Comparative study on the therapeutic effects of bone marrow mesenchymal stem cells versus platelet rich plasma on the pancreas of adult male albino rats with streptozotocin-induced type I diabetes mellitus

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Comparative study on the therapeutic effects of bone marrow mesenchymal stem cells versus platelet rich plasma on the pancreas of adult male albino rats with streptozotocin-induced type I diabetes mellitus

H. El-Haroun, R.M. Salama, BMSCs versus PRP on TIDM

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Abstract

The optimal treatment for autoimmune type I diabetes mellitus (TIDM) is endogenous regeneration of the pancreatic β-cell. This can be achieved either by transplanting mesenchymal stem cells (BMSCs) from the bone marrow or injecting platelet-rich plasma (PRP). Current research reviewed a TIDM model and compared the effect of BMSCs on exocrine and endocrine pancreas portions versus PRP. Rats divided into four groups: Control group, Diabetic group (single STZ dose 60 mg / kg I.P), Diabetic + PRP group (PRP, 0.5 ml/kg SC twice weekly/4 weeks given to diabetic rats) and Diabetic + BMSCs group (1 ml of PKH26 labeled MSCs suspension in BPS (3x10⁶ cells/ml) IV to diabetic rats. Glucose, amylase and lipase levels were calculated and pancreases were designed for light, electron microscopic, immunohistochemistry, morphometry and statistical analysis. Diabetic rats exhibited elevated glucose, decreased amylase and lipase compared to control rats. In addition, variable histological degenerative changes in the form of congested blood vessels have been identified with a significant increase in the mean area percentage of collagen, a significant decrease in the diameter of the islets, the number of islet cells in Langerhans and the number of zymogen granules. Ultrastructural findings exhibited distorted Golgi,
degenerated mitochondria, pyknotic nuclei, and few secretory B-cell granules. Administration of BMSCs to diabetic group significantly increased the number of cells and diameter of Langerhans islets and the number of zymogen granules compared to Diabetic group as well as Diabetic + PRP group. BMSCs could be considered more efficient than PRP in the treatment of type I diabetes.

Key words: type I diabetes mellitus, streptozotocin, BMSCs, PRP, anti-insulin antibody

INTRODUCTION

As a major health concern in the 21st century, diabetes mellitus (DM) is a chronic metabolic disease causing hyperglycemia, it is categorized into two major types (I and II). Type I diabetes mellitus (TIDM) is an autoimmune disease distinguished by an absolute insulin deficiency due to immune-mediated beta-cell-decimation of the islet pancreas [34]. Type II diabetes mellitus (TIIDM) is distinguished by insulin resistance, reduced insulin production, and pancreatic beta-cell failure [45].

Diabetes has the potential to cause severe morbidity associated with microvascular damage and neuropathy affecting the retina, kidney, peripheral arteries and peripheral nerves due to numerous organ and body system complications [5, 10].

Insulin injections, hormone replacement, and oral hypoglycemic drugs are main treatment choices for diabetic patients to stabilize their blood glucose [6] and to prevent deterioration of the condition [40]. However, these available treatments only manage symptoms or patient's condition and can only ameliorate hyperglycemia [32]. Consequently, they are less effective in reversing insulin resistance, and in altering the progression of DM, and they do not prevent the progressive beta islet cell degeneration, and cannot cure diabetes [27].
Another way of treatment was reliance on beta-cell renewal therapy utilizing either the pancreas or the islet transplantation [47]. However, these procedures were constrained by risks of major invasive surgery, donor shortages and rejection complications [44].

Therefore, regeneration of the endogenous β-cells would be the ideal therapeutic approach for DM control [36].

Platelet-rich plasma (PRP), other names, growth factor-rich plasma or autologous concentrated plasma is now a low-cost procedure and has acted as an attractive area for clinicians and researchers over the two last decades in tissue repair [48]. PRP is an aggregation of the homologous platelets that is 3 to 5 times exceeding physiological constituent of thrombocytes suspended in a small volume of plasma in the entire blood [31]. Marx et al. [38] was the first to research PRP, suggested that plasma can only be called platelet-rich if the concentration of condensed platelets exceeds the baseline (150-400 thousand per microliter). For autologous plasma to have high healing properties, the platelet levels should be at least 1 million in 5 ml of plasma.

