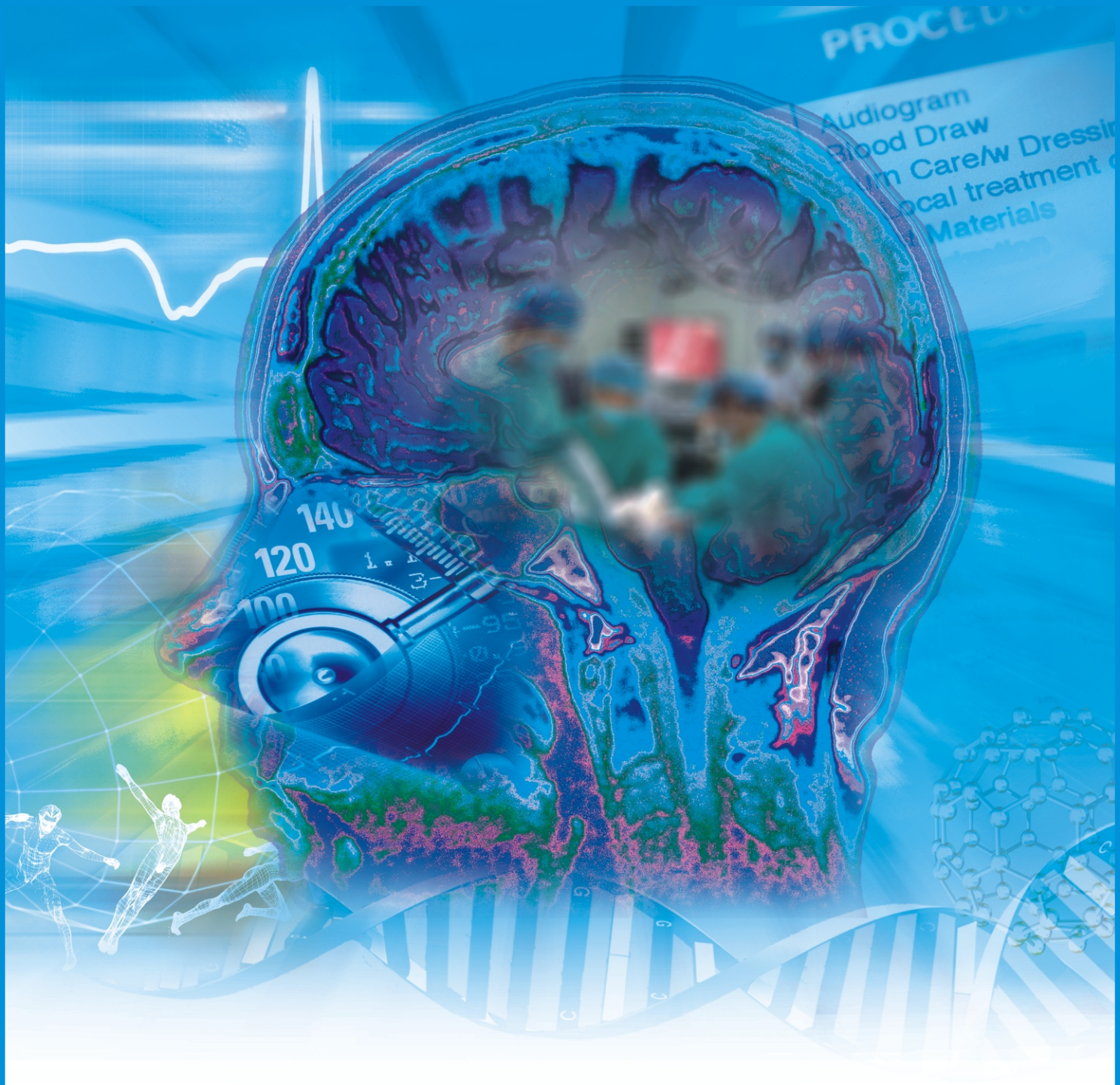

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Nanog overexpression allows Human Mesenchymal Stem Cells to Differentiate into Neural Cells*

——Nanog transdifferentiates Mesenchymal Stem Cells

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ABSTRACT

Although stem cell therapies have been proposed as a candidate for treating neurological diseases, the best stem cell source and their therapeutic efficacy remain uncertain. Embryonic stem cells (ESCs) can efficiently generate multiple cell types, but pose ethical and clinical challenges, while the more accessible adult stem cells have a limited developmental potential. Following included-expression of Nanog, an ESC gene, adult human mesenchymal stem cells (HMSCs) are able to develop into cells exhibiting neural cell-like characteristics based on morphology, cell markers, and gene expressions. After expansion, Nanog overexpressed HMSCs differentiated into cells immunopositive for βIII-tubulin and glial fibrillary acidic protein, lineage markers for neurons and astrocytes, respectively, under the influence of conditional media from differentiated human neural stem cells. This result indicates that the Nanog expression increased the ability of HMSCs to become a neural cell lineage. We further demonstrated that Nanog-overexpressed HMSCs were able to survive, migrate, and undergo neural cell-like differentiation after transplantation in vivo. This data offers an exciting prospect that peripheral adult stem cells can be modified and used to treat neurological diseases.

Keywords*: *Stem Cells*, *Dedifferentiation*, *Transplantation*, *Nanog

1. Introduction

The central nervous system is one of the most limited systems in the human body in terms of regeneration and recovery after cell loss [1]. While many pharmacological treatments mediate symptoms, they fail to cure neurological diseases. Today, many researchers are investigating stem cells as potential therapeutics to overcome this issue.

The use of embryonic stem cells (ESCs) has been proposed as the most promising strategy for treatment of neurological diseases because of their pluripotency to become a variety of tissues, but concerns over ethics [2-4], immune response [5,6] and tumor formation [7-9] have been major barriers for their clinical use. Adult stem cells are known to exist throughout the body, and they can be harvested from a patient and autologously transplanted back to the patient. The autologous approach will eliminate the issues associated with the use of ESCs. However, the ability of adult stem cells to develop along multiple lineages is limited by their tissue origin. Thus to regenerate neural tissue, neural stem cells (NSCs) isolated from brain tissue are needed. However, finding the tissue within a patient from which to isolate them may be difficult. Utilization of other easily accessible adult stem cells, such as mesenchymal stem cells (MSCs) found in the bone marrow, could eliminate the difficulty of acquiring transplantable material. Although studies have claimed that human MSCs (HMSCs) transdifferentiate into cells outside their restricted germ layer, the transdifferentiation could have been from a very limited population of HMSCs [10,11] or due to the low frequencies of cell fusion, which allow HMSCs to acquire characteristics of multiple cell types by fusing to somatic cells [12,13]. Therefore, a strategy to increase the transdifferentiation abilities of adult stem cells is requisite for

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their use in neuroreplacement therapies.

In a previous study, we demonstrated that HMSCs treated with bromodeoxyuridine (BrdU), which is incorporated into the DNA as a thymidine analog, undergo neural differentiation following transplantation in the brains of rats and improve cognitive function [14]. This result indicates that epigenetic modification of the adult stem cells may increase their potency. However, efficiency of transdifferentiation and concerns with the non-specific epigenetic modification, led us to explore other strategies.

One possible strategy is cell fusion that would alter the characteristics of adult stem cells based on the exogenous cell used for merging. This method could change the transdifferentiation ability of cells allowing them to develop into cells beyond their respective lineage [15-17]. The fusion of somatic cells to ESCs prompts expression of the ESC gene Oct-4 [17,18]. Thus, the expression of stem cell genes that regulate self-renewal and pluripotency may play an integral role in reprogramming the cell lineage.

Earlier studies have indicated that the expression of critical stem cell genes is capable of maintaining ESCs in a pluripotent state. The over-expression of ESC genes, including Nanog [19,20], Pem [21] and Rex1 [22], suppressed differentiation of ESCs, while the presence of elevated levels of Oct-4 was insufficient to guard against ESCs differentiation [23]. In this study, we tested our hypothesis that developmental ability of HMSCs can be increased by changing the gene expression profile through the over- expression of Nanog, and the resulting cells can be transdifferentiated into neural cells. This technology may allow us to perform autologous therapies to treat neurological diseases using patients' own HMSCs.

2. Materials and Methods

Cell culture: Adult human bone marrow-derived HMSCs (Cambrex) were cultured in DMEM (Invitrogen) supplemented with 1% antibiotics (Invitrogen) and 10% FBS for improved HMSC growth (StemCell Technologies). Per Cambrex product information, mesenchymal stem cells are harvested and cultured from normal human bone marrow. Cell purity is far higher than cells from traditional Dexter cultures. Cells are tested for purity by flow cytometry and for their ability to differentiate into osteogenic, chondrogenic and adipogenic lineages. Cells are positive for CD105, CD166, CD29, and CD44. Cells test negative for CD14, CD34 and CD45. Media systems are available to support growth of HMSCs, and their differentiation into adipogenic, chondrogenic, and osteogenic lineages. Cells were cultured in T75 tissue culture treated flasks (BD Biosciences) and incubated in a CO_2 chamber at 37℃ with 5% CO_2 (NuAire). Co-culture experiments were carried out using differentiated NSCs in Falcon tissue culture treated 6-well plates (BD Biosciences). Prior to co-culture, fetal-derived human NSCs (Cambrex) were expanded in serum-free NSC medium of DMEM/F12 (Invitrogen) supplemented with B27 (1:50, Invitrogen), basic Fibroblast Growth Factor (bFGF, 20 ng/ml, R&D Systems), Epidermal Growth Factor (EGF, 20 ng/ml, R&D Systems), heparin (0.18 U/ml, Sigma), and 1% antibiotics (Invitrogen). Cells were allowed to spontaneously differentiate for one week in tissue culture treated 6-well plates containing serum-free neural basal medium.

For co-culture, cell culture inserts with a semi-permeable membrane with 0.4 μm pores (BD Biosciences) were used to separate the Nanog-transfected HMSCs from the differentiated HNSCs. This allowed for the dynamic exchange of secreted factors and eliminated direct cell contact to avoid possible cell fusion. Nanog- or mock-transfected HMSCs were then transferred to co-culture to promote neural differentiation. To eliminate Nanog expression in viral-loxP-Nanog-transduced HMSCs prior to the co-culture, plasmids containing the Crere-combinase gene regulated by an EF1α promoter (Addgene, plasmid 11918) were transfected into the cells using the FuGene 6 reagent. Cells were allowed to differentiate for 10 days and then stained for early (βIII-tubulin) and mature (MAP2) neuronal markers and astrocytic markers, GFAP and S100.

Cloning of Nanog gene: Nanog was originally cloned from male genomic DNA that was pre-digested with the restriction enzymes NotI, XbaI, and SpeI, then amplified by PCR using Nanog-specific primers, (CGTTCTGCTGGACTGAGCTGGTT, CGGGCGGATCACAAGGTCAG). PCR conditions consisted of pre incubation at 94℃ for three minutes, 30 cycles consisting of 94℃ for one minute, 52℃ for 30 seconds, and 72℃ for three minutes, and post dwells at 72℃ for 10 minutes. The PCR product was then placed into a mammalian expression vector (TopoHisMax 4.1, Invitrogen) according to manufacturer's protocol. The cloned sequence was confirmed by DNA sequencing.

Production of lentivirus containing Nanog: The gene encoding for Nanog (gift from Austin Smith, MD University of Cambridge) was amplified using the Herculase II fusion DNA polymerase (Promega) and gene-specific primers containing a BamHI enzyme-cutting site in the forward primer and a SalI-cutting site in the reverse primer (ATAGGGATCCACATGAGTGTTGACCCAGCTT, ATAGGTCGACTCACACGTCTTCAGGTTGCA). The PCR amplified Nanog was sub-cloned into the pLox lentiviral vector (gift from Didier Trono, MD and Patrick Salmon, MD, LVPU, Centre MédicalUniversitaire, Genève, Switzerland).

Production of a lentiviral vector containing the Nanog

sequence was carried out using a vector containing a LoxP site. The pLoxNanog vector, the packaging vector pCMVΔR8.91 (AddGene) encoding for regulatory proteins Tat and Rev as well as the Gag and Pol precursors, and a vector for the envelope protein VSV-G (Clontech) were used for viral production. The aforementioned vectors and lentiviral vectors pLoxNanog and pLoxGFP, combined with the packaging and envelope plasmids at a ratio of 2:1:1 (pLox:pCMVΔR8.91:pVSV-G) [24,25], were transiently transfected into the HEK293T/17 cell line (ATCC) using Lipofectamine (Invitrogen) at a DNA (20μg) to Lipofectamine ratio of 1:2.5. The cell culture media was removed at 24 hours and collected every 12 hours thereafter for the next two days to harvest the viral supernatant.

Non-viral and viral gene delivery: For non-viral gene transfection, 75% confluent HMSCs were transfected with 3 μg of Nanog vector using two different reagents, Neuroporter (Gene therapy systems) or FuGene 6 (Roche), at DNA to reagent ratios of 1:15 and 1:3, respectively. Proliferative clusters began to emerge after one week and grew large enough for expansion typically by three weeks. Clustered cells that resembled Nanog-transfected HMSCs were passed by mechanical dissociation from the feeder layer and subsequently plated with a feeder cell layer of HMSCs.

For lentivirus-mediated transfection, viral supernatant was transferred to HMSC cultures for viral transduction. Delivery of Nanog was analyzed through fluorescent microscopy for positive green fluorescent protein (GFP) expression. Differentiation was induced through the deletion of the Nanog-containing proviral sequence with a vector encoding for Crerecombinase (Addgene pBS513) [26]. The Cre vector, which contains an EF1α promoter [26,27], was delivered to the cells through chemical transfection. Following Cre-transfection, cells were used for neural differentiation or gene expression analysis at 72 hours post-transfection. All recombinant DNA research was performed in accordance with NIH guidelines.

Gene expression analysis: RNA extraction was performed using TRIzol (Invitrogen) according to manufacturer's instructions. Media was removed from cultured cells and incubated with 1ml of TRIzol for five minutes at room temperature. Reverse transcription was performed using an iScriptcDNA synthesis kit (Bio-Rad).

Primers used for RT-PCR were GAPDH (AGCCACATCGCTCAGACACC, GTACTCAGCGGCCAGCATCG), β-actin (TCCTGAGCGCAAGTACTCC, AAGCATTTGCGGTGGACGA), Nanog (ACAACTGGCCGAAGAATAGC, AGTGTTCCAGGAGTGGTTGC), Oct-4 (CTTGCTGCAGAAGTGGGTGGAGGAA, CTGCAGTGTGGGTTTCGGGCA), TERF1 (GCAACAGCGCAGAGGCTATTATT, AGGGCTGATTCCAAGGGTGTAA), Sox-2 (ATGCACCGCTACGACGTGA, CTTTTGCACCCCTCCCATTT), ZFP342 (GAAGGCATCACCCAAAAAGA, GCGGTTGAGCTTACTGCTCT), TERT (CGGAAGAGTGTCTGGAGCAA, GGATGAAGCGGAGTCTGGA), and eGFP (CCTGAAGTTCATCTGCACCA, GGTCTTGTAGTTGCCGTCGT).

Real-time two-step RT-PCR was performed using a SYBR green PCR mix (Bio Rad), carried out in a My-IQiCycler (Bio Rad) and then analyzed by the ΔCt method as previously described [28,29].

Stem cell transplantation: Two different transplantation studies were performed with C57/Black mice at four months of age in accordance with approved protocols from the University of Central Florida's Institutional Animal Care and Use Committee.

The animals were fixed in a stereotaxic apparatus and approximately $1x10^5$ cells in 10 μl of phosphate-buffered saline (PBS) were slowly injected into the right lateral ventricle (coordinates: AP -1.4, ML 1.8, DV 3.8). Experiments were carried out independently using HMSCs dedifferentiated through non-viral transfection or lentiviral transduction.

Brain sample preparation: Animals were deeply anesthetized and perfused using a 10% sucrose solution followed by fixation with a 4% paraformaldehyde PBS (pH 7.2). Following fixation, brains were removed and placed inside a 20% sucrose/4% paraformaldehyde solution and left overnight at 4℃. When the brain settled to the bottom of the container, it was froze in isopentane pre-cooled by submerging the beaker into liquid nitrogen. The brains were mounted using a cryomedium, sliced into 20 μm sections using a cryostat at –20℃ and collected in PBS and stored at 4℃ until antibody staining.

Immunocytochemistry and immunohistochemistry: Cultured cells were washed with PBS then fixed with a 4% buffered paraformaldehyde (Sigma) solution overnight at 4℃. Following fixation, cells were washed with PBS (Sigma) then permealized with PBS-Tween (Sigma) containing 0.1% Triton-X (Fisher Scientific) for one hour at room temperature. Brain sections were washed with PBS then permealized by incubation in PBS-Tween with 0.1% Triton-X at room temperature for one hour. The samples were then incubated for one hour at room temperature in a blocking solution of PBS-Tween with 3% donkey serum (Jackson ImmunoResearch). Primary antibodies TRA-1-60 (MAB4360), SSEA-3 (mab4303), Sox-2 (AB5603), MAP2 (AB5622), and Oct-4 (mab4305) all from Chemicon, Nanog (AF1997, R&D Systems), βIII-tubulin (T8660, Sigma), S100 (S2644, Sigma), and GFAP (G9269, Sigma) were added to blocking solution and incubated overnight at 4℃. The next day, samples were washed with PBS and incubated in the dark with

FITC- or TRITC-conjugated secondary antibodies at room temperature. Samples were washed with PBS, cover-sliped with water-based mounting solution containing DAPI (Vector Laboratories), and sealed using clear nail polish.

3. Results

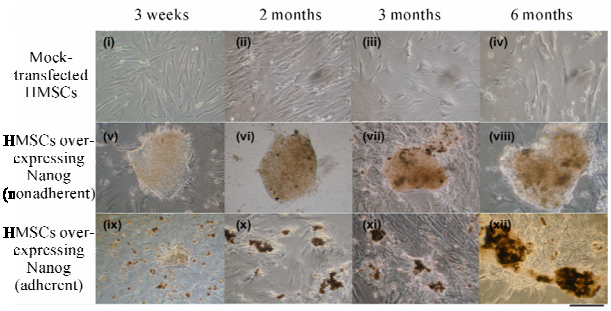
Cloning of Nanog: Sequence analysis of the clone established from genomic DNA showed over 99% sequence identity with Nanog but did not contain introns, suggesting that it may be Nanogpseudogene 8 (NANOGP8) [30], one of twelve Nanog variants [30-32]. The high homology and intact coding region suggests that the cloned sequence should be indistinguishable from Nanog and the translated product virtually identical to the actual Nanog protein, with the exception of substitutions occuring in residues 16 and 253, changing alanine and glutamine for glutamate and histidine, respectively. The cloned gene sequence can be segmented into seven distinct regions: the 5' untranslated region (UTR), N-terminal domain, homeodomain, C1 domain, Cw domain, C2 domain, and the 3' UTR. The 5' region contains binding sites for ESC genes Oct-4 and Sox-2, which are part of a transcriptional regulatory loop [33-35]. The 5' region also contains a p53-binding site within the Nanog promoter region that facilitates ESC differentiation [36] and is possibly responsible for the shift in replication timing observed with neural differentiation [37]. The N-terminal region of Nanog has transcriptional activity [38] and encodes for a sequence containing a SMAD-binding domain [31,39]. The homeodomain portion is similar to the NK-2 and ANTP family of homeodomain transcription factors, but comparing 120 different homeodomain proteins using BLOcksSUbstitution Matrix (BLOSUM) and Point Accepted Mutation (PAM) matrices suggests that Nanog represents a distinct protein family divergent from both the NK-2 and distal-less gene family (data not shown). The C-terminal domain contains no apparent transactivation motifs, but has greater transactivation activity compared to the N-terminal and homeodomain [38,40].

The C-terminal domain can be subdivided into three regions: the portion immediately following the homeodomain region (C1), the subregion containing a unique repeated motif of tryptophan flanked with four polar-uncharged amino acids (Cw), and a more distal sequence (C2). Cloning of Nanog inside the pLoxlentiviral vector was successful and DNA sequencing confirmed a match for the actual Nanog gene. The Nanog sequence was properly inserted into the vector containing a LoxP sequence within the long tandem repeat, allowing for efficient proviral deletion following delivery of Crerecombinase [41,42].

