Original Article

Stem Cell Transplantation in Egyptian Patients with Cerebral Palsy

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ABSTRACT

Background: Stem cell-based therapies have been developed for various CNS diseases including perinatal hypoxic ischemic insults of the brain. Stem cells have the capacity to proliferate in culture, ability to migrate and disseminate following implantation within the adult CNS. **Objective:** To study the impact of stem cell transplantation (STC) on psychomotor functions in patients with cerebral palsy. **Methods:** Fifty two Egyptian patients with cerebral palsy were divided into: group I (26 patients who underwent stem cell transplantation) and group II (26 patients who did not undergo stem cell transplantation). Both groups were assessed, initially and after one year, by a group of clinical scales to assess motor, communication and independence skills. **Results:** In group I, using Boyd's developmental progress scale revealed a statistically highly significant improvement in motor, independence and communication skills after SCT (P value < 0.01). Also, 100 points scale revealed a statistically significant improvement after SCT (P value < 0.05). **Conclusion:** Autologous stem cell transplantation could be a useful tool for the management of patients with cerebral palsy as it may help in improvement of motor, independence and communication skills. **[Egypt J Neurol Psychiat Neurosurg. 2012; 49(2): 117-122]**

Key Words: Stem cell transplantation, cerebral palsy.

INTRODUCTION

Cerebral palsy (CP) is an "umbrella" term that describes non-progressive brain lesions involving motor or postural abnormalities that are noted during early development[1]. Cerebral palsy is caused by an insult to the immature brain; the period during which the insult can occur ranges from any time before birth up to the postnatal period[2].

A link exists between various prenatal, perinatal, postnatal factors and CP. However, prenatal factors play a predominant role contributing to 70-80% of cases of CP[3]. Treatment of cerebral palsy is aimed at improving infant-caregiver interaction, as well as at promoting motor and developmental skills[4]. Various modalities of treatment have been proposed for cerebral palsy patients, including physical therapy, occupational therapy, speech therapy, recreational therapy and surgical intervention for associated skeletal deformities[5]. In 2001, Collet and his colleagues[6] tried the use of hyperbaric oxygen, but showed no benefit.

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Regenerative medicine is the process of creating living, functional tissues to repair or replace tissue or organ function lost due to age, disease, damage or congenital defects[7]. Stem cell-based therapies have been developed for various CNS diseases; some are accepted; others are still under investigations[8]. The success of any attempted repair will depend on the severity of the insult, the ability of the environment where the neural stem/ progenitor cells (NSPs) live to sustain them and the ability of these cells to migrate to the site of injury, mature and survive. Because of the limited replacement of brain cells that occurs naturally, it is likely that the number of resident neural stem/ progenitor cells (NSPs) available is insufficient to repopulate the brain fully after an injury. Strategies to expand the regenerative potential of the neural stem/progenitor cells (NSPs) of the individual or exogenous stem cell transplantation may be necessary[9]. Because a person's own (autologous) cord blood stem cells can be safely infused back into that individual without being rejected by the body's immune system, they are an increasing focus of regenerative medicine research[10]. Given the inaccessibility of conventional neuronal stem cells, marrow stromal cells may therefore eventually have applications in the treatment of neurological disease[8].

Several studies, using stem cell therapy, were conducted on cerebral palsy patients[11-14]. 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[14]. 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[14].

The aim of this work is to study the impact of stem cell transplantation (STC) on psychomotor functions in patients with cerebral palsy.

SUBJECTS AND METHODS

Subjects

This phase I study was conducted on 52 Egyptian patients with cerebral palsy from Neurology Out-Patient Clinic, Kasr El-Eini Hospital and Physiotherapy clinic Abo-Elreesh Hospital, from November 2007 till February 2009. They were 26 male patients and 26 female patients. Their age ranged from 1 to 8 years. They were divided into 2 groups :

1) **Group I (study group):** 26 patients with cerebral palsy who underwent stem cell transplantation.

   Inclusion criteria:
   1. Patients with cerebral palsy (all clinical types).
   2. Age between 1-8 years.

