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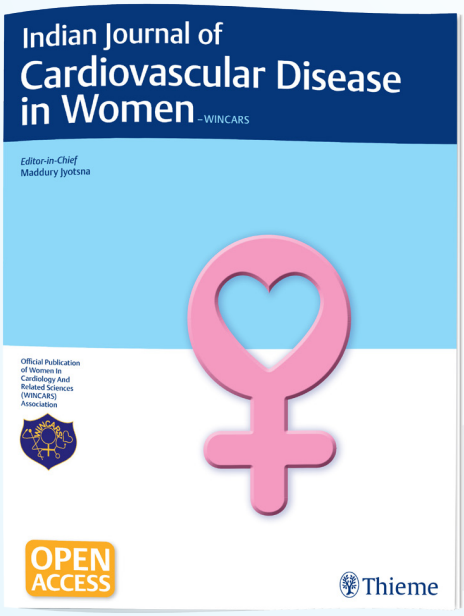

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Editorial

Quality Improvement in Medical Education

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Sanjeev Misra

During our medical school days, one of the professors used to ask us to keep a notebook labeled as *Jigyasa Diary* (Curiosity Diary), and one had to note down any question that came to our mind. Questions could be related to any field. It was not mandatory but optional. This strategy worked, and when few students started maintaining and posing questions, the others followed the suit. Such an exercise leads to the development of a curious mind. This strategy by our medical teacher led us to believe that if offered a choice, it can inculcate a habit of lifelong learning among students. This is called change management or quality improvement (QI) in the teaching process.

Medical education globally has come to an age and is still developing driven by the changing needs of the society and societal expectations. The ultimate aim of medical education is to develop health resources, which, in turn, help develop a healthy society. Toward this end, medical institutions have been entrusted to prepare and nurture the physicians of tomorrow. They are also entrusted with the responsibility of developing and sustaining a habit of continuous professional development among health care workers. To promote this development, institutions, societies, and associations organize continuing medical education activities. It is a well-known fact that the impact of education on behavioral changes is difficult to assess in a short term as the outcomes are visible much later. In fact, medical education has become a process, has evolved much more with time, and still offers opportunities for positive change.

The Medical Council of India (MCI) was established in 1934 under the Indian Medical Council Act, 1933, with the main function of establishing uniform standards of higher qualifications in medicine and recognizing medical qualifications in India and abroad. The apex body was mandated for the promotion and maintenance of excellence in medical education. Through a lot of deliberations and discussions among the stakeholders and experts during the past 6 years, the MCI has recently introduced "Competency-based Medical Education Curriculum" throughout India from July 2019 session with an emphasis on skill and proficiency development aligned with the need of the society. The National Medical Commission Bill was introduced in 2019 and seeks to repeal the Indian Medical Council Act, 1956. It will provide for a medical education system that ensures (1) availability of adequate and high-quality medical professionals, (2) adoption of the latest medical research by medical professionals, (3) periodic assessment of medical institutions, and (4) an effective grievance redressal mechanism.

It is amply clear that there is a need for continuous QI in medical education. When we talk of QI in medical education, it can have two connotations: (1) to have QI curriculum in medical training for both undergraduates and postgraduates and (2) to improve the process of medical education through the principles of QI. There is lot of debate on the first one: whether we should introduce QI in UG training so early? Although the evidences are in favor, understanding QI may be complex for undergraduates. What I am referring to QI in medical education is teaching and learning processes.

Let us define QI in health care. There are many definitions but most refer to it as "systematic and continuous actions that lead to measurable improvement in health care services and the health status of targeted patient groups." The Institute for Healthcare Improvement (IHI) says: "the science of improvement is an applied science that emphasizes innovation, rapid-cycle testing in the field, and spread in order to generate learning about what changes, in which context(s), produce improvement(s)." IHI further states that "It is characterized by the combination of expert subject knowledge with improvement methods and tools." It is multidisciplinary—drawing on clinical science, systems theory, psychology, statistics, and other fields.[1] QI also involves developing theories for change, testing them, measuring their impact with data that is collected in "real time," and refining theories of change using an iterative trial and learning methodology, for example, PDSA (Plan, Do, Study, Act) cycles.[2] Moreover, QI differs from audit as it does not need to be based on a standard and may even be based on a hunch or intuition. However, it does require a lot of training.

Used earlier in agriculture and manufacturing environments that are built on processes, many strategies and tools

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for QI are in existence now, and any one of them can be used in health care and medical education. The basic tenets of QI are based on following principles: (1) adoption by institutes through hands-on projects, (2) defining quality and have consensus, (3) measure improvement, (4) use QI tools and PDSA cycles, and (5) learn from variation in data (control charts, run charts, pareto charts, etc.).

In medical education, there are multiple processes, such as institutional policies, classroom environment, social diversity of the class, the facilitator qualities, teaching methods, use of the type of teaching media, assessment method, assessment environment, and various psychological factors facilitating learning. It is not necessary to change everything drastically in one go. It is recommended, as per the QI principles, to start small and make big difference.

Curriculum for undergraduation is being implemented in all medical colleges in India from July 2019 coupled with faculty development programs. At the same time, reasonable flexibility is maintained since the MBBS program is still time-bound. Even then, there is scope for QI in our curriculum and its delivery.

The University of Vermont proposed a new approach in moving away from didactic lectures.[3] The concept of flipped classroom is rapidly expanding to medical education and is partly replacing traditional lectures. The most important cited reason is that it offers increasing student engagement, whereby basic and fundamental information is provided to the students before the class, and the class time is then used for deep learning and application of knowledge.[4] When introducing the flipped classroom concept, many challenges may be encountered. Content distributed before class may not be studied or viewed. Using principles of QI, e-mail reminders, SMS, and coaxing may be used and their impact can be seen. These small changes will make flipped classroom more rewarding and also provide better impact on student learning. These same QI change management can be shared with faculty members as best practices to improve learning.

The same concept can be used for student attendance, which can be a problem with the advent of technology. If we perform root cause analysis, many factors may emerge. Any one or more may be chosen for the QI process keeping in mind what we are trying to accomplish, how we will know that change is improvement, and to use the PDSA cycle to ascertain what changes we make that results in improvement.

For change management to be successful, stakeholders' involvement and their engagement is very crucial. The Head of Institutions, Principals, Deans, and Heads of the Departments and medical education unit teams assume importance as their involvement augment the process.

Richman et al have demonstrated that the systematic process used by their team to collect data from students and faculty helped facilitate QI in a key course in phase 1 of their Learning-focused, Experiential, Adaptive, Rigorous and Novel (LEARN) curriculum introduced in 2015.

They have shown that a structured QI process can enable the faculty to raise the level of student satisfaction and course grades by better integrating basic and clinical sciences, engaging course faculty in closer collaboration, and improving assessments.[5]

In India, a highly dedicated working group is mentoring health care professionals and promoting QI on a large scale in health care.[6] Efforts ranging from simple measures to improve newborn care, waiting time in emergency and designing learning platform for QI training, the group is proactively involved in innovations and capacity building with support from various national and international agencies.

We hope that using QI principles in medical education, small changes will yield high dividends and will catalyze our health care system toward optimal performance.

Conflict of Interest
None declared.

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Cutaneous Anthrax—Still a Reality in India

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Abstract

Anthrax, a toxigenic zoonosis, incidentally affecting humans has become rare but endemic outbreaks still continue to occur in tropical countries like India, parts of South America, and Europe where veterinary control of livestock is marginal and environmental conditions favor an animal–soil–animal cycle. India, with its largest population of livestock in the world, continues to have anthrax outbreaks with highest incidence reported from south, and the authors have reported an outbreak of 23 cases from 1998 to 2001 from south India. Children outnumbered adults and most of them had lesions on the exposed sites. However, there is a limited documentation of anthrax outbreaks from India warranting the need for sensitizing and creating awareness among health care professionals to identify and report these cases at the earliest so that appropriate actions are taken. Anthrax continues to retain a certain fascination and notoriety because of the potential for use of the bacillus spores in biologic warfare.

Keywords

- chemical extract of anthrax
- *Bacillus anthracis*
- inhalational anthrax
- bioterrorism
- injectional

Introduction

Anthrax occupies an important place in the history of infectious diseases, as it is the first human disease to be attributed to a specific pathogen.[1,2] It is a zoonotic infection with *Bacillus anthracis*. The name anthrax comes from a Greek word for "coal," a reference to black eschar that is eventually formed in cutaneous anthrax.

Human anthrax is of four clinical forms depending on the route of transmission: cutaneous (the most common form accounting for nearly 95% of all anthrax cases), inhalational, ingestion form, and injectional form.[3]

History

In 1876, Robert Koch (1843–1910) reproducibly transmitted anthrax to mice by inoculating them with blood from the cattle and subsequently, he then recovered the same rod-like bacteria from the sick mice as came from the cattle. He could pass the disease from one mouse to another by inoculating them with these pathogens. Based on his experiments, he proposed the "Henle–Koch postulates" for proof that a microorganism was the cause of an infectious disease.[4]

Robert Koch's landmark work on anthrax helped to establish the germ theory of disease, and an 1877 report on *B. anthracis* included the first published photomicrographs of any bacteria.[3]

B. anthracis is a large (1–5 micron), spore forming aerobic, or facultative anaerobic gram-positive rod, usually arranged in a box-car pattern.[1,5] When cultured on 5% sheep blood agar, 2 to 5 mm colonies have ground glass appearance with nonhemolytic, tenacious colonies (beaten egg-white appearance) after 15 to 24 hours. On India-ink staining, poly-D glutamic acid capsule can be visualized. On methylene blue staining, the bacterial cell is stained blue, whereas the surrounding capsule is pink. This is described as the M'Fadyean's reaction. Koch's postulates were proved on this organism for the first time.[5]

Epidemiology

Being a zoonotic infection anthrax is mainly linked to goat, sheep, cattle, antelope, kudu, pigs, horses, zebu, and other animals.[6] Animals are infected by ingesting the spores from contaminated soil, feed, or even meat (in case of wild animals). It is transmitted to humans by meat, wool, hides, bones, and hairs and rarely from person to person.

Infection in animals commonly occurs due to ingestion of spores or inoculation into abraded perioral areas while grazing in contaminated area or eating contaminated feed

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or meat. Subsequent death of the animal causes recontamination of the environment.[6,7] After such multiple events, the area might become endemic. Other modes of spread in animals are fomites, by insects or legs of vultures.[6,7]

Anthrax should be considered as possible cause of death in herbivorous animals that have died suddenly and unexpectedly, especially if hemorrhage from the nose, mouth, or anus has occurred.[3] Failure of the blood to clot, the absence of rigor mortis, and the presence of splenomegaly are the most significant necropsy findings in such animals.[6,7]

The disease has a global distribution but incidence in livestock and humans varies with local ecology, implementation of control strategies, and sociocultural practices that determine spillover from animals to humans. Although most developed countries report few sporadic cases in livestock and humans, the disease is still enzootic in parts of Africa (e.g., Zimbabwe and Chad), the Middle East, Central Asia, China, Pakistan, Bhutan, Bangladesh, and India.[1,2,6-15]

The geographic distribution of anthrax is associated with certain ecological factors. In some ecosystems, outbreaks occur late in the hot–dry season, whereas in others, outbreaks are associated with the end of heavy rains, suggesting that weather extremes may be an important trigger of outbreaks.[6]

One of the largest outbreaks of anthrax occurred in Zimbabwe during 1979 to 1985 where approximately 10,000 cases of cutaneous anthrax were reported. Inhalational anthrax historically referred as "wool-sorters" disease because it occurred in industrial settings where spore contaminated wool or animal hides are handled.[5,16]

Anthrax is also mentioned prominently as potential agent of biowarfare and bioterrorism. In the 1950s and 1960s, both the United States and former Soviet Union developed anthrax as a biological weapon, as have other countries.[5]

An epidemic of inhalation anthrax occurred among persons living in Sverdlovsk, Union of Soviet Socialist Republics, in April and May 1979 resulting in at least 96 cases and 66 deaths.[17-19] This outbreak also affected cattle within the city. It was concluded that this largest outbreak of human inhalation anthrax was due to an infectious aerosol emanating from the military facility. This outbreak raised considerable concern among scientists and policymakers about the potential for the use of aerosolized *B. anthracis* spores as an agent of biological terrorism. Indeed, these fears were confirmed in 2001 when an outbreak of 22 cases of anthrax (11 inhalational and 11 cutaneous) occurred in the United States from intentional contamination of the U.S. mail delivered to several persons by the U.S. Postal Service. Five deaths and 22 cases of anthrax occurred.[17-19]

Since 2009, anthrax has emerged among heroin users in Europe, presenting a novel clinical manifestation, "injectional anthrax," which has been attributed to contaminated heroin distributed throughout Europe; before 2009, only one case was reported.[20,21] During 2012 and 2013, new cases of injectional anthrax were diagnosed in Denmark, France, Germany, and the United Kingdom. Overall 70 confirmed cases were reported, with 26 fatalities (37% case fatality rate). The latest two confirmed cases occurred in March 2013.[20,21]

Anthrax in India

A few sporadic cases and endemic outbreaks have been reported from India.[1,2] During the last two decades, 70 cases of human anthrax were encountered at the Christian Medical College at Vellore in Tamil Nadu of which 26 cases had cutaneous anthrax. A review of Indian literature in 1996 found 112 cases of anthrax (71 cutaneous anthrax cases) in places, other than Vellore. The authors have recorded 23 cases of cutaneous anthrax over a period of 3 years with a single mortality due to septicaemia.[1,2] Recently, outbreaks of cutaneous anthrax have been recorded from Andhra Pradesh (in the year 2005, 2009, and 2012), and West Bengal (2009).[22-25] These data confirm the endemicity of anthrax, besides in the state of West Bengal, in other three southern states of India, that is, Andhra Pradesh, Karnataka, and Tamil Nadu.

Life Cycle of *Bacillus anthracis*

►**Table 1** depicts the life cycle of *B.anthracis*.[1,20,21]

Pathogenesis

Cutaneous inoculation occurs at the site of minor trauma, insect bite or preexisting skin lesions whereas inhalational (pulmonary or Woolsorter's disease) and intestinal forms result from inhalation or ingestion of spores.[1,3,5]

Major virulence factors in *B. anthracis* known for inflammatory response with hemorrhage and necrosis and gelatinous edema in the tissue are as follows:

1. Edema factor (EF).
2. Lethal factor (LF).
3. Antiphagocytic poly-D glutamic acid capsule.

EF and LF bind with protective antigen (PA) to form EF-PA binary toxin and LF-PA binary toxin. EF causes water and calcium dysregulation resulting into characteristic edema

Table 1 Cycle of *Bacillus anthracis*

Soil cycle	Anhrax spores in soil/ vegetation	Multiplications (soil contamination) spread to the herbivorous animals
Animal cycle (primarily herbivorous)	Animal anthrax	The infected animals die and contaminate the soil and other water resources
Human cycle	Direct contact with the animals	Clinical anthrax (cutaneous, pharyngeal, and intestinal)
	Indirect contact with the contaminated animal products, such as hair, hides, bones (Industrial contact)	Clinical anthrax (cutaneous, inhalation)
Insect cycle	Insects play role in the transmission of *B. anthracis* to humans (cutaneous anthrax) and domestic animals	
Injection cycle	Contaminated heroin injections in Europe (due to use of animal skin for smuggling)	

of anthrax. It also impairs function of polymorphonuclear leucocytes. LF interferes with normal T-cell function causing excessive production of cytokines and dysregulation of cytokine network leading to cytokine storm resulting in multiorgan failure, shock, and death. It is also responsible for bleeding diathesis.[1,3,5]

Clinical Features

The lesion of cutaneous anthrax follows the introduction of endospores into the skin. Common sites involved are exposed parts of the body.[1,3,5,8,10] All ages and both genders may be affected. In rural settings, children minding cattle may be affected.[26-33] Generally, cutaneous anthrax presents with single lesion but they may be multiple. Fatalities in cutaneous anthrax are mainly due to obstruction of the airways by the edema that accompanies lesions that form on the face or neck but can also occur when cutaneous disease progresses to systemic infection.[3]

Clinical features of various types of anthrax are tabulated in the ►**Table 2.**[1,3,5,20,21]

Table 2 Clinical types, diagnostic features, and prognosis of anthrax

Type	Source of infection	Incubation period	Clinical features	Laboratory diagnosis	Prognosis
Cutaneous anthrax	1. Inoculation into abraded skin during skinning and butchering of infected animals 2. Insect bites	1–12 days	The initial skin lesion is papule develops into vesicle which rupture to produce necrotic ulcer surrounded by smaller peripheral vesicles Later central black eschar form and heal with scarring in 1–2 weeks Distinctive features are as follows: 1. Lesion is painless 2. Edema out of proportion 3. Lack of neutrophilic response 4. Regional lymphadenopathy	Smear from edge of eschar or vesicular fluid for • Gram stain • PCR Skin biopsy (full thickness punch biopsy from papule, vesicle, or eschar). Blood culture Serodiagnosis (when culture fails owing to the previous treatment) Guinea pig or mouse inoculation	Sepsis is rare and mortality is less than 1% with adequate antibiotic therapy
Inhalational anthrax	1. Contaminated wools and hides 2. Bioterrorism	2–43 days	Flu like symptoms and nonproductive cough followed by respiratory distress and respiratory failure	Chest radiograph/ CT scan: mediastinal widening due to hemorrhagic lymphadenopathy, hemorrhagic pleural effusion, infiltrate or consolidation Blood culture Serodiagnosis	
Gastro-intestinal anthrax	1. Consumption of raw or under-cooked meat	2–144 hours	Oropharyngeal: severe sore-throat, swelling of neck, regional lymphadenopathy, dyspnea and fever Intestinal: present with hemorrhagic gastro-enteritis which may lead to obstruction, or perforation. It is due to hemorrhagic ulceration which appear in mucosa of terminal ileum or cecum	Gram staining in infected fluids or blood Blood culture Serodiagnosis Guinea pigs or mouse inoculation	Case fatality is very high
Injection anthrax	Contaminated heroin injections due to use of animal skin for smuggling		Serious soft tissue infection with significant edema	Tissue biopsy Blood culture Serodiagnosis	Progression to septic shock can be rapid
Anthrax meningo-encephalitis	Usually associated with inhalational and GI anthrax, rarely with cutaneous		• Cerebral edema • Parenchymal brain hemorrhage • Vasculitis • Subarachnoid hemorrhage	CSF and blood culture Gram staining	Nearly always fatal

Abbreviations: CSF, cerebrospinal fluid; CT, computed tomography; GI, gastrointestinal; PCR, polymerase chain reaction.

Differential Diagnosis

Cutaneous anthrax needs to be differentiated from vaccinia, Milker's nodule, orf (ecthyma contagiosum), and furuncle. Painless eschar, with profound edema and lack of neutrophilic response gives clue to the diagnosis of cutaneous anthrax supplemented by Gram's stained smear and culture of the *B.anthracis*.[1,3,5] The anthraxin skin test, consisting of subdermal injection of a commercially produced chemical extract of an attenuated strain of *B.anthracis*, is now available for the diagnosis of acute and previous cases of anthrax.[1]

Treatment

The most commonly used antibiotics are mentioned below:

- Ciprofloxacin.
- Erythromycin.
- Tetracycline/doxycycline.
- Chloramphenicol.

In case of extensive edema, meningitis, or swelling in the head and neck region, corticosteroid may be given. One or more additional antimicrobials (rifampicin, vancomycin, ampicillin, imipenem, clindamycin, or clarithromycin) are required in cases of inhalational or gastrointestinal anthrax.[1,3,5,32,33]

The treatment may be modified in the light of drug sensitivity pattern, once these are available. Surgical interventions are not beneficial as it can exacerbate the injury.

Two types of anthrax toxin antibodies, anthrax immune globulin and humanized monoclonal antibody can be given as adjunctive therapy.[5]

Prevention

1. Control of animal anthrax.[5,32,33]
2. Use of proper sterilization techniques in industrial settings dealing with animal products like hides and wools.
3. Immunization in high-risk population with anthrax vaccine adsorbed (AVA).
4. In suspected event of bioterrorism event, exposed individuals should take preexposure prophylaxis consisting of 60 days of antibiotic (ciprofloxacin or doxycycline) with or without AVA.[5,32,33]

Note

The author was selected for Dr. R.V. Rajam Oration for the year 2018–2019.

Conflict of Interest

None declared.

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Restorative Therapies after Stroke: Drugs, Devices, and Robotics

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Abstract

Restorative therapies aim to improve outcome by salvaging threatened brain, as with reperfusion or neuroprotective drugs and also by promoting plasticity within surviving neural tissue. Restorative therapies typically have a therapeutic time window measured in days and weeks and so have the potential to be assessed by a large fraction of patients with a new stroke. Examples of such brain repair therapies include growth factors, cell-based therapies, and devices. Positive clinical trials have been reported in human studies for several classes of restorative therapy after stroke. These include robotics, constrain-induced movement therapy (CIMT), and pharmacological therapy, such as levodopa and selective serotonin reuptake inhibitors. In addition, several forms of noninvasive cortical stimulation, such as rapid transcranial magnetic stimulation, transcranial direct current stimulation, and theta-burst stimulation, have shown promise in early phase studies. The current review gives a glimpse of the existing strategies, those on the anvil of implementation and those with a hope of launch in near future.

Keywords
- stroke
- stem cell therapy
- vascular endothelial growth factor
- physiotherapy

Introduction

Increased understanding of pathogenesis and pathophysiology of stroke in the last few decades has paved way for path breaking advances in bettering stroke outcomes.[1]

The injury, repair, and recovery after stroke have been extensively defined.[2-5] The first epoch is related to acute injury and takes place in the first initial hours after stroke when changes in blood flow, edema, metabolism rate, and diaschisis occurs. A second epoch is related to repair which starts days after stroke and lasts for several weeks and is referred to as endogenous repair suggesting a golden period for initiating restorative therapies. A third epoch occurs weeks to months after stroke when spontaneous recovery gains have plateaued and this represents a stable but modifiable late phase.[6,7]

Unimodel targeting of key events in stroke pathophysiology has not been effective in providing long-term benefits, leading to negative results in previous clinical neuroprotective stroke trials.[8] A successful future stroke therapy needs to approach multiple pathophysiological mechanisms besides revascularization/reperfusion including thrombolytics related adverse side-effects, prevention of apoptosis (programmed cell death), stimulation of neuroregeneration, and neuronal plasticity.[8,9]

Review

Acute Reperfusion Therapies after Ischemic Stroke

Thrombolytic therapy is an inherently attractive treatment for acute ischemic stroke (AIS), based on the known pathologic and angiographic substrates of ischemic cerebrovascular disease.[10,11]

Emerging strategies include those that have the potential to extend cerebral reperfusion therapy beyond 4.5 hours of time window, as well as the means to bridge the "stroke recovery gap" (defined as the difference observed between the clinical response to thromboytic therapy in a given population of patients presenting with ischemic stroke and the potential clinical recovery if all of the penumbra were salvaged under ideal circumstances).[12] Approaches to this include the following: (1) intra-arterial pharmacological reperfusion

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approaches, combined intravenous–intra-arterial fibrinolysis, and combined fibrinolytics and glycoprotein IIb-IIIa agents[12-14]; (2) emerging endovascular mechanical reperfusion strategies including intra-arterial thrombectomy (clot retrieval devices and suction thrombectomy devices), mechanical disruption (microguide wire passage, laser photo acoustic emulsification, and primary intracranial angioplasty); (3) augmented fibrinolysis by endovascular ultrasound; (4) multimodal imaging with magnetic resonance (MRI) or computed tomography (CT) to rapidly assess the infarct core, penumbra, site of vessel occlusion, and tissue hemorrhagic propensity, enabling improved selection of patients for reperfusion therapy beyond any arbitrary fixed time window; (5) newer thrombolytic agents; (6) adjunctive therapies such as neuroprotectants.[12]

Endovascular treatment of acute ischemic stroke (AIS) is a therapy with a visible effect. With reperfusion, we know that in the right patient, our hemiplegic patients can walk out of hospital back into their lives. All the recent trials, for example, MR CLEAN, EXTEND-1A, SWIFT-PRIME, and REVASCAT, have all given unequivocal results in favor of endovascular intervention in selected patients. We have entered a new era of stroke therapy for major acute ischemic stroke.[15-17] Endovascular treatment has become a new standard of care for large vessel AIS. We will need to adapt triage rules and process and train new and existing personnel. We will need to assess the medical aspects of care including the thrombolytic agents in combination with endovascular thrombectomy, anesthesia use, adjuvant antithrombotic therapy, and medical management of blood pressure.[17,18] We need to properly identify the best imaging selection techniques because the association with outcome will not be confounded by the lack of reperfusion.

