Evaluation of Stem Cell Transplantation In Cerebral palsy

Mohamed S. A. Abd El Kader,[1] Maisa N. Farid,[1] Khaled H.Taman,[1] George S. Kobinia,[2] and Reham S. Tarkhan[1]

[1] Faculty of Postgraduate Childhood Studies, Faculty of Medicine- Ain Shams University, Cairo, Egypt

[2] Stem Cell Therapy Group Vienna Medical University of Vienna Vienna- Austria

Abstract

Background: Perinatal complications may result in life- long morbidities, among which cerebral palsy (CP) is the most severe motor disability. Once developed, CP is a non- progressive disease with a prevalence of (1- 2) per 1000 live births in developed countries. It demands an extensive and multidisciplinary care. Therefore, it is a challenge for our health system and a burden for patients and their families. Recently, stem cell therapy emerged as a promising treatment option and raised hope in patients and their families, Aim and objectives: the aim of the study is to evaluate the role of stem cells transplantation in CP.

Patients, Subjects& Methods: This is a follow up study, that was carried out at Waady Elneel hospital, Cairo, the study was conducted on 20 diagnosed Cerebral Palsy patients classified according to Age into Two Groups: Group 1: one year to five years, and Group 2: five years to fifteen years, the study was carried out from January 2018 to January 2020, Follow up the patient with convulsions was done before injection of stem cell and 3 months, 6 months, 9 months, 12 months after injection of stem cell.

Results: The results of the study revealed that there is no significant difference at GMS between the two groups as regard injection score, there is significant in gross motor scale from pre- injection to final injection score in group A and there is no significant difference at GMS between the two groups as regard injection score, Conclusion: gross motor function and muscle tone in children with CP were remarkably improved at 6 months and 12 months after stem cell transplantation,

Keywords: Transplantation, Gross Motor Function, Stem Cells, Cerebral Palsy, Convulsions.

تقييم زرع الخلايا الجذعية فى الشلل الدماغى

الخلفية: قد تؤدى المضاعفات التى تحدث فى الفترة المحيطة بالولادة إلى إعاقة تدوم مدى الحياة، ومن بينها الشلل الدماغى هو الإعاقة الحركية الشديدة. ما إن يتم تطويره، يعتبر مرض الشلل الدماغى مرضا غير متطور مع انتشار ١- ٢ لكل ١٠٠٠ مولود حى فى البلدان المتقدمة. يتطلب رعاية واسعة ومتعددة التخصصات. لذلك، إنه تحد لنظامنا الصحى وعبء على المرضى وأسرهم. فى الآونة الأخيرة، برز العلاج بالخلايا الجذعية كخيار علاج واعد وأثار الأمل فى المرضى وأسرهم،

الهدف: الهدف من الدراسة هو تقييم دور زرع الخلايا الجذعية فى مرضى الشلل الدماغى.

الأشخاص والمناهج والطرق: هذا متابعة الدراسة، التى أجريت فى مستشفى وادى النيل، القاهرة، أجريت هذه الدراسة على ٢٠ مريضا مصابا بالشلل الدماغى تم تشخيصهم حسب العمر إلى مجموعتين: المجموعة تضم افراداً اعمارهم سنة واحدة إلى خمس سنوات، والمجموعة تضم افراداً اعمارهم خمس سنوات إلى خمسة عشر عاما، أجريت الدراسة فى الفترة من يناير ٢٠١٨ إلى يناير ٢٠٢٠، وتمت متابعة المريض مع التشنجات قبل حقن الخلايا الجذعية و٣ أشهر، ٦ أشهر، ٩ أشهر، ١٢ شهرا بعد حقن الخلايا الجذعية.