Transfection of Nanog: In the current study, human bone marrow HMSCs were cultured and grown to 75% confluency and then treated with either a plasmid containing Nanog or a mock-transfected control. Following optimization, we achieved transfection rates of less than 5% using non-viral transfection. Nanog transfection altered the morphology of cells, producing smaller, proliferative cells that themselves formed clusters (**Figure 1 (a)**).

Two basic cell types were observed; namely, the proliferative clusters tended to form either an adherent mass of cells, resembling the morphology of an ES cell cluster (**Figure 1(a)**, **v-viii**); or, more spherical, non-adherent/ loosely adherent clumps, somewhat resembling embryoid bodies morphology (**Figure 1(a)**, **ix-xii**). The former cells extensively proliferated for a long period. The latter type cells originated as small, scattered clumps, but formed larger aggregates within weeks. These larger clusters seemed to be mainly the result of clump aggregation rather than cell proliferation. Normal MSCs have a doubling time of approximately 70 hours in our conditions. After Nanog transfection of HMSCs, their doubling time changed to approximately 16 hours.

Transfection with the neuroporter reagent appeared to have more toxicity and was more likely to produce more HMSCs; therefore FuGene 6 was the preferred transfec



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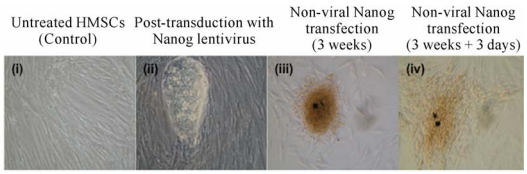


(b)

Figure 1. (a) HMSCs over-expressing Nanog displayed ESC-like (v-viii) or EB-like morphology (ix-xii). Morphological changes seen at three weeks (i,v,ix), two (ii,vi,x), three (iii,vii,xi), and six months (iv,viii,xii) post-transfection. (b) HMSCs nine days post-transduction with Nanog lentivirus (ii). Three weeks following non-viral Nanog transfection (iii) and three days later (iv). Untreated HMSCs showed as a control (i).

tion reagent. Cells that displayed the flattened, ES cell-like morphology were detected as early as one week, but were usually distinct at two to three weeks. The number of colonies produced did not appear to directly correspond to transfection rates. Following one week, one or two colonies could be observed in the wells. No colonies were able to expand without a feeder layer, and only a few colonies were able to expand into larger colonies of thousands of cells for subsequent passaging.

Moreover, the colonies resembling morphology of an ES cell cluster were only found within the Nanog-transfected HMSC cultures. Both of the previously defined cell types either adhered and differentiated or underwent cell death when transferred to separate culturing flasks with no feeder layer (data not shown). The inability of isolated colonies to continually proliferate on their own indicates that the majority of non-transfected HMSCs served as a feeder layer, helping provide growth factors and aid in cell survival. Nanog-transfected HMSC colonies were less homologous and displayed greater propensity for differentiation than has been reported with ESCs (**Figure 1(a)**).

There was little difference in the morphologies between Nanog-transfected HMSCs and ESCs for up to two months in culture, and while they were able to proliferate, they did not appear to grow past 1000 μm. However, by three months, gradual changes became evident as heterogeneity within the structures became more apparent. It is uncertain whether this phenomenon is the result of cells undergoing differentiation, reaching a proliferative limit, or the result of changes in the underlying feeder layer of un-transfected HMSCs. Control HMSCs showed changes in morphology and displayed little or no proliferation at three months in culture.

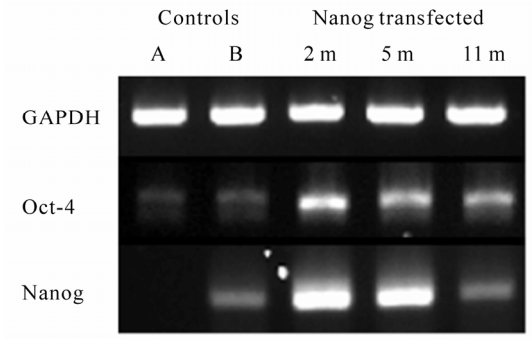
Beyond three months, the number of Nanog-transfected HMSCs diminished, and mock-transfected HMSCs showed age-related alterations. HMSCs could be cultured for longer periods of time through continuous passages, but late-passaged HMSCs displayed changes in morphology, including increased cell size, larger cytoplasm, and no detectable proliferation. Following one year of culturing and expansion, HMSCs failed to survive and few Nanog transfected-HMSC remained.

Cells co-transduced with Nanog lentiviruses showed prominent cluster formation (**Figure 1(b)**, **ii**). Colonies formed by transduction with Nanog were easier to maintain and grew much larger than HMSCs chemically transfected with Nanog. Colonies produced through chemical transfection were difficult to maintain as the colonies tended to disperse (**Figure 1(b)**, **iii**, **iv**).

Gene expression and immnohistochemistry of Nanog-transfected HMSCs: Exploring biochemical changes following Nanog transfection, we performed RT-PCR for Nanog and Oct-4 to compare with mock-transfected HMSCs, as well as immunostaining for known ESC markers. Expression levels of Nanog and Oct-4 were absent or low in two different batches of mock-transfected HMSCs (**Figure 2(a)**).

This illustrates the heterogeneity of HMSCs in culture and is consistent with the data showing a subpopulation of pluripotent HMSCs [10,11,43,44]. Following Nanog transfection, expression of both Nanog and Oct-4 were highly elevated at two, five, and eleven months. It was unexpected that either Nanog or Oct-4 would be expressed following long-term expansion since the few remaining cell clusters did not appear to proliferate at one year. Interestingly, levels of Oct-4 did not directly correlate with expression of Nanog, which is consistent with findings that Oct-4 is not directly controlled by Nanog [33,45,46].

Quantitative gene expression analysis was difficult given the heterogeneous population and relatively low frequency of Nanog-transfected cells. We therefore at-



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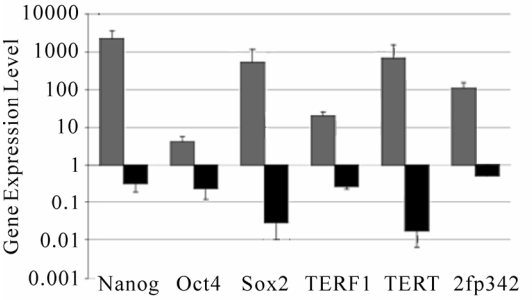

(b)

Figure 2. (a) RT-PCR shows little or no expression of Nanog and Oct-4 in mock-transfected HMSCs but up-regulation of both at two, five, and eleven months following Nanog transfection up to 11 month in a culture. GAPDH was a control. (b) qRT-PCR of Nanog lentiviral-transduced cells. Up-regulation of multiple ESC genes after Nanog transfection (grey) were observed and these genes were down-regulated after (black) delivery of Cre recombinase vectors to remove Nanog expression.

tempted to select out Nanog-dedifferentiated cells using a lentiviral system co-expressing GFP. Using lentivirus to deliver Nanog and GFP, we created identifiable clusters of cells resembling the morphology of ES C clusters that were more homogenous, highly proliferative, and easily expandable. In fact, the cells were able to grow with or without feeder cells for over 40 passages.

Quantitative real-time PCR was performed on lentiviral-transduced cells and showed a dramatic increase in most of the ESC genes tested. We were able to detect low levels of both Nanog and Oct-4 in two of three HMSC batches tested, but telomerase expression was absent. Following forced expression of Nanog, we measured dramatic increases in Nanog, Sox-2, zinc-finger protein 342, TERF1 and telomerase. Given the earlier lack of telomerase expression, we assigned the lowest value for detection in order to perform an analysis that does not allow for "zero" expression. We observed only a modest, yet statistically significant, increase in levels of Oct-4 to four times the normal level (**Figure 2 (b)**). The measured changes, particularly the sudden expression of telomerase and increase in TERF1, demonstrate fundamental changes in the HMSCs following delivery of Nanog. Previous work has revealed that HMSCs fail to express telomerase and have a unique telomerase biology compared to other stem cells [47,48], so the link between Nanog and telomerase is an exciting area that warrants exploration.

Removing Nanog and GFP using a vector encoding for the Cre recombinase enzyme should reverse gene expression changes in the viral-transduced cells. Recombination and gene excision were successful, as most viral-transduced cells were negative for GFP post Cre transfection, allowing for neural differentiation. Additionally, real-time PCR reveals an 89% decrease in GFP expression in viral-transduced cells 72 hours following delivery of Cre (data not shown). We compared changes in gene expression in Cre-transfected, virally-de-differentiated cells and found reduction in most stem cells genes. Nanog, Oct4 and TERF1 showed a 70% to 80% decrease in expression, while Sox-2 and telomerase showed decreases of approximately 98% each (**Figure 2(b)**).

Immunohistochemical staining was performed using antibodies against the ESC markers Nanog, Oct-4, stage-specific embryonic antigen-3 (SSEA3), and keratan sulphate-associated antigen TRA1-60 (**Figure 3**). If Nanog increases the transdifferentiation ability of HMSCs, transfected cells should stain positive for these markers. The vast majority of untreated cells failed to stain for any of the markers, but a small population (approximately 1%) of cells, did show positive staining for transcription factors Oct-4 and Nanog, while faint staining for surface markers was seen in about 5% of cells (**Figure 3**). Alternatively, Nanog-transfected cells did display positive staining for the transcription factors Nanog and Oct-4 within the proliferative cell clusters, although not in the surrounding feeder layer of HMSCs. Nanog-transfected HMSCs clusters also showed positive expression of surface markers SSEA3 and TRA1-60 (**Figure 3**).

This staining pattern was more apparent with the use of the GFP and Nanoglentiviral-transduced cells. Following transduction with Nanog and GFP, large colonies morphologically similar to ES cell colonies began to form. GFP expression appeared localized to these colonies and showed positive staining for Nanog and Sox-2, unlike non-treated HMSCs (**Figure 4**). Taken together, it appears that forced expression of Nanog results in the increased transdifferentiation ability of HMSCs and induces the expression of genes related to pluripotency. Immunostaining against ESC markers in HMSCs has

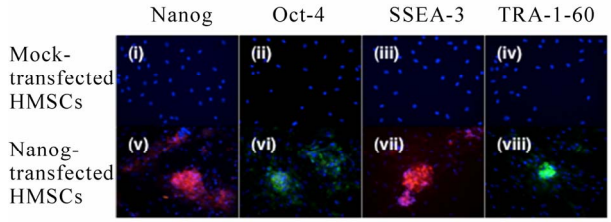

Figure 3. Immunocytochemistry of Nanog transfected HMSC colonies showed strong immunoreaction for ES cell markers after15 weeks in a culture (v-viii) while there is no expression of ES cell markers in mock-transfected HMSCs (i-iv). The cells were immunostained for Nanog (i, v), Oct-4 (ii, vi), SSEA-3 (iii, vii), and TRA-1-60 (iv, vii).

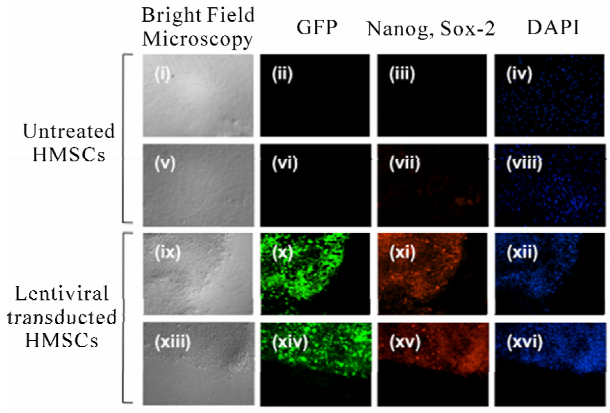

Figure 4. With mock-transfected HMSCs showed no ESC-like colony formation (i,v) nor GFP expressions (ii,vi) and immunoreactivities for Nanog (iii) and Sox-2 (vii) are not detected. Lentiviral transduction with Nanog and GFP induced colony formation (ix, xiii) and GFP expression (x, xiv), and positive for Nanog (xi) and Sox-2 (xv) immunoreactivities. DAPI is used counter stain of nuclei (iv, viii, xii, xvi).

previously been done [49].

We next tested the ability of Nanog-transfected cells to undergo neural differentiation using a previously established co-culture system. Cells were placed in co-culture consisting of a feeder cell layer separated by a semi-permeable membrane to eliminate direct cell contact. The feeder cells used were neurons and glial cells derived from HNSCs, and were grown as neural spheres and cultured in serum-free basal media. This system allows for the exchange of growth factors and eliminates the concern over cell fusion since it prevented direct cell contact between the HMSCs and underlying feeder cells. Cell clusters adhered to the membrane surface and differentiation occurred as cells radiated outwards. Control HMSCs adhered to the membrane surface but failed to differentiate into neurons and astrocytes. The differentiation pattern was tested by immunostaining against βIII-tubulin and GFAP, early neuronal and astrocytic lineage markers, respectively (**Figure 5**, **i-iii**). LentiviralNanog-transfected HMSCs were able to undergo neural differentiation following transfection using a Crerecombinase vector. The un-transfected HMSCs did not show positive staining for the neuronal early lineage marker βIII-tubulin, but approximately two percent of the cells did show weak expression of GFAP (**Figure 5**, **i**). This may represent a subpopulation of HMSCs [10,43] that is capable of differentiating into astrocytes. Modified cells formed spherical clusters with a similar appearance to differentiated NSCs, forming a web-like network of neurons and astrocytes that stained positive for both βIII-tubulin and GFAP (**Figure 5**, **ii**). Since, βIII-tubulin and GFAP are early lineage commitment markers, so we examined the expression of MAP2 and S100 to determine if these mature neuronal and astrocytic markers would be expressed following neural differentiation to our modified cells. Cells were cultured for two weeks and stained for mature neural markers. Cells stained positive for MAP2 and S100, indicating the cells were able to express mature neuronal and astrocytic makers, respectively (**Figure 5**, **iv**). Induction of differentiation in the non-viral transduced cells was achieved by first transfecting the cells with 3μg of Nanog vector using FuGene6 at DNA to reagent ratio of 1:3, then placing the cells in conditioned media for neural differentiation. We found that some cells did show positive staining for both markers, and most cells were negative for GFP (**Figure 5 iii**). Immunostaining shows expression of early neuronal (βIII-tubulin) and astrocytic (GFAP) markers at three days in neural differentiation culture. Additionally, we conducted transdifferentiation studies *in vitro* and *in vivo* with naive HMSCs, however we could not find significant production of GFAP positive cells nor β–III tubulin cells.

Transplantation results: We tested the cell fate and migration of non-viral and lentiviral dedifferentiated HMSCs *in vivo* three weeks post-transplantation. Following sacrifice, the brains were sectioned and examined. None of the animals displayed evidence of tumors. Immunohistochemical staining for human βIII-tubulin and GFAP did reveal evidence of both *in vivo* early neuronal and glial differentiation. Moreover, the presence of transplanted cells, marked by the expression of human neural markers or GFP in the *CA*1 regions of the hippocampus proper and dentate gyrus regions, is encouraging given the role of these structures in learning and memory (**Figure 6**).

4. Discussion

This study demonstrates a novel method to increase the transdifferentiation ability of adult stem cells by overexpressing genes regulating pluripotency, with the end goal of facilitating neural transdifferentiation of HMSCs, which may allow us to perform autologous cell therapies for individuals with neurodegenerative diseases and other neurological disorders. Nanog transfection of HMSCs produced proliferative cells with morphological and gene expressions resembling ES cells, though their pluripotency has not been confirmed.

We previously demonstrated that treatment with the nucleotide derivative BrdU allows for transdifferentiation [14], and other groups have demonstrated that fusion of stem cells and somatic cells can alter cell properties [13,17,50]. In the current study, we show that transdiffe-

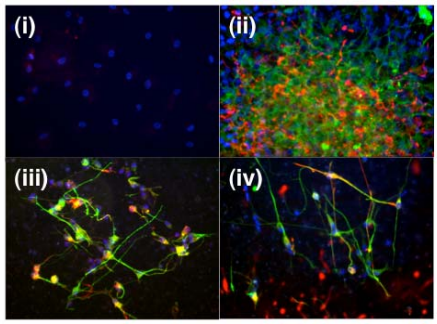

Figure 5. After co-culture with differentiated human neural stem cells, naive HMSCs showed few GFAP (red) but no βIII-tubulin (green) immunoreactivites (i). While Nanog-transfected HMSCs forming clusters of cells co-cultured with differentiated human neural stem cells attached to the culture insert membrane and migrated outward. They were positive for GFAP (red) and βIII-tubulin (green) immunoreactivites indicating neural differentiation of the cells, (low magnification in ii, high magnification in iii). Differentiated Nanog-transfected HMSCs are also stained positive for MAP2 (green) and S100 (red) at two weeks, indicating differentiation into mature neurons and astrocytes, respectively.

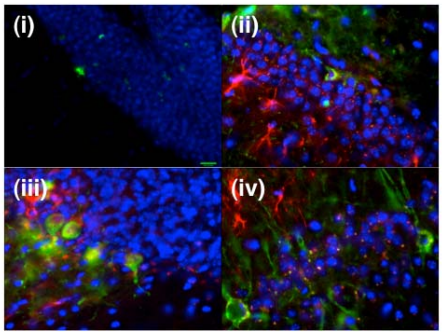

Figure 6. After transplantation into mice, Nanog-GFP transfected HMSCs HMSCs are capable of migration into hippocampus dentate gyrus (i). Immunostaining specific to human βIII-tubulin (green) and GFAP (red) indicated that the transplanted Nanog-transfected HMSCs after Cre recombinase treatment differentiated into neurons and astrocytes in the dentate gyrus (ii, iii) and CA1 regions (iv) of the hippocampus, respectively.

rentiation ability of HMSCs can be increased by gene delivery of only Nanog, although other factors already present in the cells may contribute to forming a cluster of cells whose morphology resembles that of an ESC cluster. Once the environment inside the cells have been changed by Nanog over-expression, ESC genes including Nanog, Sox2, and Oct4 is increased and maintained for longer periods due to each of the genes ability to influence each other. Recently, delivery of four factors induced pluripotency in somatic adult, fibroblasts. Their use of transcription factors Oct-4, Sox-2, and KLF4 along with the oncogenec Myc was sufficient to induce pluripotent transformation [51-54].These results have been independently achieved by different labs using human cells with the same set of genes [55-57] or with a combination of Nanog, Oct-4, Sox-2, and Lin28 [58].

We found levels of Oct-4, Sox-2, and other genes related to pluripotency and self-renewal were significantly increased after Nanog over-expression. Previous reports failed to show production of cells expressing these genes using any single ESC gene when delivered to stem cells or fibroblasts [53,59], nor by combining Oct-4, Sox-2, Klf4 and cMyc in adult HMSCs [56]. The use of additional vectors encoding for the simian virus large T antigen (SV40T) and the catalytic subunit for telomerase (hTERT) produced a few colonies resembling ES cell clusters but still showed cellular loss with expansion [56]. Such discrepancy between the studies analyzing ESC genes in HMSCs could be because those cells expressing ESC genes are a very minor sub population in HMSCs and could be altered by culture conditions.

We observed that the number of colonies resembling the morphology of ES cell cluster that formed after Nanog transfection did not directly correspond to the number of cells receiving the gene. This may be likely the result of a number of critical factors. First, MSCs have a limited capacity for expansion and vary in their ability to proliferate and differentiate, qualities that decrease with age and vary among sources. They are sensitive to culturing conditions, particularly plating density, supplements and serum quality [60-63]. Thus, heterogeneity in the culture may be responsible for this existence of responder and non-responder in the culture. Gene delivery is also challenging in these cell types given their difficulty to transfect, death from toxicity [64-67], their propensity to undergo senescence after several passages [47,68,69], or toxicity associated with viral transductions [67,70]. Additionally, original heterogeneity within HMSCs and variation between cultures may account for t lesser efficiency. Previous studies are inconsistent regarding the expression of pluripotency transcription factors Nanog, Oct-4, and Sox-2 in adult stem cells. Oct-4 is present at low levels in HMSC *in vitro* cultures or can be induced in a subtype of cells using various culture conditions [10,60,71,72]. However, low levels of Sox2 and Nanog are detected in some, but not all HMSCs [44,60,73-75]. This inconsistency extends to telomerase activity and the ability to immortalize HMSCs.