The single unifying theme will be speed. Onset to reperfusion time is the new bottom line process metric and we cannot compromise on this. This will remain the fundamental principle for AIS now and into the future.[18]

Stroke infrastructure must now adapt to endovascular therapy. As with intravenous (IV) recombinant tissue plasminogen activator (rTPA), only a small percentage of patients with stroke will require endovascular therapy (estimates are 10%), but this small percentage will drive the reorganization of systems of stroke care.[18]

The ultimate aim of any therapeutic strategy is maximum restoration possible and eventual return to normalcy of function. The nonregenerating aspect of an injured adult brain has been challenged in the recent past and neural plasticity documented in both global and focal models of animal ischemia.[6]

Cell Based Therapies

Biological basis for neurorestorative therapy poststroke are as follows:

- Neurorestoration poststroke is achieved by enhancing neurogenesis, angiogenesis, and oligodendrogenesis which in concert promote neurological recovery.[19]
- Neurogenesis, the generation of new parenchymal cells from neural stem cells (NSC), and progenitor cells stimulates plasticity.
- Oligodendrogenesis restores neuronal signal transduction and promote myelination.
- Angiogenesis and arteriogenesis increases cerebral blood flow perfusion and mediates the generation of important restorative trophic factors and proteases.[19]

Cell-based therapies under investigation include use of bone-marrow mesenchymal cells, cord blood cells, fetal cells, and embryonic cells. The common restorative characteristic of these therapies is that they target many types of parenchymal cells (including neural stem cells, cerebral endothelial cells, astrocytes, oligodendrocytes, and neurons), leading to enhancement of endogenous neurogenesis, angiogenesis, axonal sprouting, and synaptogenesis in ischemic brain tissue. These events collectively improve neurological function after stroke.[20]

Stroke poses special conditions that impact the potential success of transplantation to enhance neurological recovery. An infarct might involve the thalamus, hippocampus, and striate cortex affecting three or more very different neuronal populations. Besides, neurons, oligodendrcytes, astrocytes, and endothelial cells are also affected. Reconstitution of the complex and widespread neuronal-glial-endothelial networks is a herculean task to say the least.

There is uncertainity about the mechanisms by which cell transplantation might improve stroke deficits. Transplanted cells would ideally replace cells that are damaged by ischemia and take over function of these cellular elements. However, it is also possible that transplanted cells secrete trophic factors that help to maintain marginally surviving cells or otherwise enhance the local environment to improve function.[21-25]

How do Transplanted Cells Work?

In most cases of neural transplantation, it is likely that therapeutic effects of the implanted neurons or their precursors would be dependent upon their functional and structural integration into the brain tissue. However, the question is whether establishment of neural circuitry is the only means of improvement. It is likely that transplanted cells release neurotransmitters or neurotrophic/neuroprotective factors which counteract degeneration or promote regeneration. Even transplanted glial cells have been used to modify response to injury and assist in structural repair and promote remyelination. Studies using bone marrow stromal cells or umbilical cord blood cells as potential donors have shown functional improvement in behavioral recovery in animal models within days of transplantation. This raises issues whether recovery observed in such short periods is related to release of trophic factors rather than engraftment and differentiation of transplanted cells into mature neurons and/or glia.[26-29] The functional benefits after neural

transplantation are likely to be mediated by one of the following mechanisms[22]:

1. Neurotransmitters released from the graft tissue act on the afferent deprived limb of the postsynaptic receptors.
2. Release of the neurotrophic/growth factors (brain derived neurotrophic factor [BDNF], glial derived neurotrophic factor [GDNF], nerve growth factor [NGF], etc., acting as local pumps to support cell function and to prevent cascade of apoptosis. Regenerating neuronal population further prevents subsequent cell death.
3. Reestablishment of local interneuronal connections and synaptic connectivity between the host and graft.
4. Cell differentiation and integration.
5. Improvement of regional oxygen tension.
6. Limit glial reaction and prevent retrograde degeneration.

Possibly, the overall success of functional outcome is mediated by a combination of the above mentioned factors.

Cell Types and Sources

A range of different cell types under investigation for transplantation in experimental and clinical stroke trials are N Tera Neuron like cells (NT2N), autologous bone marrow derived stem cells (BMSC), human umbilical cord blood cells, NSC, and adipose tissue cells.[23,29]

Adult stem cell therapy for stroke can be divided in an endogenous and exogenous approach. The aim of the endogenous stem cell therapy is to exploit the population of adult stem cells already physiologically present either in the central nervous system (CNS) or hematopoetic system derived adult stem or precursor cells are administered locally or systemically after purification and propagation in culture.[23,3031]

Interestingly, acute cerebral ischemia in human individuals leads spontaneously to a three-fold increase in CD34+ cell count in the peripheral blood. Considering this change as an insufficient self-repair mechanism, it is a logical consequence to further promote CD34+ cell mobilization pharmacologically by the administration of granulocyte–colony stimulating factor (G-CSF). In addition, G-CSF has been described to exert neuroprotective effects following cerebral ischemia. A recent preclinical study found functional improvement in rats with focal G-CSF. There are ongoing clinical studies with G-CSF in acute ischemic stroke.[23,31,32]

Currently guidelines are being formulated to guide further research into the role of stem cell therapy in both translational and basic research areas.

Over the past decades, convincing evidence emerged that neurogenesis in the adult CNS is a continuous physiological process. Neurogenesis is present in two regions, the subventricular zone (SVZ) and the subgranular zone of the dentate gyrus. Additionally, recent studies also indicated the existence of NSCs in other regions of the CNS, namely, the striatum, spinal cord, and neocortex. External global stimulants, such as enriched environment, physical activity and stress, or application of defined molecules, such as fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor (VEGF), BDNF, and erythropoietin differentially modulate adult neurogenesis and have been tried in experimental models of stroke.[31]

Some of the major public sector tertiary care centers and institutes are presently conducting peer reviewed scientific studies on various aspects of stem cell therapy in stroke patients, as well as in animal models of stroke.

Another study recently completed under the aegis of department of science and technology of India (DST; Padma et al) found benefit with autologous bone marrow derived and expanded marrow stromal cells infusion given intravenously in patients with chronic stroke (3 months to 2 years after the event). The autologous bone marrow derived mononuclear cells and ex vivo culture expanded mesenchymal stem cells were found to be safe and feasible mode of treatment in chronic stroke in their study of 40 patients.[33-35] Combining physiotherapy with autologous stem cells (mono nuclear cells and meschenchymal stem cells) lead to clinical and functional improvements as assessed on functional MRI (fMRI) and diffusion tensor imaging (DTI) and the lasting effects of the same could be observed till 24 weeks. The administration of stem cells lead to cortical reorganization as evidenced by measurement of laterality index (LI), fractional anisotropy ratios (FA), and signal intensity change of the activated hemisphere and the fiber tract density and length on fMRI and DTI studies.[36]

Translation to the Clinic

The essential difference between neuroprotective and neurorestorative treatments is that the former treat the lesion and the latter, whether they are cell based or pharmacological therapies treat the intact tissue. The therapeutic time window and treatment protocols will thus be very different.[20] Restorative therapies are effective when initiated 1 month after stroke onset and cerebral perfusion is not problematic because the therapeutic target is cerebral tissue with normal perfusion. Restorative treatments are expected to reduce some of the impediments to the translation of laboratory proven therapies to patients. However, restorative treatments have their own sets of complicating factors. The treatments must be clearly proven to be safe in patients; this is particularly challenging for cell-based therapies. The interactions between restorative interventions and different environments, comorbidities, and rehabilitations strategies must be taken into account.[19,20,37]

Role of Growth Factors in Post Stroke Recovery

Neurotrophic Agents and Growth Factors

Basic FGF was shown to protect against excitatory amino acid toxicity in vitro, basic FGF chimeric peptide was highly effective in reducing infarct volume in a rodent model of permanent focal ischemia. FGF has been investigated in phase II/III trials. The results of the Clinical Safety Trial of Intravenous Basic FGF in Acute Stroke did not report any serious adverse events. The European–Australian phase II/III safety and efficacy trials were halted; no significant improvement was noted, although a trend toward treatment advantage was observed with the agent.[38]

Angiogenesis is the key feature of neuronal poststroke reorganization and stroke recovery. Brain ischemia itself induces angiogenesis through hypoxia inducible factor 1. A transcription factor that responds to the changing intracellular O2 concentration and induces erythropoietin expression. Angiogenesis is activated through release of polypeptide growth factors and cytokines and specific upregulation of the angiogenic factors involves transforming growth factor β, platelet derived growth factor, VEGF and basic FGF-2 in response to ischemia. VEGF is the postpotent hypoxia inducible angiogenic factor amongst all and is secreted by endothelial cells and pericytes. VEGF is upregulated by other growth factors within hours of stroke and has a strong influence on growth of new blood vessels in the injured areas of the brain. Its production constitutes adaptive response to hypoxia which promotes angiogenesis in poststroke events and eventually leads to functional recovery.[39,40]

Role of VEGF in Postischemic Stroke Recovery

Endogenous VEGF

In the ischemic brain, the macrophages, neurons, and glial cells appear to contain VEGF. Many cytokines and growth factors have been shown to modulate VEGF gene expression. Erythropoietin (EPO) plays an important role in angiogenesis through upregulation of VEGF/VEGF receptor system, both directly by enhancing neovascularization and indirectly by recruiting endothelial progenitor cells (EPCs).[38]

Exogenous VEGF

Hypoxia itself induces an increase of VEGF expression in ischemic area of brain but this endogenous VEGF secretion is inadequate to entirely protect the brain injury. In humans, expression of VEGF was found to be significantly increased after AIS. VEGF reached a peak 7 days after stroke and remained elevated up to 14 days. Mean VEGF expression was lowest in serum of patients with small infarct, increased in moderate infarct, and was greatest in large infarct. Serum VEGF levels also correlated with the long-term prognosis in AIS. Elevated VEGF levels were found proportional to improved NIHSS scores after 3 months.[38]

Exercise and VEGF

Exercise induces neurogenesis and angiogenesis through growth factors cascade. Endurance exercise, that is, running up regulates BDNF and synapsin one mRNA which helps to facilitate better outcome in patients with stroke. Exercise also strengthens the micro vascular integrity after cerebral ischemia and upregulates endothelial nitric oxide (NO) synthesis which improves endothelium function again up regulating VEGF expression.[38]

Repetitive Transcranial Magnetic Stimulation and VEGF

The repetitive transcranial magnetic stimulation (rTMS) has been known to upregulate neurotrophins like VEGF. The purpose of the study was to investigate the effect of high-frequency rTMS with constrain-induced movement therapy (CIMT) on serum VEGF level in chronic stroke patients with upper extremity motor deficits. The ongoing RCT recruited 35 chronic stroke patients from 3 to 18 months of index event with Brunnstrom's stages 2 to 4 and NIHSS of 4 to 20. Patients were randomized to CIMT alone and rTMS with CIMT. The rTMS (10 Hz, 750 pulses with 110% RMT) was administered for 3 weeks (5 days/week). Serum level for VEGF was estimated along with assessment of Fugl Meyer (FM), Barthel's index (BI), and modified Rankin's scales at base line; 15th and 90th day. Significant improvement was seen in patients treated with rTMS with physiotherapy on FM (50.25 vs. 40.9; $p = 0.001$) and BI (89.38 vs. 77.86; $p = 0.001$). VEGF levels were upregulated (845.51 vs. 450.07 pg/mL) in the combination group as compared with only physiotherapy group. A positive correlation of VEGF with FM score ($r = 1$) was observed in the combination group. Increased serum VEGF after rTMS may help in enhancing neuroplasticity leading to significant improvement in upper extremity motor function.[41-43]

Pharmacotherapy for Neurointervention

Role of Nitric Oxide

Nitric oxide (NO) received attention when it was discovered that endothelial derived relaxing factor was infact NO, an integral molecule involved with maintaining endothelial cell integrity, as well as participating, in hemodynamic homeostasis. The administration of NO donors increases neurogenesis in the adult rat SVZ and dentate gyrus suggesting an expanded role for the NO cascade beyond embryogenesis. Treatment with NO donors beginning 24 hours poststroke in rat models is associated with increased neurogenesis and improvement in functional outcome despite no change in infarct volume.[44,45]

Phosphodiesterase Inhibitors

The cGMP levels may be increased by inhibiting its metabolism by the phosphodiesterase enzyme. The strategy of increasing the downstream mediator cGMP without affecting NO levels may be preferred due to the mixed outcomes in stroke reported in animal models. A major phosphodiesterase 5 inhibitor is sildenafil. Animals treated with sildanefil poststroke achieved significant and substantial increase in neurological functional recovery. Sildanefil demonstrated improved cerebral blood flow, neurogenesis, angiogenesis and synaptogenesis following experimental stroke, even when therapy is delayed for up to 1 week. In these studies, once again, the improvements in functional outcome that occur despite no change in infarct volume are intriguing.[46,47]

Statins

Drugs which increase high-density lipoproteins, such as slow release niacin have also been employed to treat stroke and have shown substantial neurological benefit when treatment is initiated days after stroke. Other neurorestorative agents

under investigation are erythropoietin, carbamylated EPO, and Thymosin B4.[21]

Role of Gamma-Aminobutyric Acid

Recovery after stroke involves remapping of the neuronal circuitry in the regions adjacent to the site of injury or the peri-infarct zone. A pharmacological approach to reestablish functional neuronal connections that are lost during stroke could enhance current physical rehabilitation therapies. Recently Clarkson showed that inhibiting tonic gamma-aminobutyric acid (GABA) ergic signaling days after stroke can improve locomotor function, suggesting a therapeutic approach that is less sensitive than acute reperfusion therapies. GABA signaling reduces neuronal excitability and thereby modulates synaptic plasticity.[48]

Role of Minocycline

Minocycline is the second generation tetracycline derivative known to have anti-inflammatory effects independent of its antimicrobial action. Recent studies have shown that minocycline prevents microglial activation, and also has notable beneficial effects in animal models of global and transient focal cerebral ischemia and other brain injuries The proposed mechanisms of minocycline include anti-inflammatory effects, reduction of microglial activation, MMP reduction, NO production, and inhibition of apoptotic cell death. In a randomized single-blinded study, we studied the effects of oral minocycline (200 mg/day) for 5 days poststroke versus placebo. Of 50 patients included into the trial, patients who received minocycline had significant improvements in stroke outcome as noted on NIHSS, mBI, and MRS scores. Larger trials are needed for confirming these results.[49]

Role of EPO

Recombinant EPO was reported to be safe and efficacious in a proof of concept study. A phase II/III study of 522 patients, however, was negative and showed a higher death rate and complications in patients receiving EPO. Possible interaction with rTPA was cited as a likely cause of increased mortality.[21,50-52]

Role of G-CSF

An IV G-CSF has also been investigated in a dose escalation phase IIa study (AXIS: 44 patients, dose administered within 12 hours). The authors reported a good tolerability and suggest further trials.[51]

Role of Cerebrolysin

Cerebrolysin, a peptide based rug is another candidate with potential for approval to be used as a restorative agent. Multiple laboratories have demonstrated the safety and efficacy of this drug in the treatment of experimental stroke. Cerebrolysin is currently in clinical trials and also in use in some countries for clinical treatment of stroke. Cerebrolysin has been proposed to induce neurogenesis, and angiogenesis in animal models f stroke and concomitantly enhances brain plasticity and recovery after stroke.[21]

NIASPAN Treatment Promotes Brain Plasticity after Stroke

There is growing body of evidence that strengthens the link between brain high-density lipoprotein cholesterol (HDL-C) metabolism and factors involved in synaptic plasticity. Scanvenger receptor class B1 binds HDL and facilitates α-tocopherol and cholesteryl esters transfer into cells from circulating HDL. Niaspan, an extended release formulation of Niacin, may be effective in reducing neurological deficits poststroke by promoting axonal remodeling, angiogenesis, and arteriogenesis. Niaspan when administered 24 hours after MACo significantly upregulates neuronal synaptic rewiring in the perinfarct region and restores connections between different cerebral areas after stroke. This increase in axonal density and synapse formation translates into long-term functional recovery after experimental stroke. Niacin induced increase in synaptic plasticity and axon growth may be mediated by the upregulation in the BDNF-TrkB (tropokinin receptor kinase B) axis.[19,53]

Enhancing Recovery with Special Reference to Walking and Aphasia after Stroke

Motor weakness and the ability to walk have been the primary targets for testing interventions that may improve after stroke. Physical therapeutic interventions enhance recovery after stroke; however, the timing, duration, and type of intervention require clarification and further trials. Pharmacotherapy, in particular with dopaminergic and selective serotonin-reuptake inhibitors, shows promise in enhancing motor recovery after stroke; however, further large scale trials are required.[54]

Pharmacotherapy may influence how the injured brain recovers. This complex array of influences and recent research increasingly confirm this concept. Many varied strategies and techniques are undergoing assessment including pharmacological therapy for aphasia, transcranial magnetic stimulation for motor recovery, and cognitive rehabilitation for attention deficits.[55] It is possible that when used in combination, these techniques may be symbiotic and synergistic. Much of the research in the area of stroke has focused on recovery of walking. Walking is a basic human function, often affected by stroke, more easily observed, more easily measured, and potentially more easily rehabilitated than other functional deficits.[55,56]

Besides loss of power in lower limb, walking also relies on the integrity of the trunk for balance, and the upper extremity for associated walking movements. In addition to motor weakness, the complex activity of walking requires the integration of sensory, visual, perceptual, and cognitive inputs.[57]

Giacino et al randomized patients with severe traumatic brain injury to amantadine, an indirect dopamine agonist, or placebo between 4 and 16 weeks after injury. Patients were treated for 4 weeks and then assessed at 6 weeks. Amantadine increased the speed of recovery during the active treatment phase. Although the Disability Rating Scale (DRS) between

baseline and at 6 weeks was similar in both groups, a post hoc analysis at the end of 4 weeks of treatment showed that more patients on amantadine had an improvement in their Disability Rating Scale scores and categories. It would now seem that another pharmacological intervention changes the course of "natural" recovery after brain injury.[58]

Dopaminergic agents and selective serotonin-reuptake inhibitors (SSRIs) have to date shown the most promise in altering the natural history of recovery after stroke.[59] Dopamine is a neurotransmitter that may promote neuroplasticity in the cerebral cortex and that may also be important in working memory and learning.[60,61] Animal studies suggest that dopamine is an important neurotransmitter for learning and memory.[62] A single-oral dose of 100 mg of levodopa and 25 mg of carbidopa can enhance the ability of patients with chronic stroke to encode an elementary motor function. Scheidtmann et al randomized 53 patients between 3 weeks and 6 months poststroke to either 3 weeks of 100 mg of levodopa with carbidopa or placebo daily, 5 days per week before physiotherapy. Patients who received levodopa had a significant improvement in motor recovery and in particular many more achieved the ability to walk early and independently. Subsequent small studies using levodopa with or without methylphenidate or levodopa with or without amphetamine could not show a difference in motor recovery or improvement in functional outcomes with treatment.[63] An ongoing study started in 2010, enrolling 572 patients, with a new stroke who cannot walk 10 m, were to receive 100 mg of levodopa and 25 mg of carbidopa, or placebo, 1 hour before physiotherapy. Patients will be treated for a maximum of 6 weeks. The primary outcome will assess the number of patients walking independently at 8 weeks after randomization.[64]

SSRIs are essential for regulation and maintenance of memory, mood, and sleep. They have also been implicated in modulating neuronal plasticity. Animal studies suggest that SSRIs may be involved in neurogenesis and activation of cortical motor areas. A single dose of citalopram can normalize the balance in cortical excitability, as measured by transmagnetic stimulation, of the affected as compared with the unaffected hemisphere in stroke patients.[65] Patients more than 6 months after stroke, in a single-dose crossover experiment with citalopram, showed improved hand dexterity as measured by the nine-hole peg test, while using the affected hand. A single dose of fluoxetine given to patients, 2 to 3 weeks after stroke showed improved motor skills on the none-hole peg test, and increased activation of the affected side on functional resonance imaging.[66]

The above studies demonstrate that SSRIs alter motor recovery and motor function. Chollet et al randomized 118 acute ischemic stroke patients within 5 to 10 days of stroke to fluoxetine (20 mg/day by mouth) or placebo. At the end of 90 days of treatment, patients were assessed using the Fugl-Meyer motor scale (motor score varies from 0 to 100, 66 points upper limb, 34 points lower limb; movements measured as none, partial, or full). The mean improvement in the total Fugl-Meyer motor scale from baseline to 90 days was significantly higher in those patients treated with fluoxetine. The improvement was present both in the arm and the leg. Patients treated with fluoxetine were more likely to reach functional independence as measured by the modified Rankin's scale.[67] A recent meta-analysis of randomized controlled trials that recruited stroke patients treated with an SSRI compared with usual care or placebo identified 52 trials for analysis. Although the use of SSRIs seems to be associated with an improvement in dependence, disability, neurological impairment, and depression, methodological limitations call for large randomized trials to derive definitive conclusions.[68]

Neurorestorative Therapy using Pharmacotherapy: Is There a Hope?

Is pharmacological restorative therapy poststroke merely a chimera? A perusal of clinical trials of neurorestorative agents certainly seem depressing at first glance. Nevertheless, if experimental evidence of neurorestoration is definite, why then has it not been replicated in clinical domains?

Translation of these restorative agents from the laboratory to the clinic has to be performed with caution and care, failing which the bench to bed-side transition will be a failure, like it happened on several previous occasions. For example, EPO was demonstrated in multiple preclinical studies to provide potent therapeutic benefit for the treatment of stroke and appeared to be a strong candidate for translation into the clinic. The phase-III clinical trial that was performed was unsuccessful and had to be terminated because of high mortality and adverse events. Of the stroke patients in the reported trial, 63.4% were administered rTPA yet prior to the performance of the clinical trial, EPO was not tested in the laboratory in conjunction with rTPA. A subsequent study with the combination of EPO with rTPA clearly demonstrated in animals the adverse events observed in the clinical trial.

