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- Clarity in Writing
- Appropriateness of the Language and Style
- Validity and logic of the Study methods, data and the statistical methods
- Support of the Conclusion by reasonable data
- Significance of the information from the readers perspective
- Compliance of the manuscript with the focus and scope of the journal.

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From the Editor's Desk.....-

I am extremely pleased to write this inaugural editorial for the first issue of the Indian Journal of Stem Cell Therapy. The inception for this project occurred last year, during the first national conference of the Stem Cell Society (India). The excitement generated in these scientific sessions re-emphasized that the field of regenerative medicine and stem cell therapeutics was in the midst of an unprecedented explosion of exciting and path breaking work. This was more heartening, since the majority of this work was happening in India. It reconfirmed that "Stem cell therapy is an idea-whose time has come".

The time to translate decades of basic research, hard work, understanding of disease pathology and stem cells has finally arrived. It is widely acknowledged that stem cells as tools for rebuilding tissues/organs has huge potential. This is a revolutionary concept, which is marking a change in the outlook of clinicians towards "incurable disorders", which were till recently, not amenable to any treatment options. Myriads of researchers, scientists, clinicians from India are already working in this field for over 2 decades now. Importantly, India is now emerging as the global leader in the translation of stem cell research. Till date, all new breakthroughs have come from the west and we have followed them. However, in the field of cellular medicine and stem cell transplant, India has taken the lead. We have the unique opportunity in our hands - an opportunity to pave the way for bringing pioneering stem cell research from "bench to the bedside".

Hence, the need of the hour is to put together, all the path breaking work happening in India and present it to the global community.

The Indian Journal of Stem Cell Therapy thereby, endeavours to provide a unique scientific forum that will capitalize on the wealth of new information in stem cell research and cellular therapy happening in India and facilitate its dissemination to the global community. The Indian Journal of Stem Cell Therapy is an initiative of the Stem Cell Society (India) to bring this futuristic field into the present.

IJSCT focuses on the work done in the field of stem cell therapeutics and regenerative medicine for a host of disorders and ailments incurable neurological conditions, haematopoietic disorders, cancers, hepatic disorders, renal disorders, bone and joint related disorders, ophthalmic conditions, cardiac disorders, etc.. The list is exhaustive and so is the potential of stem cell therapeutics.

In the first issue, IJSCT invited the speakers of the 2nd annual conference of the Stem Cell Society (India) to contribute with respects to the work that they have done and reviews on

some of the important progress and new understanding in selected key aspects of stem cell therapeutics. In the following issues, our journal seeks to publish original, high quality, peer-reviewed papers including research articles, reviews, short communications and case reports that will provide comprehensive coverage on all aspects and subspecialties of stem cell therapeutics and basic research which has the potential to translate into therapy. Submissions related to studies on the stem cell transplantations, possible mechanisms of action, route of delivery, objective evidences and outcome measure, side effects monitoring, and related basic research work which has the potential to translate into clinical application will be particularly encouraged.

To ensure the quality of the science we have assembled an outstanding Board of Editors who will be responsible for rapid peer review of all articles. The board comprises of the pioneers in stem cell therapeutics in the country.

In addition, as mentioned above, IJSCT is supported by the Stem Cell Society (India), which brings together all the stakeholders, who have the onus of taking ahead the pioneering work of stem cell translation in India and also is successfully promoting the exchange of stem cell science in India and globally.

I am extremely proud to be the Founder Editor in Chief of this exciting new endeavor and am confident that it will become a highly respected and trusted resource of leading knowledge in stem cell therapeutics, in India and around the world. We trust that the information presented in this publication will serve to make further advances in stem cell therapy and eagerly look forward to seeing your research in our pages.

Dr. Nandini Gokulchandran

The need to review the existing guidelines and proposed regulations for stem cell therapy in India based on published scientific facts, patient requirements, national priorities and global trends

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Abstract

India was amongst the first countries in the world to create a set of dedicated guidelines for stem cell therapy in the year 2002. These guidelines which were created by the Indian Council of Medical Research were comprehensive and progressive when one considers that the field was at that time in its infancy. Subsequent developments including the more recent guidelines created in the year 2007 and 2013 did not keep up with the progressiveness of the initial guidelines. In fact it would not be incorrect to state that the most recent set of guidelines (2013) is regressive and if implemented will destroy the emerging field of regenerative medicine in the country. This paper highlights the limitations and flaws in the existing guidelines and proposed regulations and makes suggestions based on published scientific data from our own clinical work, national priorities, patient requirements and global trends, which could form a basis for a review of our national guidelines and regulations.

Keywords : Ethics, Regulations, Cell Therapy, Stem Cells, Regulatory challenges.

Introduction

Regulations, guidelines, ethics and principles of evidence based medicine form the foundation of modern medical practices. There is no questioning the value of these in maintaining public safety in connection with medical practice. However the emergence of the field of regenerative medicine and cellular therapy has raised new questions about the limitations of the existing systems in the development and availability of emerging technologies. Despite tremendous increase in and availability of both basic science data as well as clinical results of safety and efficacy, the benefits of regenerative medicine are still not available to millions of patients who are potential beneficiaries. A decade ago patients of Duchenne Muscular dystrophy, Motor neuron disease and other incurable

illnesses were dying because there was no treatment available. In 2015 thousands of these patients are dying but the tragedy is that this is happening despite the availability of a treatment whose results have been documented and published. This treatment is Stem cell therapy and it is the guidelines and regulations that are preventing the wider availability of this life saving treatment. It would therefore not be an exaggeration to state that the current regulations and guide lines instead of saving life are actually resulting in the loss of life. Whereas regulatory bodies are correct in having stringent standards to ensure patient safety, we believe there are two sides to this issue. The other side is that many patients are being deprived of treatments that could potentially save their lives, reduce their disability or ease their suffering. A good analogy of what is currently happening would be looking

at a coin. A coin has two sides. But at any one time we can see only one side. Presently the regulators are seeing only one side of the coin and the practitioners of stem cell therapy are seeing the other. But a coin to be whole needs both sides. It is important therefore that both sides (regulators and doctors) agree to look at both sides of the coin and come to a balanced approach. Regulators need to be open to the reality that stem cell therapy is here to stay and should be open to accepting newer indications for this treatment based on safety records, evolving trends and published literature. They need to recognize that stopping or preventing the practice of stem cell therapy in newer indications could do more harm and result in more deaths than its being more easily available. On the other hand Stem cell experts need to accept there need to be checks and balances in place through guidelines and regulations to ensure patient safety and prevent patient exploitation.

The present situation in India with regards to guidelines for stem cell therapy

1. The National guidelines for stem cell research have been formulated by the Indian Council of Medical Research and the Department of Biotechnology in 2013 [1]. These guidelines have retained the 2007 classification of stem cell research into 3 categories namely permitted, restricted and prohibited [2]. However, it has introduced an additional layer of oversight besides the institutional ethics committee (IEC) in the form of Institutional Committee for Stem Cell Research (IC-SCR) and the National Apex Committee for Stem Cell Research and Therapy (NAC-SCRT). A major recommendation has been to omit the word therapy from the title of the guidelines.
2. The Ministry of Health and Family Welfare, Government of India, established a High Powered Committee in June 2013 to suggest a road map for regulation of stem cells and other cell based therapies being practiced in India. Under the chairmanship of Professor Lalji Singh it submitted a Guidance Document for Regulatory Approvals of Stem Cell and Cell Based Products (SCCPs) in December 2013. This Guidance Document

is based on the recommendations of that committee and it is subsidiary to the amendments made in 2013 to the Drugs and Cosmetics Act (DCA), 1940 and the new rules proscribed there under. As per these amendments it has been decided that Government of India, through the DCG (I) and CDSCO, shall regulate all practices related to the use of stem cells, and other cells, for therapeutic purposes in India. The amendment in DCA also mandates that all stem cells and cell based products that can be used for therapeutic purposes shall be referred as Stem Cell and Cell Based Products (SCCPs) and all activities related to their usage i.e. manufacture/isolation/ collection, storage and transplantation into patients must be done only under a license or permission that would be granted by the DCG(I)/CDSCO [3].

3. Another important and major development has been the proposal of the Drug Controller General of India DCG(I) to include "stem cells" in the definition of new drugs in the proposed bill titled "Drugs and Cosmetics (Amendment) Bill 2015" [4].

The flaws in the existing guidelines & proposed regulations

1. The current guidelines are uniformly applicable to "Autologous" and "Allogenic" Stem Cells. Autologous Stem Cell Therapy has been practiced for various hematological conditions for the last 3 decades and for other incurable conditions over the last 5 to 10 years. They have a proven track record of safety. On the other hand allogenic stem cells are manufactured commercially by companies and so it may be appropriate to consider them as a new drug. The use of autologous stem cells is a form of therapy and since they are not manufactured commercially, they should not be considered as a product or drug.

We, therefore, suggest that instead of including 'stem cells' in the category of new drugs, it should be changed to "allogenic stem cells". By doing this, stem cells that are being manufactured and sold by companies and are therefore a product will come under

the category of new drug whereas autologous stem cells which are being used by individual doctors will not be included in this category.

2. All medical research is done with the intention of developing newer therapies. So we should be moving from research to therapy. Our 2007 guidelines include therapy but our 2013 ones don't. By dropping the word therapy in the 2013 guidelines, we have taken a step backwards instead of moving forwards. This needs to be reviewed.
3. The foreword to the 2013 National guidelines for stem cell research makes sweeping unjustified and unsubstantiated statements about the clinical indications for stem cell therapy. Deciding what therapy is and what is not therapy comes under the purview of the medical community and treating doctors and this keeps changing with newer developments and publications. The strong inappropriate words and statements made in the foreword would effectively stop all stem cell therapy advancements in the country. The foreword itself reveals the anti stem cell therapy bias that the guidelines have. This needs to be withdrawn and corrected based on the existing scientific medical literature.
4. Whereas the guidelines do make a distinction amongst different stem cell types however when it comes to the practical implementation of policy, all the cell types seem to get bundled into one. This would be like having a common set of regulations for alcohol, aerated drinks like Coke / Pepsi and homemade orange juice calling them all beverages. Stem cell could be broadly divided into three types. Embryonic, umbilical and adult stem cells. Embryonic stem cell could be compared to alcohol, Umbilical cord stem cells to Cold drinks like Pepsi / Coke and Adult autologous stem cells to homemade fruit juice. Whereas alcohol is potentially dangerous and there should definitely be tight regulations so also embryonic stem cell work should be tightly regulated. Cold drinks may not be dangerous but can be harmful so there should be quality checks in place, so also for Umbilical cord cells there should be quality checks in place and these types of cells should be treated like drugs/ medicines and the same regulations and quality control systems should be in place for them. However there is no need for any strict regulations for home made orange juice and so autologous adult cells should be freed up from regulations and their availability in fact encouraged since they are completely safe and have shown clinical benefits in many conditions in various published scientific papers. If one were to use a traffic light as an analogy we would suggest that there be a green light for autologous, orange light for umbilical cord and red light for embryonic stem cell therapy.
5. The views of the main stakeholders i.e. [1] patients who are suffering from diseases that stem cell therapy could benefit & [2] Doctors who are practicing stem cell therapy have not been taken. Guidelines should not be framed by a handful of researchers and academicians without taking into account the views of the people directly involved in the field. The present committees (including NAC-SCRT) lack clinical experts from different specialties with experience and publications in this field. It is important that any review takes into account their views. Publications in this field particularly from India but from the rest of the world as well should be studied whilst drafting/reviewing any guidelines.

The scientific basis for the need to relook at the current guidelines

There is enough published scientific evidence in medical journals and textbooks to justify the need to make the newly developed cellular therapies more readily available to the patient population. This is more specifically required for those medical conditions for which there are no other treatments available. Whereas, there is a lot of evidence from across the world as well as from India; we now present our own published clinical results in various incurable neurological conditions.

Our own documented and published scientific work is summarized to establish the scientific basis to seek a review of the existing guidelines

1. Autism

Sharma et al 2013, published a clinical study which was an open label proof of concept study in 32 patients of autism. They administered autologous bone marrow mononuclear cells (BMMNCs) intrathecally in 32 patients with autism followed by multidisciplinary therapies. All patients were followed up for 26 months (mean 12.7). The outcome measures used were Childhood Autism Rating Scale (CARS), Indian Scale for Autism Assessment (ISAA), Clinical Global Impression (CGI), and Functional Independence Measure (FIM/Wee-FIM) scales. Positron Emission Tomography-Computed Tomography (PET-CT) scan recorded objective changes. It was found that out of 32 patients, a total of 29 (91%) patients improved on total ISAA scores and 20 patients (62%) showed decreased severity on CGI-I. On CGI-II 96% of patients showed global improvement. The efficacy was measured on CGI-III efficacy index. Few adverse events were reported, including seizures in three patients, but these were reversible and easily controlled with medications. The encouraging result of this leading clinical study provides future directions for application of cellular therapy in autism [6].

In addition to the above case series, 5 separate case reports by Sharma et al, [7,8,9,10,11] have also been published documenting the safety, efficacy and objective radiological improvements in patients of Autism following cell therapy.

The results of Sharma et al are coherent with findings of Siniscalco et al. 2012 and Yang-Tao et al, 2013 [12,13].

2. Cerebral Palsy

Sharma et al in 2015 carried out an open label, nonrandomized study on 40 cases of all types of cerebral palsy treated with autologous bone marrow mononuclear cells intrathecral administration. Three months after intervention, 14 patients showed improvement in oromotor activities, 11 in neck control, 17 in sitting balance, 15 in standing balance, 9 in walking balance, and 12 in speech. At six months, 38 out of 40 (95%) patients showed improvements and 2 did not show any improvement but remained stable without any deterioration. No major adverse events were noted except for seizures in 2

patients which were self limiting and were controlled by medications. The study, thus demonstrated the safety, feasibility, and efficacy of the intervention [14].

Apart from the above case series, 3 separate case reports by Sharma et al, [15,16,17] have also been published documenting the safety, efficacy and objective radiological improvements in patients of Cerebral Palsy following cell therapy.

The findings of Sharma et al. are similar to those of Chen et al., 2010 [18] & Chernykh et al., 2014 [19] that demonstrate the safety and efficacy of stem cell therapy in cerebral palsy.

3. Muscular dystrophy

Sharma et al 2013, carried out a study in 150 patients with muscular dystrophy which included Duchenne Muscular Dystrophy, Limb Girdle Muscular Dystrophy and Becker Muscular Dystrophy variants. They were administered with autologous bone marrow derived mononuclear cells intrathecally and intramuscularly. On a mean follow up of 12 months \pm 1 month, overall 86.67% cases showed symptomatic and functional improvements, 53% cases showed increase in trunk muscle strength, 48% showed increase in upper limb strength, 59% in lower limb strength and about 10 % showed improved gait. Patients showed shift on assessment scales such as Functional Independence Measure (FIM) and Brooke & Vignos scale. 6 patients showed changes on musculoskeletal Magnetic Resonance Imaging (MRI) with respect to muscle regeneration and decrease in fatty infiltration and 9 showed improved muscle electrical activity on Electromyography (EMG). The results show that this treatment is safe, efficacious and also improves the quality of life of patients suffering from Muscular Dystrophy. No significant adverse events were noted [20].

In another study, Sharma et al 2015, carried out a study in 59 patients with limb girdle muscular dystrophy (LGMD) who underwent autologous bone marrow mononuclear cells intrathecal transplantation and extensive rehabilitation. At a follow up of 9 months to 4.5 years, there was a statistically significant improvement in the muscle strength of major body muscles. The results showed maintained FIM scores and thus,

maintained function in patients over time which suggested achievement of plateau phase in the disease progression [21].

Safety and efficacy of cellular therapy has been documented with 4 other case reports by Sharma et al, [22,23,24,25] apart from the case series mentioned above.

4. Traumatic spinal cord injury

In a study published by Sharma et al in year 2013, fifty six chronic cervical spinal cord injury patients were treated with autologous bone marrow mononuclear cells intrathecally. On a mean follow up of 2 years \pm 1 month, out of the affected patients improvement was seen in 92.31% in trunk stability, 87.5% in sitting balance, 77.78% in trunk muscle strength, 52% in upper limb strength, 48.21% in standing balance, 21.57% in sensation, 20.59% in bladder sensation, 18.37% in spasticity and 14.29% in walking balance. All the patients who suffered from postural hypotension showed an improvement

On ASIA scale, two patients showed a change from level B to C and one from level A to B and one from C to D. On FIM scale, 24 out of 56 patients showed an increase in the score[26].

Sharma et al carried out a study which was published in 2013, 110 patients with thoracolumbar SCI were administered autologous bone marrow derived mononuclear cells intrathecally. On a mean follow up of 2 years \pm 1 month, 100 cases (91%) showed symptomatic, investigational, and functional improvement. A reduction in spasticity was found in 26% of cases, partial sensory recovery in 28%, improved trunk control in 96%, and less postural hypotension in 100%. There was also an improvement in bladder management with respect to a shift from indwelling and condom catheters to intermittent self-catheterization in 33% of cases. Further, 22% of wheelchair-bound cases started walking and 60% of patients whose activities of daily living were affected showed improvement. Two of the 110 patients shifted from grade A to C on the ASIA scale, one shifted from grade B to C, and eight shifted from grade A to B. The median preintervention FIM score was 71 and after intervention was 79.5. Fifty-nine patients showed a significant change in FIM score [27].

The safety and efficacy of cellular therapy is

further supported with 2 additional published case reports by Sharma et al [28,29].

Clinical studies conducted in various parts of the world by Huang et al 2012 [30], Saberi et al 2008 [31], Moviglia et al 2009 [32] and Tabakow et al 2013 [33] also highlight the safety and efficacy of stem cell therapy.