   Exclusion criteria:
   1. Patients with history of seizures.
   2. Patients with severe fixed deformities.

2) **Group II (non-intervention group):** 26 patients with cerebral palsy who did not undergo stem cell transplantation.

Both groups were allowed to continue their usual medical treatment (nootropic drugs e.g. piracetam, ginko biloba.etc) and physiotherapy.

Methods

1. **A written informed consent**, from the parent(s) upon enrollment of their child into the study.
2. **Clinical evaluation:** including:
   * Full history taking with special emphasis on prenatal, natal, postnatal, developmental history.
   * Full neurological examination.
   * Clinical measures of disability, including:
     - Gross Motor Function Classification System (GMFCS)[15].
     - Boyd's developmental progress scale[16].
     - A 100 points scale[14].
3. **Stem cell transplantation (STC) (in group I only) :**

   Bone marrow aspiration from the posterior iliac crest. 10-15 ml of bone marrow was aspirated on preservative-free heparin.
   a. Isolation and cultures of mesenchymal stem cell (MSCs).
   b. Separation of mononuclear cells.
   c. Enumeration of the percentage of CD 34 +ve and CD 44 +ve cells.
   d. Cell culture.
   e. Cells were resuspended in sterile saline for injection in the patients.

   Reinjection of MSCs intrathecal: in another sitting 3-5 days after bone marrow aspiration. The target dose of mesenchymal stem cells was 2×10^6 cell/kg BW; in 16 patients such dose was achieved after a single aspiration, only in 10 patients we needed to repeat the procedure in order to achieve the target dose.
4. **Follow up of patients after one year.**

Statistical analysis: The data were coded and entered using the statistical package social science (SPSS) version 12. Descriptive analyses were conducted using mean and standard deviation for quantitative variables. To test the significance of difference between quantitative variable of the same group (pre and post SCT) Wilcoxon sign rank test was used, while Mann Whitney test was used in comparison of quantitative variables between cases and controls. When P was <0.05 this was statistically significant, when P was <0.01 this was statistically highly significant.

RESULTS

I) Clinical Data:

Distribution of clinical syndromes: Clinical presentation of patients in both groups is summarized in Table (1).

II) Severity Scales:

1. **Boyd's developmental progress scale (BDPS):**
   A. Results of initial assessment of both groups: No significant difference was observed between both groups, as regards motor, independence and communication skills (P>0.05) (Table 2).

B. Results of follow up assessment of both groups: No significant difference was observed between both groups, as regards motor, independence and communication skills (P>0.05) (Table 3).

C. Results of assessment of group I pre and post SCT: A statistically highly significant improvement was observed in motor, independence, and communication skills after STC as compared to pre STC scores in group I(P<0.01) (Table 4).

D. Initial and follow up assessment of group II: No statistically significant improvement was found in the follow up assessment of group II (P>0.05) (Table 5).

2. The 100 points scale:

A. Results of initial and follow up assessment of both groups: No significant difference was observed between both groups on initial or follow up assessment (P>0.05) (Table 6).

B. Results of assessment of group I pre and post SCT: A statistically highly significant improvement was observed after STC as compared to pre STC scores in group I (P<0.01) (Table 7).

C. Initial and follow up assessment of group II: No statistically significant improvement was found in the follow up assessment of group II (P>0.05) (Table 8).

3. Gross Motor Function Classification System (GMFCS):

A. Results of initial and follow up assessment of both groups: No significant difference was observed between both groups on initial or follow up assessment (P>0.05) (Table 9).

B. Results of assessment of group I pre and post SCT: No significant improvement was observed on comparing scores of group I before and after SCT (P>0.05) (Table 10).

C. Initial and follow up assessment of group II: No statistically significant improvement was found in the follow up assessment of group II (P>0.05) (Table 11).

III) Correlations:

1. No significant correlation was found between age of patients, in both groups, and scores of any of the used severity scales.
2. No apparent correlation was found between the dose of transplanted cells per kilogram and percent of change in any of the used clinical severity scales.

Table 1. Distribution of different clinical syndromes in both groups.