Similar to genes associated with pluripotency, telomerase activity has been detected by some groups [76,77], but not by others [47,48,78,79]. Conflicting results are also observed when groups attempt to immortalize HMSCs through viral delivery of telomerase. Overexpression of telomerase appears to overcome early senescence and generate immortalized cell lines [32,80-83] while other groups report hTERT alone is insufficient [78,79]. Alternatively, only a subpopulation of HMSCs may increase transdifferentiation ability by Nanog. Presumably cells that endogenously express other necessary stem cell genes would be responsive to Nanog over-expression. This hypothesis is supported by the ability to convert NSCs, which already express many stem cell factors including Sox2 and cMyc, to pluripotent cells through forced expression of two factors, Oct-4 and Klf-4 [84].

In addition to the presence of critical stem cell genes, the level of expression is likely to be imperative in determining cell conversion. Since the combination of Oct-4 and Sox-2, which may up-regulates Nanog, is reported to fail increasing transdifferentiation ability of the adult cells [34,35], high levels of Nanog may be the critical factor. Other research found that selection of the cells expressing high levels of Nanog after transfection with a combination of Oct-4 and Sox-2 has yielded ESC-like colonies [52].

Cells receiving a non-viral transfection of Nanog tended to lose proliferative capability while lentiviral-transfected Nanog cells can be maintained over forty passages. HMSCs show extremely low rates of stable transfection using non-viral transfection [65]. Thus, differences between cells receiving Nanog through chemical transfection compared to those receiving it through viral delivery might explain the lack of stable expression of Nanog in the non-viral transfected cells. However, stable Nanog expression may not only increase their rate of proliferation but also prevent them from differentiating into functional cells.

The presented work demonstrates that forced expression of Nanog in HMSCs interacts with endogenous factors to induce neural transdifferentiation ability in cells committed to a mesoderm lineage and increase the developmental potential of the cells. We repeated the experiments with both Nanog pseudogene 8 and Nanog clone from Dr. Austin Smith and we found the same results. The ability to generate human neural cells from adult bone marrow derived stem cells may allow us to perform autologous regeneration therapies for neurological diseases using the patient's own cells. This will eliminate the issues associated with use of ESCs or adult NSCs. Although we observed both early glial and neuronal differentiation in aged wild-type mice, functionality of the cells needs to be confirmed and we have to consider effects of pathological condition on the cells in each disease [85,86]. This technology may also open a door for the possible production of disease-specific functional cells from patients that can be used to create disease models for research of the disease mechanisms and the development of personalized drugs. However, further detail studies are needed to prove pluripotency by formation of teratoma and/or chimera of the Nanog-transfected HMSC-forming colonies resembling the morphology of ES cell clusters and to show functionality of neural cells derived from the Nanog-transfected HMSCs by head-to-head comparison with the primary neural cells and neural cells derived form NSCs and/or ESCs.

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6. Author Disclosure Statement

No competing financial interests exist.

REFERENCES

[1] D. C. Hess and C. V. Borlongan, "Stem Cells and Neurological Diseases," *Cell Proliferation*, Vol. 41, Suppl. 1, 2008, pp. 94-114.

[2] E. Juengst and M. Fossel, "The Ethics of Embryonic Stem Cells-Now and Forever, Cells Without End," *The Journal Of the American Medical Association*, Vol. 284, No. 24, 2000, pp. 3180-3184.

[3] A. McLaren, "Important Differences between Sources of Embryonic Stem Cells," *Nature*, Vol. 408, 2000, pp. 513.

[4] A. McLaren, "Ethical and Social Considerations of Stem Cell Research," *Nature*, Vol. 414, No. 8859, 2001, pp. 129-131.

[5] R. A. Barker and H. Widner, "Immune Problems in Central Nervous System Cell Therapy," *NeuroRx*, Vol. 1, No.4, 2004, pp. 472-481.

[6] J. A. Bradley, E. M. Bolton and R. A. Pedersen, "Stem cell Medicine Encounters The Immune System," *Nature Reviews Immunology*, Vol. 2, No. 11, 2002, pp. 859-871.

[7] S. Arnhold, H. Klein, I. Semkova, K. Addicks and U. Schraermeyer, "Neurally Selected Embryonic Stem Cells Induce Tumor Formation after Long-Term Survival Following Engraftment into the Subretinal Space," *Investigative Ophthalmology & Visual Science*, Vol. 45, No. 12, 2004, pp. 4251-4255.

[8] E. Bieberich, J. Silva, G. Wang, K. Krishnamurthy and B. G. Condie, "Selective Apoptosis Of Pluripotent Mouse And Human Stem Cells By Novel Ceramide Analogues Prevents Teratoma Formation and Enriches For Neural Precursors in Es Cell-Derived Neural Transplants," *The Journal of Cell Biology*, Vol. 167, No. 4, 2004, pp. 723-734.

[9] D. Rubio, J. Garcia-Castro, M. C. Martin, R. de la Fuente, J. C. Cigudosa, A. C. Lloyd and A. Bernad, "Spontaneous Human Adult Stem Cell Transformation," *Cancer Research*, Vol. 65, No. 8, 2005, pp. 3035-3039.

[10] Y. Jiang, B. N. Jahagirdar, R. L. Reinhardt, R. E. Schwartz, C. D. Keene, X. R. Ortiz-Gonzalez, M. Reyes, T. Lenvik, T. Lund, M. Blackstad, J. Du, S. Aldrich, A. Lisberg, W. C. Low, D. A. Largaespada and C. M. Verfaillie, "Pluripotency of Mesenchymal Stem Cells Derived from Adult Marrow," *Nature*, Vol. 418, No. 6893, 2000, pp. 41-49.

[11] Y. Jiang, B. Vaessen, T. Lenvik, M. Blackstad, M. Reyes and C. M. Verfaillie, "Multipotent Progenitor Cells Can be Isolated From Postnatal Murine Bone Marrow, Muscle, and Brain," *Experimental Hematology*, Vol. 30, No. 8, 2002, pp. 896-904.

[12] M. Alvarez-Dolado, R. Pardal, J. M. Garcia-Verdugo, J. R. Fike, H. O. Lee, K. Pfeffer, C. Lois, S. J. Morrison and A. Alvarez-Buylla, "Fusion of Bone-Marrow-Derived Cells with Purkinje Neurons, Cardiomyocytes and Hepatocytes," *Nature*, Vol. 425, 2003, pp. 968-973.

[13] N. Terada, T. Hamazaki, M. Oka, M. Hoki, D. M. Mastalerz, Y. Nakano, E. M. Meyer, L. Morel, B. E. Petersen and E. W. Scott, "Bone Marrow Cells Adopt the

Phenotype of Other Cells by Spontaneous Cell Fusion," *Nature*, Vol. 416, No. 6880, 2002, pp. 542-545.

[14] T. Y. Qu, X. J. Dong, I. Sugaya, A. Vaghani, J. Pulido and K. Sugaya, "Bromodeoxyuridine Increases Multipotency of Human Bone Marrow-Derived Stem Cells," *Restor Neurol Neurosci*, Vol. 22, No. 6, 2004, pp. 459-468.

[15] J. T. Do and H. R. Scholer, "Cell-Cell Fusion as a Means to Establish Pluripotency," *Ernst Schering Research Foundation workshop*, Vol. 60, 2006, pp. 35-45.

[16] M. Tada and T. Tada, "Nuclear Reprogramming Of Somatic Nucleus Hybridized with Embryonic Stem Cells by Electrofusion," *Methods in Molecular Biology*, Vol. 329, 2006, pp. 411-420.

[17] M. Tada, Y. Takahama, K. Abe, N. Nakatsuji and T. Tada, "Nuclear Reprogramming of Somatic Cells by in Vitro Hybridization With Es Cells," *Current Biology*, Vol. 11, No. 19, 2001, pp. 1553-1558.

[18] J. T. Do and H. R. Scholer, "Comparison of neurosphere Cells with Cumulus Cells after Fusion with Embryonic Stem Cells: Reprogramming Potential," *Reproduction, Fertility and Development*, Vol. 17, No. 1-2, 2005, pp. 143-149.

[19] I. Chambers, D. Colby, M. Robertson, J. Nichols, S. Lee, S. Tweedie and A. Smith, "Functional Expression Cloning of Nanog, a Pluripotency Sustaining Factor in Embryonic Stem Cells," *Cell*, Vol. 113, No. 5, 2003, pp. 643-655.

[20] K. Mitsui, Y. Tokuzawa, H. Itoh, K. Segawa, M. Murakami, K. Takahashi, M. Maruyama, M. Maeda and S. Yamanaka, "The Homeoprotein Nanog is Required for Maintenance of Pluripotency in Mouse Epiblast and Es Cells," *Cell*, Vol. 113, No. 5, 2003, pp. 631-642.

[21] Y. Fan, M. F. Melhem and J. R. Chaillet, "Forced Expression of the Homeobox-Containing Gene Pem Blocks Differentiation of Embryonic Stem Cells," *Developmental Biology*, Vol. 210, No.2, 1999, pp. 481-496.

[22] R. Eiges, M. Schuldiner, M. Drukker, O. Yanuka, J. Itskovitz-Eldor and N. Benvenisty, "Establishment of Human Embryonic Stem Cell-Transfected Clones Carrying a Marker for Undifferentiated Cells," *Current Biology*, Vol. 11, 2001, pp. 514-518.

[23] H. Niwa, J. Miyazaki and A. G. Smith, "Quantitative Expression of Oct-3/4 Defines Differentiation, Dedifferentiation or Self-Renewal of Es Cells," *Nature Genetics*, Vol. 24, No. 4, 2000, pp. 372-376.

[24] N. al Yacoub, M. Romanowska, N. Haritonova and J. Foerster, "Optimized Production and Concentration of Lentiviral Vectors Containing Large Inserts," *The Journal of Gene Medicine*, Vol. 9, 2007, pp. 579-584.

[25] B. Mitta, M. Rimann and M. Fussenegger, "Detailed Design and Comparative Analysis of Protocols for Optimized Production of High-Performance Hiv-1-Derived Lentiviral Particles," *Metabolic Engineering*, Vol. 7, No. 5-6, 2005, pp. 426-436.

[26] Y. Le, S. Gagneten, D. Tombaccini, B. Bethke and B. Sauer, "Nuclear Targeting Determinants of the Phage P1 Cre DNA Recombinase," *Nucleic Acids Research*, Vol. 27, No. 24, 1999, pp. 4703-4709.

[27] Y. Le, J. L. Miller and B. Sauer, "Gfpcre Fusion Vectors with Enhanced Expression," *Analytical Biochemistry*, Vol. 270, 1999, pp. 334-336.

[28] K. J. Livak and T. D. Schmittgen, "Analysis of Relative Gene Expression Data Using Real-Time Quantitative Pcr and the 2(-delta delta c (t)) Method," *Methods*, Vol. 25, 2001, pp. 402-408.

[29] J. S. Yuan, A. Reed, F. Chen and C. N. Stewart, Jr., "Statistical Analysis of Real-Time Pcr Data," *BMC Bioinformatics*, Vol. 7, No. 85, 2006, pp. 1471-2105.

[30] H. A. Booth and P. W. Holland, "Eleven Daughters of Nanog," *Genomics*, Vol. 84, No. 2, 2004, pp. 229-238.

[31] A. H. Hart, L. Hartley, M. Ibrahim and L. Robb, "Identification, Cloning and Expression Analysis of the Pluripotency Promoting Nanog Genes in Mouse and Human," *Developmental dynamics*, Vol. 230, 2004, pp. 187-198.

[32] D. Pain, G. W. Chirn, C. Strassel and D. M. Kemp, "Multiple Retropseudogenes from Pluripotent Cell-Specific Gene Expression Indicates a Potential Signature for Novel Gene Identification," *Journal of Biological Chemistry*, Vol. 280, No. 2, 2005, pp. 6265-6268.

[33] J. L. Chew, Y. H. Loh, W. Zhang, X. Chen, W. L. Tam, L. S. Yeap, P. Li, Y. S. Ang, B. Lim, P. Robson and H. H. Ng, "Reciprocal Transcriptional Regulation of Pou5f1 and Sox2 Via the Oct4/Sox2 Complex in Embryonic Stem Cells," *Molecular and Cellular Biology*, Vol. 25, No. 14, 2005, pp. 6031-6046.

[34] T. Kuroda, M. Tada, H. Kubota, H. Kimura, S. Y. Hatano, H. Suemori, N. Nakatsuji and T. Tada, "Octamer and sox Elements are Required for Transcriptional Cis Regulation of Nanog Gene Expression," *Moecular and Celluar Biology,* Vol. 25, No. 6, 2005, pp. 2475-2485.

[35] D. J. Rodda, J. L. Chew, L. H. Lim, Y. H. Loh, B. Wang, H. H. Ng and P. Robson, "Transcriptional Regulation of Nanog by Oct4 and Sox2,"*Journal of Biological Chemistry,* Vol. 280, No. 26, 2005, pp. 24731-24737.

[36] P. Deb-Rinker, D. Ly, A. Jezierski, M. Sikorska and P. R. Walker, "Sequential DNA Methylation of the Nanog and Oct-4 Upstream Regions in Human Nt2 Cells during Neuronal Differentiation," *Journal of Biological Chemistry*, Vol. 280, No. 8, 2005, pp. 6257-6260.

[37] P. Perry, S. Sauer, N. Billon, W. D. Richardson, M. Spivakov, G. Warnes, F. J. Livesey, M. Merkenschlager, A. G. Fisher and V. Azuara, "A Dynamic Switch in the Replication Timing of Key Regulator Genes in Embryonic Stem Cells Upon Neural Induction," *Cell Cycle*, Vol. 3, No. 12, 2004, pp. 1645-1650.

[38] G. J. Pan, D. Q. Pei, "Identification of Two Distinct Transactivation Domains in the Pluripotency Sustaining Factor Nanog," *Cell Research*, Vol. 13, No.6, 2003, pp. 499-502.

[39] A. Suzuki, A. Raya, Y. Kawakami, M. Morita, T. Matsui, K. Nakashima, F. H. Gage, C. Rodriguez-Esteban and J. C. Izpisua Belmonte, "Nanog Binds to Smad1 and Blocks Bone Morphogenetic Protein-Induced Differentiation of Embryonic Stem Cells," *Proceedings of the National*

Academy of Sciences, Vol. 103, No. 27, 2006, pp. 10294 -10299.

[40] G. Pan and D. Pei, "The Stem Cell Pluripotency Factor Nanog Activates Transcription with Two Unusually Potent Subdomains at Its C Terminus," *Journal of Biological Chemistry*, Vol. 280, No. 2, 2005, pp. 1401-1407.

[41] M. Eberhardt, P. Salmon, M. A. von Mach, J. G. Hengstler, M. Brulport, P. Linscheid, D. Seboek, J. Oberholzer, A. Barbero, I. Martin, B. Muller, D. Trono and H. Zulewski, "Multipotential Nestin and Isl-1 Positive Mesenchymal Stem Cells Isolated from Human Pancreatic Islets," *Biochemical Biophysical Research Communications*, Vol. 345, No. 3, 2006, pp. 1167-1176.

[42] P. Salmon, J. Oberholzer, T. Occhiodoro, P. Morel, J. Lou and D. Trono, "Reversible Immortalization of Human Primary Cells by Lentivector-Mediated Transfer of Specific Genes," *Molecular Theraby*, Vol. 2, No. 4, 2000, pp. 404- 414.

[43] M. Reyes and C. M. Verfaillie, "Characterization of Multipotent Adult Progenitor Cells, A Subpopulation of Mesenchymal Stem Cells," *Annals of the New York Academy of Sciences*, Vol. 938, 2001, pp. 231-233, discussion 233-235.

[44] F. Ulloa-Montoya, B. L. Kidder, K. A. Pauwelyn, L. G. Chase, A. Luttun, A. Crabbe, M. Geraerts, A. A. Sharov, Y. Piao, M. S. Ko, W. S. Hu and C. M. Verfaillie, "Comparative Transcriptome Analysis of Embryonic and Adult Stem Cells With Extended and Limited Differentiation Capacity," *Genome Biology*, Vol. 8, No. 8, 2007, pp. R163.

[45] Y. H. Loh, Q. Wu, J. L. Chew, V. B. Vega, W. Zhang, X. Chen, G. Bourque, J. George, B. Leong, J. Liu, K. Y. Wong, K. W. Sung, C. W. Lee, X. D. Zhao, K. P. Chiu, L. Lipovich, V. A. Kuznetsov, P. Robson, L. W. Stanton, C. L. Wei, Y. Ruan, B. Lim and H. H. Ng, "The Oct4 and Nanog Transcription Network Regulates Pluripotency in Mouse Embryonic Stem Cells," *Nature Genetics*, Vol. 38, No. 4, 2006, pp. 431-440.

[46] G. Pan, J. Li, Y. Zhou, H. Zheng and D. Pei, "A Negative Feedback Loop of Transcription Factors That Controls Stem Cell Pluripotency and Self-Renewal," *The Federation of American Societies for Experimental Biology*, Vol. 20, No. 10, 2006, pp. 1730-1732.

[47] M. E. Bernardo, N. Zaffaroni, F. Novara, A. M. Cometa, M. A. Avanzini, A. Moretta, D. Montagna, R. Maccario, R. Villa, M. G. Daidone, O. Zuffardi and F. Locatelli, "Human Bone Marrow Derived Mesenchymal Stem Cells Do Not Undergo Transformation After Long-Term in Vitro Culture and Do Not Exhibit Telomere Maintenance Mechanisms," *Cancer Research*, Vol. 67, No. 19, 2007, pp. 9142-9149.

[48] S. Zimmermann, M. Voss, S. Kaiser, U. Kapp, C. F. Waller and U. M. Martens, "Lack of Telomerase Activity in Human Mesenchymal Stem Cells," *Leukemia*, Vol. 17, No. 6, 2003, pp. 1146-1149.

[49] U. Riekstina, I. Cakstina, V. Parfejevs, M. Hoogduijn, G. Jankovskis, I. Muiznieks, R. Muceniece and J. Ancans, "Embryonic Stem Cell Marker Expression Pattern in Human Mesenchymal Stem Cells Derived From Bone Marrow, Adipose Tissue, Heart and Dermis," *Stem Cell Review*, Vol.5, No. 4, 2009, pp. 378-386.

[50] L. M. Eisenberg and C. A. Eisenberg, "Stem Cell Plasticity, Cell Fusion, and Transdifferentiation," *Birth Defects Research C Embryo Today*, Vol. 69, No. 3, 2003, pp. 209-218.

[51] A. Meissner, M. Wernig and R. Jaenisch, "Direct Reprogramming of Genetically Unmodified Fibroblasts into Pluripotent Stem Cells," *Nature Biotechnology*, Vol. 25, No. 10, 2007, pp. 1177-1181.

[52] K. Okita, T. Ichisaka and S. Yamanaka, "Generation of Germline-Competent Induced Pluripotent Stem Cells," *Nature*, Vol. 448, No. 7151, 2007, pp. 313-317.

[53] K. Takahashi and S. Yamanaka, "Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors," *Cell*, Vol. 126, No. 4, 2006, pp. 663-676.

[54] S. Yamanaka, "Induction of Pluripotent Stem Cells from Mouse Fibroblasts by Four Transcription Factors," *Cell Proliferation*, Vol. 41, Suppl. 1, 2008, pp. 51-56.

[55] W. E. Lowry, L. Richter, R. Yachechko, A. D. Pyle, J. Tchieu, R. Sridharan, A. T. Clark and K. Plath, "Generation of Human Induced Pluripotent Stem Cells from Dermal Fibroblasts," *Proceedings of the National Academy of Sciences*, Vol.105, No. 8, 2008, pp. 2883-2888.

