Case Reports

Treatment of a Type-1 diabetic adolescent male with mitral valve prolapse with his mother's insulin secreting adipose tissue derived mesenchymal stem cells and bone marrow derived haematopoietic stem cells

Umang G Thakkar[1], Aruna V Vanikar[2], Hargovind L Trivedi[1,3], Shruti D Dave[2]

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Introduction

Type-1 diabetes mellitus (T1DM) results from a cell-mediated autoimmune attack against pancreatic beta cells confirmed with positive antibodies against glutamic acid decarboxylase (GAD)[1]. Symptoms of the disease appear when insulin making β-cell mass gets reduced by approximately 90% leading to severe insulin deficiency and hyperglycaemia[1]. At present, the only therapeutic option for management is life-long exogenous insulin. Population studies show that more than one half of the patients with MVP are asymptomatic and usually have a benign course[2]. We report the treatment of a16 year old adolescent male with MVP and 10 years of T1DM with his mother's insulin-secreting adipose-tissue derived mesenchymal stem-cells (IS-AD-MSC) along with her bone-marrow (BM) derived haematopoietic stem-cells (HSC).

Case report

A 16 year old adolescent male, diagnosed as having T1DM since 2004, presented with weakness, fatigue and uncontrolled raised blood sugar for the past 12 months. His weight was 46 kg and his height 168 cm. He was admitted to G.R. Doshi and K.M. Mehta Institute of Kidney Diseases & Research Centre- Dr. H.L. Trivedi Institute of Transplantation Sciences for stem cell therapy (SCT) in 2009. His fasting blood sugar (FBS) and post-prandial blood sugar (PPBS) were 280 mg/dl and 380 mg/dl respectively. The serum C-peptide was 0.05ng/ml and the glycosylated haemoglobin 11.3%. He had a biphasic-isophane insulin requirement of 104 IU daily. His urine sugar was +4 with absent serum acetone. He had 300 IU/ml (normal <10IU/ml) GAD antibody by ELISA (Euroimmun, UK), 8.8 U/ml (normal <12U/ml) insulin antibody and absent anti-islet cell antibody. He had unremarkable general and vital examinations. On cardiovascular examination, a mid-systolic click was audible at the cardiac apex. Electrocardiogram suggested bigemini/trigemini due to premature atrio-ventricular contractions and echocardiography suggested MVP and tab propranolol was started 2.5 mg twice a day. He was subjected to SCT (figure 1) after informed consent and approval by the Institutional Review Board.

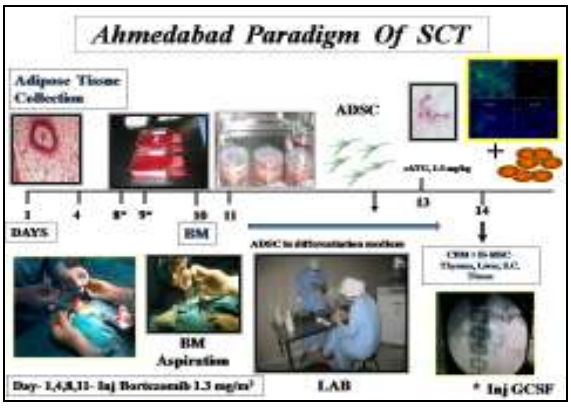

Figure 1: ***Paradigm of stem cell therapy***

IS-ADMSC were generated as per our previously described technique[1] from 10g of allogenic adipose tissue from anterior abdominal wall on day-1 under local anaesthesia (LA) from his mother. This was subjected to generate *in-vitro* MSC on day-11, which further differentiated into insulin-secreting cells on day-14. This was quantified and tested for sterility,

[1]*Department of Regenerative Medicine and Paediatrics,* [2]*Department of Pathology, Laboratory Medicine, Transfusion Services and Immunohaematology,* [3]*Department of Nephrology and Transplantation Medicine, G. R Doshi and K.M. Mehta Institute of Kidney Diseases & Research Centre (IKDRC)-Dr. H L Trivedi Institute of Transplantation Sciences (ITS)*

