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A Preliminary Safety and Efficacy Evaluation of Direct Intra-pancreatic Tail Delivery of Autologous Bone Marrow-Derived Mononuclear Cells in Egyptian Patients with Type 1 Diabetes Mellitus

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Abstract

Background: Type 1 diabetes (T1D) results from the autoimmune destruction of islet β-cells. Recent studies have shown that cell therapy shows promise in combating this. However, many problems are associated with the systemic administration of these cells, including nonspecific accumulation of cells in an organ other than the target organ. The objective of this study was to evaluate the safety and efficacy of autologous bone marrow-derived mononuclear cells (A-BMMNCs) via Direct Trans-gastric Intra-pancreatic (DTI) delivery as a potential treatment for Egyptian patients with T1D.

Methods: 17 patients whose ages ranged between 2 and 30 years were assigned to receive a single administration of A-BMMNCs via DTI delivery and followed up for 1 year. The main outcome was assessment of safety. We also assessed islet cells and insulin antibodies pre-transplantation and, in patients who were serologically positive for these, again 6 months post-transplantation. Glycated hemoglobin (HbA1c) and fasting and 2 h postprandial C-peptide levels (FCP, 2h-CP) were also measured to evaluate β-cell function. Finally, we determined insulin doses.

Results: No complications were recorded during the study across all patients. Islet cell antibodies were undetectable pre-transplantation for all patients. 10/17 patients were positive for anti-insulin antibodies pre-transplantation and 7/17 were negative; of those who were positive, 6 converted to being negative for anti-insulin antibodies and 4 were still positive for anti-insulin antibodies) within 1 year post-transplantation. HbA1c and insulin doses were significantly lower at 5 months post-transplantation compared with baseline. FCP levels rose significantly in the first 2 months with little change in 2h-CP levels.

Conclusions: A-BMMNCs transplantation via DTI delivery route is a simple, safe and innovative procedure. It can temporarily modify the course of T1D, could be a beneficial treatment modality in the future to control the progression of the disease and potentially opening a new way to cure diabetes (ISRCTN15591075).

Keywords: Cell therapy; Bone marrow; Diabetes

Abbreviations A-BMMNCs: Autologous Bone Marrow-Derived Mononuclear Cells; BMT: Bone Marrow Transplantation; CT: Computed Tomography Scan; DTI: Direct Trans-gastric Intra-Pancreatic; e.g.: For Example; FCP, 2h-CP: Fasting and Postprandial 2-h C-Peptide Levels; HbA1c: Glycated Hemoglobin; HSC: Hematopoietic Stem Cells; IDDM: Insulin Dependent Diabetes Mellitus; JCI: Joint Commission International; MSC: Mesenchymal Stem Cells; T1D: Type 1 Diabetes.

Background

Type 1 diabetes mellitus is a complex disease resulting from autoimmune destruction of the majority of islet β-cells [1]. Although exogenous insulin therapy is effective in protecting type 1 diabetic patients from diabetic ketoacidosis, diabetes is still considered one of the major causes of morbidity and mortality worldwide [2,3]. Some researchers have suggested that C-peptide deficiency may be implicated in this dilemma. C-peptide is not only a by-product and marker of endogenous insulin secretion, but it is also a biologically active molecule which is able to protect against the development of long term complications [4]. Despite advanced efforts to simulate the normal function of pancreatic β-cells by islet transplantation, a shortage of donor supply and the necessity of lifelong immunosuppressant use have been considered as limiting factors for success; thus transplantation is selectively reserved for certain patients [5,6]. Another approach has been the use of systemic immune-modulators. However, side effects and relapse after discontinuation remain challenges for this strategy [7]. Alternatively, recent therapeutic studies have aimed to induce tolerance and restore β-cell function by using hematopoietic stem cells (HSCs) or mesenchymal stem cells

(MSCs) derived from bone marrow or other sources. Stem cells may offer new opportunities for the treatment of patients with insulin dependent diabetes mellitus (IDDM) [8-10].

Bone marrow is a diverse source of stem cells. Its transplantation can assist in achieving tissue repair, regeneration and/or modulating the immune response of various immune diseases [11-13]. Systemic delivery routes (intravenous/intra-arterial injections) are commonly used to administer the cells in many preclinical studies and clinical trials. However, there may be problems associated with these systemic delivery routes [14,15]. Moreover, little is known about the homing mechanism of stem cells to the damaged tissue [16,17]. Nonetheless, there is a theoretical possibility that administration of autologous bone marrow-derived mononuclear cells (A-BMMNCs) directly to the tail of the pancreas may be a safe and feasible approach. The aim of the current study was to evaluate the safety of a new strategy (designed to enhance localization of the injected cells to the diseased pancreas along with avoidance of nonspecific organ entrapment) by a single administration of A-BMMNCs via a computed tomography (CT) scan-guided direct trans-gastric intra-pancreatic (DTI) delivery route. Furthermore, it aimed to explore whether this treatment leads to improved β-cell function in IDDM patients.

