Islet Transplantation in Type I Diabetes Patients with Hypoglycemia

Ramiza Sami

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Handledare (eller flera handledare): Martin Burman
Examinator: Staffan Tavelin
ABSTRACT

Introduction: The islets of Langerhans are cell cluster in the pancreas that produces insulin. Islet cell transplantation represents a promising treatment option for patients with Type 1 diabetes mellitus (T1DM). Whole pancreas transplantation is favored in patients who need kidney transplantation or have exocrine deficiency; whereas islet cell transplantation is performed in patients with brittle diabetes and hypoglycemic awareness. Today islet transplantation is the only treatment without risking episodes of hypoglycemia it even provides improved glycemic control and potentially insulin independence. The main benefit of successful islet transplantation is that it can eliminate the need of frequent blood sugar measurements and also insulin injections. It also protects against many serious long term complications of diabetes including kidney disease, heart disease, stroke, and nerve and eye damage.

The aim of this literature study is to find out how effective islet cell transplantation is during treatment of type 1 diabetes, what happens after islet cell transplantation and what are the outcomes and future developments.

Method: The information was collected between January and August 2016. Some original articles are used but mostly review articles with a publishing date no more than ten years old, internet sources have also been used. The criteria used in selecting articles or review articles was based on reading the abstract and the full article based on if the content matched the survey questions. Sources from the internet such as American diabetes Association and National institute of health (NIH) have been used. The results were drawn from different articles and review articles; total 7 studies from articles are included in this work.

Result: Currently islet cell transplantation offers protection from severe hypoglycemia and freedom from the need for high rates of exogenous insulin. Developments in islet transplantation and improved choice of immunosuppression was introduced in the “Edmonton protocol” which consisted of a more potent, less diabetogenic and corticosteroid-free immunosuppressive treatment for type 1 diabetic patient. The benefits of successful islet transplantation are that it can eliminate the need for frequent blood sugar measurements and also insulin injections. It protects against serious long term complications of diabetes including kidney disease, heart disease, stroke, and nerve and eye damage. A common risk which occurs at any organ or tissue transplantation is the rejection of the donor cells. To avoid toxicity and side effects it is necessary to adjust the drug doses. After the transplantation the patient is frequently followed-up during the first six months where complete blood counts and basic metabolic parameters are controlled to minimize the risk of developing early side effects such as neutropenia, anemia and electrolyte imbalances. After the transplantation the pancreatic islets begin to release insulin. However the blood vessel growth from the injected islets takes time. During this period the transplant recipients continue with their insulin injections until the cells become fully functional. Researchers are trying to develop new strategies to achieve immune tolerance of the transplanted islets so that the immune system does not recognize the new islets as foreign.

Discussion: To treat type 1 diabetes there are two transplantation options: whole pancreas transplantation or islet cell infusion. The results were consistent in the different studies providing a strong result that islet transplantation has the ability to reduce hypoglycemia in patients with T1DM. Nevertheless one of the study done in Korea shows that the islet transplantation was not much successful as the result indicates that insulin-independence was not achieved but the patients with a marginal dose of islets and reduced immunosuppressant acquired stabilized blood glucose, reduced insulin dosage and improvement of hypoglycemic unawareness. Islet transplantation has the disadvantage that several donors are needed to obtain enough islets to accomplish insulin-independence.

Conclusion: In summary islet transplantation can provide insulin independence in selected type 1 diabetes patients with the aim of improving the quality of life. Once implanted the beta cells in the islets begin to release insulin.
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INTRODUCTION

Diabetes mellitus is a chronic autoimmune disease characterized by high blood glucose levels that result from relative deficiency of an anabolic hormone called insulin. Insulin is essential to process carbohydrates, fat and protein. This specific hormone is produced by beta cells of the islets of Langerhans located in the pancreas. Insulin has the function to move blood sugar into cells, where it is stored and later used as energy source. The main symptoms of diabetes are: increased fatigue, often thirsty, increased urination and weight loss (1). Diabetes has become treatable by the discovery of subcutaneous bolus injection of insulin since 1922 by Nobel Laureates Banting, McCleod, and co-workers Best and Collip (2). However with optimal insulin therapy there are common complications resulting in developing different health problems like retinopathy, neuropathy, nephropathy, vasculopathy and heart diseases (3). During 2014 the global prevalence of diabetes was estimated to be 9% among adults 18 years and older. In 2012 diabetes was the direct cause of 1.5 million deaths according to the World Health Organization (WHO). It is estimated that in Sweden nearly 50,000 persons have Type 1 diabetes mellitus (T1DM) (4).

Type 1 diabetes mellitus (T1DM) results from autoimmune destruction of beta cells where the immune system attacks and destroys the insulin producing beta cells in the pancreas. T1DM is usually diagnosed in children and young adults. Without insulin blood glucose levels rise and glucose cannot enter the cells and be used as energy as a result it remains in the bloodstream. High levels of glucose over a long period of time can lead to health complications including kidney disease, heart disease, stroke, and nerve and eye damage. Type 1 diabetes is developed based on a combination of a genetic predisposition and an autoimmune process that leads to destruction of the beta cells causing an absolute insulin deficiency. Other factors may include viruses, dietary factors, environmental toxins and physical or emotional stress (5). Symptoms may include increased thirst and urination, constant hunger, weight loss, blurred vision, and extreme fatigue. The most common treatment is by injecting insulin or either insulin pumps and inhaled insulin, besides an individually adjusted diet and exercise program (6). Diabetes medication has been individualized; today the standard of treatment is by self-measuring and a combination of long and short acting subcutaneously administered insulin. However treatment with insulin can be
life threatening for insulin-dependent T1DM patients and overdose can cause severe hypoglycemia unawareness- the inability to sense low blood glucose (7).

