

STEM CELL TRANSPLANTATION IN PATIENTS WITH CEREBRAL PALSY

THESIS

**Submitted for partial fulfillment of M.D. Degree in
Neurology**

By

Amr Hassan El Sayed El Hassany

M.B.B.CH.,M.Sc., Cairo University

Supervised by

Prof. Dr. Mahassen Ali Hassan

Professor of Neurology – Cairo University

Prof. Dr. Hala Gabr

Professor of Clinical Pathology – Cairo University

Dr. Shereen Fathi

Ass. Professor of Neurology – Cairo University

Dr. Gehan Ramzy

Ass. Professor of Neurology – Cairo University

Department of Neurology – Faculty of Medicine

Cairo University

2010

”

”

(:)

ACKNOWLEDGEMENT

I would like to express my deepest gratitude and sincere thanks to Prof. Dr. **Mahassen Ali Hassan**, Professor of Neurology, Cairo University, the unique Neurologist, for her continuous guidance, supervision and suggestion of this work. I appreciate her hard support and powerful push and above all acceptance of my annoyance with endless patience, she was really the heart of this work.

I wish to express my deepest thanks and gratitude to Prof. Dr. **Hala Gabr**, Professor of Clinical Pathology, Cairo University, for her kind support, constructive criticism and valuable assistance specially In performing the lab work, without which, this work could not have been accomplished.

I am extremely grateful to Dr. **Shereen Fathi**, Ass. Professor of Neurology, Cairo University, for her great care, continuous guidance, excellent supervision and valuable suggestion, saving no effort or time during the whole work.

I would like to extend my appreciation and deep thanks to Dr. **Gehan Ramzy**, Ass. Professor of Neurology, Cairo University for her kind, calm and extremely helpful attitude.

I wish to express my deepest thanks to Dr. **Nermin Adel**, Ass. Professor of Neurology, Cairo University, for her kind support and valuable assistance.

I would like to extend my appreciation to Dr. **Heba Fathi**, Lecturer of Psychiatry, Cairo University for her kind help in choosing scales used in the study.

I would like to thank Dr. **Mariam Thabet**, a Clinical Pathologist, for her kind help in performing the lab work .

I wish to thank all members of Neurology Department, Cairo University, for their support and encouragement throughout the work.

Finally, I am really grateful to all patients who participated in this work.

Amr Hassan

2010

TO MY BELOVED
FATHER
(GOD BLESS HIM)

TO MY GREAT
MOTHER

TO MY FAMILY

CONTENTS

	Page
List of Abbreviations.....	
List of Tables.....	
List of Figures.....	
Introduction.....	1
Aim of the Work	4
Review of Literature:	5
. Cerebral palsy.....	5
. Biology of stem cell.....	31
. Stem cell transplantation	56
 Subjects and Methods	80
Results.....	94
Discussion.....	121
Summary and Conclusions.....	133
Recommendations.....	137
References.....	139
Appendices.....	161
Arabic Summary.....	