النتائج: كشفت الدراسة أنه لا يوجد فرق كبير في مقياس الحركة العامة بين المجموعتين فيما يتعلق بدرجة الحقن، وهناك فرق كبير فى الحجم الحركى الإجمالى من مرحلة ما قبل الحقن إلى درجة الحقن النهائى فى المجموعة أولا يوجد فرق كبير في مقياس الحركة العامة بين المجموعتين فيما يتعلق درجة الحقن.

الخلاصة: تم تحسين وظيفة الحركية الإجمالية ونبرة العضلات فى الأطفال الذين يعانون من الشلل الدماغى فى ٦ أشهر و١٢ شهرا بعد زرع الخلايا الجذعية.

الكلمات الرئيسية: زرع، وظيفة المحرك الإجمالى، والخلايا الجذعية، الدماغ الشلل والتشنجات.

Introduction:

Cerebral Palsy is the commonest cause of severe neurological disability in children. The general prevalence is 2- 3 per 1000 live births and has slightly increased in recent years. This is due to the decreased mortality of low- birth- weight infants together with an increased rate of cerebral palsy in the survivors (Mutch et.al, 1992). CP describes a group of permanent disorders of the development of movement and posture, causing activity limitations. The motor disorders of cerebral palsy are often accompanied by disturbances of sensation, perception, cognition, communication, and behavior, (Rosenbaum et.al., 2007).

Woodbury et.al. claimed that adult rat and human bone marrow stromal cell differentiate into neurons (Woodbury et.al., 2000). Stem cell transplantation has been reported to be effective in animal models as well as in patients with other degenerative neurological disorders such as stroke and demyelination (Park et.al., 2009).

Stem cell research originated in the 1980s within the field of biotechnology. Since then, progress has been made towards developing a number of potential treatments for what are still considered untreatable diseases. However, controversy regarding the means and methods of study has slowed further progress within the field. This article will address the medical benefits of stem cell research and the controversy surrounding its practice (Lepore et.al., 2005).

Researchers are using several different types of stem cells to study brain injury to explore the possibilities for developing new treatments. In some of these studies, researchers do appear to have been able to reduce damage to the brain have shown some improvements in their symptoms. However, these effects in patients are not yet fully understood, much more work is needed and it will be many years until such research can be applied to treating cerebral palsy (Korzeniewski et.al., 2008).

Subjects And Methods:

This study was a follow up study. This study was carried out in Waady Elneel Hospital, Cairo, from January 2018 till January 2020.

The study included 20 diagnosed Cerebral Palsy patients classified according to Age into Two Groups:

- Group 1: One year to five years.
- Group 2: Five years to fifteen years.

Exclusion criteria:

1. Children with other physical disabilities, Children below 1 Year or above 15 Years and Children their weight below 10 Kg.
2. The study included 20 diagnosed Cerebral Palsy patients.

Methods:

1. Clinical Assessment:
   a. History: Prenatal History, Natal History, Post Natal History, Mode of Delivery, History of Motor, Mental Developmental milestones.
   b. Clinical examination: General examination, Neurological examination, Gross Motor Scale was done before injection of stem cell and 3 months, 6 months, 9 months, 12 months after injection of stem cell.
   c. Intervention: On the day of the procedure, the bone marrow was collected under strict sterile conditions in the operating room under general anesthesia. Two to five milliliters per kg of marrow were collected from the anterior or posterior iliac crests depending on the patient posture and contractures. Six to eight puncture sites were used to aspirate the bone marrow. The marrow was aspirated using a twenty ml syringe with citrate or heparin sodium as anticoagulants in a 10% solution. The BMMCs were collected and then washed and separated using the density gradient centrifugation method before counting the cells using the Neubauer chamber. The cells were subjected to microbiologic, microscopic, and serologic testing to characterize them, and secure their safety. The resulting cells were re- suspended to prepare the 5~10 ml BMMC to be injected. The injected cells were adjusted to two million cells per kilogram of body weight± 10%. The patients were injected intrathecally once at the L4~L5 level.
2. Laboratory procedure: Bone marrow was harvested (2- 3ml) by puncturing the iliac crest with bone marrow puncture needle (Komiyasshiki needle gauge 12) that was inserted.
   a. The mandrill was removed and bone marrow was aspirated with a 5 ml syringe containing 5000 UI/ ml of heparin immediately after puncture.
   b. Blood was transferred to a vacum tube containing 5000 UI/ ml of heparin.
   c. The total volume of bone marrow (2ml) was used for mononuclear cell isolation by gradient centrifugation at 2000 rpm for 30 minutes at room temperature.
   d. The mononuclear cell layer was aspirated with a pipette washed twice and resuspended in DMEM medium. Cells was prepared with 1 ul of cell suspension and placed in a neubauer chamber. The number of cells was estimated as a quadrant delimited the area observed under the microscope in order to convert the number of cells counted in quadrant into cell/ ml a correction factor of 104 was used. After counting cell viability was checked by the tripan blue dye exclusion method which transverses the membrane in a dead cell and dyes its blue cells were prepared by adding 1 ul of cell suspension to 1 ul of tripan blue 1: 1.
   e. The patients were anesthetized with an i.m injection of zoletal (0.4ml/ ug) and ketamine (0.4ml/ ug) for maintenance this dose was dilated into 10 ml of sterile saline i.m injected (1- 2 m) when necessary. The site of injection scrubed and covered with as sterile adhesion film to reduce the risk of contamination.
   f. Local hemostasis was achieved by infiltrating lidocaine 2% during injection.
3. Follow up: Follow up the patient with convulsions was done before injection of stem cell and 3 months, 6 months, 9 months, 12 months after injection of stem cell.

4. Statistical analysis: Analysis of data was done using Statistical Program for Social Science version 20 (SPSS Inc., Chicago, IL, USA). An informed written consent from all parents of participants was taken and confidentiality of information was assured. Permission from the Ethical Committee of Institute of Postgraduate Childhood Studies was obtained.

Results:

This table shows that mean age in group A is 3.3± 1.3 with range 1.5-5 while, mean age in group B is 10.1± 2.3 with range of 6.5- 14. 30% OF Cases were males for Group A and 60% for Group B While 70% were females in Group A and 40% in Group B. Noticed that there is a significant difference between blood pressure of Group A and Group B, SBP of Group A was 102.2± 1.3 and DBP was 60.2± 2.5 while Group B were 110.4± 4.4 and 72.7± 3.7. Table (1).

This table and figures shows that% of Maternal Age more than 40 years was 45% and less than 40 years was 55% in total of CP cases. The most percentage of cases maternal subjected to cesarean delivery with percentage 60% while only 15% needed to assistant Instrument (forceps) during birth and 25% of Maternal born by Natural childbirth. So that the percentage of causes reported as Natal (during birth) 50% but postnatal (after birth) was 15% and before birth 35% Table (2).

The changes in gross motor scale as shown in previous table when pre injection in both group A& B was (100%) of cases 5 scale and after the 3 months post injection this gross motor scale changed in (80%) of cases to scale 4 in both Groups then after 6 months post injection reported that (70%) of cases changed to 3scale and 30% changed to scale 4 at Group A while 50% of cases changed to 3 scale and 50% changed to scale 4 and finally after 1 year reported that most of cases at both Groups (90%) reached to 2 scale and the rest (10%) reached to 3 scale. Fig (1).

This table shows that there is high significant change in gross motor scale over time F. Table (3).

This table shows that there is significant in gross motor scale from preinjection to final injection score in group A. Fig (2).

This table shows that there is high significant change in gross motor scale over time. Table (4).

This table shows that there is no significant no correlation between age, weight, SBP, DBP, Hypoxia, and Exposure to toxic material with final post injection score and there is no significant association between cause, type CP, Consanguinity and hypoxia with final post injection score but Jaundice and Consanguinity affected on class with non- significant negative correlation. Table (5).