Type 2 diabetes mellitus is a heterogeneous disorder; patients with this disorder have insulin resistance. The pancreas does not have the ability to make enough insulin to keep blood glucose levels normal often the body does not respond well to insulin. Type 2 diabetes mellitus is more common because of increases in the prevalence of a sedentary lifestyle and obesity; it can be prevented by changes in the lifestyles of high-risk subjects. Symptoms may include fatigue, frequent urination, increased thirst and hunger, weight loss, blurred vision, and slow healing of wounds and sores. (6).

To maintain good blood glucose level the blood sugar level should be before eating at 5-7 mmol/l (90-130mg/dl) and about 1-2 h after food intake less than 8-10 mmol/l (140-180mg/dl) (8).
1.1. The Pancreas
The pancreas is a grandular organ and is part of the digestive and endocrine system. In the human body it is the second largest gland. (3)

1.2. Anatomy of the pancreas
The pancreas is located under the left rib cage in the back of the abdominal cavity behind the stomach. It is the size of the palm of your hand and shaped like a flat pear extended horizontally across the abdomen (9).

1.3. The Histology of the pancreas
Islets of Langerhans contain beta cells that produce insulin and alpha cells that produce glucagon. Stained section (figure 2) shows the islet cells that have been stained by using antibodies to insulin, glucagon and somatostatin is used to demonstrate beta cells (pink), delta cells (blue) and alpha cells (brown). In the figure 2 the predominance of the beta cells that produce insulin is obvious whereas alpha and delta cells are mainly located at the periphery of clusters beta cells.
Isolating islet cells from the pancreas is not easy because the pancreas contains nearly 1-14.8 million islets of mean diameter 157 µm consisting only of 0.8-3.8% of the total mass of the gland (10).

**Figure 2.** Islet of Langerhans was stained using antibodies to insulin, glucagon and somatostatin to demonstrate beta cells (pink), alpha cells (brown), and delta cells (blue). α and δ-cells are typically located at the periphery of clusters β-cells (figure brought from reference 11).

### 1.4. Physiology of the pancreas

The pancreas serves endocrine and exocrine functions that are vital for the human survival. The exocrine tissues produce enzymes that helps digest food. These enzymes include amylase for the digestion of carbohydrates, trypsin and chymotrypsin to digest proteins and lipase to break down fats. The endocrine function, the production of insulin and glucagon regulates the blood sugar levels.

### 1.5. Hypoglycemia

Hypoglycemia is also called low blood glucose or low blood sugar; it is the most common adverse event of insulin treatment. When the blood sugar level falls below 2.5 mmol/l this results in activation of the autonomous nervous system that stimulates the suprarenal gland to release adrenaline. Glucagon activates the liver to release glucose which increases blood sugar level and adrenaline stimulates the production of glucose by catabolizing glycogen depots (8). The patient experiences
waring signals such as sweating, tremors, restlessness and hunger. During these symptoms it is important for patient to immediately eat or drink a small amount of glucose rich food. If left untreated hypoglycemia can get worse and cause loss of cognitive function, seizures, coma, cerebral infraction and even death. In addition 10% of patients with type I diabetes has the risk to die from hypoglycemia. The patient during this condition potentially loses consciousness and the inability to awake from sleep a syndrome known as “dead in bed syndrome” (7). The primary goal with islet transplantation is not to achieve insulin-independence but to reduce severity of hypoglycemic events (3). In following sections more discussions will take place in effort to restore glucose levels by beta cell replacement either by whole pancreas or by isolated islet transplantation. Figure 3 presents reduction in hypoglycemia clearly observed in patients after the islet transplantation.

![Figure 3](image_url)

**Figure 3.** Islet cell transplantation presents reduction in hypoglycemia. Shortenings: pre-Tx: patients selected for islet transplantation before procedure, post-Tx: patients after islet transplantation; T1DM: patients attending the clinic with Type 1 diabetes mellitus. (Figure brought from reference 10).

1.6. Whole pancreas transplantation and islet transplantation
The first surgical technique with whole pancreas transplantation was simultaneous kidney-pancreas transplant performed in two patients with end-stage diabetic
nephropathy during 1966 by the Minnesota surgeons William Kelly and Richard Lillehei (3, 7). After the transplantation one of the patient achieved near-normal glycemia for two months (7). Pancreas transplantation is an option for patients with: T1DM, selected patients with T2DM and for patients with diabetes caused by surgical removal of the pancreas (1). Simultaneous pancreas/kidney transplantation is an accepted therapy for TDM1 patients with end stage renal failure. Since first pancreas transplantation more than 42,000 pancreas transplants have been reported to the International Pancreas Transplant Registry (3). Over the past two decades, development in transplantation approaches and improvement in the safety and efficacy has been applied through great improvements in surgical technique, immunosuppressant and management. Improved outcome at 1 year post-transplant now presents patients survival rates >95% and graft survival rates approaching 85% (3). The pancreas transplantation requires intra-abdominal surgery causing risk for cardiac morbidity, pancreatitis, infections, and graft loss due to thrombosis. Whole pancreas transplantation has better long-term outcomes compared to islets transplantation. Although the transplantation has highly risk for peri-operative infections, graft pancreatitis and graft thrombosis.

The first clinical attempt to treat diabetes by islet transplantation occurred in 1893. This was 28 years before insulin was discovered; the following year the first clinical attempt was published in the British Medical Journal. Dr. Watson Williams and his surgical colleague Mr. Harsant transplanted pieces of sheep’s pancreas into subcutaneous tissues to a 15-year old boy, who died after three days from uncontrolled ketoacidosis. Transplantation took place without the use of immunosuppressant believing that the immune system would tolerate the xenogeneic tissues naturally. Furthermore developments have occurred within the 50 years including the mechanisms underlying allografts rejection, the concept of immunological tolerance and how the immune system would react with powerful non-specific immunosuppressant (10).