Criticisms of animal studies include the following: (1) small sample size (underpowered), (2) lack of randomization, (3) variable injury levels, (4) interspecies variations, (5) confounding variables (hypothermia and use of anesthetic agents), (6) lack of evaluation of the dose–response relationship and side-effects (therapeutic index), (7) inadequate outcome measures or biomarker end-points, and (8) flawed statistical analysis. On the basis of these observations, the Stroke Therapy Academic Industry Roundtable (STAIR) recommendations were developed for providing a stronger preclinical database for potential therapeutic agents.[69]

Role of Robotics

As demonstrated by two recently published clinical trials, the key to improve rehabilitation outcomes might be found in new assistive technologies, such as robotic exoskeletons and brain-machine interfaces.[70,71]

In the first study, published in Lancet Neurology this year, Verena et al describe how the ARM in exoskeleton can facilitate the rehabilitation of hemiparesis caused by stroke.[72] Therapy robotics have the potential to enhance recovery of a paralyzed arm or leg beyond what seems to be possible with conventional therapies. Myoelectric computer interface (MCI) is another technique being developed.

Machines assisting recovery from stroke (MARS) is a rehabilitation engineering research center in the United States which is also developing several assistive devices which have potential to enhance recovery with different exercise regimes.

Neurorestoration is a concept that has been proven emphatically in several experimental models of stroke. The lack of proof in clinical settings will continue to be discouraging until the reasons for failure in this endeavor are examined. The trials of the past cannot be termed as failures as they definetly have contributed to our understanding of the complex biology of brain injury. This knowledge must provide an impetus for the development of superior candidate molecules and methodological interventions that will enhance drug development, as well as clinical testing.

Note

The author was selected for Dr. Baldev Singh Oration for the year 2017-2018.

Conflict of Interest

None declared.

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Pathogenetic Mechanism of Type 2 Diabetes Mellitus and its Clinical Implications

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Abstract

Oxidative stress is an important pathogenetic mechanism for the development of type 2 diabetes mellitus (T2DM) and its complications. Oxidative stress is an imbalance of the generation of free radicals (reactive oxygen species [ROS] and reactive nitrogen species [RNS]) and their neutralization by the antioxidant mechanisms. Increased levels of ROS and RNS lead to damage of lipids, proteins, and DNA, ultimately causing the destruction of the islet cells of pancreas through apoptosis. Another important factor in the development of diabetes mellitus and metabolic syndrome is inflammation. We studied oxidative stress in type 2 diabetic patients, patients with obesity, metabolic syndrome, and T2DM with iron-deficiency anemia. The elevation of oxidative stress in these conditions along with the increase in inflammation suggests that both oxidative stress and inflammation may heighten the risk for the development of T2DM and its complications.

Keywords
- oxidative stress
- diabetes mellitus
- obesity
- anemia

Introduction

Type 2 diabetes mellitus (T2DM) is a rapidly increasing problem in India. The prevalence has been estimated at 8.8%.[1] This has been attributed to an underlying high genetic risk, which is abetted by the environmental factors of obesity, lifestyle, and stress. The role of the initiating factors like oxidative stress and inflammation has been studied in T2DM. The risk factors of the metabolic syndrome, which is one of the main pathogenetic factors apart from insulin deficiency, should be the target to address before the onset of glucotoxicity and lipotoxicity. Metabolic syndrome encompasses a cluster of risk factors like glucose intolerance, hyperinsulinemia, dyslipidemia, and clinical features such as increased abdominal circumference, acanthosis nigricans, and polycystic ovarian disorder. Variations in the definitions have led to difficulties in determining the prevalence of metabolic syndrome in the population.[2]

In the progression of metabolic syndrome to T2DM, there are inherent genetic factors, like polymorphism of the apolipoprotein E4, aldose reductase, angiotensin converting enzyme, and toll receptor genes. The initiating events include inflammation, oxidative stress, activation of protein kinase C, selectins, vascular cell adhesion molecules and interleukins, tumor necrosis factor α and nuclear factor kappa β (NF-κB) and nitrotyrosine. If these initiating events are not targeted early, they progress to functional and pathologic changes of lipotoxicity and glucotoxicity to diabetes and its complications.[3]

Oxidative stress is a state in which there is an excessive generation or incomplete removal of reactive oxygen radicals (ROS) and reactive nitrogen species (RNS), resulting in the damage to cellular macromolecules by these reactants. The ROS include superoxide, hydrogen peroxide, hydroxyl radicals, and singlet oxygen and RNS include nitric oxide and peroxynitrite. In a healthy human being, the endogenous sources of oxidative stress include mitochondria, peroxisomes, lipoxygenase, nicotinamide adenine dinucleotide phosphate oxidase, and cytochrome P450 system; the exogenous sources include ultraviolet light, ionizing radiation, chemotherapeutics, inflammatory cytokines, and environmental toxins.[4] Low levels of ROS act as physiological and normal cell signaling molecules that mediate cellular differentiation, survival, and metabolism.[5] However, increased levels of oxidative stress lead to impaired physiological conditions of

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decreased proliferative response and defective host response. A balance is maintained by the antioxidant defenses of enzymatic systems of catalase (CT), glutathione peroxidase, superoxide dismutase, and nonenzymatic systems of glutathione, vitamins A, C, and E and molecules like uric acid and bilirubin.[5] If the antioxidant mechanism is defective then there is cellular damage and specific signaling pathways are affected leading to aging, disease, and cell death.

T2DM, Oxidative Stress, and Inflammation

The fundamental abnormality in T2DM is hyperglycemia, which is associated with oxidative stress. The metabolic pathways induced by hyperglycemia are: the polyol pathway, the hexosamine pathway, activation of protein kinase C, and the advanced glycation end products pathway; alterations in these pathways cause enhanced oxidative stress.[6] Increased ROS lead to the damage of DNA, lipids, and proteins. As the diabetes progresses, the ROS can cause β-cell failure and insulin resistance. β-cells are particularly susceptible to ROS due to low expression of antioxidants like catalase and superoxide dismutase 2.[7] Oxidative stress at the cellular level leads to pancreatic islet cell damage as well as the microvascular complication of the eyes, nerves, and kidney.

Oxidative stress and inflammation associated with obesity and metabolic syndrome probably contribute to the progression of the clinical manifestations of the metabolic syndrome.[8] Insulin has been shown to exhibit anti-inflammatory activity: it suppresses several proinflammatory transcription factors like NF-κB, Egr-1, and activating protein-1 and their corresponding genes that mediate inflammation.[8] However, insulin resistance would cause the activation of these transcription factors leading to inflammation.

Oxidative Stress in T2DM

To understand oxidative stress in diabetes and the link between hyperglycemia and enhanced free radical activity, we studied the lipid peroxidation and protein carbonyl levels in diabetics. We studied 60 diabetics and age- and sex-matched controls. Malondialdehyde, measured as thiobarbituric acid reactive substances; an index of lipid peroxidation) and protein carbonyl levels (index of protein damage by free radicals), was studied and compared with normal healthy individuals. Both malondialdehide (MDA) and protein carbonyl levels were significantly increased in the type 2 diabetics. The enhanced lipid peroxidation leads to increase in free radical activity and together with protein damage increases insulin resistance and cell damage.[9,10]

Obesity, Metabolic Syndrome, and Oxidative Stress

Obesity, a growing problem in India and the world over, is associated with oxidative stress and low-grade inflammation.[11] We studied oxidative stress in obesity.

Obese patients were divided into two groups of obesity and metabolic syndrome and compared with 30 age- and sex-matched controls. As expected, weight, waist and hip circumference, and the waist hip ratio were more in patients with obesity and metabolic syndrome. The systolic and diastolic blood pressure was also high in patients with metabolic syndrome. In the metabolic parameters, the triglyceride and very low density lipoproteins were significantly more in both the obese and the metabolic syndrome groups and there was a trend of increasing cholesterol in the metabolic syndrome group but was not significant. We measured lipid soluble and water soluble hydroperoxides and the total antioxidant capacity. While the water soluble hydroperoxide level was significantly increased in the obese and metabolic syndrome patients, the lipid soluble hydroperoxides and total antioxidant capacity did not show any change.[12] In another study, the levels of high-sensitivity C-reactive protein (hs-CRP), a nonspecific marker of inflammation was measured in obese women and compared with nonobese age-matched controls. The enhanced levels of hs-CRP in obese women indicate an increase in the levels of inflammation.[11] We concluded that early identification of at-risk obese patients by markers like increased oxidative stress, insulin resistance, inflammation, dyslipidemia, and some anthropometric parameters may decrease the progression of complications of metabolic syndrome.

T2DM, Anemia, and Oxidative Stress

Iron-deficient anemia is a common global nutritional disorder highly prevalent in the developing countries. There is a close association between the metabolisms of iron and glucose: while insulin is required for the uptake of iron in cells, in the liver, iron influences glucose metabolism and the uptake and metabolism of insulin. Iron plays a direct and causal role in diabetes pathogenesis by mediating both β-cell failure and insulin resistance. Iron (Fe^{2+}) is a prooxidant and catalyzes the generation of highly reactive hydroxyl radical, resulting in damage to cellular macromolecules. Iron overload states increase the incidence of T2DM, which may be reversed by the reduction of iron load.[13] Oxidative stress due to the prooxidant role of iron may contribute to tissue damage and enhance the risk for diabetes.[13] Iron-containing proteins like catalase and peroxidase function as antioxidants and reduction in the levels of iron would impair the antioxidant defense system. Hence, the role of iron on oxidative stress in iron deficiency together with diabetes was studied. We studied two groups of 30 patients, each having diabetes with and without iron deficiency, and compared them to normal individuals. We studied the parameters of anemia, namely, iron profile, and markers of oxidative stress (MDA levels as a marker of lipid peroxidation and serum uric acid levels).[14] Serum iron was significantly low in the patients with iron deficiency anemia. A significant increase in the malondialdehyde levels and decrease in the uric acid levels in the iron-deficient diabetic patients when compared with diabetics without iron deficiency, were observed. We concluded that diabetes is a state of lower antioxidant defenses because in iron deficiency the enzymes involved in the antioxidant defense system would be functionally defective due

to decreased levels of iron-containing enzymes like catalase and peroxidase, which are important free radical scavengers.[15] Thus, iron deficiency also results in enhanced oxidative stress. Elevated serum ferritin levels observed in diabetes without iron deficiency suggest the increase in inflammation. Increased oxidative stress and inflammation progress to the development of T2DM and its complications.

Discussion

There are many studies on diabetes and oxidative stress in the world literature.[16] The association of obesity and oxidative stress has also been studied. Their findings are similar to our studies and they have found oxidative stress to be increased in obese (independent of glycemic intolerance) and diabetic patients. Although oxidative stress with iron overload has been studied extensively, there are few reports of oxidative stress in iron-deficiency anemia.[15] We have found that iron-deficiency with diabetes also leads to enhanced oxidative stress due to decreased antioxidant levels.

In the presence of hyperglycemia, there is enhanced oxidative stress in several tissues. The ROS, in turn, activate stress sensitive signaling pathways that regulate gene expression resulting in the deterioration of the islets β-cells of the pancreas, thus resulting in reduced release of insulin.[17] Also, interference with the insulin signaling pathways results in the development of insulin resistance.[18]

Another risk factor of diabetes mellitus is inflammation.[17] The inflammatory condition triggers the development of insulin resistance and diabetes mellitus through a very complex mechanism consisting of several kinases and signaling pathways.[18] Mechanistically, the adipocytes and immunocytes produce various proinflammatory cytokines including interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) that are involved in the pathogenesis of diabetes mellitus.[19] These cytokines are involved in the activation of the NF-κB pathway leading to serine phosphorylation of insulin receptor substrate resulting in the insulin resistance.[20] Further, diabetes is also induced by the islets β-cells dysfunctioning caused by excessive IL-6 and TNF-α.[21]

Conclusion

Oxidative stress and inflammation are important pathogenetic factors in the initiation of diabetes and its complications. To control the epidemic of diabetes in our country we will have to look at the factors generating oxidative stress for primary prevention of diabetes and secondary prevention of complication of diabetes.

Note

Mala Dharmalingam was selected for Dr. J.S. Bajaj Oration for the year 2018–2019.

Conflict of Interest

None declared.

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Success and Challenges in the Management of Chronic Myeloid Leukemia

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Abstract

Chronic myeloid leukemia (CML) is one of the most common myeloproliferative neoplasms characterized by the presence of Philadelphia chromosome, that is, t(9:22), a reciprocal translocation between long arms of chromosomes 9 and 22. In its natural course CML has three phases, that is, chronic phase, accelerated phase, and blast crises phase. Peripheral blood shows marked leukocytosis and left shift. Diagnosis is confirmed by demonstration of specific molecular abnormality by polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH) method or cytogenetics. The drug of choice is tyrosine kinase inhibitor (TKI); imatinib. Other TKIs are dasatinib and nilotinib. Most patients respond and have almost normal life span. However, challenges remain. At present the drug is prescribed for lifelong. Recent studies have shown that the drug may be stopped in certain groups of which around 50% remain in long term remission (operational cure). However, around 20% did not respond and showed resistance. Research is in progress to find out the mechanism of resistance and newer therapeutic modalities or agents.

Keywords

- chronic myeloid leukemia
- tyrosine kinase inhibitor
- imatinib

Introduction

Chronic myeloid leukemia (CML) is one of the most common myeloproliferative neoplasms and commonest type of leukemia in India. It was the first malignancy found to be associated with a cytogenetic abnormality.[1] It is the first disease to have a successful molecularly targeted therapy. CML is characterized by the presence of Philadelphia chromosome, that is, t(9:22), a reciprocal translocation between long arms of chromosomes 9 and 22. Shortened chromosome 22 is known as Philadelphia chromosome.[2]

It is amyeloproliferative neoplasm that originates in an abnormal pleuripotent bone marrow stem cell and is consistently associated with a Philadelphia (Ph) chromosome and/or the BCR-ABL fusion gene. t(9:22) leads to fusion of c-abelson gene on chromosome 9 and breakpoint cluster region (BCR) on chromosome 22 leading to formation of BCR-ABL transcript. This leads to the formation of BCR-ABL fusion protein which has tyrosine kinase activity. This tyrosine kinase activity leads to phosphorylation of substrates and activates many downstream signaling pathways leading to cell proliferation, prolonged survival, and decreased apoptosis. Tyrosine kinase inhibitors including imatinib bind to the ATP binding pocket of BCR-ABL transcript and inhibit of substrate phosphorylation.[3]

Clinical Presentation

Median age of CML varies between 32 and 38 years in India compared with 50 to 55 years in the West.[1,2] Usually patients present with heaviness and dragging sensation in left hypochondrium due to enlarged spleen. Often diagnosis is incidental while routine workup or workup for some other unrelated disease. Patients also complained about weakness, fatigue, and weight loss. Rarely can it present with infection, thrombosis, bleeding, priapism, and visual disorders. In its natural course, CML has three phases, chronic phase, accelerated phase, and blast crises phase. About 80 to 90% patients present in chronic phase. About 10 to 20% patients present with either accelerated phase or blast crises. Criteria for accelerated phase are, blast 10 to 20%, basophils > 20%, platelets < 100,000 without treatment, or > 100,00,00 on

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treatmentor cytogenetic clonal evolution. Blast crises are characterized by blasts > 20% in peripheral blood or bone marrow or extramedullary blast collection. Blast crises can be myeloid, lymphoid, or mixed. Survival in blast phase or accelerated phase is very dismal without treatment. With availability of various tyrosine kinase inhibitors (TKIs) and SCT, there is possibility of better outcomes in these scenarios.[2]

Diagnosis

Although we have to do molecular test to diagnose CML, peripheral blood and bone marrow examination gives some very important clues. Peripheral blood shows marked leukocytosis and left shift, eosinophilia, and basophilia. Bone marrow shows marked myeloproliferation with myelocyte bulge in routine bone marrow differentials. Diagnosis is confirmed by demonstration of molecular abnormality by polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH) method, or cytogenetics.[1] We have seen a tremendous rise in number of CML patient in our outpatient department. Number of CML patient in 2007 were around 100 which increased to 500 in 2014 and 1,400 in 2019. Less than 10% of our patients present with upfront accelerated phase/blast crisis (AP/BC). Before 2008, median duration of symptoms before diagnosis was around 20 months which is now <5 months. This is possibly due to increased awareness about the diseased and availability of diagnostic facilities in primary and secondary health care set up.

Treatment

Busulphan, interferon, and hydroxyurea have all been used extensively in past for treatment of CML. They were able to control counts in CML, reduce size of spleen, and relieve symptoms of this disease to a great extent but were unable to prolong the survival. Since 1973, bone marrow transplant for CML is available. Although it cured the disease, it is associated with high mortality. Targeted therapy became available in 1999 and imatinib (Gleevac) was the first molecule to be available for use.[3] Dasatinib became available in 2006 and nilotinib in 2007. TKI in CML is the success story of molecularly targeted therapy. Treatment with TKI and response assessment is guided by the European Leukemia Network (ELN) guidelines 2013. In due course around 25 to 30% of patient on imatinib show resistance. Mutation analysis can identify the underlying abnormality and can tell about the sensitivity and MIC of other TKI like nilotinib and dasatinib.[4] On imatinib, approximately 10% do not tolerate, all do not respond, and those who respond may eventually fail. Mutations leading to resistance to imatinib are the most common cause for imatinib failure. We studied pattern of kinase domain mutations in 40 patients of CML who either lost their response or did not achieve it in defined time points.[4] Loss of molecular response was the most common indication for mutation analysis. Sixteen patients were found to have detectable mutations. M351T was the most common tyrosine kinase mutation followed by Y253H and H396R. Two patients had two mutations simultaneously. M351T is the most common mutation in our patient population. Need for prolonged treatment (usually lifelong) and long-term toxicities are other concerns with tyrosine kinase inhibitors.[5]

Challenges

CML is the most common leukemia in India and it occurs in India in younger age. The cause for this is not known. We studied various CYP 3A5 polymorphisms (CYP 3A5 *1/*1, CYP 3A5 *1/*3,CYP ⅗ *3/*3) and could not found any difference in cases and controls.[5] We also studied glutathione S transferase (GSTM1 and GSTT1) null type in patients and controls. Although null type was slightly more common in CML cases but not statistically different from cases.[6-8] It is not clear if the early onset of disease and its higher incidence in India could be attributed to genotypic variations in xenobiotic enzymes activity.

We have performed studies to know the predictive factors in CML, such as S-phase fraction (SPF) and aneuploidy. SPF was significantly higher in CML-chronic phase (CP) and CML-AP compared with controls.[9] Those patients with higher SPF converted more commonly to accelerated phase. Seventy five percent of patients with SPF ≥7% converted to accelerated phase. Similarly patients with aneuploidy were more likely to convert to accelerated phase compared with no aneuploidy patients. Status of lipid peroxidation is also a predictive factor in CML. Plasma levels of malondialdehyde and protein carbonyl were studied and found to be significantly elevated in accelerated phase compared with controls and CML-CP.[10-12]

Another challenge in the management of CML patient is that around 20% patients do not optimally respond to TKIs. Hence we saught to understand the mechanism of imatinib resistance using K562 (BCR-ABL+) cell lines. Nitric oxide (NO) is known to regulate cell proliferation, as well as apoptosis. Free radical generation (uperoxide, Mitochondrial reactive oxygen species [ROS], ROS and/or reactive nitrogen species) and H_2O_2 level were more in Drug naïve and imatinib resistant in patients. Recovery in these parameters was observed in patients showing optimal response to imatinib. NO level was less in drug naïve and imatinib resistant CML patients (cell proliferation potential enhanced), while NO level was augmented in imatinib responders (optimal proliferation and enhanced poptosis). inducible nitric oxide synthase (iNOS) mRNA and protein expression was less in drug naïve and imatinib resistant CML patients. NO generation and iNOS expression was enhanced in those CML patients who exhibit optimal response to imatinib. Less NO/iNOS seems to be associated with cell proliferation and reduced apoptosis in BCR-ABL+ cells.[13,14]

NF-kB, a transcription factor regulates expression of iNOS. Expression of NF-kB (p50/p65) is several folds more in CML cells. Binding of NF-kB to iNOS promoter is less in BCR-ABL+ cells. Imatinib increased binding of NF-kB to iNOS promoter. Increased binding augmented NO generation/iNOS expression and apoptosis and decreased cell proliferation. In drug naïve and imatinib resistant patients low NO/iNOS is due to less binding of NF-kB to iNOS promoter.[14]

We also studied newer compound Aryl NaphthylScafold (MND) for imatinib resistant CML. MND was able to cause

apoptosis in CML cell lines, as well as CML cells from the patients. CD34+ hematopoetic stem cells known to be responsible for disease relapse were isolated from imatinib resistant patient sample and were treated as indicated for cell viability assay. T315I is multidrug resistant mutant and many other mutations form and PBMCs from Imatinib resistant CML patient were found to be more sensitive to MND than drugs already in use.

Conclusion

CML is cytogenetically defined chronic leukemia with available treatment options which target the molecular defect. Even after availability of TKIs, many patients still progress to accelerated phase and blast crises. Need for search of various mechanisms other than tyrosine kinase mutations exists. Average survival of patients with CML has improved and it is shown that most patients with CML live normal life span on TKI treatment. Operational cure is possible in 40 to 50% of patients who have shown prolonged and very good response (deep molecular response) to TKIs and are adherent to the monitoring protocol.

Note

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Conflict of Interest

None declared.

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Cancer Therapy: A Brief Outline

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Abstract

Modern cancer treatment has evolved over several years to reach the current era of precision therapy. Exciting developments in all modalities of cancer treatment and rapidly growing arena of translational research are contributing to the steady improvement in clinical outcomes. Although several old and new challenges have to be overcome, parallel technological advances in the tools and techniques of drug discovery has promise for future. An outline of the overall approach to cancer management and a broad perspective of multimodality treatment methods are discussed in this brief review.

Keywords
- cancer
- treatment
- review

Introduction

History of modern cancer treatment dates back to about 200 years, although cancer is as old as humankind or even life.[1-3] The incidence rate for all cancers in all age groups combined is progressively rising, from 182.3 per 100,000 in 2012 to 197.9 per 100,000 in 2018 globally. Nevertheless, mortality rates overall have been marginally but steadily declining over the past few decades, from 102.4 per 100,000 in 2012 to 101.1 per 100,000 in 2018.[4] GLOBOCAN 2018 estimated an incidence of 18.1 million new cancer cases and 9.6 million cancer deaths worldwide for 2018.[4] Growing understanding of cancer biology, parallel advances in diagnosis and risk stratification, improved cancer treatment modalities, new drug discoveries and better supportive care, and cooperative group trials, all have resulted in significant rise of survival for both childhood and adult cancers. In this very brief review on cancer therapy, we attempt to summarize the principles of cancer treatment and their application and challenges in clinical practice for the beginners in oncology.

Cancer Treatment Modalities

Cancer is broadly divided into solid tumors and hematological malignancies. The intent of cancer therapy may be curative or palliative depending on the disease and patient characteristics. Solid tumors of different organs are generally staged as localized, locoregional, or metastatic disease. Localized and regional solid tumors are primarily treated with locoregional treatment modalities, like surgery and radiotherapy. Also, depending on the stage and disease extent, systemic chemotherapy is added as adjunct to local treatment to prevent recurrences and thereby improve survival. Metastatic solid tumors and all hematological malignancies are principally treated with systemic chemotherapy. Locoregional treatment with surgery or radiotherapy is also considered for certain metastatic solid tumors (e.g., germ cell tumor, colorectal cancer, renal cell carcinoma, pediatric solid tumors) and hematological malignancies (e.g., lymphomas with bulky or residual disease, plasmacytoma) as component of main treatment plan or for palliation. Other components of systemic therapy include hormone therapy, various targeted agents, monoclonal antibodies, and immunotherapy which are used in the course of treatment of different solid and hematological malignancies. Thus, treatment of cancer generally requires multimodality approach which has to be tailored as per the cancer type, stage, and biology, and according to the patient's clinical risk group and demographic characteristics. ►**Fig. 1** summarizes the available modalities and multimodal approach to cancer treatment.

