Evaluation of the Efficacy of Autologous Bone Marrow Stem Cell Transplantation in Type 1 Diabetes Mellitus.

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Abstract

Diabetes mellitus (DM) is characterized by hyperglycaemia that is often associated with long- term complication, including microvascular (retinopathy, nephropathy, and neuropathy) macro vascular damage. The present study was designed to evaluate the possible role of autologous bone marrow stem cell transplantation for cases of type 1 DM, which could differentiate into insulin- producing β cells and its role in modulating the immune response from T helper 1 to T helper 2. This study was conducted on 10 cases of autoimmune type 1 diabetes mellitus, both males and females. They were selected according to inclusion and exclusion criteria. We observed that 70% of studied subjects decreased in their insulin requirements. Fasting and postprandial blood glucose significantly decreased after transplantation, while levels of C- peptide were significantly increased at the end of 9 months of autologous bone marrow- derived stem cell transplantation (SCT). But glycated haemoglobin significantly decreased after transplantation. No side effects were noted in liver and kidney functions. Our observations indicated that SCT is a safe and effective modality of treatment to improve β- cell function in patients with T1DM.

Key Words: Type 1 Diabetes Mellitus / Stem cell therapy / Transplantation

1. Introduction

Diabetes mellitus (DM) is a common metabolic disorder resulting from defects in insulin secretion or action or both, resulting in impaired metabolism of carbohydrates, lipids, proteins, water and electrolytes **(Rasineni *et al*.,2010** and **Saumya and Basha, 2011).** Chronic hyperglycemia was found to increase the production of free radicals that is associated with long-term damage, dysfunction and failure of various organs, especially kidney, nerves, heart and blood vessels **(Heidari *et al*., 2008; Arora *et al*.,2009** and **Teoh *et al*.,2010).**

Type 1 diabetes mellitus (DM) results from a cell-mediated autoimmune attack against pancreatic beta cells **(American Diabetes Association, 2004),** Type 1 diabetes only 5% to 10% of all diabetic etiologies but is associated with a high frequency of vascular complications and compromises quality and expectancy of life.

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There are two distinct phases in progression of type 1 diabetes. The first phase called insulitis, is characterized by invasion of islet tissue by a population of leukocytes (T-lymphocytes) in the islet tissue. Insulitis occurs only when β-cell is present, indicating that this is a specific β-cell-targeted process. The second phase corresponds to β- cell destruction and the subsequent lack of insulin **(Mathis *et al*., 2001 and Roep., 2003)** . The pathogenic immune response is mediated by T helper 1 (Th1) subset of T cells, whereas the protective immune response is mediated by a T helper 2 (Th2) subset of T cells **(Rabinovitch *et al*., 1998).**

Stem cells have two important characteristics that distinguish them from other types of cells. First, they are unspealized cells that renew themselves for long periods through cell division **(Watt and Hagan, 2000).** The second is that under certain physiologic or experimental conditions, they can be induced to become cells with special functions such as the beating cells of the heart muscle or the insulin – producing cells of pancreas. There are two types of stem cells, embryonic stem cells and adult stem cells **(Anne *et al*., 2010).**

Bone Marrow is an important and easily accessible source of adult stem cells .Use of Autologous bone marrow –derived stem cell is safe and devoid of any ethical issues .Bone Marrow Transplantation (BMT) is becoming a powerful strategy in treating autoimmune diseases such as rheumatoid arthritis (RA), insulin dependent diabetes mellitus (IDDM) and chronic glomerulonephritis. Bone marrow (BM) contains hematopoietic stem cells and mesenchymal stem cells , both of which exhibit considerable developmental plasticity, An initial report that BM stem cells could engraft into pancreatic islets in vivo and differentiate to insulin - expressing phenotype **(Ianus *et al*.,2003)** could not be confirmed by other groups **(Efrat.,2008) .**

On the other hand, studies of Watada and colleagues suggested that bone marrow derived cells are a distinct cell population from islet cells and that transdifferentiation from bone marrow derived cells to pancreatic β cells is a rare event **(Choi *et al*.,2003 ; Lechner *et al*.,2004)** .

The present study was taken to evaluate the possible role of autologous bone marrow stem cell transplantation for cases of type 1 DM, which could differentiate into insulin- producing β cells, and its role in modulating the immune response from T helper 1 to T helper 2.

2. Material and Methods

This study was conducted on cases of auto immune type 1 Diabetes Mellitus. The selected groups of patients were enrolled in this study in **(Ain Shams University Hospitals in the Pancreatic Islet Transplantation and diabetes Research Unit)**. They were selected according to inclusion and exclusion criteria and who agreed to sign a written consent.