5. Stroke

Sharma et al in 2014 published a study in which the effect of intrathecal administration of autologous bone marrow mononuclear cells (BMMNCs) was analyzed on the recovery process of patients with chronic stroke. 24 patients diagnosed with chronic stroke were administered cell therapy, followed by multidisciplinary neurorehabilitation. They were assessed on functional independence measure (FIM) objectively, along with assessment of standing and walking balance, ambulation, and hand functions. Out of 24 patients, 12 improved in ambulation, 10 in hand functions, 6 in standing balance, and 9 in walking balance. Further factor analysis was done. Patients of the younger groups showed higher percentage of improvement in all the areas. Patients who underwent cell therapy within 2 years after the stroke showed better changes. Ischemic type of stroke had better recovery than the hemorrhagic stroke. This study demonstrates the potential of autologous BMMNCs intrathecal transplantation in improving the prognosis of functional recovery in chronic stage of stroke [34].

Apart from the above case series 2 separate case reports are published by Sharma et al that provide evidence about the safety and efficacy of stem cell therapy [35,36].

A Li et al in 2013 conducted a controlled clinical study with 60 patients who received stem cell therapy and 40 patients who did not and found that there was neurological and functional improvement in higher percentage of the patients that received stem cell therapy as compared to the control group [37].

6. Traumatic Brain Injury

Sharma et al in 2015 published a study carried out in 17 patients with Traumatic brain Injury (TBI) who had attained a plateau stage, treated with autologous bone marrow mononuclear cells

transplantation. Symptomatic analysis was done for the common symptoms observed in these patients and was graded as no change, mild moderate and significant improvements. The symptoms included higher mental functions, posture, trunk activity, upper limb activity, lower limb activity, coordination, oromotor, ambulation and Activities of Daily Living. Mild improvement was defined as improvements till 3 of the symptoms mentioned. Moderate was considered when 4 to 6 symptoms showed improvement, whereas significant improvements were considered when there were improvements recorded in 7 to 9 of the symptoms.

Analysis revealed that out of 17 patients, 29.41% of patients showed significant improvements in higher mental functions, posture, trunk activity, upper limb activity, lower limb activity, coordination, oromotor, ambulation and Activities of Daily Living; 23.52% of patients showed moderate improvement, 41.17% of patients showed mild improvements and 5.88% of patients showed no improvements in any of the symptoms. There were 4 patients that showed improvement in brain metabolism on PET CT scan of brain. Since TBI causes widespread brain damage to multiple areas of the CNS, most of the patients showed mild improvements [38].

7. Motor neuron disease

Sharma et al analyzed the survival duration of 46 ALS patients treated with intrathecal autologous bone marrow mononuclear cells transplantation since August 2008 till February 2014 compared with 20 patients who did not undergo intrathecal autologous BMMNCs transplantation using Kaplan-Meier survival analysis. Comparison of the survival duration suggested that the mean survival duration of the patients treated with intrathecal autologous BMMNCs transplantation was longer [104.069 months] than those who were not treated with intrathecal autologous BMMNCs transplantation [57.38 months]. A clinically significant difference of 47 months in the survival duration suggests the potential of intrathecal autologous BMMNCs transplantation in the treatment of ALS [39].

In addition to the above case series, 1 separate case report by Sharma et al also provides evidence about safety and efficacy of stem cell therapy [40].

Clinical study published by Prabhakar et al. 2012 [41], Blanquer et al. 2012w [42], Mazinni et al [43,44] & Martinez et al [45,46] also demonstrated a similar effect on progression of the disease. Also the case reports by Huang et al. [47,48,49] demonstrate the slowing down of the progression of the disease.

8 other publications by Sharma et al, document clinical outcomes in various other incurable neurological conditions [50,51,52,53,54,55,56,57]

Objective neuroradiological and electrophysiological improvements that document the improvements of cell therapy

An argument given by critics of stem cell therapy is that the clinical improvements reported after stem cell therapy may be the result of the placebo effect. To counter this argument we present from our own clinical work a few representative cases where objective improvements have been clearly documented on investigations. This scientifically shows that biologically beneficial changes are occurring in damaged tissues following stem cell therapy.

1. Autism

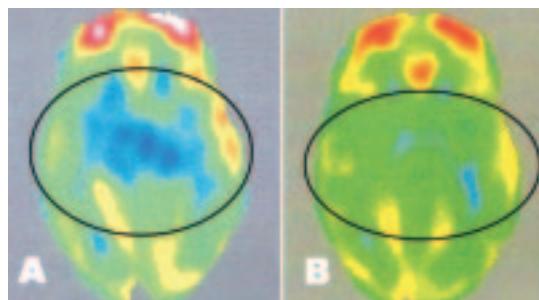


Figure 1: In the figure, A & B show PET-CT scan images before and after stem cell therapy, respectively. PET-CT scan after Stem cell therapy shows increase in the metabolism as outlined by the circles. Blue areas depicting hypometabolism in the pre SCT image which have changed to green areas depicting normal metabolism.

2. Cerebral Palsy

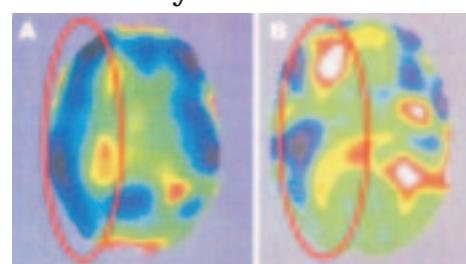


Figure 2: (A) Before stem cell therapy blue areas representing severe hypometabolism (B) Reduction in the blue areas suggesting increase in the metabolism and a positive response to the treatment.

3. Muscular dystrophy

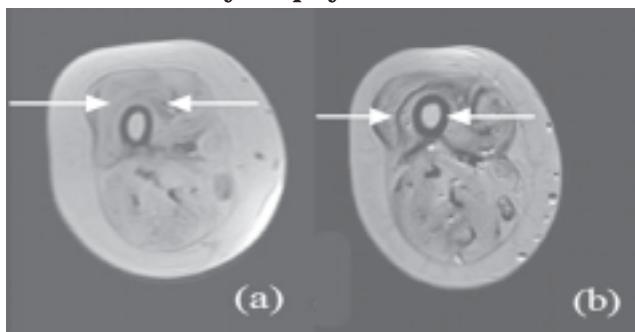


Figure 3: In the figure, A & B show MRI Musculoskeletal images before and after stem cell therapy, respectively showing regeneration of muscle in vastus medialis and vastus radialis.

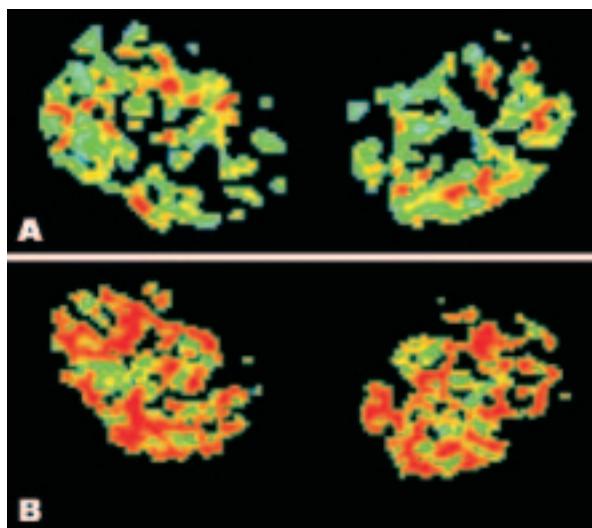


Figure 4: In the figure, A & B show MRI MSK DTI images before and after stem cell therapy, respectively showing increased red areas depicting increased activity of muscles.

4. Traumatic spinal cord injury

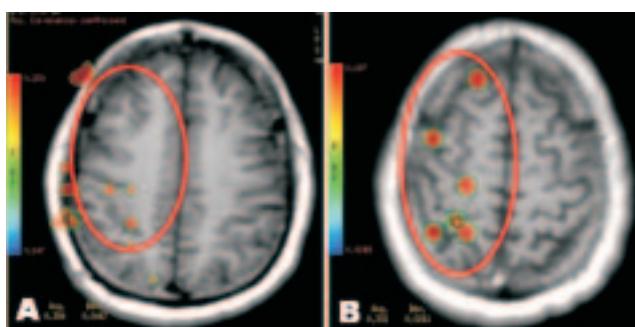


Figure 5: Figure A, shows the areas activated in the brain before stem cell therapy; Figure B shows increased activation of brain areas post stem cell therapy, new areas of activation are recorded in red and green color in the functional MRI of the brain.

5. Stroke

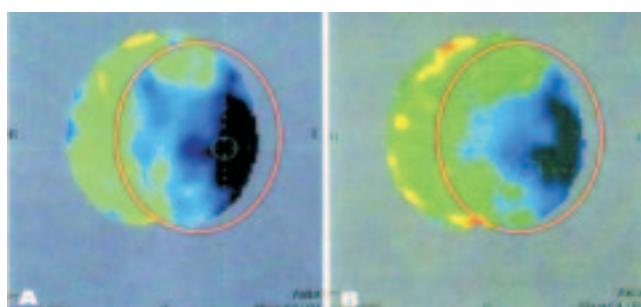
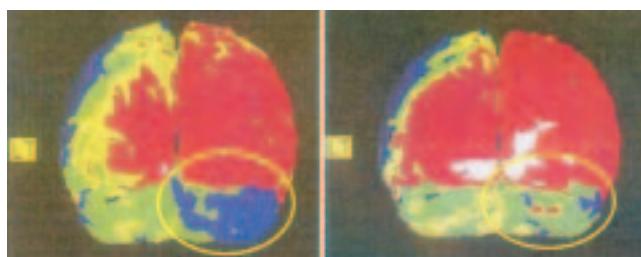


Figure 6: In the figure, A & B show PET-CT scan images before and after stem cell therapy, respectively. PET-CT scan after Stem cell therapy shows increase in the metabolism as outlined by the circles. Blue/black areas depicting hypometabolism in the pre SCT image which have reduced after Stem cell therapy.

6. Traumatic Brain Injury



(A) Before stem cell therapy (B) After stem cell therapy

Figure 7: Figure A, reduction in the metabolism of the brain, shown in blue color on the PET CT scan. Figure B, showing improved metabolic activity post stem cell therapy which is indicated by decrease in blue areas and increase in the green areas.

Review of internationally published work documenting the safety and efficacy of stem cell therapy in various neurological disorders.

A review of International scientific literature reveals a large number of published articles that clearly document the safety and efficacy of stem cell therapy in various conditions. In spinal cord injury (SCI) there are over 66 published papers

in which 1599 patients have been treated using various different types of cellular therapies and in these 844 patients have shown functional and neurological improvements and with no major adverse events reported. In cerebro vascular accident, there are more than 11 published studies including over 334 patients. In motor neuron disease, there are 9 studies evaluating the effects of cellular therapy in 203 patients (41-49). There are over 19 published studies in cerebral palsy including 344 patients. These published clinical results are only part of a larger group of work that definitively establishes the role of stem cell therapy as a safe and effective treatment option.

Medical Textbooks too have started including chapters on stem cell therapy. The Internationally accepted "Harrisons Principles of Internal Medicine, 19th Edition" has a separate section on stem cell therapy and mentions several indications for the clinical use of stem cells [58]. An international book on cerebral palsy "Cerebral palsy challenges for the future" has a chapter on "stem cell therapy in cerebral palsy" written by Sharma et al 2014 [59].

International documents that need to be referred to before formulating guidelines & regulations for stem cell therapy

1. World Medical Association' declaration of Helsinki for Ethical Principles for Medical Research Involving Human Subjects [60]

Clause 37 of the Helsinki declaration states that, *"In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available."*

This implies that it is ethical for a physician to treat patients with an unproven therapy when no other treatments are available. This part of the Helsinki Declaration should be considered as

an important inclusion for any regulations regarding stem cell therapy. This would mean that clinical work with stem cell therapy being done by centers following this aspect of the Helsinki declaration would be ethical and legitimate.

2. The International Society for Cellular Therapy (ICST) "White paper" published in 2010 in Cytotherapy [61]

The following important aspects of this paper need to be taken into consideration for any stem cell therapy regulations.

a. Distinction between clinical trials and medical innovation

Regulations should make a distinction between clinical trials and medical innovation. According to the ICST white paper the distinction can be made as follows,

"Medical innovation in cellular therapy may be viewed as ethical and legitimate use of non-approved cell therapy by qualified healthcare professionals in their practice of medicine. Patients not eligible for controlled clinical trials should be able to choose unproven but scientifically validated cell therapy medical innovations, if the researchers are competent and those seeking treatment are truthfully and ethically informed. There is a place for both paradigms in the cell therapy global community."

As per this it can be said that non approved cell therapy should be considered as ethical and legitimate part of medical innovation if (i) It is done by qualified healthcare professionals in their practice of medicine (ii) The researchers are competent & (iii) Those seeking treatment are informed truthfully and ethically. There is a role for both, clinical trials and medical innovation, in the cell therapy global community.

b. Distinction between legitimate cell therapy medical services and fraudulent services.

Regulations need to differentiate between legitimate cell therapy medical services and fraudulent services. ICST "White paper" highlights how this can be done,

"The following guidelines are useful in assessing scientific rigor and for differentiating between legitimate cell therapy medical services (including clinical trials and medical innovation) and fraudulent cell therapies."

1. *Peer review and transparency: consumers of cell therapy medical innovation should evaluate evidence from peer-reviewed publications, professional society presentations and scientific recognition. They should be encouraged to seek multiple professional opinions and have all questions answered to their satisfaction.*
2. *Safety and regulatory history: patients should consider the reputation of the investigator and clinic, as well as the record of disciplinary activities against these entities.*
3. *Informed consent: patients should expect to be informed fully and accurately of the risks, benefits, costs, safety, compensation for injury, investigator conflicts of interest and alternative therapies, as a minimum."*

Therefore institutes whose clinical results are peer reviewed and transparent through peer reviewed publications, professional society presentations & scientific recognition, who have a good safety & regulatory history and who take informed consent may be considered as centers offering legitimate cell therapy.

c. The basic right of a patient to seek treatment should be respected

Regulations may violate the basic bioethical principle of autonomy of the patients with reference to cellular therapy. Implementing evidence based guidelines alone implies that the patients' rights of choosing a treatment which is safe are being denied. The ICST White Paper is in agreement with this and states,

"Patients seeking medical treatment for cellular therapies have the following rights that must be respected by healthcare providers and all associated with their care.

- a. *The right to seek treatment: patients and their families/partners have the right to seek treatments for their diseases. No entity should withhold this fundamental right unless there is a high probability of harm to the patients.*
- b. *The right to information: patients have the right to an accurate representation regarding the safety and efficacy record of the cell treatment. This includes probable side-effects and a truthful record of efficacy.*
- c. *The right to informed consent: patients have a*

right to a true informed consent process that includes all the elements described above."

This implies that the right to seek treatment is the fundamental right of the patients and their families and this should not be taken away by any regulatory or professional body. Patients also have a right to information and a right to informed consent which should be made available to the patients and their families by the treating physicians.

d. Distinguishing various centers offering cellular therapy

The ICST White Paper distinguishes various centers offering cell therapy as follows

1. *Approved/standard therapies (e.g. hematopoietic stem cell transplant and other cellular therapies approved for marketing)*
2. *Controlled clinical trials*
3. *Valid compassionate use of unapproved therapies*
4. *Treatments not subject to independent scientific and ethical review.*"

At present most regulations only recognize (1) approved/standard therapies (e.g. hematopoietic stem cell transplant and other cellular therapies approved for marketing) (2) controlled clinical trials. Anything apart from this is not recognized as ethical or legal. It is important that as per the ICST white paper, (3) Valid compassionate use of unapproved therapies, be recognized as a separate, ethically accepted and legitimate alternative.

3. The United States Food and Drug Administration article 1271 15B (Human cells, Tissues, and Cellular and Tissue based products) [5]

This states that :- *"You are not required to comply with the requirements of this part if you are an establishment that removes Human cells, Tissues and cellular and tissue-based products from an individual and implants such products into the same individual during the same surgical procedure"*

What this implies is that autologous and minimally manipulated cell therapy should not have regulations that are in place for other human cells, tissues, tissue based products and drugs. This is the single most important distinction that

any guideline or regulation for stem cell therapy should make. Having common regulations for all cellular therapy would be like having common regulations for alcohol, sodas and homemade orange juice covering them all under the category of beverages.

4. The new Japanese legislation on stem cell therapies [62]

In their recent amendment to their pharmaceutical law, Japan has created a separate approval channel for regenerative medicine. Instead of, using phased clinical trials, researchers will have to demonstrate efficacy in pilot studies of as few as 10 patients in one study if the change is dramatic enough or a few hundred if the improvements are marginal. If the efficacy can be surmised it will be approved for marketing. At that stage the treatment would be approved for commercial use as well as national insurance coverage. Thus the Japanese government has lowered the bar for regenerative therapies dramatically by requiring limited safety and efficacy data. Other regulatory bodies should study the new Japanese legislation and incorporate the relevant aspects of this path breaking regulation for regenerative medicine [44].

National Policies: Views of the Government and the Prime Minister of India

The importance the Government of India and our Honorable Prime Minister gives to the field of stem cell therapy is highlighted by the following :-

[1] In the address by President of India to the joint session of the Lok Sabha at the central hall of parliament where the President shared the governments agenda for the country in paragraph 38 it states "*My government will build world class research centers in the fields of nanotechnology, material sciences, thorium technology, brain research, stem cells etc*".

[2] The Prime Minister of our country, Shri Narendra Modi has shown special interest in the field of Stem Cell Therapy as is evident from the fact that :-

When he visited Japan in August 2014, he visited the Stem Cell Institute in Kyoto and personally met with Stem Cell pioneer and Nobel Prize winner Prof. Shinya Yamanaka.

In February 2015 he visited the NCBS and inStem in Bangalore and visited the laboratories to see Stem cell research. He also had a vibrant and truly interactive engagement with the faculty and students on the use of stem cell based therapies.

