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RESEARCH ARTICLE

Improved survival in amyotrophic lateral sclerosis patients following autologous bone marrow mononuclear cell therapy: a long term 10-year retrospective study

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ABSTRACT

Background: Promising results from previous studies using cell therapy have paved the way for an innovative treatment option for amyotrophic lateral sclerosis (ALS). There is considerable evidence of immune and inflammatory abnormalities in ALS. Bone marrow mononuclear cells (BMMNCs) possess immunomodulatory properties and could contribute to slowing of disease progression.

Objective: Aim of our study was to evaluate the long-term effect of autologous BMMNCs combined with standard treatment on survival duration in a large population and to evaluate effect of type of onset and hormonal status on survival duration in the intervention group.

Methods: This controlled, retrospective study spanned over 10 years, 5 months; included 216 patients with probable or definite ALS, 150 in intervention group receiving autologous BMMNCs and standard treatment, and 66 in control group receiving only standard treatment. The estimated survival duration of control group and intervention group was computed and compared using Kaplan Meier analysis. Survival duration of patients with different types of onset and hormonal status was compared within the intervention group.

Results: None of the patients reported any major adverse events related to cell administration or the procedure. Kaplan Meier analysis estimated survival duration in the intervention group to be 91.7 months while 49.7 months in the control group (p = 0.008). Within the intervention group, estimated survival was significantly higher (p = 0.013) in patients with limb onset (102.3 months) *vs.* bulbar onset (49.9 months); premenopausal women (93.1 months) *vs.* postmenopausal women (57.6 months) (p = 0.002); and preandropausal men (153.7 months) *vs.* postandropausal males (56.5 months) (p = 0.006).

Conclusion: Cell therapy using autologous BMMNCs along with standard treatment offers a promising and safe option for ALS with the potential of long term beneficial effect and increased survival. Limb onset patients, premenopausal women and men ≤ 40 years of age demonstrated better treatment efficacy.

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1 Introduction

Amyotrophic lateral sclerosis (ALS) is the commonest motor neuron disease in human adults and is characterized by a rapid spread of muscle weakness resulting from progressive degeneration and loss of spinal cord, brainstem and cortical motor neurons [1]. Extensive preclinical studies have resulted in growing knowledge and understanding of the underlying disease process. However, it continues to be a fatal disease with a mean survival of 2–3 years for bulbar onset cases and 3–5 years for limb onset cases [2]. The available treatment is essentially supportive with limited effect on survival and quality of life of patients. The underlying disease pathology is highly complex and contributes to the unsatisfactory treatment results. Moreover, only 5%–10% of cases are familial. The only standard treatment, Riluzole gives a very modest benefit of increasing the patient's lifespan by 2–3 months [3]. Edaravone is another drug that has demonstrated limited clinical efficacy in slowing down disease progression [4, 5] and two studies failed to reach statistical significance on the primary endpoint [6, 7]. Moreover, studies on Edaravone are limited to a follow-up duration of 6 months. Whether or not the benefits are sustained beyond this time period and its effect on survival is not known. Also, the procedure is expensive and laborious considering the low quality of life of these patients. Gene therapy approaches are still in development phases [8].

In view of lack of available effective treatment and on the other hand promising outcomes of preclinical and clinical studies using stem cells [9], cell therapy may serve as an innovative approach for ALS treatment. Research has shown a relationship between an inflammatory pathway and disease progression in both animal models [10] and human patients [11]. For any treatment to be effective, slowing of the rapid disease progression resulting from neuroinflammation needs to be targeted. Owing to their immunomodulatory properties, neuroprotective and neurotrophic effects, bone marrow mononuclear cells (BMMNCs) could contribute to slowing disease progression. Previous studies have demonstrated the beneficial effects of BMMNCs in ALS [12–14]. These studies demonstrated cell-associated safety [12], the ability of these cells to effectively modify the motor neuron microenvironment [13] and stabilization of the disease [14]. Our previous study also demonstrated improved survival duration in patients that had received BMMNCs [15]. Moura et al. conducted a systematic review and meta-analysis to assess the efficacy of stem cell therapy in preclinical and clinical studies which showed improved survival and absence of serious adverse events respectively [9]. The studies, however, had small sample sizes and did not assess the long-term effect of cell therapy on ALS patients.

