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Encouraging effect of autologous bone marrow aspirate concentrate in rehabilitation of children with cerebral palsy

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Abstract. – OBJECTIVE: In this study, we used autologous bone marrow aspirate concentrate (BMAC) transplantation to treat children with cerebral palsy (CP) to improve their motor and cognitive functions.

PATIENTS AND METHODS: Forty-two patients with CP received BMAC. The transplantation of stem cells *via* the intrathecal route includes three BMAC applications. The patients' examination was before the injection of stem cells, with follow-ups on 1, 3, 6, and 12 months after the injections. The assessments included the gross motor function classification scale, the Ashworth scale, and the Learning accomplishment profile-diagnostic scale.

RESULTS: This study included 42 patients with CP who received three BMAC intrathecal administrations. A personalized home rehabilitation program was designed and included for each patient in the study. After the treatment, we observed a reduction of spasticity in 58% of patients and significant cognitive improvement in 35% of patients.

CONCLUSIONS: The outcome of this study indicates that stem cell therapy and personalized training can improve the development of children with CP. The crucial goal of this therapeutic intervention is to substitute injured tissue with new tissues by activating the regenerative capacity of stem cells.

Key Words:
Cerebral palsy, Stem cells, Bone marrow aspirate concentrate, Intrathecal administration, Rehabilitation.

Introduction

Cerebral palsy (CP) is a neurological insufficiency that results from brain injury occurring before full neurological development[1]. CP involves impairment of movement, muscle, and cognitive functions. This condition is accompanied by impairments in speech, cognition, epilepsy, secondary muscle contraction, and deformity of limbs. In addition, seizures, mental retardation, speech disorders, and auditory visual impairments often coexist in CP[2].

The old acceptance about the nature of neurological disorders is based on the failure of brain cells to regenerate. Current progress in regenerative medicine has confirmed that stem cells can restore the injured brain[3]. Cell therapy is a promising treatment for several neurological disorders acting by replacing dead cells, releasing protective factors to the damaged cells, and modulating the lesion's microenvironment in the nervous system[4-6]. Stem cells stimulate the repair process by homing to the injured sites of the brain and carrying out regeneration[7]. The action mechanism of stem cells involves cell replacement and cell repair *via* the paracrine effect. Different stem cells have been applied, such as embryonic stem cells, mesenchymal stem cells, neural precursor cells,

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and induced pluripotent stem cells[8]. An umbilical cord blood cells, bone marrow cells, and adipose tissue cells have been used to treat CP[9,10]. Bone marrow (BM) is a source of collected autologous cells with regenerative properties, applicable to treating chronic and acute diseases. Mechanism of autologous bone marrow aspirate concentrate (BMAC) action includes a process that involves neuromodulation of neurons, axon growth, neuroregeneration, and replacement of neurons[11]. The ability of neuroplasticity, neural repair, and neurogenesis restores the functioning of the injured cells. Thus, this allows healthy adjacent cells to compensate for the functions of injured cells[12]. Neuroplasticity is maximal during childhood. Stem cell intervention is more effective in children. The reason is more active repair process after assimilation of new cells in the brain[13]. Stem cells express the homing affinity toward the injured sites of the brain, guided by the chemoattractant pathway[14]. It is speculated that exosomes may be one of the major substances in the stem cell treatment of damages[15]. Exosomes can transmit genetic materials – such as messenger ribonucleic acid to recipient cells – and effectively regulate various physiological functions of recipient cells[15]. The properties of cellular therapy are improving brain tissue repair and regeneration of neural tissue. Stem cells secrete neurotrophic factors that regulate cell proliferation and cytokines in the microenvironment and stimulate endogenous stem cell differentiation. The secreted factors may support stem cell survival by introducing other cell types. These cells help to reestablish missing enzymes in an otherwise deficient environment[13].

Bone marrow mesenchymal stem cells (MSCs) include a significant fraction of MSCs and hematopoietic stem cells. Evidence from preclinical and clinical studies has confirmed that MSCs have the characteristics of self-renewing, differentiation potential, low immunogenicity, and inherent tumor or inflammatory tropisms[10]. These stem cells have the potential to duplicate indefinitely, produce more than 50 types of growth factors and cytokines[16], and differentiate into neuron-like cells[17,18]. The mechanism of action seems to be through chemical signaling with a range of growth factors and cytokines. MSCs secrete key factors that promote brain function recovery[7]. The factors include growth factors, such as basic fibroblast (bFGF), vascular endothelial (VEGF), fibroblast (FGF), in addition to connective tissue growth factor. Bone marrow MSCs also reduce the levels of harmful chemicals raised due to activating cells forming scar tissue in the brain, enhancing the endogenous brain repair[7]. MSCs growth factors initiate neoangiogenesis. MSCs can potentiate angiogenesis *via* cell contact interaction or paracrine effects. MSC-secreted cell factor triggers proangiogenic and antiapoptotic activity. MSCs can secrete a composite of angiogenic factors and stromal-cell-derived factors, which promote local angiogenesis and local blood flow recovery. This process starts forming a new blood vessel network that will provide blood circulation and support recovery of damaged tissue functions[19]. MSCs are easily available and do not cause an immune rejection after transplantation[20]. Bone marrow is a proficient source of rapidly harvested autologous cells.