Methods

Patients

Participants in this study were males and females, with ages ranging from 2 to 30 years of age, who had confirmed T1D based on the American Diabetes association (ADA) 2015 criteria for diagnosis [18].

Body mass index (BMI) was ≤ 29.9. Patients were excluded if they had active infections; any chronic or acute illness, antibodies to hepatitis B surface antigen, hepatitis C, human immunodeficiency virus or evidence of diabetic complications at baseline, or if they were pregnant, were breast-feeding or intended to become pregnant during the study. Subjects with clinically relevant uncontrolled medical conditions not associated with diabetes (such as hematologic, renal, hepatic, neurologic, cardiac and respiratory conditions), evidence of active malignancy or a prior history of active malignancy were also excluded. Seventeen Egyptian patients were assessed for eligibility and assigned to receive A-BMMNCs transplantation; adult participants gave consent to participate in the study and the parents of child participants gave consent on their behalf. Before being asked to give their consent, all adult patients and the parents of child patients received detailed information about the potential risks of the procedure during interviews conducted when they arrived at the hospital and were also given written material at that time. During the study, subjects had to be willing to comply with "insulin management" as directed by the endocrinologist with the goal of maintaining blood glucose as close to normal as possible and maintaining HbA1c levels within the age-specific ranges recommended by the ADA 2015 guideline as much as possible. All patients were given the same type of insulin (rapid and long acting) as standard medical therapy and they came to the hospital according to the schedule of study visits and protocol requirements. The trial was approved by the institutional review board of Wadi El-Neel hospital (JCI accredited hospital).

Procedure

Under sedoanalgesia, the trial was designed so that percutaneous aspiration of bone marrow from right posterior iliac crest puncture could be performed in the left lateral position, as described by the guidelines of the European Society of Blood and Marrow Transplantation. To optimize cell yield, 2-3 punctures were performed and a total of 2.2 ml of bone marrow per kg of body weight up to a maximum of 220 ml was aspired. The aspired marrow was immediately mixed with heparin and processed using a cell processing system (SEPAX 2, Biosafe, Eysins, Switzerland) and a bone marrow separation set. Briefly, the separation is based on gravity centrifugation and the sample was separated into red blood cell, plasma and mononuclear cell fractions according to the manufacturer's instructions. The mononuclear fraction was set to be 10% of the total bone marrow aspirate volume. In this way, only minimal processing of bone marrow was performed. In the supine position, the location of a suitable trans-gastric intra-pancreatic puncture site was performed with a spinal needle under CT scan guidance. The total count of the transplanted A-BMMNCs was 1×10^6 cells/kg. The count of CD34+ cells was $1.04 \times 10^6 \pm 0.0045 \times 10^6$ (mean value ± SE) cells/ml. In total, 5 ml of the bone marrow mononuclear cell fraction was injected *via* (DTI) delivery to the pancreatic tail.

Clinical and laboratory follow-up

Pre-procedure: Sex, age, duration from the onset of diabetes to the transplantation and clinical parameters including weight and height were recorded. Weight and height were expressed as BMI. Blood samples for assessment of HbA1c, fasting C-peptide (FCP) and 2h-C-peptide (2h-CP) levels (as indirect measures of endogenous insulin secretion), levels of islet cells and insulin antibodies were collected and insulin doses were determined.

During the procedure

Before discharge, clinical assessment of bleeding from the bone marrow and trans-gastric puncture sites and assessment of abdominal pain and other clinical complaints (e.g. nausea, vomiting, pain and fever) were assessed. Blood amylase levels were measured (to exclude acute pancreatitis complication). Hematocrit values were obtained only in suspected bleeding cases.

Follow-up at 1–2, 3–5, 6–8 and >8 months

Patients were evaluated by a physician for abdominal pain and any clinical complaints. After 6 months, if islet cell and insulin antibodies were abnormal and serologically positive before the transplantation, they were re-measured to record any improvement or decline in these antibodies. If the patients were negative for islet cell or insulin antibodies before the transplantation, the test was not repeated after 6 months, as antibody testing and regular follow-up on a scheduled basis are not recommended as routine clinical practice for T1D patients [19]. At 1-2, 3-5, 6-8 and >8 months post-transplantation, HbA1c, FCP, 2h-CP, insulin doses and frequency of hospitalization for hypo- and hyper-glycemic attacks (common acute short-term complications of uncontrolled diabetes that can lead to hospitalization and account for the majority of costs associated with diabetes care) were assessed [20,21]. All patients were encouraged to self-monitor blood glucose at least twice daily.