Transplantation of islet cells is less risky compared to whole pancreas transplantation. Nearly 447 patients with T1DM have been treated with islet transplantation between 1974 and 2000 (2). Unfortunately only 10% maintained insulin-independence and less than 28% having C-peptide detectable by one year.
C-peptide is a byproduct of insulin production by the pancreas. The level of C-peptide indicates how much insulin the body is producing. In the year 2000 the development of the “Edmonton protocol” dramatically transformed the clinical outcomes in islet transplantation and was seen as a milestone success as all seven first treated patients achieved and maintained 100% insulin-independence at one year. Over the past 12 years (year 2012) clinical islet transplantation has progressed considerably and more than 750 patients with TD1M have received islet cells internationally (12). An essential component was through the use of two immunosuppressant- sirolimus and low-dose tacrolimus and anti-interleukin-2 receptor antibody daclizumab (2, 13). While insulin-independence rates were highly successful the patients had to stay on lifelong immunosuppression.

**Figure 4.** The table illustrates the number of islet transplant recipients since 1999-2013. (Figure brought from reference 13).

**1.7. Islet cells transplantation:**

There are two types of pancreas islet transplantation:

- allo-transplantation
- auto-transplantation
Pancreatic islet allo-transplantation is an experimental procedure in which the beta cells that produce insulin are removed from the pancreas of a deceased organ donor and transferred into a person with diabetes. Specialized enzymes are used to remove the islets and these are purified and counted in a lab. The patient receives two infusions with the average of 400,000 to 500,000 islets per infusion. Once this is performed the donor has the ability to release and make insulin (14).

Islet cell transplantation is suitable for: Type 1 diabetes patients who have severe hypoglycemia, type 1 diabetes patient with a functioning kidney transplant and impaired hypoglycemia awareness or patients with poor blood glucose control despite having the best medical treatment.

People not suitable for islet transplantation: People with high insulin requirements (e.g. more than 50 units per day for a 70kg person), body weight >85 kg, poor kidney function, women planning a pregnancy (15).

Different factors are linked to a better outcome for the patients:

- Age > 35 years old
- Lower blood fat levels
- Lower pre-transplant insulin

**Figure 5.** The procedure of islet transplantation. Islets are taken from the patient’s own pancreas and then infused through the catheter into the portal vein of the liver. (Image brought from reference 16).
Pancreatic islet auto-transplantation (figure 5) is a procedure followed by surgical removal of the whole pancreas from the patient. The aim of this procedure is to infuse the patient with healthy islets to make insulin. This is the last option for patients with chronic pancreatitis and also when other treatments are not effective. The surgery is performed in a hospital; the patient’s own pancreas is removed and then the islets are extracted and purified from the pancreas. After few hours the patient’s own islets are infused through catheter into the liver (17).

**1.8. Human islet isolation**

Figure 6 below shows the isolation of islets. The pancreas is placed in a cold storage solution and transported to the islet isolation laboratory. The aim is to isolate the islets from the extracellular matrix through a combination of mechanical and enzymatic digestion. First the excess fat is trimmed from the pancreatic capsule. The pancreatic duct is cannulated by an intravenous catheter and then the collagenase enzymes are injected through the system allowing distension of the pancreas (6B). The collagenase enzymes start to digest the pancreas where the islets are separated from the exocrine tissue for 10 minutes. The pancreas is cut into 7-9 pieces and loaded into the Ricordi Chamber for digestion (6C) (18). In the Ricordi Chamber warmed (at 37 °C) collagenase enzyme solution is recirculated around the gland as well the digesting pancreas is mechanically fragmented. The chamber is mechanically shaken at this period. Once adequate numbers of islets are obtained by monitoring samples taken from the recirculating system then the pancreas digestion is stopped by cooling (19). At defined intervals the samples are assessed with Dithizone stain and counted (6D) (18). The digested pancreas contains many connective and exocrine tissues thus removing these cellular components is important as it can cause portal vein hypertension and thrombosis. Through current isolation techniques it is impossible to obtain all islets from the pancreas since many islets are lost in pancreas digestion stage. In the purification stage islets are lost with other cells such as acinar and ductal cells. Over 11000 islets per kilogram are required to treat diabetes. To acquire this number of islets from single donor is impossible-multiple donors are needed (18).
Figure 5. (A) The pancreas is placed in a cold storage solution and transported to the islet isolation laboratory. (B) The pancreas is trimmed and the pancreatic duct is cannulated. (C) The pancreas is loaded into the Ricordi Chamber for digestion. (D) At defined intervals the samples are assessed with Dithizone stain and counted (Image brought from reference 18).

The procedure before the transplantation is to maintain the recipient’s blood pressure, heart rate rhythm and oxygen saturation (8). The transplant is performed by a radiologist. In the patients upper abdomen a catheter is inserted and threaded into the portal vein of the liver. The portal vein is the major vein to supply blood to the liver and has been recognized as the most efficient site with high vascularity, proximity to islet-specific nutrient factors and physiological first pass-insulin delivery to the liver. Once the islets are infused into the liver they then undergo a process of angiogenesis and neovascularization to form a microvascular network and reestablish nutritional blood supply (10). Through the catheter a teaspoonful of islet cells are injected. During this period the patient is at a conscious stage but anesthetized during the procedure; and can return home within some days. More than one transfusion of islet cells may be needed and the patient must remain on immune-suppressant therapy for life-long to prevent the transplanted tissue from being rejected by the body (18). Researchers are in the hope that islet transplantation will help type 1 diabetes patients to live without daily injections and eliminate hypoglycemia unawareness (21).
The evolution of immunosuppressive therapy by the “Edmonton Protocol”

Earlier it has been a formidable challenge to select an optimal immunosuppressive drug for islet transplantation since the aim was to overcome both autoimmune and allo-immune barriers and to minimize toxicity to the islet graft. The drug selection was based on similar to those used for kidney transplantation protocols which consisted of azathioprine, cyclosporine and corticosteroids (3), (22). Corticosteroids induced hyperglycemia by reducing insulin-mediated glucose uptake; earlier medications were themselves diabetogenic and were damaging the transplanted islets (23). As mentioned earlier fewer than 10% of the patients were able to achieve insulin-independence. Developments in islet transplantation and improved choice of immunosuppression was introduced in the “Edmonton protocol” which consisted of a more potent, less diabetogenic and corticosteroid-free immunosuppressive treatment for type 1 diabetic patient. The protocol was based on high-dose sirolimus used for maintenance therapy, low-dose tacrolimus and replaced glucocorticoids with daclizumab an anti-interleukin-2 receptor antibody (3), (22).

