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Effect of Stromal Cell and Bone Marrow Stem Cell Fusion in Patients with Mild Alzheimer's Disease: A Proof of Concept Study

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Abstract

Objective: This article presents a proof of concept study on the efficacy of autologous treatment with adipose tissue stromal cells and bone marrow stem cells on patients with mild Alzheimer's disease.

Methods: Eligible patients were selected on the basis of SPECT test, NINCDS-ADRDA criteria and specific inclusion and exclusion criteria. Cognitive status of the patients was assessed before and after autologous intrathecal administration of stem cells through different assessment tests. Neurological status was determined using PET scan, SPECT imaging and CSF protein analysis.

Results: Steady improvement in the cognitive capability of patients was observed in the first six months of treatment. Later, there was a decline in the neurological ability which was revealed in the cognitive assessments. PET scan results were not changed.

Conclusion: In mild AD patients, the effect was obvious although short lived. The enhancement of the general status of the patients revealed the therapeutic potential of the therapy. Future large-scale clinical trials with more study subjects to determine the safety and efficacy of the therapy in different clinical settings are warranted.

Keywords: Alzheimer's disease; Stem cell therapy; Cognitive assessment; Dementia

Introduction

Alzheimer's Disease (AD) is a general geriatric neural debility which widely affects the intellectual discretion and cognitive potentiality of individuals. It claims huge worldwide expense of more than 818 billion US dollars with an alarming disease progression every year. The 2015 World Alzheimer report claims that more than 46 million people worldwide are living with the disease and the number is likely to increase to 131.5 million people by 2050 [1]. The disease pathogenesis is quite complicated and so the treatment methods. Diverse treatment modalities have been dowsed in the past to minimize the severity of the disease. The disease exhibits a unique pathway with multiple molecular loops and therefore, finding a drug which untangles these knots is indispensable. Development of AD is characterized by consecutive β and γ secretase cleavage of the amyloid-β protein precursor leading to accumulation of the amyloid-β plaques in the brain. These deposits lead to sequential tau phosphorylation, entanglement of the neurofibrils and immature neuronal cell senescence.

Risk factors and AD associations

About 95% of AD cases are non-familial. External factors like low education, low economic or social status and a strong history of head injury along with familial AD history are major predisposing factors to AD [2]. Many researchers have been studying on the strong relationship between AD occurrence and their genetic alteration. Major molecular changes are observed in the orientation and function of the amyloid precursor protein (APP, chromosome 21), presenilin 1 (PS1, chromosome 14) or presenilin 2 (PS2, chromosome 1) genes [3,4]. The genetic modification of apolipoprotein E4 (APOE4) is one of the major risk factors for non-familial spasmodic AD. Casserly and Topol reported on the strong relationship between AD, vascular disease and atherosclerosis [5]. Several studies on the association between hippocampus ischemia, cerebral vasculopathy and AD have been reported [6,7].

Evidence to microbiological association with AD have been reported by many researchers. Smith et al. claimed that majority of AD patients' brains have been infected with Chlamydia pneumonia [7]. Reports claiming the strong association between pathogens like herpes simplex virus, Borelia spirochetes and AD are also prevalent [8,9]. It has been reported in many a study that there is a strong attraction between microglia and infirm brain plaques in AD samples of humans and transgenic rodent models. The infirmed plagues caused due to AD infection have attracted the colonization and deposition of microglia in both human brain samples and rodent transgenic models [10,11].

Therapeutic tools

AD is a neural disorder that vitiates the episodic and semantic nexus of the brain and reducing its capability. The basal forebrain cholinergic neural system undergoes neurodegeneration. Myriad of potential drug candidates have been tested down the years to treat the

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disease. The Pharmaceutical Research and Manufacturer of America (PhARMA) has reported 123 failed research attempts with different drug molecules. Following this extensive research in the early 1990s, the FDA identified four drugs namely, donepezil, rivastigmine, galantamine and memantine as potential drug candidates in treating early stage AD [12]. Diverse baseline research studies on the pathogenesis, clinical pathways of the disease lead to identification of efficacious drugs. Many types of therapeutic drugs including cholinesterase inhibitors, muscarinic agonists, non-cholinergic drugs, metabolic enhancers were prescribed to treat symptomatic AD. Vaccines and pathway inhibitors like Aβ vaccine, β-site amyloid precursor protein cleaving enzyme 1 (BACE-1), β-secretase inhibitors, anti-tau drugs, tramiprosate, scyllo-inositol, γ- secretase modulator taren-flurbil, semagacestat, avagacestat, Aβ antibodies-bapineuzumab, solanezumab and immunoglobulins are based on the amyloid cascade hypothesis proposed in 1991. Many of the human trials based on these hypothetical pathways produced negative or null results [13]. Amidst all the diverse drugs targeting different molecular pathways, a potential drug therapy to treat the disease has not been identified till date. Researchers therefore attempt other treatment modalities targeting neuro-regeneration and stem cell research.