Biochemical and clinical measurements

Serum levels of anti-islet cell antibodies were measured by ELISA technique using commercial kits DRG® Islet Cell Autoantibodies (ICA)

ELISA, Ref EIA-1594 (DRG International, Inc., USA) and the results were considered positive if
they were greater than 1.05 U/mL. Serum levels of anti-insulin antibodies were measured by ELISA technique using commercial kits, Ref DRG® Anti-Insulin EIA-3608 (DRG International, Inc., USA) and the results were considered positive if they were greater than 10 U/mL. HbA1c was measured by high performance liquid chromatography (HPLC) technique. Serum C-peptide levels were measured by ELISA technique using commercial kits DRG® C-Peptide ELISA, Ref EIA-1293(DRG International, Inc., USA). The normal range for fasting c-peptide 0.5-1.9 ng/ml and 2 h postprandial c-peptide 1.5-3.4 ng/ml. Serum amylase activity was measured as per the routine procedure which was followed in the Laboratory Department of Wadi EL-Neel hospital, Cairo, Egypt.

Study endpoints

The morbidity, mortality and unwanted side effects from the transplantation were the key end points of the study to assess safety. Secondary outcomes were serological changes in positive islet cell and insulin antibody levels, decreased HbA1c and increases in FCP and 2h-CP levels to assess β-cell function. Insulin doses were also determined.

Statistical analysis

Data analysis of the study was conducted using Statistical Package for Social Sciences, IBM software (SPSS) version 22.0 (IBM Corp., New York, USA). Continuous variables with normal distribution were analyzed using one way repeated measure ANOVA and expressed as mean ± standard error (SE). Confounders were tested between baseline and treatment groups using two-way ANOVA with one repeated measure statistical model. For categorical variables, results were compared using McNemar's test and expressed as count. P-value ≤ 0.05 was considered statistically significant. Finally, the confounding factors between the control and treatment groups have been corrected before the indicators are compared. Confounders Sex (Male *vs.* Female); Age (>18*vs.*<18 years); Duration from onset to the transplantation (>24*vs.*<24 Months); BMI (>18.5*vs.*<18.5 kg/m^2) were tested between the baseline group and treatment groups before the indicators are compared using two-way ANOVA with one repeated measure statistical model. No significant differences were met between the control and the treatment time points for the four confounders.

Results

30 patients were entered into the screening phase based on the inclusion criteria. 17 patients (8 male and 9 female) were finally enrolled who agreed to sign the informed consent to participate in the study. Sample size in our study was determined upon the revision of similar studies with the same concept (clinical trials using stem cells in diabetes patients). Our 17 patients after excluding patients who didn't participate in the study were still higher than many of the similar studies. Although it would add to the study to have a control group but in a self-control study, each patient is considered to be his or her own control and that's why no separate control group was established. The scope of our study was to follow the consequences of injecting stem cells by this delivery route on the improvement or deterioration of type I diabetes patients' status over time. Before treatment results were considered as a base line or control group for the rest of time points [22,23]. Self-control design has its advantages: It should eliminate subject to subject variability; it is very useful in dealing with sample size limitation as it can produce results that are statistically and clinically valid with far fewer patients than it would otherwise be required. Mean (± SD) age was 14.7 ± 8.24 years and mean (± SD) disease duration was 33.9 ± 36.8 months. Baseline demographic and biochemical parameters are summarized in Table 1. All 17 included patients received exogenous insulin with a mean dose ± SE (37.735 ± 5.151) as described in Table 2. All patients underwent A-BMMNCs transplantation via DTI delivery at the bone marrow transplantation unit at Wadi El-Neel hospital. The patients received no other treatments in addition to their routine insulin regimen, and they maintained their regular diets and lifestyle habits during their hospitalization and follow-up. All of the patients were followed up for 12 months. No significant differences were met between the control and the treatment time points for the four confounders (sex, age, duration and BMI) that mentioned in Table 1 before the results are compared.

Transplantation-related complications

There were no documented complications during or after the procedure (up until the completion of the study), with no cases of suspected bleeding or abdominal pain recorded. Also, for all 17 patients, no visible hematoma or other clinical complains which occurred in other studies using intra-pancreatic endovascular (through the pancreatic arteries) delivery route were reported [22,23]. Amylase test for all patients was normal post-transplantation.

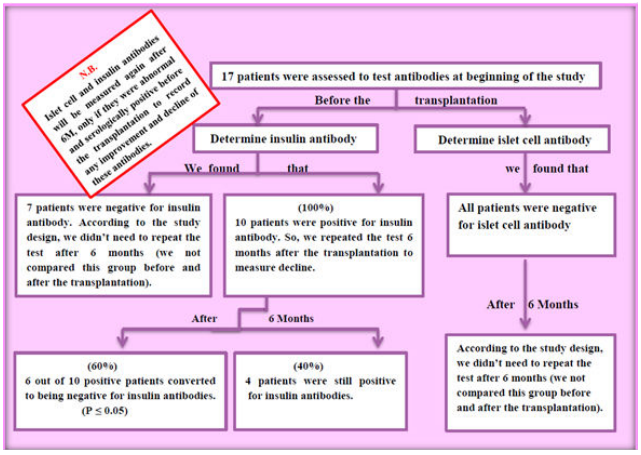

Figure 1: Schematic representation of changes in antibodies during the 12-month study period.