Locoregional Therapy

Earliest treatment of cancer in the 1800s for patients with localized tumor growths consisted of radical anatomical dissection based on Halstedian concepts of orderly contiguous tumor spread and consideration of cancer as locoregional disease autonomous of its host. In early 1900s, radiotherapy emerged as a modality of cancer cell kill through

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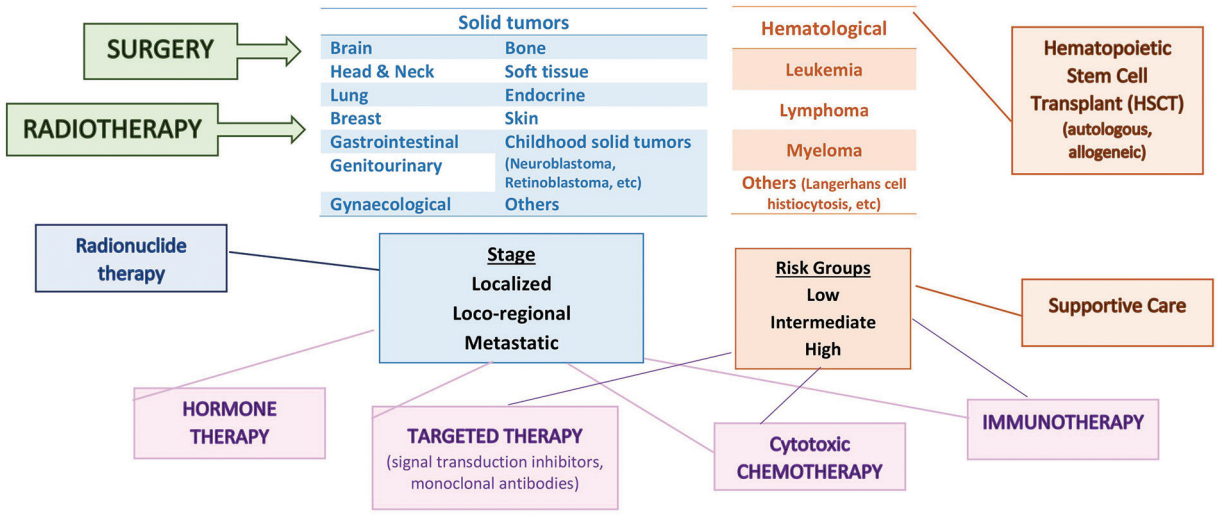


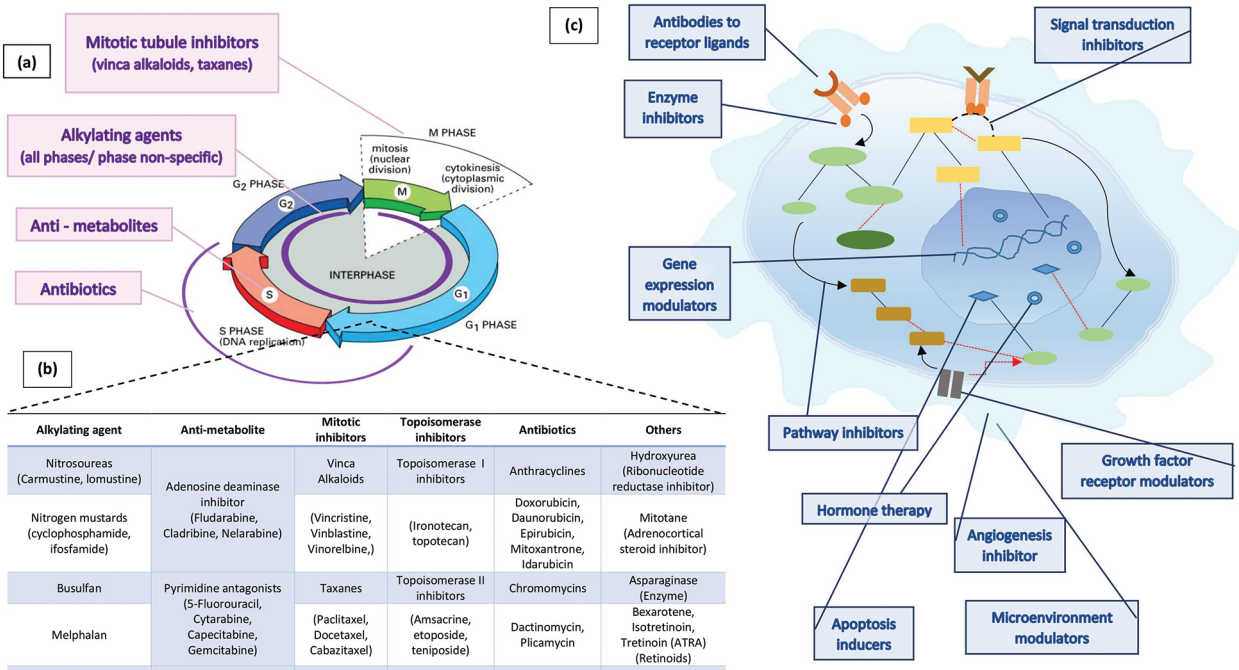
Fig. 1 Cancer treatment modalities: Locoregional treatment modalities such as surgery and radiotherapy are used to treat early stage solid tumors. Systemic therapy such as cytotoxic chemotherapy, hormone therapy, targeted drugs, and immunotherapy are used alone or in different combinations to treat hematological malignancies, as adjunct therapy for early or locally advanced solid tumors and as palliative therapy for metastatic solid tumors. Hematopoietic stem cell transplant is used for various indications in the treatment of hematological malignancies. Advances in supportive care have supplemented the administration of all these intense treatment modalities.

ionizing radiation that disrupts various pathways of cell cycle.[5] Radiotherapy thus provided an alternative or adjunct modality of locoregional treatment for various solid tumors. In the past two to three decades, significant technological advances in the conduct of surgery (e.g., endoscopic surgery, laparoscopic surgery, robotic surgery) and delivery of radiotherapy (e.g., intensity-modulated radiotherapy, volumetric modulated arc therapy, stereotactic body radiotherapy) have led to more precision in locoregional treatment, more organ preservation methods, and reduced morbidity. Parallel advances in reconstructive surgeries and various rehabilitation procedures have improved quality of life for patients with early stage solid tumors.

Systemic Therapy

Systemic anticancer treatment started with the discovery of cytotoxic chemotherapy in the late 1940s, the first few drugs being nitrogen mustard compounds and antifolates used in the treatment of leukemias and lymphomas. Since then, from 1949 to 2014, a total of 150 medicines has been approved including cytotoxic drugs and targeted agents with an indication for at least one type of cancer.[6] Most of the cytotoxic drugs are alkylating agents, antimicrotubule agents, antimetabolites, and topoisomerase inhibitors which work in different phases of cell cycle, while most of the targeted drugs belong to signal transduction inhibitors, gene expression modulators, apoptosis inducers, hormone therapies, angiogenesis inhibitors, immune modulators, and monoclonal antibodies which targets one or more of the hallmarks of cancer pathogenesis. In the past 5 years from 2015 to 2019, approximately 60 new anticancer medicines, latest being the immunotherapy group of drugs, have been approved and several older drugs are being approved for newer indications, underscoring the steadily escalating efforts in drug discovery and translational cancer research. ►**Fig. 2** summarizes the major classes of cytotoxic drugs and targeted agents. Additionally, several supportive care drugs used to treat various side effects of cancer therapy as nausea and vomiting, myelosuppression, febrile neutropenia, gastrointestinal toxicities, neuropathy, and others have developed in parallel, allowing for timely and adequate delivery of intensive treatment protocols. Systemic chemotherapy is administered as cycles or periodic courses, with interval between two doses of an average 3 to 4 weeks, to allow adequate time for normal cells to recover from collateral cytotoxicity.

Combination therapy with enterally or parenterally administered cytotoxic drugs with different mechanisms of action and differing dose limiting toxicities forms the mainstay of treatment of hematological malignancies. Optimization of drug dose, regimen, and schedule over decades through conduct of cooperative group trials have led to significant cure rates in acute leukemias and lymphomas and prolonged progression-free survival in myeloma. Novel cytotoxic drugs, targeted agents, and monoclonal antibodies are used either as single agent or in combination for treatment of relapsed/refractory diseases and for particular indications have moved to the first line therapy (e.g., rituximab in B cell lymphomas). A few targeted agents have changed the treatment paradigm of some diseases, for example, imatinib, a tyrosine kinase inhibitor targeting *BCR-ABL*, introduced in 2001 in the treatment of chronic myeloid leukemia have obviated the need for upfront allogeneic stem cell transplant in this disease. Similarly, all-trans-retinoic acid and arsenic trioxide targeting and releasing the differentiation block in acute promyelocytic leukemia caused by the *PML-RARA* translocation have resulted in cure rates of 80 to 90% with a chemotherapy-free protocol.



Alkylating agent	Anti-metabolite	Mitotic inhibitors	Topoisomerase inhibitors	Antibiotics	Others
Nitrosoureas (Carmustine, lomustine)	Adenosine deaminase inhibitor (Fludarabine, Cladribine, Nelarabine)	Vinca Alkaloids	Topoisomerase I inhibitors	Anthracyclines	Hydroxyurea (Ribonucleotide reductase inhibitor)
Nitrogen mustards (cyclophosphamide, ifosfamide)		(Vincristine, Vinblastine, Vinorelbine,)	(Irinotecan, topotecan)	Doxorubicin, Daunorubicin, Epirubicin, Mitoxantrone, Idarubicin	Mitotane (Adrenocortical steroid inhibitor)
Busulfan	Pyrimidine antagonists (5-Fluorouracil, Cytarabine, Capecitabine, Gemcitabine)	Taxanes	Topoisomerase II inhibitors	Chromomycins	Asparaginase (Enzyme)
Melphalan		(Paclitaxel, Docetaxel, Cabazitaxel)	(Amsacrine, etoposide, teniposide)	Dactinomycin, Plicamycin	Bexarotene, Isotretinoin, Tretinoin (ATRA) (Retinoids)
Platinums (Cisplatin, Carboplatin. Oxaliplatin)	Purine antagonists (Mercaptopurine, Thioguanine,)			Miscellaneous	
Imidazole Carboxamide (Dacarbazine, Procarbazine)	Anti-folates (Methotrexate, Pemetrexed)			Mitomycin, Bleomycin	

Fig. 2 (**A**) Cytotoxic class of drugs acting on different phases of cell cycles. (**B**) Table illustrating the major classes and components of cytotoxic chemotherapy. (**C**) Simplified illustration of families of targeted agents and their respective subcellular targets in the cell, nucleus, and microenvironment.

In solid tumors, the evolving concept of operable cancer being systemic disease with potential for dissemination through lymphatics and blood stream even in early stages and recognition of complex host–tumor interrelationship affecting disease biology, which were contrary to the old Halstedian principles, led to the experiments for adjuvant systemic therapy in the treatment of localized disease. These experiments of systemic therapy as adjunct to surgery led by Fisher and colleagues in the 1970s, concluded that two paradigms govern the management of cancer, first is related to the use of surgery to eradicate local and regional disease; the second is related to the eradication of systemic disease (micrometastases).[7] The treatment of patients who has no identifiable metastatic disease with systemic adjuvant therapy (after surgery) or neoadjuvant therapy (before surgery) with either hormonal agents (e.g., tamoxifen in breast cancer), targeted or cytotoxic chemotherapy, or both have resulted in decreased local and regional recurrences as well as distant metastases after minimal conservative surgery and have improved survival in patients of various solid tumors to the tune of 4 to 15% absolute benefit at different stages.

Chemotherapy, along with locoregional therapy, is also an integral component of curative treatment of certain metastatic solid tumors such as germ cell tumors, choriocarcinoma, and neuroblastoma, which are highly chemo-sensitive. For most of the other solid tumors with advanced and metastatic disease, systemic chemotherapy and targeted agents are used for palliative treatment. However, with the advent of combination chemotherapy, targeted agents, monoclonal antibodies, and immunotherapy, several sequential lines of treatment can be administered safely even for advanced diseases with resultant improvement in clinical outcomes for many of the common malignancies such as breast, prostate, lung, and colorectal cancers. Yet, treatment intent still remains palliative and not curative in majority of metastatic solid tumors, nevertheless, provides better quality of life and considerable prolongation of survival.

Multimodality Approach

Multidrug, multiphase combination chemotherapy regimens comprising of cytotoxic drugs, targeted agents, monoclonal antibodies, etc. in defined schedule forms the basis of treatment of hematological cancers. Radiotherapy is generally given for bulky or residual disease sites in lymphoma, for prophylactic or therapeutic cranial irradiation in leukemias, and as single modality radical treatment for plasmacytoma. ►**Table 1** shows an outline of some of the most widely used treatment protocols for some common hematological malignancies. Hematopoietic stem cell transplant (HSCT) either autologous or allogeneic is used for consolidation treatment as part of frontline therapy in certain high-risk hematological malignancies, for example, multiple myeloma (autologous HSCT), Philadelphia positive acute lymphoblastic leukemia in adults (allogeneic HSCT), intermediate and high-risk acute myeloid leukemia (allogeneic HSCT), and for salvage treatment of refractory/relapsed hematological cancers.

Table 1 Common chemotherapy regimens used in hematological malignancies

Disease	Common chemotherapy regimens
Hodgkin's lymphoma	ABVD (adriamycin, bleomycin, vinblastine, dacarbazine)
Non-Hodgkin's lymphoma	CHOP ± R (cyclophosphamide, doxorubicin, vincristine, prednisone) / Rituximab
Multiple myeloma	VRD (bortezomib, lenalidomide, dexamethasone)
Acute myeloid leukemia	3+7 (daunorubicin and cytarabine)
Acute promyelocytic leukemia	ATRA + ATO (all-trans-retinoic acid, arsenic trioxide)
Acute lymphoblastic leukemia	Intensive multiagent chemotherapy (steroid, 6-MP, vincristine, daunorubicin L-asparaginase, cytarabine, cyclophosphamide, high dose methotrexate) in induction and consolidation phase followed by maintenance with 6-MP and methotrexate
Chronic lymphocytic leukemia	BR (bendamustine, rituximab)
Chronic myeloid leukemia	Imatinib (BCR–ABL tyrosine kinase inhibitor)

In solid tumors, depending on the stage, all the three main modalities—surgery, radiotherapy, and chemotherapy—are used in the frontline treatment. Further, hormonal therapy, targeted agents are added to the protocol in certain tumors depending on the biological characteristics and risk group. We will discuss two tumors—breast carcinoma and neuroblastoma (common childhood tumor)—as prototype for multimodality treatment plan. ►**Fig. 3** highlights the usual treatment modalities and course for breast carcinoma and neuroblastoma. For stage II/III breast carcinoma, general course of treatment includes neoadjuvant combination chemotherapy (mainly with anthracyclines and taxanes) followed by surgery (either breast conservation or mastectomy depending on the baseline stage) and followed by radiotherapy (depending on type of surgery and baseline stage). Hormonal therapy for a duration of 5 to 10 years is added for patients with estrogen or progesterone receptor positive tumors and anti-Her 2 therapy (monoclonal antibody directed at the epidermal growth factor receptor Her 2) for Her 2 positive tumors. For a subset of very early stage, hormone receptor positive, Her 2 negative tumors, with low recurrence score by molecular tests treatment can be done by only surgery followed by hormonal therapy without the need for radio- or chemotherapy. In high-risk metastatic neuroblastoma, treatment is done with all modalities as combination chemotherapy, surgery, autologous HSCT, radiotherapy, and posttransplant maintenance treatment with differentiation agent (isotretinoin) and immune modulators (anti-GD2 antibody and interleukin-2). Thus, majority of the malignancies require a multimodality treatment approach for curative outcomes. Treatment decisions are generally taken in a multidisciplinary tumor board consisting of surgeons, anesthetist, radiation oncologist, and medical oncology experts. Further, the multidisciplinary team should also consist of nutritionist, physiotherapist, speech therapist, palliative care physicians, infection disease expert, psychosocial counselors, and other specialists (endocrinologist, cardiologist, etc., depending on the age, cancer, and treatment type) for guiding supportive care during the course of treatment, rehabilitation posttreatment, and for monitoring and management of late side effects.

In metastatic solid tumors, for most of the common malignancies of lung, breast, prostate, colorectal, renal, ovary, etc., a multitude of treatment options are now available for the first line and subsequent lines of therapy that have resulted in a significant increase in overall survival, up to 12 to 18 months on average over historical outcomes, in particular patient subsets in these cancer subtypes. These treatment options include besides conventional cytotoxic chemotherapy, targeted therapy related to the specific driver genomic alteration, hormonal therapy for hormonally driven cancers, drugs targeting the angiogenesis pathway and tumor microenvironment, and immunotherapy targeting the immune checkpoints involved in tumor cell to immune cell interactions. ►**Table 2** outlines the common therapies currently available for metastatic castrate-resistant prostate cancer which can be ordered in numerous schedules for sequential use. Current challenge in management of these metastatic solid tumors is in optimizing the right combination and sequencing of treatment. Another important clinical challenge is in evaluating cost effectiveness of the newer drugs for palliative treatment and in identifying futility of further treatment or when to stop further treatment for patients with poor general condition and progressive disease.

Other aspects of cancer care such as prevention, screening, early diagnosis, toxicity management, and rehabilitation are important areas, but are beyond the scope of the current review. Discussing the details on each cancer modality and drug, and treatment for individual cancers, is also outside the space of this brief summary. Many comprehensive international guidelines are available that summarizes the treatment approach and algorithm for management of all common malignancies and serve as useful resource.[8,9] Finally, in clinical practice, treatment decisions require the expertise and experience of the oncology team.

Treatment Adaptation: Based on Prognostic and Predictive Biomarkers

Prognostic factor is defined as measurement taken at the time of diagnosis or treatment that is associated with the outcome, determining a patient's ability to fare in the absence of treatment, for example, age of the patient, stage determined by tumor size, nodal involvement and distant spread, grade, cytogenetic or molecular profile, etc. Often a combination of clinical pathological and genetic changes are taken together to determine risk groups and based on the individual risk group treatment can be tailored—either intensified for high-risk group patients or de-intensified for low risk.

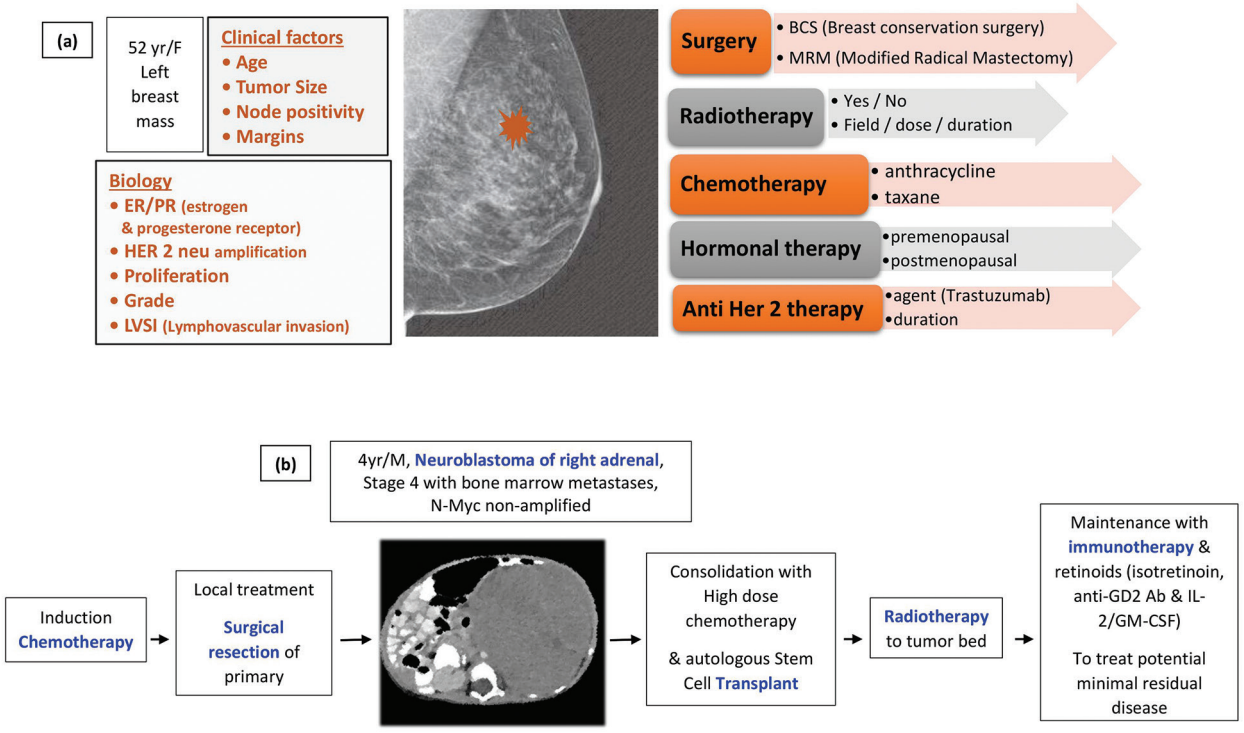


Fig. 3 Multimodality treatment approach for solid tumors, two prototype examples: (**A**) Breast carcinoma, depending on the clinical and pathological factors, locoregional and systemic treatment are used to different extent and combination. (**B**) Neuroblastoma, for high-risk patients all treatment modalities of combination chemotherapy, surgery, radiotherapy, autologous stem cell transplant, immunotherapy, and differentiation agent are used in sequence.

Table 2 Treatment options for metastatic castrate resistant prostate cancer: clinical conundrum

Drug (chemotherapy, hormonal therapy, immunotherapy, radio-ligand therapy)	First line options	Second line options	Third line options	Third line: Beyond and novel agents
Abiraterone	Sipuleucel-T	Abiraterone	Cabazitaxel	^{177}Lu-PSMA therapy
Enzalutamide				Pembrolizumab
Docetaxel	Abiraterone	Sipuleucel-T	Radium 223	PARP (poly ADP ribose polymerase) inhibitors
Cabazitaxel				VEGF inhibitors
Sipuleucel-T	Enzalutamide	Docetaxel	Enzalutamide	Src inhibitors
Radium 223				HSP90 inhibitors
^{177}Lu-PSMA therapy	Docetaxel	Enzalutamide	Abiraterone	AKT inhibitors
Pembrolizumab (for MSI high tumors)				PI3 kinase inhibitors
		Radium 223		MTOR inhibitors

Predictive factor is a measurement that predicts response or lack of response to a specific treatment. Some common examples include epidermal growth factor receptor (EGFR) mutation in lung cancer that determines response to EGFR inhibitor like gefitinib, Her2/neu amplification in breast cancer that determines response to anti-Her2 therapy like trastuzumab or lapatinib, and K-RAS/N-RAS mutations in colorectal cancer which are a negative predictive factor for response to EGFR monoclonal antibody such as cetuximab. Several other biomarkers (mutations or polymorphism) either in the tumor genome or in germline deoxyribonucleic acid involving the drug metabolizing pathways are known that can affect responses to particular drugs.[10,11] A plethora of biomarkers have been studied in different diseases but clinical utility has been established only for a handful of them. With growing understanding of cancer biology and disease pathogenesis, molecular classification is evolving for all cancer types which when clinically validated will help in further prognostication and identification of predictive markers and thereby in personalizing cancer treatment.