The aim of this study was to evaluate the long-term effect of autologous BMMNCs along with standard treatment on survival duration in a larger population over an extended follow-up period. Also, within the intervention group, the effect of treatment on onset type and hormonal status were studied.

2 Materials and methods

2.1 Study design and patient selection

This is a retrospective controlled cohort study including 216 patients. Patient records from December 2008 to November 2020 were gathered. All patients had been followed up regularly either by a physical examination or telephonically with attention to the progression of primary symptoms and Revised ALS

Functional Rating Scale (ALS FRS-R). Date and cause of death if any were recorded during telephonic follow-ups. 150 patients that had received cell therapy formed the intervention group. 66 patients who did not receive cell therapy formed the control group.

2.1.1 Inclusion criteria

Records of patients of age 35 years and above with the diagnosis of definite or probable ALS based on Revised El-Escorial Criteria were included for analyses.

2.1.2 Exclusion criteria

For record gathering, patients with a diagnosis of progressive lateral sclerosis (PLS), progressive bulbar palsy (PBP), progressive muscular atrophy (PMA), madras motor neuron disease and other neuromuscular disorders that mimic ALS symptoms were excluded.

Patients with the presence of acute infections and other medical conditions such as anemia (hemoglobin < 8 g/dl), bleeding tendencies, malignancy and severe renal or liver dysfunction had been excluded for intervention with cell therapy.

2.2 Pre-intervention assessment

As a routine, a comprehensive neurological evaluation had been performed including a functional assessment on ALS FRS-R. Medical fitness was established by routine biochemical, serological and hematological tests. Electromyography/nerve conduction studies, electrocardiogram, 2-dimensional echocardiography, chest X-ray, arterial blood gas test. Morning plasma total testosterone level tests were also performed in male patients between October 2016 and November 2020.

2.3 Intervention

Patient selection for the procedure was based on the Revised World Medical Association Helsinki Declaration for Ethical Principles for Medical Research involving human subjects [16]. Ethical approval was obtained from the Institutional Ethics Committee (IEC). Written informed consent was obtained from each patient. 48 and 24 hours before transplantation, patients were administered Granulocyte Colony Stimulating Factor (GCSF) to promote mononuclear cell mobilization.

2.3.1 Bone marrow aspiration

On the day of cell therapy, 100–120 mL of bone marrow was aspirated using a bone marrow aspiration needle from the iliac bone at the anterior superior iliac spine, in the operation theatre under aseptic conditions and under local anesthesia.

2.3.2 Cell separation

This procedure was done in the stem cell laboratory under aseptic conditions. Mononuclear cells were isolated by density gradient separation and were checked for viability count using trypan blue vital dye mixed in 1:1 proportion and loaded on haemocytometer. This was then confirmed on the TALI machine. CD34+ count was checked by fluorescence-activated cell sorting (FACS) using CD34 PE antibody.

2.3.3 Administration of BMMNC

In patients with a bulbar onset with absence of limb and/or trunk involvement, all the cells diluted in the patient's cerebrospinal fluid (CSF) were injected intrathecally. Until March 2017, 47 patients had received the cells, by both intrathecal and intramuscular routes. In patients with limb and/or trunk involvement, two-thirds of the separated mononuclear cells were injected intrathecally at the level between 4th and 5th lumbar vertebrae. One-third of the amount was diluted in the patient's CSF and injected intramuscularly at the motor points of muscles

that were weak and of functional importance. Since no significant benefit was observed in the injected muscles, targeting all the separated cells to the brain and spinal cord by intrathecal route was considered a more viable option. Hence, all patients after March 2017, received BMMNCs intrathecally only. Patients were simultaneously administered 20 mg/kg body weight methylprednisolone in 500 mL ringer lactate intravenously to reduce the immediate inflammation and to enhance cell survival.

Cell therapy was followed by an individualized multidisciplinary neurorehabilitation program over the next 4-day hospital stay. This included physiotherapy, occupational therapy, speech therapy and psychological counseling. Patients were monitored for immediate and long-term adverse events.