Sirolimus (Rapamune) is characterized as macrocycle lactone produced by the bacterium Streptomyces hygroscopicus; it is known as a potent immunosuppressive drug used to prevent rejection in organ transplantation. Following the entry into cytoplasm sirolimus binds the cytosolic protein FK-binding protein 12 (FKBP12). The sirolimus-FKBP12 complex apparently inhibits the activity of protein mammalian target of rapamycin (mTOR). This results cell-cycle arrest G1-S phase. Sirolimus prevents the activity of B-cells and T-cells by inhibiting the production of cytokine-interleukin-2 (IL-2). The process leads to prevent cell-cycle proliferation and progression and potential effects on islet graft. The dose is either given to the patient one day before transplant or on the day of transplantation. The transplant recipient receives a single oral dose of 0,2mg/kg, thereafter followed by 0,1-0,15 mg/kg per day (10).

Tacrolimus is a calcineurin inhibitor (CNI). It has the efficacy in preventing acute organ rejection and improves short-term graft survival (23). It binds an intracellular protein FKBP-12; created as complex FK-FKBPK it generates into active drug. The activated drug inhibits the function of calcium-regulated phosphatase calcineurin and prevents the T-lymphocyte activation and cytokine transcription. Dose: treatment
with tacrolimus is started at 1 mg orally and later adjusted to reach 12 hr trough levels of 4-6 ng/ml (10).

After the transplantation daclizumab is given intravenously to the patient and then discontinued; whereas sirolimus and tacrolimus must be taken for life-long until the islets continue to function (16). After patent expiry of daclizumab it has been removed from the market; other T-cell depletional agents have been used such as thymoglobulin (antithymocyte globulin), basiliximab (an monoclonal antibody that works by blocking the immune response that causes transplant rejection) and more newly alemtuzumab a humanized IgGI monoclonal antibody it targets CD25 antigen (a glycoprotein found on cell surfaces), monocytes and lymphocytes. Currently in clinical further modifications are being done to the “Edmonton protocol” where the aim is to completely eliminate the calcinerurin inhibitors. Calcinerurin inhibitors inhibit the action of calcineurin a protein phosphatase which is involved in activating the T-cells (10). In addition different protocols have added etanercept a TNF-a receptor antagonist for induction and glucagon-like peptide-1 analogue. Secondly other protocols use rabbit antithymocyte globulin (RATG), methylprednisolone, etanercept, mycophenolate mofetil, everolimus (a derivate of sirolimus) for maintenance (3).

-Rabbit antithymocyte globin (RATG) (acetaminophen, diphenhdramine, pentoxifylline): is used to treat acute transplant rejection with other medicines. It is an immune globin and works by suppressing the body’s immune response.

-Methylprednisolone is a steroid in the body it prevents the release of substances that cause inflammation.

-Etanercept relieves the symptoms of autoimmune disorders as it interferes with tumor necrosis factor (TNF) and acts as TNF-inhibitor.

-Mycophenolate mofetil belongs to a class of medications called immunosuppressant. It is used to prevent the body from rejecting a transplanted organ (10).
Side effects from immunosuppressive medications may include:
- Mouth sores and gastrointestinal problems, diarrhea and upset stomach, increased blood cholesterol, high blood pressure, anemia, fatigue, decreased white blood cell counts, decreased kidney function and bacterial and viral infections (24).

What are strategies to prevent immunosuppressive side effects?
To avoid toxicity and side effects it is necessary to adjust the drug doses. After the transplantation the patient is frequently followed-up during the first six months where complete blood counts and basic metabolic parameters are controlled to minimize the risk for developing early side effects such as neutropenia, anemia, electrolyte imbalances, etc. Monthly toxicity assessments are also done to identify more subjective side effects including tremor and memory loss, which can mainly affect the patient’s daily function and quality of life (10). Researchers are developing modifications to the Edmonton protocol; trying to improve medication regimens for successful transplants. Medication regimens differ from one transplant center to another.
AIM

The aim of this literature study is to find out how effective islet cell transplantation is during treatment of type 1 diabetes and the possibilities to avoid hypoglycemia. The following questions will be addressed in this work:

- What are positive and negative effects from islet transplantation?
- What happens after islet transplantation?
- What are the new studies?
METHOD

In this literature study the information is brought between January and August 2016. Some original articles are used but mostly review articles with a publishing date no more than ten years old. Some books are used about islet cell transplantation as one of them titled “Islet transplantation and beta cell replacement therapy” by James Shapiro and James Shaw (2007). The criteria’s used in selecting articles or review articles was based on reading the abstract and the full article based on if the content matched with the survey questions. The selection was done based on relevant content of the articles and to include information based on the title. TD1M and hypoglycemia was the main focus in articles. While selecting articles about “Edmonton protocol” the aim was to discover the recent improvements in outcomes of islet transplantation methods. Sources from the internet have been used such as American diabetes Association and National institute of health (NIH). The result was brought from different articles and review articles; total 7 studies are included in this work. The aim was to provide results that answers the questions required including islets transplantation and improvement with hypoglycemia in type 1 diabetes patients.