Advances in stem cell therapy

Stem cells with their self-mitotic ability can develop into varied cell types like totipotent, pluripotent, multi potent, unipotent and oligopotent cells. Stem cell therapy is an upcoming research tool and its efficacy in treating varied conditions like cancer, auto immune disorder and AD is explored recently. Scientists hope that stem cell therapy can control or even cure AD to a greater extent in comparison to other available AD treatment methods. Both pre-clinical and clinical studies have proved the safety and efficacy of the therapy in treating AD, although the unifying hypothesis to prove the mechanism is unknown. These stem cell studies reflect on the paracrine effects through releasing neurotropic factors and immunomodulatory activity of transplanted cells (Figure 1).

Therefore, we endeavoured to study the efficacy of stem cells in treating early Alzheimer's disease in a few patients. The results showed immediate restoration in cognitive capability and general activity after which there was a pause and sudden deterioration in the improvements. This is a pioneer study and molecular changes after administration has to be deciphered successively. The methodology we employed in preparing the stem cell fusion and administration are given as follows.

Materials and Methods

Patients with AD attending the Neurology Clinic, Anupum Hospital, India between September 2011 and September 2016 were evaluated for eligibility. The study was approved by the Institutional Ethics Committee and written informed consent was taken from all the patients. AD was confirmed upon a positive SPECT scan and NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke, Alzheimer's disease and Related Disorders Association) criteria. (Clinical Trials.gov identifier no: NCT03117738).

Inclusion criteria

- Age of the subjects should be 50 years or above at the time of signing the consent form.
- Subjects who have discretion to understand and provide written informed consent (assent).
- Subjects with probable mild-to-moderate Alzheimer disease or symptoms according to NINCDS-ADRDA criteria
- Subjects with MMSE Scores greater than 15 and less than 26 at the time of screening.
- Subjects who are on medications (donepezil, galantamine, memantine, rivastigmine or their combinations) regularly for the past 3 months.
- Subjects who are accompanied by a care giver who can read, write and communicate with the investigator. The care giver should visit the clinical site along with the patient during every visit and live with the patient or be with the patient at least ≥ 2 h/day ≥ 4 days/week.

Exclusion criteria

- Subjects who have signs of delirium
- Pregnant females or lactating women.
- Subjects who have had stroke complaints in the past 2 years.
- Subjects who had a long QTc interval, >450 m/sec in male or >470 m/sec in female at screening
- Subjects who had white matter hyper intensity (WMH) (≥ 25 mm of the deep white matter and ≥ 10 mm of the periventricular capping/banding in lengths).
- Subjects who are diagnosed with cognitive impairment other than Alzheimer's disease which has possibilities to confound misinterpretation of the drug effect during the course of the study.
- Subjects who are known to have autosomal dominant mutation-associated presenile AD.
- Subjects who had previous history of more than 4 cerebral micro haemorrhages or a single area of superficial siderosis or evidence of a prior micro haemorrhage as assessed by MRI.
- Subjects who have AIDS (Acquired Immunodeficiency Syndrome), HBV (Hepatitis B Virus), HCV (Hepatitis C), active lung disease, history of malignant cancer within the last 5 years,
- Subjects who are administered drugs like immunosuppressant's, cytotoxic drugs, corticosteroids etc. regularly for other ailments.
- Subjects for whom the investigator judges the liposuction can cause any problems.
- Subjects who are prone to local anaesthetic allergy.

Isolation of adipose derived stromal cells

The liposuction on the patients was done under general anaesthesia in a sterile environment. About 100 ml abdominal fat was aspirated in 3 mm cannula containing tumescent solution and replaced in sterile centrifuge tubes (size-250 ml). Liberal quantity of phosphate buffered saline was used to wash the adipose tissue and incubated with 75 µg/ml collagenase at 37°C for 30 min to enable tissue digestion. The stromal vascular fraction was obtained after centrifugation for 5 min at 500 xg. The suspended pellet was washed twice in normal saline and washed with PBS. The contents were then strained through a 100 µm strainer and centrifuged again for 5 min at 500 xg. Samples were taken to determine the cell quantity, viability, to culture and characterize the stem cells [14,15]. The flow cytometric analysis was done following the protocol as given by Francis et al. [16].