Antibody analyses

Islet cell antibodies were undetectable pre-transplantation for all patients. 7/17 patients were negative for anti-insulin antibodies pre-transplantation. In accordance with the study design, we did not repeat the test 6 months post-transplantation for patients who were negative for islet cell and insulin antibodies pre-transplantation because there are no recommendations for regular monitoring of auto-antibodies in type 1 diabetic patient. Insulin antibody analysis was conducted 6 months post-transplantation for the ten patients who were positive for insulin antibodies pre-transplantation. We found that of these regardless of pre-transplantation C-peptide levels, duration from onset to the transplantation and other parameters such as sex and age 6 (60%) converted to being negative for anti-insulin antibodies while 4

(40%) remained positive (Figure 1 and Table 1). For example, as shown in Table 1, patient no. 1 was positive for anti-insulin antibodies and had high C-peptide levels and short duration of disease; patient no. 2 was positive for anti-insulin antibodies and had low C-peptide levels and long duration. Both, however, converted to being negative for anti-insulin antibodies after 6 months regardless of their other dissimilar parameters. Insulin antibody analysis 6 months post-transplantation for the 4 patients who remained positive for insulin antibodies showed that patients no. 9 and 10 had higher insulin antibody titers than their respective pre-transplantation values (patient no. 9: baseline 31 U/mL, 6 months 50 U/mL; patient no. 10: baseline 18.2 U/mL, 6 months 61.8 U/mL). Patient's no. 7 and 8, in contrast, had the same titers for insulin antibodies as pre-transplantation.

Patients No./Sex	Age (Years)	Duration (Months)	BMI (KG/m2)	HbA1c (%)	FCP (ng/ml)	2h-CP (ng/ml)	Insulin AB	Insulin AB after 6M
1\F	16	7	23.3	12.5	0.4	0.7	P	N
2\M	11	64	26.7	8.9	0.1	0.1	P	N
3\M	29	72	22.4	7.1	0.3	0.8	P	N
4\F	18	5	23.4	10.6	NA	3.3	P	N
5\F	20	131	26.6	7.8	0.01	0.01	P	N
6\F	19	48	22.9	9.6	0.1	0.1	P	N
7\F	9	24	17.1	8.2	0.5	0.8	P	P
8\M	22	35	23.8	6.7	1.1	2.9	P	P
9\M	2	3	20	7.7	0.01	0.01	P	P
10\M	2	1	20	9.1	0.1	0.20	P	P
11\M	25	2	24.7	8.7	NA	2.6	N	NA
12\F	4	1	15.6	8.3	0.5	2.1	N	NA
13\F	6	27	14.3	9.3	0.1	0.18	N	NA
14\M	15	4	19.3	9.8	NA	NA	N	NA
15\M	20	20	24.3	8.9	0.1	0.4	N	NA
16\F	10	48	25.3	9.7	0.01	0.01	N	NA
17\F	22	84	18.2	11.2	0.1	0.1	N	NA
Mean (SD)	14.7 8.24	33.9 36.8	21.5 3.84	9.1 1.47	0.20 0.29	0.84 1.13		

Note: P: Positive; N: Negative; HbA1c: Glycated Hemoglobin; FCP: Fasting C-peptide; 2h-CP: 2-hour Postprandial C-peptide; AB: Antibody; BMI: Body Mass Index; NA: Not Available; M: Month.

As the study was designed to monitor only abnormal and positive antibodies to record any decrease after 6 months we did not repeat the test after 6 months for the patients who were negative for insulin antibody pre-transplantation; thus those data are not available. Islet cell antibody data are not included in this table because it was negative pre-transplantation for all patients. N.B. antibody testing and regular monitoring to follow up type 1 diabetic patient is not recommended (Bingley). Duration refers to the length of time from onset of diagnosis of diabetes to transplantation.

Table 1: Characteristics of type 1 diabetic patients pre-A-BMMNC transplantation and insulin antibody levels pre- and post-transplantation.

β-Cell Function and insulin doses

DTI transplantation of A-BMMNCs had a positive impact on HbA1c, FCP and 2h-CP levels. HbA1c levels declined for the first 5 months post-transplantation; they increased thereafter, but did not become higher than those observed pre-transplantation (Figure 2 and Table 2). FCP levels were maximal within 2 months post-transplantation; they then decreased slightly up to 6-8 months, although not to levels less than those observed pre-transplantation.