Clinical Syndrome	Group I (n= 26)	Group II (n= 26)	Total
Athetoid CP	1(4%)	0(0%)	**1**
Diplegia	5(19%)	2(8%)	**7**
Hemiplegia	1(4%)	1(4%)	**2**
Quadriplegia	19(73%)	23(88%)	**42**
Total	**26(100%)**	**26(100%)**	**52**

Table 2. Initial assessment of study and control groups using (BDPS).

	Group I Mean (SD)	Group II Mean (SD)	P-value
Motor skills	8.19 (8.75)	8.09 (9.57)	0.11
Independence skills	9.23 (8.55)	9.16 (8.73)	0.18
Communication skills	10.19 (8.99)	10.08 (8.39)	0.32

Table 3. Follow up assessment of both groups using BDPS.

	Group I Mean (SD)	Group II Mean (SD)	P-value
Motor skills	9.19 (8.99)	8.46 (8.63)	0.77
Independence skills	10.19(8.99)	9.5(8.59)	0.78
Communication skills	11.5(8.27)	10.46(7.21)	0.63

Table 4. Assessment of patients in group I pre and post SCT using BDPS.

	Pre SCT Mean (SD)	Post SCT Mean (SD)	P-value
Motor Skills	8.19 (8.75)	9.19 (8.99)	*0.001**
Independence Skills	9.23 (8.55)	10.23 (7.11)	*0.001**
Communication Skills	10.19 (8.99)	11.5(7.39)	*0.004**

Table 5. Initial and follow up assessment of group II using BDPS.

	Initial assessment Mean (SD)	Follow up assessment Mean (SD)	P-value
Motor skills	8.09 (9.57)	8.46 (8.63)	0.11
Independence skills	9.16 (8.73)	9.5 (8.59)	0.183
Communication skills	10.08 (8.39)	10.46 (7.21)	0.320

Table 6. Initial and follow up assessment of both groups using 100 points scale.

	Group I Mean (SD)	Group II Mean (SD)	P-value
Initial assessment	47.31 (32.69)	38.65 (25.24)	0.29
Follow up assessment	50.58 (34.01)	38.85 (24.95)	0.16

Table 7. Assessment of group I pre and post SCT using 100 points scale.

	Pre SCT	Post SCT	P-value
Mean (SD)	47.3 (32.68)	50.57 (34.00)	0.002*

*statistically significant at $p<0.05$

Table 8. Initial and follow up assessment of group II using 100 points scale.

	Initial assessment	Follow up assessment	P-value
Mean (SD)	38.65(25.24)	38.85(24.95)	0.23

Table 9. Initial assessment of both groups using GMFCS.

	Group I Mean (SD)	Group II Mean (SD)	P-value
Initial assessment	4.23 (1.37)	4.62 (0.75)	0.21
Follow up assessment	4.15 (1.46)	4.58 (0.76)	0.2

Table 10. Assessment of group I pre and post SCT using GMFCS.

	Pre SCT	Post SCT	P-value
Mean (SD)	4.23 (1.37)	4.15 (1.46)	0.16

Table 11. Initial and follow up assessment of group II using GMFCS.

	Initial assessment	Follow up assessment	P-value
Mean (SD)	4.62 (0.75)	4.58 (0.76)	0.19

DISCUSSION

Stem cell therapy based on a stem cell transplantation, which is aimed directly to augmenting reparative abilities of an injured brain, opens new opportunities in the cerebral palsy treatment. By experimental and clinical investigations, it is firmly established that when grafted into the injured brain, stem cells are able to ameliorate greatly injury-caused and neurological defects in children with cerebral palsy[17].

Cerebral palsy by definition is a non progressive condition so it was possible to design the current trial to be single-armed and to compare condition of the patients after SCT to their condition prior to SCT, however, it was preferred to design it to be a double-armed study in order to nullify the effect of the confounders that could affect the treatment outcome such as other lines of therapy the patient received (e.g. medical treatment, physiotherapy, speech therapy, etc.) specially that it was difficult from the ethical point of view to ask the patient to stop all other lines of the therapy they receive for one year(the duration of the follow up).