Articles are brought from the database of Umeå University’s library. From the references of the articles further research in seeking valuable information has been done and the sources have been brought in this work. As islet transplantation is still under experimental trail it was therefore difficult to find latest articles from 2010.

Table 1. Search design and results of the literature from Umea University’s library database:

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RESULTS

Here is a summary of the seven articles that try to answer the issues in this work.

*Simultaneous Islet and Kidney Transplantation in Seven Patients with Type 1 Diabetes and End-Stage Renal Disease Using a Glucocorticoid-Free Immunosuppressive Regimen with Alemtuzumab Induction* (25). In this study seven patients with type 1 diabetes and with end-stage renal failure received simultaneous islet and kidney transplantation. To prevent organ rejection the patient received alemtuzumab, sirolimus and tacrolimus. No glucocorticoids were used in the study. The patients were followed up during 18.3 months. The results show that kidney survival was 100% whereas four patients became insulin independent for one year; and the other three had less than 25% insulin use. After the transplantation the serum C-peptide levels were greater in all seven patients this significantly indicates that the islets were functioning well. The transplantation had no major complications. Type 1 diabetes can damage the kidney function therefore often combined islet and kidney transplantation is performed.

Research design: Study was preformed from June 2005 to December 2006; it includes seven patients -three men and four women. The patients were experiencing serve hypoglycemia since 3 years (blood glucose level 50 mg/dl) including end-stage renal disease and type 1 diabetes. Serum C-peptide level was 0.3 ng/ml. The age of the patients was at the range of 32-49 and with duration of diabetes for 14.8 years. Hemodialysis treatment was done 10 months ago.

The number of islets used for transplantation were 450,000 –760,000. In islet preparation no bacteria or viruses were found. Patient 3, 6, and 7- received single islet transplantation. After the transplantation patient 3 become insulin-independent after 34 days, thereafter was observed free of the need for exogenous insulin. Patient 6 and 7 did not become insulin- independent whereas their insulin dose reduced to more than 60%. Patient 2, 4, and 5 received two islet infusions from other pancreas donors; the second infusion took place after 45 days. Patient 2 and 4 became insulin
independent however patient 5 needed 8 IU insulin daily. Lastly the patient 1 required three islet infusions and became insulin-independent. After the first transplantation the need for insulin decreased in all seven patients and four patients (57.1%) became insulin-independent.

**Reversal of hypoglycemia unawareness with a single-donor, marginal dose allogeneic islet transplantation in Korea (26).**

Case description: Patient- 59-year old women diagnosed type 1 diabetes since 25 years. Body weight 52 kg and BMI 20.31 kg/m². She suffered from severe hypoglycemia- unawareness. The C-peptide concentration of the patient was 0.07 ng/mL and 0.23 ng/mL respectively. When her blood glucose level dropped to 50 mg/dL symptoms from hypoglycemia was experienced. Total insulin requirement was 34 U/day and the glycated hemoglobin (HbA1c) that reflects blood sugar levels over the past two to three months ranged at the patient from 7.8% to 9.6% with a mean value of 8.54% in the preceding 2 yr. Total purified islets was 216,500 islets. The purity of the isolated islets was 79.6 %, and the islet viability was > 90%. In November 2013 the islet transplantation was performed. The immunosuppressive drugs were based on the Edmonton protocol. Basiliximab dose of 20 mg was given intravenously two hours before and four days after the transplantation. Sirolimus was given once daily the aim was to achieve a therapeutic range of 12-15 ng/mL after 3 months and the target range was planned to be lowered to 7-12 ng/mL. Tacrolimus was administered two times a day and adjusted to achieve a target trough level of 3-6 ng/mL. One month later the patient experienced systemic mucositis and leucopenia and so the dosage of sirolimus was reduced at the trough level of 5-9 ng/mL. Insulin requirement decreased to 22U/day after the transplantation it was 65% of the previous requirement. After two months the C-peptide concentrations improved to 0.25 ng/mL and 0.44 ng/mL, respectively. The HbA1c level was low before the transplantation took place as it is a requirement for better outcome for the patient. Whereas mentioned earlier patients with high insulin requirements are not suitable for islet transplantation. Three months after the transplantation HbA1c level improved to 7.3%. Before each meal and at bed time self-monitored blood glucose concentration was measured, after the transplantation improvements in results were seen including both extreme hyperglycemia and hypoglycemia.
Single-Donor, Marginal-Dose Islet Transplantation in Patients with Type 1 Diabetes (27).

This is a one year follow-up study preformed from July 2001 to August 2003. In the first year the recipients achieved insulin-independence after a single-donor islet transplant. Insulin-independence was observed mainly when the patients maintained fasting blood glucose levels below 126 mg/dL (7.0 mmol/L) and 2-hour after meal levels below 180 mg/dL (10.0 mmol/L) after discontinuation of insulin. In the study 8 patients were included coincidentally all women.

Inclusion criteria: Age 18 years or older, C-peptide–negative and type 1 diabetes for 5 years complicated by 1 of the following:

Complications including:
- Proliferative retinopathy, macular edema or photocoagulation, urinary albumin excretion.
- Metabolic lability/instability- (2 episodes of severe hypoglycemia or 2 hospital admissions for ketoacidosis in the past year)
- Hypoglycemia unawareness

Figure 7. (A) Average dosage of insulin requirement for 2 weeks before, 3 and 9 months after islet transplantation. (B) Fasting and stimulated C-peptide level before and after islet transplantation. (C) Changes in HbA1c level before and after islet transplantation. (Tx, transplantation). (Diagrams from reference 26)
After accessing the portal vein islets were infused through a percutaneous transhepatic portal venous catheterization. The number of islets infused were 7271 (SD, 1035) islet equivalents/kg of recipient body weight. Intravenous heparin treatment was given for 48 hours including enoxaparin 30mg given two times daily till day 7. Induction immunosuppression consisted of rabbit antithymocyte globulin (RATG), methylprednisolone, daclizumab and etanercept. Premedication for RATG included acetaminophen and diphenhydramine as well as pentoxifylline. Maintenance immunosuppression was initiated with sirolimus and tacrolimus. The dosage of the immunosuppressant was gradually adjusted either discontinued or dosed to a target trough level at 1 month post-transplantation; tacrolimus was gradually replaced with mycophenolate mofetil and minimized calcineurin inhibitor exposure. Post-transplantation no procedural complications observed. Five recipients experienced adverse events as lymphopenia and transient neutropenia. No clinically changes observed in creatinine clearance or urinary albumin. Results present that all 8 recipients became insulin-independent and free from hypoglycemia. Out of 8 recipients; 5 recipients remained insulin-independent for more than one year whereas 3 were insulin-independent for 121, 76, and 7 days.