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viability and insulin-secreting markers (pax-6, ipf-1 and isl-1) by immunofluorescence. C-peptide and insulin secretion were tested by chemiluminescence assay (Lumax, USA). 100 ml BM was aspirated from posterior superior iliac-crest under LA from his mother on day-10, followed by administration of 300µg granulocyte colony stimulating factor subcutaneously on day-8 and 9 for *in-vitro* generation of HSC. On day-14, 4ml IS-ADMSC of 2×10^3/µl with $CD90^+$-29.70%, $CD73^+$-15.12% and 100 mL HSC of 2.64×10^4/µl with $CD34^+$-0.11% were infused into subcutaneous tissue, 20ml (IS-AD-MSC, 1ml+BM-HSC, 19ml) and superior mesenteric artery to portal route, 81ml (IS-AD-MSC, 2ml+BM-HSC, 79ml) + brachiocephalic artery to thymic, 3ml (IS-AD-MSC, 1ml+BM-HSC, 2ml) circulation via femoral artery catheterization after infective endocarditis prophylaxis under LA uneventfully.

Patient's blood sugar levels were monitored 4 hourly for the first 2 days after stem cell infusion and patient was discharged at the end of the 3rd day with advice to take insulin according to sliding scale according to blood sugar and monitoring the FBS and PPBS daily for 5-days and then weekly for the first month, fortnightly for the next 2-months and monthly thereafter till the end of 1-year. Subsequently he was advised to check HbA1c every 3-months.

Over a follow-up of 24 months (Figure 2), patient is maintaining FBS and PPBS, 203 and 225 mg/dL respectively, serum C-peptide increased to 0.34ng/mL from 0.05ng/mL, glycosylated hemoglobin reduced to 7.4% from 11.3% and daily biphasic-isophane insulin requirement reduced to 48IU from 104IU. There were no untoward side effects recorded. This was safe, effective, reproducible and viable therapeutic option.