A detailed home program was prescribed at discharge and patients were advised to follow up every 3 months. Standard Riluzole medication was continued. Lithium 300 mg once/twice a day for 6 weeks was prescribed, to bring levels between 0.5–0.8 mEQ/L, to those who could tolerate it. Other medications included medications to control symptoms such as drooling, spasticity, etc.

Post-October 2016, patients with levels below mean age-matched healthy levels of plasma testosterone levels were prescribed injection testosterone enanthate 250 mg, once a month for 3 months.

2.3.4 Follow up

Patients had been followed up regularly either physically or telephonically until November 2020. A detailed neurological assessment was performed at each physical and/or telephonic follow up follow up. Symptomatic or ALS FRS-R change and the date of death, wherever applicable, had been recorded.

2.4 Outcome measures and statistical analysis

2.4.1 Demographic assessment

Baseline information about age at disease onset, gender, percentage of patients that had taken Edaravone injections and patients that had a limb or bulbar onset for the treatment and control groups was obtained. Within the intervention group, the percentage of patients that had received testosterone enanthate injections and lithium was obtained.

The intervention group was subdivided based on the type of onset, as bulbar and limb onset; hormonal status of women, as premenopausal and post-menopausal women and; hormonal status of men, as preandropausal men (≤ 40 years of age) and postandropausal men (> 40 years of age). Since testosterone levels decline after the age of 40 years, it was chosen for studying the effect of hormones on survival duration in males [17, 18].

2.4.2 Adverse events

Patients had been monitored for immediate and long-term adverse events. Adverse events were categorized as procedure-related or cell-related. Percentage of patients that exhibited adverse events were computed (Table 2).

2.4.3 Percentage distribution of mortality

Percentage mortality was compared between intervention and control groups. Within the intervention group, percentage mortality in the subgroups (premenopausal women, postmenopausal women, preandropausal and postandropausal) was calculated as: number of deaths in each group/total number of deaths in the intervention group × 100%.

2.4.4 Survival duration

Comparison of survival duration. Survival duration was used as an outcome measure. Time in months until mortality was obtained for the intervention and control groups and compared using Kaplan–Meier survival analysis. Also, within the intervention group, months

until mortality for bulbar and limb onset patients, pre- and post-menopausal women, pre- and post-andropausal, were computed and compared using Kaplan–Meier survival analysis.

2.4.5 Statistical analysis

Demographic data analysis. Descriptive statistics were used for demographic data. Data are described using means and standard deviation and percentages.

Survival analysis. Kaplan–Meier survival analysis was used to estimate the survival duration. Comparison between the groups was made using a log-rank test. All the statistical tests were performed with a significance level of *p*-value less than 0.05. SPSS (version 20.0) was used for the analysis.

Figure 1 shows the patients included in the study and the outcome measures used.

3 Results

3.1 Demographic assessment

The demographic description has been shown in Table 1.

3.2 Adverse events

None of the patients reported any major adverse events related to stem cells or the procedure. All the adverse events were mild and transient and resolved with medical treatment. The common minor adverse events included back pain or pain at the injection site, constipation, headache, loose motions, nausea and/or vomiting, and pain at the aspiration site (Table 2).

3.3 Percentage distribution of mortality

The total number of deaths was 102 of 150 (68%) patients in the intervention group and 52 of 66 (78.8%) patients in the control group. The mean survival duration in the intervention group was 56.1 months (minimum: 8 months; maximum: 433 months). The mean survival duration in the control group was 43.8 months (minimum: 3 months; maximum: 121 months).

Percentage mortality in the premenopausal, postmenopausal, preandropausal and postandropausal subgroups within the intervention group are shown in Table 3.