**Improvement in Outcomes of Clinical Islet Transplantation: 1999–2010 (28).**

The Collaborative Islet Transplant Registry (CITR) from 2007 to 2010 significantly shows improvements in safety and efficacy outcomes comparable to recipients who received transplant with fewer islet cell infusions during 1999-2006. CITR monitors progress and safety of islet transplantation by using data from centers in the U.S., Canada, Australia and Europe and the registry represents complete information from the last decades on islet transplantation. Total of 677 recipients were included in the analysis with 214 recipients in 1999–2002, 255 in 2003–2006, and 208 in 2007–2010. The results indicate that insulin-independence at three years after the islet transplantation improved from 27% in 1999-2002 to 37% in 2003-2006 and to 44% in 2007-2010. The transplantation included islet alone in 575 recipients and simultaneous islet kidney transplantation in 102 recipients. They received 1,375 islet infusions from 1,502 donors. Since 12-years-period there have been substantial shifts in strategies for using immunosuppressive drugs. During 1999-2006 Edmonton
The protocol was dominated which included daclizumab an interleukin 2 receptor antagonist for induction and sirolimus a mammalian target of rapamycin (mTOR) inhibitor combined with tacrolimus a calcineurin inhibitor (CNI). Since 2007-2010 there has been a shift to induction with a T-cell depleting antibody with or without etanercept an inhibitor of tumor necrosis factor-a. For maintaining immunosuppression mTOR inhibitors have been used in combination with a CNI.

The criteria’s applied for patient selection for the islet transplantation was: older age of the patient with type 1 diabetes at long duration period, less insulin requirement and having better kidney function. Improvements in HbA1c and fast blood glucose had been discovered in nearly all islet recipients. The effect of T-cell depleting antibody with etanercept (an inhibitor of tumor necrosis factor-a) proves to have long term insulin-independence whereas 50-60% of the recipients receiving this induction were insulin-independent at 3-5 years. Currently islet cell transplantation offers protection from severe hypoglycemia and freedom from high rates of exogenous insulin need (28).

1999- 2002
First year: 51% were insulin-independent
Second year: 36% were insulin-independent
Third year: 27% were insulin-independent

2007-2010
First year: 66% were insulin-independent
Second year: 55% were insulin-independent
Third year: 44% were insulin-independent (28).
Islet auto transplantation following total pancreatectomy (29).

In Leicester England, a total of 46 patients underwent total pancreatic resection followed by immediate islet auto transplantation where the patient received own islet cells. The study took place during the period from September 1994 to December 2006. None of the patients had earlier received islet auto transplantation and the patients received a median of 2246 islets/kg body weight. The final option for the patients suffering from severe chronic pancreatitis is surgical resection or either part of the pancreas. In the study 29 patients were female with the mean age of 42.5 years at time of surgery and body mass index (BMI) was 21.8 kg/m2. The islets were prepared and infused into the liver or spleen. The patients received 5000 IU of intravenously administered heparin before the infusion. Forty-two patients had islets infused into the liver, two patients transplanted only into the spleen and two patients had islets infused both into the liver and spleen. After the transplantation all 46 patients were transferred to intensive care or to high-dependency unit and the total hospital stay was 20 days. Out of 46 patients, 12 patients have shown significant periods of insulin-independence for 16.5 months and 5 remain insulin-free at the time of writing. The patients included in this study had no significant relationship between age, BMI, sex, family history. Evidence indicates that islets infused into the liver within hours of the operation had better metabolic function within 10 years after the transplantation comparable to islet infused into the spleen. Islet auto-transplantation is a valuable procedure with pancreatic resection. The majority of patients received tightly regimen of insulin right after the transplantation this is because the islets do not function optimally until one year after transplantation. Through experimental models it is known that directly after the transplantation the exogenous insulin protects the newly transplanted islets from exhaustion and serves in addition to prolong the islet function. Six patients out of the twelve patients received insulin nearly three months after the transplantation and periods of insulin-independence was observed (29).

Phase 3 Trial of Transplantation of Human Islets in Type 1 Diabetes Complicated by Severe Hypoglycemia (30).

This phase 3, single-arm study was conducted at eight centers in North America where human pancreatic islets have been purified as investigational product. In this study 48 adults age between 18-65 years were included with T1DM for more than 5 years, absent stimulated C-peptide and suffering from impaired awareness of
hypoglycemia (IAH) and severe hypoglycemic events (SHEs). The patients received immunosuppression followed by one or more transplantation of the purified human pancreatic islets (PHPI). For the first transplantation induction immunosuppression consisted of rabbit anti–thymocyte globulin and etanercept. Basiliximab replaced the rabbit anti–thymocyte globulin. For maintenance immunosuppression sirolimus and low-dose tacrolimus were used. This study was manufactured in accordance under good manufacturing practice conditions. The end points from this study was the achievement after the first transplant of HbA1c <7.0% (53 mmol/mol) at day 365 and freedom from SHEs from day 28 to day 365. The 48 adults received 75 purified human pancreatic islets infusions as follows: 22 adults (45.8%) received one infusion, 25 (52.1%) received two infusions, and 1 subject (2.0%) received three infusions. Insulin independence was achieved by 23% of the patients at day 75 and by 52.1% at day 365. Insulin independent was observed at 25 patients at 1 year among them 13 received one islet infusion and 12 received two islet infusions.