Discussion

Stem Cell Therapy is an upcoming and developing field in modern medicine in which India has played a pioneer and leadership role. Many medical conditions that were earlier considered incurable or untreatable can now be treated with stem cell therapy. This has resulted in Indian Scientists and Doctors playing a lead role in the field due to which many patients from all over the world are now coming to India to take this treatment. The primary job of the various regulatory authorities is to ensure the safety of patients with the country and to see that there is no violation of scientific and ethical principles. Our regulators have done a great job in this. However with reference to the field of stem cell therapy, this has also resulted in a slowness in the availability of this form of treatment to people at large. It is our view that whilst ensuring safety the regulatory authorities should also permit and in fact encourage the wider availability of the safer forms of this treatment (such as adult stem cell therapy) since stem cell therapy has shown significant benefit to children of autism / cerebral palsy / mental retardation / blindness as well as patients who are paralyzed because of spine injury / brain stroke. We believe that there are thousands of patients deteriorating and dying presently with serious neurological and other diseases. (motor neuron disease, muscular dystrophy, heart failure, liver failure etc etc) whose lives could be saved if this treatment was more easily available. There are many scientific publications in international medical journals (many of them from India) that have shown the safety and efficacy of Stem Cell therapy. The existing guidelines are not in our National interest. These will have a negative impact on the health of the people of this country since many people are today dying or suffering from serious diseases who could be treated by a treatment that is available but cannot be given since the regulatory bodies will not permit its greater availability. Whilst thousands of people are dying or suffering today because they cannot get stem cell therapy

for their otherwise incurable diseases on the other hand there are no patients who have either died or suffered due to stem cell therapy. There may be a few cases of complications or adverse events but then which established medical treatment is 100 % free from complications or side effects. As compared to all other treatment forms cell therapy is one of the safest forms of therapy and there are now many scientific papers that have clearly established the safety of cell therapy. These draft guidance have been prepared with a anti stem cell therapy bias as well as without study of the present literature available in international and national journals that show its efficacy and safety. of cell therapy in various diseases. The drafting committee did not have practicing doctors from the clinical specialties who are the appropriate people to judge whether stem cell therapy is useful in their fields of specializations. The main stakeholders (i.e. patients who have received stem cell therapy and doctors practicing stem cell therapy) have not been consulted. We are suggesting simple easy to implement methods that will simultaneously achieve two goals. We will have strict regulations as well as greater availability of this form of treatment.

Conclusions

We are proposing that the existing guidelines and proposed regulations for stem cell therapy for India be reviewed and those reviewing these should consider the following:

1. Make a distinction between autologous and allogenic stem cell therapy by having a more permissive approach towards autologous stem cell therapy. In continuation with this, in the definition of new drugs "stem cells" be replaced by " allogenic stem cells." This will ensure that stem cells that are been manufactured and are marketed commercially as products (allogenic stem cells) are kept under strict regulations whereas autologous stem cells which are safe, have a published track record of efficacy in several incurable conditions and which are not a product are easily available for patient's benefit in different medical conditions.
2. Study the new Japanese legislation that ensures a fast track approval for regenerative medicine.

3. Evaluate published clinical results of individual practitioners from within the country as well as those from other countries and take these into consideration for deciding approved indications.
4. Criteria be evolved for recognizing legitimate cell therapy medical services, medical innovation and the valid compassionate use of unapproved therapies.
5. The patients' right to seek treatments for their diseases and suffering be respected.
6. The socioeconomic conditions of India be kept in mind. The majority of our population cannot afford expensive treatments. Therefore a more permissive approach be taken to the less expensive autologous therapies that can be easily done in our public hospitals and smaller private hospitals as compared to the more expensive allogeneic therapies manufactured by the large corporates.
7. The views of the people as reflected by the views of the elected representatives in particular our Honorable Prime Minister Shri Narendra Modi be taken into consideration whilst reviewing the guidelines and regulations.
8. That the various expert committees, including NAC-SCRT, be reconstituted with greater representation from different clinical specialties and practicing doctors with clinical experience and publications in stem cell therapy.
9. That free and frank discussions are held with the main stakeholders who are the patients suffering from diseases that stem cell therapy could benefit and the doctors who have expertise and are practicing this therapy and their views be respected and reflected in the reviews.

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Stem Cell Therapy as a Treatment Modality for Neurotrauma

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Abstract

Neurotrauma is a common cause of chronic severe neurological deficits. No available treatment can reverse the neural damage. In recent advances, cell transplantation has emerged as a promising treatment modality to repair the damage. This is supported by numerous published animal studies. There have also been published human clinical trials which showed significant improvement in the neuro deficits. We have previously published three clinical studies and three case reports on the use of autologous bone marrow mononuclear cell (BMMNC) therapy in neurotrauma. Here we present a review of these studies which substantiate the use of cell therapy for neurotrauma. The clinical studies included a total of 179 cases of chronic neurotrauma which had 166 cases of spinal cord injury and 13 cases of traumatic brain injury. 146 out of 179 cases showed improvements after receiving cell therapy (82% cases improved) in combination with neurorehabilitation. These cases showed clinical improvements which were also recorded on objective scales. Functional neuroimaging performed before and after intervention has also shown improvements in the neural activity. No major adverse events were noted during any of these clinical studies. We conclude that the autologous bone marrow mononuclear cells in combination with rehabilitation is safe and feasible treatment strategy for chronic neurotraumatic conditions.

Keywords : Neurotrauma, autologous, BMMNC, rehabilitation, spinal cord injury, traumatic brain injury

Introduction

Neurotraumatic injuries are one of the leading causes of death and disability all over the world. It majorly involves the damage of the central nervous system which is made up of brain and spinal cord. The initial injury to the CNS is the primary damage which leads to a cascade of deleterious events known as secondary damage which further leads to loss of function and prolonged degeneration due to cell death. (1) This has devastating effects on quality of life of the affected individual. Hence, researchers are actively seeking a treatment strategy which can reverse as well as stop further damage caused to the CNS due to trauma or disease.

For a very long time, it was believed that damage to the CNS is irreversible. (2) However, growing

research has shown that stem cells have the ability to restore the CNS. Stem cell therapy is emerging as a potential treatment strategy for conditions such as spinal cord injury, traumatic brain injury, brachial plexus, etc. (3,4) Effects of various types of cells such as embryonic stem cells, adult stem cells, umbilical cord blood cells and induced pluripotent stem cells have been actively studied in these disorders. (5,6) It is essential to select suitable cells to achieve optimal therapeutic efficacy. However, adult stem cells are the most preferred type of cells. The underlying mechanism of action of these cells is that they help in neuromodulation, neuroprotection, axon sprouting, neural circuit reconstruction, neurogenesis, neuroregeneration, neurorepair, and neuroreplacement. (7,8)

To demonstrate the therapeutic benefits of these cells in chronic neurotraumatic conditions, we administered 179 cases of chronic neurotrauma (spinal cord injury and traumatic brain injury) with autologous bone marrow mononuclear cells intrathecally. These cells are easily obtainable, do not involve any immunogenic complications and ethical issues. Here, we present a review of our previously published research data on the use of stem cell therapy as a treatment modality for neurotrauma.

Material and Method

Study Design

We performed a study to demonstrate the effect of intrathecal autologous bone marrow mononuclear cells in patients with chronic neurotraumatic conditions such as spinal cord injury and traumatic brain injury. This is a review of three case series and three case reports.

Intervention Protocol

The protocol of the study was reviewed and approved by The Institutional Committee for Stem Cell Research and Therapy (IC-SCRT) in accordance to the Indian Council of Medical Research (ICMR) guidelines. Patients were selected based on the World Medical Association Helsinki Declaration for Ethical Principles for medical research involving human subjects (9). A written informed consent was obtained from the patients and their families depending on the patient's cognitive status. The inclusion criteria were diagnosed cases of chronic neurotraumatic conditions and age above 1 year. The exclusion criteria were presence of acute infections such as HIV/HBV/HCV, malignancies, bleeding tendencies, renal failure, severe liver dysfunction, severe anemia [$Hb < 8$], pregnancy, lactation, any bone marrow disorder and other acute medical conditions such as respiratory infection and pyrexia.

Before the intervention, every patient underwent a detailed neuroevaluation by medical experts. Serological, biochemical and hematological tests were also performed. Functional independence of all the patients was evaluated using Functional Independence Measure (FIM). Electroencephalography (EEG), Electromyography (EMG),

Nerve conduction velocity (NCV), Somatosensory evoked potentials (SSEP), Magnetic Resonance Imaging (MRI) of brain, with Diffusion tensor imaging (DTI), functional Magnetic Resonance Imaging (fMRI) of brain and Positron Emission Tomography- Computed Tomography (PET-CT) brain scans were performed in patients before the treatment depending on the disorder.

Bone marrow aspiration, separation and injection

Granulocyte Colony Stimulating Factor (G-CSF) injections were administered 48 hours and 24 hours prior to the procedure as it stimulates and mobilizes the bone marrow stem cells (10).

Approximately 80-100 ml bone marrow was aspirated from the anterior superior iliac crest under local anesthesia with or without mild sedation (depending on the case scenario), using the bone marrow aspiration needle. MNCs were separated using density gradient centrifugation method. Their viability is checked manually using trypan blue dye and confirmed with propidium iodide dye in TALI (Life Technologies. Invitrogen). Average viability was found to be 97%. CD34+ counting was also performed by fluorescence activated cell sorting (FACS) using CD34 PE antibody.

The separated autologous BMMNCs (body weight $\times 10^6$) were immediately injected intrathecally using a 25G spinal needle between fourth and fifth lumbar vertebrae under local anesthesia with or without mild sedation (depending on the case scenario). Simultaneously, 20mg/kg body weight methyl prednisolone in 500 ml Ringer Lactate was given intravenously to enhance survival of the injected cells.

Neurorehabilitation

The intervention included neurorehabilitation along with stem cell therapy. A multidisciplinary neurorehabilitation plan was customized for every case which included physiotherapy, occupational therapy, speech therapy and psychological intervention. The regime was commenced immediately after stem cell therapy and was advised to continue as a home program. Patients were followed up regularly at 3 months,

6 months and yearly thereafter after the intervention. A complete neurological evaluation was performed.

Result

The 179 cases of chronic neurotrauma included 166 cases of spinal cord injury and 13 of traumatic brain injury. The average age of the study sample was 33 years.

Amongst the 166 cases of spinal cord injury, 110 cases were of dorsolumbar level while 56 were of cervical level. Overall, 100 out of 110 (91%) patients showed improvements. (Figure 1) Improvement in trunk control was observed in 95.6% cases, bladder management in 33% with respect to shift from indwelling and condom catheter to self intermittent catheterization, partial sensory recovery in 27% and reduction of spasticity in 26%. All the patients showed

improvement in postural hypotension. 38% wheelchair bound patients started walking with assistance. Functionally, 27% showed improved activities of daily living (ADLs) and 53.6% showed a positive change in FIM score. 10% cases showed a shift in ASIA scale. On electrophysiological studies, 2 showed improvement and 1 showed change in functional MRI.

However, in cervical SCI patients, 37 out of 50 (74%) showed improvements. (Figure 2) Sensation recovery was observed in 26% cases, improved trunk control in 22.4%, spasticity reduction in 20% and bladder sensation recovery in 14.2%. All the 50 cases had improvement in postural hypotension. 12.24% wheelchair bound patients started walking with assistance. Functionally, 20.4% patients showed improved ADLs and 48% showed a positive change in FIM score. 6% cases showed a shift in ASIA scale.

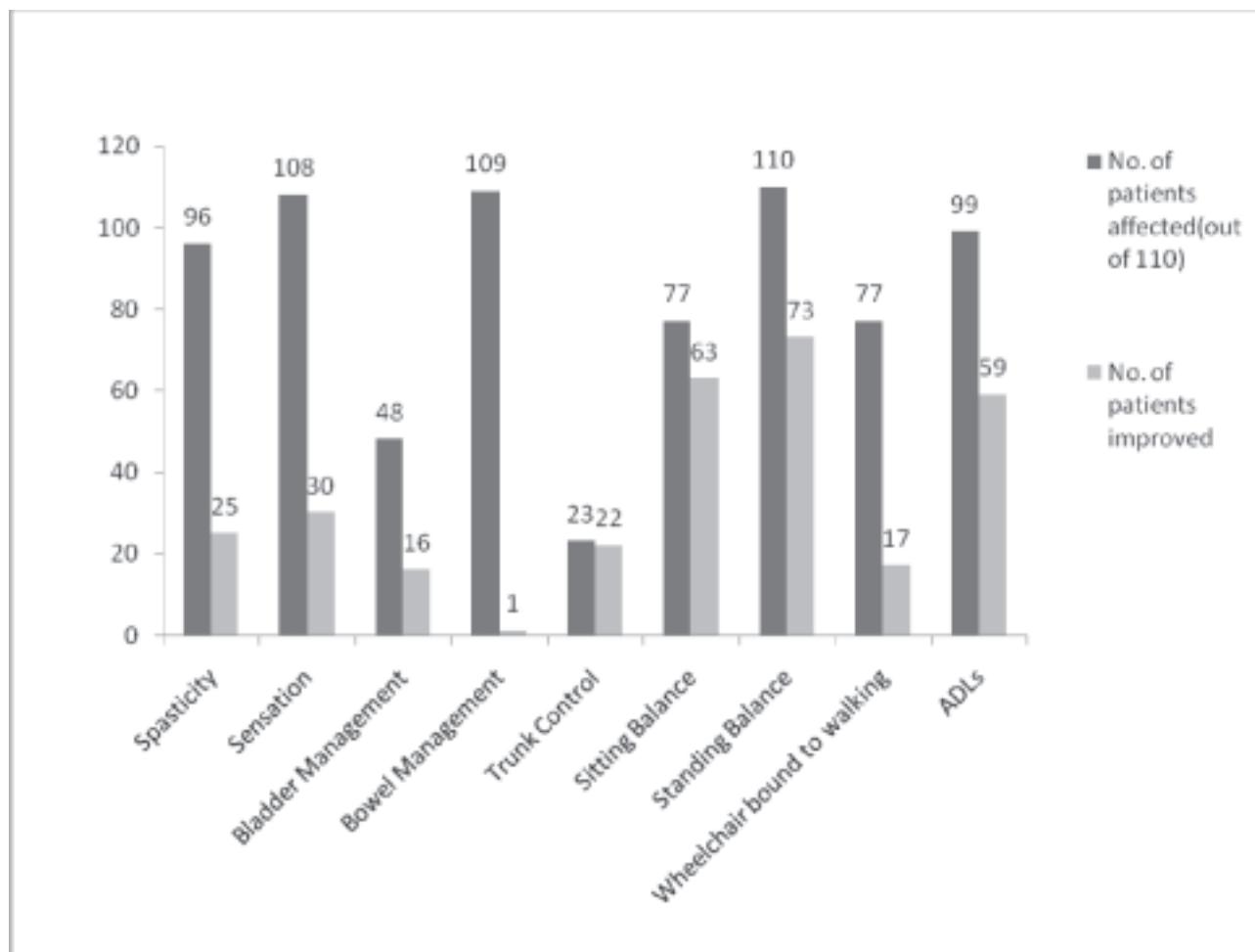


Figure 1: Graph representing symptomwise improvements in dorsolumbar spinal cord injury patients after stem cell therapy

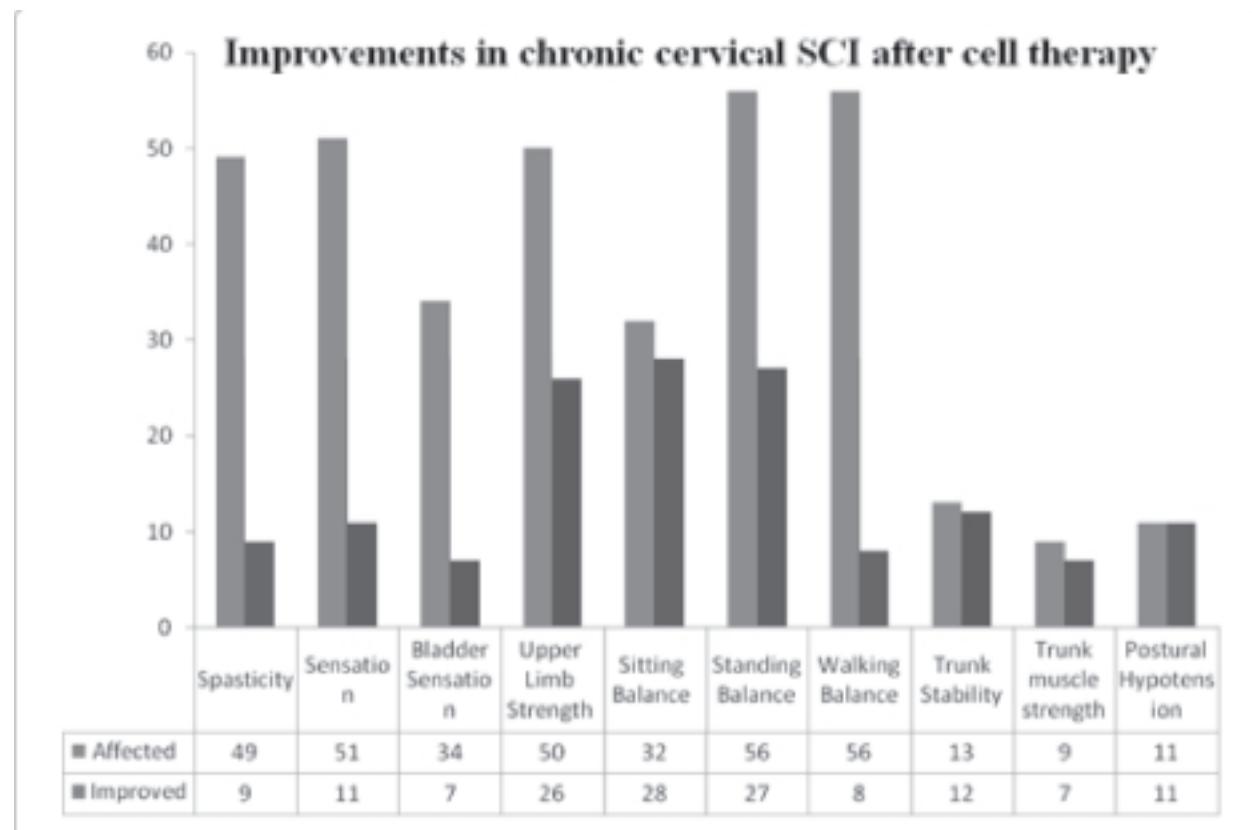


Figure 2: Graph representing symptomwise improvements in cervical spinal cord injury patients after stem cell therapy