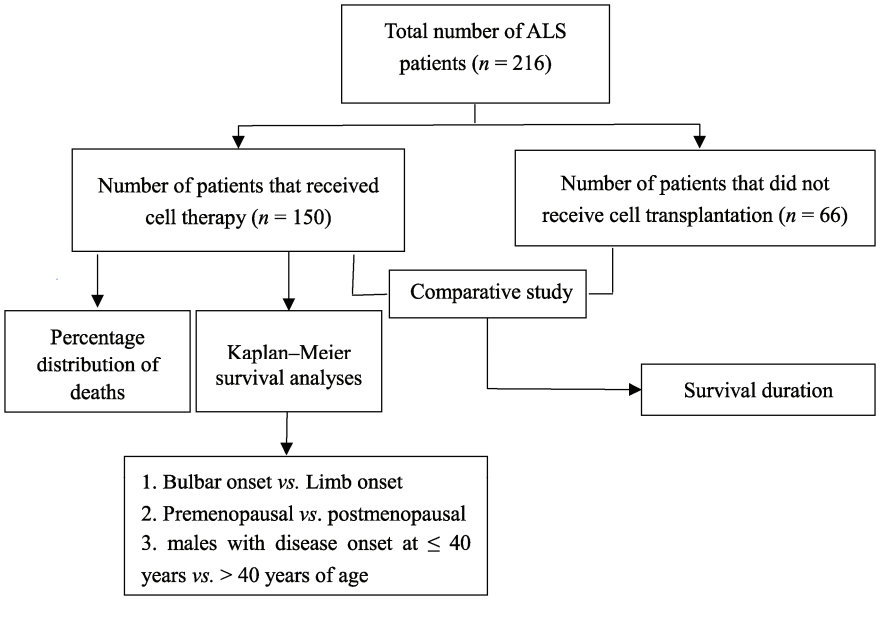

Fig. 1 Study protocol and outcome measures.

Table 1 Demographic data of the study population.

Demographics	Intervention group	Control group
Gender		
No. of females (percentage)	51 (34%)	19 (28.79%)
No. of males (percentage)	99 (66%)	47 (71.21%)
Intramuscular Injection		
Patients received intramuscular transplantation of cells (percentage)	47 (31.3%)	0 (0)
Patients not received intramuscular transplantation of cells (percentage)	103 (68.7%)	66 (100%)
Lithium		
Patients prescribed lithium (percentage)	125 (83.3%)	0 (0)
Patients not prescribed lithium (percentage)	25 (16.7%)	66 (100%)
Edaravone		
Edaravone taken (percentage)	19 (12.7%)	4 (6.01%)
Edaravone not taken (percentage)	131 (87.3%)	62 (93.94%)
Testosterone		
Testosterone injection prescribed (percentage)	23 (23.2%)	0 (0)
Testosterone injection not prescribed (percentage)	76 (76.8%)	66 (100%)
Type of Onset		
Patients with bulbar onset (percentage)	34 (22.7%)	16 (24.2%)
Patients with limb onset (percentage)	116 (77.3%)	50 (75.8%)
The average age at onset in years (SD)	50 (10)	54 (9)

Table 2 Adverse events observed in the post-intervention period.

Adverse events	Percentage adverse events immediately post-intervention	cell-related adverse events
Minor		
Spinal headache	13.3 %	None
Nausea and/or vomiting	7.0 %	
Pain at the aspiration site	3.8 %	
Backache/pain at the injection site	10.1 %	
Fatigue	2.7 %	
Constipation	8.2 %	
Loose motion	1.9 %	
Major		
Sudden onset of respiratory discomfort	None	None
Neurological deficits	None	
Paresthesia/loss of sensation in lower limb	None	
Cardiac failure	None	
Hematoma at the injection site	None	

Table 3 Percentage distribution of deaths within intervention group in premenopausal and postmenopausal women, preandropausal and postandropausal men.

	Premenopausal women	Postmenopausal women	Preandropausal men	Postandropausal men
Distribution of mortality	6 (5.9%)	31 (30.4%)	14 (13.7%)	51 (50%)

3.4 urvival analyses

3.4.1 Comparison of survival duration between intervention and control group

The mean survival duration in the intervention group was estimated to be 91.7 months and in the control group, it was estimated to be 49.7 months. The survival duration in the intervention group was estimated to be 42 months higher than in the control group (p = 0.008) (Table 4, Fig. 2).

3.4.2 Comparison of survival duration between subgroups within the intervention group

The estimated survival duration for limb onset patients (102.3 months) was significantly higher (p = 0.013) than those with bulbar onset (49.9 months). Likewise, survival duration was significantly higher (p = 0.002) in premenopausal women (93.1 months) when compared with postmenopausal women (57.6 months) and in preandropausal men (153.7 months, p = 0.006) compared to postandropausal men (56.5 months) (Table 5, Fig. 3).