Cerebral white matter injury is common in CP, resulting in oligodendrocytes loss. The consequences are damaged myelin and disrupted nerve conduction[21]. Stem cells help in changing the microglial reaction and improve axonal growth. Bone marrow MSCs have the prospect of differentiating into oligodendrocytes and astroglial cells. These cells carry out the repair process by remyelinated axons[22]. It is possible to restore lost myelin with stem cell therapy, i.e., dead cells are replaced with new oligodendrocytes and their progenitors[13]. Improvements in autologous BMAC therapy allow optimism to increase effective treatment in CP. An essential success requirement is recruiting many MSCs to the injury site to achieve regeneration. Magnetic resonance imaging (MRI) scan and microscopy monitoring of magnetically labeled MSCs showed that stem cells migrated away from the injection site toward lesion zones in both hemispheres[23]. Chen et al[23] suggested that MRI scans and microscopy have a capacity for precise migration monitoring to even widespread and distant, damaged areas of the central nervous system.

Intrathecal administration of cells is a minimally invasive procedure. This procedure includes the delivery of cells *via* lumbar puncture. This injection mode allows effective delivery of cells and the opportunity of migration of cells to the tissues other than the damaged ones is avoided[24].

Treatment that combines stem cell therapy with physiotherapy provides a healing opportunity for patients with CP. The rehabilitation itself cannot repair the damaged nerve function, but it could prevent the process of muscle atrophy and joint stiffness[25]. Included personalized rehabilitation programs optimized recovery as exercise plays a role in the success of musculoskeletal regeneration.

The study aimed to evaluate the usefulness of the intrathecal application of autologous BMAC on patients with CP. We also monitored potential treatment-based adverse events.

Patients and Methods

Patient Selection Criteria

This study included 42 CP patients, ages from one to twelve years. The study duration was from March 2018 until March 2021. The Institutional Ethical Committee of Parks Dr. Dragi Hospital approved the study following the world medical association Declaration of Helsinki[26].

We explained the procedure to the patient's parents. The parents signed an informed consent form (ICF) after the explanation. The exclusion criteria were active infections, hydrocephalus with ventricular drain, chromosomal abnormalities, hereditary metabolic diseases of the nervous system, tumors, diseases of the heart, blood, lung, liver, or kidney, and allergy to anesthetic agents. The patients underwent a complete physical and neuropediatric examination before the intervention. The examination included serological, biochemical, and hematological tests. The brain injury's extent was assessed *via* the MRI and electroencephalography (EEG) examinations.

We used a grading system to evaluate the functional outcome in the subjects. The system is based on improvements in the symptoms and graded as 1. no improvement for improvements observed in less than 10% of symptoms, 2. mild for improvements in 10-35% of symptoms, 3. moderate for improvements in 35-70% of symptoms, and 4. significant for improvements in over 70% of symptoms[27].

The improvement of motor function is evaluated using the gross motor function classification system (GMFCS), and child development was measured by the Learning Accomplishment System-Diagnostic (LAP-D). We used the Modified Ashworth Scale (MAS) to measure the muscle tone increase.

The GMFCS is a five-level classification system that describes the gross motor function of children with CP based on their self-initiated movement with an emphasis on sitting, walking, and wheeled mobility. This tool considers the patient's age, their mobility aids usage, and the movement quality[28]. At levels I and II, the patients have almost independent mobility, and at level III can move with assistive devices. Levels IV and V have minimal mobility with high dependence on the helpers.

The MAS is a five-point rating, ranging from 0 to 4, which measures resistance to passive movement. The lower scores or reduced muscle tone represent motor function improvements[29].

The LAP-D was used to evaluate fine locomotor skills, cognitive skills, and speech skills. This scale measures progress for various stages of a child's development[30].

Procurement of Autologous Bone Marrow Cells

The intervention included three intrathecal administrations of BMAC per subject. The first intrathecal injection was given immediately after processing. The second injection was given 30 days after the first administration, and the third intrathecal injection was 30 days after the second. The cell therapy procedure is a one-day procedure. Bone marrow aspiration was done under general anesthesia. Patients were placed in the prone position in the operating room, with their right anterior iliac crests exposed. After the drilling place was prepared, a small incision was made with surgical blade No. 11. Bone marrow aspiration was done through the iliac crest using a 22G special harvest. We applied Acid Citrate Dextrose (ACD) formula A in ratio 7:1 for bone marrow anticoagulation[31].