[56] I. H. Park, R. Zhao, J. A. West, A. Yabuuchi, H. Huo, T. A. Ince, P. H. Lerou, M. W. Lensch and G. Q. Daley, "Reprogramming of Human Somatic Cells to Pluripotency with Defined Factors," *Nature*, Vol. 451, No. 7175, 2008, pp. 141-146.

[57] K. Takahashi, K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda and S. Yamanaka, "Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors," *Cell*, Vol. 131, No. 5, 2007, pp. 861-872.

[58] J. Yu, M. A. Vodyanik, K. Smuga-Otto, J. Antosiewicz-Bourget, J. L. Frane, S. Tian, J. Nie, G. A. Jonsdottir, V. Ruotti, R. Stewart, I. I. Slukvin and J. A. Thomson, "Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells," *Science*, Vol. 318, No. 5858, 2007, pp. 1917-1920.

[59] M. J. Go, C. Takenaka and H. Ohgushi, "Forced Expression of Sox2 or Nanog in Human Bone Marrow Derived Mesenchymal Stem Cells Maintains Their Expansion and Differentiation Capabilities," *Experimental Cell Research*, Vol. 314, No.5, 2008, pp. 1147-1154.

[60] V. L. Battula, P. M. Bareiss, S. Treml, S. Conrad, I. Albert, S. Hojak, H. Abele, B. Schewe, L. Just, T. Skutella and H. J. Buhring, "Human placenta and Bone Marrow Derived Msc Cultured in Serum-Free, B-Fgf-Containing Medium Express Cell Surface Frizzled-9 and Ssea-4 and Give Rise to Multilineage Differentiation," *Differentiation*, Vol. 75, No. 4, 2007, pp. 279-291.

[61] I. Sekiya, B. L. Larson, J. R. Smith, R. Pochampally, J. G. Cui and D. J. Prockop, "Expansion of Human Adult Stem Cells from Bone Marrow Stroma: Conditions That Maximize the Yields of Early Progenitors and Evaluate Their Quality," *Stem Cells*, Vol.20, No. 6, 2002, pp. 530-541.

[62] P. A. Sotiropoulou, S. A. Perez, M. Salagianni, C. N. Baxevanis and M. Papamichail, "Characterization of the Optimal Culture Conditions for Clinical Scale Production of Human Mesenchymal Stem Cells," *Stem Cells*, Vol. 24, No. 2, 2006, pp. 462-471.

[63] M. Yokoyama, H. Miwa, S. Maeda, S. Wakitani and M. Takagi, "Influence of Fetal Calf Serum on Differentiation of Mesenchymal Stem Cells to Chondrocytes During Expansion," *Journal of Bioscience and Bioengineering*, Vol. 106, No.1, 2008, pp. 46-50.

[64] D. Baksh, R. Yao and R. S. Tuan, "Comparison of Proliferative and Multilineage Differentiation Potential of Human Mesenchymal Stem Cells Derived from Umbilical Cord and Bone Marrow," *Stem Cells*, Vol. 25, No. 6, 2007, pp. 1384-1392.

[65] H. Haleem-Smith, A. Derfoul, C. Okafor, R. Tuli, D. Olsen, D. J. Hall and R. S. Tuan, "Optimization of High-Efficiency transfection of Adult Human Mesenchymal Stem Cells in Vitro," *Molecular Biotechnology*, Vol. 30, No. 1, 2005, pp. 9-20.

[66] A. Hamm, N. Krott, I. Breibach, R. Blindt and A. K. Bosserhoff, "Efficient Transfection Method for Primary Cells," *Tissue Engineering*, Vol. 8, No. 2, 2002, pp. 235-245.

[67] J. M. McMahon, S. Conroy, M. Lyons, U. Greiser, C. O'Shea, P. Strappe, L. Howard, M. Murphy, F. Barry and T. O'Brien, "Gene Transfer into Rat Mesenchymal Stem Cells: A comparative study of viral and nonviral vectors," *Stem Cells and Devlopment*, Vol. 15, No. 1, 2006, pp. 87-96.

[68] M. M. Bonab, K. Alimoghaddam, F. Talebian, S. H. Ghaffari, A. Ghavamzadeh and B. Nikbin, "Aging of Mesenchymal Stem Cell in Vitro," *BMC Cell Bioloty*, Vol. 7, 2006, pp.7-14.

[69] F. Moussavi-Harami, Y. Duwayri, J. A. Martin and J. A. Buckwalter, "Oxygen Effects on Senescence in Chondrocytes and Mesenchymal Stem Cells: Consequences for Tissue Engineering," *Iowa Orthopaedic Journal*, Vol. 24, 2004, pp. 15-20.

[70] X. Y. Zhang, V. F. La Russa and J. Reiser, "Transduction of Bone-Marrow-Derived Mesenchymal Stem Cells by Using Lentivirus Vectors Pseudotyped with Modified Rd114 Envelope Glycoproteins," *Journal Virology*, Vol. 78, No. 3, 2004, pp. 1219-1229.

[71] S. J. Greco, K. Liu and P. Rameshwar, "Functional Similarities among Genes Regulated by Oct4 in Human Mesenchymal and Embryonic Stem Cells," *Stem Cells*, Vol. 25, No. 12, 2007, pp. 3143-3154.

[72] S. Wislet-Gendebien, G. Hans, P. Leprince, J. M. Rigo, G. Moonen and B. Rogister, "Plasticity of Cultured Mesenchymal Stem Cells: Switch from Nestin-Positive to Excitable Neuron-Like Phenotype," *Stem Cells*, Vol. 23, No. 3, 2005, pp. 392-402.

[73] R. Gonzalez, C. B. Maki, J. Pacchiarotti, S. Csontos, J. K. Pham, N. Slepko, A. Patel and F. Silva, "Pluripotent Marker Expression and Differentiation of Human Second Trimester Mesenchymal Stem Cells," *Biochemical Biophysical Research Communication*, Vol. 362, No. 2, 2007, pp. 491-497.

[74] R. Izadpanah, T. Joswig, F. Tsien, J. Dufour, J. C. Kirijan and B. A. Bunnell, "Characterization of Multipotent Mesenchymal Stem Cells from the Bone Marrow of Rhesus Macaques," *Stem Cells Development*, Vol. 14, No. 4, 2005, pp. 440-451.

[75] M. S. Tsai, S. M. Hwang, Y. L. Tsai, F. C. Cheng, J. L. Lee and Y. J. Chang, "Clonal Amniotic Fluid-Derived Stem Cells Express Characteristics of Both Mesenchymal and Neural Stem Cells," *Biology Reproduction*, Vol. 74, No. 3, 2006, pp. 545-551.

[76] L. Liu, C. M. DiGirolamo, P. A. Navarro, M. A. Blasco and D. L. Keefe, "Telomerase Deficiency Impairs Differentiation of Mesenchymal Stem Cells," *Experimental Cell Research*, Vol. 294, No. 1, 2004, pp. 1-8.

[77] D. Parsch, J. Fellenberg, T. H. Brummendorf, A. M. Eschlbeck and W. Richter, "Telomere Length and Telomerase Activity during Expansion and Differentiation of Human Mesenchymal Stem Cells and Chondrocytes," *Journal of Molecular Medicine*, Vol. 82, No. 1 2004, pp. 49-55.

[78] T. Mori, T. Kiyono, H. Imabayashi, Y. Takeda, K. Tsuchiya, S. Miyoshi, H. Makino, K. Matsumoto, H. Saito, S. Ogawa, M. Sakamoto, J. Hata and A. Umezawa, "Combination of Htert and Bmi-1, E6, Or E7 Induces Prolongation of the Life Span of Bone Marrow Stromal Cells From an Elderly Donor without Affecting Their Neurogenic Potential," *Molecular and Cellular Biology*, Vol. 25, No. 12, 2005, pp. 5183-5195.

[79] T. Okamoto, T. Aoyama, T. Nakayama, T. Nakamata, T. Hosaka, K. Nishijo, T. Nakamura, T. Kiyono and J. Toguchida, "Clonal Heterogeneity in Differentiation Potential of Immortalized Human Mesenchymal Stem Cells," *Biochemical Biophysical Research Communications*, 295, 2002, pp. 354-361.

[80] B. M. Abdallah, M. Haack-Sorensen, J. S. Burns, B. Elsnab, F. Jakob, P. Hokland, M. Kassem, "Maintenance of Differentiation Potential of Human Bone Marrow Mesenchymal Stem Cells Immortalized by Human Telomerase Reverse Transcriptase Gene Despite [Corrected] Extensive Proliferation," *Biochemical Biophysical Research Communications*, Vol. 326, 2005, pp. 527-538.

[81] E. S. Jun, T. H. Lee, H. H. Cho, S. Y. Suh and J. S. Jung, "Expression of Telomerase Extends Longevity and Enhances Differentiation in Human Adipose Tissue-Derived Stromal Cells," *Cellular Physiology Biochemistry*, Vol. 14, 2004, pp. 261-268.

[82] S. K. Kang, L. Putnam, J. Dufour, J. Ylostalo, J. S. Jung and B. A. Bunnell, "Expression of Telomerase Extends the Lifespan and Enhances Osteogenic Differentiation of Adipose Tissue-Derived Stromal Cells," *Stem Cells*, Vol. 22, No. 7, 2004, pp. 1356-1372.

[83] J. L. Simonsen, C. Rosada, N. Serakinci, J. Justesen, K. Stenderup, S. I. Rattan, T. G. Jensen and M. Kassem, "Telomerase Expression Extends the Proliferative Life-Span and Maintains the Osteogenic Potential of Human Bone Marrow Stromal Cells," *Nature Biotechnology*, Vol. 20, No. 6, 2002, pp. 592-596.

[84] J. B. Kim, H. Zaehres, G. Wu, L. Gentile, K. Ko, V. Sebastiano, M. J. Arauzo-Bravo, D. Ruau, D. W. Han, M. Zenke, H. R. Scholer, "Pluripotent Stem Cells Induced from Adult Neural Stem Cells by Reprogramming with Two Factors," *Nature*, Vol. 454, 2008, pp. 646-650.

[85] K. Sugaya, "Neuroreplacement Therapy and Stem Cell Biology under Disease Conditions," *Cell Mol Life Sci*, 60 (2003) 1891-1902.

[86] K. Sugaya, A. Alvarez, A. Marutle, Y. D. Kwak and E. Choumkina, "Stem Cell Strategies for Alzheimer's Disease Therapy," *Panminerva Medica*, Vol. 48, 2006, pp. 87-96.

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Discounting Future Pain: Effects on Self-Reported Pain

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ABSTRACT

Empirical results are presented showing that people who acknowledge pain anticipation when expecting an injury experience higher sensitivity to pain (*GREP, Robinson et al.,* 2001)*. The positive correlation between sensitivity and anticipation is highly significant. However, no relationship is found between anticipation and pain endurance.*

***Keywords*:** *Discounting*, *Pain Anticipation*, *Sensitivity and Endurance*

1. Introduction

"He who suffers before it is necessary, suffers more than is necessary"

Lucius Annæus Seneca (c. 4 BC-65 AD)

Humans are biologically programmed to anticipate threats, a subtle aid to survive the extant challenges in their environment. Recent studies in the field of behavioural economics have shown that people differ in the way they anticipate or value the future (technically, they "discount" the future) in their inter-temporal choices.[1] Thus, we may find two diametrical personalities: 1) those who are present-oriented and therefore show a more impatient behaviour; and 2) those who are future-oriented and show a self-controlled behaviour.

For instance, people with unhealthy lifestyles (that is, people who smoke, eat junk food, are sport-averse, etc.) are said to seldom highly value the future quality of their lives, thus typifying present-oriented personalities. In other words, they prefer good lives in the present to higher quality of life in the future (see [1]). The common explanation is that fulfilment of their expectations about future lifestyles or events remains highly uncertain due to life's contingencies, and this makes maximizing their current lives more desirable.

Savers typify the future-oriented group. Saving habits provide a good indication of how individuals discount the future. Savers, by keeping part of their current income for future use, show that they highly value the future as this deferral is apparently in anticipation of greater benefits accruable from the future use of such income. Thus, they tend to hold a more optimistic view about the fulfilment of their expectations about future lifestyles or events.

Recent research has explored the idea that time perception guides intertemporal choice ([5]). When making decisions, subjects use subjective delays and predict outcomes. Therefore, the subjective time perception regarding the delay of an event affects present behaviour. Thus, those subjects who perceive a shorter delay will be more affected by future events.

Similar arguments may be extended to loss scenarios. For instance, some people may suffer today in anticipation of future worse-case scenarios and anticipatory feelings of future losses or pain affect their present well-being. In other words, those who are aware of the likelihood of future pain and perceive a shorter subjective delay could start to "feel" the pain even prior to experiencing the real pain. Thus, anticipation would be more likely for impatient subjects.

This study links pain anticipation and self-reported pain latency. Questionnaires were administered to 122 experimental subjects. Data were collected on individual experience of pain in anticipation of expected injuries and also on pain sensitivity and pain endurance as defined in the Gender Role Expectations of Pain questionnaire (GREP [6]). The subjects reported their pain sensitivity and endurance levels on the Visual Analog Scale

[1]Viscusi, Huber & Bell [2] estimated discount rates and examine how they vary with individual characteristics. See also [3] and [4].

(VAS), which enabled comparisons with a typical woman and also with a typical man. This task was straightforward and clear to the subjects as it never referred to any specific type of pain, but only to the subject's general attitude towards pain. The results allowed us to measure the subjects' awareness of pain sensitivity and endurance.

The correlation of the GREP measurements with real pain is a critical issue. Defrin *et al.* [7] showed that pain thresholds and tolerance to real heat pain stimulus correlate significantly with GREP sensitivity measurements but not with endurance ones. Using thermal pain, Wise *et al.* [8] found that a higher GREP score is associated with both a lower pain threshold and less tolerance to real pain.

Recent literature on pain perception ([9,10,11]) noted that pain sensitivity and endurance are mediated by socio-cultural factors (e.g. age, ethnicity), psychological factors (e.g. anxiety, depression) and biological factors (e.g. genetics, gonad hormones, endogenous pain inhibitors). Edwards & Fillingim [12] using a hypothetical test different from GREP, likewise found that self-reported pain sensitivity is uncorrelated with tolerance to real pain but highly correlated with anxiety.

Our paper contributes to the existing literature by advancing the idea that subjects' inter-temporal valuation of the consequences of pain (anticipation of future events) has a nexus with their perception of pain tolerance and endurance. Thus, this research adds a new psychological factor that mediates pain sensitivity.

Our empirical analysis yielded a conclusive result: those who felt pain in anticipation before suffering the real injuries had significantly higher sensitivity values in all categories (that is, with respect to women, men, one's own sex, intersex and the average). In contrast, no differential effect on endurance levels was found. Furthermore, we find that anticipation is related to impatience, which is consistent with the idea of a subjective perception of time.

2. Research Question

There is a sequential process following an injury. It begins with pain *sensitivity*, which refers to the period of time that has elapsed before a person experiences pain after an injury. This is followed by the level of pain *endurance*, which refers to the period of time that has elapsed before a person experiencing pain will seek relief from their symptoms. These definitions are summarized in **Figure 1**:

Our hypothesis is that there is an additional step in this sequential process: pain is not only experienced after the injury, but produces anticipatory discomfort before and immediately after the injury occurs. Once subjects are aware of the future injury, they start to foresee pain and this anticipation shortens the period between injury and pain. Hence, the hypothesis can be formulated as follows: anticipation decreases pain latency. This idea is illustrated in **Figure 2**.

We conjecture that those who "feel" the pain *ex-ante* will have shorter pain latency. Hence, we expect sensitivity and anticipation to be positively correlated. Note that anticipation occurs prior to the onset of pain. Hence, endurance should be independent of anticipation.

We test the following hypotheses:

H1: anticipation and sensitivity are positively correlated.

H2: anticipation and endurance are uncorrelated.

A hypothetical experiment was used to check the validity of our hypothesis. The experiment and the dataset are described in the next section.

3. Data & Methodology

3.1. Variables

Subjects responded to a two-part questionnaire. Part I was a GREP test (*Gender Role Expectation of Pain;* [13,14,6,8]) in which subjects were requested to report their pain sensitivity and endurance levels on a Visual Analog Scale (VAS). Part II included questions on personal characteristics (age, sex, health, anticipatory behaviour) and social habits related to pain.

Part I. GREP

Subjects were asked to report their level of sensitivity and endurance as compared to a typical woman and then to a typical man.[2] The questionnaire was identical to Ro-

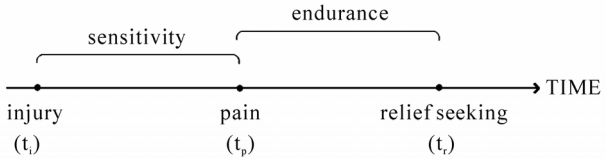

Figure 1. Sensitivity and endurance.

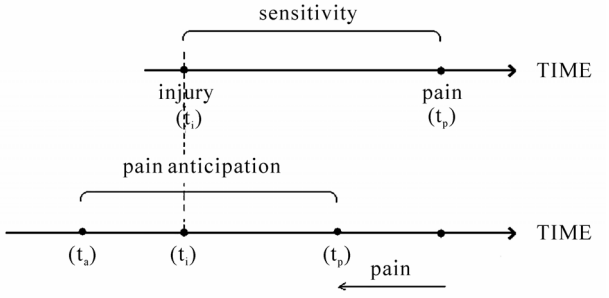

Figure 2. The effect of pain anticipation on sensitivity.

[2]There were also many other items not used in this research study, for example "propensity to talk to other people about painful experiences."

binson *et al.* ([13]).[3] Subjects place a mark on a 10 cm line to estimate their level of sensitivity and endurance.

The mark is then transformed into an integer number in the interval [-50,50] to measure the subjects' relative position with the center of the interval corresponding to a value equal to the reference point (the typical man or the typical woman). The GREP test provides the following variables:

In **Table 1**, "same-sex" (sensitivity or endurance) denotes that the comparison is within the responder same sex (s_w and e_w for women, and s_m and e_m for men). "Opposite-sex" denotes that the comparison is made with the other sex (s_m and e_m for women, and s_w and e_w for men).

We also compute the average values, $s_{mean} = (s_w + s_m)/2$ and $e_{mean} = (e_w + e_m)/2$. These variables are used to compare each individual to the rest of the society, regardless of gender, and they are obtained to check the robustness of the estimation. However, these average measurements do not have a straightforward interpretation, since sensitivity to pain is gender-related and the typical person is therefore not well defined.

The GREP test is appropriate for our purposes as it captures personal history, that is, the individual compares her own experience (regarding pain) to that of the typical person. The information conveyed refers to the average past pain experiences, presumably of different types, and is therefore not specific to a particular type of pain.

The information obtained from the GREP test is then interpreted as the overall measurement of individual pain sensitivity and endurance. Given previous results by Wise *et al.* [8] and Defrin *et al.* [7] regarding the correlation between results in the GREP test and real pain experiments, those who declare themselves to be more sensitive than the average will therefore be considered as having greater sensitivity. Identical argument is used for endurance.

Part II. Pain anticipation and personal characteristics

The question aimed at measuring pain anticipation was formulated as follows:[3]

When you know you are going to suffer a painful experience, do you start feeling pain even before the experience actually takes place? YES (= 1) or NO (= 0).

The independent variable, pain anticipation, was derived from this question; 44% of the subjects stated that they felt pain in advance.

Anticipation could be related to impatience, which is consistent with the idea of a subjective perception of time as the basis for temporal discounting ([5] and [15]). In a parallel study, subjects were asked the following hypothetical question:[3]

Suppose that we offer you 100 *euros that you will receive after* 30 *days. How much money are you ready to pay in order to get that money tomorrow? That is, which part of the* 100 *euros you would be willing to sacrifice to get the money in advance*? *I will pay* _____ *euros of the* 100.

This question was posed to obtain the subjects' level of impatience; 101 subjects complete both the anticipation and the time preference questions.[4] The amount of money to be paid in order to receive an earlier payment varies substantially across subjects (*mean* = 14.65; *st. dev*. = 17.91; *min* = 0; *max* = 80). A simple Pearson test between anticipation (yes/no) and impatience ($X^2 = 0.29$; $p-value = 0.002$) shows that the distribution of impatience among the subjects who anticipate pain is different from the distribution among those who do not. In particular, subjects who anticipated pain were also willing to pay more to obtain the payment immediately than those who did not. Hence, pain anticipation and impatience are positively correlated.