Table 4 Kaplan–Meier analysis of survival duration for patients with and without cell therapy.

	Intervention group	Control group	p value
Estimated survival duration (months)	91.7	49.7	0.008*

*Indicates a statistically significant difference between the groups.

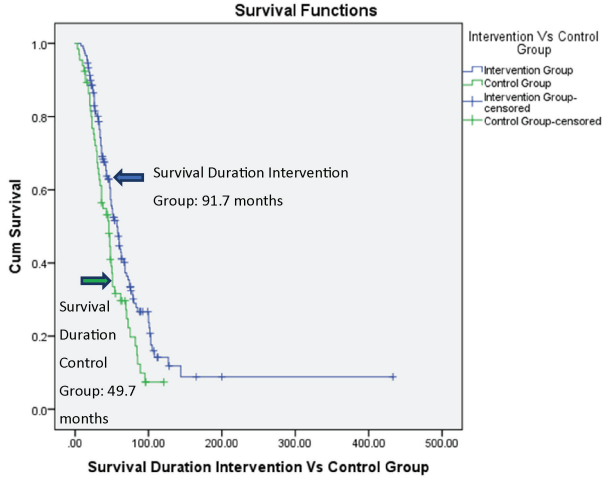

Fig. 2 Kaplan–Meier graph showing comparison of the estimated survival duration in intervention and control groups. Mean estimated survival duration was 91.7 months in the intervention group while in the control group it was 49.7 months (p = 0.008*). *Indicates a statistically significant difference between the groups.

4 Discussion

4.1 Management of ALS

With an incidence of between 1.2 and 4.0 per 100,000 persons per year, ALS continues to be a fatal neuromuscular disease [19]. The only standard treatment, Riluzole gives a very modest benefit of increasing the patient's lifespan by 2–3 months [3]. Edaravone is another drug that has received FDA approval, but studies are limited to a follow-up duration of 6 months. Whether or not the benefits are sustained

Table 5 Effect of prognostic factors on survival duration within the intervention group.

Prognostic factors	Estimated survival duration (months) as calculated by Kaplan–Meier survival analysis	Statistical significance
Onset type		
Bulbar onset	49.9	0.013*
Limb onset	102.3	
Hormonal status (Women)		
Premenopausal women	93.1	0.002*
Postmenopausal Women	57.6	
Hormonal status (Males)		
Preandropausal men	153.7	0.006*
Postandropausal men	56.5	

*Indicates a statistically significant difference between the groups.

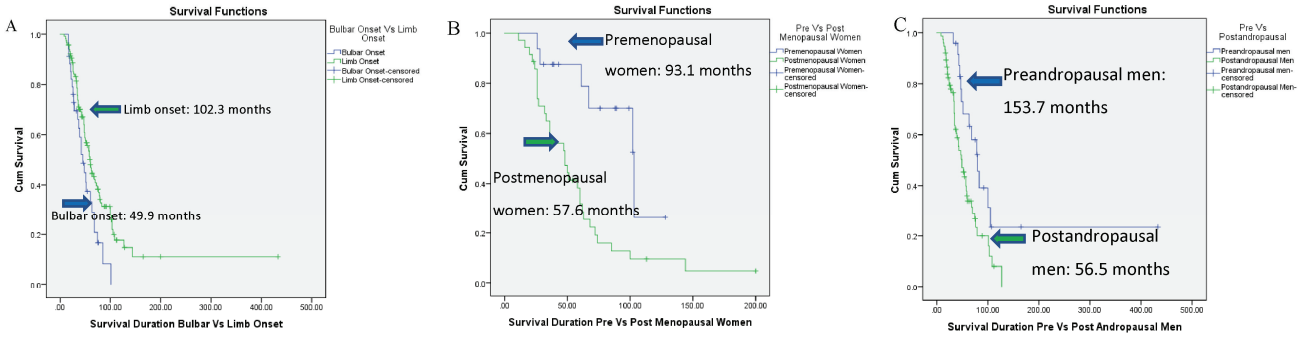

Fig. 3 Comparison of survival duration among the subgroups within the intervention group. (A) Bulbar onset *vs.* limb onset groups; (B) premenopausal *vs.* postmenopausal women; and (C) preandropausal *vs.* postandropausal men.

beyond this time period and its effect on survival is not known. Gene therapy approaches are still in development phases.