Clinical Endpoints

Treatment results in oncology are generally defined by response rates, disease-free remission, morbidity or late sequel of treatment, quality of life, and survival. In other words, a clinically relevant endpoint is a characteristic that reflects how a patient feels, functions, or survives. In cancer patients, the risk for death from a specific neoplasm is highest in the initial years after diagnosis; it decreases progressively thereafter. To apply the word "cured," the time from the cancer diagnosis must be such that the patient's risk of death does not, because of cancer, exceed that of a sex- and age-matched general population.[12,13] However, in oncology, use of word "cure" is debatable in view of late and very late relapses in certain malignancies and the more commonly used terminology is long-term survivor. For some of the early stage and good risk malignancies, for example, testicular germ cell tumor, thyroid cancer, Hodgkin's lymphoma, childhood acute lymphoblastic leukemia, and gestational trophoblastic neoplasm, 5- and 10-year disease-free survival (that is the period the disease remains in continuous clinical remission and without any recurrence) is close to 85 to 90% which may be taken as functional "cure." For other tumors, available treatment modalities have prolonged survival with a fairly better quality of life making many cancers a chronic disease. Ongoing translational research in cancer biology and treatment may further help to improve their outcomes.

Challenges and Future of Cancer Treatment

Cancer therapy is a continuously evolving field and every year there is considerable upsurge in new drug discovery and approvals, in drug repurposing and approval of newer indications for older drugs, in newer methods of drug delivery and optimized management of toxicities, in discovery of new predictive biomarkers and new treatment approaches, and also in technological advances in locoregional treatment modalities of surgery and radiotherapy. However, there remain several challenges in the path to translational of all these new developments in practice of real precision medicine and into clinically meaningful benefit in cancer survival. Some of the important challenges are dealing with tumor heterogeneity, handling drug resistance either due to pharmacogenomics differences in drug metabolism and transport or more commonly due to acquired mutations/alterations in cancer genome or its downstream pathway, finding of actionable alterations in the tumor tissue or its microenvironment (currently druggable genomic alterations represent only a small subset in certain cancer types), and identification and validation of predictive markers of immune therapy. Also, the disconcerting background of genomic variability creates issues regarding clinical interpretation, application, and validation of enormous and complex genomic data. Another practical challenge is in finding the optimal combination regimen, targeting several molecular alterations concurrently or in precise sequence, and validating them in clinical trials for demonstrating final benefit in survival.[14]

Some of the additional challenges pertaining to resource-limited settings in the Indian context are wide disparity in the access to cancer treatment, delayed presentation with higher disease burden, heterogeneity in resources, available expertise and treatment cost and payment structure across centers, poor social support system, significant financial constraints as most of the treatment expenses are met out of pocket, poor understanding of the disease and its treatment course and consequently higher treatment abandonment rates, restrictive access, nonavailability or prohibitive cost of the latest anticancer drugs, and very low rates of recruitment into well-designed clinical trials.

Besides development of newer generations of older targeted agents, discovery of new drugs targeting single molecular abnormality or pathway, and the expanding field of immune checkpoint inhibitors, the following novel approaches to cancer treatment which have already been studied in early phase clinical trials are making headway into mainstream therapy. These include cellular immune therapy such as CAR-T cell (chimeric antigen receptor) therapy, anticancer vaccines, and new therapeutic approaches based on genomic editing.[15] Also, so far the approach to cancer treatment had been reductionist, which is targeting single molecular abnormality or cancer pathway that have modestly improved outcomes, but to move toward potential cure, systems biology or multipronged approach targeting several driver molecular pathways or cancer hallmarks of etiopathogenesis simultaneously might be a promising therapeutic strategy.[14] As mentioned above, challenge for this approach is in optimizing the right combination or sequence, and in finding valid biomarkers; however, with better comprehension of next-generation precision oncology tools and data this should be attainable in the near future. Further, to find answer to locally relevant clinical problems in the Indian context, well-designed clinical trials through multicenter collaboration at the regional or national level is a pressing need that would be vital to improve outcomes close to that seen in the western developed world.

Conclusion

In this concise review, we have attempted to outline the major modalities of treatment in oncology, their evolution in brief, recent advances and challenges, and multimodality approach to cancer management in clinical practice with some common examples. Overall, treatment has to be evidence- and value-based, cost effective, and guided by local problems, expertise, and resources. We hope this would be a useful summary on cancer therapy for a new induct into oncology or for anybody who is keen on understanding the basic principles of cancer treatment, and encourage them to read and explore further and contribute their bit to cancer management.

Conflict of Interest

None declared.

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Imaging Spectrum of Pediatric Nonspecific Aortoarteritis on CT Angiography: A Retrospective Study

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Abstract

Introduction Takayasu's arteritis, a chronic, idiopathic, inflammatory panarteritis, is the major cause of Aortoarteritis in pediatric age group. The purpose of this study is to analyze the patterns of involvement and imaging findings of pediatric Takayasu's arteritis.

Materials and Methods We retrospectively reviewed the CT angiographic images of 11 pediatric cases reported as "Takayasu's arteritis" or "nonspecific arteritis" over the period of last 3 years.

Results Mural thickening with luminal stenosis was the most common findings. Aneurysmal dilatation of the descending thoracic aorta was observed in one case. Vessel stenoses without mural thickening, thrombosis and collateral vessel formation were the other findings. Pattern of involvement ranged from variable involvement of thoracic and abdominal aorta and their branches.

Conclusion Awareness of its CT angiographic appearances and distribution pattern can help the clinicians and radiologists to have high index of suspicion in aiding early diagnosis and better management of this disease

Keywords
- Takayasu's arteritis
- pediatric
- nonspecific aortoarteritis
- CT angiography

Introduction

Aortoarteritis, is a blanket term that accommodates a wide array of infectious and noninfectious inflammatory conditions which involve aorta and its major branches. The multitude and nonspecific nature of its clinical symptoms often lead to delayed diagnosis and thereby compromised management of these patients. Large vessel vasculitides such as Takayasu disease and giant cell arteritis are the major noninfectious causes; however, other collagen vascular diseases such as rheumatoid arthritis and ankylosing spondylitis also can lead to aortoarteritis. Infectious aortitis was usually a complication of infectious endocarditis in preantibiotic era. Aorta is normally very resistant to infection and infectious aortitis is nowadays usually associated with pre-existing aortic aneurism, diabetes, cystic medial necrosis, or surgery. *Staphylococcus aureus*, salmonella species, and tuberculosis are the usual culprits.[1] Takayasu's arteritis, a chronic, idiopathic, inflammatory panarteritis, is the major cause of aortoarteritis in pediatric age group. In children, it is the most common cause of renovascular hypertension.[2] The disease is common in India which befits its proposed causal association with tuberculosis. The nature of this association is however not completely understood yet. In 1994 the Takayasu Conference in Tokyo proposed an angiographic classification, which divides the disease in six subgroups (►**Table 1**). In addition, an appendage of a "C+" or "P+" was used to denote coronary or pulmonary involvement.[3,4]

Ultrasound with Color Doppler is the first diagnostic modality used for screening of the disease which can assess branches of aortic arch, abdominal aorta, and its branches and can show downstream flow changes in the extremities. Conventional angiography has been traditionally considered gold standard for diagnosis.[5] However, they have been largely replaced by noninvasive modalities such as CT angiography

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Table 1 Angiographic classification of Takayasu's arteritis

Type I	Branches from the aortic arch
Type IIa	Ascending aorta, aortic arch, and its branches
Type IIb	Ascending aorta, aortic arch, and its branches, thoracic descending aorta
Type III	Thoracic descending aorta, abdominal aorta, and/or renal arteries
Type IV	Abdominal aorta and/or renal arteries
Type V	Combined features of Types IIb and IV

and MRI which can demonstrate mural changes even before luminal narrowing occur.

Materials and Methods

We searched our CT scan center archives to look for the word "Takayasu's arteritis" or "nonspecific arteritis" reported over the period of last 3 years. From the total cases, we selected patients with age less than 18 years. The diagnosis of Takayasu's arteritis was based on criteria given by Sharma et al according to which presence of two major, or one major and two minor, or four minor criteria suggested the diagnosis (►**Table 2**).[6] CT angiography was performed on all the cases by 64-channel multidetector CT (Lightspeed Ultra, GE Medical Systems, Milwaukee, WI). A 20-gauge needle was placed into the antecubital vein, and nonionic contrast material, iohexol (Omnipaque 300; GE Healthcare) was injected at a rate of 2.5 to 3.0 mL/s using a power injector (OptiVantage DH; Tyco, Mallinckrodt) in amount of 2 mL/kg. Slice thickness of 1.25 mm was used during arterial and venous phase with pitch value of 1.

Results

A total of 23 patients were reported as Takayasu's arteritis or nonspecific aortoarteritis. Of these 23 patients 11 were of pediatric age group (<18 years) and we retrospectively reviewed CT angiographic findings of these 11 pediatric patients of Takayasu's disease. Three of them were males and 8 were females. Their age ranged from 12 to 18 years (mean age 15 years). In 5 out of 11 patients, both abdominal and thoracic aorta with their branches were involved. Isolated thoracic and isolated abdominal aorta with their branches were involved in three cases each. Pattern of distribution of disease is summarized in ►**Table 3**. Two of the patients showed pulmonary artery dilatation and coronary arteries were involved in none of the cases. Ten out of 11 patients were started on prednisone. One was given prednisone with antitubercular regimen as CT raised suspicion of tuberculosis revealing mediastinal and retroperitoneal lymphadenopathy and cavitary lesions in lungs along with bronchiectasis which was confirmed on histopathological examination. Antihypertensive drugs were given in the patients presenting with hypertension. No follow-up CT scan was performed predominantly due to financial issues and patients were followed up mainly by clinical examination, erythrocyte sedimentation rate levels, and Doppler studies wherever possible.

Typical CT angiographic features of the patients are summarized in ►**Table 4** and ►**Table 5** in thoracic and abdominal lesions. Mural thickening with luminal stenosis was the most common finding (►**Figs. 1** and **2**). Aneurysmal dilatation of the descending thoracic aorta was observed in one case (►**Fig. 2**). Vessel stenosis without mural thickening, thrombosis and dilatation were the other findings. Collateral vessels were observed in many cases both in thoracic and abdominal vessels (►**Fig. 3**). In thorax these vessels were visible around subclavian and carotid arteries predominantly derived from intercostal vessels. In abdomen multiple retroperitoneal, and abdominal wall collateral vessels were

Table 2 Diagnostic criteria for Takayasu's arteritis

Major criteria	Minor criteria
• Left mid-subclavian artery lesion • Right mid-subclavian artery lesion • Characteristic signs and symptoms of at least 1-month duration (including limb claudication, pulselessness or pulse differences in limbs, an unobtainable or significant blood presence difference, fever, neck pain, transient amaurosis, blurred vision, syncope, dyspnea, or palpitations)	• High erythrocyte sedimentation rate • Carotid artery tenderness Hypertension • Aortic regurgitation or annuloaortic ectasia • Pulmonary artery lesion • Left mid-common carotid lesion • Distal brachiocephalic trunk lesion • Descending thoracic aorta lesion • Abdominal aorta lesion • Coronary artery lesions

Table 3 Pattern of involvement in 11 pediatric cases of Takayasu's disease

Involved segment	No. of cases	%
Ascending aorta	2	18.2
Arch of aorta	3	27.3
Descending aorta	7	63.6
Proximal abdominal aorta	4	36.4
Mid abdominal aorta	4	36.4
Distal abdominal aorta	3	27.3
Right common carotid artery	2	18.2
Left common carotid artery	4	36.4
Brachiocephalic artery	2	18.2
Right subclavian artery	2	18.2
Left subclavian artery	6	54.6
Superior mesenteric and/or celiac artery	3	27.3
Right renal artery	4	36.4
Left renal artery	7	63.6

Table 4 Sites of involvement and findings on CT angiography in eight pediatric patients of Takayasu's arteritis with thoracic lesions

Artery involved	Mural thickening		Dilatation		Thrombi		Ulcer like lesion		Collateralization	
	No.	%	No.	%	No.	%	No.	%	No.	%
Ascending thoracic aorta	2	25	0	0	0	0	0	0	1	12.5
Arch of aorta	3	37.5	0	0	0	0	0	0	1	12.5
Descending thoracic aorta	7	87.5	1	12.5	1	12.5	1	12.5	0	0
Brachiocephalic	2	25	0	0	0	0	0	0	0	0
Common carotid (right)	2	25	0	0	0	0	0	0	1	12.5
Common carotid (left)	4	50	0	0	1	12.5	0	0	3	37.5
Subclavian (right)	2	25	0	0	0	0	0	0	2	25
Subclavian (left)	5	62.5	0	0	1	12.5	0	0	6	75
Vertebral (right)	1	12.5	0	0	0	0	0	0	1	12.5
Vertebral (left)	2	25	0	0	0	0	0	0	2	25
Pulmonary artery	0	0	2	25	0	0	0	0	0	0

Table 5 Sites of involvement and findings on CT angiography in eight pediatric patients of Takayasu's arteritis with abdominal lesions

Segment involved	Mural thickening		Ostial narrowing		Stenosis without thickening		Thrombosis	
	No.	%	No.	%	No.	%	No.	%
Proximal abdominal aorta	4	50	0	0	0	0	1	12.5
Mid abdominal aorta	4	50	0	0	0	0	0	0
Distal abdominal aorta	1	12.5	0	0	2	25	0	0
Celiac trunk and superior mesenteric artery	0	0	2	25	1	12.5	0	0
Right renal artery	0	0	4	50	3	37.5	0	0
Left renal artery	1	12.5	7	87.5	4	50	2	25

observed. Paravertebral and inferior mesenteric artery also contributed to the formation of collateral around renal artery in one case. One of the cases also revealed pontine infarct. In abdomen, involvement of aorta and renal arteries was the most commonly involved site with cases presenting with diffuse mural thickening leading to diffuse narrowing as well as ostial stenosis. Ostial stenosis was also seen at the origin of celiac and superior mesenteric artery (►**Fig. 4**). Two cases of vessel lumen thrombosis were seen, one involving left subclavian and other involving right common carotid artery (►**Fig. 5**). One of the cases of descending thoracic aortic aneurysm was partially thrombosed (►**Fig. 2**).

Discussion

Takayasu's disease, also known as Martorell syndrome, occlusive thromboaortopathy, or pulseless disease, has a more aggressive course in pediatric age group with a reported 5-year mortality rate of 35 to 40%.[7-9] Many studies have shown it to be associated with tuberculosis; however, the nature of association is not clear.[7] One out of 11 of our cases also had tuberculosis and was treated with steroid as well as antitubercular regimen. Takayasu's arteritis is difficult to diagnose in children as most of the cases present with generalized systemic manifestations and nonspecific complaints. However, high degree of suspicion is mandated in our country given the disease has relative frequency in this region. The disease has a predilection for young female as also found in our study.

The clinical symptoms correlated well with the pattern of vessel involvement of the disease in our study. Eight out of 11 cases presented with hypertension, seven of which revealed renal artery involvement on CT angiography. One of them was associated with renal infarct. Cases with symptoms like headache, dizziness, neck tenderness revealed carotid and vertebral artery involvement on imaging. One of the cases also revealed pontine infarct. Cases with abdominal aortic involvement are related with vague abdominal pain and other abdominal symptoms. Different investigation modalities have been used to evaluate Takayasu's arteritis. Sonography remains primary modality to evaluate carotid and subclavian arteries and was used in follow up of the patients in our study. CT angiography has largely replaced conventional angiography in diagnosis and work up of these patients because besides luminal changes CT can also assess mural thickening, intraluminal thrombus, collateral vessels,

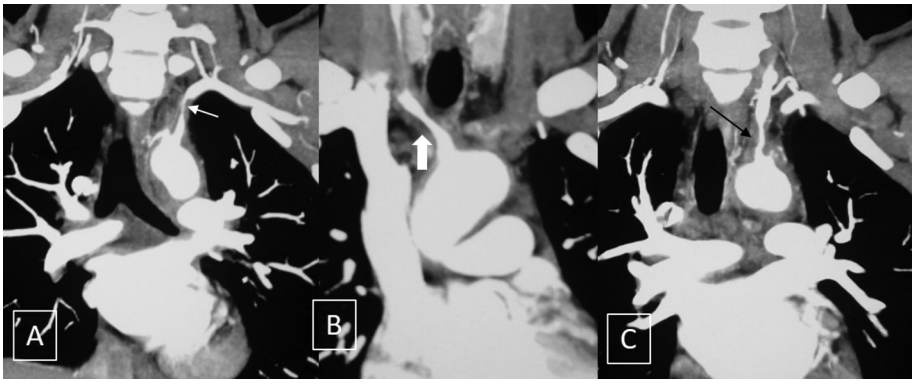

Fig. 1 Short segment mural thickening with luminal narrowing is seen in left subclavian (*white arrow* in **A**), brachiocephalic (*solid white arrow* in **B**) and left common carotid (*black arrow* in **C**) arteries.

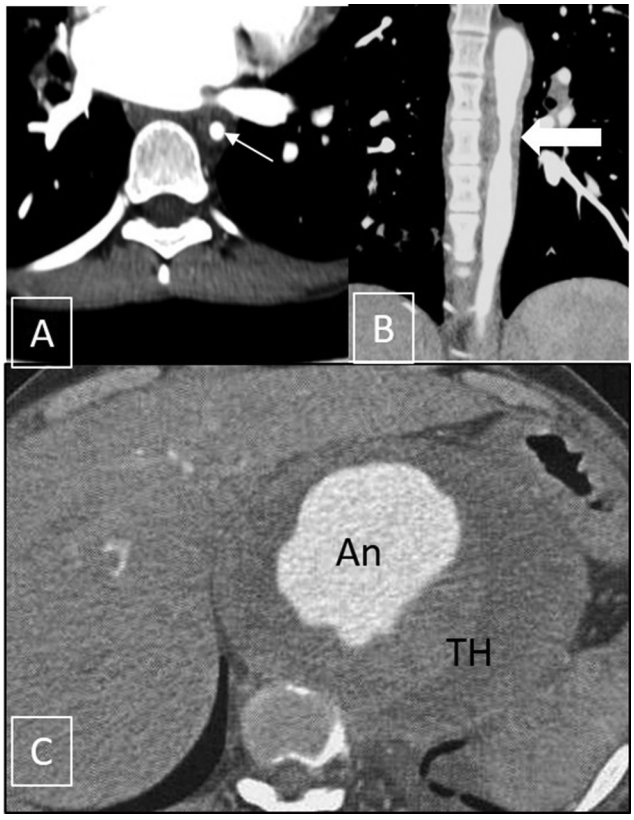

Fig. 2 (**A**, **B**) These figures show circumferential mural thickening in descending thoracic aorta causing significant luminal narrowing (*white arrows*). Another patient (**C**) showing aneurysmal dilatation of the involved aortic segment (An) with partial luminal thrombosis (TH).

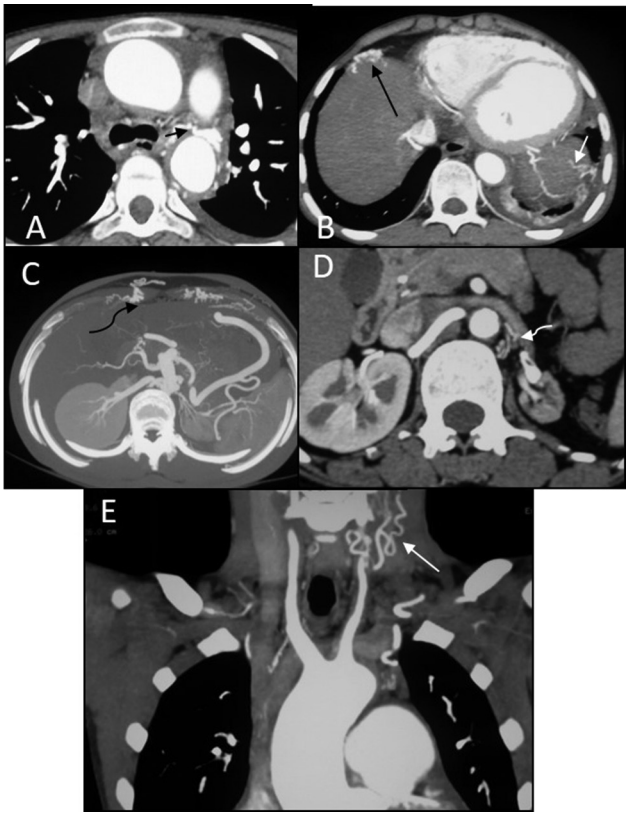

Fig. 3 Multiple collateral vessels are seen in various patients in CT angiographic images: aortopulmonary (**A**, *short black arrow*), left phrenic (**B**, *small white arrow*), anterior perihepatic (**B**, *large white arrow*), anterior abdominal wall (**C**, *curved black arrow*), left cervical collaterals (**E**, *large white arrow are seen*). (**D**) This shows paravertebral collaterals replacing left renal artery in a case (*curved black arrow*).

and other end organ changes unlike conventional angiography.[10-12] In our study we came across following findings on CT angiography.

Vessel Wall Thickening

Mural thickening, with or without luminal stenosis, and increased vessel wall enhancement of the involved segment are the most common findings that are associated with this disease. Various studies have shown the decrease of mural thickening and enhancement with treatment.[10-12] Paul et al studied the role of electron beam CT in follow up of 16 patients and demonstrated the decrease of vessel wall thickening with appearance of wall calcification with treatment.[5] In our study all the cases were associated with enhancing soft tissue density thickening of vessel walls. Transmural calcification of thickened arterial wall is also an important feature of Takayasu's arteritis and is seen in approximately one-third of patients. Few studies have reported double ring pattern enhancement in the thickened vessel wall.[13]

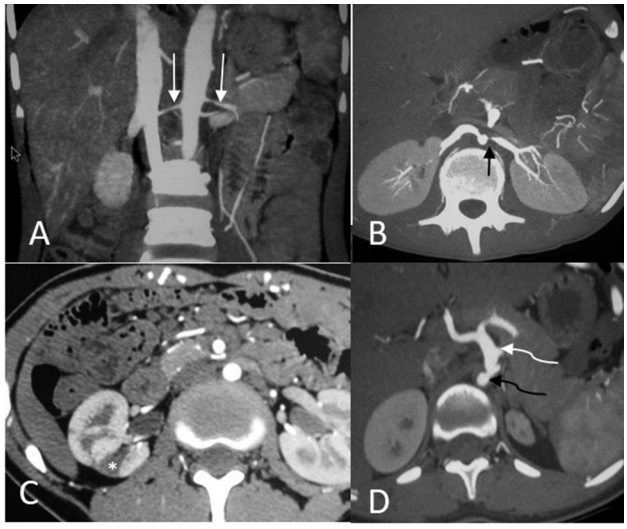

Fig. 4 Diffuse mural thickening and narrowing of bilateral renal artery in a patient (**A**, *white arrows*). Another patient shows ostial stenosis of the origin of left renal artery (**B**, *black arrow*). Small renal infarct (*) is depicted in (**C**). A patient with abdominal involvement (**D**) shows thickening and stenosis of proximal abdominal aorta with ostial stenosis (*curved black arrow*) of origin of celiac trunk with poststenotic dilatation (*curved white arrow*).

