such as IVM and Alb as listed holds promise for integrated approaches for treatment. Similarly opportunity exists for tackling multiple diseases in area of morbidity management, vector control and preventive chemotherapy. Availability of several partnerships between the WHO, MoH, NGO and many other donors also provide important opportunities for developing integrated approach. However, several challenges for integration still remain. These include ensuring high compliance, management of drug interaction and identifying possible drug resistance.

Apart from integrated approach with existing wide network for drug distribution the success of elimination also depends on simultaneous social and economic development that includes availability of clean water and improved sanitation. All these efforts need to be strengthened with adequate monitoring and evaluation and strong IEC effort. Ultimately, despite the advances in various fields the success of the programme will depend on strong political and administrative support and heightened advocacy and social mobilization to ensure complete participation by the communities. When these occur we can promise that young citizens of our country and land will be free of filariasis.

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Telemedicine: An Era Yet to Flourish in India

Shilpa Sharma
Department of Pediatric Surgery,
All India Institute of Medical Sciences, New Delhi.

With the rapid expansion of internet facilities, there is already a growing tendency amongst the lay men and the patients to gather information about their symptoms, diagnosis, investigations, medicines, diseases and prognosis. Hence it is but natural that the health care professionals around the world have become very careful of the information they are providing online with each hospital trying to dissipate disease related information through its official websites. Advances in telecommunications technology has paved the way for telemedicine.

Telemedicine is described as a provision of remote clinical services, via real-time two-way communication between the patient and the healthcare provider, using electronic audio and visual means. It is a subset of telehealth that refers to wholesome concept of more general health services; a collection of means or methods for enhancing health care, public health, and health education delivery and support using telecommunications technology.

Today, phone calls, video calls, live video telemedicine, smart phones, computers and smart televisions with a network of internet access are available even in remote areas of the country. Telemedicine has already taken a place in people's lives. A simple phone call from a patient to seek a doctor's advice about a non emergency ailment is a form of telemedicine. Telemedicine usually employs a desktop computer, with a special video card. The computer stores data securely. High-speed telephone lines or satellite connections allow interaction between two sites or locations. On occasions, the patient is at the originating site, and receives the service via telecommunications service, with the mediation of a telepresenter.

Types of Telemedicine-based on Connections

1. Networked Programs

These use networked connections with high speed internet to link remote health clinics to larger health facilities like tertiary level hospitals.

2. Point-to-point Connections

These link small, understaffed remote health centers to specialists at one, large, central health facility via high speed internet. These are popular in the developed counres; telepsychiatry, teleradiology and emergency services.

3. Monitoring Center Links

These are used for remote patient monitoring. This link creates a digital connection between a patient's house and a remote monitoring facility, so that a patient's medical data can be measured at home and transmitted electronically to a distant medical monitoring facility. These links may be in the form of internet, short message service (SMS), or telephone connections. They are most commonly used for monitoring of pulmonary, cardiac, or fetal medical data. Electrocardiogram data is easily transferred using these links.

Correspondence: Dr. Shilpa Sharma, Associate Professor, Department of Pediatric Surgery, All India Institute of Medical Sciences, New Delhi. Email: drshilpas@gmail.com.

Types of Telemedicine-based on Data or Information Transferred

1. Store-and-forward Telemedicine Solutions

They are asynchronous meaning that the specialist, patient, and primary doctor does not need to be communicating at the same time. They enable the primary doctor to forward and share patient medical data like laboratory results, images, videos, records with a specialist at a different location in a sophisticated and secure way. The best use is in the field of teleradiology that allow technicians and healthcare professionals at smaller hospitals to share patient x-rays for diagnosis by a specialist at another location. Other fields include teledermatology, teleophthalmology and pathology.

2. Remote Patient Monitoring or Telemonitoring or Home Telehealth

These allow healthcare providers to track a patient's vital signs and other health data from a distance. They felicitate recognition of warning signs that need intervention. They are useful chronic diseases like diabetes; hypertension and also post operative follow- up.