Participants were also asked to report information on personal characteristics. *Age*, *gender* and *health status* were used as control variables. The descriptive statistics are presented in **Table 2**.

The health status variable is self-reported; 5 levels are considered, from very bad (0) to excellent (4). Interest-

Table 1. Variables.

Original variables	Sens.	Endur.
With respect to a typical woman	s_w	e_w
With respect to a typical man	s_m	e_m
Transformations		
With respect to a typical person (same sex)	s_{same}	e_{same}
With respect to a typical person (oppos. sex)	s_{op}	e_{op}
Average values	s_{mean}	e_{mean}

Table 2. The descriptive statistics.

	Obs.	*Mean*	*sd.*	*min*	*max*
Age	122	24.40	3.19	20	46
Woman	122	0.55	0.49	0	1
Health	122	2.81	0.84	1*	4
Anticipation	122	0.44	0.49	0	1
s_w	122	-9.76	23.26	-50	50
s_m	122	-0.29	23.00	-50	50
e_w	122	6.90	23.15	-50	50
e_m	122	0.75	22.08	-50	50

[3]Translated into Spanish.

[4]Only 57 subjects of this sample also completed the GREP test.

ingly, no subject reported the minimum level of health (very bad).

We observe that, on average, subjects declared negative values for s_w which indicated that individuals (both males and females) consider themselves less sensitive than the typical woman. This is in contrast with s_m, where the mean value was not significantly different from zero.

Hence, women are stereotyped as more pain-sensitive than men (as shown in [6]). Identical results were found for pain endurance: on average, both female and male subjects considered their levels of endurance to be higher than that of a typical woman, but not higher than that of a typical man.

3.2. Experiment and Methodology

The experiment was conducted in four sessions at the University of Granada (Spain) in September and December 2009. All the sessions were run by the second co-author.

Questionnaire data were collected from 122 university students (55% women), who voluntarily participated in the experiment. The subjects were Economics and Business students (graduate students and undergraduates in the last two years of their degree). **Table 2** reports the main descriptive statistics of the population.

A censored regression model (Tobit) was used to estimate the relationship between pain anticipation and pain sensitivity, on the one hand, and pain anticipation and pain endurance on the other, thereby testing the two main hypotheses. A censored regression or Tobit is similar to a linear model (OLS regression) except that it takes into account that the dependent variable is censored, that is, it cannot take values outside a specified interval. Note that in this study, the values for dependent variables: pain sensitivity and pain endurance were restricted to the interval [−50,50].

The list of independent variables also includes age, gender and health status. The data were processed using STATA10 for MAC.

4. Results

Table 3 shows the estimation results for both pain sensitivity and endurance and at the different reference points (typical woman, typical man, typical person of the same sex, typical person of the opposite sex and the average person, respectively). Pain anticipation was significant for pain sensitivity. Subjects who felt pain in anticipation of future injuries also reported higher pain sensitivity values after controlling for sex, general level of health and age.

The bottom part of the table shows the estimation results for pain endurance. In sharp contrast to the pain sensitivity results, pain endurance is not correlated with pain anticipation in all the reference groups。

Hence, pain anticipation appears to be a key psychological factor mediating pain sensitivity. The subjects' inter-temporal valuation of the consequences of pain is related to their perception of pain tolerance. Furthermore, since pain anticipatory behaviour is prior to the onset of pain, we hypothesized that endurance should be uncorrelated to anticipation, which was confirmed by the data.

Figure 3 shows the Box-Plots of pain sensitivity for same sex (on the left) and for opposite sex (on the right) for two groups of subjects, those anticipating pain and those who did not. The Box plots show the first quartile, the median and the third quartile.

We observe that the group of subjects who anticipated (on the right-side of each figure) showed higher values for the three statistics than their colleagues who never anticipated pain (on the left-side of each figure). These results illustrate how pain anticipation can increase the perception of pain sensitivity.

Table 3. Estimation results.

Sensitivity	s_w	s_m	s_{same}	s_{op}	s_{mean}
Age	0.73 (0.28)	-1.67 (0.01)	-1.09 (0.01)	0.18 (0.81)	-0.53 (0.33)
Woman	12.29 (0.00)	10.29 (0.01)	-0.11 (0.97)	22.96 (0.00)	10.73 (0.00)
Health	4.89 (0.05)	1.71 (0.49)	1.20 (0.57)	5.39 (0.06)	3.25 (0.12)
Anticipation	13.98 (0.00)	8.10 (0.05)	11.14 (0.00)	11.05 (0.02)	10.48 (0.00)
Endurance	$\boldsymbol{e_w}$	e_m	e_{same}	e_{op}	e_{mean}
Age	1.10 (0.11)	-0,26 (0.70)	0.08 (0.89)	0.76 (0.29)	0.46 (0.42)
Woman	-5.54 (0.22)	1.48 (0.73)	4.45 (0.28)	- 8.61 (0.07)	-1.88 (0.61)
Health	1.50 (0.56)	-0.85 (0.73)	0.24 (0.91)	0.41 (0.88)	0.28 (0.89)
Anticipation	2.24 (0.61)	2.36 (0.57)	5.22 (0.20)	-0.70 (0.87)	2.44 (0.50)

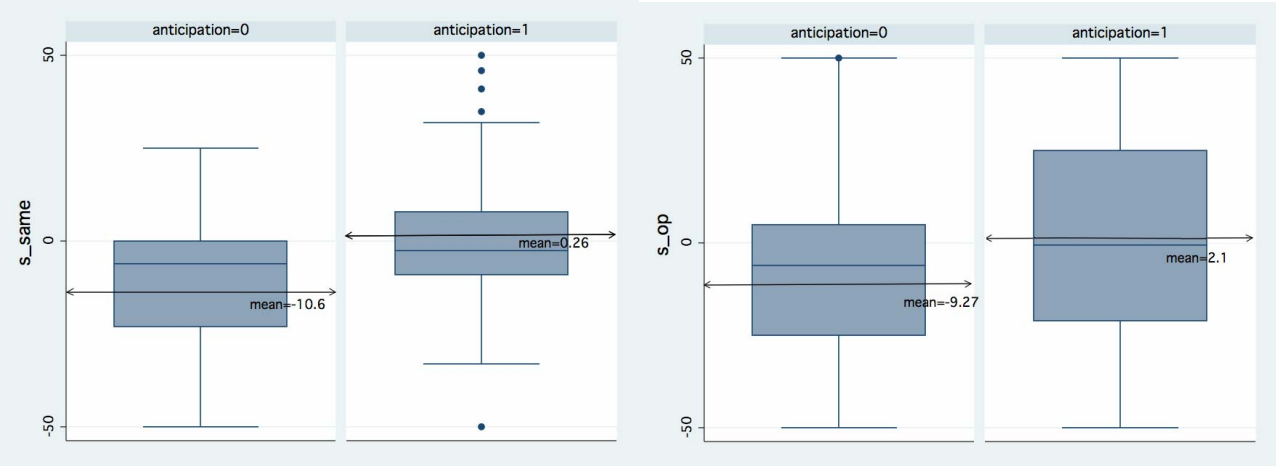

Figure 3. Pain sensitivity and anticipation

5. Discussion

We tested the hypothesis that pain is not only experienced after the injury but produces anticipatory discomfort that shortens the period between injury and pain. Our results indicated that there is a positive relationship between pain sensitivity and pain anticipation, thus suggesting that an anticipatory feeling of pain reduces pain latency by increasing awareness of the future pain.

It may also be argued that the positive correlation between pain anticipation and pain sensitivity is due to the fact that people with lower pain thresholds are more concerned about pain, and therefore likely to suffer more in anticipation of it. However, the fact that pain anticipation and pain endurance are unrelated suggests that higher pain sensitivity is indeed caused by the awareness of the likelihood of future injury.

An interesting question which deserves further analysis is whether the correlation found in the literature between anxiety and pain sensitivity (see [12]) could be related to the psychological factor highlighted in this paper, as individuals who anticipate future pain could also exhibit ensuing anxiety. Finally, a critical issue is how relevant this result is to clinical practice. Previous research has shown that perceptions of pain sensitivity, as measured in the GREP, may predict pain thresholds and tolerance to real pain stimulus ([8] and [7]). Our research suggests that anticipatory behaviour and the resulting perception of pain sensitivity could be useful to predict real pain tolerance and pain thresholds of patients.

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REFERENCES

[1] D. Cutler and E. Glaeser, "What Explains Differences in Smoking, Drinking and Other Health-Related Behaviors?" *American Economic Review*, Vol. 95, No. 2, 2005, pp. 238-242.

[2] W. K. Viscusi, J. Huber and J. Bell, "Estimating Discount Rates for Environmental Quality from Utility-Based Choice Experiments," *Journal of Risk and Uncertainty*, Vol. 37, No. 2-3, 2008, pp. 199-220.

[3] S. A. Golub, D. T. Gilbert and T. D. Wilson, "Anticipating One'S Troubles: The Costs and Benefits of Negative Expectations," *Emotion*, Vol. 9, No. 2, 2009, pp. 277-281.

[4] E. Fehr, "Behavioral science: The Economics of Impatience," *Nature*, Vol. 415, 2002, pp. 269-272.

[5] M. Wittmann, and M. P. Paulus, "Intertemporal Choice: Neuronal and Psychological Determinants of Economic Decisions," *Journal of Neuroscience, Psychology, and Economics*, Vol. 2, No. 2, 2009, pp. 71-74.

[6] M. E. Robinson, J. L. Riley III, C. D. Myers, R. K. Papas, E. A. Wise, L. B. Waxenberg and R. B. Fillingim, "Gender Role Expectations of Pain: Relationship to Sex Differences in Pain," *The Journal of Pain*, Vol. 2, No. 5, 2001, 251-257.

[7] R. Defrin, L. Shramm, and I. Eli, "Gender Role Expectations of Pain is Associated with Pain Tolerance Limit but Not with Pain Threshold," *Pain*, Vol. 145, No. 1-2, 2009, pp. 230-236.

[8] E. A. Wise, D. D. Price, C. D. Myers, M. W. Heft and M. E. Robinson, "Gender Role Expectations of Pain: Relationship to Experimental Pain Perception," *Pain*, Vol. 96, No. 3, 2002, pp. 335-342.

[9] Z. Wiesenfeld-Hallin, "Sex Differences in Pain Perception," *Gender Medicine*, Vol. 2, No. 3, 2005, pp. 137-145.

[10] S. J. Gibson and M. Farrell "A Review of Age Differences in the Neurophysiology of Nociception and the Perceptual Experience of Pain," *The Clinical Journal of Pain*, Vol. 20, No. 4, 2004, pp. 227-239.

[11] R. B. Fillingim, C. D. King, M. C. Ribeiro-Dasilva, B. Rahim-Williams, and J. L. Rilley III, "Sex, Gender and Pain: A Review of Recent Clinical and Experimental Findings," *The Journal of Pain*, Vol. 10, No. 5, 2009, pp. 447- 485.

[12] R. R. Edwards and R. B. Fillingim, "Self-Reported Pain Sensitivity: Lack of Correlation with Pain Threshold and Tolerance," *European Journal of Pain*, Vol. 11, No. 5, 2007, pp. 594-598.

[13] M. A. Robinson, and E. A. Wise, "Prior Pain Experience: Influence on the Observation of Experimental Pain in Men and Women," *The Journal of Pain*, Vol. 5, No. 5, 2004, pp. 264-269.

[14] M. E. Robinson, C. Gagnon, J. L. Riley III and D. D. Price, "Altering Gender Role Expectations: Effects on Pain Tolerance, Pain Threshold, and Pain Ratings," *The Journal of Pain*, Vol. 4, No. 5, 2003, pp. 284-288.

[15] K. Kim and G. Zauberman, "Perception of Anticipatory Time in Temporal Discounting," *Journal of Neuroscience, Psychology, and Economics*, Vol. 2, No. 2, 2009, pp. 91-101.

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Normalizing the Arm Reaching Patterns after Stroke through Forced Use Therapy – A Systematic Review

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ABSTRACT

Hemiparesis is common following stroke. The ability to reach and grasp is a necessary component of many daily life functional tasks, hence reduced upper limb function has an impact on the ability to perform activities of daily living. In hemiparetic patients, the unrestricted and unguided repetition of a motor task may reinforce compensatory movements. Trunk restraint allowed the patients to use joint ranges that were present but not recruited during unrestrained reaching. Later, studies combined the trunk restraint training with additional therapeutic interventions. With the growing number of studies on this intervention in the stroke population, there is the need to consolidate this evidence to determine the potential use of trunk restraint training in improving arm reaching in neurological rehabilitation particularly for stroke patients. A considerable research effort had assessed the effects of trunk restraint training on the recovery of reaching movements in hemiparetic patients. This review identified 5 *relevant trials in which one trial is a pilot study. Among* 5 *trials, three trials recorded the movement kinematics* (*outcome measure*) *by Optotrak Motion analysis System, in the other two trials the movement kinematics* (*outcome measure*) *were analysed by a* 6 *– camera,* 3*D Motion analysis system and* 10 *– camera Motion Analysis System respectively. The effect size for the intervention was calculated by Cohen's d. In this review, for the meta-analysis we used trunk displacement, trunk flexion, elbow extension, Smoothness and hand trajectory straightness* (*movement variables in kinematic analysis*). *The results of our review demonstrated that the use of trunk restraint as a treatment paradigm aimed at decreasing compensatory strategies has the potential of becoming an effective therapy. Further studies are necessary to determine the long term effect of the trunk restraint training.*

Keywords*: *Stroke, Trunk Restraint, Reaching

1. Introduction

Research on the effectiveness of rehabilitation techniques for patients with stroke is important not only for stroke survivors but also for care givers, treatment providers and society alike. The ability to reach and grasp is a necessary component of many daily life functional tasks, hence reduced upper limb function has an impact on the ability to perform activities of daily living [1], which is likely to reduce independence and increase burden of care. In the months after stroke, function of the paretic arm can improve as reaching; grasping and manipulating ability is regained. Improvements in function can occur in 2 ways. In one way, premorbid movement patterns may be regained because of true motor recovery. In another way, because of the redundancy in the number of degrees of freedom (DFs) of the body [2], actions can be accompanied by substitution of other DFs for movements of impaired joints. These alternative movements or motor compensations [3] are also observed in animals recovering from experimental stroke [4,5].

In patients with hemiparesis, the unrestricted and unguided repetition of a motor task may reinforce compensatory movements.[6] Patients with severe impairment tend to improve performance (defined as movement speed, precision and smoothness) of a pointing movement after

1 day of intensive training by incorporating trunk anterior displacement, a movement not normally needed for the task. Thus, in the short term, although compensatory movements may improve performance of the paretic arm, in the long term, these may be maladaptive by preventing recovery or reappearance of more efficient arm movement patterns [7].

Although neurorehabilitation research has recently demonstrated that structured, specific, and intensive training protocols increase the amount of hemiparetic limb use, less attention has been given to normalizing movement strategies poststroke [8]. Hence, Michaelsen *et al.* evaluated movement patterns of the hemiparetic arm made with or without restriction of compensatory trunk movements during reach to grasp tasks and found restriction of compensatory trunk movements may encourage recovery of 'normal' reaching patterns in the hemiparetic arm when reaching for objects placed within arm's length. During trunk restraint, patients improved active elbow extension, shoulder ranges and interjoint coordination when reaching [9-11]. Trunk restraint thus allowed patients to use joint ranges that were present but not recruited during unrestrained reaching. Later, studies combined the trunk restraint training with additional therapeutic interventions [12,13].

With the growing number of studies on this intervention in stroke population, there is a need to consolidate this evidence to determine the potential use of trunk restraint training in improving arm reaching in neurological rehabilitation particularly for stroke patients.

2. The Review

2.1. Aim

1) The primary aim is, to assess the effectiveness of trunk restraint training on the recovery of reaching movements in stroke patients.
2) Secondary aim is, to find out the effectiveness of trunk restraint training combined with other therapeutic interventions.

2.2. Inclusion Criteria

2.2.1. Type of Studies

To determine the evidence of the effectiveness of trunk restraint practice in neurological rehabilitation of stroke patients, randomised controlled clinical trials (RCTs) was the study design of choice. Clinical controlled trials (CCTs), quasi experimental studies, descriptive studies were also considered in the absence of RCTs.

2.2.2. Types of Participants

This review included any study involving any adult person with stroke, except those with pathology of the cerebellum or the basal ganglia **(Table 1)**.

2.2.3. Types of Interventions

Trunk restraint training, Trunk restraint training combined with other interventions **(Table 2)**.

2.3. Outcome Measures

2.3.1. Clinical

- Fugl—Meyer Assessment (arm section).
- TEMPA Scale.
- Manual Dexterity (Box and Block Test).
- Isometric Force (Handheld & handgrip Dynamometers).
- AROM (Active Range of Motion).
- Composite Spasticity Index.
- Wolf Motor Function Test.
- Motor Activity Log—Amount of Use Scale and Quality of Movement Scale.

2.3.2. Kinematic

- Motion analysis system.

2.4. Search Strategy for Identification of Studies

2.4.1. Language

Published English language studies were sought.

2.4.2. Keywords

Chronic stroke, arm, reaching, trunk restraint training.

Studies identified during the database searches from 2000 to 2009 were assessed for relevance from a review of the title, abstract and descriptors of the study. The databases that were searched included:

- CINAHL,EMBASE, MEDLINE, Cochrane library, PEDro, Pubmed, Ovid **(Figure 1)**.

2.5. Data Extraction

Study outcome data were collected by one reviewer and

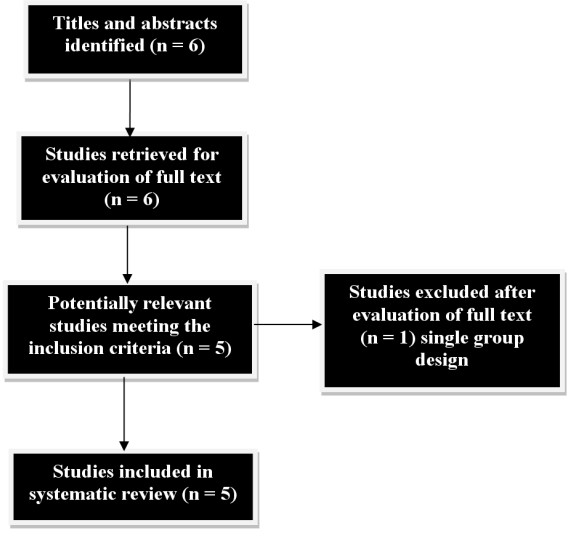

Figure 1. Search outcome.

Table 1. Criteria for inclusion of participants in studies.

Criteria	SM Michaelsen *et al.* 2001	SM Michaelsen *et al.* 2004	SM Michaelsen *et al.* 2006	G Thielman *et al.* 2008	ML Woodbury *et al.* 2009
Time since onset of stroke	5 to 69 months	7 to 94 months	6 to 48 months	7 to 36 months	6 to 101 months
Age (Years)	> 20	< 80	< 85	< 90	18 to 90
Specified diagnosis	X	-	-	X	-
Specified side of Hemiplegia	X	X	X	X	X
No evidence of excessive Spasticity	X	X	-	-	-
No excessive pain	X	X	X	-	X
Measurement of reduced upper limb function	X	X	X	X	X
Specified level of Balance	X	-	-	-	-
Not participating in an active rehabilitation program	-	X	-	X	-
Not part of other experimental studies	-	-	-	X	-
No upper limb conditions limiting use before stroke	X	X	X	X	X
No other significant medical conditions	X	X	X	X	X
Specification of hand dominance	-	X	-	-	X
No evidence of severe perceptuocognitive deficits	X	X	X	X	X
Able to perform reach to grasp movement	-	X	X	X	X
No limitations in passive range of motion	-	X	-	-	X

Table 2. Description of trunk restraint procedures.