ABSTRACT

Background: Cerebral palsy is an "umbrella term covering a group of non-progressive motor impairment syndromes secondary to lesions or anomalies of the brain arising in the early stages of its development". Stem cells are well known to be capable of differentiating into specialized cells performing different functions in the body. A stem cell transplantation (SCT) technology represents a new feasible approach to the treatment for a number of diseases including cerebral palsy. **Purpose of study:** was to study the impact of SCT on psychomotor functions in patients with cerebral palsy. **Subject and Methods:** This randomized control trial was conducted on 52 Egyptian patients presenting with cerebral palsy, they were divided into 2 groups; Group I: Patients who underwent stem cell transplantation and Group II (control group): Patients who did not undergo stem cell transplantation. All patients (Group I & II) were subjected to the following battery of assessment after parent/s consent; history taking, examination and clinical measures of disabilities that include Gross Motor Function Classification System (GMFCS), Boyd's Progress Developmental Scale and the 100 Points Scale. SCT was done group I through bone marrow aspiration then isolation and culturing of MSCs followed by reinjection into the subarachnoid space via lumbar puncture. All patients were reassessed 1 year after the initial assessment. **Results:** Assessment of the patients in study group (group I) using Boyd's developmental progress scale revealed that mean score for the motor skills was 8.19 ± 8.75 prior to SCT & 9.19 ± 8.99 after SCT, score for the independence skills was 9.23 ± 8.55 prior to SCT & 10.19 ± 8.99 after SCT, mean score for the communication skills was 10.19 ± 8.99 prior to SCT & 11.5 ± 7.39 after SCT, all these differences were statistically significant (P value < 0.05). Assessment of the patients in study group (group I) using 100 points scale revealed that mean score was 47.3 ± 32.68 prior to SCT & 50.57 ± 34.00 after SCT, this difference was statistically significant (P value < 0.05). As regard GMFCS, mean score was 4.23 ± 1.37 prior to SCT & 4.15 ± 1.46 after SCT, this difference was statistically insignificant (P value > 0.05). **Conclusion:** Autologous stem cell transplantation could be a safe & helpful tool in the management of patients with cerebral palsy.

Keywords:

- Autologous stem cell transplantation
- Cerebral palsy.

LIST OF ABBREVIATIONS

AABB	:American Association of Blood Banks
AAN	:American Academy of Neurology
BDNF	:Brain derived neurotrophic factor
BM	:Bone marrow
BW	:Body weight
CD	:Cluster of differentiation
CNS	:Central nervous system
CP	:Cerebral palsy
CSF	:Cerebrospinal fluid
CT	:Computerized tomography
DMEM	:Dulbecco's Modified Eagle Medium
DNA	:Deoxy Nucleic Acid
EDSS	:Expanded disability status scale
EEG	:Electroencephalography
EG	:Embryonic germ
EGF	:Endothelial growth factor
ES	:Embryonic stem cells
FACS	:Fluorescence-activated cell sorting
FDA	:Food and Drug Administration
FFT	:Fast Fourier transformer
FITC	:Fluoresce isothiocyanate
FGF-2	:Fibroblast growth factor-2
G-CSF	:Granulocyte colony stimulating factor
GMFCS	:Gross Motor Function Classification System
HLA	:Human leukocyte antigen
hNSCs	:Human neural stem cells
HSC	:Human stem cells
hUCSC	:Human umbilical cord stem cell
IMDM	:Iscove's modified Dulbecco's medium
LRP	:Lineage restricted precursors
MNC	:Mononuclear cells
MRI	:Magnetic resonance imaging
MS	:Multiple sclerosis
MSCs	:Mesenchymal stem cells
NGF	:Nerve growth factor
NSCs	:Neural stem cells
NSPs:	:Neural stem/progenitor cells
NT3:	:Neurotrophin-3

P	:Probability
PBS	:Phosphate buffer saline
PE	:Phycoerythrin
PET	:Positron emission tomography
PMT	:Photomultiplier tubes
RMS	:Rostral migratory stream
ROM	:Range-of-motion
rpm	:round per minute
SCs	:Stem cells
SCID	:Severe combined immunodeficiency
SCT	:Stem cell transplantation
SD	:Standard deviation
SP	:Side population
SPSS	:Statistical Package Social Science
SVZ	:Subventricular zone
TBI	:Traumatic brain injury
TNF	:Tumor necrosis factor
UCB	:Umbilical cord blood

LIST OF TABLES

Table	Title	Page
1	100 Points Scale	85
2	Gender distribution in study and control groups	94
3	Mean age in study and control groups	95
4	Distribution of different clinical syndromes in study and control groups	96
5	Initial assessment of study and control groups using Boyd's developmental progress scale	99
6	Follow up assessment of study group and control groups using Boyd's developmental progress scale.	101
7	Comparison between improvement of study and control groups in Boyd's developmental progress scale	102
8	Comparison of assessment of patients in study group using Boyd's developmental progress scale pre and post SCT.	103
9	Comparison of initial and follow up assessment of patients in control group using Boyd's developmental progress scale.	105
10	Comparison of percent of change of Boyd's developmental progress scale in study and control groups.	106
11	Results of assessment of study and control groups using 100 points scale.	108
12	Comparison between patients of study and control group regarding functions improved in 100 points scale.	109
13	Comparison between patients of study and control groups regarding improvement in total score of 100 points scale	110
14	Comparison of assessment of patients in study group using 100 points scale pre and post SCT.....	110
15	Comparison of initial and follow up assessment of patients in control group using 100 points scale	111