Bone marrow aspiration

The whole process was performed under local anaesthesia. About 240 ml of bone marrow was aspirated from the iliac crest from multiple points. The BM was transferred to transfer bags (Baxter, R4R 2001) and HES (Baxter B5084 6% in 0.9% Sodium Chloride) was added at a concentration of 20% blood volume. The bag was centrifuged (Multifuge Therma) at 125 xg for 10 min with the brake off, to stop disruption of the RBC pellet. The supernatant containing the nucleated cells were transferred to another bag and centrifuged for 10 min. The supernatant was removed for the third time using plasma expressor leaving the concentrated pellet (Fenwal BM-1. Lake Zurich, IL, USA). The pellet was flooded with normal saline and centrifuged again at room temperature for 15 min at 800 g. This removes majority of RBCs and the VSELs (very small embryonic-like stem cells) rich supernatant was again centrifuged at 1000 g for 10 min at room temperature. The centrifuged contents were mixed with PBS to make up the volume to 20 ml. This suspension pellet is rich in mononuclear cell population (comprising of haemopoietic stem cells and mesenchymal stem cells, endothelial progenitor cells, VSELs). The supernatant may contain platelets and growth factors [17].

Administration

Combination of ADSC (Adipose derived stromal cells) and BMAC (Bone marrow aspirate concentrate) was administered intrathecally along with mannitol. This procedure was repeated 3 times at 8 week interval. Subjects were scheduled for two follow-up visits at Weeks 4 and 8 to evaluate the safety and efficacy through primary and secondary outcome endpoints.

Treatment related adverse events

Any adverse event associated with the treatment was evaluated by analysing abnormalities in symptoms, vital signs, ECG, laboratory results and abnormal findings on physical examination. SPECT imaging was done at baseline and at one year. A PET scan of time frame between Baseline and 32 Weeks was also done. CSF protein analysis was performed at baseline than repeated after every treatment.

Cognitive assessment tests

The cognitive ability of the patients was tested using the following tests: Mini-mental status examination, Geriatric Depression Scale, FRSSD scale (Functional Rating Scale for Symptoms of Dementia) to assess the patients' daily activity parameters, Neuropsychiatric Inventory (NPI) to test the psychopathological status, Functional Cognitive Assessment Scale (FUCAS) to determine the executive cognitive function of day to day activities [18-22]. These Tests and scales were performed by trained staff member to ensure repeatability and reliability.

ADAS-Cog (Alzheimer's Disease Assessment Scale-cognitive subscale)

The version used in this study is the basic ADAS-Cog with 11 items: It was designed to measure cognitive areas commonly seen to decline in Alzheimer's disease (AD), like learning and reading, naming objects, perceiving commands (1 to 5 elements), ideational praxis (mail a letter), constructional praxis (copy 4 figures) orientation (person, time and place), recognition memory (from a second word list) and remembering test instructions (from the recognition subtest). Other subjective scales like testing the spoken language ability, word finding difficulty and comprehension. This test takes about 30 min. The ADAS-Cog is scored from 0 to 70 and higher score indicates greater cognitive impairment [23].

Results

Three AD patients (2 M, 1Fe, 70-78 y/o) were recruited as per inclusion criteria. Written consent was received from both the patients and relatives before commencing the study.

All the three patients completed the entire protocol with positive benefits in all scores at 6 months. But the scores declined at 9 months and reached near baseline levels at one year (Table 1).

There was a gradual difference in almost all the cognitive tests within 6 months of treatment. Changes were observed in MMSE by 3 (3^{rd} month) and 5 points (6^{th} month), NPI values improved by 6 (3^{rd} month) and 13 points (6^{th} month). Mild changes were observed in FRSSD by 1 & 2 points, GDS by 2 (3^{rd} month) and 6 (6^{th} month), FUCAS by 2 (3^{rd} month) and 1 (6^{th} month) ADAS-Cog by 4 (3^{rd} month) and 6 (6^{th} month) points. During the first six months of treatment, patients showed significant improvement in attention and general memory. Speech was expressive, patients were able to do daily activities better and began to execute more complicated tasks. The changes were evident and caregivers requested repeated administration of autologous stem cells. Beta amyloid levels and tau levels during the commencement of the study, 3 months and 6 months were analysed with baseline reduction. Percentage reduction from baseline showed gradual reduction over 6 months of the study period (Tables 2 and 3). The administration of dosage was done as given in Table 4.

Bone marrow

The typical yield on an average per 60 ml of bone marrow varies from 320-380 million mononuclear cells (MNCs) [15 to 18 million CD34+ cells, 0.5 million MSCs and 1 million VSELs].

Cognitive tests	Baseline	3 months	6 months	9 months	12 months
MMSE	9	12	14	12	9
NPI	21	15	8	12	18
FRSSD	22	21	20	20	22
GDS	9	7	3	5	7
FUCAS	96	98	99	98	96
ADAS-Cog	38	36	32	34	36

Abbreviations: MMSE: Mini Mental State Examination; NPI: Neuropsychiatric Inventory; FRSSD: Functional Rating Scale for Symptoms of Dementia; GDS: Geriatric Depression Scale; FUCAS: Functional Cognitive Assessment Scale; ADAS-Cog (Alzheimer's disease Assessment Scale-cognitive subscale). Score of 30-34 is mild impairment. An improvement of 3 points is considered significant.