Growth factors (GFs) are among the innate biological peace-makers that have a role in regulating processes of tissue repair and regeneration, growth, and differentiation [35]. Platelets contain not only proteins needed for hemostasis but also convey many growth factors (GFs) [54], related to vasculature and tissue repair such as transforming growth factor (TGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), cytokines and insulin-like growth factor (IGF) [37].

PRP can be used as an inexpensive technique for delivering high levels of homologous GFs. [14]. It has been used in a number of medical and surgical specialties, including orthopedics, neurosurgery, ophthalmology, maxillofacial surgery, cosmetic surgery and dentistry [59]. Nevertheless, there was a dearth of research that detected the influence of PRP injection on diabetic rat pancreas.

Mesenchymal stem cells (MSCs) have recently attracted considerable attention as a more promising method of treating DM. MSC are multipotent bone marrow stem cells,
adipose tissue, umbilical cord and many other tissues. Such cells are used in regenerative medicine as they are capable of renewing themselves and differentiating into a wide range of cells and have a high capacity for cultural expansion [51, 60].

Stem cell transplantation can delay diabetes progression and prevent the complications of hyperglycemia side effects and was successfully implemented in diabetes [63]. Similarly, BMSCs transplant could retrieve pancreatic β-cells and control blood glucose levels [43]. In addition, there are angiogenic, anti-apoptotic, anti-inflammatory and immunomodulatory effects [13].

The purpose of this study was to compare the impact of BMSCs and PRP on the biochemical and structural changes in the pancreas of adult male albino rats with STZ-induced TIDM in order to shed light on the potential for clinical use of such applications.

MATERIALS AND METHODS

 Animals

In this study, seventy adult male albino rats were used, each weighing 180-200 gm. The animals were purchased from and kept in the Medical Research Institute's animal house, Alexandria University, Egypt, and the experiment was carried out there. Until the beginning of the experiment, animals were housed in separate wire mesh cages and acclimatized at room temperature for one week, with a light and dark duration of 12:12, with access to food and water ad libitum. In this research, ethical principles for the treatment of animals were applied and monitored by an animal facility, Medical Research Institute, Alexandria University.

 Chemicals

Streptozotocin (STZ): a vial of 1.5 g STZ powder and 220 mg citric acid (Sigma Aldrich, Egypt), dissolved in 0.1 M sodium citrate buffer (pH 6) in accordance with the directions of manufacture and was used within 5 minutes of preparation.

 Platelet-Rich Plasma
**PRP Preparation:** At the Medical Research Institute's animal house, Alexandria University, Egypt, PRP was freshly prepared. The preparation method was accomplished by adjusting the protocol of the double centrifugation tube [49]. With the rats under ether anesthesia, 2 ml of blood was collected from the retro-orbital plexus under sterilization. The blood was submitted for 10 minutes centrifugation at 1600 revolutions/minute, resulting in 3 different layers; red blood cells below, white blood cells buffy coat in the middle, and plasma above. The plasma was then pipetted and the part over the buffy coat was gained. After that, the plasma was submitted for 10 minutes centrifugation at 2000 rpm, resulting in 2 portions: platelet poor plasma (PPP) above and platelet button at the bottom. To allow platelet resuspension, a portion of the PPP was removed and another portion remained with platelets that were slightly agitated. To verify the platelet concentration, 80 μL of the PRP was counted in an automatic apparatus to ensure that the platelet number was greater than 1,000,000/μL. Finally, aspirate 0.5 ml/kg PRP with micropipette and dissolve into the saline phosphate buffer (PBS) (PRP 1:1 PBS), and subcutaneously injected with a sterile insulin syringe.

**BM-MSCs (labelled PKH67 (Green Fluorescent Cell Linker))** were supplied from the stem cell research unit of the Department of Biochemistry of the Faculty of Medicine, Cairo University, Egypt. According to [9], BM-MSCs were harvested from the bone marrow of femurs and tibiae of 6-week-old male rats.