The bone marrow was processed using the Angel whole blood separation system (Arthrex, Naples, FL, USA). BMAC was produced from the aspirate using the density gradient centrifugation, which separated BMAC, hematopoietic stem cells, and platelet-poor plasma. The collected volume is based on the patient's body weight as follows 7-8 ml/kg for patients under 10 kg; 4-5 ml/kg body weight, but no more than 160 ml in total[32]. We counted the BMAC, hematopoietic stem cells (CD34+), and platelet-poor plasma. The BMAC end volume varied from 1.5-4 ml and depended on a patient's baseline bone marrow count[33].

Isolation of BMAC

The cytokines measured from the BMAC and cerebrospinal fluid (CSF) samples for all subjects were adiponectin, adipsin, RBP4, MCP-1, IL-1β, IP-10, IL-10, IL-8, leptin, IL-6, IFN-γ, resistin, TNF-α. We were measuring the levels of Stro-1, CD133, CD73, CD146, CD105, CD45, CD34, CD90, 7AAD. The flow cytometry method is used to check the viability and count the BMAC and hematopoietic stem cells (CD34+). We injected

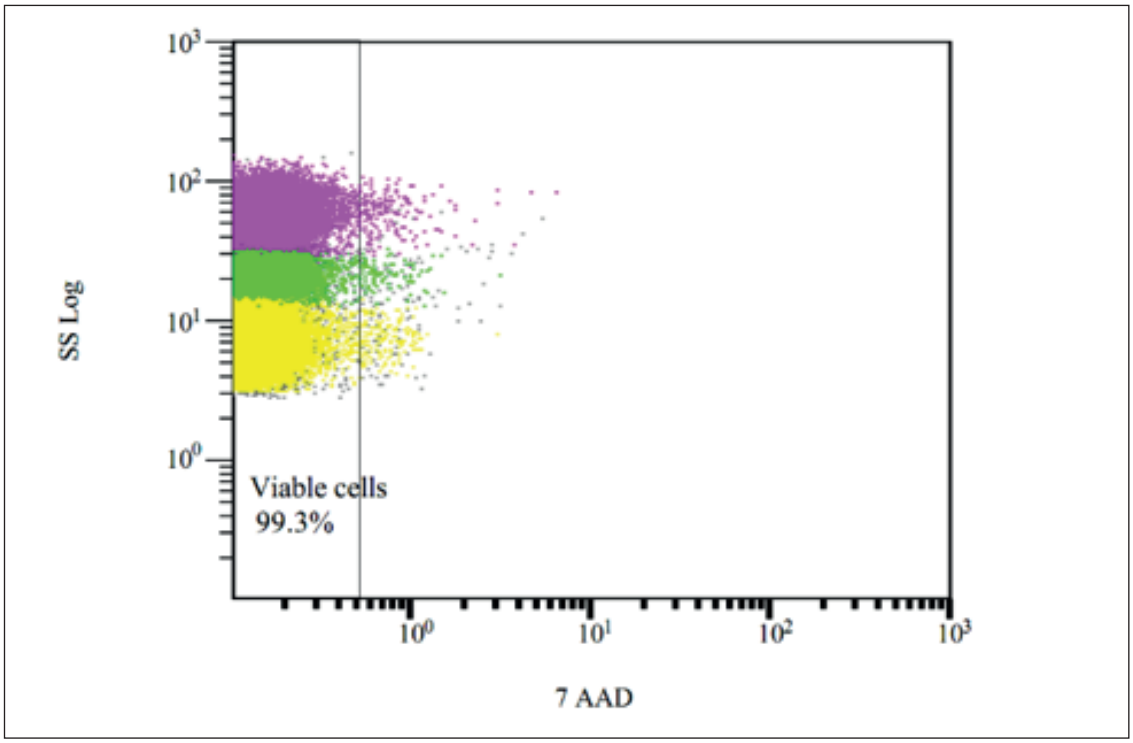

Figure 1. Percentage of viability cells in the sample.

these cells into the subarachnoid space intrathecally. In the 1st intrathecal injection, the average BMAC count for the total nucleated cells (TNC) was 54x106 mL, and viability was 98%. In the 2nd injection, the TNC was 45x106 mL, and viability was 99%. In the 3rd injection, the TNC was 50x106 mL, and viability was 99.3% (Figure 1).

Transplantation of BMAC

Before the injection, a sample of the cerebrospinal fluid (CSF) was taken to match the BMAC solution volume. This is needed to avoid disturbance of the CSF circulation. We used a 25-gauge spinal needle to inject the BMAC intrathecally between the L4 and L5 vertebra. The total procedure time was 30-45 minutes. After the procedure, we observed the patient for any procedure-related adverse events.

We calculated the absolute number of cells expressed per milliliter of the BMAC sample based on the total number of cells in the BMAC sample and the percentage of CD90 positive cells. We determined an increase in their numbers at the follow-up (Figure 2).

The absolute number of events recorded on the flow cytometer (Figure 3) was processed by Friedman's test for paired samples. The confirmation of this observation ($p = 0.0281$) is statistically significant.