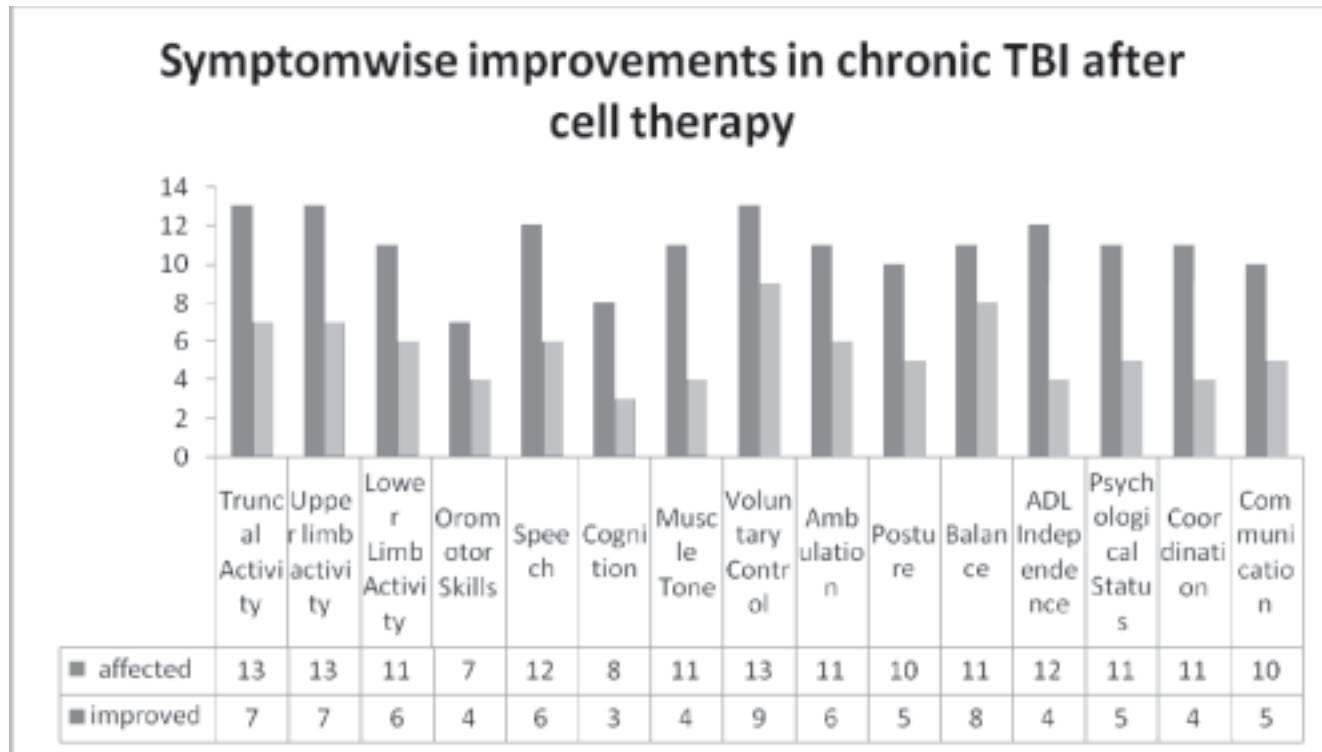


Figure 3: Graph representing symptomatic improvements in traumatic brain injury after cell therapy

In Traumatic Brain Injury, (Figure 3) amongst 13 patients, 73% showed improvement in balance, 69% in voluntary control, 60% in memory, 57% in oromotor activities, 55% in lower limb activity and ambulation and gait patterns, 54% in trunk and upper limb activity, 50% in speech, posture and communication, 45% in psychological status, 38% in cognition, 36% in muscle tone and coordination and 33% in ADLs. PET CT scan was repeated in 3 patients at the end of six months and they showed improved metabolism after intervention. The changes were consistent with the clinical and functional improvements demonstrated by these patients.

No major side effects were recorded in the duration of follow up. Minor procedure related side effects such as headache, nausea, vomiting, and backache were observed in a few cases which were controlled with medications.

Discussion

Neurotrauma is not only characterized by focal abnormalities but also multifocal, global structural and functional damage of the brain and spinal cord network. (11) The trauma results in loss of neuronal and oligodendroglial cells, reactive astrogliosis, and proliferation/activation of microglia. (12) Due to loss of functions, these conditions highly affect the quality of life of the injured patients. There are currently no treatments available to reverse the damage to the CNS.

Stem cell therapy has shown to be a potential treatment strategy for chronic neurotraumatic conditions. These cells either directly or indirectly help in reversal of the damage. These cells have anti-inflammatory, immunomodulatory and neuroprotective effects. (13,14) They have the ability to multiply and engraft diffusely to replace the lost cells of the CNS. They replace neurons and oligodendrocytes lost to necrosis or apoptosis and help to remyelinate axons. They also impart neuroprotective and neuroregenerative functions by releasing various growth factors. These growth factors stimulate the endogenous resident stem cells to multiply and replace the lost cells. These mechanisms might help in reconstructing the molecular and cellular milieu of the injured brain and spinal cord. (15,16)

We conducted an analysis on 179 cases of chronic neurotrauma to study the effect of autologous bone marrow mononuclear cells, administered intrathecally. These cells are a heterogeneous mixture of hematopoietic cells, mesenchymal cells, very small embryonic like cells (VSELs) and endothelial progenitor cells. (17) It has been observed that use of BMMNCs is more successful than the sub fractionated cell preparations. The cell mixture promotes angiogenesis and vascular repair. (18) These cells have the capacity to mobilize to the damaged areas and exert their reparative effects. We chose to administer the cells intrathecally as they are minimally invasive and are more targeted than intravenous transplantation. It has been observed that the cells administered intravenously get trapped in the lungs hence affecting the number of cells reaching the target tissue. (19) Direct injection to the site of the injury may be the most efficient route, but it involves an invasive procedure which could result in secondary damage.

All the patients included in the study underwent neurorehabilitation after stem cell therapy. It has been observed in animal studies that the combination of rehabilitation along with stem cell therapy results in a positive functional outcome.

On follow up, significant symptomatic improvement was observed in all the conditions. Improvement was also recorded on the outcome measures such as FIM and ASIA. Objective improvements were also observed in EMG, PET CT scan and fMRI of brain. These improvements in neurological functions indicate interaction between the microenvironment of the CNS and the implanted cells.

Conclusion

We conclude that the autologous bone marrow mononuclear cells in combination with rehabilitation is safe and feasible treatment strategy for chronic neurotraumatic conditions. The reparative effect of these cells is exhibited in the form of symptomatic, functional and objective improvements. However, to establish stem cell therapy as a standard treatment for these disorders, multicentre, large-scale, randomized clinical trials are required.

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Role of cord Derived MSCs & IGF1 In The Management of Duchenne Muscular Dystrophy

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Abstract

Duchenne muscular Dystrophy (DMD) is a lethal musculodegenerative disease with an underlying genetic defect. It is clinically manifested as progressive weakening of the skeletal muscles due to lack of Dystrophin production. Currently, glucocorticoid therapy is the only established treatment to contain muscular inflammation but with a lot of side effects on long term use, while gene replacement, exon skipping and allogenic stem cell transplantation have been relatively unsuccessful in the past. As IGF-1 plays a crucial role in the regeneration of skeletal muscles by proliferation and differentiation of satellite stem cells, we carried out an investigational therapy in 11 children between January 2012 to November 2014 to establish the role of IGF 1 and cord derived mesenchymal stem cell transplantation in the management of DMD. We have shown, for the first time, that Cord derived MSCs + IGF 1 is a safe and viable option not only to contain the disease but also in improving the quality and longevity of life by definitive improvement in muscle power.

Keywords : Duchenne muscular dystrophy, dystrophin, cord derived MSCs, IGF1

Introduction

Duchenne Muscular Dystrophy is a lethal musculodegenerative disease. Basically it is a Genetic disorder characterized by lack of Dystrophin production. Only Boys are affected as it is an x - linked recessive disorder. Children are affected in early childhood and present with Delayed standing/ walking, Frequent falls, Toe Walking, Difficulty in climbing stairs and getting up from the ground. Later they present with waddling gait & lordosis. (1,2)

Such children are diagnosed by Calf hypertrophy, Positive Gower's sign, raised CK & LDH levels. Immunohistochemistry (IHC) shows absence of Dystrophin and analysis of Dystrophin gene shows Deletion/duplication of exons (1-79) . In the advanced stages they present with Poor lung functions (Decreased FEV1) & Dilated cardiomyopathy (low EF)

Current Treatment options are Glucocorticoid therapy, to slow down muscle inflammation but a lot of side effects occur on long term use, Physiotherapy, use of orthotics and respiratory supports like BIPAP & CPAP. (3) Treatments

under trial are use of exon skipping drugs like Ataluren (Translarna), Allogenic MSCs, Autologous mononuclear cells (Bone marrow), Gene therapy, IGF1 therapy & Myoblast transplant but all above methods have limited source/ efficacy . (4-6)

An investigational therapy was conducted between Jan 2012 to Dec.2014, using Cord derived MSCs and IGF1 in DMD patients. Approval was taken from Institutional Ethics committee and detailed informed consent was taken from parents. 11 patients of DMD of 5 to 18 year age group were given stem cell transplantation (SCT), 5 Patients were observed as controls .

Inclusion Criteria

Boys of age 5 to 18 years with Calf hypertrophy, positive Gower's sign, raised CK and LDH levels, absence of Dystrophin in IHC & Exon deletion/ duplication in Dystrophin gene were included in the study. No one was on glucocorticoid therapy in last 6 months, registered on other trials & suffering from infective or any other life threatening disease.

Exclusion Criteria

Boys were excluded if on glucocorticoid therapy, suffering from infective or any other life threatening disease & registered on other trials.

Material and methods

Total patients included in the study were 16, where 11 patients were in group A who received SCT and 5 in group B were not given SCT and observed as controls. Group A patients were given 4 sessions of cord derived MSCs and IGF1 on monthly interval while Group B patients were kept on Calcium & Vitamin D only. Each patient in group A were administered 2 million MSCs per kg body weight per session through IM and IV route.

Parameters of Assessment

Patients were assessed Pre SCT and Post SCT on clinical, radiological and biochemical parameters. Muscle power was assessed on MRC scale. Other parameters were assessment of activities of daily life(ADL), Gower's time, amount of fatty degeneration of skeletal muscles in MRI scan, CK and LDH levels . Pulmonary function tests were done to see change in FEV1 while Echocardiography done to assess ejection fraction (LVEF)

Results

Muscle Power on MRC scale showed definite increase in 6 (11) patients so 56% responded positively in group A, while 5(11).. 44% did not show increase in muscle power although did not worsen as well. In group B the decrease in muscle power noted in all 5 patients. Gower's Time(Group A)decreased from average 30 seconds to 8 seconds in 3 months but increased to 15 seconds in 1 year. Positive response noted in 6 children only while rest could not get up from the ground. 2 D ECHO showed increase in LVEF from 45% average to 50% average in 3 months. PFT showed dramatic improvement of 20 TO 25% increase in FEV1 value in just 1 month and 25 to 30% increase in 3 months. MRI scan showed decrease in fatty degeneration of muscles while Ck levels did not have a definite pattern .Immediate Post SCT Random blood sugar (RBS) was in normal range.

Complications

Only minor G I side effects like nausea &

vomiting were noted with transient fever.No serious or life threatening complications were observed.

Observations

Combination of Cord Derived MSCs and IGF1 did not cause any major complication where 56% patients responded favourably in group A. Those patients who did not show increase in muscle power in group A, showed decrease in the decline rate. Increase in muscle power in responder' group was very good in first 3 months and then decreased slowly over a period of time but still better than day 1 even after 3 years of SCT. All patients in group B showed decline in muscle power over a period of time. These results were without corticosteroid therapy

Conclusions

Cord derived MSCs and IGF1 combination is safe and effective in DMD patients in the age group of 5 to 18 years. It can be used as an alternative to Glucocorticoid Therapy to control decline in muscle power. Patient needs repeated booster sessions of SCT to maintain muscle power. Lastly CK levels can be used in establishing the diagnosis of DMD but no relevance noted as monitoring tool in the progression of disease.

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Stem Cells Overview and Research in Diabetes

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Abstract

Diabetes mellitus is a major health concern of the originating and developed nations across the world. This devastating disease accounts for the 5% deaths around the globe each year. The current treatment methods do not come up to the underlying causes of the disease and have severe limitations. Stem cells are unique cells with the potential to specialize into whatever case of specialized cells. The different type of stem cells induced pluripotent stem cells (iPSCs), embryonic stem cells (ESCs) and adult stem cells shows to be powerful in treating diabetes with certain restrictions.

Pancreas or islet transplant only provides partial exogenous insulin independence and induces several adverse effects, including increased morbidity and mortality. Mesenchymal stem cells (MSCs) have been envisioned as a promising tool for T1DM treatment over the past few years, since they could differentiate into glucose-responsive insulin-producing cells.

This article precisely reviews the resources and progress gained in the area of stem cell research for diabetic treatment. This review focuses on recent pre-clinical data supporting MSCs use in regenerating β -cell mass and also in treating diabetes. Clinical trial results and the ongoing obstacles which must be addressed regarding the widespread use of such therapy are also discussed.

Keywords: Stem Cell, Diabetes, Mesenchymal stem cell, Clinical therapy

Introduction

Diabetes mellitus (DM) is most devastating, chronic, common non-transmitted illness (NCD) and has become a significant downside globally. The physical structure of the diabetic population around, the planet is frequently increasing with a current estimation of 371 million cases in 2012 and it is anticipated to reach 552 million by 2030 (1). It's additionally calculable that fifth of all deaths within the universe is caused by diabetes and also the range is apace increasing. This polygenic malady is quick gaining the standing of a possible epidemic in India with over sixty two million diabetic people presently diagnosed with the disease (2). It is foretold that by 2030 DM could afflict up to 79.4 million people in India, whereas China (42.3 million) and also the USA (30.3 million) will see important will increase in those laid low with the diabetes. India presently faces associate unsure future in reference to the potential burden that diabetes could impose upon the country.

The chemical ways of treatment for unwellness don't address the causes of the disease and cause facet effects. Thus, in this location is a visible hunt for the appropriate different treatment ways. The present cellular based mostly therapeutic methodology for the treatment of diabetes is aimed along the transplantation of either the duct gland or islet-cells to restructure the hypoglycemic agent secreting purposeful β -cells. Nevertheless, this method is hampered by a lack of donor organs. Of these problems fixed-up the trail to explore the analysis potentialities of generating exocrine gland β -cells from stem cells. The distinctive regenerative properties of stem cells may well be an important tool which might be worked within the discussion of diabetes. This text reviews the progress of the stem cell analysis performed within the space of diabetes treatment and sensible hurdles related to it.

Stem cell and their therapeutic potential

The stem cells are more gifted and are responsible

for the formation of different types of cells during the early embryonic life and the later growth of the organism. Stem cells possess an exceptional quality to refill itself associated to supply any specialized cell sorts underneath acceptable microenvironment. A rapidly dividing stem cell produces 2 new cells, ever having two decisions relying upon the need of the organism. Thus, a

fresh created cell either might remain as a stem cell or it may bear any differentiation to become a lot of specialised cell with specific functions. The stem cells possess the potential to turn whatever type of specialized cell such as a myocyte, blood cell, hepatocyte and brain cell (Figure 1).

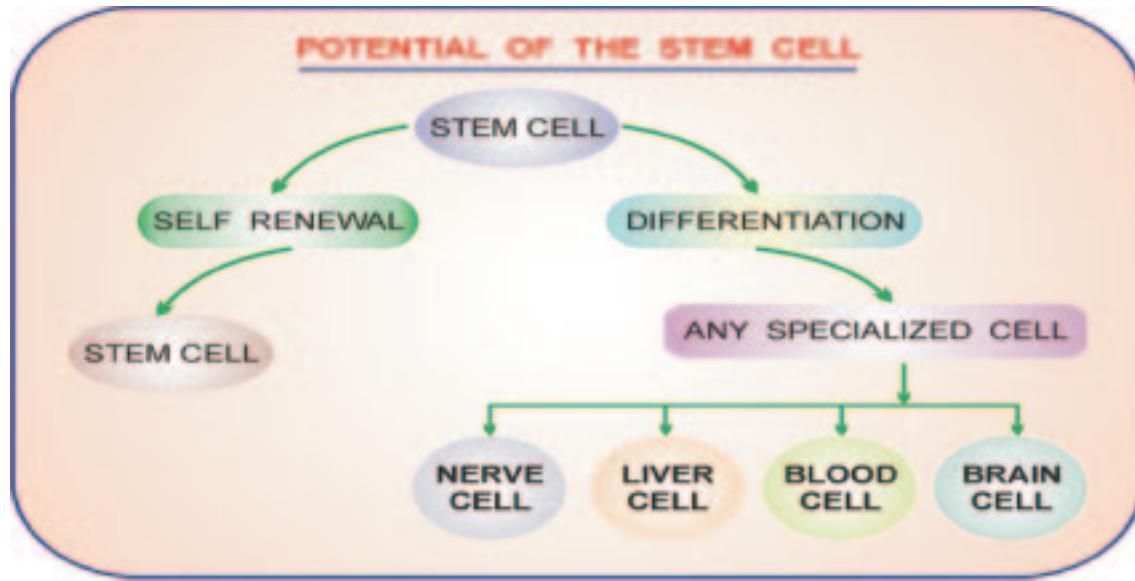


Figure 1: Differential potential of stem cells

The stem cells can rapidly divide in some organs such as bone marrow and gut to repair and put back the damaged tissues. Nevertheless, in some other organs such as in the heart and pancreas, the stem cells stay on as a resident cell and undergo division only under specific requirement. The stem cells can be sorted in many

ways based on the origin, potential, source and method of derivation, etc. Scientists around the world are gaining a considerable exertion to constitute utilization of different type stem cells for the handling of several medical conditions (Figure 2) (3).