Author	Restraint Procedures
SM Michaelsen *et al.* (2001)	Trunk was secured to the chair back with the harness minimizing shoulder girdle movement and preventing trunk flexion and rotation
SM Michaelsen *et al.* (2004)	Participants wore a harness consisting of breast and back plates connected by adjustable straps. An electromagnet attached to the wall was locked to the back electromagnetic plate at the interscapular level
SM Michaelsen *et al.* (2006)	Trunk movements were prevented by body and shoulder belts attached to the chair back. Scapular elevation / protraction were not restricted.
G Thielman *et al.* (2008)	Restraining device (LL Bean Co, Freeport, ME) was attached to the chair's back and had 2 padded shoulder straps that come across the glenohumeral joint, permitting approximately 3cm of scapula motion but limiting trunk flexion.
ML Woodbury *et al.* (2009)	To discourage anterior trunk displacement a custom designed trunk restraint was placed between the participant and the table. The restraint was placed between the participant and the table. The restraint was constructed on a stable base designed to fit around the outside of a chair while allowing the chair to slide under it to the table. The restraint was adjusted in height so that a padded shield was located anterior to and lightly touching the participant's sternum.

checked by a second reviewer. The following study characteristics were recorded on a data extraction form: setting and phase, study design and population, intervention, outcome and measurement.

2.6. Data Analysis

The effect size for the intervention was calculated by Cohen's d [14,33]. The effect sizes are especially important because they allow us to compare the magnitude of experimental treatments from one experiment to another. Estimates from individual studies are combined to reflect the overall size of the effect of the independent variable. The larger the difference, the greater the 'effect' of the intervention. If the effect size is >= –0.15 and < .15, the effect is negligible, if the effect size >= .40 and < .75, it is said to be medium effect, while the effect size is > 1.45, the effect is said to be huge. The size of percentage change is also calculated.

In this review, for the meta-analysis we used the following movement variables in kinematic analysis (outcome measure),

- ❖ trunk displacement,
- ❖ trunk flexion,
- ❖ elbow extension,
- ❖ Smoothness and hand trajectory straightness.

2.7. Results

The search yielded 6 full text articles, following the exclusion based on the criteria; 5 articles were included. Among the 5 articles included, one was done in Canada with 11 healthy and 11 hemiparetic individuals [9], the another two studies were studied in Canada with 28 hemiparetic patients and 30 patients respectively [10,11]. The fourth one was done in New York with 11 patients [12]; fifth study was done with 12 strokes, 5 health individuals in Florida [13].

Michaelson SM *et al.* [9-11] studied the effect of trunk restraint training on the recovery of reaching movements in chronic hemiparetic patients. Thielman G. *et al.* [12] studied the task related training and resisted exercise combined with trunk restraint training. Woodbury ML *et al.* [13] combined the trunk restraint training with intensive task practice and studied its effects on reach and function.

Participants across all the trails had similar diagnosis of hemiparesis with more upper limb involvement. All the trails exclude the patients with hemispatial neglect or apraxia, shoulder pain or neurologic or orthopaedic conditions affecting the arm or trunk.

The duration of the trail, setting type, intensity and type of therapy varied across trails which are described in **Table 3**. The effect sizes and percentage change for the outcome measure was calculated for both treatment and control groups. It was described in the **Tables 4**, **5**, **6**, **7**, **8**, which revealed negligible effect to very large effect sizes and negligible change to large decrease respectively.

2.7.1. Trunk Restraint Training and Recovery of Reaching

Three of the five trials studied the effect of trunk restraint training on reaching movements in patients with chronic stroke. All the three trials were done by SM. Michaelson *et al.* [9-11]. In the first trial, he included 11 healthy and 11 hemiparetic individuals. Data was collected with the use of an Optotrak Motion Analysis System. He concluded that trunk restraint decreases the number of joints involved in reaching. The effect sizes for elbow extension is 1.24 (Very large effect) in trunk restraint training. The percentage changes from comparison to treatment groups for elbow extension are –15 (Medium decrease). In this study, elbow extension was increased on an average of 14 degrees.

In the second study, 28 hemiparetic were assigned into two groups. The outcome measure was Optotrak motion Analysis system. The author concluded that restriction of compensatory trunk movements during practice may lead to greater improvements in reach – to – grasp movements in patients with chronic stroke than practice alone. The effect sizes for trunk displacement is 0.58 (Medium effect) and elbow extension is 0.29 (small effect). The percentage changes from comparison to treatment group for trunk displacement is –33 (Large decrease) and elbow extension is 7 (Small increase). Individual analysis of hemiparetic patients in trunk restraint group showed an increase in elbow extension (> 10 degrees) between pre-test and retention test; Anterior trunk displacement was decreased significantly more (by 52 mm) in trunk restraint patients.

In the third trial, Michaelsen randomly assigned 30 patients into trunk restraint group and control group. The author concluded that treatment should be tailored to arm impairment severity with particular attention to controlling excessive trunk movements if the goal is to improve arm movement quality and function. The effect sizes for smoothness (in whole group) is 0.22 (Small effect) and hand trajectory straightness (in whole group) is 0 (negligible effect). The percentage changes from comparison to treatment group for smoothness is –10 (Small decrease) and hand trajectory straightness is 0 (negligible change). Kinematic analysis revealed that trunk restraint decreased mean trunk displacement by 32.8 mm at post-test; at the same time trunk restraint increased elbow extension by 5.9 degrees at post-test.

Table 3. Summary of trial design features.

Study (First author)	Setting	Intensity of therapy	Type of therapy	Additional therapy
Michaelsen 2001	Inpatient	20 trials	With trunk restraint and with full vision, participants reached toward, grasped and returned the cone to the midchest region at a comfortable self paced speed. Reaches to target 1 and target 2 repeated with trunk secured to the chair back with a harness.	Nil
Michaelsen 2004	Inpatient	60 trial training period on day 1 and in a single session on day 2	Participants reached and grasped a cylinder in response to an auditory signal. Both groups were instructed not to move the trunk and to use as much as elbow extension	Nil
Michaelsen 2006	Inpatient	1 hour therapist supervised program 3 times per week for 5 weeks(total = 15 sessions)	With trunk restraint, repetitive functional uni- and bimanual reach to grasp tasks using objects varying in size, weight and shape.	Nil
Thielman 2008	Inpatient	12 sessions (3 per week), 45 minutes per session, 200 movements for each session.	For task related training, participants reached to contact or grasp objects variably placed to require arm movements of different amplitudes across all quadrants of the table top. Common objects were used that varied in size, shape and weight (eg, cups, mugs, writing, eating utensils). For resisted exercise, repetitive movements that required proximal and distal arm muscles were carried out against the resistance of the theraband. Trunk was restrained in both groups.	Task related training and resisted exercises.
Woodbury 2009	Outpatient/ inpatient	14 day mCIMT protocol and 10 days of inclinic task practice for 6 hours per day	Modified CIMT protocol along with trunk restraint training. Tasks progressed in difficulty as a participant demonstrated success	mCIMT

Table 4. Effect sizes for the trial 1 (SM Michaelsen *et al.* 2001).

Outcome measure	Effect sizes		Percentage change From comparison to treatment	
	Trunk free	Trunk restrained	Trunk free	Trunk restrained
Elbow extension	**1.32 very large effect**	**1.24 very large effect**	**−27 medium decrease**	**−15 medium decrease**
Shoulder horizontal adduction	1.89 huge effect	2.19 Huge effect	−41 large decrease	−44 large decrease
Shoulder flexion	1.63 huge effect	1.27 very large effect	−54 very large decrease	−30 large decrease
Peak velocity	0.84 large effect	1.40 very large effect	−22 medium decrease	−38 large decrease
No. Of peaks	1.26 very large effect	1.89 huge effect	87 huge increase	100 huge decrease
Index of curvature	0.83 large effect	0.76 large effect	12 small increase	11 small increase
Slope of angle	1.17 very large effect	0.44 medium effect	−54 very large decrease	−17 medium decrease
Angle correlation	0.9 large effect	0.53 medium effect	−22 medium decrease	−5 small decrease

2.7.2. Trunk Restraint Training Combined with Task Related Training and Resisted Exercise

G. Thielman *et al.* [12] in his study included 5 stroke patients in task related training group and 6 stroke patients in resisted exercise group. After the training, 3D Motion analysis system was used for testing. He concluded that training done by restricted truncal motion during task related training improved the precision of reaching more than during resisted exercise. The effect sizes for elbow extension (in midline) is 0.08 (Negligible effect) and for trunk flexion (in midline) is 0.8 (Large effect). The percentage change from comparison to treat-

Table 5. Effect sizes for the trial 2 (SM Michaelsen *et al.* 2004).

Outcome measure	Effect sizes	Percentage change From comparison to treatment
Performance outcome measures		
Velocity peaks	0.04 negligible effect	2 negligible change
Movement time	0.02 negligible effect	1 negligible change
Wrist peak velocity	0.16 small effect	4 negligible change
Time to peak velocity	0.46 medium effect	17 medium increase
Movement variable		
Trunk displacement	**0.58 medium effect**	**-33 large decrease**
Trunk rotation	0 negligible effect	0 negligible change
Elbow extension	**0.29 small effect**	**7 small increase**
Shoulder horizontal adduction	0.33 small effect	19 medium increase
Shoulder flexion	0.31 small effect	19 medium increase

Table 6. Effect sizes for the trial 3 (SM Michaelsen *et al.* 2006).

Outcome measure	Effect size	Percentage change From comparison to treatment
Clinical		
Elbow extensor strength		
1. Whole group	0.25 small effect	8 small increase
2. Mild group	0.23 small effect	−6 small increase
3. Moderate group	0.58 medium effect	21 medium increase
BBT		
1. Whole group	0.1 negligible effect	5 small increase
2. Mild group	0.08 negligible effect	2 negligible change
3. Moderate group	0.21 small effect	13 small increase
Kinematic		
Shoulder flexion		
1. Whole group	0.17 small effect	9 small increase
2. Mild group	0.23 small effect	6 small increase
3. Moderate group	0.19 small effect	13 small increase
Peak velocity		
1. Whole group	0.07 negligible effect	2 negligible change
2. Mild group	0.39 small effect	10 small increase
3. Moderate group	0.22 small effect	−6 small decrease
Smoothness, # peaks		
1. Whole group	0.22 small effect	−10 small decrease
2. Mild group	0.15 small effect	− small decrease
3. Moderate group	0.28 small effect	−10 small decrease
Hand trajectory straightness		
1. Whole group	0 negligible effect	0 negligible change
2. Mild group	0.84 large effect	8 small increase
3. Moderate group	0.2 small effect	−3 negligible change

Table 7. Effect sizes for the trial 4 (Thielman *et al.* 2008).

Measures	Effect sizes			Percentage change		
	Ipsilateral	Midline	Contra lateral	Ipsilateral	Midline	Contra lateral
Movement time	0.33 small effect	0.12 negligible effect	0.03 negligible effect	13 small increase	4 negligible change	1 negligible change
First velocity peak	0.41 medium effect	0 negligible effect	0.49 medium effect	8 small increase	0 negligible change	7 small increase
Elbow extension	**0.27 small effect**	**0.08 negligible effect**	**0.29 small effect**	**9 small increase**	**2 negligible change**	**11 small increase**
Trunk flexion	**0.37 small effect**	**0.8 large effect**	**0.71 medium effect**	**−17 medium decrease**	**−32 large decrease**	**−42 large decrease**
Trunk rotation	0.74 medium effect	0.4 medium effect	0.65 medium effect	−33 large decrease	−15 medium decrease	−36 large decrease
Scapula	1.9 huge effect	1.15 very large effect	0.9 large effect	−186 huge decrease	−300 huge decrease	90 huge increase
Independent arm	0 negligible effect	0.49 medium effect	0.39 small effect	2 negligible change	16 medium increase	9 small increase

Table 8. Effect sizes for the trial 5 (Woodbury *et al.* 2009).

Outcome measure	Effect sizes	Percentage change
Clinical evaluation		
FMA	0.42 medium effect	7 small increase
WMFT	0.43 medium effect	−16 medium decrease
MAL – AOU	0.91 large effect	−28 medium decrease
MAL – QOM	0.56 medium effect	−19 medium decrease
Kinematic results		
Peaks	2.4 huge effect	−34 large decrease
Index of curvature	3.33 huge effect	−32 large decrease
Trunk displacement	**5.53 huge effect**	**−36 large decrease**
Shoulder flexion / extension excursion	**0.21 small effect**	**7 small increase**
Elbow flexion / extension excursion	**0 negligible effect**	**11 small increase**

ment group for elbow extension is 2 (Negligible change) and trunk flexion is –32 (Large decrease). The elbow extension increased significantly from pre-test to post test in trunk restraint group (55 degrees to 64 degrees).

2.7.3. Trunk Restraint Training Combined with Intensive Task Practice

ML. Woodbury *et al.* [13] did a pilot study in which he included 11 chronic stroke patients and 5 healthy individuals. Data were collected with 10 – camera motion analysis system. He concluded that intensive task practice structured to prevent compensatory movements and promote shoulder flexion – elbow extension coordination may reinforce development of normal reaching kinematics. The effect sizes for trunk displacement are 5.53 (Huge effect). The percentage changes from comparison to treatment group trunk displacement are –36 (Large decrease). In mCIMT +Trunk restraint group, the trunk displacement is 0.13(0.01) in pre-test & 0.04(0.01) in post-test; the elbow flexion/extension excursion is –7.48 (6.69) in pre-test & 1.87 (1.93) in posttest.

3. Discussion

A considerable research effort has assessed the effects of trunk restraint training on the recovery of reaching movements in hemiparetic patients. This review identified 5 relevant trials in which one trial is a pilot study. Among 5 trials, three trials recorded the movement kinematics (outcome measure) by Optotrak Motion analysis System, in the other two trials the movement kinematics(outcome measure) were analysed by a 6 – camera, 3D Motion analysis system and 10 – camera Motion Analysis System respectively. The effect sizes for the outcome measure (Kinematic analysis) were analysed which shows medium effect to very large effect favouring the trunk restraint group. There is variability in the result of the studies included in this review, since the studies included mildly to moderately affected patients there is lack of improvement resulted from the training not being challenging enough.

Reaching ability is an important component for independent living. However, survivors of stroke often rely on compensatory movement strategies to accomplish reaching tasks. Carr and shepherd [15] suggest that compensatory strategies are the result of using available movements given the poststroke state of the central system, which leads to long-term functional limitations. Hence Michaelsen *et al.* studied the effectiveness of

trunk restraint training on arm recovery in stroke patients and demonstrated that trunk restraint is a treatment paradigm which decreases the compensatory strategies.

Since task related training [19,20] and resisted exercise[21] demonstrated enhanced recovery in stroke patients, Thielman *et al.* [12] compared the effects of task related training and resisted exercise combined with trunk restraint training in his recent trial. His results added one more stone in the crown of trunk restraint training. Extensive practice using task related training with truncal restraint appears to be a more effective approach to rehabilitate reaching with the hemiparetic arm.

3.1. How Trunk Restraint Training Improves Arm Reaching?

The effects of trunk restraint indicate that hemiparetic patients did not use their potential joint range for free arm movements. A likely explanation stems from the findings of Levin *et al.* [16]. They defined articular ranges in which hemiparetic patients could make isolated elbow flexion and extension movements by using a reciprocal muscle activation pattern. The increase in joint ranges with trunk restraint may be partly due to an adaption involving anticipation of changed external load conditions.

Another possibility is that the adaptation was triggered by somatosensory input from the trunk or shoulder caused by the trunk restraint [17]. In other words, patients are forced to make movements "out of synergy", which probably involves a focussed and greater effort on their part. This is similar to the strategy of constraining the unaffected arm [18] to force the patient to make more use of the affected arm with the additional feature that reduction of compensatory movement patterns is also targeted. This was proved by the recent findings of ML. Woodbury *et al.* [13]. Physical trunk restraint can be considered similar to "Manual guidance" in which spatial constraints are used to promote use of more optimal movement patterns [10].

In this training paradigm, external feedback, that is, explicit information was inherently built into the task practice with trunk restraint context both as knowledge of results (KR) and knowledge of performance (KP). For example, the participant received KR by either achieving or failing to achieve the task- goal. Additionally, the participants received KP via an afferent cue from the trunk restraint if he/she leaned forward [13].

3.2. Trunk Restraint Training in Future

Findings of Hsu WL *et al.* [22] suggest that the muscles in the affected ankle cannot be recruited timely and efficiently for the reaching task in stroke patients, as with relative recovery in lower limbs. In other words, they do not generate normal motor recruitment patterns to accomplish the motor task. It has been reported that even when the recovery is scored high in test situations, stroke patients do not spontaneously use their paretic side in daily living situations [23]. Future studies should emphasize some interventions to the hemiplegic lower limb while giving trunk restraint training to the hemiplegic upper limb.

The interaction between arm and trunk movements may also be altered in patients with hemiparesis due to the excessive displacement of the trunk for arm transport as has been previously reported during unimanual reaching and grasping [9-26]. The increased role of the trunk for arm transport and problems of trunk control in individuals with hemiparesis may represent additional challenges to inter-segment coordination and result in a destabilization of posture during tasks requiring arm movements from a standing position [27]. In daily living, reaching is more likely to be performed in a standing position [28,29]. In future, we recommend to study the influence of trunk restraint training in arm reaching in standing position.

It has long been recognized by clinicians [7,30] that once compensation has been learned, it is very difficult to modify. Indeed, prolonged use of compensatory trunk movements to reach targets placed within arm's length may result in the system learning not to use arm joints for reaching and grasping (learned nonuse) [18]. So that recovery of independent use of these joints would be discouraged. Compensatory movement strategies may be very difficult to unlearn [31], frustrating efforts to improve movement for both patient and therapist. Though all the trials included in this review demonstrated positive results for trunk restraint training on arm reaching movements, for maintaining the training effects for the rest of the day we suggest to study the effects of additional usage of strapping or splints or brace with trunk restraint training.

It is already known that stroke patients are deconditioned; hence training programs should combine physical conditioning and motor learning principles which will give the best and most permanent effect on motor recovery [32]. Trunk restraint training didn't address whether the intervention improved functional capacity of the arm, because it was expected that longer term practice would be necessary to affect change in this dimension. Hence in future, studies on trunk restraint training can also include physical conditioning program along with long term practice.

Young and Schmidt showed that less retention of learning occurs when continuous feedback is given compared with less frequent feedback. Hence further studies are necessary to determine the efficacy of faded trunk restraint program [34].

4. Limitations

The limitations of this review are,

1) Among the studies included in this review, only one study is randomized controlled trial which is suitable to find out the clinical efficacy.

2) The study quality of included papers is not evaluated, because we included descriptive studies in the absence of randomized controlled trials.

3) The kinematic analysis done in the studies included in this review used different movement variables; hence it is difficult to summarize the results.

5. Conclusions

This review identified 5 full text trials of trunk restraint training in stroke patients. The results of our review demonstrated that the use of trunk restraint as a treatment paradigm aimed at decreasing compensatory strategies has the potential of becoming an effective therapy. It shows that under lying "normal" patterns of movement coordination are not entirely lost after stroke and that appropriate treatments may be applied to uncover them to maximize function. One cost of this recovery may be a short-term decrease in movement speed. Our review also suggests that trunk restraint training + other rehabilitation program encouraged near to normal coordination patterns which were not seen in the absence of trunk restraint training in the rehabilitation protocol. Further researches with randomized control trials are necessary to determine the long term effect and the clinical efficacy of the trunk restraint training.

REFERENCES

[1] S. J. Page, S. Sisto, P. Levine and R. E. Mcgrath, "Efficacy of Modified Constraint-Induced Movement Therapy in Chronic Stroke: A Single-Blinded Rendomized Controlled Trial," *Archives of Physical Medicine and Rehabilitation*, Vol. 85, No. 1, 2004, pp. 14-18.

[2] N. A. Bernstein, "The Coordination and Regulation of Movement," Pergamon Press, Oxford, UK, 1967.

[3] M. C. Cirstea and M. F. Levin, "Compensatory Strategies for Reaching in Stroke," *Brain*, Vol. 123, No. 5, 2000, pp. 940-953.