We tracked surface markers in BMAC samples: CD34, CD45, CD73, CD90, CD105, and CD271 based on the minimal criteria for identifying mesenchymal stem cells proposed by Dominici et al[33]. We observed an increase in the number of CD90 positive cells in each subsequent measurement, both as a percentage of the total nucleated cells in the sample and as the absolute number of events recorded on the flow cytometer. The average volume of aspirated bone marrow was 92 ml, and of intrathecal applied BMAC substrate was 2.26 ml. Quality control assessment showed a very high level of viability in the BMACs samples. The cells were analyzed by multi-parameter flow cytometry, with 98% of cells showing no staining for 7AAD. No serious adverse events or severe complications during the transplantation procedure were reported.

Post-Therapy Assessment

An experienced pediatric psychologist and psychiatrist did clinical examinations using the GMFCS, modified Ashworth scale, and the LAP-D score during the baseline, at 3rd, 6th, and

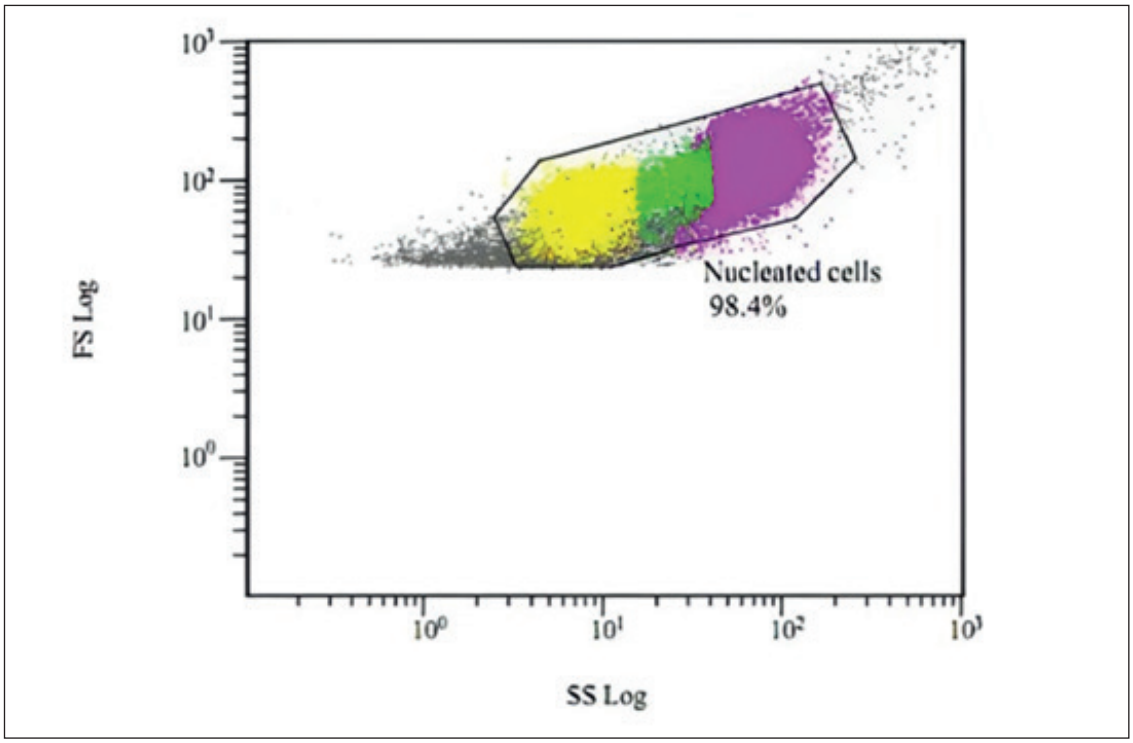

Figure 2. Percentage of total nucleated cells in the sample.

12th months after the first transplantation. Our goal was to perform as many assessments as possible during three-month intervals. A planned grading system to evaluate the functional outcome in every individual was based on mild, moderate, and significant improvements in the symptoms.

The GMFCS was used before and after therapy as a classification tool to evaluate the changes in gross motor function. The levels from one to five describe the motor function limitations concerning age, usage of mobility aids, and the movement quality[28]. Levels 1 and 2 have almost independent mobility, and level 3 can move with assistive devices. Levels 4 and 5 represent significant limitations and dependence on the helpers for minor movements.

The modified Ashworth scale was used to assess spasticity before and after therapy[34]. An expert therapist verified the level of spasticity based on the current practice.

The learning accomplishment system-diagnostic (LAP-D) is a developmental screener that provides a snapshot of whether a child might be at risk for a developmental delay[35]. LAP-D focuses on gross motor, fine motor, pre-writing, cognitive, language, and social/emotional aspects of early childhood development[35].

Neurorehabilitation

After stem cell transplantation, children had extensive rehabilitative therapy for 12 days (1-2 h per day) at our rehabilitation center. Simultaneously, the parents were instructed on home rehabilitation, based on their child's personalized home rehabilitation program. The program included personalized physiotherapy, occupational and speech therapy, and continued for at least 12 months.

