

# Use of Human Embryonic Stem Cells in the Treatment of Diabetes Mellitus: A Case Series

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## Abstract

**Introduction:** Diabetes mellitus (DM), a metabolic disorder, is known to be highly prevalent in people aged 40 - 60 years in developing countries whereas in developed countries, it mostly affects people above the age of 60 years. It is of two types: DM type I, an autoimmune disorder that mostly onsets after an infection and DM type II that is commonly associated with obesity. Several treatments are available for the treatment of DM, but none has successfully cured diabetes. Nowadays, stem cell therapy is being investigated for use in the treatment of DM and has shown positive results. **Case Report:** Our study presented results of three diabetic patients who were treated with human embryonic stem cell (hESC) therapy. Following the therapy, blood glucose levels were reduced. An improvement was observed in eye sight, stamina, gait pattern endurance, mental focus ability and muscle strength. There was a reduction in secondary side effects of high blood sugar such as affectation of cardiac, kidneys, polyneuropathy, vision etc. No adverse events and teratoma formation were observed after the treatment. **Conclusion:** It was concluded that hESCs showed good therapeutic potential in the treatment of patients with diabetes.

## Keywords

Diabetes, hESC Therapy, iPSCs

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## 1. Introduction

Diabetes mellitus (DM) is estimated to affect around 285 million people worldwide and this number is expected to reach 439 million people by 2030. It is highly prevalent in people aged 40 - 60 years in developing countries whereas in developed countries, it mostly affects people aged above 60 years [1]. DM is also simply referred to

as diabetes that occurs due to the defects in insulin secretion, insulin action, or both leading to hyperglycemia [2]. Diabetes has been classified into two types depending upon the defect leading to it. DM type I is caused by a cellular-mediated autoimmune destruction of the  $\beta$ -cells of the pancreas that results in significant drop in the insulin secretion and thus, the insulin deficiency. DM type II, also known as non-insulin-dependent diabetes or adult onset diabetes, is caused by multiple factors like insulin resistance in which the cells of the body do not utilize insulin effectively [3]. DM type II is responsible to be the cause of diabetes in 90% - 95% of the affected people [2]. Multiple factors are involved in the onset of DM type II which include genetic factors and non-genetic factors such as obesity because of high calorie intake, increasing age, sedentary lifestyle and central adiposity [4]. There is a wide list of complications associated with chronic hyperglycemia such as cardiovascular disease (CVD), coronary artery disease (CAD), myocardial infarction and peripheral arterial disease with the consequence of limb amputation, neuropathy, retinopathy, nephropathy, cardiomyopathy and diabetic foot ulcers [5]-[9].

Drugs used to manage DM include antidiabetic drugs like sulfonylureas, metformin, dipeptidyl peptidase-4 (DPP-4) inhibitors, colesevelam, thiazolidinediones, meglitinides,  $\alpha$ -glucosidase inhibitor and rosiglitazone [10]. Other treatments include insulin therapy and glucagon-like peptide-1 receptor agonists (GLP-1 RAs) [11]. But none has been able to cure diabetes.

Transplantation therapies that have been investigated include whole pancreas transplantation, islet cell transplantation and stem cell transplantation [12]. However, in the last decade stem cell therapy has gained a greater momentum than other treatments. We presented three cases of DM patients who were treated with hESC therapy.

In our previous studies, we have shown improvement in the condition of patients suffering from cerebral palsy or/and cortical visual impairment, spinocerebellar ataxia, spinal cord injury, and Friedreich's ataxia after hESC therapy [13]-[17].

## 2. Methods

hESCs are cultured and maintained as per our proprietary in-house patented technology in a Good Manufacturing Practices (GMP), Good Laboratory Practices (GLP) and Good Tissue Practices (GTP) compliant laboratory at Nutech Mediworld (Patent-WO 2007/141657A PCT/IB 2007 Published 13 Dec. 2007). Establishment and characterization of the hESCs is elaborated elsewhere [18]. The evidence for the use of hESCs at Nutech Mediworld has also been submitted in written and accepted by the House of Lords, Regenerative Medicine, Science and Technology Committee [19]. The study protocol was approved by an independent Institutional Ethics Committee (IEC). The institutional committee for stem cell research and therapy of Nutech Mediworld, New Delhi, India reports to the National Apex Committee for Stem Cell Research and Therapy (NAC-SCRT). The safety and efficacy of this cell line is established [20]. The cell lines are free of animal and microbial product and are chromosomally stable.