16	Comparison of percent changes of the 100 points scale in study and control groups.....	112
17	Results of initial and follow up assessment of study and control groups using GMFCS.....	114
18	Comparison between patients of study and control groups regarding improvement in GMFCS	115
19	Comparison of assessment of patients in study group using GMFCS pre and post SCT.....	115
20	Comparison of initial and follow up assessment of patients in control group using GMFCS.....	116
21	Comparison of percent of change of GMFCS in study and control groups.....	117

LIST OF FIGURES

Fig	Title	Page
1	Stem cell self-renewal.....	32
2	Unidirectional stem cell differentiation.....	34
3	The stem cell life cycle.....	36
4	Fate of the stem cell.....	37
5	The origin, isolation and specialization of stem cells.....	39
6	The blastocyst.....	40
7	Hematopoietic and stromal stem cell differentiation.....	44
8	Autologous transplant process.....	57
9	Potential therapeutic uses of stem cell.....	59
10	Basic approaches for bone marrow aspiration.....	86
11	Gender distribution in both groups.....	95
12	Mean age in both groups	96
13	Distribution of different clinical syndromes in study group.....	97
14	Distribution of different clinical syndromes in control group.....	97
15	Immediate complications of SCT in the study group.....	98
16	Initial assessment of study group control groups using Boyd's developmental progress scale.....	100
17	Follow up assessment of study and control groups using Boyd's developmental progress scale.....	101
18	Comparison of assessment of patients in study group using Boyd's developmental progress scale pre and post SCT.....	104
19	Comparison of initial and follow up assessment of patients in control group using Boyd's developmental progress scale.....	105
20	Comparison of percent of change of Boyd's developmental progress scale in study and control groups.....	

Fig	Title	Page
21	Results of initial and control assessment of study and control groups using 100 points scale.....	107
22	Comparison of assessment of patients in study group using 100 points scale pre and post SCT.....	108
23	Comparison of initial and follow up assessment of patients in control group using 100 points scale.....	111
24	Comparison of percent changes of the 100 points scale in study and control groups.....	112
25	Results of assessment of study and control groups using GMFCS.....	113
26	Comparison of assessment of patients in study group using GMFCS pre and post SCT	114
27	Comparison of initial and follow up assessment of patients in control group using GMFCS.....	116
28	Comparison of percent of change of GMFCS in study and control groups.....	117

Introduction

INTRODUCTION

Cerebral palsy is "an umbrella term covering a group of non-progressive, but often changing, motor impairment syndromes secondary to lesions or anomalies of the brain arising in the early stages of development." (*Mutch et al., 1992*).

This condition has devastating consequences for the individual and for society. Multiple factors can cause injury to the developing brain leading to cerebral palsy. Many preconceptional, prenatal and perinatal factors (oxidative damage, perinatal hypoxia/ischemia and maternal infection among others) are known to be associated with brain injury (*Stanley et al., 2000*).

There are no effective means to repair the brain once damage has occurred. Moreover, since many of these insults occur in utero, prevention may prove difficult, and regenerative strategies may be a better alternative to reduce the damage to the brain (*Plane et al., 2004*).

Neural stem/progenitor cells (NSPs) have been recently identified in the mammalian central nervous system, including humans, at all stages of life. Defined as self-renewing, primordial cells with the capacity to give rise to all cell lineages in all regions of the nervous system, Neural stem/progenitor cells (NSPs) are found in the germinal zone in the brain of the embryo and fetus where they participate in central nervous system formation. Cells