Table 1: Cognitive ability tests for the three AD patients.

S. no	Beta amyloid (pg/ml)				
	Baseline	6 months	% reduction from baseline (%)	12 months	% reduction from baseline (%)
Patient 1	1640	780	52.4	1420	13.4
Patient 2	1448	440	69.6	1108	23.5
Patient 3	1190	800	32.8	1050	11.8
Range: % to % decrease in beta amyloid					

Table 2: Results of beta amyloid with their percentage reduction from baseline.

tau ng/l					
S. No	Baseline	6 months	% reduction from baseline (%)	12 months	% reduction from baseline (%)
Patient 1	989	672	32.10%	941	4.9
Patient 2	867	745	14.10%	770	11.2
Patient 3	724	586	19.10%	537	25.8
Range: % to % decrease in Tau					

Table 3: Results of tau with their percentage reduction from baseline.

Dosage concentration per patient	Adipose tissue derived stromal cells (ADSC)	Bone marrow mononuclear stem cells (MNC)
Patient 1	100 million	1280 million
Patient 2	112 million	1400 million
Patient 3	120 million	1320 million
2% of adipose tissue cells contained MSCs approximately In Bone marrow cells 5% were CD 34 positive and 0.4 % was MSCs approximately		

Table 4: Stem cell concentration/dosage.

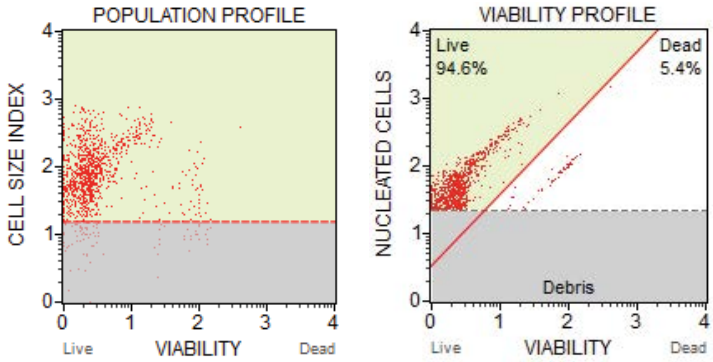


Figure 1: Viability test. The percentages of viable cells in SVF fraction 92 to 94.6%, respectively.

Cell characterisation

Total and dead cells were counted in all samples using the automated NucleoCounter® NC-100™ system following manufacturer's instructions. Cell viability was measured as previously described (Figure 1).

The stromal vascular fraction

Copious population of ASCs can be harvested using this isolation methodology. Besides sharing a mesenchymal morphology with other MSCs, the ASCs isolated in this streamlined manner were shown to share multipotent differentiation capability and an immune phenotype with the traditionally isolated ASCs. They possess characteristics which are also indistinguishable from Bone Marrow Stem Cells (BMSCs). Rapidly isolated ASCs have a CD14-CD29+, CD31-, CD34 low/+, CD45-, CD73+ and CD105+ immune phenotype, consistent with classically isolated ASCs and BMSCs. The Vegf levels values ranged from 1274 to 368029 pg/ml, with a mean value of 1970 pg/ml.

According to the preliminary data obtained in our lab, the stromal vascular fraction [SVF] represents 50-70% in volume of a lipoaspirate specimen. The SVF hosts a heterogeneous cell population [110 x 103 cells/ml on average] comprising mainly CD105+ mesenchymal stem cells [MSC, 20%], plus a wide number of CD34+ hematopoietic cells [40%]. As ADVF represents a heterogeneous cell population, 6 approximately 50%-70% of the total number of isolated cells were anticipated to be of mesenchymal origin, meaning CD29-, CD105-, CD90- and CD73-positive and CD34- and CD45-negative cells.

The pet scan hypo metabolism in periventricular white matter of both hemispheres, as well as in both temporal and parietal lobes was done. Follow up Pet scan revealed the same picture with no significant changes. No adverse responses were observed in the four patients. Haematological parameter and CSF analysis did not reveal any abnormality. The percentage of viable cells isolated by the MyStem EVO® device and the standard protocol, were 75.87% and 85.29%, respectively (Figures 2-6) (Table 5).