Three scales were used to assess patients in both groups (study and control groups) to obtain a genuine idea about their level of the disability to be used as a baseline for comparison with follow up assessment measures for motor, independence and communication abilities.

Our patients were followed up one year after the initial assessment, this duration is relatively satisfactory to assess whether patients are actually gaining benefit from the SCT or not. Other studies also agreed with us in such follow up period[14,18].

The current study showed that stem cell transplantation had a positive effect on motor, independence and communication skills in the study group patients using Boyd's developmental progress scale and 100 points scale and such improvement was statistically highly significant. Our findings were congruent with other studies that reported functional and psychomotor improvement in cerebral palsy patients following SCT[11,13,14,18].

Although our patients achieved mild improvement in Gross Motor Function Classification System (GMFCS), this improvement did not reach statistical significance which could be explained by the wide stratification of the levels of the scale, so that mild improvement in motor functions, that could be detected by the previous scales (Boyd's developmental progress scale & 100 points scale), was not able to move patients from a level to a higher one.

The age of our patients was not correlated with the response to SCT in the used scales. However, it was noticed the mean age of patients who had improved was younger than those who did not. This is consistent with results of Seledtsov et al.[18], who noticed that the improvement was more marked in infants and toddlers.

Furthermore, in our study, the dose of the injected cells was not correlated with the response to SCT in the used scales. This was also reported by others[14]. This could be explained by that only a small number of cells is required to perform the desired functions.

Finally, we recommend further similar studies using larger number of patients ,extending the period of follow up in order to assess long term effects whether positive (more clinical improvement) or negative (long term complications).

Conclusion

Autologous stem cell transplantation could be a useful and safe tool for the management of patients with cerebral palsy, resulting in improvement of motor, independence and communication skills which is not correlated with the age of the patient or the dose of the injected cells.

[Disclosure: Authors report no conflict of interest]

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الملخص العربي

يعد مرض الشلل الدماغى عند الأطفال من أهم أسباب الإعاقة لدى الأطفال وينتج فى معظم الأحيان عن تعثر الولادة والذى يؤدى إلى نقص وصول الأكسجين للمخ مما يؤثر على نمو الخلايا و قدرتها عند الطفل. وتعد زراعة الخلايا الجذعية من الوسائل المستحدثة والتى يتم دراسة جدواها فى علاج مثل هؤلاء الأطفال اعتمادا على قدرة الخلايا الجذعية على التحول إلى أنواع أخرى من الخلايا ومنها الخلايا العصبية. وقد أجرى هذا البحث على ٥٢ من الأطفال المصريين المرضى المصابين بمرض الشلل الدماغى. وقد تم تقسيمهم إلى مجموعتين :

* المجموعة الأولى : مجموعة الدراسة وتضم ٢٦ مريضا تم إجراء زراعة للخلايا الجذعية الذاتية لهم.
* المجموعة الثانية : المجموعة الضابطة وتتكون من ٢٦ مريضا لم يتم علاجهم باستخدام الخلايا الجذعية.

وقد أجريت الفحوصات الآتية لجميع المرضى المشاركين بالبحث، وهى كالاتى :

١. أخذ التاريخ المرضى و إجراء الفحص السريري.
٢. تطبيق معايير قياس شدة المرض الآتية :
   - مقياس بويد لتدرج النمو.
   - مقياس المائة نقطة.
   - نظام تقسيم الوظائف الحركية.

ثم تم إجراء زراعة الخلايا الجذعية لمرضى المجموعة الأولى.

وقد أظهر البحث النتائج الآتية :

١- تحسن مرضى مجموعة الدراسة بالمقارنة بالمجموعة الضابطة وذلك باستخدام مقياس بويد لتدرج النمو (تحسن ذو دلالة إحصائية).
٢- تحسن مرضى مجموعة الدراسة بالمقارنة بالمجموعة الضابطة وذلك باستخدام مقياس المائة نقطة (تحسن ذو دلالة إحصائية).
٣- عدم تحسن مرضى مجموعة الدراسة بالمقارنة بالمجموعة الضابطة وذلك باستخدام نظام تقسيم الوظائف الحركية.