Laboratory and Imaging Diagnostics

All patients had MRI (Siemens Avanto Tim 1.5 T) and electroencephalography of the brain exams before the intervention. The exams are repeated six months later to detect the improvements in the brain. Routine hematologic and biochemistry examinations were performed at baseline and 3, 6, and 12 months later.

Statistical Analysis

The data were coded and processed using the statistical package social science (SPSS), version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Comparisons of variables before and after transplantation for each patient were based on a *t*-test. A significant difference was indicated by $p < 0.05$.

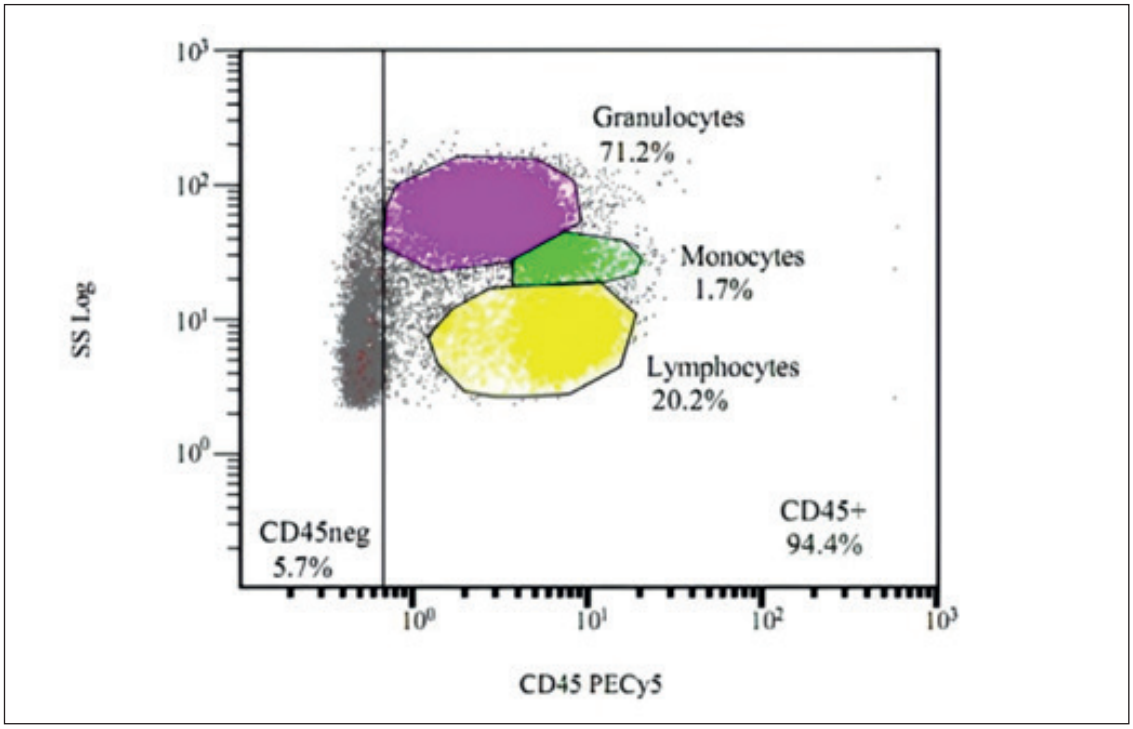

Figure 3. The absolute number of events recorded on the flow cytometer.

Pre-therapy Observations

The study enrolled 42 patients with CP, ages 1 to 12 years, 24 males (57%) and 18 females (43%). The CP patients were classified into spastic hypertonic and non-spastic types (dyskinetic and hypotonic) based on the presence or absence of spasticity, coordination, and muscle tone. The motor function was hypertonic in 24 (60%), dyskinetic in 10 (25%), and hypotonic in 8 (19%) patients. The distribution of gender, age, and clinical presentation of patients is summarized in Table I.

Post-Therapy Observations

No complications were observed during the procedures. However, a few patients showed minor procedure-related adverse events during the hospital stay. The reported adverse events were limited to mild headaches (5%), transient fever (6%), local pain at the site of injection (16%), and vomiting (3%). Patients' adverse events generally occurred on the first operative day and resolved to normal levels within one hour to 3 days in one week.

Motor Movements

Improvements that followed the BMAC therapy were noted within the first seven days. The improvements included decreased muscle tone and involuntary limb movements, improved head control, and reduced salivation. The improvement

Table I. Gender and age group, type of CP and number of patients.

Demographic characteristic	Demographic group	No. of patients (N=42)
Gender	Male	24
	Female	18
Age	< 3 Years	6
	3-8 Years	26
	>8 Years	8
Type of CP	Hypertonic	24
	Dyskinetic	10
	Hypotonic	8

Table II. Number of patients showing improvements based on gender and age of the patients.