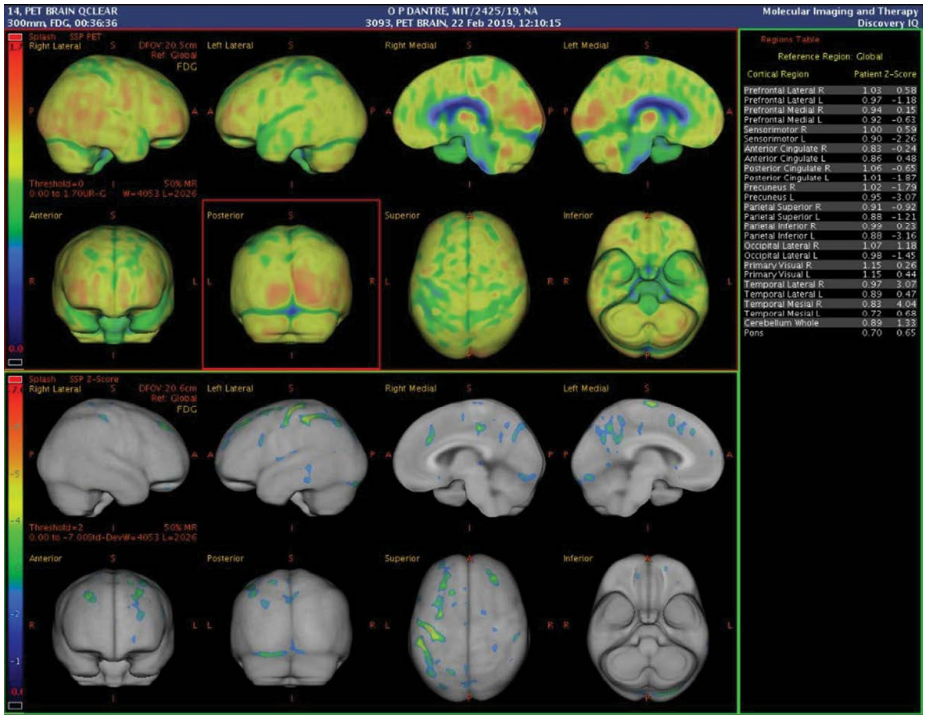


Figure 2: PET scan image of the cortical region of the brain showing no clinical changes in hypometabolic area.

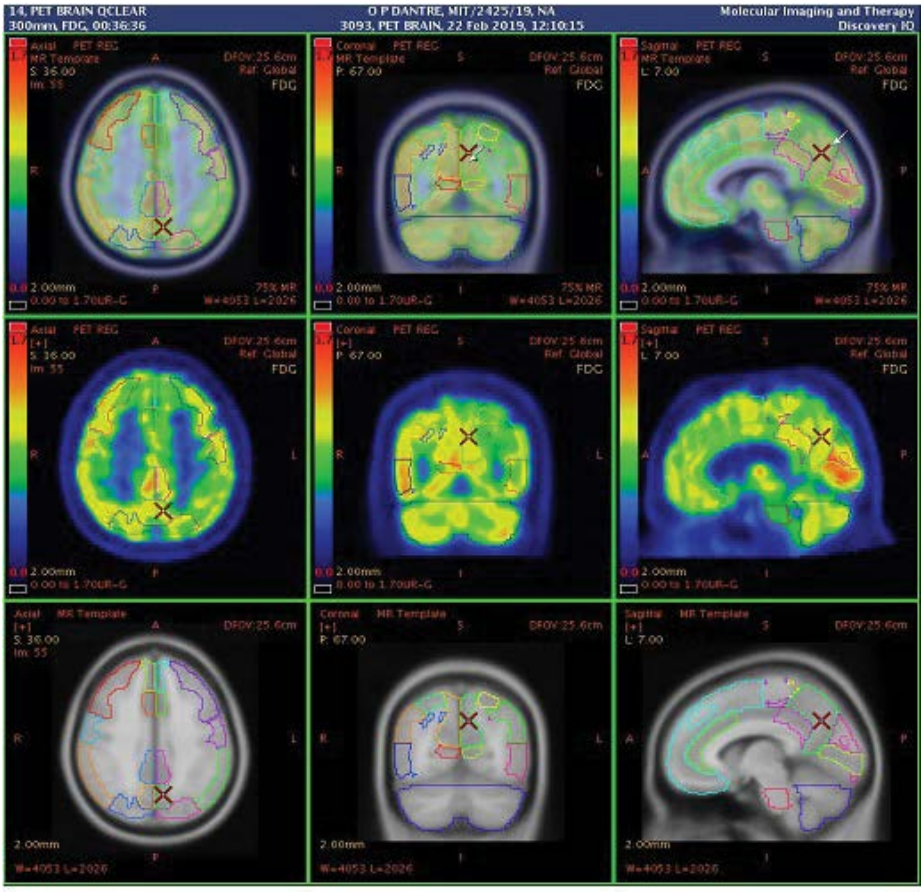


Figure 3: Figure showing PET scan images showing no change in hypometabolic areas.