Our study demonstrated that administration of autologous BMMNC possesses long term safety and efficacy. In our study, we found that there was a significantly higher survival duration of 42 months in patients that received cell therapy and lithium along with standard treatment as compared with patients that received standard treatment only.

4.2 Effect of onset type, age, and hormonal status on survival duration

Within the intervention group, limb onset patients, premenopausal women and preandropausal men had significantly higher survival duration compared to the bulbar onset, postmenopausal women and postandropausal men at disease onset respectively. Previous studies have shown patients with bulbar-onset to have lower survival than patients with the limb-onset disease, and this, independent of the age factor [20–24]. Increasing age is also a known risk factor for ALS. The average age of disease onset is 61.8 ± 3.8 years (ranging from 54–67 years of age) [25]. At higher ages, the difference in the proportion of males and females with the disease drops significantly. In the younger age groups, the male/female ratio of ALS incidence is about 2.5:1 but in the older age group it has shown to decline to 1.4:1 [26]. In ages above 60 years, the male/female ratio of ALS incidence decreases further and becomes 1:1 [27]. Our earlier studies showed better treatment outcome in premenopausal women and in patients younger than age 50 years of age [15, 28]. This suggests a possible protective role of sex hormones in the younger age groups. Several studies have suggested a role of sex hormones in the pathogenesis of the disease. Estradiol has shown to have a direct protective effect on spinal motor neurons by preventing glutamate and nitric oxide-induced neuron death [29]. Ovariectomy in mice models of ALS caused an acceleration of the disease progression [30]. Further, when these mice were subsequently treated with 17β-estradiol, disease progression was slowed. Likewise, progesterone levels have shown to possess a positive correlation with survival and length of time between disease onset and diagnosis both of which are factors of a better prognosis [31]. Treatment of animal models of the disease with progesterone showed less severe symptoms and even reversal of histopathological abnormalities as compared to those mice that didn't receive progesterone [32, 33]. Though in humans, the association between estrogen and ALS incidence has not been proven and the results of studies are conflicting [34, 35], our present study showed better survival duration in premeno-

pausal women than postmenopausal women with a difference of 35.5 months which was statistically significant.

Androgens have also been suggested to have a possible role in the pathogenesis of ALS. Deficient synthesis of testosterone, ADIONE, its precursor and 5α-DHT was observed in testes of animal models of ALS [36]. Further, low plasma testosterone levels were shown to correlate with low testosterone levels in the spinal cord. Administration of anti-androgen in mice resulted in acceleration of disease onset and motor deficits [37]. In our earlier study including patients with ALS, a correlation between lower levels of plasma testosterone and disease severity on ALS FRS-R scale and King's staging was seen [38]. Therefore, post-October 2016, patients with levels below mean age-matched healthy levels of plasma testosterone levels were prescribed injection testosterone enanthate 250 mg, once a month for three months. In the present study also, we found better survival duration in men aged 40 years and below as compared with men above 40 years of age. The difference of 97.2 months was statistically significant.