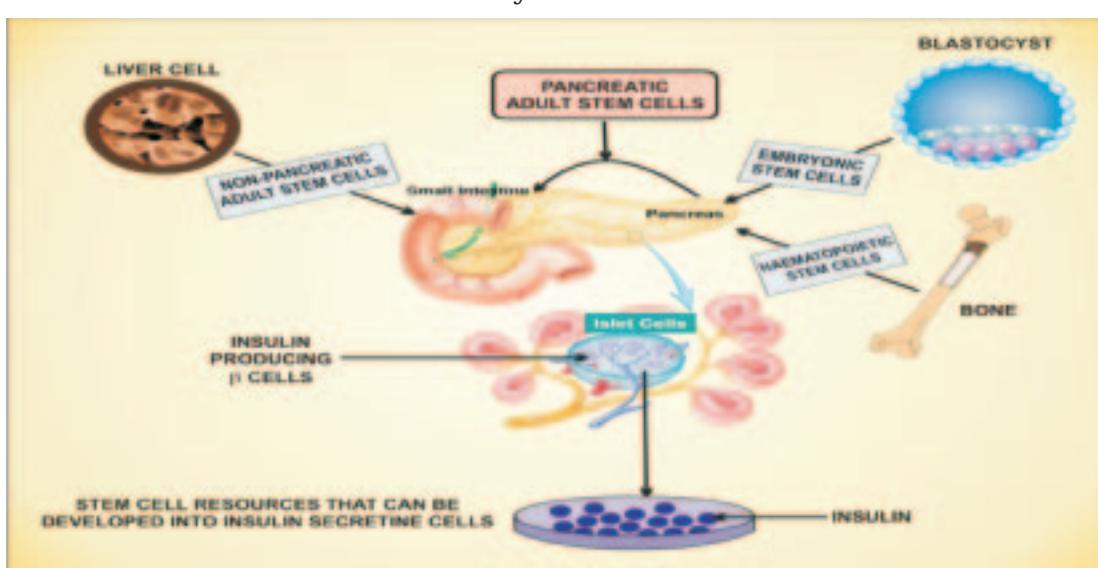


Figure 2: Types of stem cell with potential to be developed into insulin secreting cell

ESCs and diabetes

The pluripotent nature of the ESCs has been hailed for long by the researchers and these cells are explored for their use in a number of medical conditions, including diabetes (4). ESCs are viewed as an excellent resource for the generation of insulin secreting islet cells through the established developmental and differentiation pathways. Theoretically, it is possible, despite the troubles, that ESCs could be directed to differentiate into pancreatic islet cells and these cells could then be implanted in patients with diabetes, thus the β -cell deficit could be beaten. A routine of other groups have also utilized both mouse (5) and human (6) ESCs for their subjects and have described the different degree of success in producing islets. All these endeavors have come across different events that include final cell homogeneity (7), immaturity of the differentiated cells (5), low numbers of insulin-making cells (8) and a poor insulin response when the cells were exposed to glucose (9). All these issues collectively forced the researchers to rethink their differentiation strategies and Kubo et al. (2004) acquired a formula to convert mouse ESCs into definitive endoderm (10). This protocol was redefined by D'Amour et al. to produce a near 100% pure, definitive endodermal cell population (11). This group transplanted their unique, differentiated cells that resemble 6-9-week-old fertilized egg into the immunodeficient mice and demonstrated that the insulin release was glucose dependent. This allowed the cells to both recover mice from STZ (streptozotocin)-induced diabetes as well as to forbid it (12). These breakthroughs may lead the way for ESCs to become a firm candidate for cellular replacement therapy in T1DM in near future.

Induced pluripotent stem cells and diabetes

The production of pluripotent stem cells from non pluripotent resource is referred as induced pluripotency. The induced pluripotent stem cells (iPSCs) exhibit high telomerase activity similar to that of ESCs and possess hypomethylated gene promoters (13). These iPSCs are preferred choices of cell based therapy for diabetes management as they can be patient specific and eliminate the possibility of likelihood rejection. The fibroblast cells are stimulated to produce iPSCs and these

cells are subsequently converted to pancreatic beta like cells by a three-stage differentiation process. Zhang and fellow workers have demonstrated that the human ESCs and iPSCs were differentiated into mature pancreatic cells that were capable of secreting both insulin and C-peptide (14). The above mentioned research innovations and recent progress in the subject area of induced pluripotency would allow the usage, patient-specific iPSCs for cell based therapies in diabetes. Still, the safe usage of iPSCs for diabetes management must be ensured as these cells exhibit irregular behavior and significant variations in reprogramming (15).

Adult stem cells and diabetes

Pancreatic stem cells

The technical advancements over a decade enabled the scientific community to derive stem cells from diverse cases of tissue sources, including bone marrow, umbilical cord blood, adipose tissue, skin, periosteum, dental pulp, etc. Animal studies have demonstrated that the availability of low quantities of pancreatic tissue would restore the maximum pancreatic β -cell mass (16). This is imputable to the replication of differentiated β -cells of pancreatic ducts and differentiation of these cells to pluripotent cells that in turn produce more β -cells. Further investigation uncovered that this population of ductal cells could, *in vitro*, be educated and guided to form insulin producing clusters (17). Xu et al. (2008) have offered a strong evidence for the existence of multipotent progenitor cells in the pancreatic ducts of mice that can give rise to new β -cells (18). More research strategies are needed to develop suitable methods to address the issue of isolation and *ex vivo* expansion of these stem cells for transplantation.

Homeopathic progenitor cells

The adult stem cells of the haemopoietic system, like HSCs and mesenchymal stem cells (MSCs) are having the ability to transdifferentiate into a figure of other cell lineages, including the liver, mental capacity, lung and even gastrointestinal tract cells (19). This multipotent differentiation of homeopathic progenitors was explored in greater detail by various groups of researchers to replenish the β -cell population in T1DM. The vivo

experiments with mouse model indicated that bone marrow cells can be aimed to the pancreas and that hyperglycemia can be reversed (20). Surveys carried out with autologous HSCs showed improvement in both type 1 (21) and type 2 diabetes mellitus (22). These trials provide promising solutions for the use of autologous HSCs in the discussion of diabetes.

Other adult stem cells

The liver and small intestine are extensively considered as possible sources of pancreatic β -cells. Irrespective of the method used, amelioration of hyperglycemia was achieved by these cellular telephones in the mouse models. This created hope among the researchers to search for extra pancreatic sources of insulin (23). Many other stem cell resources have been explored for the output of insulin secreting β -cells and different degrees of success have been attained with them. In the age to come, the hepatic production of insulin has the potential to become a feasible source for β -cells replacement. This is possible not before addressing the practical hurdles associated with these cell lines, culture conditions, complete differentiation, and islet structure formation etc.

Mesenchymal Stem Cells for Regenerating Pancreatic β -Cell Mass

The primary challenge for successful stem cell therapy to treat T1DM lies in producing functional β -cells and overcoming the autoimmune reaction. In theory, β -cell mass and function could be preserved and/or restored in at least three different ways (Figure 03): replacing damaged β -cells by direct stem cell differentiation, modifying the pancreatic microenvironment allowing endogenous β -cell regeneration and abrogating the autoimmune response to β -cells. Multipotent mesenchymal stromal cells (also referred to as mesenchymal stem cells-MSCs), a heterogeneous adult stem cell population, seems to represent an ideal tool, since they can be easily isolated from bone-marrow and other mesenchymal tissue, like adipose tissue, dental pulp, placenta, Wharton's jelly and umbilical cord, and rapidly expanded ex vivo (24). MSCs is hypo-immunogenic, allowing allogeneic transplant without histocompatibility or recipient

conditioning being required (25). When MSCs are systemically administered they can selectively migrate and engraft in damaged tissue (26) and differentiate into insulin-producing cells (27).

Furthermore, while proof of concept has demonstrated that MSCs can differentiate into insulin-secreting cells (at least in vitro), such an approach is limited by an inability to achieve a fully-differentiated β -cell phenotype, the relatively low amount of insulin secreted in vivo and inefficacy in rapidly adapting to day-to-day physiological requirements.

Modifying the pancreatic micro-environment to allow endogenous regeneration of pancreatic β -cells

It is well-known that MSCs secrete a broad range of proactive growth factors in their vicinity (i.e. VEGF, bFGF, IGF, HGF and EGF) (28). Therefore, MSCs could provide trophic support for injured tissue by modifying the microenvironment to induce local precursor proliferation and differentiation, improve damaged tissue irrigation and prevent parenchymal cell apoptosis (29).

Adipose-derived MSCs represent a very hopeful approach to diabetes since they are invested with a great number of bioactive mediators, such as leptin, adiponectin and visfatin, which are recognized to regulate glucose homeostasis (30). The secretion of the aforementioned growth factors could therefore make a tissue microenvironment assisting endogenous β -cell regeneration and damaged islet revascularisation. The β -cell regeneration in humans has been indicated by observing residual β -cells in T1DM patients after onset (31) or even many years after diagnosis (32); however, it remains unclear whether residual β -cells or the remaining endogenous precursors could be stimulated to amass great enough to control glaucoma.

Abrogating autoimmunity to pancreatic β -cells

It has been shown that diabetes develops by dendritic cell and macrophage invasion of the pancreas, followed by CD4 and CD8 T-lymphocyte, natural killer (NK) cell and B-lymphocyte infiltration (33). β -cell death

during the course of firing is probably mediated by direct contact with activated macrophages and self-reactive T-lymph cells and by exposure to soluble mediators secreted by these cells, including pro-inflammatory cytokines, nitric oxide and oxygen free radicals (34).

MSCs may thus prove to be useful in treating autoimmune diseases, such as T1DM, owing to their immunosuppressive and anti-inflammatory properties which could promote immunological tolerance (35). MSCs have a wide range of

immunomodulatory features as they can secrete anti-inflammatory cytokines and form cell to cell inhibitory interactions (36); they can also affect dendritic cell function by inhibiting monocyte precursor differentiation (37). Interestingly, both Fiorina et al., have shown murine MSC preferential migration to secondary lymphoid organs, including pancreatic lymphoid nodes, thereby suggesting that such homing ability could be crucial in modulating an immune response (35).

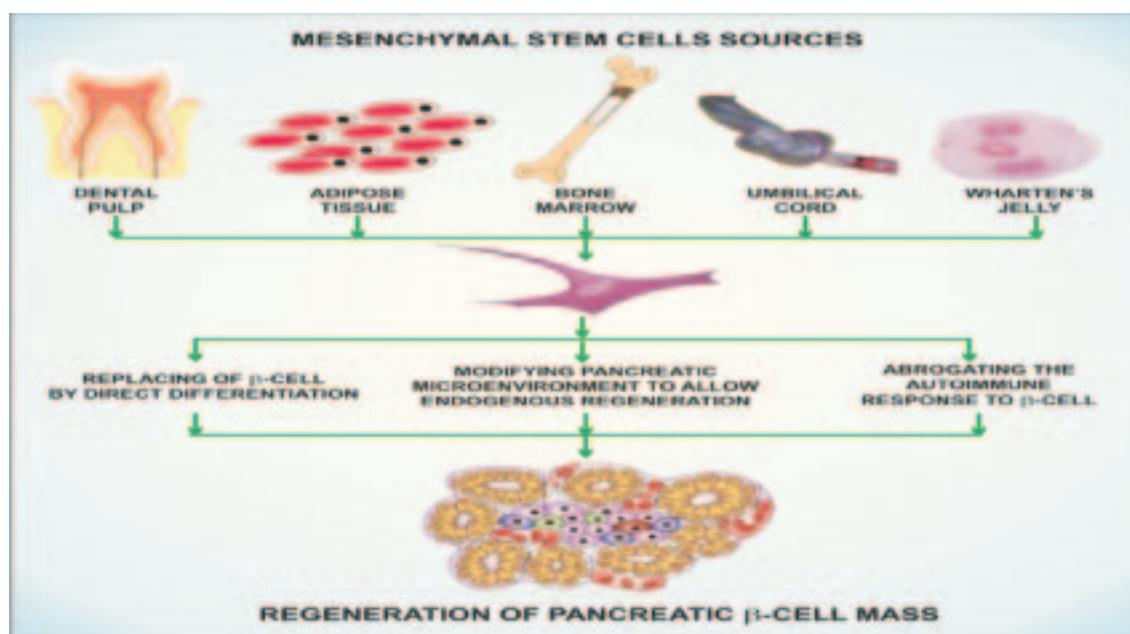


Figure 3: Regeneration of β -cell mass by mesenchymal stem cell

Mesenchymal stem cell: from preclinical data to clinical practice

Despite the great amount of preclinical data supporting a therapeutic role for MSCs in β -cell mass regeneration and treating diabetic complications, few clinical tests have involved using MSCs in diabetes. In one such trial, Haller et al. studied the safety and efficacy of MSC-containing autologous cord blood infusion concerning DM in children. There were no substantial adverse effects, suggesting that cord blood infusion was feasible and safe in the aforementioned conditions (38). However, the therapeutic effect disappeared two years after cell infusion; no patient thus achieves long-term preservation of the remaining β -cell volume (39). The concept of immunomodulation by cord blood-derived MSCs was recently brought up by

Zhao et al., who developed a fresh procedure for re-educating patients' lymphocytes through co-culturing with allogeneic MSCs (40). This device consisted of a stack of Petri dishes containing cord blood-derived MSCs functioning as part of a closed-loop system that circulates the patient's line of descent through a blood cell separator, briefly co-cultured a patient's lymphocytes with MSCs *in vitro* and returned the lymphocytes to the patient's circulation. In this setting, through secreted and cell-surface signalling molecules, the MSCs educated the lymphocytes passing through the device. Outcomes from this trial highlighted the fact that such treatment led to a clinically-relevant improvement in T1DM patient's metabolic control (increased C peptide levels and reduced daily insulin requirement in T1DM patients having or lacking residual β -cell volume),

which survived for months following a single treatment (40). Hu et al. reported the results of a randomized controlled trial in another study purporting to assess the long-term effects of injecting Wharton's jelly-derived MSCs for new-onset T1DM patients. Treated T1DM patients had better glycemic control and increased C peptide levels after two years' follow-up compared to

people having the same age, diabetes' duration and receiving intensive insulin therapy (41). The role of allogeneic bone marrow-derived MSCs (Prochymal) has been tested for determining whether MSCs could halt autoimmunity progression and restore glycemic control in newly-diagnosed T1DM patients. List of Clinical trial has been listed in Table 01.

Table 01: Clinical Trial using Stem Cells:

S.No.	Status	Clinical trial No.	Source	Disease
1	Recruiting	NCT01219465	Umbilical Cord Mesenchymal Stem Cells	Type1 Diabetes Mellitus
2	Completed	NCT01068951	Mesenchymal Stem Cells	Type1 Diabetes
3	Completed	NCT00788827	Autologous CD34+ Stem Cells	Type 2 Diabetes
4	Completed	NCT00690066	PROCHYMAL®; Drug: Placebo	Type1 Diabetes Mellitus
5	Active, Not recruiting	NCT00807651	Immunosuppression and Autologous Stem Cell Transplantation	Type1 Diabetes Mellitus
6	Completed	NCT01786707	Autologous Stem Cells and Hyperbaric Oxygen Therapy	Type 2 Diabetes Mellitus
7	Ongoing	NCT01374854	Umbilical Mesenchymal Stem Cell (UC-MSCs) Infusion	Type1 Diabetes Mellitus
8	Ongoing	NCT00644241	Stem cell Harvest; Procedure: Angiographies Transplantation of Stem Cells	Type 2 Diabetes Mellitus
9	Ongoing	NCT00315133	Autologous Hematopoietic Stem Cell Transplantation	Type1 Diabetes Mellitus
10	Ongoing	NCT01413035	Umbilical Cord/Placenta-Derived Mesenchymal Stem Cells	Type 2 Diabetes
11	Ongoing	NCT01694173	Autologous Bone Marrow Derived Stem Cells	Type 2 Diabetes Mellitus
12	Ongoing	NCT01954147	Umbilical Cord Mesenchymal Stem Cells	Type 2 Diabetes Mellitus
13	Ongoing	NCT01142050	Mesenchymal Stem Cells	Type 2 Diabetes Mellitus
14	Ongoing	NCT01143168	Autologous Bone Marrow Mononuclear Cells and Umbilical Cord Mesenchymal Stem Cells	Type1 Diabetes Mellitus
15	Completed	NCT01121029	Autologous Hematopoietic Stem Cell Transplantation	Type1 Diabetes Mellitus
16	Recruiting	NCT02057211	Autologous Mesenchymal Stem Cell Transplantation	Type1 Diabetes Mellitus
17	Recruiting	NCT01285934	Autologous Hematopoietic Stem Cell Transplantation;	Type1 Diabetes Mellitus
18	Ongoing	NCT02287831	Umbilical Cord Mesenchymal Stem Cells	Diabetes; Peripheral Arterial Disease
19	Recruiting	NCT01759823	Mesenchymal Stem Cell Transplantation	Type 2 Diabetes Mellitus
20	Ongoing	NCT00703599	Autologous Adipose Derived Stem Cells	Type1 Diabetes Mellitus
21	Completed	NCT00539851	Allogeneic Stem Cell Transplantation	Diabetes Mellitus

22	Recruiting	NCT01322789	Intravenous Mesenchymal Stem Cell Infusion	Type1 Diabetes Mellitus
23	Completed	NCT00665145	Stem Cells in Peripheral Blood	Diabetes Mellitus Type 1 Diabetes Mellitus Type 2
24	Recruiting	NCT02302599	Umbilical cord Mesenchymal Stem Cells	Type II Diabetes
25	Recruiting	NCT01157403	Autologous Mesenchymal Stem Cell	Diabetes Mellitus Type 1
26	Recruiting	NCT01453751	Autologous Adipose derived Stromal Cells	Diabetes Mellitus Type II
27	Completed	NCT01102699	Bone Marrow Derived Stem Cells	Diabetes Mellitus
28	Completed	NCT00968006	Endothelial Progenitor Cells	Type 2 Diabetes Mellitus
29	Ongoing	NCT01257776	Autologous Adipose Derived Mesenchymal Stem Cells	Critical Limb Ischemia (CLI); Diabetes
30	Completed	NCT02056210	Bone Marrow Derived Stem Cells	Diabetes Mellitus
31	Recruiting	NCT02240381	Allogeneic Stem Cell Transplantation	Diabetes Mellitus
32	Enrolling by invitation	NCT02138331	MSC Exosomes	Diabetes Mellitus Type 1
33	Recruiting	NCT01677013	Autologous Bone Marrow Mononuclear Cell Transplantation	Diabetes Mellitus Type 2
34	Ongoing	NCT00767260	Bone Marrow Mononuclear Cell	Diabetes Mellitus Type 2
35	Ongoing	NCT01686139	Bone Marrow Mesenchymal Stromal Cells (BM-MSCs)	Type I &II Diabetes Mellitus with Ulcer
36	Recruiting	NCT01832441	Bone Marrow Derived Autologous Cells	Diabetes Mellitus

Hurdles in the progression

Despite the valiant strides made in the area of stem cell biology over a decade, the use of stem cells in diabetes treatment is all the same in its crude phase. The effective and full-fledged usage of stem cells relies upon how well the associated issues and hurdles are solved. The stem cell based cure of diabetes will become reality only when all the difficulties are properly set and effectively directed. The predominant concerns associated with the development of a potential source of stem cells for the treatment of diabetes have been discussed below

Safety aspects

The power to form teratomas and the possible risk of malignancy are the key features associated with the utilization of ESCs (42). Therefore, on that point should be a strict testing and showing for potential side effects before applying it in clinical trials and for treatment in human population.