[4] I. Q. Whishaw, "Loss of the Innate Cortical Engram for Action Patterns Used in Skilled Reaching and the Development of Behavioural Compensation Following Motor Cortex Lesions in the Rat," *Neuropharmacology*, Vol. 39, No. 5, pp. 788-805.

[5] K. M. Friel and R. J. Nudo, "Recovery of Motor Function after Cortical Injury in Primates: Compensatory Movement Patterns Used During Rehabilitative Training," *Somatosensory and Motor Research*, Vol. 15, No. 3, 1998, pp. 173-189.

[6] M. C. Cirstea, A. Ptito and M. F. Levin, "Arm Reaching Improvements with Short Term Practice Depend on the Severity of the Motor Deficit in Stroke," *Experimental Brain Research*, Vol. 152, No. 4, 2003, pp. 476-488.

[7] L. Ada, C. Canning, J. H. Carr, S. L. Kilbreath and R. B. Shepherd, "Task—Specific Training of Reaching and Manipulation," *In: Bennet KMB, Castiello u, eds. Insights into Reach and Grasp Movement*, Elsevier–Biosoft, Cambridge, UK, 1994, pp. 239-265.

[8] J. W. Krakauer, "Motor learning: Its Relevance to Stroke Recovery and Neurorehabilitation," *Current Opinion in Neurology*, Vol. 19, No. 1, 2006, pp. 84-90.

[9] S. M. Michelsen, A. Lutta, A. Roby-Brami and M. F. Levin, "Effect of Trunk Restraint on the Recovery of Reaching Movements in Hemiparetic Patients," *Stroke*, Vol. 32, No. 8, 2001, pp. 1875-1883.

[10] S. M. Michaelsen, D. E. A. and M. F. Levin, "Short Term Effects of Practice with Trunk Restraint on Reaching Movements in Patients with Chronic Stroke," *Stroke*, Vol. 35, No. 8, 2004, pp. 1914-1919.

[11] S. M. Michaelsen, R. Dannenbawn and M. F. Levin, "Task–Specific Training with Trunk Restraint on Arm Recovery in Stroke," *Stroke*, Vol. 37, No. 1, 2006, pp. 186-192.

[12] G. Thielman, T. Kaminski and A. M. Gentile, "Rehabilitation of Reaching after Stroke: Comparing 2 Training Protocol Utilizing Trunk Restraints," *Neurorehabil Neural Repair*, Vol. 22, No. 6, 2008, pp. 697-705.

[13] M. L. Woodbury, D. R. Howland, T. E. Mcguirk, S. B. Davis, C. R. Senesac, S. Kautz and L. G. Richards, "Effects of Trunk Restraint Combined with Intensive Task Practice on Poststroke Upper Extremity Reach and Function: A Pilot Study," *Neurorehabil Neural repair*, Vol. 23, No. 1, 2009, pp. 78-91.

[14] J. Cohen, "Statistical Power Analysis for the Behavioural Sciences," 2nd Edition, Academic Press, New York, 1977.

[15] J. Carr and R. Shepherd, "Movement Science: Foundations for Physical Therapy in Rehabilitation," 2nd Edition, Aspen Publishers, Gaithersburg, 2000.

[16] M. F. Levin, R. W. Selles, M. H. G. Verheul and O. G. Meijer, "Deficits in the Range of Regulation of Central Control over Arm Movement in Stroke Patients," *Brain Research*, Vol. 853, 2000, pp. 352-369.

[17] S. V. Adamovich, P. Archambault, M. Ghafouri, M. F. Levin, H. Poizner and A. G. Feldman, "Hand Trajectory Variance in Reaching Movements Involving the Trunk," *Experimental Brain Research*, Vol. 138, No. 3, 2001, pp. 288-303.

[18] E, Taub, N. E. Miller, T. A. Novack, E. W. Cook III, W. C. Fleming, C. S. Neomuceno, J. S. Connell and J. E. Crago, "Technique to Improve Chronic Motor Deficit after Stroke," *Archives Physical Medicine Rehabilitation*, Vol. 74, No. 4, pp. 347-354.

[19] C. M. Dean, C. L. Richards and F. Malouin, "Task–Related Circuit Training Improves Performance of Locomotor Tasks in Chronic Stroke: A Randomized Controlled Pilot Trial," *Archives Physical Medicine Rehabilitation*, Vol. 81, No. 4, pp. 409-417.

[20] C. M. Dean and R. B. shepherd, "Task Related Training Improves Performance of Seated Reaching Tasks after Stroke," *Stroke*, Vol. 28, No. 4, 1997, pp. 722-728.

[21] S. L. Morris, K. J. Dodd and M. E. Morris, "Outcomes of Progressive Resistance Strength Following Stroke: A Systematic Review," *Clinical Rehabilitation*, Vol. 18, No. 1, 2004, pp. 27-39.

[22] W. L. Hsu, Y. R Yang, C. T. Hong and R. V. Wang, "Ankle Muscle Activation during Functional Reach in Hemiparetic and Healthy Subjects," *American Journal of Physical Medicine and Rehabilitation*, Vol. 84, No. 10, 2005, pp. 749-755.

[23] K, Andrews and J, Stewart, "Stroke Recovery: He Can But Does He?" *Rheumatology Rehabilitation*, Vol. 18, No. 1, 1979, pp. 43-48.

[24] C. M. Cirstea and M. F. Levin, "Compensatory Strategies for Reaching in Stroke," *Brain*, Vol. 123, No. 5, 2000, pp. 940-953.

[25] M. F. Levin, S. M. Michaelsen, C. M. Cirstea and A. Roby–Brami, "Use of the Trunk for Reaching Targets Placed within and Beyond the Reach in Adult Hemiparesis," *Experimental Brain Research*, Vol. 143, No. 2, pp. 171- 180.

[26] A. Roby–Brami, A. Feydy, M. Combeaud, E. V. Biryukova, B. Bussel and M. F. Levin, "Motor Compensation and Recovery for Reaching in Stroke Patients," *Acta Neurologica Scandinavica*, Vol. 107, No. 5, 2003, pp. 369-381.

[27] K. L. Ustinova, V. M. Goussev, R, Balasubramaniam and M. F. Levin, "Disruption of Coordination between Arm, Trunk and Centre of Pressure Displacement in Patients with Hemiparesis," *Motor control*, Vol. 8, No. 2, 2004, pp. 139-159.

[28] J. Carr and R. Shepherd, "Stroke Rehabilitation: Guidelines for Exercise and Training to Optimize Motor Skill," NY, Elsevier, New York, 2003.

[29] P. Vanvliet, M. Sheriddan, D. G. Kerwin, *et al*., "The Influence of Functional Goals on the Kinematics of Reaching Following Stroke," *Neurology Report*, Vol. 19, No. 1, 1995, pp. 11-16.

[30] N. A. Bernstein, "The Coordination and Regulation of Movement," Pergamon Press, Oxford, UK, 1967.

[31] N. A. Bernstein and G. R. Buravtseva, "Coordination Disturbances and Restitution of the Biodynamics of Gait after Brain Damage," Thesis, 7th session, *Academy of Medical Science*, Institute of neurology, Moscow, 1954.

[32] J. Whitall, "Stroke Rehabilitation Research: Time to Answer More Specific Questions?" *Neurorehabil Neural Repair*, Vol. 18, No. 1, 2004, pp. 3-8.

[33] W. Thalheimer and S. Cook, "How to Calculate Effect Sizes from Published Research: A Simplified Methodology. A Work-Learning Research Publication," November 2009. http://work-learning.com/effect_sizes.htm

[34] D. E. Young and R. A. Schmidt, "Augmented Kinematic Feedback for Motor Learning," *Journal of Motor Behavior*, Vol. 24, No. 3, 1992, pp. 261- 273.

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Comparison between Auditory and Visual Simple Reaction Times

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ABSTRACT

Objective: *The purpose of this study was to find out whether the simple reaction time was faster for auditory or visual stimulus and the factors responsible for improving the performance of the athlete. Methodology*: 14 *subjects were assigned randomly into groups consisting of* 2 *members. Both the members from each group performed both the visual and auditory tests. The tests were taken from the DirectRT software program from a laptop. The DirectRT software consists of Testlabvisual and Testlabsounds to test the reaction times to visual and auditory stimuli. The* 2 *members from each group completed both the visual and auditory reaction times, the data was taken and the mean reaction time was calculated excluding the first and last values. Results: The results show that the mean visual reaction time is around* 331 *milliseconds as compared to the mean auditory reaction time of around* 284 *milliseconds. Conclusion: This shows that the auditory reaction time is faster than the visual reaction time. And also males have faster reaction times when compared to females for both auditory as well as visual stimuli.*

Keywords*: *Reaction Time, Auditory Stimuli, Visual Stimuli, Neuromuscular-Physiological Response, Auditory Cortex, Visual Cortex, Muscle Contraction

1. Introduction

Reaction time (RT) is the elapsed time between the presentation of a sensory stimulus and the subsequent behavioral response. Simple reaction time is usually defined as the time required for an observer to detect the presence of a stimulus. It is a physical skill closely related to human performance. It represents the level of neuromuscular coordination in which the body through different physical, chemical and mechanical processes decodes visual or auditory stimuli which travel via afferent pathways and reach the brain as sensory stimuli.

Simple reaction time can be determined when an individual is asked to press a button as soon as a light or sound appears.Research done by Pain & Hibbs, 2007 [1], shows that simple auditory reaction time has the fastest reaction time for any given stimulus. A study done by Thompson *et al.*, 1992 [2] has documented that the mean reaction time to detect visual stimuli is approximately 180 to 200 milliseconds, whereas for sound it is around 140-160 milliseconds. On the other hand, there are also researches done by Yagi *et al.*, 1999 [3], that show that reaction time to visual stimuli is faster than to auditory stimuli. Research by Verleger, 1997 [4] also confirms that visual reaction time is faster than auditory reaction time during or after exercise.

There are various factors that affect the reaction time to a stimulus. Factors like intensity and duration of the stimulus, age and gender of the participant, effect of practice can affect the reaction time of an individual to a particular stimulus. For example, there are relative differences between the reaction time to visual and auditory stimuli between genders. Male athletes tend to be faster than their female counterparts in responding to different stimuli. Researches done by Engel, 1972 [5], show the reaction time to sound to be faster in males when compared to females. Studies done by Dane et al, 2003 [6], show the difference in reaction time in eye-hand reaction time among male and female handball players.

The purpose of this study was to find out whether the simple reaction time was faster for auditory or visual stimulus and the factors responsible for improving the performance of the athlete.

2. Methodology

14 subjects were randomly divided into groups consisting

of 2 members. Both the members from each group performed both the visual and auditory tests. The tests were taken from the DirectRT software program in the laptop. The tests for visual reaction time were taken from the 'testlabvisual' file in the DirectRT program. Before starting the test, the subjects were asked to give individual file numbers under the 'enter codes' menu, in order to access the data after the test. In the testlabvisual test, the subjects were asked to press the 'space bar' key, every time they saw a yellow box on the screen. Once the test was completed, the data was taken from the output file, the mean reaction time was calculated excluding the first and last values. After both the subjects from each group completed the visual test, they undertook the auditory reaction test. This was taken from the 'testlabsounds' file in the DirectRT program. In the testlabsounds test, the subjects were asked to press the 'spacebar' key, every time they heard a 'beep' sound. Once the test was completed, the data was taken from the output file, the mean reaction time was calculated excluding the first and last values. After both the members of a group completed the visual and auditory tests, the mean reaction time data for both the visual and auditory tests were entered in the laptop.

3. Results

The results show that the auditory reaction time is faster than the visual reaction time. And also males have faster reaction times when compared to females for both auditory as well as visual stimuli.

4. Discussion

As the result shows, in **Figure 1**, the mean visual reaction time is around 331 milliseconds as compared to the mean auditory reaction time of around 284 milliseconds. This confirms that the auditory reaction time is definitely faster compared to the visual reaction time. This finding is similar to the studies done by Pain & Hibbs, 2007 [1] and Thompson *et al.*, 1992 [2], which also show that auditory reaction time is faster than visual reaction time.

Reaction time is dependent on several factors like arrival of the stimulus at the sensory organ, conversion of the stimulus by the sensory organ to a neural signal, neural transmissions and processing, muscular activation, soft tissue compliance, and the selection of an external measurement parameter (Pain & Hibbs, 2007 [1]). Researches by Kemp *et al.*, 1973 [7], show that an auditory stimulus takes only 8-10 milliseconds to reach the brain, but on the other hand, a visual stimulus takes 20-40 milliseconds. This implies that the faster the stimulus reaches the motor cortex, faster will be the reaction time to the stimulus. Therefore since the auditory stimulus reaches the cortex faster than the visual stimulus, the auditory reaction time is faster than the visual reaction time.

Reaction times are widely used to evaluate neuromuscular-physiological responses in sports. Studies by Pain & Hibbs, 2007 [1], have shown that the neuromuscular-physiological component of an auditory reaction time for sprint athletes can be around 85 milliseconds. Faster reaction times are significant for better performance of athletes. The faster the stimulus reaches the brain, the faster the signal is processed and the necessary responses are sent for the necessary motor reaction. Van den Berg *et al.*, 2006 [8], also found that fatigue due to sleep deprivation caused subjects to have slower reaction times. Studies by Ando *et al.*, 2002 [9], reported that reaction times reduced with repeated practice. Therefore reaction times to a particular stimulus can be made faster with repeated practice with a particular stimulus and with adequate rest in between stimuli.

In this study, as seen in **Figure 2**, it was also found that the male subjects had faster reaction times compared to the female subjects for both auditory as well as visual stimuli. This finding is similar to the research done by Dane *et al.*, 2003 [6]. The reason for this difference could

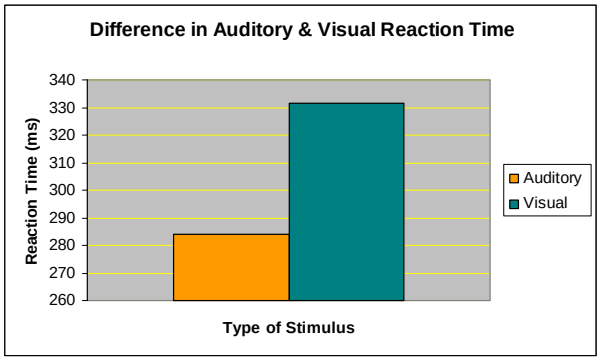

Figure 1. Graph showing faster simple reaction time for auditory stimulus compared to visual stimulus.

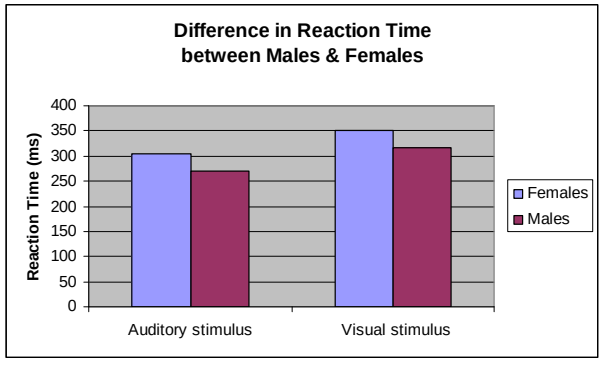

Figure 2. Graph showing males having faster simple reaction time compared to females for both auditory and visual stimuli.

be that it takes the same time for both the auditory and visual stimuli to reach the cortex but the time taken for the corresponding motor response and muscle contraction might differ. This was documented in the study done by Silverman, 2006 [10], that the motor response is faster in males when compared to females because they are comparatively stronger than females. This explains why males have faster simple reaction times for both auditory as well as visual stimuli.

5. Conclusions

From the above study it can be concluded that simple reaction time is faster for auditory stimuli compared to visual stimuli. Auditory stimuli has

- The fastest conduction time to the motor cortex.
- Fast processing time in the auditory cortex.
- Therefore faster reaction time and quick muscle contraction.
- And on the whole improves the performance of the athlete.

As exercise physiologists, our main aim is to improve the speed, skill and performance of the athlete. The above evidences suggest that speed and performance of an activity can be improved with faster reaction time to a stimulus. From the above findings of the study, faster reaction times can be achieved by providing repeated auditory stimuli and with adequate periods of rest between the stimuli.

A performance enhancing program can look like this:
- Exposure to adequate auditory stimuli;
- Repeated exposure to stimuli during practice;
- Adequate periods of rest between practices.

REFERENCES

[1] T. G. Matthew Pain and A. Hibbs, "Sprint Starts and the Minimum Auditory Reaction Time," *Journal of Sports Sciences*, Vol. 25, No. 1, 2007, pp. 79-86.

[2] P. D. Thompson, J. G. Colebatch, P. Brown, J. C. Rothwell, B. L. Day and J. A. Obeso, "Voluntary Stimulus Sensitive Jerks and Jumps Mimicking Myoclonus or Pathological Startle Syndromes," *Movement Disorders*, Vol. 7, No. 3, 1992, pp. 257-262.

[3] Y. Yagi, K. L. Coburn, K. M. Estes and J. E. Arruda, "Effects of Aerobic Exercise and Gender on Visual and Auditory P300, Reaction Time, and Accuracy," *European Journal of Applied Physiology*, Vol. 80, 1999, pp. 402-408.

[4] R. Verlager, "On the Utility of P3 Latency as an Index of Mental Chronometry," *Journal of Psychophysiology*, Vol. 34, No. 2, 1997, pp. 131-156.

[5] B. T. Engel, P. R. Thorne and R. E. Quilter, "On the Relationship Among Sex, Age, Response Mode, Cardiac Cycle Phase, Breathing Cycle Phase, And Simple Reaction Time," *Journal of Gerontology*, Vol. 27, No. 4, 1972, pp. 456-460.

[6] S. Dane and A. Erzurumluoglu, "Sex and Handedness Differences in Eye-Hand Visual Reaction Times in Handball Players," *International Journal of Neuroscience*, Vol. 113, No. 7, 2003, pp. 923-929.

[7] B. J. Kemp, "Reaction Time Of Young And Elderly Subjects in Relation to Perceptual Deprivation and Signal-on Versus Signal-off Condition," *Developmental Psychology*, Vol. 8, No. 2, 1973, pp. 268-272.

[8] B. J. Van den and G. Neely, "Performance on a Simple Reaction Time Task While Sleep Deprived," *Perceptual and Motor Skills*, Vol. 102, No. 2, 2006, pp. 589-600.

[9] S. Ando, N. Kida and S. Oda, "Practice Effects on Reaction Time for Peripheral and Central Visual Fields," *Perceptual and Motor Skills*, Vol. 95, No. 3, 2002, pp. 747-752.

[10] I. W. Silverman, "Sex Differences in Simple Visual Reaction Time: A Historical Meta-Analysis (Sports Events)," *Sex Roles*: *A Journal of Research*, Vol. 54, No. 1-2, 2006, pp. 57-69.

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Association of Human Herpesvirus 6 and 8 in Relapsing Remitting Multiple Sclerosis Type during Exacerbation

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ABSTRACT

Multiple Sclerosis (*MS*) *is a chronic demyelinating disease of the CNS with assumed autoimmune etiology. Human herpes viruses have probable effects on relapsing-remitting MS pathogenesis presumably through molecular mimicry and/or bystander mechanisms. In this study we probed the possible contribution of the two herpes viruses, human herpesvirus* 6 (*HHV*-6) *and human herpesvirus* 8 (*HHV*-8), *in clinically definite multiple sclerosis* (*CDMS*) *patients-relapsing remitting type* (*RRMS*) *during clinical exacerbations. All patients had no history of immune modulating or suppressing drugs intake in the last* 6 *months. The peripheral blood samples, from CDMS patients* (*n* = 20) *(13F/7M, age* (*y*) = 30.3 ± 3.21) *and other immune mediated neurological disorders* (*OIND*) (11*F/9M, age* = 25.2 ± 12.1), (*Myasthenia Gravis, Guillain Barré Syndrome, ischemic stroke in adolescent and young adult with no clear risk factors*), *as a control group, had been enrolled within* 15 *months* (*January*-2007-- *March* -2008). *We investigated the existence of specific deoxyribonucleic acid* (*DNA*) *sequences belonging to HHV*-6 *and HHV*-8, *using polymerase chain reaction* (*PCR*) *in the isolated peripheral blood mononuclear cells* (*PBMCs*) *and in plasma. PCR demonstrated HHV*-6 *DNA in* 7 *cases* (35%), *HHV*-8 *sequences in only one cases* (5%) *in PBMCs from* 20 *relapsed CDMS patients; all HHV*-6 *positive cases showed positive plasma results, while the blood samples from* 20 *OIND patients showed negative results except one case* (5%) *out of* 9 *cases of GBS was positive for HHV*-8 *in PBMCs. We consequently concluded that there is considerable evidence in this study that proposed the roles of HHV*-6 *and HHV*-8 *in MS pathogenesis and clinical exacerbation.*

***Keywords*:** *Multiple Sclerosis, HHV*6, *HHV*8, *Antiviral Drugs*

1. Introduction

Multiple Sclerosis (MS) is a chronic demyelinating disease of the CNS with assumed autoimmune etiology that for many years have been supposed to be of potential viral triggering. Generally speaking, 85% of MS patients have relapsing remitting MS type (RRMS) in the early disease course that converted to secondary progression within 15 years in the majority of MS victims. Therefore it is considered as the most important cause of nontraumatic disability disease among young adults [1,2].