Table (1) Demographic characteristics of the study Groups

Group		Cerebral Palsy Patient Groups		P-value
		Group B (n=10)	Group A (n=10)	
Age (Years)		3.3± 1.3	10.1± 2.3	0.000
	(mean±SD)	3.5	7.5	
	Range	1.5- 5	6.5- 14	
Weight		14.4± 2.8	30.2± 5.6	0.000
	(mean±SD)	7.6	18.8	
	Range	10.5- 17.9	21.2- 40	
Sex	Male	3	6	0.3
	n(%)	(30%)	(60%)	
	Female	7	4	
	n(%)	(70%)	(40%)	
SBP (mmHg)		102.2±1.3	110.4±4.4	0.000
	(mean±SD)	4	14	
	Range	100-104	106-120	
DBP (mmHg)		60.2±2.5	72.7±3.7	0.000
	(mean±SD)	9	12	
	Range	55-64	68-80	
Types of CP	Spastic	8	8	0.9
	n(%)	(80%)	(80%)	
	Dyskinetic	1	1	
	n(%)	(10%)	(10%)	
	Mixed	1	1	
	n(%)	(10%)	(10%)	

Table (2) Distribution of Birth risk factors among the studied Cases:

Variables		Total CP Cases
Maternal Age	<40 n (%)	11 (55%)
	≥40 n (%)	9 (45%)
Mode of Birth	Cesarean n (%)	12 (60%)
	Forceps n (%)	3 (15%)
	Natural n (%)	5 (25%)
Causes	Prenatal n (%)	7 (35%)
	Postnatal n (%)	3 (15%)
	Natal n (%)	10 (50%)

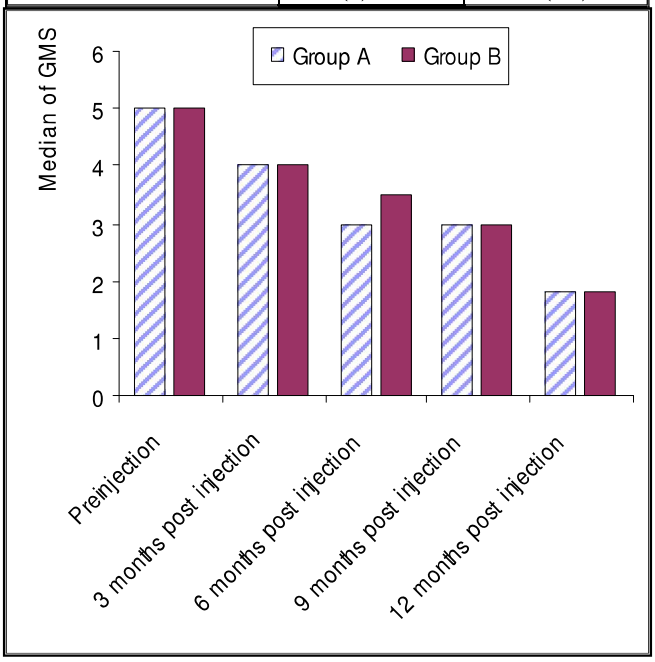

Fig (1).Comparison mparison between median changes in gross motor scale between Groups A and Group B

Table (3): Pre- injection and over one year injection changes in gross motor scale Group A

Variable	Pre-injection (n=10)	3 months post injection (n=10)	6 months post injection (n=10)	9 months post injection (n=10)	12 months post injection (n=20)	p
GMS (%)	5 (100%)	5(20%)	4(50%)	4(10%)	3(10%)	<0.001 (HS)
4 (80%)	4 (80%)	3(50%)	3(60%)	2(30%)	2(90%)	
Median	5	4	3	3	2	
Range	(5-5)	(4-5)	(3-4)	(2-4)	(2-3)	