Included patients:

Study Group: ten patients were enrolled in this study.

Inclusion criteria:

- Diabetic cases type 1 within 5-10 years of diagnosis
- Age above 15 years.
- Both males and females.

Exclusion criteria:

- Receiving medications that depress patient's immune system.
- Patients with recurrent diabetic ketoacidosis.
- Pregnancy and lactation (history and pregnancy test in suspicious cases)
- Presence of other auto immune disorder eg. Extrinsic asthma, Graves' disease (history and clinical examination).
- Presence of allergic conditions eg. Atopy
- Patients having congestive heart failure (clinically and by echocardiography).
- Patients with chronic liver disease or renal impairment (clinically and by liver and kidney function tests).

Study procedures

a) Pre-Transplantation assessment :

All patients were subjected to:

1-Complete medical history taking and thorough clinical examination
2-Fasting and post prandial blood sugar
3-Hemoglobin A_1C
4- C- peptide
5-Liver function tests (AST, ALT and serum albumin).
6-Kidney function tests (serum urea, BUN and Creatinine).

b) Transplantation

- ♣ Bone marrow blood aspiration under local anaesthesia
- ♣ Harvesting
- ♣ Mononuclear layer to be injected intra venously.

c) Post –injection follow up:

Follow up of temporal changes in exogenous insulin requirements (daily dose and duration of usage). Secondary end points: Serum levels of hemoglobin A1C, C-peptide levels for 3 months post injection.

Serum was separated for the assessment of carbohydrate related parameters (glucose according to the method adapted by **(Trinder,1969)**, liver function parameters (Albumin according to **Doumas *et al.*(1971)**, Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) according to **Bergmeyer *et al.*(1978)**. Kidney function parameters (serum urea according to **Fawcett and Scott (1960)** and creatinine according to **Seeling and Wust (1969)**. C-peptide **(Krause *et al.*,1981),** Also, fresh whole blood was obtained in heparinized test tubes to determine the glycoslated haemoglobin (HbA1c) according to **(Klenk, 1991)**.

Statistical analysis

Data were analyzed using the SPSS for windows soft ware, version 17

3. Results and Discussion

Results

Table (1): Change in insulin requirement after transplantation during 9 months of follow up. This table shows that 70% of studied subjects decreased in their insulin requirement while 10% of studied subjects no changed, but still 20% of studied subjects increased.

Table (2): Liver function parameters pre and post transplantation. This table shows no change in ALT, AST and Albumin pre and post transplantation.

Table (3): Kidney function parameters pre and post transplantation. This table shows no change in urea, blood urea nitrogen (BUN) and creatinine pre and post transplantation.

Table (4): Fasting and post prandial blood sugar parameters pre and post transplantation in patients with type 1 DM. This table shows that fasting blood sugar and post prandial blood sugar significantly decreased post transplantation with (-176% and -25.5%) respectively after 9 months of follow up period.

Table (5): Comparison of C-peptide parameter pre and post transplantation in patients with type 1 DM. This table shows that C-peptide highly significant increased after 3, 6 and 9 months with (215%, 360% and 533%) respectively.

Table (6): Comparison of HbA1c parameter pre and post transplantation in patients with type 1 DM. This table shows that HbA1c slightly decreased after 3 months and significantly decreased after 6 and 9 months with (-2.8%, -4.6% and -7.6%) respectively.

Table (1): Change in insulin requirement after transplantation during 9 months of follow up.

Change in total insulin requirement after transplantation.		
	N	**%**
Increased	2	20.00
Decreased	7	70.00
No change	1	10.00
Total	10	100.00

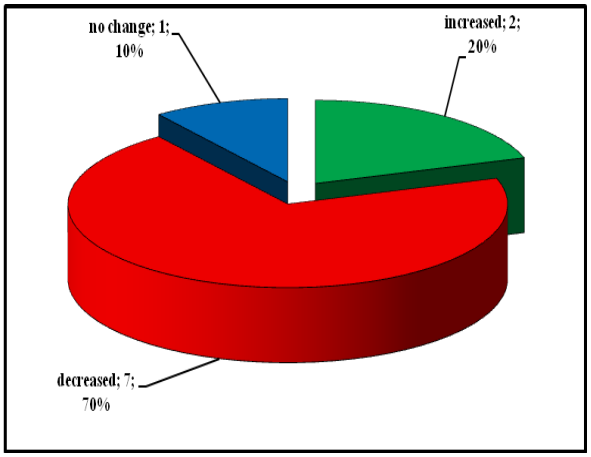

Table (2): Liver function parameters pre and post transplantation.