4.3 Pathophysiology and mechanism of action of BMMNCs in ALS

The discovery of mutations in Cu/Zn superoxide dismutase (mSOD1) as the commonest cause of familial ALS led to the development of experimental animal models and an increase in the understanding of the underlying pathogenesis [39]. Mitochondrial dysfunction, increased reactive oxygen species (ROS), misfolded and aggregated proteins, and dysfunction of the ubiquitin proteasome pathway have been suggested as the possible events that promote neurodegeneration [40]. Though the exact cause of motor neuron degeneration is still unclear, considerable evidence exists to demonstrate the presence of immune and inflammatory abnormalities in ALS. Irrespective of the cause of the disease, neuroinflammation at the sites of motor neuron injury is evident as the presence of microglial activation, astrogliosis and monocyte and T-cell infiltration. The early immune responses to signals of motor neuron injury are to rescue and repair damaged tissue [41]. However, as the disease progresses, there is a shift from the beneficial M2 microglia and regulatory T-cells to the deleterious effect of immune responses involving activated M1 microglia. Activated M1 microglia is cytotoxic due to release of more pro-inflammatory factors such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β and nitric oxide (NO) and ROS which is neurotoxic and mediate motor neuron death [42–44]. The pro-inflammatory factors further activate the microglia in a self-propagating cycle causing a further increase in neurotoxicity [45]. Activated microglia also show enhanced phagocytic activity and can phagocytose apoptotic neural cells as well as the normal neurons [46]. Microglial activation is more prolonged and to a greater degree in the aged brains as compared to the adult brain [47]. Thus, uncontrolled inflammatory responses mediated by activated microglia can have detrimental effects, particularly in the older age groups. The M2 microglia, on the other hand, are anti-inflammatory and neuroprotective [48]. In order to effectively alter the rapid disease progression, neuroinflammation needs to be targeted. Multiple compounds with anti-inflammatory properties have been tested in clinical trials but unfortunately, have failed to show positive results [49–55]. Inflammation may be better modulated by cell therapy. Adult BMMNCs contain a mixture of cells including hematopoietic progenitor cells, lymphoid cells, monocytes, macrophages [56] and cells from the non-hematopoietic lineages including, side

population cells [57], mesenchymal stromal cells [58], very small embryonic stem cells [59], multipotent adult progenitor cells [60], hemangioblasts, endothelial progenitor cells [61] and tissue committed stem cells [62]. BMMNCs have demonstrated the ability to decrease proinflammatory cytokines and increase antiinflammatory cytokines [63]. Also, mesenchymal stem cells can alter the polarization status of microglia as evidenced by an increased expression of M2 markers and a decreased expression of M1 markers, *in vitro* [64–67]. Further, bone marrow cells release a host of trophic factors such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and glial cell line derived neurotrophic factor (GDNF) [68–71] many of which have shown to alleviate motor neuron degeneration in animal models [12, 72]. GDNF has shown to protect neurons from entering apoptosis [73, 74]. Blanquer et al. demonstrated evidence of decreased ubiquitin deposits in the spinal motoneurons post autologous BMMNC transplantation [13]. BMMNCs may significantly increase the level of vascular endothelial growth factor (VEGF) [75]. Oosthuyse et al. implicated VEGF in the pathogenesis of ALS [76]. VEGF is a key angiogenic factor that also confers neuroprotection by the promotion of neuron survival [76–79] and neurogenesis by neuronal migration, axon guidance [80, 81] and increases life expectancy in animal models of ALS [82–84].

4.4 Rationale for lithium administration

Lithium has demonstrated neuroprotective effects in animals and humans. Preclinical studies have shown improved motor function in animal models of ALS post-treatment with lithium [85]. Lithium has shown to promote neuronal survival, enhance mitochondrial respiratory rate, decrease oxidative stress and modulation of calcium influx in mitochondria [86–92]. It has also shown to induce synaptogenesis [93]. A clinical study demonstrated the safety and efficacy of lithium in slowing disease progression and improving survival in ALS patients [94]. Our previous pilot study also demonstrated higher survival in patients that received lithium as compared to those who did not receive lithium [15]. Patients were hence prescribed short term lithium.

Route of administration

Intrathecal application of cell therapy offers several advantages. It is less invasive and allows for a simple procedure. It is thus possible to perform the procedure multiple times. Also, since ALS involves widespread degeneration along the entire length of the neural axis, intrathecal application of stem cells may be a more viable option. Due to the dynamics of CSF flow, it may be a more effective method to influence multiple brain and spinal cord areas that are affected by the disease [95]. Wu et al. and Bai et al. reported that cells delivered through the CSF migrate to the injury site in the spinal cord [96, 97]. Additionally, intra-arterial and intravenous administration results in the trapping of the injected cells in organs such as the lungs and liver [98, 99]. This can be avoided using intrathecal application of stem cells.

Application of cells into the muscles may be advantageous as trophic factors released by stem cells may provide support to the neuromuscular junctions [100]. Intramuscular injection of bone marrow derived stem cells has shown to cause an increase in size of endplates and a resultant greater survival of motor neurons in animal models of motor neuron degeneration [101]. However, in our initial experience of 47 patients, additional intramuscular injection of bone marrow stem cells did not demonstrate any significant improvement in

strength of those muscles. Hence, intramuscular injections were discontinued. Since the disease involves progressive degeneration along the entire length of the neural axis, targeting all the separated BMMNCs to the brain and spinal cord by intrathecal route may be more effective and beneficial. Hence, after March 2017, all patients in the intervention group were given cells by intrathecal route only.