Indeed, no animal species other than humans develop MS. Even so, MS may be the consequence of a chronic virus infection, but, so far, a definitive role for HHVs in MS pathogenesis has not been established. Both HHV-6 and 8 have the same distinctiveness features (neurovirulence, neuroinvasiveness and neurotropism) of many viruses but till now there are no data that concluded in solid base the role of these viruses in development of demyelinating diseases [3-5].

Viral neuroinvasion takes place commonly in primary acquired HHV-6 and 8 infections, although demyelination has been notified as remarkable complications. The HHVs have been isolated from MS plaques that denote a potential relation with MS pathogenesis, accordingly hindering these factors could be considered as parallel management pathway in prevention or decreasing the frequency of MS relapses, as well as, long-term disability progression [6].

Several thoughts have been sophisticated to clarify the

molecular mimicry or nonspecific bystander hypotheses of viruses. In agreement with this, two of the hallmarks of MS are sensitized auto-reactive T-cell and high auto-antibodies titers that are measurable in MS patients but not in healthy individuals. Nonetheless, many investigators using epidemiological observations as well as the pathological characteristics consider these viruses as participants in launching and perpetuation of the disease [7,8].

The rationale of this study was to investigate whether HHV-6 and 8 have a role in MS pathogenesis or not and their significant levels in relation to patients who have other immune mediated neurological disorders (Myasthenia Gravis, Guillain Barré Syndrome, ischemic stroke in adolescent and young adult with no clear risk factors).

2. Patients and Methods

This study prospectively enrolled 20 individuals who have been diagnosed RRMS with exacerbation and seen by two consultant neurologists in Mansoura university hospital throughout 15 months (January 2007, and March 2008). All MS cases showed clinical, radiological and electrophysiological (visual evoked potential (VEPs) and Brainstem Auditory evoked potentials (BAEPs)) findings that confirmed RRMS diagnosis [10,11].

The patient population was RRMS during exacerbation, the mean duration (y) of MS disease was 6.3 ± 1.86 and EDSS score was 2.88 ± 1.06. The demographic data of our RRMS- patients were 13 females and 7 males; their mean age when they included in this study was 30.3 ± 3.21years old. As regard the control groups, twenty cases with other immune mediated neurological disorders included in this study, their mean age when blood samples withdrawn was 25.2 ± 12.1 years old.

Above all, we used nested PCR (nPCR) to investigate the genomic DNA of HHV-6 and PCR for HHV-8 in peripheral blood mononuclear cells (PBMC) samples of 20 relapsing-remitting MS (RRMS) patients during exacerbation as well as control group. Two blood specimens were obtained for PCR examination from every patient. One sample was taken for PBMC separation and the second sample for plasma. Specimens from OIND were taken by the same way.

Isolated PBMCs and plasma samples were aliquoted and stored at −70℃. Blood samples from OIND patients were managed on the same technique. PBMC isolation were separated by Ficoll-Hypaque (Biotest-Germany) density gradient centrifugation and stored at −70℃. Total DNA was extracted from PBMCs and blood plasma of positive cases according to the manufacturer's instructions (Gentra, Minneapolis, Minnesota). PCR was used for the detection of both HHV-6 (nPCR) and HHV-8 (PCR) sequences in PBMC-HHV-6 and 8-DNA and blood plasma for HHV-6-DNA.

NPCR-HHV-6, a 100 μL reaction (EzWay PCR master mix, Koma Biotech) contained 0.5μM of each primer and 0.5μg of DNA as a template. Negative controls were performed with sterile deionized water. The detection of HHV-6 DNA was carried out according to Bandobashi *et al.* 1997 with a nested primer set, complementary to the gene coding the major capsid protein, which recognizes of the virus. The nucleotide sequence of HHV-6 primers 5'-G C G T T T T CA GTG TGTA G TTCGGCAG-3' and 5'- T G G C C G C A T T C G T A C A G ATACGGAGG- 3′(outer pairs); 5'-G C T A G A A C G T ATTTGCTGCAGAACG-3' and 5'-A TC CG AAA CA A CTGTCTGACTGGCA- 3' (inner pairs) for the first and second round of PCR, respectively [9]. The inner primers amplify a 258-bp fragment of HHV-6.

The first round of PCR was carried out at 94℃ for 3 min, followed by 30 cycles of 94℃ for 1 min, 57℃ for 1 min, and 72℃ for 1 min. Terminal extension of 72℃ for 5 min was carried out after completion of the 30 cycles. A sample (10 μl) of the first round product was used as template for the second round using the conditions described for the first round.

PCR was used to amplify 233-bp fragment of HHV-8-DNA with the primers forward (AGC-CGA-AAG-GAT-TCC-ACC-AT) and reverse (TCC-GTG-TTG-TCT-ACG-TCC-AG). A 100 μL reaction (EzWay PCR master mix, Koma Biotech) contained 0.5 μM of each primer and 0.5 μg of DNA as a template. Negative controls were performed with sterile deionized water. Reaction mixtures were subjected to 40 cycles of the following incubations: initial denaturations at 94℃ for 2 min (1 cycle), denaturation at 94℃ for 1min, annealing at 58℃ for 1 min, extension at 72℃ for 2 min (40 cycles) and final extension at 72℃ for 5 min.

A thermal cycler (Peltier-effect cycling, MJ Research, Inc.) (Watertown Mass) was used for the both PCR reactions. Detection of amplified DNA of both HHVs ten μL from the amplicon were analyzed by electrophoresis (Haefer HE_{33}) in 2% agarose gel with 1x TBE buffer stained with ethidium bromide, visualized under UVL (Fisher Biotech, FBTIV-88) and photographed (FB-PDH-1216).

All positive PBMC-HHV-6-DNA-PCR cases were analyzed again for plasma HHV-6-DNA for more confirmation. In this study we will discuss the results of PBMC samples because it is done for all patients but PCR for plasma samples were done for HHV-6-MS positive cases only.

Statistical analyses of data are given as mean ± SD. Continuous data were analyzed by the independent t test, as appropriate. P value of less than 0.05 was regarded as statistically significant.

3. Results

Between January 2007, and March 2008, 40 patients' blood samples were collected in Neurology department, Mansoura University, There were 20 patients who had been classified as CDMS according to published Poser and McDonald criteria of MS diagnosis, all of them are RRMS type and investigated during relapse. The control group included twenty patients who had other immune related neurological diseases.

Indeed, this prospective study significantly estimated the proportion of HHV-DNA sequences positive cases in comparison with prospectively classified OIND. The OIND spectrum comprised 9 cases with Guillain Barré Syndrome, 5 for Myasthenia Gravis, and 6 patients had ischemic stroke in adolescent and young adult with no clear risk factor. The clinical characteristics of each group in our study were compatible with so-called immune based neurological disorder, either B-cell or T-cell mediated, just to exclude the possibility of cross immunity, dominating one type of immune reaction or B-T cell conversion immunoprocess.

Among RRMS cases there were seven patients (35%) showed positive results for HHVs-DNA sequences. Pin pointing to the type of HHVs, The study implicated the relatively high frequency of HHV-6 DNA sequence in 7 cases (35%), HHV-8 sequence in only one case (5%) in PBMCs that drawn from 20 RRMS patients throughout exacerbation; all patients had no history of immunomodulatory or immunosuppressive treatments intake 6 months prior to the last attack (**Table 1**).

There was one female patient who showed positive DNA sequence for both HHV-6 and 8 and her EDSS score was 5.5. This patient also experienced high frequency of MS exacerbation.

The PCR of the blood samples that had been drawn from twenty OIND patients showed no positive DNA sequence for HHV-6 or 8 except one case out of 9 cases of GBS (11.1%) was positive for HHV-8 that represent 5% of the total OIND patients (**Table 1**). There was no significant predilection to females. Three out of the seven patients (42.8%) who had positive HHV-6-DNA sequences as well had high EDSS scores (more than 4).

Table 1. Comparison between positive PCR-HHVs* cases.

Diagnosis / *HHVS*	*RRMS* (*n*** = 20)		*OIND*(*n* = 20)	
	n	*%*	*n*	*%*
***HHV*-6**	7	**35**	0	**0**
***HHV*-8**	1	**5**	1	**5**
Both HHVs	1	**5**	0	**0**

P value < 0.001, *t Test* 0.33

*HHV, human herpesvirus; n**, number

4. Discussion

Indeed, this prospective study significantly estimated the proportion of HHV-DNA sequences positive MS cases in comparison with prospectively classified OIND. It was detected significantly (35%) in blood samples of RRMS patients more than control group (%5) ($p < 0.05$). This is indicating that viral agents could contribute to demyelination plaques and MS pathogenesis [5,6,9,11,12].

Moreover there is significant increase in frequency of relapses and severity of MS disease among HHVs positive patients (42.8%) ($p < 0.05$) who showed relative high EDSS. The percentage of cases with positive HHV-6 is higher thanHHV-8 [5,13].

In harmony with our results, Simmons 2001 and others stated that HHV-6 DNA correlates with active HHV-6 infection and is not found in healthy individuals. In this distinctive study, IgM titers showed a considerable raise to the p41/38 early antigen in patients with RRMS, in comparison to healthy controls and individuals with other neurological disease [14-16].

Two further studies have established the existence of increased IgM responses to HHV-6 in patients with MS, Additionally, HHV-6 DNA was identified in 30% of MS patients (15 of 50) and in 0% of 47 controls consisting of healthy individuals, patients with other inflammatory diseases, and patients with other neurological diseases, despite the fact that no correlation was demonstrated in Enbom, 2001 study [17-19].

Knox *et al.*, 1998, stated that HHV-6 may host CNS of MS patients and activated, as measured by DNA in plasma and CSF, from time to time. Anyhow, HHV-6 A variant was detected in the PBMC of MS patients but not in controls. [20]

It has been proposed that disagreements in these results may be caused by variations in reagents, procedures, racial and patient selection. Further serological, cellular/immune response, molecular, and clinical studies are required to explain the task of HHV-6 and 8 in MS pathogenesis [5,6,12,17,21].

Clearly, there is one patient who had HHV-8 (5%) with high EDSS that did not show significant differences with control group, which denotes the modest role of HHV-8 in disease progression and relapses frequency more than MS pathogenesis [22].

However, it is of interest that, when other investigators studied HHV-6 and 8 in RRMS, they gave attention to virus immunoglobulin titers and DNA but they did not correlate both of them with exacerbation, [23,24] there-

fore our distinctive study focus on the role of HHVs during relapses, this higher percentage of positive cases in relation to other studies may denote a considerable relation between exacerbation and HHVs, in addition to HHVs potential task in MS pathogenesis, plus all cases in this study had not receive any of beta-interferon drugs that have potential antiviral effects. As well racial factor may play also an important role in this high percentage [25,26].

5. Conclusions

We concluded that there is sizeable evidence in this study that put forward the association of HHV-6 and HHV-8 with RRMS during exacerbation. At the same time we have to be watchful to explain this mysterious disease very simply on the base of chronic viral infection, but we support extremely Egyptian therapeutic trials which put forward the role of antiviral drugs (acyclovir or valacyclovir) in treatment of MS exacerbation via evaluation of viral serology, radiological as well as immunological outcomes and the effect of these drugs on long term disease disability.

REFERENCES

[1] D. E. McFarlin and H. F. McFarland, "Multiple Sclerosis," *The New England Journal of Medicine*, Vol. 307, 1982, pp. 1183-1188.

[2] J. E. Friedman, M. J. Lyons, G. Cu, D. V. Ablashl, J. E. Whitman, M. Edgar, M. Koskiniemi, A. Vaheri and J. B. Zabriskie, "The Association of the Human Herpesvirus-6 and MS," *Multiple Sclerrosis*, Vol. 5, No. 5, pp. 355-362.

[3] H. Lassmann, "Comparative Neuropathology of Chronic Experimental Allergic Encephalomyelitis and Multiple Sclerosis," Springer Verlag, Berlin, 1983.

[4] J. F. Kurtzke, "Epidemiological Evidence for Multiple Sclerosis as an Infection," *Clinical Microbiology Review*, Vol. 6, No. 4, 1993, pp. 382-427.

[5] S. S. Solden, R. Berti, N. Salem, *et al.*, "Association of Human Herpes Virus 6 (HHV-6) With Multiple Sclerosis: Increased Igm Response to HHV-6 Early Antigen and Detection of Plasma HHV-6 DNA," *Nature Medicine*, Vol. 3, 1997, pp. 1394-1397.

[6] S. F. Hunterand D. A. Hafler, "Ubiquitous Pathogens. Links between Infection and Autoimmunity in MS," *Neurology*, Vol. 55, No. 2, 2000, pp. 164-165.

[7] A. K. Abbas, K. M. Murphy and A. Sher, "Functional Diversity of Helper T Lymphocytes," *Nature*, Vol. 383, No. 6603, 1996, pp. 787-793.

[8] M. Allegretta, J. A. Nicklas and S. Sriram, R. J. Albertini and O. T. Abramsky, "Cells Responsive to Myelin Basic Protein in Patients with Multiple Sclerosis," *Science*, Vol. 247, No. 4943, 1990, pp. 718-721.

[9] K. Bandobashi, M. Daibata, M. Kamioka, Y. Tanaka, I. Kubonishi, H. Taguchi, Y. Ohtsuki and I. Miyoshi, "Human Herpesvirus 6 (HHV-6)-Positive Burkitt's Lymphoma: Establishment of a Novel Cell Line Infected with HHV-6," *Blood*, Vol. 90, No. 3, 1997, pp. 1200-1207.

[10] W. L. McDonald, D. A. S. Compston, G. Edan, D. E. Goodkin, H. P. Hartung, F. D. Lublin, *et al*., "Diagnostic Criteria for MS: Guidelines from the International Panel on the Diagnosis of MS," *Annals of Neurology*, Vol. 50, No. 1, 2001, pp. 121-127.

[11] C. M. Poser, D. W. Paty, L. Scheinberg, W. I. McDonald, F. A. Davis, G. C. Ebers, *et al*., "New Diagnostic Criteria for Multiple Sclerosis: Guidelines for Research Protocols," *Ann Neurol*, Vol. 13, No. 2, pp. 227-231.

[12] S. Beer, K. M. Rösler and C. W. Hess, "Diagnostic Value of Paraclinical Tests In Multiple Sclerosis: Relative Sensitivities and Specificities for Reclassification According to the Poser Committee Criteria," *Journal of Neurology Neurosurgery Psychiatry*, Vol. 59, No. 2, pp. 152-159.

[13] M. L. Opsahl and P. G. Kennedy, "Investigating the Presence of Human Herpesvirus 7 And 8 in Multiple Sclerosis and Normal Control Brain Tissue," *Journal of the Neurological Sciences*, Vol. 240, No. 1-2, pp. 37-44.

[14] A. Simmons, "Herpesvirus and Multiple Sclerosis," *Herpes*, Vol. 8, No. 3, pp. 60-63.

[15] E. Norrby, H. Link and J. E. Olsson. "Comparison of Antibodies Against Different Viruses in Cerebrospinal Fluid and Plasma Samples from Patients with Multiple Sclerosis," *Infect Inmunology*, Vol. 10, 1974, pp. 688.

[16] R. T. Johnson, "The Virology of Demyelinating Diseases," *Annals Neurology*, Vol. 36, 1994, pp. 54-60.

[17] C. Martin, M. Enbom, M. Söderström, S. Fredrikson, H. Dahl, J. Lycke, *et al.*, "Absence of Seven Human Herpesviruses, Including HHV-6, By Polymerase Chain Reaction In CSF And Blood From Patients With Multiple Sclerosis And Optic Neuritis," *Acta Neurological Scandinavica*, Vol. 95, No. 5, 1997, pp. 280-283.

[18] M. Enbom, F. Z. Wang, S. Fredrikson, C. Martin, H. Dahl and A. Linde, "Similar Humoral and Cellular Immunological Reactivities to Human Herpesvirus 6 in Patients with Multiple Sclerosis and Controls," *Clinical Diagnostic Lab Immunology*, Vol. 6, No. 4, 1999, pp. 545-549.

[19] M. Enbom, "Multiple Sclerosis and Kaposi's Sacoma-Chronic Diseases Associated with New Human Herpesviruses?" *Scandinavial Journal Infectious Diseases*, Vol. 33, No. 9, 2001, pp. 648-658.

[20] K. K. Knox, D. Harrington and D. R. Carrigan, "Active Human Herpesvirus Type 6 Infections are Present in the Central Nervous System, Lymphoid Tissues and Peripheral Blood of Patients with Multiple Sclerosis," *Annals Neurology*, Vol. 44, 1998, p. 485.

[21] M. Mayne, J. Krishnan, L. Metz, *et al.*, "Infrequent Detection of Human Herpesvirus 6 DNA in Peripheral Blood Mononuclear Cells from Multiple Sclerosis Patients," *Annals Neurology*, Vol. 44, No. 3, 1998, pp. 391-394.

[22] E. Merelli, R. Bedin, P. Sola, P. Barozzi, G. L. Mancardi, G. Ficarra *et al.*, "Human Herpes Virus 6 and Human

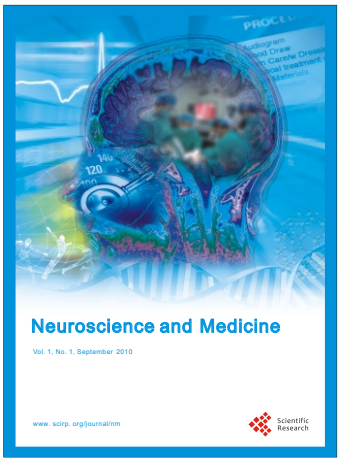
Herpes Virus 8 DNA Sequences in Brains of Multiple Sclerosis Patients, Normal Adults and Children," *Journal Neurology*, Vol. 244, No. 7, 1997, pp. 450-454.

[23] K. Felgenhauer and H.Reiber, "The Diagnostic Significance of Antibody Specificity Indices in Multiple Sclerosis and Herpesvirus Induced Diseases of the Nervous System," *The Journal of Clinical Investigation*, Vol. 70, No. 1, 1992, pp. 28-37.

[24] A. Rotola, E. Cassai, M. R. Tola, E. Granieri and D. Di Luca, "Human Herpesvirus 6 is Latent in Peripheral Blood of Patients with Relapsing-Remitting Multiple Sclerosis," *Journal of Neurology Neurosurgery Psychiatry*, Vol. 67, No. 4, pp. 529-531.

[25] J. Lycke, B. Svennerholm, E. Hjelmquist, *et al.*, "Acyclovir Treatment of Relapsing Remitting Multiple Sclerosis. Randomized Placebo-Controlled, Double-Blind Study," *Journal Neurology*, Vol. 243, No. 3, pp. 214-224.

[26] K. R. Beutner, "Valacyclovir: A Review of Its Antiviral Activity, Pharmacokinetic Properties, and Clinical Efficacy," *Antiviral Research*, Vol. 28, No. 4, 1995, pp. 281-290.



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