Transplantation issues

The autoimmune rejection is a major issue during transplantation and that demands a stable and

appropriate immunosuppression regime. Stem cell transplantation requires a number of disciplines to talk about the issues connected with the transplanted cell survival, stability and durability in the new microenvironment with appropriate vascular and neural support.

Scale up issues

Once the appropriate developmental procedures are optimized then comes the scale up issues. The amount of cells needs to fill the needs for further research including clinical trials. Therefore, it calls for effective techniques to maximize the yield by adjusting the culture requirements. To keep the equilibrium between the demand and usage, the weighing machine-up potential of stem cells requires further exploration to provide an excess of transplanted cellular reserves.

Legal and ethical issues

Referable to the nature of its source, the ESCs is the hot target for the partisans. Generally, ESCs are usually derived from fresh and/or unfertilized embryos at in vitro fertilization clinics. These ESCs need to be secured from the

donor on the foundation of informed consent before using them for any clinical study. However, in most of the cases the cells from the embryo are obtained by destroying the embryo and that rises the question about the origin of life and ethical rights to destroy the embryo. The answer will lead into the never ending debate and it is always advisable to follow the set of rules that are laid by the governing bodies around the globe based on the sentiments and beliefs of people from that particular geographical location. The adult stem cells are preferred over their embryonic counterpart as there is no much controversy about the usage of adult stem cells. The recent technological advancements in the field of induced pluripotent stem cell research allow the usage of persons own stem cells for different purposes (43).

Conclusion

Despite the achievements and advance with the stem cells, the central issues like safety concerns, teratoma formation, transplantation issues and autoimmune response, and ethical dilemmas of ESCs limit their further exploration in clinical tests. Likewise, the events associated with the scale up production, hamper the exploration of adult stem cells and iPSCs to be used as a choice of healing resources. The scientific effort of a past decade enabled us to produce insulin secreting cells and the future years may come up with the answer to use stem cells as a therapeutic agent to cure diabetes.

A research team headed by Douglas Melton (left) has made insulin-secreting cells using human stem cells. Each year, surgeon Jose Oberholzer frees a few people with type 1 diabetes, from daily insulin injections by giving them a transplant of the insulin-secreting β -cells that the disease attacks. It has been a frustrating process. Gleaned from a cadaver's pancreas, then β -cells are in short supply and vary in tone. And the patients must take drugs to suppress their immune response to the foreign cells, which fire in turn cause kidney failure. On 9 October, 2014 stem-cell researcher Douglas Melton of Harvard University in Cambridge, Massachusetts, and his colleagues reported an advance that has the potential to overcome Oberholzer's frustrations and let many more people with type 1 diabetes

to get transplants. Melton and his squad have reached a long-term goal of stem-cell science: they have created matter β -cells using human stem cells that can be turned from a potentially unlimited supply, and that behave like the genuine thing (44). The next challenge is to solve out how to shield these β -cells from the body's immune reaction. However, for those people with diabetes who face life-threatening changes in blood-sugar level each day, mature β -cells could provide a big improvement without protective devices (encapsulation), says Oberholzer who is working with Melton's team to test these cells in non-human primates. Many of his patients are relieved to be free of insulin injections: "They would much rather take immunosuppression," he states.

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Cerebral Palsy: A Case Report

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Abstract

Cerebral palsy (CP) is a severe disabling disease with worldwide incidence being 2 to 3 per 1000 live births and it is the most common motor disability in childhood. CP is considered as a noncurable, nonreparative disorder, but stem cell therapy offers a potential treatment for CP. In this study, one patient diagnosed with Cerebral palsy and for who autologous umbilical cord blood stem cell was stored underwent for transplantation. Five infusions of autologous cord blood stem cells were injected intravenously. Changes in neurological deficits and improvements in function were assessed for 6 months. Significant improvement in muscle tone leading to decrease in dystonia, improvement in oromotor control leading to decrease in drooling, neck holding, improved gaze fixation and improved trunk control were observed. In this study, we report that intravenous infusion of autologous cord blood stem cells seems to be feasible, effective, and safe with encouraging functional improvements in CP patient.

Keywords: *Cord Blood, Stem cells, Autologous Transplantation, Cerebral Palsy.*

Introduction

As the most common childhood physical disability, cerebral palsy (CP) strikes approximately 2 to 3 of every 1000 live term births and increases to 22 of every 1000 live premature births, with more boys affected than girls. Cerebral palsy (CP) is not a single entity but rather an overarching term describing a group of permanent disorders that cause a range of life-long motor and posture-related impairments. Although a diagnosis of CP refers to problems with neural control of motor function, individuals may also have complications in behaviour, learning, epilepsy, communication, vision, hearing, perception and sensation.

Conventional therapies for cerebral palsy include physical and occupational therapy, oral medications, and orthopaedic surgery for supportive and rehabilitative approaches. Treatment programs for CP encompass physical and behavioral therapy, pharmacologic and surgical treatments, mechanical aids, and management of associated medical conditions. Cord blood stem cells are used to treat children with cancerous blood disorders such as leukaemia, or genetic blood diseases like breast

cancer (1), prostate cancer, ovarian cancer, aplastic anemia (2), Fanconi's anemia (3), immune disorders, and bone marrow reconstruction after cancer irradiation. The cord blood stem cells are transplanted into the patient, where the HSCs can make new, healthy blood cells to replace those damaged by the patient's disease or by a medical treatment such as chemotherapy for cancer. Cord blood stem cells produce significantly less graft-versus-host disease (GVHD) than transplantations with bone marrow or adult hematopoietic cells (4-7). Risks are significantly reduced even with ABO blood group incompatibility (8).

Stem cell therapy is considered as a novel approach in the treatment of cerebral palsy via replacing injured or dead neuronal cells and has proven effective in restoring injured organs and tissues in animal models. Here we present a paediatric case to intravenous administration of umbilical cord blood is safe and effective in a patient with cerebral palsy.

Case Report

Autologous umbilical cord blood stem cells of subject had been stored in Reelabs Private

Limited stem cell laboratory. The subject's autologous umbilical cord blood was thawed and washed as per standard operation procedures of the ReeLabs Private Limited stem cell laboratory. An aliquot of cells was analyzed for viability and CD34 percentage. The thawed umbilical cord blood stem cells were then infused through peripheral intravenously. After infusion, subjects were observed closely for at least 6 h prior to being discharged.

The major symptoms observed in the subject were partial neck holding, poor trunk balance/control, drooling, delayed speech, squint, no grip, behavioural issues, dystonia. Autologous cord blood stem cells were isolated using density gradient centrifugation method. Briefly, the whole process of cord blood stem cells preparation was performed in a good manufacturing practice (GMP) facility in ReeLabs Private Limited stem cell laboratory. The releasing criteria included cell viability (>95%), free from bacterial and viral contamination, absence of endotoxin and immunophenotyping showing expression of CD34, CD133, and CD 45. For each treatment, a total of $10-20 \times 10^6$ cord blood stem cells in 4 ml solution were administered intravenously. During the treatment period, the patient had one episode of temporary fever without needing an additional treatment. No other medical treatment except rehabilitation training was performed. The patient was followed up for 6 months since the last transplantation of cord blood stem cells. Symptoms before and after cord blood stem cells treatment were carefully compared (Table 1). The major symptoms were improvement in muscle tone leading to decrease in dystonia, improvement in oromotor control leading to decrease in drooling, neck holding, improved gaze fixation and improved trunk control.

Table 1: Symptoms before and after therapy

Before therapy	After therapy
Dystonia	Decreased dystonia
Partial neck holding	Neck holding improved
Poor trunk balance /control	Improved Trunk control
Drooling	Oromotor control improved, decreased drooling
Squint	Improved Gaze fixation/vision
No Grip	Improved grip/ability to hold objects
Behavioural issues	Behavioural issues decreased considerably

Discussion

Medically untreatable neurological disorders are an area where stem cell (SC) therapy has generated hope in the last decade (9). Previous clinical trials showed that subarachnoid placement of umbilical cord stem cells was safe without long-term side effects (10). Satisfactory outcomes have not been achieved to date in treating CP by traditional therapies. CP represents a complex disorder and therefore series of interventions of effective therapeutics strategies are needed. Extensive research carried out in stem cell therapeutics has offered hope for conditions such as CP. By conducting this study, we provide the evidence of feasibility and efficacy of BMMNCs transplantation in CP patients (11). Umbilical CB stem cells have shown promise in the treatment of CP in both animal models and early human trials. In current study, umbilical cord blood stem cells transplantation had one episode of temporary fever but no additional treatment was required. The risks of the procedure are extremely low since the infusion is made up of the autologous cord blood cells. Some children may react to preservatives from the cells, but otherwise few side effects are expected. After the infusion, it may take some time for the cells to engraft and begin to have effects. Most children begin to see some effects within a week and further changes in the first three months. These may include increased strength and mobility, improved feeding, reduction of seizures, improved speech, and improved vision, as well as progress in other areas (12). Human umbilical cord blood cells (hUCBCs) have been explored to a great extent in cerebral palsy. hUCBCs have been administered in rat models of neonatal hypoxia/ ischemia. They protect the mature neurons in the neocortex from injury, bring about near-normalization of brain damage in the subventricular zone (SVZ) leading to significant improvement in behavioral functions. The long lasting effect of these cells is due to the paracrine effects of hUCBCs which stimulate recovery in the injured brain and protect against further brain damage. (13) On transplantation, hUCBCs have shown to ameliorate neurological and motor deficits in CP model by reducing the levels of pro-inflammatory cytokines (Interleukin-1 α (IL-1 α), Interleukin-1 β

(IL-1 β), and Tumor necrosis factor α (TNF α)) (14-15).

Conclusion

Umbilical cord blood stem cells transplantation showed the potential promise of, at least partially, improving the gross motor dysfunction of children with cerebral palsy. The result suggests that umbilical cord blood stem cells transplantation may be a safe and effective way to treat cerebral palsy. Efficacy and adverse effects in long term in a large-size cohort merit further investigation.

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Opportunities with Regenerative therapy in Orthopaedics : An Overview

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Abstract:

Stem cell therapy in orthopaedics holds a positive future in treating certain conditions which faces lot of challenges in presently available treatment modalities. These conditions like AVN head of femur, moderate stage of progressive OA, uncontrolled Rheumatoid Arthritis, DMD give enormous challenges to the treating orthopaedic Surgeon. Cell therapy is the most important strategy in the management of above mentioned conditions. Defiantly autologous minimally manipulated MNCs should be ideally used. As minimal manipulation is being done so there should not be any safety concern issues. We should plan clinical trials not only from clinical perspective but also to generate sufficient evidences of cellular therapy as treatment options in such conditions. Ultimately angiogenesis and chondrogenesis are required in AVN and OA patients respectively. Hip Avascular necrosis results from interruption of normal blood flow to the femoral head which if diagnosed at earlier stage, have the option of halting the progression with stem cell therapy. Clinical grade mesenchymal stem cells with or without PRP can be used not only in treatment of OA but also in chondral lesions due to sports injury. This is the possible correct way to avoid further cartilage degeneration leading to Sec OA. MRI changes post therapy in AVN, OA must be clinically correlated. The importance of rehabilitation for achieving expected recovery after the therapy must not be forgotten and regular follow up must be maintained to access the outcomes. Regenerative Science is thus a affective approach based on unique ability of stem cells to reproduce, repair & rejuvenate the affected damaged tissue.

Key words : Adult Stem cells, Osteoarthritis, Avascular necrosis, head of femur.

Introduction

Stem cells are receiving a great deal of scientific attention as well as coverage in the orthopaedic treatment. One of the many reasons for the attention is the potential of these cells to regenerate tissues without the production of scar tissue. Many new technologies are coming up in orthopaedic management but they are poorly defined with regard to stem cells.

We face lot of challenges in management of AVN head of femur, moderate stage of progressive OA, uncontrolled Rheumatoid Arthritis, Duchenne Muscular dystrophy etc. Most of the research

aimed at clinical treatments which have been carried out using autologous MSC's, mainly from bone marrow (1). Specifically, bone marrow derived stem cells have been used in vitro to generate bone, cartilage, tendon, ligament, meniscus, intervertebral disc, fat, muscle, and nerve. Because of the availability of adipose tissue, it too has received a fair amount of recent research as a source of MSC's (2). A clear delineation of the pros and cons of fat derived verses bone marrow derived MSC's is lacking. Ease of collection procedure, number of stem cells recovered, capacity and efficiency to differentiate into various mesenchymal tissues, as well as

morbidity associated with the collection procedure are all important points to consider when discussing bone-marrow versus adipose derived stem cells. Because MSC's treatments are being used from both fat and bone marrow, it is important to point out that few direct comparisons have been published, and at this point a definitive answer is lacking on which population of cells is better. Typically, aspirated bone marrow is described to contain 40 million nucleated cells, of which 2,000 are stem cells per milliliter (or 1 stem cell per 20,000 cells). In contrast, fat is far less cellular (approximately six million cells per cubic centimeter of tissue compared to 40 million in bone marrow aspirates), but the prevalence of stem cells in fat has been described as high as one per 4000 cells, which is higher than that in bone marrow (1).

Adult Stem cells & Osteoarthritis

Osteoarthritis (OA) and related degenerative joint disorders have a heavy disease burden and affects millions of people annually around the world. Once damaged articular cartilage lacks the ability to properly repair and regenerate itself. Various surgical procedures are being tried to restore joint functions starting from minimal procedures like shaving after lavage, laser abrasion, micro fracture of subchondral bone to more extensive procedures include autologous or allogenic osteochondral transplantation, autologous chondrocyte implantation (3). The ultimate procedure of total joint replacement remains the treatment of choice today for extensively damaged joint. Other than total joint replacement above mentioned extensive procedures are effective to varying degrees in treating chondral defects of limited sizes. Thus, there is need for improved cartilage repair modalities.

Adult stem cells, because of the ease with which they can be isolated, their capacity to self-replicate, their ability to differentiate along multiple connective tissue lineages, have become the cell type of choice for cartilage tissue repair. In vivo studies have confirmed MSCs ability to localize and participate in repair of damaged joint structures, including cruciate ligaments, menisci, and cartilage lesions (3). Most of the in vivo studies utilizing MSCs has focused on

meniscal repair, in some cases using MSCs in a carrier or scaffold while in others utilizing direct injection into the joint (4-6).

Intra articular technique:

The best approach to intra-articular injection is the path having least obstruction and maximal access to the synovial cavity which could be medial, lateral retropatellar approach or suprapatellar approach or even anterior (anteromedial or antrolateral) approach. Medial retropatellar approach is frequently being used. Position generally used is supine with knee flexed to varying degrees. Usually 20/18 gauge sterile needle is carefully inserted into the joint space with aseptic technique. Advance the needle carefully in the joint space with limited resistance. If there is significant resistance or hitting the bone then one has to again redirect the needle. To prevent damage to articular cartilage do not insert the needle deep. Then syringe having mesenchymal cells is being attached to the inserted needle and fluid is slowly injected in PRP may or may not be injected along with this.

Osteonecrosis or Avascular Necrosis Head of Femur

Autologous bone marrow transplantation is a treatment modality in the early stages that creates the regeneration of the femoral head. Surgical treatment of early avascular necrosis of the femoral head remains controversial. Avascular necrosis (AVN) of the femoral head can be a devastating disease most commonly affecting patients younger than 40 years of age. Absence of a history of hip trauma and acute onset of deep groin pain is typical of patients who present with this condition. Development of this disease process is multifactorial, but there are accepted risk factors that have been shown to affect the relative risk of disease development. These include corticosteroid use (both amount and duration of exposure are of importance), alcohol use, smoking, hemoglobinopathy (most commonly sickle cell disease), and a variety of medical conditions (7,8). Steroid exposure remains one of the more common risk factors. In case series of patients receiving steroid treatments for autoimmune therapy or transplantation suppression, 10% to 30% develop AVN within

12 months of exposure. However, idiopathic occurrence is still very common. Core decompression remains one of the most commonly used procedures in early AVN as its description by Ficat and Arlet in 1964 (9). Careful patient selection may be the most important factor in achieving success in patients who are in these so-called precollapse stage. Percutaneous aspiration of bone marrow stem cells from the iliac crest, and their concentration by centrifugation methods provides an autologous hematopoietic augmentation to the potential beneficial effects of core decompression. It is important to reemphasize that while the mesenchymal stem cells in the concentrated bone marrow are believed to be essential in leading to the regeneration of bone in the AVN lesions, the use of concentrated whole bone marrow, which includes other cells in the stromal and hematopoietic lineages, may also be critical to the therapeutic effect as they provide an optimized physiological and cellular environment for the promotion of both osteogenesis and angiogenesis.