	No improvement	Mild improvement	Moderate improvement	Significant improvement
Gender				
Male	2	4	12	6
Female	1	4	8	5
Age				
<3 years	0	0	4	0
3-8 years	3	8	12	11
>8 years	0	0	4	0

in the voluntary control of the limb parts is observed at the 1st, 3rd, 6th, and 12th months after the first transplantation (Table II).

After the three intrathecal applications of BMAC, out of 42 CP patients, 7% showed no improvement, 19% showed mild improvement, 48% showed moderate improvement, and 26% showed major improvement. One month after the intervention, 30% showed improvement in speech and standing balance, 20% in walking balance, 20% in speech, 30% in sitting balance and muscle tone of lower limb and trunk, and 28% in muscle tone of the upper limb. Three months after the intervention, 26% improved neck control, 40% in sitting balance, 36% in standing balance, 27% in walking balance, and 29% in speech. Six months after the intervention, 60% showed improvement in speech and standing balance, 67% in walking balance, 50% in sitting balance and muscle tone of lower limb and trunk, and 37% in muscle tone of the upper limb. In general, 12 months after the intervention, 93% of patients showed mild, moderate and significant improvements, and 7% of patients did not show any improvement, but they remained stable.

We used the gross motor function, the standardized observational tool to evaluate the motor function, and a classification system to assess muscle function (Table III). The GMFCS values measured at baseline were 2 (5%) with level I, 4 (9%) with level II, 8 (19%) with level III, 18 (43%) with level IV, and 10 (24%) with level V. The GMFCS score was calculated before therapy and 3, 6, and 12 months after the first transplantation. Gross motor function improved after stem cell transplantation (POST-GMFCS) compared to the baseline scores (PRE-GMFCS). The average improvement was 1.5 points with a zero to three points span. No patients showed a regression.

Further analyses included the comparison of changes in gross motor function and muscle tone before and 12 months after stem cell transplantation. The recorded improvements included the opening and closing of hand fingers. The patients testified improvements in hand coordination, head movements control, and gross motor controls. Muscle hypertonia was reduced from mild spasm to major reduction, improving sitting and balance maintenance. The patients were observed and tested: we noted improvements in muscle tone and motor function.

Three months after cell transplantation and rehabilitation the POST-GMFCS score had significant improvement (p <0.05), compared with the score before transplantation. Six months after cell transplantation and rehabilitation, the POST-GMFCS score had significant improvement (p <0.05) than before transplantation. Twelve months after cell transplantation and rehabilitation, the POST-GMFCS score had significant improvement (p <0.01) compared with the baseline.

The modified Ashworth scale was used to assess spasticity before and after therapy. A physio-

Table III. Improvements based on GMFCS levels of the patient's 12th months after intervention.

GMFCS levels	PRE-GMFCS	POST-GMFCS Mild improvement	POST-GMFCS Moderate improvement	POST-GMFCS Major improvement
I	2	0	2	0
II	4	0	4	0
III	8	0	4	4
IV	18	2	11	5
V	10	6	4	0

Table IV. Improvements based on the modified Ashworth scale (MAS) of the patients 12 months after the first transplantation.

MAS score	Mild improvement	Moderate improvement	Major improvement
0	0	0	0
1	4	0	0
2	8	8	4
3	0	9	3
4	0	6	0

therapist assessed the functional and muscle tone at baseline, three, six, and twelve months after the first transplantation (Table IV).

Twelve months after cell transplantation and rehabilitation, the muscle spasticity was significantly reduced from 3.6 at baseline to 2.1 (p <0.001) on the modified Ashworth scale. Abnormal posture and contracture deformities were better by approximately 1-2 scale levels. Reduction of spasticity was observed in 58% of patients.

Cognitive and Speech Improvements

Changes in cognitive function (counting and matching) and language (naming and comprehension) were further analyzed by the LAP-D. Total raw scores were calculated for the different age categories before and three, six, and twelve months after stem cell transplantation.

The study enrolled 42 CP patients with chronological age from 1 to 12 years, but the examination revealed their current developmental level from 6-11 months. The pre-therapy average developmental level of patients within a month was 9.8 (SD=0.643), and the post-therapy average developmental level of patients was 14.1 (SD=7.069). The average developmental level of the CP patients after the intervention was 23 months for cognition, 21 months for fine motor skills, and 22 months for speech skills. Improvements in speech skills showed a statistically significant difference with a correlation of $r > 0.7$ (Table V). The cognitive function assessment revealed significant improvement in 35% of patients.

Imaging Diagnostics

The MRI diagnostics revealed the three most common patterns. The patterns included cortical/sub-cortical lesions (58%), periventricular white matter lesions (12%), and dilated third ventricle, lateral ventricle, and subarachnoid space (66%). Brain MRI from 27 (64%) subjects showed slightly decreased dilated third ventricle, lateral ventricle, and subarachnoid space compared with pre-transplantation brain MRI. Fifteen (36%) patients had no significant changes in brain MRI before and after the first transplantation.