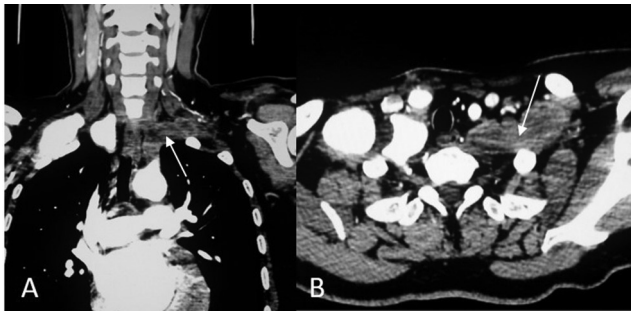

Fig. 5 Coronal (**A**) and axial (**B**) images showing complete thrombotic occlusion of left subclavian artery (*white arrows*).

Segmental Vessel Narrowing and Ostial Stenosis

Luminal narrowing can be observed in 90% of the patients.[13,14] The narrowing can be either due to arterial wall thickening or can be associated with vessel wall inflammation and fibrosis. In our study, segmental stenosis was found most commonly in abdominal aorta, descending thoracic aorta, and branches of aortic arch predominantly in subclavian and carotid arteries. Narrowing at the origin of renal arteries (7 out of 11), celiac trunk, and superior mesenteric arteries (2 out of 11) was also found. Takayasu's arteritis is the most common cause of renal artery stenosis. Carotid and extremity artery stenosis can lead to cerebrovascular stroke or limb claudication.[13,14] Recently a case of supravalvular aortic stenosis caused by Takayasu's arteritis has been reported.[15]

Intraluminal Thrombus

Thrombus formation in Takayasu's arteritis can be attributed to raised platelet count and raised plasma levels of platelet factor 4, beta thromboglobulin, thrombin/antithrombin complex, fibrinopeptide A, and D-dimer as demonstrated by various studies.[10,16] In our study thrombosis of subclavian, carotid, and renal arteries was observed. A partially thrombosed aneurysm was also seen in descending thoracic aorta. Besides the thrombosis of the involved arterial lumen, various reports have described venous thrombosis due to hypercoagulable states. Isolated case reports have described cases of cerebral venous thrombosis presenting as acute onset headache and superior mesenteric vein thrombosis presenting as acute abdomen in Takayasu's arteritis.[17] However, due to the rarity of venous thrombosis it has not been established whether thromboembolic event have an association with arteritis or is just anecdotal.

Collateral Vessels

Where there is blood, there is a way. Chronic luminal occlusion instigates formation of vascular collaterals and their assessment is useful in planning therapy. Various collateral pathways get recruited to supply the ischemic tissues depending upon the stenosed vessel. In our study we came across various intercostal, paravertebral, abdominal and chest wall, retroperitoneal and mesenteric collaterals. A notable collateral pathway that has been described in the obstruction of aorta is Winslow pathway, which connects internal thoracic artery, superior and inferior epigastric artery, and external iliac artery. Prominent Winslow pathway can lead to limb claudication due to stealing effect as well as systemic arterial hypertension.[18] An isolated case report has described a coronary-carotid collateral pathway originating from left anterior descending artery and left circumflex arteries. CT angiography can provide an accurate understanding of the anatomy of collaterals in every patient. Depending on the symptomatology, CT angiography also may aid in therapeutic decision of percutaneous embolization of the collateral responsible for the symptom.[19]

Other Miscellaneous Findings

Additionally CT angiography can also demonstrate aneurismal dilatation of involved vessel, pulmonary and coronary artery involvement. Pulmonary and coronary artery involvement has been reported in 63.3 and 44.4%, respectively.[10] However, in our study 2 out of 11 (18.2%) cases involved pulmonary artery and none of the cases revealed coronary artery involvement. CT also helps to evaluate end organ changes such as renal parenchymal atrophy, renal and cerebral infarcts and thus helps in modifying treatment protocol. Rare manifestations of Takayasu's arteritis include brain leptomeningitis, intracranial arteritis, and pyodermagangrenosum.[20-22]

Recently there has been interest in the role of MRI in Takayasu arteritis as it can detect very early mural changes with better sensitivity and can give useful information about disease activity.[23] [18F]-Fluorodeoxyglucose positron emission tomography has also been proposed to evaluate response to treatment in Takayasu arteritis.

Conclusion

Takayasu disease follows more hostile course in pediatric age group with high mortality. Awareness of its CT angiographic appearances and distribution pattern can help the

clinicians and radiologists to have high index of suspicion in aiding early diagnosis and better management of this disease.

Conflict of Interest
None declared.

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Bacteriophage Therapy: An Alternative to Antibiotics—An Experimental Study in Mice

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Abstract

The present study was planned to evaluate the efficacy of *Pseudomonas aeruginosa* specific phages in immunocompromised septicemia animal model as an alternative to antibiotics. Five different sets of experiments were performed: prophylactic administration of phage cocktail (3 lytic and unique) before and simultaneous with bacterial challenge; and therapeutic, that is, administration of phage cocktail 6, 12, and 24 hours after the bacterial challenge. No mortality was observed when simultaneous and late administration of phages was done with respect to the bacterial challenge. Contrary to this, administration of phage cocktail 100 µL (10^{12} PFU/mL) of volume after 6 hours of the infection resulted in a mortality rate of 60%. However, no mortality could be observed with reduced dose of cocktail, that is, 10^{8}, 10^{9}, and 10^{10} PFU administered 6 hours after bacterial challenge. Phage therapy in acute infections initiated with very small dosage under strict supervision may give better results. However, further studies to determine the quantity and frequency of dosage of phage cocktail for septicemia of various durations is strongly indicated.

Keywords
- *Pseudomonas aeruginosa*
- bacteriophage cocktail
- multidrug resistant
- mouse model

Introduction

Pseudomonas aeruginosa is a metabolically least demanding gram-negative bacterium that can cause a wide range of opportunistic infections. Individuals with open wounds (burn and trauma), cancer, immunocompromised cystic fibrosis, and septicemia are particularly susceptible to *P. aeruginosa* infections.[1-3] Environmental signals during infection cause several genotypic and phenotypic changes enabling *P. aeruginosa* to survive in the form of planktonic cells, colonies, or biofilms.[4] The increasing frequency of multidrug-resistant strains is particularly concerning as treatment options are severely limited in the absence of effective antibiotics.[5,6] The problem has reached such a dimension that, at global level, United Nations General Assembly was called in New York in September 2016 to decide the plan for fighting antimicrobial resistance together. This was only the fourth time in the history of the UN that a health topic was discussed at the General Assembly. Other three were HIV, noncommunicable diseases, and Ebola. The delegates and heads of the nations addressed the seriousness and scope of the situation and agreed on sustainable, multisector approaches to address the issue of antimicrobial resistance. The different alternatives suggested are; newer antibacterial molecules, antibacterial peptides, bacteriocins, probiotics, prebiotics, and bacteriophage therapy. Even if a given antibiotic molecule is effective in vitro against the infecting strain, it may fail in vivo because of biofilm formation leading to poor permeation of antibiotics at the infection site. In case of *P. aeruginosa*, it has been observed that in biofilm profile, it may resist biocides up to 100 times greater than the planktonic or free swimming cells.[7] Rigorous research activities are going on to develop alternatives toward the treatment of infections caused by *P. aeruginosa.* Ironically, active immunization against *P. aeruginosa* in immunocompromised patients has got no relevance.[8] Of these, the phage therapy

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has emerged as one of most promising alternative to overcome the problem of bacterial resistance.[9] The capacity of phages through production of highly specific enzymes like polysaccharide depolymerases or alginate lyases to eradicate bacterial biofilms is an important aspect toward their successful implementation in *in vivo* treatments. Phage cocktails have been applied as alternative or as supportive treatments simultaneously with antibiotics for *P. aeruginosa* eradication causing various infections, such as purulent wounds, septicemia, urinary tract, or lung infections. Many of the previous studies have given conflicting results due to several confounding factors.[9-17] However, the commercialization of the phage therapy is still far away due to lack of convincing preclinical and clinical trials. Hence, prior to translation of phage therapy into clinical settings, vigorous experimental authentications with extensive *in vitro* and *in vivo* studies are needed. The present study, therefore, was planned to see the efficacy of *P. aeruginosa* specific bacteriophage cocktail in septicemia in burn induced immunocompromised mouse model in different experimental settings.

Materials and Methods

Bacterial Strains and Their Identification

P. aeruginosa isolates were isolated from clinical specimens (pus, blood, urine, cerebrospinal fluid [CSF], wound swabs, etc.) collected from the patients admitted to intensive care unit of a tertiary level university hospital of Banaras Hindu University on *Pseudomonas* selective media (cetrimide agar). The strains were identified by the methods already described in standard text. The study period extended from December 2012 to July 2014.

All the clinical isolates were identified as *P. aeruginosa* by using standard biochemical and molecular methods. Further study was conducted only on *P. aeruginosa* confirmed isolates. Antimicrobial susceptibility test of *P. aeruginosa* was done by the standard Bauer–Kirby disc diffusion method. The size of inhibition zones were recorded and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) breakpoint guideline 2012. All the *P. aeruginosa* strains were tested for their susceptibility to gentamicin (GEN, 10 µg), amikacin (AK, 30 µg), netilmicin-sulfate (NET, 30 µg), carbenicillin (CB, 100 µg), piperacillin/tazabactam (PTZ, 100/10 µg), ceftriaxone (CTR, 30 µg), ceftazidime (CAZ, 30 µg), cefepime (CPM, 30 µg), imipenem (IPM, 10 µg), meropenem (MRP, 10 µg), ertapenem (ETP, 10 µg), ciprofloxacin(CIP, 5 µg), levofloxacin (LE, 5 µg), ofloxacin (OF, 5 µg), cotrimoxazole (COT, 25 µg), polymyxin-B (PB, 300 unit), chloramphenicol (C, 30 µg), colistin sodium methanesulfonate (Cl, 25 µg), and azithromycin (AZM, 15 µg).

Phage Isolation and Purification

Isolation of bacteriophages was done from different water sources (river, ponds, and sewer) by using double agar overlay method with slight modification as described earlier.[17] In brief, for isolation of bacteriophages, the *P. aeruginosa* was plated as lawn culture (10^8 CFU/mL) on Mueller–Hinton agar (MHA). Water specimens from different water bodies were treated with 1% chloroform (v/v) for 20 minutes and centrifuged for 15 minutes at 10,778 × *g*. The supernatant in the volume of 1 mL was flooded on the 5-hour old lawn culture growth (log phage) of the *P. aeruginosa* (isolated strains from different hosts) on 90-mm nutrient agar plate and incubated overnight at 37°C. Next day the lawn was washed with 3 mL TMG (Tris-HCl, magnesium sulfate, and gelatin pH 7.4) buffer and centrifuged at 10,778 × *g* for 15 minutes. The supernatant (1 mL) was transferred to a 1.5 mL microcentrifuge tube. One drop of chloroform was added and mixed well by vortexing or by inversion for 15 minutes. Centrifugation was done at 10,778 × *g* for 10 minutes. The lawn culture in log phase of the host was again prepared and the supernatant collected as mentioned above was inoculated in the volume of 100 µL at 10 to 12 places to screen for lysis. The surface with clear plaque was cut and collected in 1 mL of the TMG buffer and propagated further and plaque counting was done by soft agar overlay method.[18] The single isolated plaque was picked up for further processing. The number of phage particle was increased by soft agar overlay method. After bulk production, the bacteria were killed by 1% chloroform and centrifuged. The clear supernatant was preserved at 4°C for further use. For purification (toxin free) and concentration of phages the harvested fluid was subjected to membrane dialysis against polyethylene glycol (PEG 6,000; 20% in 2.5 M NaCl) for overnight and then washed with PBS (phosphate buffer saline) at 4°C. This process was repeated twice at 4°C.

Assessment of Anti-*P. aeruginosa* Activity of Bacteriophages

Bacteriophage Host Range Determination

All the 35 isolated phages were subjected to the assessment of their antibacterial activity on a total of 100 clinical isolates of *P. aeruginosa*. The lawn culture of *P. aeruginosa* (10^8 CFU/mL) was made on MHA. Each of the phages having concentration of 10^9 plaque forming unit (PFU)/mL was spotted on the plate in the volume of 10 µL. The plates were observed for the clear zone after overnight incubation at 37°C. Each phage was tested against all the bacterial strains in duplicate in independent experiments.

Isolation of Bacteriophage DNA

Isolation of phage DNA was performed with phenol/chloroform and ethanol precipitation method. Briefly, purified phage particles (10^{10}–10^{12} PFU/mL) were treated with 1 µg of DNase I and RNase A (Bangalore Genei, Bangalore, India) at 37°C for 30 minutes. To the mixture, proteinase-K (Bangalore Genei, Bangalore, India), and SDS were added at a final concentration of 0.05 mg/mL and 0.5% respectively and incubated at 56°C. After 1 hour of incubation, an equal volume of phenol:chloroform was added to remove proteinaceous material. The extraction was repeated thrice with phenol–chloroform–isoamyl alcohol (25:24:1). The nucleic acid was precipitated with chilled ethanol and suspended in 20 µL of TE buffer (10 mM Tris-HCl, pH = 7.0; 1.0 mM EDTA, pH = 7.0) according to standard procedure.[19]

Genotyping of Bacteriophages by ERIC-PCR

All the bacteriophages were subjected to genotyping by ERIC-PCR (used on the principle of RAPD). This test was used to genotype the phages to see whether they were genotypically similar or different. This test enabled us to pick up the phages which were not only different in antibacterial activity wise but different genotypically also. The primer sequences used for ERIC and PCR conditions are given in ►**Table 1**. The ERIC primers were used like RAPD with 49°C as annealing temperature rather than 61°C.[20] PCR was performed in 25 µL volume using 10 ng of genomic DNA, 1 U of Taq polymerase (Bangalore Genie, India), and 15 pmol of each primer (Bangalore Genie), 200 mmol/L (each) deoxynucleotide triphosphate (Bangalore Genie, India), and 2 mmol/L $MgCl_2$ in standard PCR buffer. Amplification reactions were performed in a thermal cycler (Biometra, Goettingen, Germany).

Preparation of Dendrogram

The gel images were analyzed under ultraviolet light using a gel documentation system (BioRad, Universal Hood II, United States). The size of DNA bands were estimated according to molecular weight markers. Cluster analysis of all the 22 bacteriophages was done on the basis of the fingerprints generated. Based on the banding patterns obtained from ERIC-PCR, dendrogram was constructed. For each phage a haplotype matrix or a binary table was manufactured by linearly composing lysis (1) and no lysis (0), data derived from gel analysis of ERIC-PCR. The resulting similarity matrix was used as the input data for cluster analysis by NTSYS pc2.0 program of UPGMA.[21]

Phage Cocktail Preparation

The three most potent bacteriophages ϕpsbhu-1, ϕpsbhu-15, and ϕpsbhu-17 were purified and made toxin free with membrane dialysis (dialysis membrane-135, HiMedia Laboratories Pvt. Ltd. Mumbai, India). A phage cocktail containing equal concentration and volume of above three phages were tittered at 1×10^{12} PFU/mL. These phages were different from each other genotypically based on ERIC PCR and also activity wise on 100 indicator strains of *P. aeruginosa*.

Animal Model Studies

The study protocol was approved by Institute Animal Ethics Committee of Banaras Hindu University.

Safety of Bacteriophage Cocktail

A group of 10 adult Swiss albino mice approximately 6 to 8 weeks old were taken and 100 µL intraperitoneal (I/P) injection of phage cocktail consisting of ϕpsbhu-1, ϕpsbhu-15, and ϕpsbhu-17 at the concentration of approximately 2×10^{12} PFU/mL was given to them without anesthesia and burn injury. These mice were observed for 1 month. None of the mice was found sick or dead.

Determination of LD100

A group of 10 adult Swiss albino mice approximately 6 to 8 weeks old on antibiotic free diet were taken and anaesthetized with help of optimum dose of ether. Care was taken to avoid deep anesthesia. Mice were placed into a template with an opening of 4.5 cm by 1.8 cm to expose their shaved backs. Third-degree thermal injury to the skin was induced by the exposed back area to the coin template dipped into 90°C water for 10 seconds. About 0.8 mL of ringer's lactate solution was administered immediately following the burn. The mice were challenged by intraperitoneal injection of 100 µL (inoculums containing1×10^6 to 1×10^9 CFU/mL) the *P. aeruginosa* strain (PS BHU-17), was resistant to gentamicin, netilmicin, amikacin ceftazidime, meropenem, ciprofloxacin, and piperacillin/tazobactam but sensitive to tobramycin, was isolated from a burn wound of a patient. The mice were kept under observation in an ambient environment. It was observed that all the mice died between 36 and 72 hours of the infection. Liver, spleen, heart, and peritoneum fluids of dead mice were collected at postmortem examinations. Individual organs were weighted and suspended in 2 mL of PBS. They were then homogenized using Wheaton overhead stirrers. The homogenate was plated on MHA plate to see the bacterial growth.

Assessment of Clinical Efficacy of the Phage Cocktail

We used phage cocktail as prophylactic, as well as therapeutic purposes. The mice experiments were set up in following groups and each group contained five mice.

Group A

Bacteriophage cocktail was given at constant dose, that is, in the volume of 100 µL containing 10^{12} PFU/mL. Further, the bacterial challenge of 100 µL of *P. aeruginosa* in the concentration of 1×10^9 CFU/mL was given in all the settings of this section of the study.

A.1) Simultaneous administration of bacteriophage and *P. aeruginosa* challenge:

The above mentioned dosage of bacterial challenge and bacteriophage cocktail was given in the different flanks through intraperitoneal route.

A.2) Bacteriophage cocktail 6 hours later to bacterial challenge:

The bacterial challenge was given 6 hours later to the initial prophylactic protection by the dose mentioned above. The mice were observed for 96 hours.

A.3) Bacteriophage cocktail 6 hours before bacterial challenge:

Table 1 Primer set, annealing temperature, amplicon size, and references of the protocol used in the study

Gene targets	Oligos	Annealing Temperature	Amplicon size (bp)	Reference
ERIC-PCR	F-5'-ATGTAAGCTCCTGGGGATTCAC-3' R-5'-AAGTAAGTGACTGGGGTGAGCG-3'	49°C	Multiple	[20]

Initially the bacterial challenge was given and 6 hours later the bacteriophage cocktail was given in the different flank of abdomen.

A.4) Bacteriophage cocktail 12 hours after bacterial challenge:

Initially the bacterial challenge was given and 12 hours later the bacteriophage cocktail was given in the different flank of abdomen.

A.5) Bacteriophage cocktail 24 hours after bacterial challenge:

Initially the bacteria challenge was given and 24 hours later the bacteriophage cocktail was given in the different flank of abdomen.

Group B

Assessment of Phage Efficacy after Decreasing the Volume of Phage Cocktail

Bacteriophage cocktail 6 hours after bacterial challenge of 20, 40, and 60 µL of approximately 2×10^{12} PFU/mL of the three different dosages were given to three different groups (five mice in each group).

Grading of diseases: The grading of the disease was done as follows: grade-I, normal when there was no obvious change seen in the experimental animals; grade II was given to those mice who had slight illness, lethargy and ruffled fur; grade-III scoring was given to the mice having moderate illness, severe lethargy, ruffled fur, and hunched back; grade IV to those having severe illness with above sign, exudative accumulation around eyes; and grade V to those who died at the point of examination.

Statistical Analysis

Data were expressed as means ± standard deviation (SD) of mean and statistical analysis was performed with Wilcoxon's signed-ranked test using student's *t*-test for calculations of mean and SD. Difference with $p \leq 0.05$ was considered as statistically significant.

Results

Safety of Bacteriophage Cocktail

When the phage cocktail was given at the concentration of approximately 2×10^{12} PFU/mL, none of the mice was found sick or dead when observed for 1 month.

Determination of LD100

The challenge bacterial strain could give 100% mortality in 36 to 48 hours at the dose of 100 µL 1×10^{9} CFU/mL.

Prophylactic Treatment

Simultaneous Administration of Bacteria and Phage Cocktail

No mortality occurred in any mice. The score of morbidity is given in ►**Table 2**.

Bacteriophage Cocktail 6 Hours before Bacterial Challenge

Result of prophylactic study states that only two (20%) out of 10 treated mice died after 12 hours. However, rest of eight mice (80%) survived and recovered to normal healthy level after 72 hours of the infection.

Administration of *P. aeruginosa* 6 Hours before Bacteriophage Cocktail

In our result, death of six mice out of 10 mice was observed within 24 hours of infection and remaining four mice recovered to normal healthy after 72 hours of infection.

Administration of *P. aeruginosa* 12 Hours before Bacteriophage Cocktail

In our result, none of the mice died in this group. Only they remain sick up to 48 hours and after 72 hours they recovered to normal healthy.

Administration of *P. aeruginosa* 24 Hours before Bacteriophage Cocktail

In our result, none of the mice died in this group. Only they remain sick up to 48 hours and after 72 hours they recovered to normal healthy.

Concentration Dependent Phage Cocktail

We observed that none of the mice died in these three groups as described above. However, the mice of given 60 µL of phage cocktail was observed more sick at 24 hours than the rest of two groups. Further recovery was very slow in the group received 20 µL of phage cocktail (►**Fig. 1**).

Assessment of Phage Efficacy after Decreasing Volume of Phage Cocktail after 6 Hours of Bacterial Challenge

Bacteriophage cocktail 6 hour after bacterial challenge of 20, 40, and 60 µL of approximately 2×10^{12} PFU/mL of the three different dosage were given to three different groups (five in each group) of mice.

Bacteriophage Cocktail Dose having 60 µL of Approximately 2×10^{12} PFU/mL

A total of three out of five mice belonging to this group had increased severity and developed additional sign of exudative accumulation around the eyes. No death was noted. In the rest of the mice severe illness persisted at 72 hours but improved and became normal at 96 hours (►**Fig. 1**).

Bacteriophage Cocktail Dose having 40 µL of Approximately 2×10^{12} PFU/mL

This group receiving 40 µL of approximately 2×10^{12} had severe illness persisting up to 72 hours with the peak at 24 hours. In this group also severe illness continued up to 72 hours. However, all of them became healthy after 96 hours.

Bacteriophage Cocktail Dose having 20 µL of Approximately 2×10^{12} PFU/mL

This group receives 20 µL of approximately 2×10^{12} of the cocktail. All the five mice had all the signs of severe infection at 24 hours after the start of the therapy. The sickness persisted up to 72 hours with complete recovery at 96 hours (►**Fig. 2**).