**Improvement in Insulin Sensitivity after Human Islet Transplantation for Type 1 Diabetes (31).**

The study indicates that islet transplantation can improve insulin sensitivity in patients with long-standing TD1M. Twelve patients with T1DM underwent evaluation in the Clinical and Translational Research Center; the transplant recipients underwent one or two intra-portal infusions of islets. The patients had 30 years of disease duration and an insulin requirement of 0.5 U/kg that was substantially reduced after the transplantation. The study was done before and between 6 to 7 months after the transplantation and was compared with other patients. The patients received low-dose calcineurin inhibitor tacrolimus which was given in combination with sirolimus that has been shown to induce insulin resistance.
DISCUSSION

Answering the questions from the results is that currently islet cell transplantation offers protection from severe hypoglycemia and freedom from high rates of exogenous insulin need. Developments in islet transplantation and improved choice of immunosuppression was introduced in the “Edmonton protocol” which consisted of a more potent, less diabetogenic and corticosteroid-free immunosuppressive treatment for type 1 diabetic patient. Benefits of successful islet transplantation are that it can eliminate the need of frequent blood sugar measurements and also insulin injections. It protects against serious long term complications of diabetes including kidney disease, heart disease, stroke, and nerve and eye damage. A common risk which occurs at any organ or tissue transplantation is the rejection of the donor cells. To avoid toxicity and side effects it is necessary to adjust the drug doses. After the transplantation the patient is frequently followed-up during the first six months where complete blood counts and basic metabolic parameters are controlled to minimize the risk for developing early side effects such as neutropenia, anemia and electrolyte imbalances. After the transplantation the pancreatic islets begin to release insulin. However the blood vessel growth from the injected islets takes time. During this period the transplant recipients continue with their insulin injections until the cells become fully functional. Researchers are trying to develop new strategies to achieve immune tolerance of the transplanted islets so that the immune system does not recognize the new islets as foreign.

To treat type 1 diabetes there are two transplantation options: whole pancreas transplantation or islet cell infusion. Edmonton protocol has been utilized in many clinical islet transplant trails since outcomes have improved using a steroid free immunosuppressive protocol with a combination of sirolimus and tacrolimus. Different questions have been answered through the seven studies included in this work. Such as the patients have become insulin-independent after successful islet transplantation and the have experienced relief from hypoglycemia some of the studies included in this work has been using the “Edmonton protocol” while choosing immunosuppressive drugs after the islet transplantation. The methods used in the studies were good and the results are reliable as the patients were followed up during long period of time and compared with other patients. The results have been affected by different factors such as older age of the patient with type 1 diabetes at long
duration period, less insulin requirement and having better kidney function. The inclusion criteria for islet transplantation as referring to the studies included in this work most of the patients are of high age and with long diabetes duration which indicates that the outcome from the transplantation have been positive and successful.

The results from simultaneous islet and kidney transplant (study 1) have been carried out with good results. What are the immunosuppressive drugs that have been used in islet cell transplantation? The patients received no glucocorticoid and the immunosuppressive protocol consisted of sirolimus, low-dose tacrolimus and alemtuzumab (monoclonal antibody) the choice of immunosuppressive medication was based on the Edmonton Protocol. Patients with end-stage renal disease and diabetes have poor survival rate and the kidney transplantation cannot completely improve the condition. However simultaneous islet and kidney transplant have been carried out with good results. The main challenge with this procedure is to avoid steroids and to prevent allo-reactivity and recurrence of autoimmunity against the islet cells. Since steroids are favorable to prevent organ rejection but they are harmful for the newly infused islets. Alemtuzumab is a humanized monoclonal antibody that targets the CD52 antigen it is mainly used in solid-organ transplantation. The patients were followed-up for 18.3 months where four patients became insulin-independent and three patients were capable of reducing their insulin dose to near 75% the amount required before transplantation. C-peptide concentrations were all above 0.5nmol/l. For the transplanted kidney there were no observations for acute or chronic rejections. After the first transplantation the need for insulin decreased in all seven patients and four patients (57.1%) became insulin-independent. Islet transplantation is a safer method since past the isolation technique has been improved and better immunosuppressant’s have been established. After the kidney transplantation no acute and chronic rejections were observed.

Study 2 was the first successful reported case on allogenic islet transplantation of type 1 diabetic patient in Korea. Although the results show that insulin-independence was not achieved but the patients with a marginal dose of islets and reduced immunosuppressant acquired stabilized blood glucose, reduced insulin dosage and improvement of hypoglycemic unawareness. However this procedure was quite successful comparable with only a marginal quantity of islets used in the experiment.
(4,163 IEQ/kg) with reduced immunosuppressant. The success rate of islet transplantation has been increased by infusing higher mean islet mass.

In study 3 the recipients achieved insulin-independence after a single-donor islet transplant during the first year where an average of 7,271 IEQ/kg had been used; these were prepared from 2 to 4 donor pancreas. Glucocorticoid-free immunosuppression was used along with potent induction immunotherapy-antithymocyte globulin, daclizumab and etanercept. It is important that insulin-independence is restored with a single donor as it has the possibility to reduce the risks and costs and even increase the availability of islet transplantation. The criteria’s used in the protocol was to limit ischemic injury of islets during pancreas storage, allow initiation of potent immunotherapy before the transplantation, and to reduce calcineurin inhibitor dose as it may minimize nephrotoxicity and cardiovascular toxicity. The seven studies used in this work all indicating a positive effect from islet cell transplantation for TD1M patients and the theme of my perspective is that the future of islet transplantation is very robust. Islet transplantation has the disadvantage that several donors are needed to obtain enough islets to accomplish insulin-independence.