Occurrences of patients' seizures decreased. One child reported the complete stop of seizures, which was confirmed using EEG.

Discussion

The limited CP therapeutic options have led to the exploration of neuroregenerative options, such as cellular therapy. The cellular therapy benefit may be due to neuroprotection, neurorestoration, or neuroregeneration[36]. The exact mechanism of stem cell therapy applied in CP treatment is still unknown. Advancement of the successful clinical translation calls for established optimal stem cell administration for CP treatment[37].

Multiple routes have been used for stem cell transplantation, such as intrathecal, intraspinal, intracerebral, intraventricular, intravenous, intraarterial, intramuscular, intraperitoneal, and intranasal[38]. Compared to invasive routes, intrathecal demon-

Table V. Improvements based on LAP-D of the patients 12 months after intervention.

LAP-D	Cognitive skills	Fine motor skills	Speech skills	Sitting	Standing
r	0.049	0.078	0.852	0.138	0.149
p	0.834	0.769	0.000	0.702	0.529
AD	23	21	22	21	23

r-Pearson's correlation coefficient (r >0.7)
p-statistical significance (p=0.000)
AD-the average developmental level of patients (in months)

Table VI. Recent clinical trials using intrathecal injection of autologous BMAC for the treatment of CP.

Reference	Number		No. of cells	No. of treatment and viability (V)	Results	Adverse events
	trial	control				
Wang et al[43]	46	0	$2x10^7$ cells $4x10^7$ cells	No. 3-4 V: no data	Gross motor functional recovery after 1,6, and 18 months.	No
Gabr et al[44]	44	50	$2x10^6$ cells	No. 2-6 V: no data	18.8% motor, cognitive improvement or both between pre and post transplantation.	No
Sharma et al[27]	40	0	$10.23x10^6$ cells	No. no data V:98%	Gross motor functional score and cognitive skills recovery after 3, and 6 months. 95% of patients showed improvements.	No
Nguyen et al[32]	40	0	1st:$27.2x10^6$ + $2.6x10^6$ cells 2nd: $17.1x10^6$ + $1.7x10^6$ cells	No. 2 V: 1st 97.8% 2nd 72%	Gross motor functional and modified Ashwort score recovery after 3, and 6 months.	No
Liu et al[45]	67	38	$2x10^6$-$4x10^6$ cells	No. 4 V: ≥ 95%	Gross motor functional and fine motor functional recovery after 3,6,12 months.	No
Thanh et al[46]	25	0	1st: $17.4 \pm 11.9x\ 10^6$ + $1.5 \pm 1.4x10^6$ cells 2nd:$15.0 \pm 12.8x10^6$ + $1.1 \pm 1.1x10^6$ cells	No. 2 V: 1st 96.9% 2nd 71%	Gross motor functional and modified Ashwort score recovery after 3 and 6 months.	No
Tarkan et al[47]	20	0	$2\text{-}5x10^6$ cells	No. 1 V: no data	Gross motor functional and fine motor functional recovery after 3,6 and 12 months. 73% of patients showed improvements.	No
Present study	42	0	$54x10^6$ cells	No. 3 V: 1st 98% 2nd 99% 3nd 99.3%	Gross motor functional score, modified Ashwort score, LAP-D score recovery after 3,6, and 12 months. 58% of patients showed reduction in spasticity, 35% of patients showed cognitive improvements.	No

strates multiple advantages: 1. Transplanted MSCs can be delivered into the entire neuraxis *via* CSF flow[39]; 2. Transplanted MSCs injected into the subarachnoid space migrate to injured thoracic spinal cord tissue, and infiltrate deeper spinal cord parenchyma[40]; 3. Lastly, some MSCs differentiated into immature neurons or glial cells[40].

The best-established protocol of MSCs therapy does not yet exist[41]. The MSC therapy impact varies with the injection dose, physiological state of cells, and cell viability. Dosage is a significant factor in the treatment success, and it should be adequate to reach the site of action. Sun et al[42] reported that those who received a higher cell dose demonstrated significant improvement in the gross motor function classification system than those who received a lower dose. Repetitive administration enhanced cell delivery and therapeutic efficacy[39].

The recent clinical reports using the intrathecal injection of autologous BMAC for the treatment of CP are summarized in Table VI.

These studies assessed the outcome after one, three, six, and twelve months. The majority of clinical trials showed a remarkable difference in motor functions in the treatment group. They recorded a significant improvement in motor function, sensory function, cognitive function, and speech on follow-up. Sharma et al[27] reported a remarkable 95% improvement in patients with CP. Our results concur with the above trend. The neurologic improvements were significant in patients with hypertonic CP. On one year follow-up, statistically, significant advancements were noticed in motor independence and communication skills. The Ashworth scale showed a significant reduction in spasticity, with higher flexibility and easiness in the patient's movements. After the treatment, a drop of spasticity was observed in 58% of patients. The cognitive assessment revealed significant improvement in 35% of patients. Speech has improved in terms of clarity, fluency, and intelligibility.