Recent advances in the understanding of the pathophysiology of osteonecrosis suggest that a decrease in the mesenchymal stem-cell pool of the proximal aspect of the femur might not provide enough osteoblasts to meet the needs of bone-remodeling in the early stage of the disease (10). An insufficiency of osteogenic cells could explain the inadequate repair mechanism that, it is postulated, leads to femoral head collapse. The effectiveness of bone-marrow mononuclear cells may be related to the availability of stem cells endowed with osteogenic properties, arising from an increase in the supply of such cells to the femoral head through bone-marrow implantation. Indeed, in the very early stages of osteonecrosis, providing sufficient repair capacity through the implantation of osteogenic cells could make these lesions reversible (11,12). Another possible explanation for the therapeutic effect of bone-marrow implantation is that injected marrow stromal cells secrete angiogenic cytokines resulting in

increased angiogenesis and subsequent improvement in osteogenesis. One study has suggested that the efficacy of such implantation was due to a supply of endothelial progenitor cells included in the CD34+ fraction as well as to

multiple angiogenic factors (vascular endothelial growth factors, basic fibroblast growth factor, and angiopoietin-1) released from the CD34+ fractions (13). Thus, the outcome of osteonecrosis of the femoral head is influenced by the size of the lesion, the stage of disease, the time from the diagnosis and etiological factors (14).

Conclusion:

This promising new approach of stem cell therapy, for the treatment of osteonecrosis femoral head could benefit from the recent advances made in the field of stem-cell biology, including the use of subpopulations of progenitors with greater therapeutic potential. Stem cell therapy in OA knee, although still at a developmental stage and not without hurdles, promises to bring hope and eventual solution to these patients.

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Role of Autologous Bone Marrow Derived Mononuclear Cells (BMMNCS) in 25 Subjects Of Cerebral Palsy

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Abstract

Cerebral Palsy is caused by tissue damage in the motor control centers of the developing brain that may result in severe motor disability and cognition problems. The regeneration capacity of the central nervous systems minimal and it is thought to be almost insignificant in the human brain. Steady advancements in bone marrow derived stem cell research have been showing their extensive capacity to regenerate and their ability to trans-differentiate into neural cells to restore functional tissue in the brain. In our current Open-label study, we have used Bone marrow derived Autologous Stem Cells to treat 25 patients under 18 years of age, diagnosed with Cerebral Palsy. Intrathecal implantation of 1 X 10⁷stem cells showed significant improvement in these patients as shown by their neuromuscular assessment studies such as improved muscle tone evidenced by their Modified Ashworth Scale scores, improved visiomotor coordination and gait pattern. The post-treatment MRI images of the brain showed decrease in leukomalacia. The medical history, administration methodology, detailed analysis of results and the conclusive effect of stem cell therapy in each case will presented and discussed.

Keywords : Cerebral Palsy, Autologous Stem Cells, Stem cell therapy, BMMNC, leukomalacia

Introduction

Cerebral palsy is a common problem and cause of disability. The worldwide incidence of CP is approximately 2-2.5 per 1,000 live births (1). Cerebral palsy is a disorder of movement, muscle tone or posture that is caused by injury or abnormal development in the immature brain, most often before birth. In CP, signs and symptoms appear during infancy or preschool years. CP can be classified according to the severity of motor deficits as mild, moderate, or severe (2). Several other classification systems exist based on the pathophysiology, etiology, and distribution of motor deficits. There is no cure for cerebral palsy, treatment will often improve a child's capabilities. Cell transplantation based regenerative medicine has been studied at length in animal models (3) of brain disease. Bone marrow and umbilical cords are rich sources of mesenchymal (from the middle layer of cells in the developing body) stem cells, which normally produce the tissues of the skeletal, muscle, and circulatory systems (4).

Materials and Methods

The present study was carried out to evaluate the effect of autologous bone marrow derived mononuclear cells (BMMNCs) stem cell transplantation in CP. This study was conducted according to the principles of the Declaration of Helsinki at Chaitanya stem cell Hospital (ISO Certified) of Pune India, which was approved by Institutional Review Boards & ICSCRT of Chaitanya Hospital. Informed consents were obtained from the patient and their parents as per ICMR and ICH -GCP guideline. In this study, children of both sexes were considered with inclusion criteria of ages 2 years to 18 years, MRI evidence of Cerebral Palsy, Normal Hb /WBC count & Fitness for G.A. No HIV / Hepatitis / Transmittable Disease No Peripheral Myopathy / Neuromuscular Disorder, willingness to undergo Stem Cell Therapy, willingness to visit the hospital for follow up examinations, physical examination of Gross & Fine Motor Skills, Co-ordination & Gait, Vision, Speech & Hearing Tests, Spasticity grading as per Modified

Ashworth Scale Various Investigation carried out as per protocol. Parents of the CP children in the study were thoroughly briefed concerning the study protocols and subsequently gave informed (written) consent for their children to have stem cell therapy using Autologous BMMNCs

Intervention:

BMMNC were derived from bone marrow of patient (Autologous) after obtaining the consent from the parents for procedure, the patient is prepared for Position which is in prone or side-lying position. After patient gets anesthetized, area exposed for aspiration and locate posterior superior iliac crest. Using aseptic technique bone marrow aspiration was done by holding bone marrow needle with stiletto in place, puncture skin and advance through subcutaneous tissue, periosteum and into marrow cavity using a steady, controlled pressure with a twisting motion ion located area. By Appling strong, quick suction 100 ml amount of bone marrow is aspirated. After the aspiration Bone Marrow is send to CSCL (Chaitanya stem cell Lab.) for processing. Using the density gradient method, MNCs (Mononuclear cells) were isolated in aseptic precautions and sterile atmosphere in class 1000 room. BMMNCs were checked before transplantation, for quality assessment, under which total count, viability at CSCL were done. The BMMNCs were administered on same day by an intrathecal injection into the spinal canal (Intrathecal space surrounding the spinal cord), at L3-L4 level.

A total of three treatments as mentioned above were processed. For each treatment, a total of 10x10⁷ MNCs were transplanted intrathecally.

After the infusion the patients were assessed for any adverse reaction. During the treatment period, the patient had one episode of temporary headache without needing an additional treatment. No other medical treatment except rehabilitation training was performed. Post therapy follow up was done on every 6 months since the last transplantation. Symptoms before and after MNC treatment were carefully compared with physical and psychological examination. Changes in neurological deficits and functional improvements were compared between pre and post therapy assessments using Standard scales for Modified Ashworth Scale, Oxford scale, Binet Kamat Scale and Gait Assessment Rating scale were used. Subjects were also assessed for change in MRI findings.

Results and Discussion

In these patients, intrathecal implantation of 1X 10⁷ stem cells showed significant improvement as shown by their neuromuscular assessment studies such as improved muscle tone evidenced by their Modified Ashworth Scale scores, improved visiomotor coordination and gait pattern. We observed that the subjects showed improvement in muscle power overall gait and balance, reduction in spasticity, remarkable improvement in overall IQ levels. We also observed mild regression in periventricular white matter accompanied with gliotic changes in the MRI scans of two subjects. The post-treatment MRI images of the brain showed decrease in leukomalacia. The medical history, administration methodology, detailed analysis of results and the conclusive effect of stem cell therapy in each case is showed in following images.

Table 1: Post-Therapy Observations

Observation	Observed in	Reduced in	%
Hypertonicity	20	14	70
Spasticity	23	23	100
Gross Motor Skills Defects (Sitting/Standing/Gait)	25	22	88
Fine Motor Skills Defects (Grasp/Release/Hand-Mouth/Transfer)	20	19	95
Speech Problems	16	10	62.5
Vision Problems	7	3	42.8
Hearing Defects	1	0	0
Low IQ	19	18	94.7

Figure 1: Comparison of Pre and Post MRI images. (A) Pre treatment MRI Image. (B) Post Treatment MRI Image.

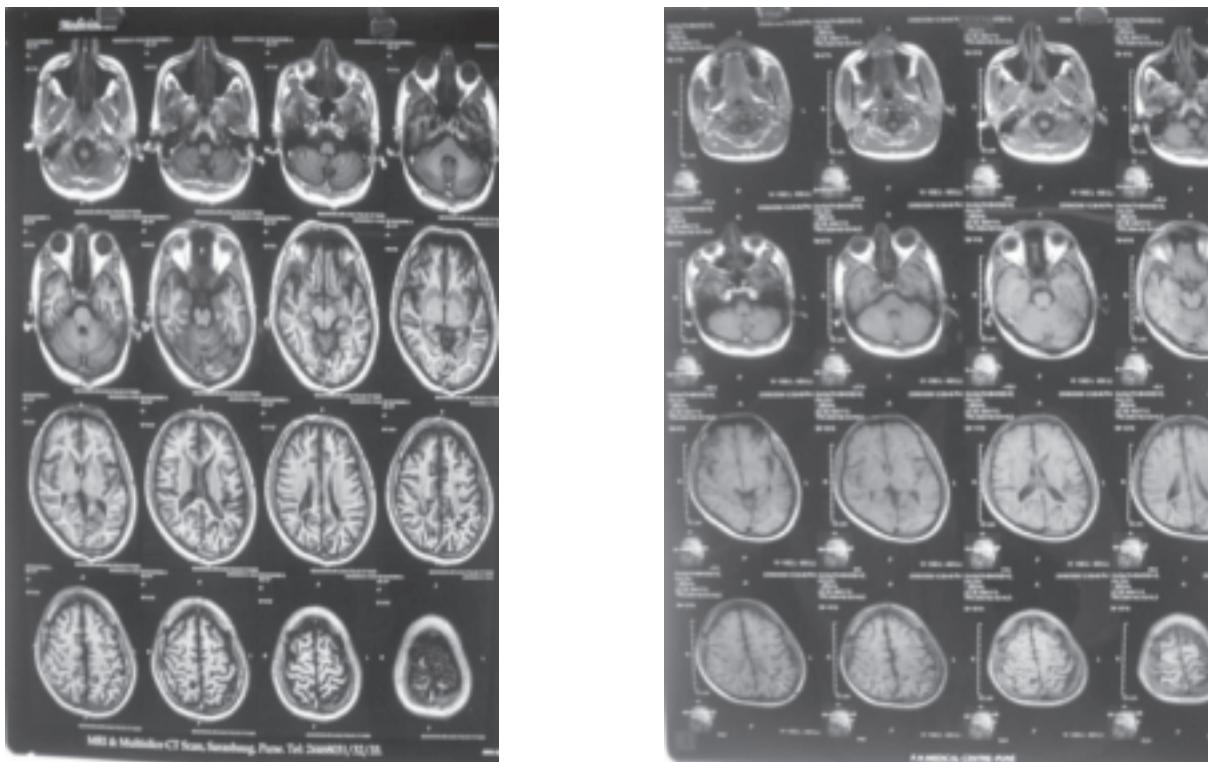
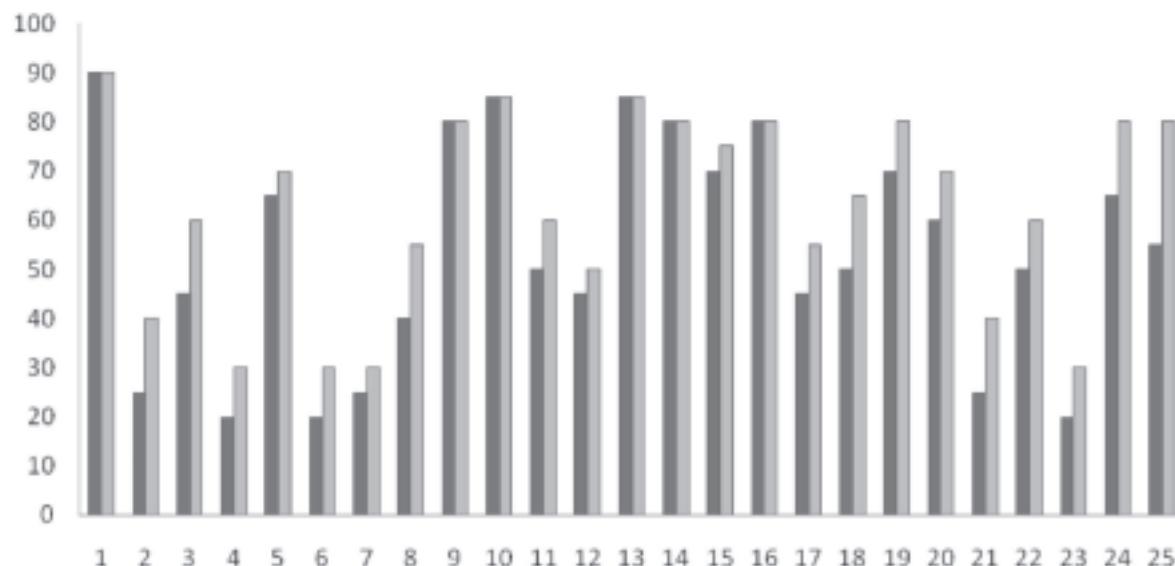


Table 2: Post-Therapy MRI Observations

Observation	Observed in	Reduced in	%
Cerebral Atrophy	9	6	66.6
Focal Atrophy	15	14	93.3
Periventricular Leukomalacia	18	13	72.22
Demyelination	12	7	58.33

Fig 3: Effect of BM-MNC therapy on IQ (Binet Kamat Test)



Conclusion

Thus, our findings indicate that BMMNCs is a viable and safe treatment alternative for treatment of CP. The definitive decrease in Spasticity and improvement in muscle tone, IQ and MRI results of the 25 cases under the present study provide pilot evidence for the efficacy of Stem Cell Therapy for currently incurable neurological disorders like Cerebral Palsy. Our findings also show that BMMNCs caused greatly increasing quality of life in such individuals. The present study demonstrated that the use of Bone marrow derived Stem cells (BMMNCs) achieve a recovery in CP. However, there are limitations to the strength of the available evidence, and more research is needed.

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Changing Paradigms in Healthcare Medicine

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Abstract

The science of healthcare medicine is changing rapidly and coming out of its shell with better prospects. The healthcare sector is now not limited to the old concept of one pathogen causing one disease and treated by one medicine but it is being replaced by the application of newer treatment modalities to treat the diseases and disorders. The concept of donor organ transplant is now changed to concept of organ regeneration (using iPSC's- induced pluripotent stem cells or even using autologous stem cells). Regenerative Medicine has played a big role in this paradigm shift. The Regenerative medicine - a branch of translational research uses stem cells in tissue engineering and molecular biology which deals with the process of reengineering or regenerating human cells, tissues or organs at the defective sites to restore or establish normal function. The applications of Regenerative Medicine are worldwide and it is now important to enhance the efficacy of these applications by merging the distinguishing fields of science using the variables those can change the paradigms of Regenerative Medicine.

Keywords: Regenerative Medicine, Stem cells, iPSC's, organ regeneration

Introduction

Stem cells are not new to us and we all know the importance of therapeutic applications of the stem cells in different disease and disorders but we are also aware about the fact that stem cell therapy has sparked much controversy over the last several years as this field is still surrounded by ethical, legal, political and social barriers. Safety and efficacy issues in use of stem cells have raised the concern about their use in the treatment of different diseases and disorders but if these issues are properly taken care of Stem Cells can play the role of wonder drug in modern medicine. Most of the medical experts are aware about the fact that old and conventional medical therapies have proven to be ineffective and useless for many of the dreadful diseases. In such cases where people are asked to compromise with their health condition, stem cells can really prove a medicinal boon to such patients. The Regenerative Medicine market has already started encroaching European and US countries with stem cells products, therapies and technologies. There is no doubt that

the upcoming era will be of Cellular medicine with more and more treatment modalities. As shown in the Fig.1, it is clear that how fast this parallel science of Regenerative medicine is showing its potential in the global healthcare market. What requires now is to understand its wide scope and to focus on the usefulness of this science in the mass.

Regenerative medicine based on the applications of the stem cells comprises a market for regenerative products and can be seen growing exponentially in coming years. Stem cells have wide use in treatments of orthopedic conditions, neurodegenerative conditions, cardiovascular diseases and even in several chronic metabolic disorders. Other disorders that will benefit from cell therapies include diabetes, inflammatory diseases, and several aging disorders. The success ratios may vary with respect to disease condition and the type of therapy used. Many a times it is seen that efficacy of the treatment is dependent on the allied treatment modalities coupled with the main stem cells therapy.

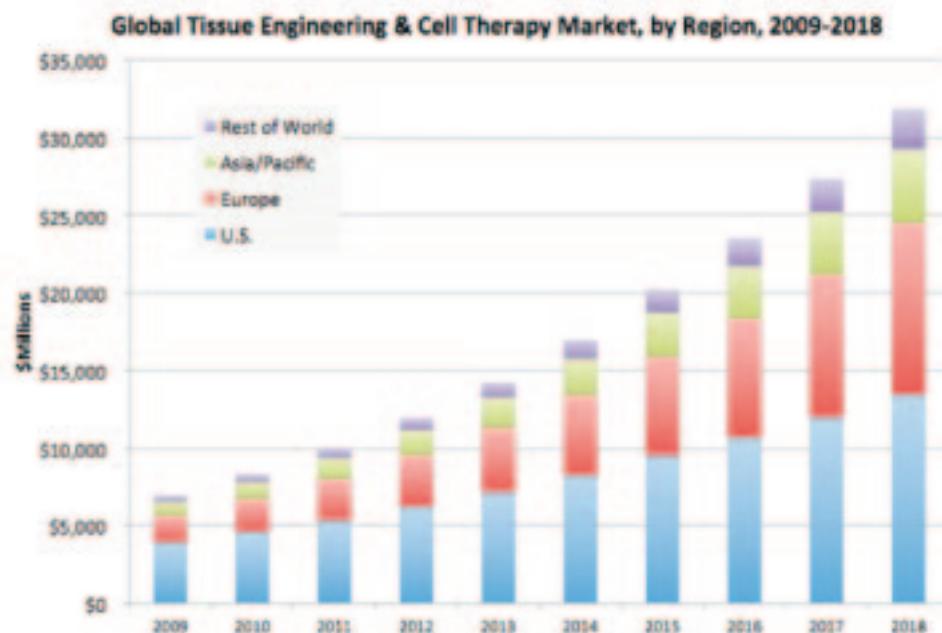


Fig 1. (Source: Report #S520, "Tissue Engineering, Cell Therapy and Transplantation: Products, Technologies & Market Opportunities, Worldwide, 2009-2018.")