3. Real-time Interactive Services or Real-time Telehealth

They are synchronous and involve a live audio-video interaction between either a health professional and patient, or between health professionals. It provides a virtual alternative to a physical visit with the use of a compatible device, internet connection, microphone, and webcam.

Telemedicine is a technology that may have both advantages and few disadvantages. Fortunately the advantages as given in Table 1 outnumber the disadvantages outlined in Table 2.

Few popular telemedicine specialties are as follows:

1. Teleradiology

This was one of the earliest specialities of telemedicine to begin way back in 1947 when images were transmitted via phone lines (1). It was developed to expand the access to read x-rays. Smaller hospitals that may not always have a radiologist on staff or all the time can send a patient's x-rays and records securely to a qualified radiologist at another location, and get a quick opinion.

2. Telepsychiatry

Qualified psychiatrists can provide treatment to patients remotely, expanding access to behavioral health services (2).

3. Teledermatology

These are usually store-and-forward

Table 1: Advantages of telemedicine

1.	Convenient to the patient; obviates physical visit to clinic or hospital.
2.	Cost-effective; saves time and money
3.	Follow-up of patients with chronic diseases such as diabetes, high cholesterol, or high blood pressure can be easily done.
4.	Advice can be sought for dose adjustment of medications, lifestyle regimens, prescription reordered or get an access to a support group.
5.	Help the doctor select urgent calls after clinic timings
6.	Easier for a busy physician to help out more patients

7.	Patients are using technology to monitor their vitals, blood sugar, blood pressure and hence would be more compliant in monitoring if they have to report their status to the physician.
8.	Especially useful for International patients to seek the best available expertise worldwide.
9.	Comfortable approach for those who feel daunted by medical professionals and hospitals.
10.	As the medical information is exchanged in strict confidence this can also encourage a good relationship to develop between patients and healthcare professionals.
11.	Telemedicine can be applied in places such as rural, remote, post disaster communities where there is no constant healthcare available or the necessary transport facilities.
12.	This can be an effective tool in health education by allowing the observation and supervision of newly-qualified healthcare professionals during clinical practice. Teaching files can also be made available in many forms, (e.g. Web casts of lectures, daily presentations of cases, educational conferences) whilst eliminating the necessity for travel.
13.	Elderly patient with mobility issues restraining to visit a doctor or hospital may be seen from home.
14.	One can get immediate access to a number of specialists.
15.	It can provide second opinions easily and faster thus making the patient and physician experience better.
16.	One can get access to renowned specialists in fields like rare cancers without restrictions of geographical location.
17.	Small remote hospitals without adequate radiology specialist on-staff can outsource evaluation of x-rays via telemedicine.
18.	It helps to increase patient engagement by allowing them to connect with their doctor more frequently, in a convenient way leading to a stronger doctor-patient relationship.
19.	Better quality patient care with improved health outcomes are provided.
20.	Telemedicine adds a dimension of clinical protection for users by eliminating the possibility of transmitting infectious diseases between healthcare professionals and patients.
21.	Telemedicine facilitates effective monitoring and treatment thereby reducing the number of unnecessary outpatient visits
22.	Computerised medical databases allow health professionals in primary care to access patient records in hospital databases. It also allows mobile collaboration between healthcare professionals from multiple locations when cases are particularly critical or might require multidisciplinary approach.
23.	The availability of patient records online has the potential to make patient prescriptions more reliable and accurate.

Table 2: Disadvantages of telemedicine

1.	Subtle unrecognized signs which the doctor may pick up on close clinical inspection; palpating or auscultation may be missed.
2.	Paying the due private doctors fees may be a problem
3.	It may be strenuous for a busy physician if the calls are not scheduled
4.	Requires some training and equipment purchasing
5.	May reduce in-person interactions with doctors
6.	Some telemedicine models may reduce care continuity

technologies that allow a local healthcare provider to send a patient picture of a rash or another skin lesion for remote diagnosis and get opinion whether further examination is needed from a dermatologist.