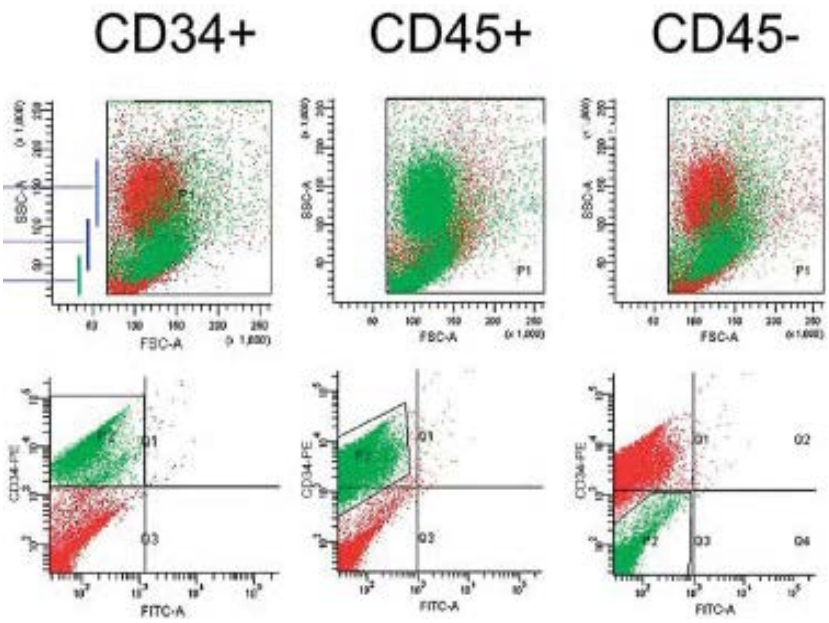
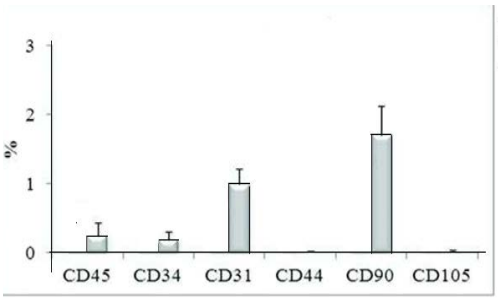


Figure 4: Flow cytometric analysis of SVF. 1-granulocytes, 2-monocytes, 3-lymphocytes CD34+cells (left) and CD45--cells (right) are shown in green CD45+cells (middle).



SINGLE MARKER EXPRESSION			
CD90	CD105	CD73	CD44
95% ± 5	96% ± 3	98% ± 2	93% ± 4

MARKER CO-EXPRESSION

Co- expressed marker	CD105+
CD73+	63% ± 8
CD90+	45% ± 13

Co- expressed marker	CD73+
CD105+	50% ± 10
CD90+	45% ± 10

Co- expressed marker	CD90+
CD105+	50% ± 10
CD73+	63% ± 8

Figure 5: Immunophenotype of SVF. The profile of cell surface antigens resembled an ASC-like Immunophenotype: CD90+, CD105+, CD44+, CD45-, CD34- and CD31-

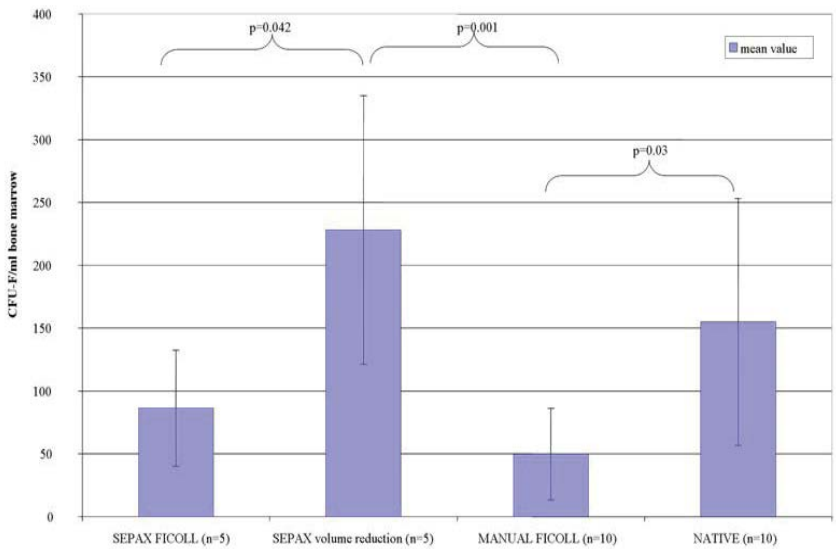


Figure 6: Incidence of CFU-F/ml bone marrow. [CFU-F/ml bone marrow] 280+64.4

Parameter	Baseline	After 1 year
RBC count, 10^6 per µl	4-4.3	4.1-4.7
Haemoglobin, g/dl	12.2-14.5	11.8-14.2
PCV haematocrit, %	36-40	38-43
Platelet count, 1000 per µl	276-337	260-395
White blood cell count, 1000 per µl	6.1-8.7	6.5-8.9
WBC differential count		
Neutrophils, %	52-73	65-77
Eosinophils, %	3-7	1-6
Lymphocytes, %	32-38	30-38
Monocytes	2-4	1-4
Basophils	0	0
CRP	<1	≤ 1
ESR	7-12	9-13
SGPT, IU/l	27-33	27-39
SGOT, IU/l	15-22	17-21
Serum creatinine, mg/dl	0.4-0.8	0.5-0.8
Glucose (F)	93-112	87-109

Table 5: Haematology parameters before and after stem cell therapy.