The written and videotaped informed consent was provided by all the patients prior to start of the treatment. The condition of the patient was video graphed before, during and after the treatment periods. The doctors and the rehabilitation team conducted a detailed examination of the patients before, during and after each treatment cycle.

The treatment approach was divided into phases followed by gap phases in between. In the first phase, T1 (6 week), 0.25 ml (<4 million cells) hESCs were administered *via* the intramuscular (i.m) route twice daily to "prime" the body and allow for the recipient immune system to be active, 1 ml hESCs (<16 million cells) were administered twice weekly *via* intravenous (i.v) route to "home in" to the required area and 5 ml hESCs were also administered intravenously after every 7 days. Succeeding a gap period of 3 - 6 months, the subsequent treatment phases, T2 (3 to 4 weeks) and T3 (3 to 4 weeks) also used the same dosage regime as T1. The treatment approach was repeated annually, if needed. Any co-existing condition was also treated accordingly.

A gap phase of 3 - 4 months between the subsequent treatment phases was decided to allow the injected hESCs to develop into mature cells and regenerate the affected part. The treatment periods T2 and T3 were incorporated to add more cells into the body, aimed at repair and regeneration of the affected tissue.

The laboratory parameters of the patients were assessed prior to the start of the treatment and then at regular intervals. Trained physicians and nurses carefully observed all the patients for antigenic or prophylactic responses.

The data for all the patients was validated by Moody's International (Document number NH-heSC-10-1), GVK Biosciences (NM-Hesc-10-1, 18 November 2010) and Quality of Austria Central Asia Pvt. Ltd. Accredi-

tation Company (Document number QACA/OCT/2013/26). These companies were allowed to examine the medical and statistical data present at the institute and were also able to meet the patients.

### Case 1

A 44-year-old male was admitted at our facility on 10 March 2006 with the diagnosis of DM. The patient was apparently well till 2001, when he developed persistent rashes on his body which were not responding to the treatment given. The patient also complained of increased thirst and increased urination frequency for a long time before the diagnosis.

On investigation, glycated hemoglobin (HbA1c) was 8.2% and fasting sugar range was 250 - 3000 mg/dl. Patient had a strong family history with mother positive for DM. He was a smoker and alcoholic. He had been taking oral hypoglycemic drugs, including metformin (250 gm, BD, Schwitz Biotech), glyzide M (80 mg, BD, Panacea Biotech) and Insulin (5 IU). He was reported to be allergic to both wheat and dairy.

At our facility, the patient was given hESC therapy as a primary treatment. He was put on strict anti-diabetic diet. Following the treatment, the patient felt stable with respect to the disease, stopped the oral hypoglycemic drugs and his blood glucose levels were under control. HbA1c reduced to 5.2%. The patient showed improved quality of life, mental focus and eye sight. He had increased energy levels and was not allergic to wheat and dairy products any more. He does not consume alcohol and his diabetes is under control. He does not take any anti-diabetics. The improvement in symptoms of the patient observed after the hESC therapy is presented in **Table 1**.

### Case 2

A 55-year-old male was admitted to our facility on 2 August 2011 with chief complaints of weakness of lower limbs (LLs), pain in LLs on walking, discomfort in LLs in supine position, inability to sit for a long time, weight loss (10 Kg) within 3 months and generalized weakness.

The patient was apparently well till December 2010 when he started feeling weakness, discomfort and occasional pain in LLs. The patient also reported to have significant weight loss. He had difficulty in initiation of sleep. He also complained of numbness in the feet which was on and off in character. He did random blood sugar testing at home (290 mg/dl). His physician put him on oral hypoglycemic drugs, including Amaryl M2 (500 mg BD, Sanofi Aventis), Diavit (OD, Franco Indian Pharmaceuticals Ltd.). Patient had a strong family history with sister positive for DM.