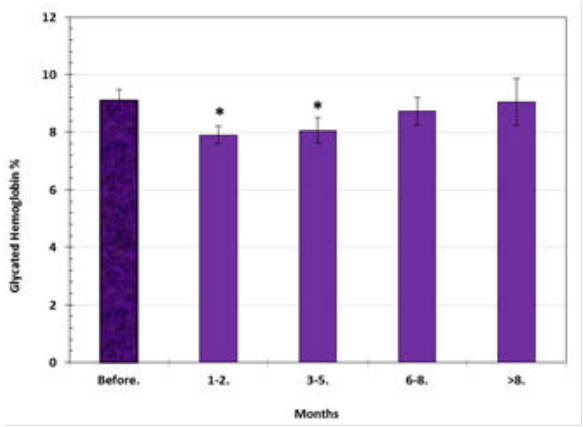

Figure 2: Changes in glycated hemoglobin levels between baseline, 1–2, 3–5, 6–8 and 8–12 months post-transplantation. Data expressed as mean ± S.E. *P ≤ 0.05 compared with baseline.

	Period (Months)				
	Before	1-2	3-5	6-8	>8
HbA1c	9.118 ± 0.358	7.906 ± 0.307	8.063 ± 0.439	8.729 ± 0.481	9.05 ± 0.816
Fasting c-peptide	0.243 ± 0.0808	0.805 ± 0.249	0.483 ± 0.211	0.514 ± 0.186	0.212 ± 0.0787
2 h C-peptide	0.893 ± 0.287	1.386 ± 0.445	0.795 ± 0.459	0.871 ± 0.354	0.45 ± 0.193
Total insulin doses	37.735 ± 5.151	35.706 ± 4.679	30.471 ± 4.255	37.735 ± 5.151	37.735 ± 5.151

Table 2: Changes from baseline to 1-2, 3-5, 6-8 and 8-12 months post-transplantation for A) glycated hemoglobin, B) fasting C-peptide levels, C) 2-hour postprandial C-peptide levels, D) exogenous insulin requirements.

Finally, FCP levels dropped below baseline levels at 8–12 months (Figure 3A and Table 2). Minor non-significant changes in 2h-CP levels were observed during 1-2 months post-transplantation. They then decreased below pre-transplantation levels (Figure 3B and Table 2). In addition, exogenous insulin doses were decreased gradually until they reached a significantly lower value at 3-5 months; they then returned to their pre-transplantation values (Figure 4 and Table 2). All patients had adequate glycemic control, and no hospitalizations due to hypo- or hyper-glycemic attacks were recorded during the 12 month study period.

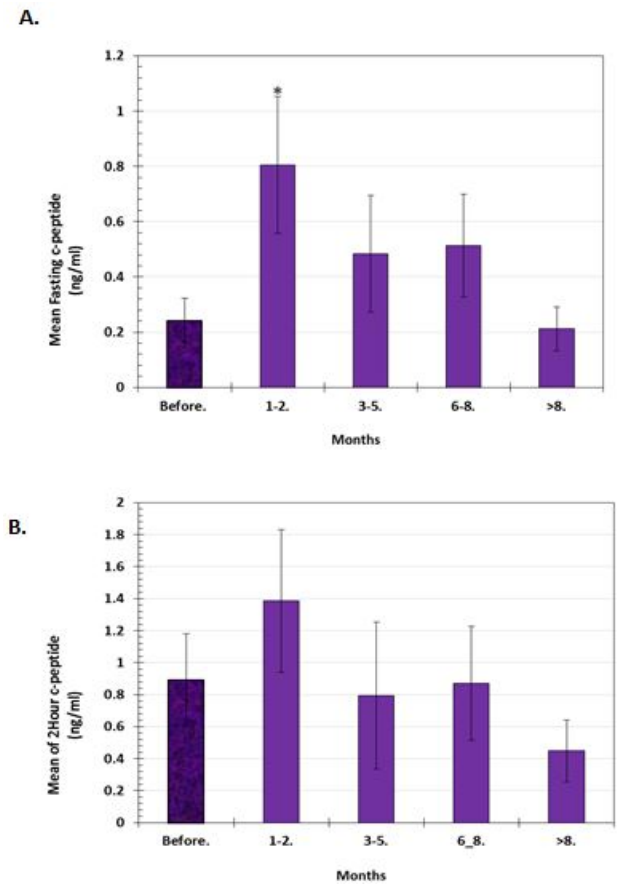

Figure 3: A: Changes in fasting C-peptide levels from baseline to 1–2, 3–5, 6-8 and 8-12 months. B: Changes in 2 h postprandial C-peptide levels from baseline to 1-2, 3-5, 6-8 and 8-12 months. Data expressed as mean ± S.E. *P ≤ 0.05 compared with baseline.