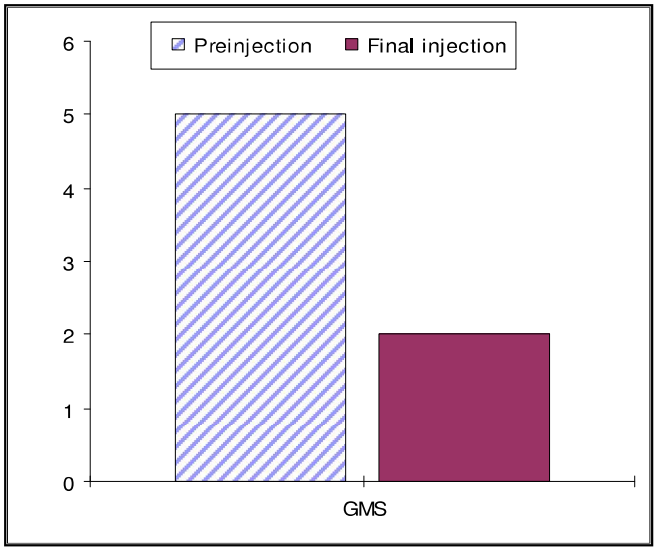

Fig. (2) Preinjection and final injection changes in gross motor scale Group A.

Table (4) Pre- injection and over one year injection changes in gross motor scale GroupB

Variable	Pre-injection (n=10)	3 months post injection (n=10)	6 months post injection (n=10)	9 months post injection (n=10)	12 months post injection (n=20)	p
GMS (%)	5 (100%)	5(20%)	4(50%)	4(10%)	3(10%)	<0.001 (HS)
4 (80%)	4 (80%)	3(50%)	3(60%)	2(30%)	2(90%)	
Median	5	4	3	3	2	
Range	(5-5)	(4-5)	(3-4)	(2-4)	(2-3)	

Table (5) Correlation between parameters and final post injection score for total cases:

final post injection score with parameters	Total	
	Spearman Coefficient (r)	P- Value
Age	0.058	0.8
Weight	0.029	0.9
SBP	0.058	0.8
DBP	0.058	0.8
Jaundice	- 0.778	0.1
Hypoxia	0.154	0.1
Exposure To Toxic Material	0.278	0.2
Consanguinity	- 0.778	0.097

r Is Spearman's Correlation

Discussion:

Several studies, using stem cell therapy, were conducted on cerebral palsy patients. The types of improvement included a decrease of spasticity, a better coordination, an increase in motor function, an increase in posture stability and an improvement in mental functions, improvement of articulation and the ability to speak better resulting in improved communication. Sitting alone, standing alone and even walking without help were reported. All the improvements started within 8 weeks after the application of autologous Stem Cells. The results show no apparent correlation between the outcome and the number of transplanted cells (Okur et.al., 2018).

This is why this study was selected to be conducted to evaluate the role of stem cells transplantation in CP Patients.

A cross sectional study was conducted in waady Elneel Hospital, Cairo including 20 diagnosed Cerebral Palsy patients classified according to Age into two groups: Group 1: One year to five years and Group 2: five years to fifteen years. The duration of the study ranges from 6- 12 months.

The mean age in group A is 3.3± 1.3 with range 1.5- 5 while, mean age in group B is 10.1± 2.3 with range of 6.5- 14. There was a significant difference between Group A and Group B regarding age, weight and blood pressure.

Our results are supported by study of Chen et.al., 2013 as they found that the studied group consisted of 14 males and 16 females with a mean age of 5.53± 1.20 years (range, 1- 32 years).

In contrary with our results, study of Shroff et.al., 2014 as they reported that a total of 91 patients were included in the study and all patients were started on intensive dosing. Most patients included in the study were males (71.4%) aged up to 18 yr.

As regard gross motor scale, the current study shows that there is no significant difference at GMS between the two groups as regard injection score. There is high significant change in gross motor scale over time F. There is significant in gross motor scale from pre- injection to final injection score in group A. There is high significant change in gross motor scale over time among group B. There is significant in gross motor scale from pre- injection to final injection score after 12 months in group B.