On investigations, HbA1c was 8.2%, fasting plasma glucose (FPG) was 218 mmol/ml, postprandial glucose (PPG) was 289 mmol/ml and level of serum insulin was 15.8 mIU/L.

At our facility, the patient was given hESC as primary treatment. He was put on strict anti-diabetic diet. He took four courses of hESC treatment. Following the treatment, the patient showed a significant improvement. He stopped all the oral hypoglycemic drugs and his blood glucose levels were under control. HbA1c was 5.2 mmol/L,

**Table 1.** The improvement in symptoms of the patient observed after the hESC therapy.

Case No.	Symptoms	
	Before	After
1.	<ul style="list-style-type: none"> <li>• Patient has been taking oral hypoglycemic drugs (Metformin, BD)</li> <li>• Low energy level</li> <li>• Poor eye sight</li> <li>• Patient is allergic to both wheat and dairy</li> </ul>	<ul style="list-style-type: none"> <li>• The oral hypoglycemic drugs were gradually stopped and his blood glucose levels are under control</li> <li>• Increased energy levels</li> <li>• Mental focus and eyesight improved</li> <li>• Not allergic to wheat and dairy products anymore</li> </ul>
2.	<ul style="list-style-type: none"> <li>• Patient has been taking oral hypoglycemic drugs</li> <li>• Weakness of lower limbs</li> <li>• Pain in lower limbs on walking</li> <li>• Discomfort in lower limbs on supine position</li> <li>• Unable to sit for a long time</li> <li>• Weight loss (10 kg)</li> </ul>	<ul style="list-style-type: none"> <li>• The oral hypoglycemic drugs were gradually stopped and his blood glucose levels are under control</li> <li>• Patient feels more energetic with improvement in generalized weakness</li> <li>• Pain in lower limbs is not there post treatment</li> <li>• Patient can sit and work for longer duration</li> <li>• Patient had regained weight</li> </ul>
3.	<ul style="list-style-type: none"> <li>• Tingling sensation and numbness of lower limbs</li> <li>• Pupils react to light, but poor vision</li> <li>• Knee and ankle: bilateral mute</li> <li>• Patient uses stick for walking</li> <li>• Bladder sphincter: impaired control</li> </ul>	<ul style="list-style-type: none"> <li>• Improvement in leg pain and burning sensation</li> <li>• He is able to see well now</li> <li>• Stamina and endurance improved with a feeling of wellbeing and less fatigue</li> <li>• Gait pattern is improved and could walk without stick</li> <li>• Muscle strength improved</li> </ul>

PPG was 110 mmol/ml and FPG was 140 mmol/L (**Table 2**). The patient felt more energetic with improvement in generalized weakness with no other complaints. The patient was able to sit and work for longer duration, and has also regained the lost weight. Now, the patient is stable with respect to the disease.

### Case 3

A 77-year-old male was admitted at our facility on 5 January 2012 with chief complaints of back pain, burning pain and weakness in LLs, stiffness of feet, profuse sweating, sleepless nights, constipation, tiredness, pause while performing the act of urination and dizziness. The patient was blind from one eye and had 20% vision from the other (right eye > left eye). The patient was reported to walk using a stick, slow gait and had a bilateral mute in knee and ankle and impaired control over bladder sphincter. On investigation, HbA1c was found to be 8.1% and the level of serum insulin was 145.2 mIU/L.

The patient was a diagnosed case of DM II since 25 yrs, hypertension until 20 yrs, diabetic neuropathy and retinopathy since 6 yrs and history of poor dietary compliance. The patient had been taking antidiabetics, including metformin (500 mg BD; Schwitz Biotech) and the lantus (40 units Sanofi-Aventis) and antihypertensives simvastatin (40 mg; Ranbaxy Laboratories Ltd.), vildagliptin (50 mg; Novartis), furosemide (40 mg; Provizer Pharma) and lisinopril (10 mg; Aventis Pharma).