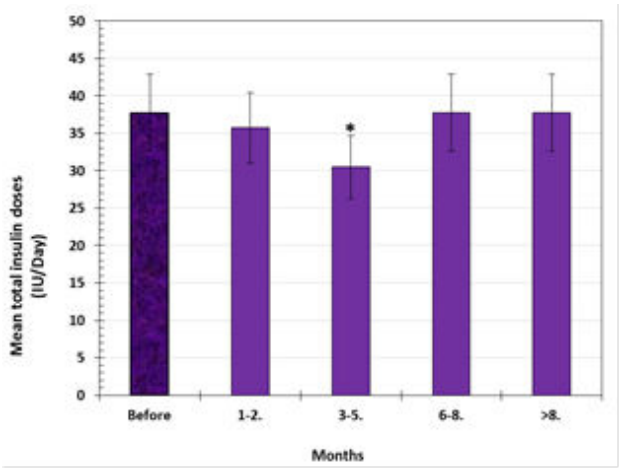

Figure 4: Insulin requirements of T1D patients at baseline and 1–2, 3–5, 6–8 and 8–12 months post-transplantation. Data expressed as mean ± S.E. *P ≤ 0.05 compared with baseline.

Discussion

To our knowledge, there are 3 possible ways to carry injected cells to the diseased pancreas. One is to inject the cells via the most convenient intravenous delivery route to allow the stem cells to reach their target with their special homing mechanism. Unfortunately, the homing mechanism of stem cells is not, as yet, fully understood; it may lead to nonspecific organ entrapment and thus needs further investigation [16,17]. The second technique involves introducing stem cells via the intra-pancreatic endovascular (through the pancreatic artery) route, which has the potential to cause severe complications, leading to impairment of the organ and loss of the benefit of the transplanted cells [22,23]. In addition, diabetes mellitus is associated with chronic vascular complications [24]. Consequently, the arteries that supply the pancreas could be affected in patients with IDDM; this may make them poor candidates for this procedure. The third way is direct delivery through part of the pancreas (direct intra-pancreatic). Data collected from many publications related to cell therapy and intra-pancreatic transplantation in animal models represent this as a promising therapeutic approach in T1D patients [25,26]. In this scenario, an innovative delivery method in T1D patients was developed by injecting the cells directly to the part of the diseased pancreas: head, tail or whole pancreas. Since similar improvements in hyperglycaemia were seen between stem cell transplantation into different pancreatic microenvironments, we selected the tail because the islet concentration in this area may be significantly greater than in other parts [27,28]. Furthermore, several modalities to replace injured pancreatic β-cells have been attempted. Despite achieving euglycemia, these could not overcome the common challenge among most of these approaches: the use of immune-modulatory regimens. The aim of these immune-modulatory regimens is to block the autoimmune nature of T1D, as B cells, T cells and other immune cells have a vital role in its pathogenesis [29-31]. However, many risks and temporary benefits have been associated with these modalities. Results from the present study suggest that the direct administration of uncultured, freshly isolated, single dose A-BMMNCs through the DTI route without an immune-modulatory regimen represents the safest strategy to treat type 1 diabetic patients. Furthermore, direct access ensures that the localization of cells in the pancreas can be achieved without possible complications that occurred with other methods [22,23].

Possible mechanisms of action of A-BMMNCs transplantation for type 1 diabetes mellitus

Bone marrow cells have previously been used in many clinical trials to improve β-cell function. Nevertheless, the exact mechanism of action of the various components of the bone marrow mononuclear cells (BMMNCs) cannot be studied by employing direct methods in humans. Consequently, their *in vivo* characteristics and functional roles have remained unclear [32,33]. Mechanistic studies either *in vitro* or using animal models of T1D and other autoimmune diseases suggest that bone marrow is a vital source of easily accessible adult stem cells. It consists of HSCs, MSCs and other BMMNCs [13]. HSCs may reconstitute the immune system towards a tolerant phenotype through its action on T cells, B cells and other regulatory immune cells. The rationale behind the use of HSCs in some studies was to preserve residual β-cell mass and support endogenous mechanism of regeneration [33-36]. Although HSCs probably do not have the capability to differentiate *in vivo* into large numbers of β-cells in patients with T1D, the best therapeutic outcome has been achieved with CD34+ HSC therapy [33,37]. MSCs are a non-hematopoietic cell type present in the bone marrow. They are multipotent stem cells that because of their ability to differentiate among many types of cells (including insulin producing cells under restricted conditions) could be a promising source from which to replenish injured tissue [38]. The differentiation capacity of MSCs into insulin producing cells *in vitro* and upon infusion *in vivo* has yielded conflicting results, both promising and discouraging [12]. However, the ability to differentiate is not the only characteristic that makes MSCs a candidate for therapeutic use in T1D. In addition, MSCs have been shown to have the potential to control major immune cells, which according to current knowledge are the main participants in the immuno-pathogenesis of T1D, owing to their immune-modulatory effects. These act to restore a balanced state between Th1/Th2, stimulate proliferation of regulatory T cells and suppress B cell activation and immunoglobulin production by plasma cells. They also inhibit differentiation, maturation and function of other autoimmune cells. Some studies have shown that injecting MSCs successfully increased β-cell mass, returning plasma glucose levels to normal values via the immune-modulatory properties of regenerated endogenous β-cells. Along with their immune-modulatory action, MSCs act via multiple other pathways, including immunosuppressive, antiapoptotic, angiogenic and anti-inflammatory. This occurs through secretion of a wide range of bioactive molecules, such as cytokines, chemokines and growth factors, which act mainly under injury circumstances to abrogate immune injury and enhance β-cell repair/regeneration [9,10,12,25,38]. Although a large amount of preclinical data supports the use of MSCs in T1D, the exact molecular mechanisms are still unclear and the number of molecules known to mediate their actions is constantly increasing, which adds to the uncertainty. However, HSCs and MSCs are not the only components of bone marrow cells implicated in treating DM. BMMNCs can combine synergistically with other bone marrow cells and may have a potential role in DM therapy. BMMNCs can secrete many bioactive elements that help to establish the tissue microenvironment that transiently supports and promotes patients' β-cell regeneration mechanism [12]. Our results, however, are in agreement with those of Wang et al. [12], as we used a heterogeneous BMMNCs mixture rather than a single type of cells. It is still unknown which specific cell type contributed to combating hyperglycemia and reversing insulin antibodies to a negative state [12]. If we had solely infused purified HSCs or MSCs, the molecular mechanism may have been illustrated more clearly. In addition, as Thakkar et al. [39] showed that autologous stem cells are more effective than allogeneic stem cells in T1D, we decided to use the autologous form.