Hypoxia and ischemia in CP lead to focal cystic necrotic lesions in the periventricular and central white matter, diffused astrogliosis, microglial activation in the surrounding white matter, diffuse myelination disturbances, and the cortex injury, basal ganglia, and thalamus in CP[2]. Cell therapy helps regulate these pathological disturbances in CP and could be an exogenous source of cells for neurogenesis. Also, the therapy has modulatory effects on the internal environment in the CNS. These exogenous and

endogenous effects of stem cells increase the plasticity, differentiation, and repair of neural tissue in cerebral palsy patients[44]. In addition, CD90 acts as a barrier in the pathway of differentiation commitment and thus controls the differentiation of MSCs[48]. Our study observed an increased number of CD90 positive cells in each succeeding measurement. BMAC contains different types of cells: platelets, erythrocytes, nucleated cells, progenitor cells, hematopoietic stem cells, MSCs. The aim is to bring hematopoiesis and mesenchymal and progenitor cells to the treatment site. Stem cells change the environment *via* paracrine secretion of neurotrophic factors, cytokines, immunomodulatory factors, and angiogenic factors. According to Jiao et al[49] these factors influence target cells to modulate inflammation/apoptosis, activating progenitor cell proliferation and tissue repair to provide a suitable environment for cell survival. This process is more direct and rapid after transplantation. The paracrine mechanism of stem cells may be the reason for the observed improvement in gross motor function[8,39]. Immune modulation is one of the principal mechanisms of action of stem cells[38]. MSCs have been reported to secrete heterogeneous lipid bilayer vesicles called extracellular vesicles, which act as mediators for intercellular communication[50]. These extracellular vesicles secreted from MSCs are known to improve neuronal functions in neurologically injured models[50]. Growth factors, stem cell factors, fibroblast growth factors, and others may reduce the ischemia volume and increase the migration and proliferation of the stem cells. The angiogenesis improvement is possible with the bone marrow endothelial precursors, up to two months post insult[51]. Different experimental studies showed that cell transplantation in CP models can lead to neurons' survival, and the differentiation of cells into neurons, oligodendrocytes, and astrocytes[52,53]. Other studies[52] demonstrated that glial cells play a vital role in the process of regeneration and functional improvement and in accelerating traumatic injuries. Stem cells can restore lost myelin by replacing dead oligodendrocytes and precursor cells. Functional cell survival can be stimulated by introducing another type of cell able to restore the lack of enzymes necessary for brain function[52]. Stem cells can reduce the levels of TNF, IL-1, and IL-6 increased due to microglial activation[53].

BMMSCs transplantation for the CP treatment is safe, minimally invasive, significantly improves gross and fine motor function, and probably the most effective route of administration[45].

Cell therapy and rehabilitation can together improve the positive effects of healing. Solopova et al[54] reported that exercise in spastic CP maintains the optimal length of the muscles and joints range of motion, which finally establishes new standing and walking stereotypes. The patient's active involvement in a movement pattern enables more efficient motor skills development and more extended maintenance compared to passive movements. Therefore, an individual therapeutic approach of cellular therapy and neurorehabilitation helps the neuroregeneration and fastens the recovery process in CP. Physical treatment accelerates stem cell mobilization, proliferation, and neurogenesis by increasing oxygen flow to the brain[3,55]. Exercise enhances the effect of injected stem cells by activating and proliferating the local stem cells, promoting muscle angiogenesis and the release of cytokines and nerve growth factors. During our study, exercise and neurorehabilitation had a synergistic effect on cell transplantation benefits.

Stem cells provide their effects through many mechanisms, and it is difficult to support a single exact action mechanism of stem cells. Sources, types, and numbers of cells administered, and frequency of transplantation are concerns that need considerable attention. It is important to standardize research protocols.

One of the major limitations of this study was that it was a non-randomized open labeled study and did not have an adequate placebo control group to compare the results. A more extended follow-up period would be required to further prove the long-term efficacy of the intervention.

Conclusions

The outcome of this study indicates that stem cell therapy and personalized training can improve the development of children with CP. Although a personalized home rehabilitation program can improve gross motor function in children with CP with age, it is not likely that the children would have improved without autologous BMAC injection. The crucial goal of this therapeutic intervention is to substitute injured tissue with new tissues by connecting with the stem cells, which have a good regenerative capacity. Nevertheless, the brain needs training

to rewire its potential for appropriate functional reorganization.

Authors' Contribution

D.M.M., Dz.A., and M.R. were the patient's surgeons and helped supervise the project. I.S. performed the measurements, analyzed the data and supervised the findings of this work. D.V. contributed to the interpretation of the results. Z.M., G.V., and S.S. reviewed the literature. D.L.M. wrote the paper with input from all authors. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

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