The patient was given hESC as a primary treatment along with extensive physiotherapy. He was also put on the strict anti-diabetic diet. Following the treatment, patient showed improvement in maintaining blood sugar levels within normal range with minimal insulin and oral hypoglycemic drugs. His HbA1c after therapy reduced to 6.2%. Patient reported reduction in leg pain and burning sensation, improvement in vision, stamina and endurance, improved gait pattern and muscle strength. The improvement in symptoms of the patient observed after the hESC therapy is presented in **Table 1**.

## 3. Discussion

Our study used in-house cultured hESCs to treat patients with DM. Following the hESC therapy, blood glucose levels and the blood pressure were within the normal range. All the patients showed improvement in their quality of life, mental focus, vision, stamina and endurance, gait pattern, LL strength and reduction in the pain in LL. One of the patients was able to move on the treadmill for 10 minutes, able to sit and work for longer duration.

Nowadays extensive research on the use of stem cell has shown a hope for cure of DM [21] [22]. Stem cells that have been investigated in the treatment of DM include pluripotent stem cells (PSCs), mesenchymal stem cells (MSCs) and embryonic stem cells (ESCs) [7] [21] [22].

Suheir and colleagues used pluripotent (undifferentiated) hESCs as a model for lineage specific differentiation of hESCs in both adherent and suspension culture. They observed *in vitro* differentiation of the cells with the trait of insulin producing  $\beta$ -cells. These cells can be validated by differentiation dependent manner and associated with the presence of other  $\beta$ -cell markers. These sighting legalize the hESCs model system as a promising base for enrichment of  $\beta$ -cell and a likely source for cell replacement therapy in diabetes [23].

Pellegrini and colleagues showed the possibility that  $\beta$ -cells might be obtained from patients *via* cell programming and differentiation. In this experiment, the human induced pluripotent stem cells (iPSCs) were derived and differentiated into pancreas obligate cells and transplanted in immune deficient mice. These transplanted pancreatic cells secreted the C-peptide in response to glucose stimulus. The study showed potential of stem cells in obtaining insulin producing cells after engrafting [24].

Another study found that MSCs could be differentiated into insulin producing cells when transfected with PDX-1 mRNA [25]. A review by Davey *et al* also concluded that MSC therapy holds a potential for treating micro vascular complications and other secondary complications associated with diabetes [7]. Embryonic and

**Table 2.** Demographics of the patient before and after the hESC therapy.

Case No.	HbA1c (%)		FPG (mmol/L)		PPG (mmol/L)	
	Before	After	Before	After	Before	After
1	8.2	5.2	-	-	-	-
2	8.2	5.2	218	110	289	140
3	8.1	6.2	-	-	-	-

adult stem cells are found to be beneficial in the long term treatment of diabetes [21]. An *in vitro* study by As-sady and colleagues showed that hESC can differentiate into cells with characteristics similar to insulin-producing  $\beta$ -cells [23]. MSCs are known to act by a mechanism of paracrine secretion that involves production of growth factors and cytokines. The secreted factors promote differentiation of progenitor cells into endogenous progenitor cells (EPCs) and homing of EPCs, thus resulting in angiogenesis and tissue regeneration in diabetic wounds [26]. hESCs used in our study might have shown their therapeutic effect by following the same mechanism.

#### 4. Conclusion

The use of hESC therapy in our study patients was safe and effective. The patients showed reduction in HbA1c, maintaining blood sugar levels within normal range with minimal insulin and oral hypoglycemic drugs. No patients experienced severe adverse events or serious adverse events during the study as a result of hESC therapy. hESC therapy was well tolerated among all the patients included in the study. No teratoma formation was seen following the treatment. Thus, hESC therapy holds a future potential to cure diabetes. But clinical trials with large population sizes are needed to be conducted to gather evidences favoring the use of hESCs in the treatment of DM.

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#### Disclosure

The author declares that she has no conflict of interest.

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