In study 5 where 46 patients underwent total pancreatic resection followed by immediate islet auto transplantation the patients received a median of 2246 islets/kg body weight. Insulin-independence is not the usual outcome of islet auto-transplantation following total pancreatectomy however over long-period it can help prevent diabetic complications.

**Positive and negative effects from islet transplantation**

Benefits of successful islet transplantation are that it can eliminate the need of frequent blood sugar measurements and also insulin injections. The islet cells are infused into the portal vein because it is the major vein to supply blood to the liver and has been recognized as the most efficient site with high vascularity, proximity to islet-specific nutrient factors and physiological first pass-insulin delivery to the liver. The liver and the spleen is the main site for transplantation with the reason that both organs receive much blood and have a nice structural building that probably reduces the risk of clot formation.
From the results in study 4 it is clearly obvious that islet transplantation offers substantial protection from severe hypoglycemic episodes and high rates of freedom from exogenous insulin requirements. Analysis of the effect of the available factors it indicates improved clinical outcomes significantly associated with the recipient age, lower initial insulin use, and the use of T-cell depletion in combination with TNF-α inhibitors.

Severe hypoglycemia is a life-threatening complication of T1DM the results from study 6 demonstrate that transplantation of human islets was effective in treating impaired awareness of hypoglycemia (IAH) and severe hypoglycemic events (SHEs) in patients with T1D. The transplantation protects against serious long term complications of diabetes including kidney disease, heart disease, stroke, and nerve and eye damage. From study 7 the results indicate that the participants after islet transplantation experienced a decrease in body weight with a resulting decrease in BMI.

A common risk which occurs at any organ or tissue transplantation is the rejection of the donor cells (14). A major obstacle to widespread use of islet transplantation is the shortage of islets (14). One million functioning islets must be transplanted to produce enough insulin. The immune system functions in a way to destroy what it recognizes as foreign including islets cells transplanted. To stop the immune system from rejecting the transplant large doses of immunosuppressive drugs are needed; there is still research ongoing trying to develop methods to reduce or eliminate the need for immunosuppressant. The drugs used become toxic to the new islet cells and put the patients at risk for infections and cancer (32). The advantage with auto-transplantation is that immunosuppressive drugs are not needed because the cells infused come from the patient’s own body are these are harmless (24). A disadvantage from the transplantation: blood-mediated inflammatory reaction-IBMIR is an inflammatory reaction triggered by tissue factor produced and expressed by the islets; the IBMIR was first demonstrated in patients with islet transplantation. The instant inflammatory reaction is quickly observed at some patients, after the human islet transplantation within the first hour more than 50% of the injected islets are lost (23). IBMIR is characterized by platelet consumption; the islets become surrounded by clots and infiltrated with leukocytes this causes damage to the
pancreatic islets. As a result when heparin and a complement inhibitor (SCRI) were added then IBMIR was suppressed and islet damage reduced (18).

**What happens after islet transplantation?**

After the transplantation the pancreatic islets begin to release insulin. However the blood vessel growth from the injected islets takes time. During this period the transplant recipients continue with their insulin injections until the cells become fully functional. The patient receives various medications before and after the transplantation for long-term functioning of the islets and to promote implantation. Since diabetic patient have an autoimmune response and can destroy the newly transplanted islets. Liver is the traditional site for infusing the islets; researchers are investigating other sites for islet infusion, alternative muscle tissue or other organ (24).

**New studies**

Researchers are trying to develop new strategies to achieve immune tolerance of the transplanted islets so that the immune system does not recognize the new islets as foreign. Immune tolerance would allow the avoidance of long-term immunosuppressive medications. Ongoing approach is to encapsulate the islets with a special coating and then transplant those to the patient; this would prevent immune rejection and to eliminate the side effects from immunosuppressive medications. From the different encapsulation systems developed since past; so far micro-encapsulations have been studied more broadly. A variety of materials has been tested for microencapsulation in various animal models; some materials used have induced immunoprotection to islet grafts without the need for immunosuppression. In clinical trials this combined procedure has yet not been successful further improvements are in the work since this technology can utilize allo- or xenogeneic cell sources to overcome limited islet cell donor supplies (19, 24).

Other selective studies have been done by collecting white blood cell from the islet donor’s spleen, and treated the cells with a chemical that masked the cells identity. Diabetic mice had been injected with these chemically treated cells before and after the mice had gone through the islet cell transplantation. As a result, the immune system of the mice didn’t try to reject the cells, because it didn’t recognize them as foreign or dangerous (33)
Furthermore new research is ongoing in *in vitro* and *in vivo* models; the major aim with modification of pancreatic islets with surface attached to heparin conjugates. Many molecules of about seventy are attached to a carrier backbone; which has the ability to protect the surface of the cells from attacks by the innate immune system after the transplantation (34). Major problem with pancreatic islet allograft transplantation is the shortage of islets from donors. Researchers are in process to solve the shortage of islets by transplanting islets from a single donated pancreas or by using islets from pigs. Pig islets have been transplanted to other animals, including monkeys. To prevent rejection the islets were encapsulated and medications were used. Other new approach is to create islets from stem cells (24).

**CONCLUSION**

In summary the seven studies used in this work are all indicating a positive effect from islet cell transplantation for TD1M patients and the theme of my perspective is that the future of islet transplantation is very robust. Islet transplantation can provide insulin independence in selected type 1 diabetes patients with the aim to improve the quality of life. Once implanted the beta cells in then islets begin to release insulin. Whereas the islet transplantation has the disadvantage that several donors are needed to obtain enough islets to accomplish insulin-independence. Further new developments in islet isolation will continue to improve mainly without the use of immunosuppressive drugs.

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