4.5 Pre-clinical studies

Intraperitoneal administration of bone marrow cells significantly delayed disease onset and increased survival in SOD1 mice [102]. Improved motor function, attributed to the trophic factors produced directly or indirectly by transplantation of bone marrow hematopoietic cells into the spinal cord of a mouse model of ALS, was reported by Cabanes et al. [73]. Intravenous combined with intramuscular administration of BMMNCs, delayed onset of functional deficits, decreased microgliosis in the lumbar ventral horn and increased the number of partially innervated neuromuscular junction [103]. Similarly, intraspinal administration of these cells in a mouse model of ALS delayed disease progression in the presymptomatic phase [104].

4.6 Clinical studies

Studies have shown autologous BMMNC transplantation to be safe and a promising treatment approach for ALS. In our previous study, survival was higher in the group of patients that underwent autologous BMMNCs intrathecally than in the control group of patients [15]. It was also higher than the survival duration of ALS patients in previous epidemiological studies. In another study by Prabhakar et al., stabilization of disease was observed, with no significant decline in ALS FRS-R score over a one-year follow-up duration, following intrathecal administration of autologous bone marrow derived stem cells [14]. A study by Martinez et al. suggested slower disease progression in ALS patients during a one year follow up post intrathecal transplantation of bone marrow derived mononuclear cells [105]. In this study, a total decrease of 4 points in the ALS FRS-R score at one year was noted against the usual average decline of 13.32 points. In a study by Deda et al., 9 of 13 ALS patients showed improvement while 1 patient remained stable on Norris scale and electroneuromyography, post-surgical transplantation of BMMNCs into the anterior part of the spinal cord, over 1 year follow up duration [106]. Blanquer et al. demonstrated that BMMNCs modified the motoneuron microenvironment and favoured their survival as was evident by spinal cord pathological analysis that showed a lack of signs of motoneuron degeneration in the treated segments [13]. Spinal transplantation of these cells also demonstrated respiratory stability over at least 1 year follow up duration. Also, the respiratory events during sleep, in these patients remained at levels lower than patients (receiving standard medical care only) with similar disease duration [107]. Systematic review and meta-analysis by Moura et al. showed improved survival and absence of major adverse effects in preclinical and clinical studies [9].

Our results and previous studies demonstrate the safety and potential benefits of autologous BMMNCs in ALS. Considering the underlying disease pathology, transplanting these cells, that have the potential of detoxifying the microenvironment around motoneurons, may be of clinical significance.

4.7 Limitations

There were some limitations to the study. Although this was a controlled study, it was

retrospective and not randomised. Not all patients in the intervention group had received lithium. Standard treatment had changes in the 10-year study duration. With the U.S. Food and Drug Administration approval of Edaravone for ALS treatment, some patients from the intervention group received the treatment. However, this was also the case for the patients in the control group. Post-October 2016 in the intervention group, males with levels below mean age-matched healthy levels of plasma testosterone levels were prescribed injection testosterone enanthate 250 mg, once a month for three months. Also, intramuscular injection of stem cells was discontinued post March 2017.

5 Conclusion

Cell therapy using autologous BMMNCs along with standard treatment offers a promising and safe option to ALS with the potential to delay disease progression and increase survival with sustained benefits over a long-term duration. Survival duration in the intervention group was estimated to be 42 months higher than in the control group. Survival was significantly higher in patients with limb onset, premenopausal women and preandropausal men as compared to patients with bulbar onset, postmenopausal women and postandropausal men. The role of hormones in the disease pathology in humans and hormone therapy as an adjuvant to cell therapy and standard treatment needs to be considered.

Ethical approval

Patient selection for the procedure was based on the Revised World Medical Association Helsinki Declaration for Ethical Principles for Medical Research involving human subjects. Ethical approval was obtained from the Institutional Ethics Committee (IEC).

Consent

Written informed consent was obtained from each patient.

Conflict of interests

All contributing authors have no conflict of interests to declare.

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