These variables may play an important role in deciding the fate of stem cells therapy in curing the signs and symptoms of disorders.

The variables of changing paradigms for regenerative medicine

The distinguishing fields of allied treatment modalities which can really revamp the Regenerative medicine comprise of game changing technologies like Robotic medicine, Tele medicine, Digital medicine, Genomic & molecular medicine and nanomedicine.

Stem Cells and Robotic Medicine

Stem cells are the repairing kits which we carry in our body. There are many sources of stem cells like cord blood, cord tissue, bone marrow, adipose tissue which can be used for the therapeutic applications. In autologous cellular treatment patients own body cells are transplanted back after processing them in vitro. Robotic Medicine is not new to healthcare. We all are aware about the Vinci Surgical system that arose in 2000. Robotic system has eased the complex surgeries and is based on the high end mechanics. In regenerative medicine stem cells harvesting procedures and transplant procedures can definitely be coupled with the use of robotic

arms and set the new perfections. Recently in 2014 Bolinger M, Wechsler L and Stein J in their studies have concluded that robotics, stem cells, and brain-computer interfaces all have tremendous potential to reduce disability and lead to better outcomes for patients with stroke (1). However continued research and funds will be required to strengthen these fields as merging capacities of these sciences are still in a nutshell.

Stem Cells and Telemedicine or Digital medicine

The wireless innovations in healthcare have revolutionized the concept of remote consultations. Using high end technologies and advanced ways of communication, now it's possible to transfer the medical information through audiovisual media. With the use of Google mirror, holographic consultations are possible indoors, sitting at home. Virtual medical visits and holographic consultations have made communication easy and will be the milestone for creating awareness about the importance of regenerative medicine in mass. The cost effective and time saving parameters further add to the importance in this area. Digital medicine is a multidisciplinary subject that arose with the merging of medicine and new digital technologies, and covers subjects such as

medicine, mathematics, informatics, electronics and mechanical engineering. It can be used for basic research, clinical studies, and for treatment of various diseases (2). Zhu Weijun et al in 2014 have shown the importance of Telemedicine and digital management in repair and regeneration after nerve injuries and other nervous system diseases (3).

Stem Cells and nanomedicine

Nanotechnology allows scientists to create, explore and manipulate nonmaterial measured in nanometers which can be used in advanced surgeries. The Clinical Potential of Targeted Nanomedicine delivering to Cancer Stem-like Cells is now world known as Sang-Soo Kim et al have shown the importance of nanomedicine treatments on cancer patients for the delivery of stem cells. They developed a tumor-targeting nanodelivery platform (scL) for systemic administration of molecular medicines. In various animal models, Post treatment with the scL nano-complex carrying various payloads, they observed exquisite tumor-targeting specificity and significant antitumor response with long-term survival benefit (4).

Stem Cells and Bioengineering

Regenerative medicine also includes use of biodegradable scaffolds with stem cells and their safe implantation at the defective sites. Combining stem cells with biomaterial scaffolds provides a promising strategy for engineering tissues and cellular delivery. Biomaterials can be natural or synthetic, protein based or polysaccharide based. Protein based natural biomaterials are collagen, silk and fibrin while Polysaccharide based natural biomaterials are agarose, alginate, hyaluronan and chitosan. Synthetic biomaterials include PLGA Poly (lactic-co-glycolic acid) and PEG (Poly ethylene glycol). The type of the biomaterial or the cues used, play an important role in deciding the fate of the stem cells implanted. Stem cells along with these biomaterials have been used in many treatment modalities for orthopedic conditions like cartilage repair, bone fractures, tendon injuries and many more. Besides orthopedic conditions, stem cells with scaffolds have proven their efficiency in cosmetics and even in cardiac operations. Cell sheets of MSC's (mesenchymal stem cells) have

seen to improve cardiac function when used with collagen (5).

Organ Development with 3D printing technology

Tissue engineering technology promises to solve the organ transplantation crisis. The assembly of vascularized 3D soft organs remains a big challenge. Organ printing defined as the computer aided, jet based 3D tissue-engineering of living human organs offers a possible solution. Organ printing involves development of blueprints for organs followed by actual organ printing and organ conditioning. Cell printers that can print gels, single cells and cell aggregates have been developed. Solidified thin layers of sequentially placed, thermo-reversible gel serves as printing paper. Combination of engineering approach with embryonic tissue fluidity concept of developmental biology enables the creation of a new rapid prototyping 3D organ printing technology, which has the potential to accelerate and optimize the tissue and organ assembly dramatically (6). 3D printing technologies are already being used in pharmaceutical research and fabrication, and look promising in bringing transformation. Advantages of 3D printing include high reproducibility, precise control of droplet size and dose, and the ability to produce dosage forms with complex drug-release profiles.

Complex drug manufacturing processes can also be standardized through use of 3D printing to make them simpler and more viable. 3D printing technology could also prove beneficial in development of personalized medicine (7).

Revamping of Healthcare Sector

Regenerative medicine coupled with all the above mentioned advancements and can revamp the entire healthcare sector. During this happening the paradigm of healthcare has shifted towards cellular medicine from the old conventional medicine. In the due course of time surgeons have done number of successful transplants with the help of stem cells. For an instance, Hip replacement is taken over by hip regeneration and Instead of spending millions of dollars on insulin injections, now it is possible to regenerate insulin factories *in vivo* to eliminate diabetes completely (8). Similarly in several orthopedic conditions,

neurodegenerative and neuro developmental conditions stem cells treatments have proven their existence. In the coming years researchers may introduce the stem cells gene therapy for immunomodulatory treatments in autoimmune disorders. Ground breaking invention of T cells educator therapy for autoimmune disorders by Dr. Yong Zhao (9) as highlighted by the American Diabetes Association at 72nd Scientific Sessions (Philadelphia, 2012) is one of the 8 major breakthroughs and initiatives which were done in 2012. Stem cell growth and migration on nano-fibrous scaffolds and micro-fluidic channels on Silicon-Chip were studied using neural stem cells isolated from adult rats. Possibly this technology of using neural stem cells will revolutionize the treatment protocols in electrical signal neural transmission system (10). The invention of VSEL's (Very small embryo like stem cells) or DC dendritic cells have already raised the standards of treatment modalities in cancer medicine (11).

Discussion

Science is so evolved that concept of developing a body organ which looked like a dream once upon a time is no more fiction but has turned into reality with the help of 3D printing and scanner technology. Now it is possible to develop organs like liver, kidney etc. This clearly indicates that Regenerative medicine is the future of the medicine and conceptually the day is no far when one can access the organ shops easily in healthcare market. However this field is under siege politically and financially so it needs an urgent attention to promote this science which has true powers of revamping the complete healthcare sector. Medical technology is growing rapidly. Several diseases can now be treated very effectively with the application of implantable devices that restores physical and mechanical function, such as replacement of hip joints or restoration of heart rhythms by pacemakers. The techniques however, are rather limited, and biological function cannot be restored through the use of inert materials and devices. Patients today demand quality health care and Health care practitioners demand stability which has to be taken care of otherwise it will lead to the problem of health crisis. This may lead to legislation expanding the scope of practice for allied health care providers, thereby

circumventing physicians and undermining our control. If we really want to avoid the health crisis problem, revamping of healthcare medicine is necessary and globally it requires an urgent attention to step towards the better healthcare system.

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Medical Regulations and issues related to Stem Cell Therapy in India from a legal perspective

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Abstract

Whilst there is no specific definition of what a "stem cell" means in any of the applicable medical laws, stem cell research and therapy is permitted in India through a national apex committee which was set up during October 2010 by the Ministry of Health and Family Welfare, Government of India. The final guidelines relating to stem cell research were issued during February 2014, by the Ministry of Health and Family Welfare, Government of India. These guidelines have classified the stem cell research into three categories, viz., permitted, restricted and prohibited. The Guidelines have also prescribed a mechanism for review and regulatory oversight of Stem Cell Research. The issues relating to clinical trials/stem cells are overseen and governed under the laws relating to the Drugs and Cosmetics Act, 1940. There is a proposal to amend this Act through a bill which was tabled in Lok Sabha. This Bill proposes to bring "Stem Cells" under the definition of "New Drugs". The proposed inclusion of the term "stem cell" under the definition of New Drugs in the proposed Bill can create problems in the research or treatment as this is not a "drug". The Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India, by an Order dated December 15, 2014, also laid down a formula to determine the quantity of compensation in case of clinical trial related injuries other than death for which the matter would be referred to an independent expert committee who will decide the quantum based on the formula providing in the said Order. In addition to the guidelines specified above, the Advertising Standards Council of India in consultation with the National Apex Committee for Stem Cell Research and Therapy has also issued guidelines to stop advertisements by clinics/organizations inviting vulnerable patients for therapies.

Keywords : Stem Cells, laws, guidelines, permitted, restricted, prohibited

Introduction

Medical laws in India cover various aspects relating to setting up hospitals, management of patients, storage of sale of drugs and medicines. Additionally, the laws also provide guidelines for qualification and conduct of medical professionals and conducting research. Given the various aspects, the number of applicable laws are immense and running and operating a hospital would require a huge amount of compliances under both Central and State laws.

Apart from the Ministry of Health and Family Welfare at a national level which oversees the national aspects relating to the medical profession, there are various bodies like the Medical Council of India, Indian Council of Medical Research, pharmaceutical authorities like the Drugs Controller, Health Research

Institutes, etc.

Over and above these, Government of India has also introduced a uniform system for maintenance of electronic medical records and electronic health records by hospitals and healthcare providers which captures the requirements at a very detailed level. These electronic health record standards can be viewed at <http://www.mohfw.nic.in>

The Government of India is also proposing to come out with detailed National Health Policy for which the draft guidelines were released on December 31, 2014. The draft guidelines can be viewed at <http://www.mohfw.nic.in>

Issues Related To Stem Cell

Insofar as the issue relating to regulations for Stem Cell is concerned, whilst there is no specific

definition of what a "stem cell" means in any of the applicable legislations, the general understanding is that it is a cell that has the ability to divide or self replicate for indefinite periods throughout the life of the organism having the potential to develop into some or many different cell types in the body, depending on whether they are multipotent or pluripotent.

Legislation governing human embryonic stem cell research is not same in all countries and varies from country to country. Whilst some countries have prohibited this, there are a few who have put certain restrictions and others who have permitted the same. Whilst this is permitted in India there is a national apex committee for stem cell research and therapy which was set up during October 2010 by the Ministry of Health and Family Welfare, Government of India, which prescribes a mechanism for review and regulatory oversight by a National Apex Committee for Stem Cell Research and Therapy.

The final guidelines relating to stem cell research were issued during February 2014, by the Ministry of Health and Family Welfare, Government of India. These guidelines have classified the stem cell research into three categories, viz., permitted, restricted and prohibited.

The areas where stem cell research is prohibited as per the guidelines is as under -

- Research related to human germ line gene therapy and reproductive cloning.
- In vitro culture of intact human embryos, regardless of the method of their derivation, beyond 14 days of fertilization or formation of primitive streak, whichever is earlier.
- Clinical trials involving transfer of xenogeneic cells into a human host.
- Clinical research on Xenogeneic-Human hybrids is also prohibited.
- Research involving implantation of human embryos (generated by any means) into uterus after in vitro manipulation, at any stage of development, in humans or primates.
- Breeding of animals in which any type of human stem cells have been introduced at

any stage of development, and are likely to contribute to gonadal cells.

The Guidelines have also prescribed a mechanism for review and regulatory oversight of Stem Cell Research by the National Apex Committee for Stem Cell Research and Therapy which was set up by an Order dated October 29, 2010, by the Department of Health Research, Ministry of Health and Family Welfare, Government of India. The role of this Committee is to monitor and oversee activities relating to stem cell research and operations at a national level.

Given that this involves research on people, the Indian Council of Medical Research (ICMR) has also issued guidelines titled "Ethical Guidelines for Biomedical Research on Human Subjects" during October 2006 which can be viewed at http://icmr.nic.in/ethical_guidelines.pdf.

The one downside to the aspect on stem cell research is that given that there is no specific legislation on this issue, any non-compliances or violations do not get covered under the medical laws but can get covered under the general law in India relating to committing of offences should any malpractices be done in such research.

However, the issues relating to clinical trials/stem cells are overseen and governed under the laws relating to the Drugs and Cosmetics Act, 1940, as amended from time to time. There is a proposal to amend this Act through a bill titled Drugs and Cosmetics (Amendment) Bill, 2015, which was tabled in the lower house of Parliament (Lok Sabha) on December 31, 2014.

This Bill proposes to bring "Stem Cells" under the definition of "New Drugs". Further, in terms of Section 18 of the Act which deals with "Prohibition of manufacture and sale of drugs and cosmetics" read with Third Schedule the Licensing Authority is empowered to issue license and permission for certain category of drugs which includes stem cells and cell based drug products as captured in Sr. No. 14 of the Third Schedule.

Additionally, the Central Drugs Standard Control Organization (CDSCO) has also been given authority to conduct visits at the facilities/ hospitals where stem cell clinical trials are conducted and should any misuse or malpractices or unethical practices are conducted to issue

notices to such hospitals/doctors and investigate such non-compliances.

The proposed inclusion of the term "stem cell" under the definition of New Drugs in the proposed Bill can create problems/impediments in either research or treatment as this is not a "drug". The definition of "drug" as captured in the Act does not include "stem cells" as a product both under the existing Act and the proposed Amendment Bill and hence an application will need to be moved to the concerned authorities to get this removed from the proposed amendment bill.

The term "Drug" as defined in Drugs and Cosmetics Act, 1940, includes -

- (i) all medicines for internal or external use of human beings or animals and all substances intended to be used for or in the diagnosis, treatment, mitigation or prevention of any disease or disorder in human beings or animals, including preparations applied on human body for the purpose of repelling insects like mosquitoes;
- (ii) such substances, other than food, intended to affect structure or any function of the human body or intended to be used for the destruction of vermin, insects or microbes which cause disease in human beings or animals, as may be specified from time to time by the Central Government by notification;
- (iii) all substances intended for use as components of a drug including empty gelatin capsules; and
- (iv) such devices intended for internal or external use in the diagnosis, treatment, mitigation, or prevention of disease or disorder in human beings or animals, as may be specified from time to time by the Central Government by notification in the Official Gazette , after consultation with the Board.

The proposed amendment to the Drugs and Cosmetics Act is proposing to delete clause (iv) of the definition and instead bring in the following clause - (iv) any new drug for which licence has been granted by the Central Licensing Authority under sub-section (2) of section 18.

The proposed Bill also imposes heavy penalties in the event of an injury or death of a patient for conducting clinical trials without permissions, where the imprisonment can extend to 10 years and the penalty will not be less than Rs.20.00 lakhs.

Further, the Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India, by an Order dated December 15, 2014, also laid down a formula to determine the quantity of compensation in case of clinical trial related injuries other than death for which the matter would be referred to an independent expert committee who will decide the quantum based on the formula providing in the said Order. A copy of the said Order can be viewed at <http://www.cdsco.nic.in>.

In addition to the guidelines specified above, the Advertising Standards Council of India (ASCI), in consultation with the National Apex Committee for Stem Cell Research and Therapy has also issued guidelines to stop advertisements by clinics/organizations inviting vulnerable patients for therapies. Additionally, the Department of Consumer Affairs, Government of India, has also tied up with ASCI to curb misleading advertisements in six priority sectors including the Health Sector and has also issued a Circular on March 18, 2015, which has been put up on http://www.ascionline.org/images/pdf/gama%20portal%20_press%20release.pdf. Additionally ASCI has also prescribed certain guidelines on advertisements which can be viewed at <http://www.ascionline.org/index.php/ascicodes.html>.

Conclusion

Given the above, the following would need to be taken up to ensure that there is a streamlining of the provisions relating to stem cell therapy -

First and foremost, the inclusion of "stem cell" from the proposed amendment to the Drugs and Cosmetics Act, 1940, to be removed.

Secondly, representation be made to ICMR, CDSO, DCGI and the Ministry of Health and Family Welfare to ensure that the shortcomings in the guidelines be removed so that the medical field relating to stem cell therapy can progress in a way which would benefit the masses.

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Authors are responsible for ensuring that the information in each reference is complete and accurate. All references must be numbered consecutively and citations of references in text should be identified using numbers in square brackets (e.g., "as discussed by Smith [9]"; "as discussed elsewhere [9, 10]"). All references should be cited within the text; otherwise, these references will be automatically removed.

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Stem Cell Society (India) is a society established to formulate guidelines and ethical principles in tandem with the advances occurring in the field of stem cell therapy. The society aims to assist and guide regulatory bodies in the formation and modification of stem cell guidelines by incorporating the voices of patient population in the formulation of newer policies. It also aims to enable practitioners to play a greater role in policy making. The society strives to set up an accreditation process for stem cell centers across the country in order to develop appropriate national standards, along with taking decisions on newer indications for stem cell therapy. The society supports fundamental research towards conditions like neurological disorders, musculo-skeletal disorders, cardiovascular disorders, malignancies, autoimmune disorders, diabetes etc. It also aims to support the setting up of stem cell, tissues and organ banks and transplantation, along with conducting clinical trials. The society endeavors to provide assistance in training medical, paramedical and other staff in all aspects of stem cell research as well as support all educational activities within India and across the world within the field. It will also provide support in patenting products developed as part of the research and development initiatives. This society will also prepare, edit, print, publish, issue and circulate literature (books, magazines, journals, circulars) in the area of stem cell research and maintain a database for the same. Stem Cell Society (India) aims to bring together every aspect of stem cell therapy which will aid in providing an impetus to the field of regenerative medicine in India.

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