Discussion

Stem cell therapy is a promising tool for the treatment of diverse conditions, including neurodegenerative diseases such as Alzheimer's disease. The hopes for stem cell therapy in Alzheimer's disease are high. Investigators believe the therapy may result in a cure for the disease, compared to existing treatments that merely slow the progression of Alzheimer's disease. Some practical reasons for choosing ASCs for regeneration are: 1) Stromal vascular fraction of adipose tissues can be regenerated *in vitro* easily, 2) Extracting them from the patient is clinically safe, 3) Implantation also is highly accessible showing minimal rejection syndrome and 4) They differentiate into myriad cell families [24].

Evident improvement after initial treatment

The changes immediately after the treatment was apparent. Caregivers could sense the changes in the daily chores of the patients like grooming themselves, watching television, eating independently etc. Similar results were observed by Van Camp et al. [25]. He reported that there were improved SLUMS scores within 90 days after treatment and sustained improvements at 216 days with corresponding decrease in beta amyloid and tau levels in csf.

Declination in neural ability after six months

All the patients displayed a transient improvement in 6 months, on behavioral problems which were verified by standardized tests. Mild improvement of MMSE and NPI suggest that cognitive abilities were better and behavioral problems were decreased. But after six months of the treatment there was a steady decline in the results. Similar trend in the cognitive status of AD patient was reported by Tsolaki et al. [15]. Tsolaki reported that there was a rapid deterioration in the mental and cognitive status on the patient after an initial transient improvement in the neuropsychiatric status of the patient. The reason for this tendency of sudden declination after evident improvements is still scientifically unfathomed. Similarly, deterioration of the patient's condition after treatment cessation poses questions regarding the long-term effectiveness of stem cell therapy. These things should be explored in detail large-scale trials. If such a clinical trial study is considered, larger doses of autologous ADSCs and a repetitive infusion for every 1-2 months may be appropriate. BMAC with adipose stromal vascular fraction" (ADVF/ADSC), is the simplest form to be used in an autologous setting, because its preparation requires minimum manipulation and has been shown to be safe.

Challenges

Mild improvement of scores in measures of general cognition and neuropsychiatric issues was observed with no changes in MRI. Since the trial is only in the experimental stage, strict duration between treatments has not been standardised. Further the minimal and maximal dosages were not fixed. There is an immediate need to standardise the treatment methods and dosages. Simultaneous MRI and neuropsychological tests can assist in understanding neural changes more vividly. Also, some practical difficulties had to be addressed. Only a general procedure for harvesting the hematopoietic and mesenchymal cells is followed. If patients have a personalised harvesting routine and if cryotherapy could be utilized, then periodic treatments for longer duration can be attained [25].

Mechanism

Mammalian brain tissues can be resuscitated through stem cell transdifferentiation. Maler et al. reported lowering levels of circulating CD34+ cells along with significant changes in the beta amyloid 1-42 (r=-0.467, P=0.025) and A beta ratio (42/40 ratio (r=-0.688, P=0.005) of CSF when treated with stem cell transdifferentiation therapy in patients at early stages of AD [26]. Hence, cell replacement therapy to compensate neural decline sounds justified. This continuing neurogenesis in the subventricular zone, olfactory bulb and hippocampal dentate gyrus is supported by the identification of Neural Stem Cells (NSCs), suggesting that the adult Central Nervous System (CNS) may be amenable to the cell intervention [24].

Simard and Rivest reported that the mesenchymal, ADSC and other bone marrow-derived stem cells are capable of passing the blood-brain barrier and populate the entire central nervous system to transdifferentiate and activate into parenchymal microglia [27]. The microglia derived from bone marrow stem cells express high levels of CD11 in comparison to native microglia.

Migration into CNS was recently confirmed by a study in which intravenously injected Human Adipose-Derived Stem Cells (hASCs) were labelled with a multimodal nanoparticle, into AD animal model,

Tg2576 mice. Strong fluorescence signals were emitted in the extracted brains of Tg2576 mice up to 12 days post treatment [28].

Hutton and McGowan reported that A beta deposits are abraded by high concentration of activated microglial cells in AD animal models [29]. It is therefore assumed that BM-MSCs restore the effects of A beta deposition by the A beta-clearance pathway by inducing the activation of microglial cells. The effect of BM-MSCs on reducing A Beta accumulation is likely attributable to restoration or enhancement of the A beta-clearance pathway *via* microglial cells enhancing the clearance of existing A beta deposits. The combined effect of co-joined BM-MSCs -activated microglia may reduce A beta deposits in the central nervous system [30]. Laske et al. demonstrated that stem cell plasma (SCF) plasma levels are significantly decreased in AD patients with fast cognitive decline (decrease [MMSE] score >4 after one year [31].