Table 2 The effect of bacteriophage therapy in different experimental groups along with criterion for scoring

Observation made after intervention with bacteriophages at 12, 24, 48, 72, and 96 hours					
A. Bacteriophage cocktail given in the volume of 100µl containing 10^{11} PFU (10 mice in each group)	12	24	48	72	96
I) Simultaneous administration of bacteriophage and *Pseudomonas aeruginosa challenge*	2[a]+2+3+3+3 +2+3+2+2+3 **(2.5)**	3+3+3+3+3+3 +3+3+3+3 **(3.0)**	2+3+3+3+2+2 +3+3+2+3 **(2.6)**	1+1+1+1+1+1+ 1+1+1+2 **(1.1)**	1+1+1+1+1+1 +1+1+1+1 **(1.0)**
II) Bacteriophage cocktail 6 h before bacterial challenge	2+2+3+3+3+3 +3+3+3+3 **(2.8)**	3+3+5+3+3+5 +3+3+3+3 **(3.4)**	2+2+5+3+3+5 +2+3+3+3 **(3.2)**	2+2+5+2+2+5+2 +2+2+2 **(2.6)**	1+1+5+1+1+5 +1+1+1+1 **(1.8)**
III) Bacteriophage cocktail 6 h after bacterial challenge	5+5+3+3+5+5 +3+2+5+3 **(3.9)**	5+5+3+3+5+5 +5+3+5+3 **(4.2)**	5+5+3+3+5+5+ 5+3+5+3 **(4.2)**	5+5+2+1+5+5 +5++5+1 **(3.5)**	5+5+1+1+5+5 +5++5+1 **(3.4)**
IV) Bacteriophage cocktail 12 h after bacterial challenge	2+3+3+3+3+3 +3+2+3+3 **(2.8)**	3+3+3+3+3+3 +3+3+3+3 **(3.0)**	2+3+3+3+3+3 +3+3+3+3 **(2.9)**	1+2+1+1+1+2+1 +2+1+1 **(1.3)**	1+1+1+1+1+1 +1+1+1+1 **(1.0)**
V) Bacteriophage cocktail 24 h after bacterial challenge	2+2+3+3+3+3 +3+2+3+3 **(2.7)**	3+3+3+3+3+3 +3+3++3 **(3.0)**	2+2+3+3+3+3 +2+3+2+2 **(2.6)**	1+2+1+1+1+1+1 +1+1+3 **(1.3)**	1+1+1+1+1+1 +1+1+1+1 **(1.0)**
B. Bacteriophage cocktail given in the volume of 100µl					
IIIA) Bacteriophage cocktail 6 h after bacterial challenge (dose $1.6x10^{10}$ PFU)[b]	2+3+2+3+3 **(2.6)**	3+3+3+3+3 **(3.0)**	1+1+1+1+1 **(1.0)**	1+1+1+1+1 **(1.0)**	1+1+1+1+1 **(1.0)**
IIIB) Bacteriophage cocktail 6 h after bacterial challenge (dose $2.5x10^{9}$ PFU)[b]	2+2+2+2+2 **(2.0)**	2+1+1+2+2 **(1.6)**	1+2+1+2+2 **(1.6)**	1+1+1+1+1 **(1.0)**	1+1+1+1+1 **(1.0)**
IIIC) Bacteriophage cocktail 24 h after bacterial challenge (dose $5x10^{8}$ PFU)[b]	2+2+2+3+3 **(2.4)**	2+2+3+3+3 **(2.6)**	2+2+3+3+3 **(2.6)**	1+1+2+2+1 **(1.4)**	1+1+1+1+1 **(1.0)**

Note:1–Normal; 2–Slight illness, lethargy, ruffled fur; 3–Moderate illness, severe lethargy, ruffled fur and hunched back; 4-Severe illness with above sign, exudative accumulation around eyes; 5- Death; Figure in parenthesis shows the average of the signs of all the 5 mice in a particular study group.

[a]Grading of diseases.

[b]Experiment done on 5 mice only.

Discussion

This study was aimed to evaluate the efficacy of bacteriophage cocktail as an alternative antibacterial therapy to deal with the MDR/XDR/PDR strain of *P. aeruginosa* leading to severe morbidity and mortality in clinical settings, especially septicemia and pneumonia. The focus of the study was to evaluate the protective effect when phage therapy is started at different time intervals after setting up the infection and also to have clues regarding dosage of the cocktail.

For the purpose, *P. aeruginosa* isolate resistant to all the available antipseudomonal drugs was picked up. The dose of 100 µL (10^{9} CFU/mL) of the bacterium was found to result into 100% mortality between 24 and 48 hours when injected intraperitoneally. On postmortem examination pure *P. aeruginosa* isolation from all the vital organs established the cause of death due to the bacterium. Further, we prepared the cocktail of the three different and the most virulent bacteriophages and tested them for safety in five healthy mice injecting through IP route. We observed no adverse effect. To assess the efficacy of cocktail at a random concentration (10^{12} PFU/mL) administered at different time points as patient may report to intensive care units at different intervals after ensuing the *P. aeriginosa* septicemia. Five different sets of experiments having 10 mice in each group were performed. The first group received bacteriophage cocktail as prophylactic antibacterial administered simultaneously with the challenge dose of the bacterium considering that infection caused by *P. aeruginosa* may occur soon after inflicting the wound. We found that the severity of disease was minimal resulting into no mortality. This observation indicates that the

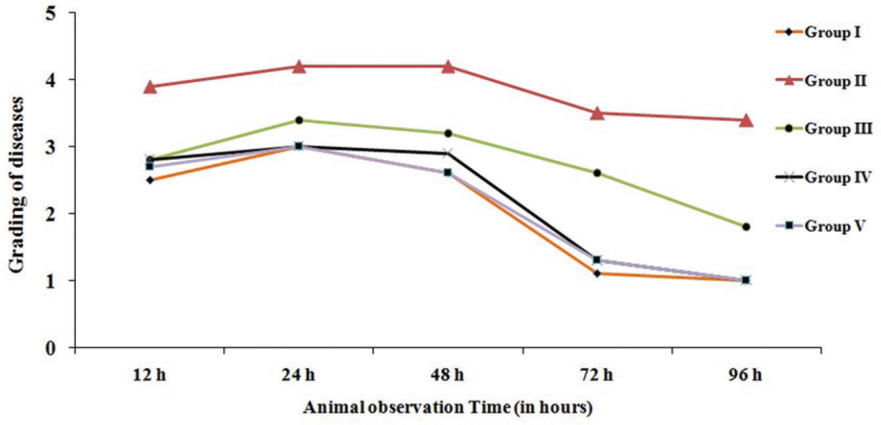

Fig. 1 Effect of pseudomonas aeruginosa and bacteriophage cocktail for clinical grading of diseases based of time of inoculation. Disease grading denoted by numbering 1: normal; 2: slight illness, lethargy, and ruffled fur; 3: moderate illness, severe lethargy, ruffled fur and hunched back; 4: severe illness with above sign, exudative accumulation around eyes; 5: death. Group I: bacteriophage (Ø) + bacteria simultaneously; group II: pretreatment of Ø 6 hours before bacterial challenge; group III: Ø 6 hours later bacterial challenge; group IV: Ø 12 hours later bacterial challenge; group V: Ø 24 hours later bacterial challenge.

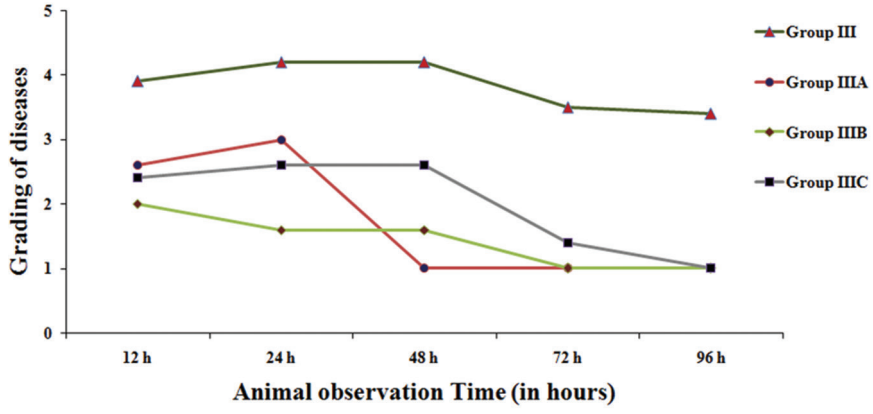

Fig. 2 Effect of pseudomonas aeruginosa at reduced dosage when bacteriophage cocktail was given 6 hours later bacterial challenge for clinical grading of diseases based on the time of inoculation. Disease grading denoted by numbering 1: normal; 2: slight illness, lethargy, and ruffled fur; 3: moderate illness, severe lethargy, ruffled fur and hunched back; 4: severe illness with above sign, exudative accumulation around eyes; 5: death. Group III: bacteriophage (Ø) cocktail (100 µL) given 6 hours later to bacterial challenge; group IIIA: Ø cocktail (60 µL) given 6 hours later to bacterial challenge; group IIIB: Ø cocktail (40 µL) given 6 hours later to bacterial challenge; group IIIC: Ø cocktail (20 µL) given 6 hours later to bacterial challenge.

P. aeruginosa specific bacteriophage cocktail prevents the death if given in advance before the infection sets in. However, in the second group when the bacterial challenge was given 6 hours before the bacteriophage cocktail, the mortality in the mice was 60%. The third experimental group, the bacteriophage cocktail given 6 hours before the bacterial challenge, led to death of 20% of the mice. However, when same dose of cocktail was evaluated after 12 and 24 hours of the initiation of the infection process, there was no mortality in both the groups. This might have occurred because of the fact that around 6 hours of the bacterial and phage challenges, the dosage of cocktail and *in vivo* multiplied *P. aeruginosa* might have reached at the optimal concentration (zone phenomenon) leading to massive lysis causing severe endotoxic shock. While in groups 4 and 5, the bacteriophages might have been adsorbed on the host bacteria replicating gradually and causing gradual lysis of the bacteria. This assumption made us to reduce the doses of cocktail in volume to in three different groups of mice to 60, 40, and 20 µL in place of 100 µL given 6 hours later to bacterial challenge. These decrease dosage delivered phages at the concentration of 1.6×10^{10}, 2.5×10^{9}, and 5×10^{8} PFU in place of 10^{11} PFU. This modification caused no death in the challenged mice 6 hours before the cocktail, although recovery was delayed with the lowest dosage. No death after decrease in dosage might have occurred on the hypothesis that the septicemia being an acute condition, even the small dose of phages replicating in the blood and other tissues where *P. aeruginosa* are actively multiplying and this will slow down the release of the endotoxins and also cure the infection without causing endotoxic shock as liver and kidney are able to tackle the situation. This suggested that the lower dosage of the bacteriophage cocktail may be better with less risk of mortality. It is preliminary study in the area of septicemia and phage therapy and therefore many more questions remain to be answered. Importantly, the question that

why there was mortality in particular group/s? Is it the sudden bacterial lysis leading massive release of endotoxins and other toxins which is stimulating release and exhaustion of cytokines (tumor necrosis factor [TNF]-α, INF-β INF-γ, interleukin [IL]-1, IL-6, etc.) and also causing multiorgan failure? This phenomenon has also been speculated in cases of clinical septicemia where antibiotics acting on cell wall (penicillin, cephalosporins, and carbapenems) are administered as a bolus.[22] There is another study in healthy rats, where intravenous bolus of 30 mg of ceftazidime per kg caused to a substantial increase in IL-6 and TNF-α concentrations in serum.[23] This observation suggests that the increase in parameters of inflammation occurring after initiation of ceftazidime therapy may be a consequence not only of the release of proinflammatory bacterial compounds. Whereas cell wall-active antibacterials can temporarily enhance the liberation of toxic or proinflammatory bacterial compounds, bactericidal antibiotics acting by the inhibition of RNA, or protein synthesis or DNA replication (rifamycins, macrolides, clindamycin, ketolides, and quinolones) delay or even circumvent bacterial lysis.[24]

However, this speculation needs to be confirmed by estimating the counting of bacteria and bacteriophages at different intervals, estimating the endotoxins levels, and proinflammatory cytokines at variable dosage of the bacteriophages. Here, it is pertinent to mention that similar observation has been made with phage therapy in *Acinetobacter baumannii* immunecompromised mouse septicemia model (unpublished data).

Therefore, it may be suggested to see the levels of bacteremia and monitoring of different cytokines and endotoxin in mouse septicemia model to translate the phage therapy in life threatening *P. aeruginosa* septicemia. The possibility of gradual increase in cocktail dosage or single small dose may be explored for its safety and efficacy also.

Conclusion

In conclusion, this study strongly supports the use of bacteriophages as therapeutic agents to combat MDR, XDR, and PDR *P. aeruginosa* infections in immunocompromised patients.

Note

The author was selected for Dr. Pran Nath Chhuttani Oration for the year 2018-2019.

Ethical Approval

The study protocol was approved by Institute Animal Ethics Committee.

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Conflict of Interest

None declared.

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Management of Laryngotracheal Stenosis: A 10-Year Study on the Role of Stents

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Abstract

Introduction Laryngotracheal stenosis (LTS) is mostly due to road traffic accidents, prolonged intubation, and tracheostomy.

Objectives This study focused on a 10-year experience on the role of stents in the management of LTS in a tertiary referral hospital. The aims of this study were to study the internal dimensions of the subglottis and upper trachea in the Indian adult population; to study the mucosal response to injury to the subglottis and the trachea; and to develop an ideal stent for use in LTS in a rabbit model.

Materials and Methods The authors have been treating patients with LTS since 2000. The present study deals with the experience of 82 cases of LTS treated over the past 10 years using stents as well as surgical procedures such as Shiann Yann Lee tracheoplasty and tracheal resection and anastomosis. The work also involved a focused research on LTS using rabbits by inducing injury to the mucosa of the upper trachea and subglottis and histological study of the response to injury. The study led to the development of new indigenous stent for use in rabbits to prevent LTS. This stent has been patented.

Statistical Analysis The study of the subglottis and upper tracheal discussion have been analyzed and proved that the dimension in the Indian adult population was smaller than in Caucasians. The mucosal inflammation following injury at the subglottis and upper trachea can be reversed by topical application of triamcinolone, which was better than Mitomycin on statistical analysis.

Results The quality of life measured after treatment of LTS using stents and other methods of reconstructive surgery has given an overall benefit of 90% with no mortality.

Conclusion LTS is a preventable disease. It can be prevented by proper care of patients in ICU and correct technique of endotracheal intubation and tracheostomy. Further, it can be avoided using medications during extubation and proper decannulation protocol. The silastic "T" tube stents are useful to improve the quality of life in these patients.

Keywords

- laryngotracheal stenosis
- upper tracheal dimensions
- postextubation laryngotracheal injury
- Montgomery T-tube laryngotracheal stents
- Shiann Yann Lee tracheoplasty
- cricotracheal resection and anastomosis

Introduction

Laryngotracheal stenosis (LTS) may be caused by trauma or by a benign or malignant lesion causing narrowing of the airway. Benign stenosis is mostly iatrogenic (prolonged endotracheal intubation, tracheostomy, etc.). Several modalities of management of LTS have been described in the literature. This in itself bears testimony to the complexity of the problem and the difficulty in restoring a normal airway. No single approach gives predictably satisfactory results. The surgical treatment has to be individualized for each patient. At present, the optimal treatment in severe LTS[1] and procedure of choice[2] is resection anastomosis. However, less severe degree of stenosis and in patients with comorbid conditions, a more conservative approach like stenting of the airway can

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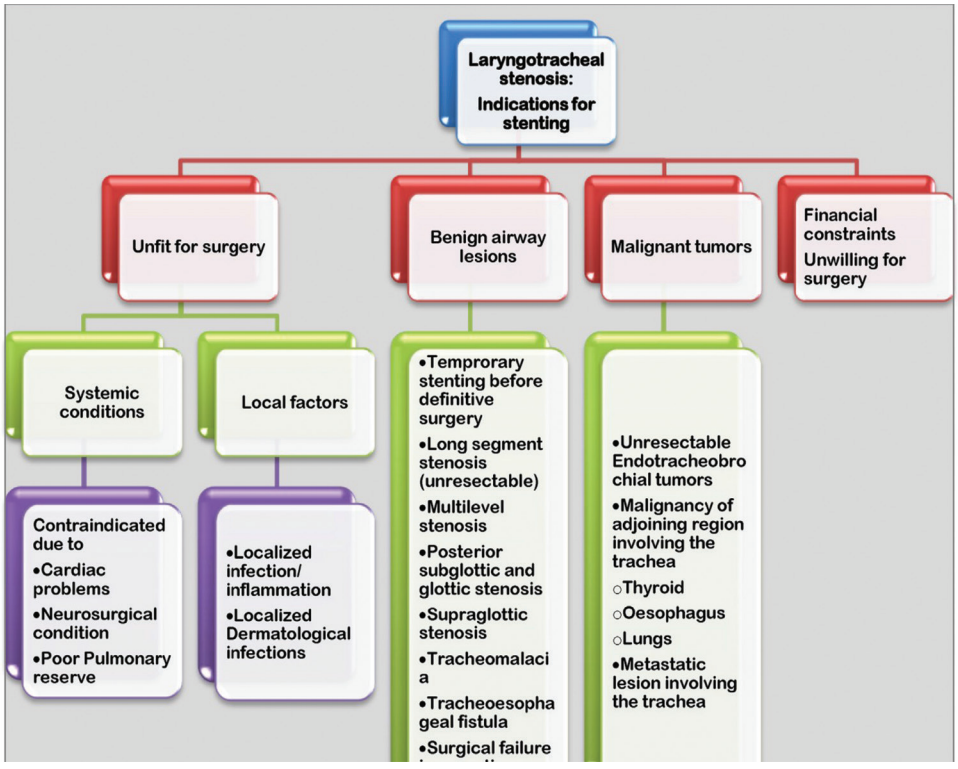

Fig. 1 The adopted flowchart for indications for stenting in laryngotracheal stenosis used in the study.

be successful. Stents need to be used intelligently.[3] They may be used for temporary stenting for restoration of airway or as a definitive permanent stenting to maintain airway.

Materials and Methods

Our experience with management of LTS dates back to 2000 when a significant number of tracheotomized patients could not be decannulated due to either subglottic or tracheal stenosis or both. Most of these patients had either been ventilated in the intensive care unit for a prolonged period or had undergone emergency tracheostomy. They were either left with permanent tracheostomy or died due to pulmonary infection leading to septicemia. An initial strategy in treating these patients was confined to excision of granulation tissue on multiple sittings or fitting the tracheostomy tube with a speaking valve. Since 2002, the senior author adopted the technique of tracheoplasty described by "Shiann Yann Lee" in selected cases of tracheal stenosis. The initial success spurred us to use it in more extensive (grade 3 and 4) cases of subglottic and/or tracheal stenosis (►**Fig. 1**). But, the success was limited due to complications arising out of the "T"-tube.

A retrospective analysis of 51 patients treated from 2004 to 2011 was done. The aim was to identify the indications, complications, and outcome of patients of LTS managed with or without Montgomery T-tube stenting and review the current literature with regard to the role of stenting in LTS. Retrospective chart reviews of 51 patients of LTS who were managed by us during the period 2004 to 2011 were included in the study. After a patient is diagnosed as a case of LTS, further evaluation is done by flexible and rigid endoscopy to assess the movement of vocal cord, type of stenosis, the grade of stenosis, length of stenosis, involvement of the posterior glottis and subglottis, etc. Computed tomography (CT) scan with three-dimensional reconstruction is also performed. A decision is then taken as to the appropriate procedure to be followed. One patient had a posterior glottic stenosis and was managed by laserization of the cicatricial scar without stenting. Two patients who had suprastomal anterior wall collapse were managed by tracheoplasty without stenting. Two patients who had posterior glottic and subglottic stenosis (SGS) had undergone excision of scar tissue followed by stenting with Hood's laryngeal stent. All these five patients were excluded from the study. Eight patients underwent tracheal resection anastomosis, of which one patient had wound dehiscence and required T-tube stenting; hence, seven patients were excluded and one was included in the study. A total of 39 patients who were stented with Montgomery T-tube either for temporary or for definitive treatment were further reviewed and analyzed in this study.

Methodology for Insertion of T-Tube

We used a very simple technique for insertion of T-tube. Initially, a tracheostomy was performed under local anesthesia to secure the airway. All attempts were made to open the trachea at the site of stenosis or just below it. One to 2 mL of 4% lignocaine is instilled in the trachea. The suprastomal trachea and the infrastomal trachea are inspected using a 70-degree nasal endoscope. Stenotic segment was opened by splitting or by tracheoplasty. Granulation within the trachea was removed under vision, using adrenaline-soaked patties over the granulations to reduce bleeding. The vertical limb of the T-tube was smeared with lignocaine gel. The lower part of the vertical limb was first

introduced into the infrastomal trachea. We generally avoid splitting of normal tracheal rings. Then, the upper limb of the T-tube was held with vascular forceps and introduced suprastomally. Using a 70-degree rigid nasal endoscope through the T-tube, the placement of the T-tube is ascertained, care is taken that the T-tube does not impinge into the glottis (subglottis was avoided if it was disease free). This could be ascertained by visual assessment of the vocal cord mobility and also listening to the patient's voice when he/she phonated. The T-tube could be readjusted, if required. Postoperatively, our patients' required only 1 or 2 days hospitalization for observation. These patients were regularly followed-up, T-tube change is done at 6 monthly intervals, and patient assessed for decannulation when the mucosal regeneration is adequate. We did not close the stoma immediately after decannulation, but observed the patient over a period of 10 days for any narrowing of tracheal lumen after removal of stent. Flap reconstruction of the tracheal stoma was done whenever required. Most of our patients required secondary repair of the tracheal stoma. The data on indications for T-tube stenting, problems/complications of stenting, duration of stenting, and outcome of management were collected, tabulated, and analyzed.

Results

Of the 39 patients, 32 had developed LTS postendotracheal intubation stenosis and 6 developed LTS posttracheostomy. One patient had thyroid malignancy with infiltration into the trachea causing stenosis and had been managed by T-tube stenting for palliation. Of the 32 patients of endotracheal intubation, 21 had emergency intubation and 11 had elective intubation. The indications for Montgomery T-tube stenting in these 39 patients is shown in ►**Fig. 1**. It is to be noted that two patients were included in both the temporary and permanent category. This is because to start with we used the T-tube stenting as the primary mode of treatment in both: but one patient developed stenosis at both ends of the tube and one patient developed tracheomalacia and required reinsertion of T-tube. Hence, they had to be included in the permanent group also. The most common site of stenosis in the postendotracheal intubation group was in the trachea (48.5%). All six tracheotomized patients had suprastomal stenosis (100%). Thirty-eight patients had normal vocal cord mobility at diagnosis. One patient had restricted mobility of the right vocal cord due to scarring and adhesions.

Complications

The problems that were encountered due to T-tube stenting is shown in ►**Table 1**. Crusting within the tube was a problem in 43.58% (►**Fig. 2**), troublesome granulation were seen in the subglottis in 33.33% (►**Fig. 3**), persistent cough in two patients (5.1%), surgical emphysema in the immediate postoperative period in two patients (5.1%) (►**Fig. 4**), tracheomalacia in one patient (►**Fig. 5**), and stenosis at both ends of the T-tube in one patient. One patient did not tolerate the T-tube because of excessive crusting, hence we had to maintain her on permanent tracheostomy tube. Thirty-two patients (82.05%) were successfully decannulated and were asymptomatic from 6 months' to 5 years' follow-up. Three patients are still stented with T-tube and one has permanent tracheostomy. Three patients died due to comorbid conditions unrelated to LTS.