Effect of administration of single dose of A-BMMNCs via DTI delivery to type 1 diabetic patients

Transplantation-related complications: In the present study, we document, for the first time, an intrapancreatic clinical trial of direct injection of A-BMMNCs into the tail of the pancreas in T1D patients. In assessing the safety of this procedure, no cases of bleeding were observed and amylase test was normal for all patients post-transplantation; there were no complications and no clinical complaints that were seen in other studies were reported from any patients during the follow-up period. The diverse characteristics (including wide age range and different disease durations) of T1D patients who were included in this study (Table 1) suggest that this technique is safely applicable across age groups (even in very young patients) and durations since diagnosis without short term risks. Very young patients were excluded from other studies because of side effects

that may be able to occur [40-58]. The safety of the direct intra-pancreatic tail delivery route is a concern and deserves further investigation. All 17 patients are still under follow-up to detect any late-onset complications.

Antibody analyses

Auto-antibodies are likely to play an important role in the pathogenesis of IDDM and its related complications [41-44]. In addition, based onADA criteria for diagnosis and classification, these autoantibodies act as one of the diagnostic markers to differentiate immune-mediated T1D (where the patient is positive for at least one of GADA, protein tyrosine phosphatase antibody, islet cell antibody or insulin antibodies) from type II diabetes [45]. Furthermore, while auto-antibodies may have a role in the prediction and prevention of T1D, the safest and most effective preventive immunosuppressant agents are still unknown [46,47]. Given this, we designed our preliminary study solely to monitor abnormal and positive pre-transplantation insulin and islet cell antibodies and to assess post-transplantation improvements or declines of these antibodies. However, the change from positive to negative status that occurred for anti-insulin antibodies Constitutes an improved outcome compared with a previous immune-modulatory report that showed only a decrease in anti-insulin antibodies during a one-year study period [5]. In addition, our observed improvement in anti-insulin antibodies was not seen in controlled clinical trials using other stem cell types in type 1diabetes during a one-year study period [57]. It may also represent a beneficial effect on humoral dysregulation and could be useful in the future as an immune intervention therapy aiding in diabetic prevention. Finally, only 2 out of 4 patients who were still positive for anti-insulin antibodies (patients no, 9 and 10) had higher anti-insulin antibody titers 6 months post-transplantation compared with pre-transplantation (patient no. 9: baseline 31 U/mL, 6 months 50 U/mL; patient no. 10: baseline 18.2 U/mL, 6 months 61.8 U/mL). These two patients were 2 years of age, suggesting that age is an important factor in elevated anti-insulin antibody titers. As diabetes is more aggressive in younger age groups, more investigation is needed to determine whether A-BMMNCs transplantation was the reason for this elevation, whether it was a consequence of diabetes in this age group or if other factors were responsible [48]. Our results are in agreement with Voltarelli et al. this change in humoral response did not have a negative influence on any other clinical parameters (since HbA1c and C-peptide levels were still at pre-transplantation levels or lower and amylase test was normal for all 4 patients who were still positive for insulin antibodies post-transplantation) [49].

β-cell function and insulin doses

Transplantation of A-BMMNCs had positive outcomes on β-cell function and transiently improved metabolic control. Furthermore, temporary improvement in β-cell function shows that impairment of the pancreas (which may occur with other delivery methods) did not occur as a result of this technique [23]. Unfortunately, when these data are compared with other successful studies in which patients became transiently insulin free, patients in this study did not achieve exogenous insulin independence. This may have been because the characteristics of our patients appear to have been different at baseline than in other studies. Most patients in compared studies had FCP and 2h-CP levels not less than 0.3 or 0.8 ng/ml respectively (there was still a larger mass of islet β-cells), even with recent onset [49-51].