Our results are supported with study of Chahine et.al., 2016 as they reported that the efficacy of the stem cell therapy and pre and post injection follow ups was available for 15 evaluable patients. Eleven of the 15 had a significant improvement (73%). The improvement ranged from 1 to 3 levels on the GMFCS scoring system. The average improvement was 1.3 points with a range of 0 to 3 points of improvement with no patients showing regression. The improvement was most pronounced in the degree of spasticity in 6 patients (40%) and in many cases it was the first feature noted to improve.

Furthermore, Thanh et.al., 2019 observed that overall, gross motor function was markedly improved at 6 and 12 months after stem cell transplantation, with median scores of 35.8 (27.6) and 53.2 (28.2), respectively, versus 18.3 (17.6) at baseline. The Wilcoxon matched- pairs signed rank test indicated that the GMFM- 66, GMFM- 88 and sub-domain median scores were significantly higher after transplantation than at baseline (p- value< 0.05). This level of improvement was higher than the study of Wang et.al., 2013 but lower than their previous study (Nguyen et.al., 2017). The GMFM- 88 score in Wang's study increased by 7.89 at 6 months after transplantation than baseline scores. The GMFM-88 in our study in using stem cell transplantation for CP related to oxygen deprivation increased by 25.1 at 6 months after the transplantation.

Liu et.al., 2017 Concludes, in terms of gross motor function, 3 months after transplantation, the BMMSC group (Bone marrow mesenchymal stem cells) and the BMMNC group (bone marrow mononuclear cells) begin to gain significant improvements (A, B, and C dimensions and GMFM (The gross motor function measure) total scores). These findings are the same as those for previous studies (Sharma et.al., 2015) using BMMNCs; Chen et.al., 2013 using neural stem cell- like cells derived from autologous BMMSCs about the timing of outcomes.

Multiple reports have demonstrated that MSCs can promote the restoration of motor function in children with CP (Gorter et.al., 2009).

In the study in our hands, there is no significant difference between GM score between male and female for Group A. There is no significant difference between GM score between male and female for Group B.

Findings of our results are in agreement with study of Thanh et.al., 2019 as they showed no relationship between improvement in gross motor function and muscle tone based on patient sex (p- value> 0.05).

The present study shows that there is significant difference between Types of CP with positive correlation between GMS and CP types. The mixed group slowly improved by GMS 4 score among both groups.

Our results are supported by study of Chen et.al., 2013 as they demonstrated that to evaluate the impact of NSC (neural stem cell) transplantation in patients with different levels of cerebral palsy, they divided the patients according to their GMFCS levels. The results indicated that patients in the transplantation group with levels IV and V had a better recovery of motor function.

The current study shows that there is no significant difference for GMS at between different modes of birth among both groups.

Findings of our results are in agreement with study of Feng et.al., 2015 as they reported that there was no statistically significant difference for GMS at different modes of birth among both groups.

The present study shows that there is no significant difference for GMS at between different causes among both groups. There is no significant correlation between Different parameters and final post injection score among both groups.

Our results are in line with study of Thanh et.al., 2019 as they showed no relationship between improvement in gross motor function and muscle tone based on patient age, sex, or GMFCS level (p- value > 0.05).

Feng et.al., 2015 as they reported that there was no statistically significant difference for GMS and causes among both groups.

This study has some limitations. There was no control group. In addition, the follow- up time of 6 months after the 2nd stem cell transplantation was relatively short.

Conclusion:

Based on the results of this study, we can conclude that gross motor function and muscle tone in children with CP were remarkably improved at 6 months and 12 months after stem cell transplantation. However, these finding should be confirmed in larger, multicenter, placebo- controlled trials.