Parameters / Pre and post Transplantation		AST	ALT	Albumin
Pre transplantation	Range	20-31	19-30	3.9-4.9
	Mean ± S.E	25 ± 1.23	24.3±1.06	4.33±0.12
Post transplantation	Range	21-30	20-30	3.9-4.8
	Mean ± S.E	25±1.09	24.8±0.93	4.28±0.11
	% of change	Zero	2.05	-1.15
	P value	N.S	N.S	N.S

Table (3): Kidney function parameters pre and post transplantation.

Parameters / Pre and post Transplantation		Urea	BUN	Creatinine
Pre transplantation	Range	17-36	14-20	0.89-1.2
	Mean ± S.E	25.9 ± 2.0	16.1±0.06	1.06±0.04
Post transplantation	Range	19-35	14-20	0.9-1.2
	Mean ± S.E	26±1.72	16.2±0.59	1.05±0.03
	% of change	0.38	0.62	-1.62
	P value	N.S	N.S	N.S

P value < 0.001 highly significant P value < 0.05 Significant
N.S: Non Significant > 0.05 S.E: Standard Error

Table (4): Fasting and post prandial blood sugar parameters pre and post transplantation in patients with type 1 DM.

Parameters / Pre and post Transplantation		Fasting blood sugar	Post prandial blood sugar
Pre transplantation	Range	20-31	19-30
	Mean ± S.E	25 ± 1.226	24.3±1.05
Post transplantation	Range	21-30	20-30
	Mean ± S.E	25±1.08	24.8±0.93
	% of change	-17	-25.5
	P value	P<0.001	P<0.001

P value < 0.001 highly significant P value < 0.05 Significant
N.S: Non Significant > 0.05 S.E: Standard Error

Table (5): Comparison of C-peptide parameter pre and post transplantation in patients with type 1 DM.

Parameter / Pre and post Transplantation			C-peptide
Pre transplantation		Range	0.5-3.6
		Mean±S.E	1.33 ± 0.29
Post transplantation	After 3 months	Range	1.5-6.3
		Mean±S.E	4.19±0.55
		% of change	215
		P value	P<0.001
	After 6 months	Range	2-9
		Mean±S.E	6.12±0.84
		% of change	360
		P value	P<0.001
	After 9months	Range	2.2-14.9
		Mean±S.E	8.42±1.36
		% of change	533
		P value	P<0.001

Table (6): Comparison of HbA1c parameter pre and post transplantation in patients with type 1 DM.

Parameter / Pre and post Transplantation			HbA1c
Pre transplantation		Range	7.0-9.2
		Mean±S.E	8.22± 0.21
Post transplantation	After 3 months	Range	7-9
		Mean±S.E	8.05±0.2
		% of change	-2.8
		P value	N.S
	After 6 months	Range	7.2-9
		Mean±S.E	7.84±0.2
		% of change	-4.6
		P value	P<0.05
	After 9months	Range	6.9-8.9
		Mean±S.E	7.59±0.23
		% of change	-7.6
		P value	P<0.05

P value < 0.001 highly significant P value < 0.05 Significant
N.S: Non Significant > 0.05 S.E: Standard Error

Discussion

The change in the daily total insulin requirement utilized by all enrolled subjects was increased in 20% of studied subjects, decreased in 70% of studied subjects this agreed with the study done by **Snarski *et al* (2011)**; who found that 11 of 13 patients required significantly lower doses of insulin for adequate glycemic control during the follow-up period.

In our present study we found that the daily insulin requirement no changed in 10% of studied subjects also all the patient enrolled in our study did not experience any time free from insulin this is against the study done by **Voltarelli *et al* (2007)**; who reported that thirteen of 15 patients experienced continuous time free from insulin and 1 patient became transiently insulin free after Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus.