Furthermore, a trial by Hu et al. which used stem cells in recent-onset type 1 diabetes mellitus [52] restricted the inclusion criteria to patients who had FCP ≥ 0.3 ng/mL, as these patients still had a large mass of islet β-cells. In contrast with the above mentioned studies, the majority of enrolled patients in the current study had longer duration of the disease with lower FCP and 2h-CP levels at baseline. However, Dor et al. suggested that the mechanism of regeneration of β-cells is a self-duplication of pre-existing β-cells rather than generation from stem cells [53,54]. Thus, β-cell mass may be considered as a critical factor in the success of A-BMMNCs transplantation to sustain endogenous β-cell function. Consequently, transient findings from the current study could have been even better if we had selected early-onset patients with high β-cell mass. As the procedure is simple, administering a second dose of A-BMMNCs has been suggested as a way to overcome these temporary results. We injected only one dose, compared with 4 doses of rituximab. Rituximab is an immune-modulatory drug which is used in autoimmune diseases with a defect in B cells, such as T1D [55]. While the present preliminary study will be of use in designing future successful trials, it had some limitations. Firstly, only two auto-antibodies were measured. Thus, individuals having autoimmune markers other than islet cell antibodies or insulin antibodies are needed to assess if A-BMMNCs transplantation has any effect on other diabetic antibodies; longer and more complete studies including other positive types of autoantibody markers should be done in future. Secondly, patients who were negative for antibodies need further analysis to determine whether the transplantation had any influence on antibody formation or whether these antibodies will be recurrent again after longer monitoring periods. Also, the duration of follow-up and sample size was relatively limited. These results should be assessed by randomized controlled trials of long duration with large number of patients to validate this method in the treatment of type 1 diabetic patients. Despite these limitations, this study has several strengths: 1) The procedure is very simple and feasible, 2) there was no tissue rejection, as we used autologous not allogeneic cells, 3) minimal processing ensured that the native components or heterogeneous populations of the bone marrow cells were not altered, 4) all the different types of stem cells and associated cells in the bone marrow were fully available to work together synergistically for immune modulation, tissue repair and/or regeneration, 5) the procedure, using adult stem cells, reduces ethical concerns such as those associated with the use of embryonic stem cells [56], 6) the procedure is more cost-effective than alternatives, as there is a short hospitalization period (only one day post-transplantation) and it does not involve use of an immune-modulatory regimen which may require special care, 7) the approach satisfies the key requirement of the FDA for such human cells in terms of minimal manipulation and/or processing before use, reducing regulatory issues, 8) the procedure optimizes compliance with the point of care of patients, as the same operating room and procedure is used for each patient and 9) the SEPAX 2 separating system is a fully-automated, mobile and efficient cell separator system which allows reproducible processing.

Conclusions

This is the first intra-pancreatic clinical trial to inject A-BMMNCs directly into the tail of the pancreas in T1D patients, a safe, feasible and cost-effective technique. In addition, this simple technique can be used to inject other specific types of stem cells to enhance regeneration of β-cells and achieve maximum regenerative benefit with the fewest side effects for T1D patients. A-BMMNCs transplantation can transiently modify the course of the disease without an

immunosuppressant regimen. The present findings raise the possibility that A-BMMNCs transplantation may be useful to treat hyperglycemia; this may work best in patients in an early diabetic stage who have sufficient β-cell mass. Furthermore, in-depth controlled mechanistic studies are needed to understand precisely how this technique affects metabolic function in T1D and to help in overcoming the transient results that have been observed in most cell therapy studies. Finally, this procedure could be promising in the field of regenerative medicine to achieve safer and more effective therapies in attempts to prevent, ameliorate or reverse IDDM and other auto-immune diseases in the future.

Ethics Approval and Consent to Participate

Wadi EL-Neel ethical committee has been approved this study; reference number (0000006555). The consent from all participants or their parents was obtained.

Consent to Publish

Written informed consent was obtained from the participant for publication of their individual details in this manuscript and Table 1. The consent form is held by the authors in the patients' clinical notes.

Availability of Supporting Data

The collected data of all patients were stored in a password-protected database in Wadi EL-Neel hospital and available upon request.

Competing Interests

The authors declare that the research was conducted in the absence of any financial or non-financial relationships that could be construed as a potential conflict of interest.

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Contributions

All the authors shared the study design. Raghda Shahin collected, analyzed, share in interpretation the data, and drafted the initial version of manuscript; Hazem Khamis contributed with intellectual input for the results interpretation and discussion and reviewed the final version of the manuscript. Azza A Ali contributed with intellectual input for the results interpretation and discussion and reviewed the final version of the manuscript. Hoda A Salem analyzed the data and contributed with intellectual input for the discussion and reviewed the final version of the manuscript; Ahmed Eldemery Contributed with intellectual input for the discussion and reviewed the final version of the manuscript. All authors read and approved the final manuscript.

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