References:

1. Chahine, N. H. A., Wehbe, T. W., Hilal, R. A., et.al. Treatment of cerebral palsy with stem cells: a report of 17 cases. **International Journal of Stem Cells** 2016; 9(1), 90.
2. Chen, G., Wang, Y., Xu, Z., et.al. Neural stem cell- like cells derived from autologous bone mesenchymal stem cells for the treatment of patients with cerebral palsy. **Journal of Translational Medicine**, 2013; 11(1), 21.
3. Feng, M., Lu, A., Gao, H., et.al. Safety of allogeneic umbilical cord blood stem cells therapy in patients with severe cerebral palsy: a retrospective study. **Stem cells international**, 2015.
4. Gorter, J, O. Verschuren, L. van Riel, et.al. "The relationship between spasticity in young children (18 months of age) with cerebral palsy and their gross motor function development", **BMC Musculoskeletal Disorders**, 2009; 10 (1): 108.
5. Korzeniewski S. J, Birbeck G, DeLano M. C, et.al. "A systematic review of neuroimaging for cerebral palsy", **Journal of Child Neurology**, 2008; 232: 216- 227.
6. Lepore A. C, Bakshi A, Swanger S. A, et.al. "Neural precursor cells can be delivered into the injured cervical spinal cord by intrathecal injection at the lumbar cord", **Brain Research**, 2005; 1045: (1- 2): 206.
7. Liu, X., Fu, X., Dai, G., et.al. Comparative analysis of curative effect of bone marrow mesenchymal stem cell and bone marrow mononuclear cell transplantation for spastic cerebral palsy. **Journal of Translational Medicine** 2017; 15(1), 48.
8. Mutch L, Alberman E, Hagberg B, et.al. "Cerebral palsy epidemiology: Where are we now and where are we going?" **Developmental Medicine and Child Neurology** 1992; 34(6): 547- 551.
9. Nguyen LT, Nguyen AT, Vu CD, et.al. Outcomes of autologous bone marrow mononuclear cells for cerebral palsy: an open label uncontrolled clinical trial. **BMC Pediatr**. 2017; 17(1): 104.
10. Okur, S. Ç., Erdoğan, S., Demir, C. S., et.al. The Effect of Umbilical Cord- derived Mesenchymal Stem Cell Transplantation in a Patient with Cerebral Palsy: A Case Report. **International Journal Of Stem Cells**, 2018; 11(1), 141.
11. Park D. H, Borlongan C. V, Willing A. E "Human umbilical cord blood cell grafts for brain ischemia", **Cell Transplantation**, 2009; 18(9): 985- 998.
12. Rosenbaum P, Paneth N, Leviton A, et.al. "A report: the definition and classification of cerebral palsy April 2006", **Developmental Medicine and Child Neurology** 2007; 49(2): 8- 14.
13. Sharma, A., Sane, H., Gokulchandran, N., et.al. A clinical study of autologous bone marrow mononuclear cells for cerebral palsy patients: a new frontier. **Stem cells international**, 2015.
14. Shroff, G., Gupta, A.& Barthakur, J. K. Therapeutic potential of human embryonic stem cell transplantation in patients with cerebral palsy. **Journal of Translational Medicine** 2014; 12(1), 318.
15. Thanh, L. N., Trung, K. N., Duy, C. V., et.al. Improvement in gross motor function and muscle tone in children with cerebral palsy related to neonatal icterus: an open label, uncontrolled clinical trial. **BMC Pediatrics** 2019; 19(1), 290.
16. Wang X, Cheng H, Hua R, et.al. Effects of bone marrow mesenchymal stromal cells on gross motor function measure scores of children with cerebral palsy: a preliminary clinical study. **Cytotherapy**. 2013; 15(12): 1549- 62.
17. Woodbury D, Schwarz E. J, Prockop D. J, et.al. "Adult rat and human bone marrow stromal cells differentiate into neurons", **Journal of Neuroscience Research** 2000; 61: 364-370.