Biometric Study of Subglottic and Upper Tracheal Dimensions in Indian Adult Population

When we analyzed the causes for failure of the "Shiann Yann Lee" tracheoplasty, it was postulated that it could be due to a mismatch in the size and shape of the "T"-tube. Hence, we did a radiological imaging study measuring the dimensions of subglottis and trachea in the Indian adult population. CT scan images of the subglottis and

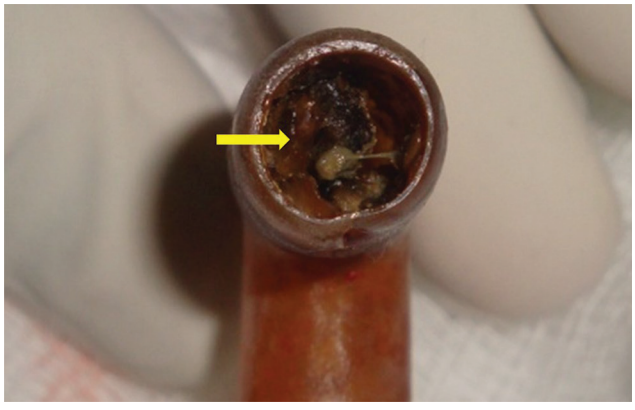

Fig. 2 Crusting within the T-tube.

Table 1 Complications due to T-tube stenting (number of complications out of 51 patients)

Immediate complications (within 24 hours)	Intermediate complications (few days to 6 weeks)	Late complications (few months)
Surgical emphysema (2) Persistent irritant cough (2) Respiratory distress The lower end of the tube entered one bronchus (1) Clots and mucus plug (1)	Hoarseness of voice (1) Respiratory distress Lower respiratory tract infection (3) Crusting and mucus plug (17) Cough with expectoration Lower respiratory tract infection (3) Tracheobronchitis (3) Aspiration (4) Granulations in subglottis and lower end of the tube (14)	Tracheomalacia (1) Restenosis at both ends of the tube (1) Displacement of the tube (1)

Note: Figure in parenthesis shows number of patients in whom the complications were noted.

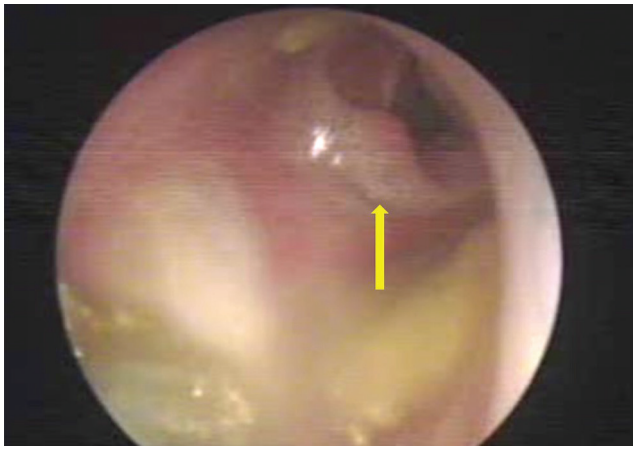

Fig. 3 Granulations in the upper end of T-tube visualized through a 70-degree endoscope.

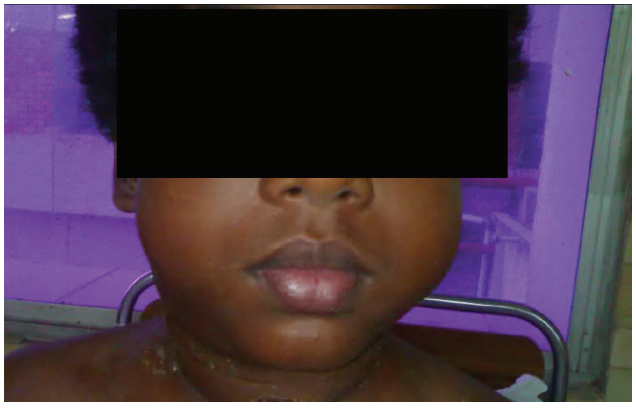

Fig. 4 Surgical emphysema.

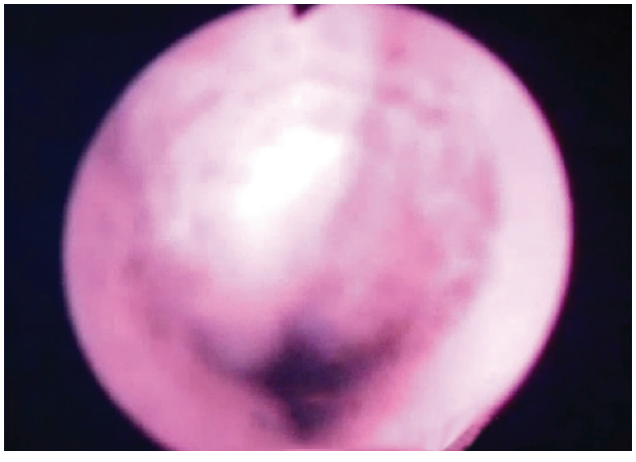

Fig. 5 Tracheomalacia.

trachea of patients undergoing CT scan of neck for other indications were obtained and analyzed by measuring the anteroposterior (AP) and transverse diameter of the trachea at various levels. After comparison with other similar studies (►**Table 2**), it was concluded that the dimensions of the trachea in Indian adult population is lesser when compared with western (Caucasian) population (►**Tables 2** and **3**).[4-10]

Effect of Thermal Injury in the Subglottis of the Rabbit Animal Model

To investigate the etiopathogenesis of the nature of injury that occurs in the subglottis and upper trachea following prolonged intubation and in emergency tracheostomy, research was performed by inducing thermal injury in the subglottis and upper tracheal mucosa of New Zealand white rabbits.[11] This study was conducted at the Central Animal Facility for Toxicology and Developmental Research, Sri Ramachandra University, Chennai, Tamil Nadu, India. Institutional Animal Ethics Committee approval was obtained. The initial studies were performed in postmortem specimens of New Zealand white rabbits weighing 2.0 to 2.5 kg. The cross-sectional diameter of the trachea and subglottis were measured. It was observed that the narrowest part of the rabbit's laryngotracheal airway was the subglottis, measuring on an average 5.8 mm in AP diameter and 5.4 mm in transverse diameter. Histopathological changes were noted at various time intervals after the injury and their response to topical mitomycin or corticosteroid application was studied.

Study on Mucosal Healing Response to Topical Application of Mitomycin versus Triamcinolone in Postendotracheal Intubation Injury to the Subglottis and Upper Tracheal Mucosa

This animal study was focused on the host tissue response to intubation-induced injury resulting in subglottic and upper tracheal stenosis and methods to prevent this complication. Also to assess the role of topically applied mitomycin-C and triamcinolone acetonide in wound healing process following postextubation subglottic and upper tracheal injury. It was a prospective randomized block, single-blinded experimental study. Forty New Zealand white rabbits were block randomized and allocated into four groups based on the type of topical medication that was applied on the mucosa of the subglottis and upper trachea postextubation. Further, these groups were subdivided into three subgroups based on the time of sacrifice (4, 6, and 12 weeks) to study the histopathological changes that occurred in a temporal sequence at the subglottis. It was observed that the rabbits in the control group and those that received mitomycin-C only had more respiratory distress compared with those treated with triamcinolone acetonide. Statistically significant histopathological changes were observed in all the four groups. Mitomycin-C applied topically did not alter the wound healing process following postextubation injury in the subglottis. Triamcinolone acetonide significantly altered wound healing in the subglottis and prevented occurrence of respiratory distress. Mitomycin-C in a dosage of 0.4 mg/mL, applied topically in four or more sittings did not seem to alter the progress of the healing process and did not prevent postextubation SGS in rabbits as evidenced in this study. Triamcinolone acetonide applied topically has shown to be a better modulator of wound healing in the laryngotracheal airway following intubation-induced injury compared with mitomycin-C.

Table 2 Internal dimensions of upper trachea by different studies

Ref no.	Authors	No. of subjects	Age range	Tracheal diameter in mm (coronal)	Tracheal diameter in mm (sagittal)
[4]	Prasanna Kumar and Ravikumar	30 (M) 18 (F)	16–60	11.4–18.2 (16.5 ± 2.38) 8.7–15.34 (17.34 ±3.5)	13.6–19.2 (12.55 ±2.31) 8.3–14.33 (13.05 +±2.35)
[5]	Katz et al	50	Not documented	13–25	Not measured
[6]	Jesseph and Merendino	21 (M) 26 (F)	13–86	15–27 13–25	Not measured
[7]	Greene	60 (M)	66.4	15–26 (19.7 ±2)	18–32 (22.5 ± 2.4)
[8]	Breatnach et al	430 (M) 378 (F)	20–79	13–25	10–23
[9]	Brown et al	40	Not documented	18.4	20.1
[10]	Kamel et al	40 (M) 20 (F)	22–88	20.1–34.5 (27.1 ± 3.4) 17.3–27.8 (22.9 ± 2.6)	16.8–28.6 (22.6 ± 2.9) 12.7–23.8 (19.2 ± 2.6)

Table 3 Adult tracheal dimensions—Sri Ramachandra study (2014)

No. of patients (48)	Age in y	Tracheal diameter in mm (coronal/transverse)	Tracheal diameter in mm (sagittal/anteroposterior)
30 (M) 18 (F)	18–60	11.4–18.2 (16.5 ±2.38) 8.7–15.34 (17.34 ±3.5)	13.6–19.2 (12.55 ±2.31) 8.3–14.33 (13.05 ±2.35)

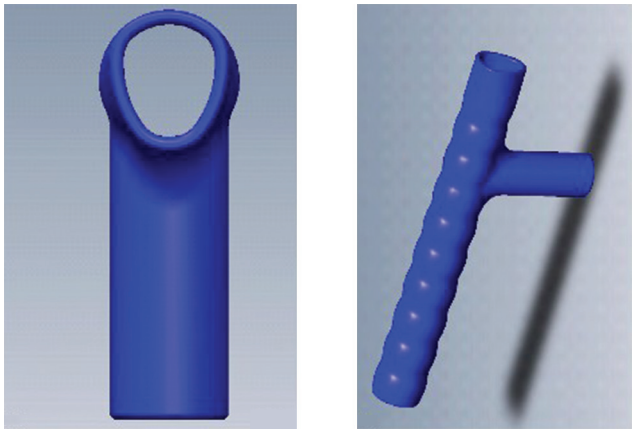

Fig. 6 Design of indigenous T-tube stent.

This research was pursued as a PhD study by the second author under the guidance of the first author.[12] The research work on rabbits led to the conclusion that the thermal injury of the mucosa of the subglottis and upper trachea increases in severity depending on the depth of mucosal damage and becomes irreversible in prolonged thermal or physical damage. This is permanent and unlikely to be reversed by topical application of anti- inflammatory agents such as mitomycin or corticosteroids, although topical application of triamcinolone acetonide had a beneficial effect in the early period after extubation.[13]

Design and Development of Indigenous T-Tube Stent for Rabbit Model

Based on the observations in the animal study and the limitations and complications noted when the Montgomery T-tube was used in patients with LTS, we started probing the alternate method of treatment using silastic stents in subglottic and upper tracheal stenosis. The time-honored Montgomery T-tube[14] made of silastic that we were using was excellent except that the shape was found to be mismatched to that of the subglottis leading to formation of inflammatory granulation tissue at the upper end in the subglottis. Several other stents were developed by other researchers which did not fulfill all the requirements of an ideal stent.[15-25]

Therefore, we decided to develop an indigenous T-tube made of silastic and coated with titanium dioxide on the outside. The raw material was chosen carefully and the shape of the tube was custom-made to suit the inner dimensions of the subglottis and upper trachea (►**Fig. 6**). The external surface was of a wavy pattern to reduce the chances of mucus accumulation and the angle of the horizontal limb was made acute to match the angulation of the trachea (►**Fig. 7**). This tube was tried out in the rabbit model after endotracheal tube-induced mucosal damage leading to stenosis of the subglottis and upper trachea. It proved to be effective and the statistical analysis of the results proved its benefits in reducing the incidence of stenosis by applying topical triamcinolone in this animal model. After adequate follow-up, it was concluded that the newly developed indigenous T-tube was more effective in subglottic and upper tracheal stenosis in rabbits that were used in this study.[13] This stent has been patented. The patent was filed on August 23, 2013 and published on February 27, 2015; Official Journal of the Patent Office, Issue no.09/2015, p. 26968; Appl. no.3728/CHE/2013 A.

We plan to continue this study by developing a stent for use in human patients affected by subglottic and upper tracheal stenosis. It is expected that this would be a cheaper and more effective T-tube than the Montgomery T-tube that is being used nowadays.

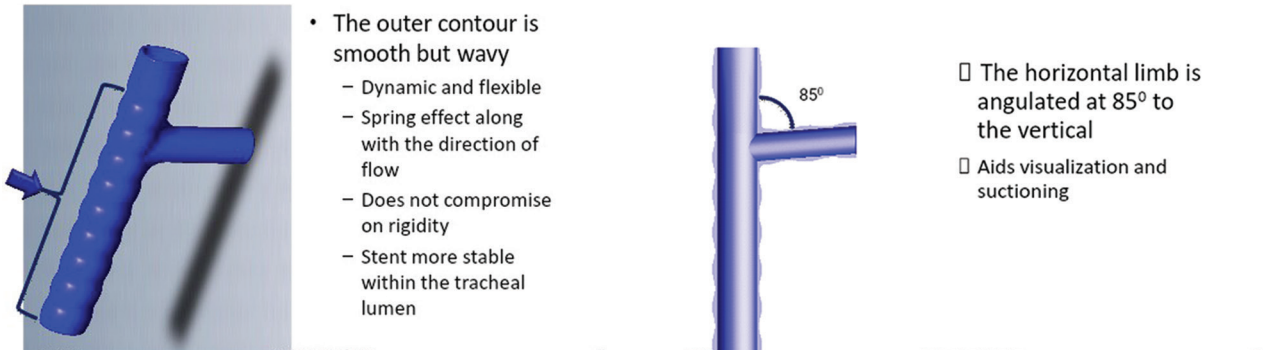

Fig. 7 Unique characteristics of the new stent.

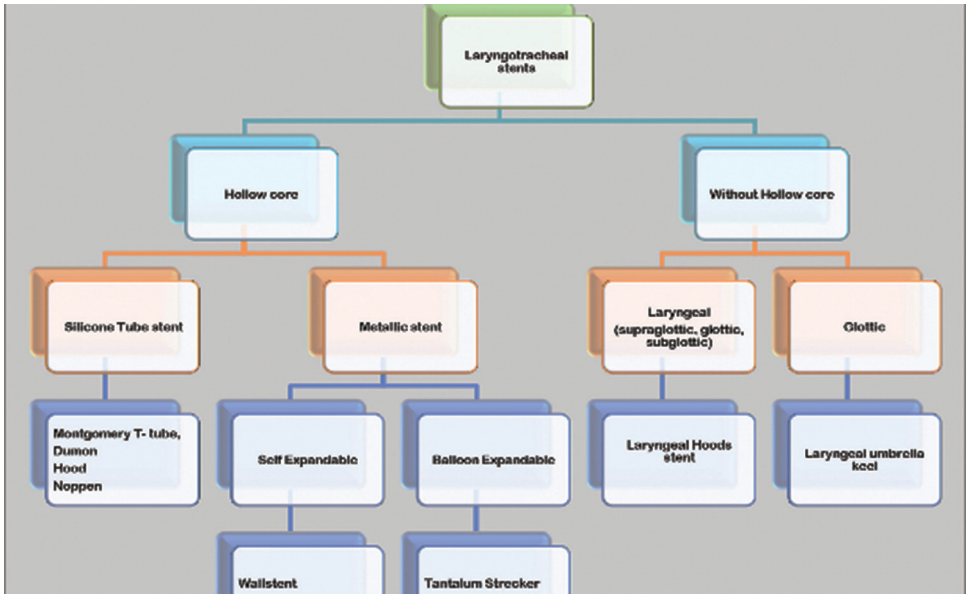

Fig. 8 Types of laryngotracheal stents.

Discussion

Laryngotracheal injuries that occur due to endotracheal intubation or due to tracheostomy may result in temporary reversible laceration and edema or leave behind a chronic scar resulting in LTS. Cooper and Grillo in 1969 analyzed and listed the types of injuries in postmortem specimens of patients who were ventilated using a cuffed endotracheal tube or cuffed tracheostomy tube.[26]

There was an alarming increase in the incidence of LTS especially in children following endotracheal intubation which Othersen called it "a new epidemic."[27] The reported incidence of laryngotracheal injuries following endotracheal intubation varied from 62[8] to 94% for temporary laryngeal injuries[28] and 10 to 20% of these result in permanent laryngeal sequelae such as LTS. The incidence of laryngotracheal injuries reportedly had reduced after the use of "high-volume low-pressure cuffed endotracheal tubes." Incidence of SGS in the 1970s and 1980s was around 0.9 to 8.3% in neonates. Schweiger et al have reported a high incidence (11.38%) of SGS in children who were endotracheally intubated.[29]

Several factors influence the severity of injury and the progress to formation of SGS. The most critical risk factor for postintubation SGS in neonates and children is the size of the tube.[30,31] To have documented evidence of normative data on the dimension of the endoluminal dimension of the subglottis and upper trachea in Indian population, a CT study was undertaken by us which showed that compared with the western literature the dimension of the subglottis and upper trachea in adult Indian population was less as shown in ►**Table 3.**

The management of LTS is varied and needs to be catered to each patient's needs, depending on the type and severity of stenosis, and also the existing comorbidity. We have identified the indication for Montgomery T-tube stenting which has been listed in ►**Fig. 1.** The complications that occurred with the use of T-tube that has been listed in ►**Table 1**, and the types of available stents for use in LTS that has been listed in ►**Fig. 8.** [32-34]

Research is on to develop an ideal stent for managing LTS. No ideal stent is available yet.[16-18] At present, the Montgomery T- tube is widely used all over the world for treating LTS.[35-38] Review of literature has shown that silastic is an ideal material that is relatively bioinert and hydrophobic; hence, less of crust

formation.[39,40] On similar lines, we had undertaken a DSIR project and successfully developed an indigenous stent that can be custom-made to the individual and also reduce the potential complications. This stent used in the rabbit model has been patented and a similar stent is being developed for human trials. Drug-eluting stents are being proposed and are on trial to reduce the recurrence of stenosis. As research in this field is minimal, we have described an animal model to produce airway injury to carry out further research in postintubation LTS.[11]

Hirshoren and Eliashar have described the various factors, chemical mediators, and the drugs that modulate wound healing. The most common drugs that are widely used are: corticosteroids and mitomycin-C. The role of both these drugs have been evaluated and debated with no concrete evidence that they influence and modulate wound healing in postintubation LTS.[41] To this end, we have studied the effect of two drugs mitomycin-C and triamcinolone as topical application in postintubation injury in New Zealand white rabbits and have found that triamcinolone showed statistically significant reduction in the occurrence and prevention of stenosis in the early stages.[12,13]

Conclusion

Regular clinical audits during the course of managing patients with LTS resulted in research which identified that the internal dimensions of the subglottis and upper trachea in Indian population is less than that reported in western literature. The management of LTS has to be individualized to cater to the need of the patient. An ideal stent and drug(s) for the management of LTS has not been developed. This opens up a requirement of Fellowship course in India. Hence, to our mind, there is a lot of scope for airway research in India.

Note

The author was selected for Col. Sangham Lal Memorial Oration for the year 2018-2019.

Conflict of Interest

None declared.

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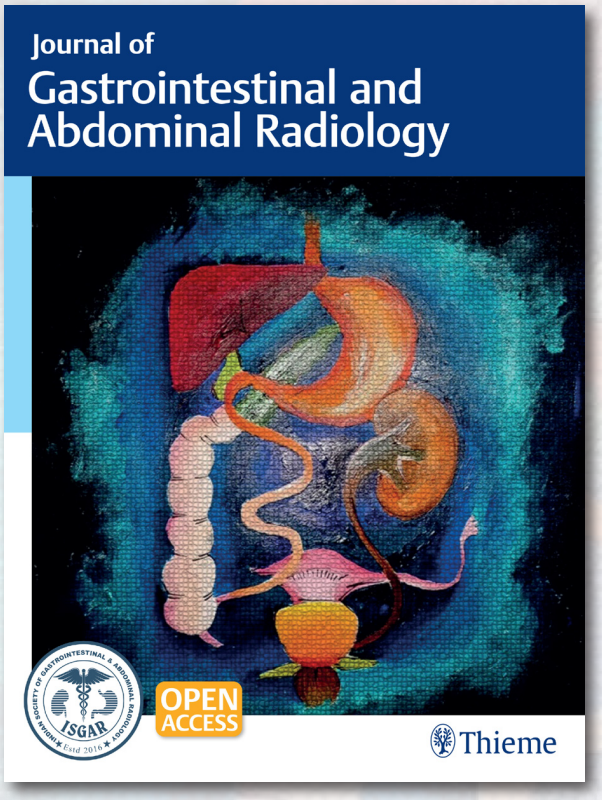

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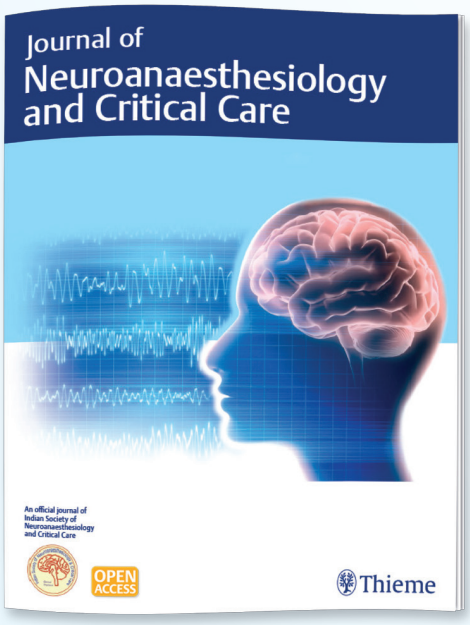

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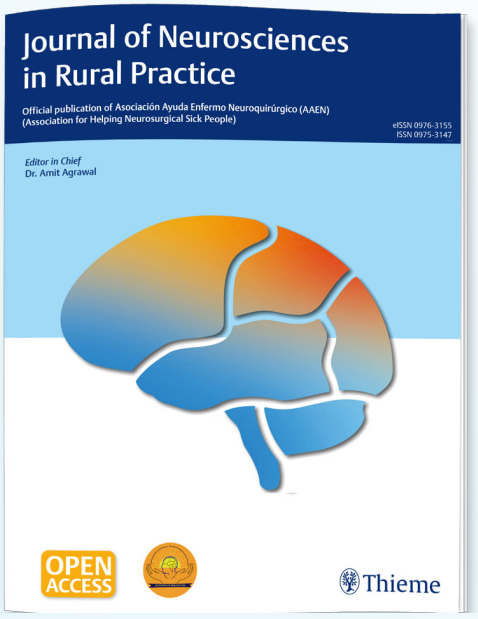

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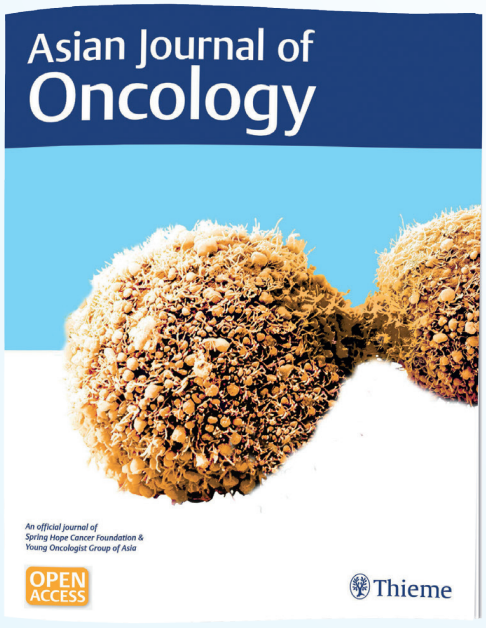

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