Brain-Derived Neurotropic Factor (BDNF) and cAMP response element-binding protein (CREB) play a pivotal role in brain-neural activities like complex memory formation, consolidation and retention. To support this hypothesis, BDNF levels were found to be decreased in comparison to healthy controls in the brain autopsy of AD patients [32]. Many studies have reported BDNF induced reversal effects of neuroprotection during neuronal culture death in specific and dose-dependent manner [33,34]. Kitiyanant et al. reported that specific Aβ42 induced neuronal cell death can be reversed by BDNF and other neurotrophins like IGF-1 and GDNF [34]. Arancibia et al. reported that BDNF exhibits neuroprotective and anti-toxigenic effects against Aβ peptides and neutralize their toxic effects [34]. High levels of BDNF leads to increased CREB phosphorylation which triggers the genes that regulate cognitive functions and memory through cellular mechanisms like neuroprotection, cell proliferation, differentiation, cell migration, synaptogenesis and neurogenesis [35].

In cerebral infarction rat models, IL-10 and TNF-α are expressed as up-regulating and down regulating factors. Transplantation with Bone marrow- Mesenchymal Stem cells showed better recovery in cerebral ischemia rats along with high levels of BDNF, neurotrophin-3 (NT-3) and vascular endothelial growth factor (VEGF) levels. VEGF exhibits neuroprotective and neurogenic effects. In the light of these findings, it can be suggested that pro-inflammatory cytokines and anti-inflammatory cytokines affect neurogenesis in a negative and positive fashion respectively [36,37]. In addition, ASCs secretes diverse growth factors like glial cell line-derived neurotrophic factor (GDNF), NT-3, Nerve Growth Factor (NGF) and Fibroblast Growth Factor (FGF) which can negatively regulate pro-inflammatory cytokine release [36-38]. Post transplantation of human Adipose tissue derived stem cells (hASC) in AD mouse models VEGF, GDNF, NT-3 and anti-inflammatory cytokine levels were increased while IL-1 beta remained unchanged. Ichim et al. reported that Neprilysin (NEP) is the most vital beta amyloid degrading enzyme in the brain which reduces the levels of both secreted and intracellular levels of beta amyloid protein [39].

Two hypothetic mechanisms which support the results are possible:

Tissue regeneration: The injected stem cells get activated as neurons through neurogenesis although we never see this phenomenon in clinical cases. No evidence-based results are available to prove adult neurogenesis in humans till date. If neurogenesis has been possible, then the results would have been permanent due to regeneration of new neurons from the passive transfer of stem cells.

Tissue rejuvenation: This phenomenon has been observed in human trials. The growth factors present in the grafted stem cells restrict the damage of the local deteriorating cells leading to tissue rejuvenation through paracrine effects exhibited by the stem cells. Here, neurogenesis does not occur, but the injected cells restrict the repercussions and lead to ameliorating effects.

As far as we could decipher, the beneficial effects of stem cells seen in clinical setting are neither due to cell replacement or new neurogenesis. Hence, PET CT or MRI do not show any significant changes. The positive outcome is probably due to tissue rejuvenation. Paracrine effect of engrafted cells makes non-functional or hypofunctional synapses and neurons to work in synergy. These cells also help oligodendrocytes to produce myelin. The paracrine effects of the MSCs also help in protective mechanisms like anti-apoptosis, anti-fibrosis, angiogenesis and anti-inflammation [14].

Besides improvement in regeneration, MSC therapy results in suppression of the underlying inflammation. The anti-inflammatory response appears to have favourable influence upon deranged neurons and neurites. These improvements may be linked with a suppressed cortical inflammatory response, improved transport or filtration of amyloid and tau from the brain/spinal fluid. These ameliorating effects are the results of neurological responses like subdued cortical inflammatory response, improved transport, amyloid and tau filtration from the spinal fluid.

Conclusion

Our small pilot study conducted with a few AD patients strongly suggests that adult stem cell therapy can ameliorate the cognitive effects of AD if the impairment is in its initial state. As AD claims many lives and imposes burden on relatives and caretakers, more treatment modalities are explored to reduce the impact of the disease on the patients and their caretakers. Any therapy which enhances the neurological status of the patient will reduce the burden on the patient and the society as well. In our study, there was significant improvement within 6 months of intervention. But the results were not stable and deteriorating after the first six months. Safe increase in the dosage level can be considered if supported by evidence studies. There were no adverse effects of any kind reported during the study period. Since stem cell therapy in AD is its nascent stage, efficacy, safety and long-term effectiveness should be studied in detail. More clinical and molecular studies are warranted to prove the effectiveness and safety of the therapy.

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