4. Teleophthalmology

These allow opthalmologists to examine the patients' eyes and also follow-up the effects of the treatment advice from a distance. These solutions are usually either live or store-and-forward telemedicine.

5. Telenephrology

These are most commonly used interprofessionally, when a family physician needs to consult a nephrologist about a patient with kidney disease. Patients with renal failure can also benefit from telemedicine by discussing the daily requirement for dialysis treatments on home dialysis with video link supervision.

6. Teleoncology

This is a rapidly growing field as more molecular tests and international consultations are being sought. These may use store-and-forward tools to forward images for diagnosis and also live video platforms to allow patient to consult with the oncologist.

7. Telepathlogy

These allow pathologists to share slides and findings at a distance for diagnosis, research, and education. Most telepathology tools are store-and-forward solutions, allowing pathologists to share and forward high-resolution images and videos.

8. Telerehabilitation

These allow medical professionals to deliver rehab services (such as physical therapy) remotely.

9. Telesurgery

A surgeon may follow up his patients during the post operative period and also later (3).

10. Telepediatric Surgery

These allow a pediatric surgeon to give opinion on patients in remote areas (4). A good surgical follow-up may also be maintained with good outcome and better patient –doctor satisfaction (5, 6).

11. Teleobstetrics and Telegynecology

Allows obstetricians to provide prenatal care to remote areas. Antenatal fetal ultrasound and heart may be monitored. A gynecologist might use a live telemedicine solution to provide birth control counselling.

12. Teleendocrinology

An endocrinologist may do live video chats with patients to discuss recent laboratory results and give advice.

13. Telepediatrics

These have been found useful to monitor cardiac care (7). They may be used to give information on vaccination to primary health centres.

14. Teleneuropsychology

Neuropsychological consultation and assessment can be done over the phone with patients for suspected cognitive disorders. Standard evaluation techniques are implemented to assess the patient via video technology.

15. Telenursing

This utilizes communicative technology to provide remote nursing services. Consultations can be made over the phone to reach a diagnosis and monitor health conditions and symptoms.

16. Telefamily Medicine

Can be used to monitor chronic health ailments. High-tech sensors, health and activity monitors, touch-screen technology and websites are used daily to record patient vitals, heart patterns, blood pressure and glucose levels. Readings are logged into personal health records, and alerts are sent wirelessly to health-care providers when readings fall beyond their normal range.

17. Telesomnology

It can be used to monitor nocturnal disorders like deprivation or sleep apnea. Via telemed devices, one can monitor both investigatory and direct treatment. The communicative nature of this technology can provide reports on sleep patterns, body positions and breathing to polish data and metrics and refine treatment courses for patients.

18. Telerobotic Surgery

Robotic surgery has been made possible by the tele-systems; Da Vinci (Intuitive), ZEUS (Computer Motion) and AESOP (Automated endoscope system for optimal positioning) (8). Robotic procedures have been shown to be quicker, have reduced complications and a shorter hospital stay than either the open or laparoscopic techniques while maintaining good cancer control in cancer of the prostate (9).

Telemedicine Clinical Guidelines

The American Telemedicine Association has put together comprehensive guidelines for a range of specialties based on a survey of over 600 studies, for professionals using telemedicine in primary and urgent care (10). They have recommended some basic protocols and rules when starting a telemedicine program are as follows:-

1. Set-up the right space for telemedicine visits
2. Create a contingency plan for emergencies and referrals
3. Patient Management and Evaluation
4. Quality Assurance
5. Billing
6. In general, follow the same standards as in-person medical services

To conclude, though face-to-face doctor patient visits are valuable and necessary in many circumstances, with growing technology the importance of supplementary telemedicine cannot be undermined. There is tremendous scope and optimism in the use of telemedicine worldwide that is becoming easier and more widely accepted.

The global telemedicine market is worth in billions. This technology has paved the way for innovations like smart Google Glasses and smart watches that can monitor patients' health data and transmit them in real time to health professionals. To keep up with this pace of progress in technology telemedicine will need to overcome all cost issues, administrative barriers, and pave the way for better primary health care in rural India in times to come.

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