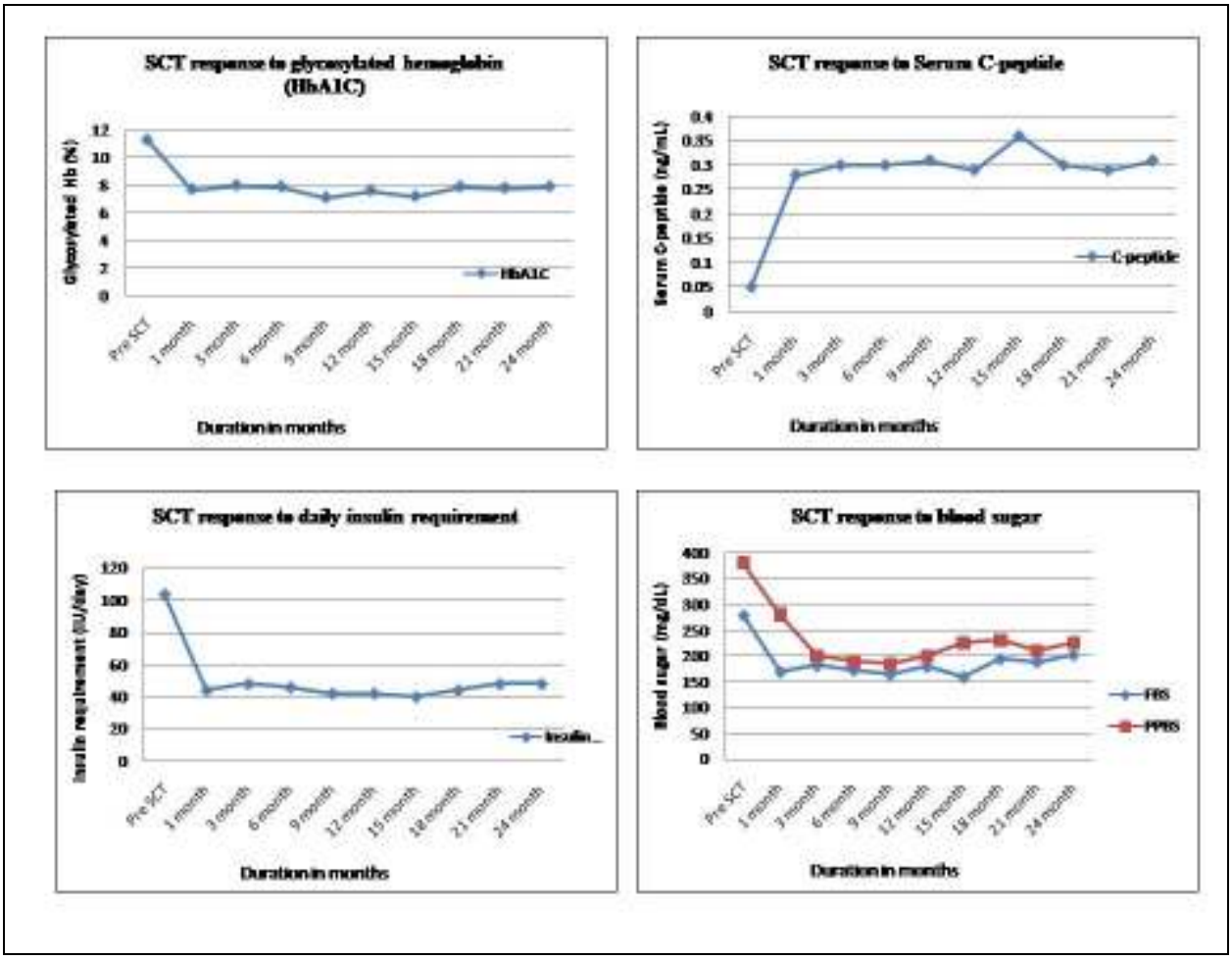

Figure 2: ***SCT response to glycosylated hemoglobin, serum C-peptide, daily insulin requirement and fasting blood sugar and postprandial blood sugar status***

Discussion

Both embryonic and adult stem cells have been used to generate surrogate beta cells or restore beta cell functioning demonstrating generation of insulin-secreting cells that normalized blood glucose values when transplanted into diabetic animal models[3].

MSCs are able to serve as a cellular vehicle for the expression of human insulin gene and have promising therapeutic role in the correction of metabolic derangement of DM and also in controlling and reverting complications in diabetics[4]. We have generated *in vitro* MSC from human adipose tissue which qualify the definition standardized by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy and further differentiated them into insulin secreting cells under defined culture conditions phenotypically identical to pancreatic β-cells without using any xenogeneic material. Cells expressed transcription factors pdx-1, pax-6, and isl-1, and are central controlling genes capable of reprogramming non-pancreatic cells to surrogate β-cell functions and up-regulate gene expression, nourishes the cells and prevents their further proliferation[1]. The HSCs were used along with IS-ADMSC because HSC infusion with conditioning is believed to create active and passive tolerance by clonal deletion/T-cell suppression[5]. Hence we decided to explore this protocol which has already given sustained benefits without any adverse effects and decided to infuse the cells in thymic circulation to achieve central tolerance[6] and portal circulation since liver is the most tolerogenic organ[7]. Subcutaneous tissue being an immunologically privileged site, we decided to inject part of the cells in abdominal subcutaneous tissue, so that it will serve as a "back-up reservoir" for insulin supply[8].

To our knowledge, this is the first case report of successfully treating Type1 DM patient with SCT associated with MVP. IS-AD-MSC and BM-HSC co-infusion could provide better diabetic control. This novel therapy is safe and effective and will open up the avenues for millions of diabetic children all across the world.

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