In type 1 diabetes there is selective destruction of beta-cells associated with severe or complete insulin deficiency, thereby making administration of exogenous insulin mandatory **(Chakraborti, 2008).** Autoimmune destruction in type 1 diabetes is precipitated by environmental factors in genetically susceptible individuals **(Muthukrishnan *et al*., 2007).** Type 1 diabetes is diagnosed when the patient's endogenous insulin secretion decreases to a level which results in hyperglycemia **(Palmer, 2009).**

Diabetes disturbs the liver function, due to which the activities of SGOT and SGPT were increased in the blood as observed by **Ahmad *et al*. (2008).**

In diabetic patients, elevated enzymatic activity of SGOT with only moderately increase in SGPT activity suggested cardiac damage while elevated activity of SGPT with only moderate increase in SGOT suggested liver damage as reported by **(Sundaram *et al*.(2009).** SGOT and SGPT enzymes are responsible for production of ketone bodies from aminoacids. The higher activities of SGOT and SGPT were suggested to be the cause for a high concentration of glucose. The gluconeogenic action of SGOT and SGPT plays the role of providing new supplies of glucose from other sources such as amino acids **(Asaduzzaman *et al*., 2010).** The normal activities of SGOT and SGPT are < 38 U/l and < 41 U/l respectively **(Tietz, 1999).**

In the present study both SGOT and SGPT activities were found to be within normal values indicating that there was no liver damage.

Diabetic nephropathy occurs in approximately one third of diabetics. A quick and simple way to check renal function in diabetics is to draw blood sample for serum creatinine and blood urea tests **(Wagle, 2010).** Elevation of the plasma urea and creatinine which are the significant renal function markers, may be due to metabolic disturbance in diabetes reflected in high activities of xanthine oxidase, lipid peroxidation, increased triacylglycerol and cholesterol levels **(Chandramohan *et al*., 2009).** Due to continuous catabolism of amino acids, high urea is likely to be formed from urea cycle **(Lal *et al*., 2009** and **Ortin *et al*., 2010).**

After many years of diabetes, the delicate filtering system in the kidney becomes destroyed. Increment of blood urea level with the increment of blood glucose level clearly indicates that the increased blood glucose level causes damage to the kidney **(Shrestha *et al*., 2008).**The reference range for blood urea is 14-50 mg/ dl **(Kohli *et al*., 2008).** The reference range for serum creatinine is 0.741.5mg/dl **(El-Shenawy and Abdel-Nabi, 2006** and **Ceriotti *et al*., 2008).**

In the present study, both urea and creatinine were within normal levels at diagnosis as well as after stem cell transplantation indicating that there was no kidney damage.

A significant decrease ($p<0.001$) was observed in fasting and Post prandial blood glucose levels among diabetic patients after stem cell transplantation.

These result came close to study done by **Feihong *et al* (2013);** who found during a 12- to 18-month follow-up for 7 patients underwent AHSCT that the fasting, post prandial blood glucose were decreased range from 3.9-6.0 mmol/L.

Insulin reduces hepatic glucose output and increases peripheral glucose utilization **(Rahman et al., 2009).** The decrease in both the fasting blood glucose and postprandial blood glucose level in the present study might be due to the hypoglycemic effects of insulin which in turn would have been mediated by decreased hepatic glucose output and increased peripheral glucose utilization.

The improvement in the glycemic status of diabetic patients in the present study might be due to expansion of β-cell mass and/or improvement of β-cell function. The present study confirmed the therapeutic effect of stem cell transplantation in patients with type 1 diabetes mellitus, which is evidenced by a significant decrease in both

fasting and postprandial glucose levels after intervention (**Monnier and Colette, 2009)**.

Glucose monitoring is a key component in diabetes **(Zhou *et al*., 2009).** Human C-peptide provides an accurate assessment of residual beta-cell function and thus has been widely used as a marker of insulin secretion in patients with diabetes **(Wiedmeyer *et al*., 2007).** Assay of HbA1c serves as a reliable measure of chronic glycemia and correlates well with the risk of long term diabetes related complications **(The International Expert Committee, 2009).**

Malini *et al.* (2011) reported that C-peptide is the product of the enzymatic cleavage of proinsulin and secreted into the circulation in equimolar concentrations. The measurement of C- peptide level has been reported to be a valuable index of insulin secretion. C- peptide has insulin-mimetic effects on its own by activating insulin receptors, and increasing glycogen synthesis and amino acid uptake. The C-peptide promotes insulin action at low concentrations and inhibits it at high levels, suggesting a modulatory effect by C-peptide on insulin signaling **(Sriram and Subramanian,2011).**

C-peptide is secreted from islet cells into the circulation in equi molar concentrations with insulin and is not extracted by the liver. Hence, C-peptide levels are used as a biomarker of β-cell function **(Ko *et al*., 2009).** A moderate correlation was observed by **Abdullah *et al*. (2010)** between body mass index (BMI), HbA1c and fasting C-peptide levels. The destruction of insulin producing β-cells in type 1 diabetes is caused by a β-cell specific autoimmune process **(Yoon and Jun, 2005).**

In our study we found that C-peptide is highly significant increased after 3, 6 and 9 months which agreed with the study done by **Ablamunits *et al* (2007)**; who found that the concentrations of serum C-peptide was significantly increased in patients for at least 6 months after autologous hematopoietic stem cell transplantation.
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Also this result agreed with the study done by **Carlos *et al* (2009);** who did a prospective study on 23 patients with type 1 DM (aged 13-31 years) recently diagnosed and confirmed by measurement of serum levels of anti-glutamic acid decarboxylase antibodies showed that the C-peptide levels increased significantly after a mean follow-up of 29.8 months following autologous nonmyeloablative HSCT.

Glycosylated haemoglobin (HbA1c) is a glycosylated derivative of haemoglobin used primarily to identify the average plasma glucose concentration over prolonged periods of time. Glycosylated haemoglobin (HbA1c) is produced by non-enzymatic condensation of glucose molecules with free amino acids on the globin component of haemoglobin **(Beissuenger *et al*.,1993).** The higher level of glucose led to elevation in HbA1c. it is useful in demonstration of glycemic control over a period of 8-12 weeks, which is the life span of RBCs.

The HbA1c level is proportional to average blood glucose concentration over the previous preceding four weeks to three months. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases in a predictable way **(Sellamuthu *et al*.,2009 and Bandawane *et al*.,2011).** Several reports indicated that

the measurement of glycosylated derivatives of haemoglobin in blood provides a good indication of the long-term efficacy of diabetic control **(Tembhurne and Sakarkar,2010 and Sriram and Subramanian,2011).**

Moreover, **Sriram and Subramanian (2011)** suggested that a high glucose concentration has been found to lead to the glycosylation of amino groups of lysine residue in proteins. Non enzymatic glycosylation of protein occurs by direct reaction between reducing sugars and amino groups in protein. This condition favors reduction in the level of total hemoglobin and elevation in glycosylated hemoglobin, which is directly proportional to blood glucose.

In present study we found that the HbA1C is slightly decreased after 3 months and significantly decreased after 6 and 9 months. This agreed with the study done by **Couri *et al*, (2009)**; who found that the mean pre-transplant HbA1C was 8% and decreased significantly ($p<.001$) at 3, 12, 24, 36 and 48 months to 5.4%, 5.7%, 5.7%, 5.5% and 6.0%, respectively.

Also this result agreed with the study done by **Otonkoski *et al* (2005)*;*** who found that the average HbA1c concentration was 11.5 % at diagnosis, 5.88% at 6 months and 5.76% at 12 months following AHSC transplantation in 15 patients (age 19 - 32) with early diagnosis of type 1 diabetes.

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الملخص العربي

تقييم كفاءة زرع الخلايا الجذعية ذاتية المنشأ المستخلصة من النخاع العظمى فى مرض البول السكرى من النوع الأول

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يتميز داء البول السكرى بإرتفاع مستوى السكر فى الدم ويصاحبه غالبا مضاعفات على المدى الطويل منها اعتلال شبكى واعتلال عصبى وكلوى وخلل فى الأوعية الدموية الكبيرة منها والدقيقة. صممت هذه الدراسة لتقييم الدور المحتمل لزراعة الخلايا الجذعية المستخلصة من النخاع العظمى لحالات السكرى من النوع الأول فى قدرتها على التميز للخلايا المنتجة للأنسولين وأيضا دورها فى اعديل السلوك المناعى من خلايا التائية المساعده 1 إلى الخلايا التائية المساعدة 2. أجريت هذه الدراسة على عشر حالات من مرضى المناعة الذاتية لمرض البول السكرى من النوع الأول وتم اختيار هذه الحالات وفقا لمعايير الاقصاء والاشتمال. أوضحت الدراسة نقص فى جرعات الأنسولين فى 70 % من الحالات. كما أوضحت انخفاض معنوى فى مستوى سكر الصائم والفاطر بعد ساعتين بعد عملية الزراعة, بينما مستويات س- بيبتيد أوضحت ارتفاع ذو دلاله معنويه خلال تسعه أشهر مدة الدراسة لكن حدوث انخفاض معنوى فى مستوى الهيموجلبين السكرى بعد عملية الزراعة. لا توجد أى أثار جانبية لوظائف الكبد والكلية. نستخلص من هذه الدراسة ان زرع الخلايا الجذعية علاج فعال وآمن لتحسين وظائف البيتا البنكرياسية فى مرضى البول السكرى من النوع الأول .