In Dulbecco's Modified Eagle Medium (DMEM) purchased from Sigma, the Isolated MSCs were cultured. 10 percent fetal calf serum (FCS) and 1 percent 100U / ml penicillin, 100mg / ml streptomycin and 2mM L-glutamine were supplemented with DMEM. In humidified CO2- 5% air at 37° C, the cells were cultured to confluence (80-90 %). Experiments were conducted on confluent cultures that were usually acquired on the 12th to 14th day of culture. In the next 3 passages, this process was repeated. For all experiments, cells at passage 3 were used. In vitro detection of MSCs was performed by culture research, using an inverted microscope (Olympus CKX53) to appear as spindle-shaped cells (Fig. 1a). The cells were centrifuged and washed twice in a serum-free medium prior to the experiment. Pancreatic tissue was studied with a fluorescent microscope (Olympus BX50F4, No. 7M03285, Tokyo, Japan) to detect and track PKH67-stained cells (Fig. 1b). The extracted
cells in the third passage were labelled with the Green fluorescent protein (PKH67- Sigma) [17].

**Induction of diabetes**

Overnight-fasted rats received a single intra-peritoneal (I.P) injection of STZ (60 mg/kg body weight) dissolved in 0.1 M sodium citrate buffer for TIDM induction. 48 hours after STZ injection (day 0 of experiment), animals were fasted overnight and blood samples were collected from the tail vein for glucose level monitoring. Rats are considered diabetic when glucose level exceeds 250 mg/dl [59].

**Experimental design**

In order to obtain PRP, twenty of the seventy male rats were used and the other fifty were randomly divided into four groups:

— **Group I (Control group), (n =20 rats):** 4 equal subgroups were subdivided into: (5 rats each)
  — **Subgroup Ia:** Rats were retained and acted as negative control without medication.
  — **Subgroup Ib:** Rats obtained an I.P injection of a single 0.1 M sodium citrate buffer.
  — **Subgroup Ic:** Rats were subcutaneously injected on a bi-weekly basis with PRP at a dose of 0.5 ml/kg for 4 weeks.
  — **Subgroup Id:** Rats received 1 ml of PKH26 labelled MSC suspension in BPS (3x106 cells/ml) IV via tail vein [25] for two successive days.

— **Group II (Diabetic group), (n =10 rats):** A single STZ (60 mg/kg body weight) I.P injection dissolved in a 0.1 M sodium citrate buffer was administered to rats.

— **Group III (Diabetic + PRP group), (n =10 rats):** PRP was administered bi-weekly to rats at a dose of 0.5 ml/kg by SC injection for 4 weeks on day 0 following STZ diabetes induction assurance.

— **Group IV (Diabetic + BMSCs group), (n =10 rats):** 1 ml of PKH26-labeled MSC suspension in BPS was administered to rats on day 0 after induction of STZ diabetes for two consecutive days (3x106 cells/ml) IV via the tail vein [25].
**Biochemical study**

Blood samples collected from the tail vein of overnight fasting rats at the end of the 4th week to measure:

Fasting glucose level, use one-touch Accu-check glucometerR and compatible blood glucose test strips, prior to grouping at the beginning of the experiment, all animals were subjected to blood glucose level measurement to ensure that they were normoglycemic. Then, for the different sample types, the measuring method was conducted with statistical analysis. Serum pancreatic amylase was performed by Landt et al. [33], an immunoabsorbent prepared by coating latex beads with a monoclonal antibody unique for pancreatic amylase. Serum pancreatic lipase was prepared by the enzyme-colorimetric method using Automated Hitachi Analyzer [57].

**Experiments in histology, immunohistochemistry and electron microscopy**

Rats from all groups were sacrificed at the end of the 4th week by being decapitated under light halothane anesthesia. Pancreases were rapidly dissected out and divided into two parts: one for light microscopic examination and the other for electron microscopic examination.

Specimens subjected for light microscopic inspection were fixed in 10% formol saline for 24h and subsequently processed for the preparation of 5-μm-thick paraffin sections for Hematoxylin and Eosin for histological validation [11], Mallory's trichrome stain for collagen fibre indication, and Toluidine blue stain [58].

**Immunohistochemical study of anti-insulin antibody**

The immune-localization technique was performed on pancreatic paraffin sections of 5um thickness with the streptavidin-biotin-peroxidase staining method [15]. Anti-sera containing rat insulin polyclonal primary antibody (Bio-Genex, cas no.: AR.295-R.) were then incubated with these parts for 1 hour. Dako-K0690; Dako Universal LSAB Kit (bio-tinylated secondary antibody) and Dako-K0690 (streptavidin horseradish peroxidase) were incubated in the components for 30 minutes. Then, they were incubated with a 3,30-diaminobenzidine 4-hydrochloride (Sigma-D5905; Sigma Aldrich Company Ltd., Gillingham, UK.) substrate
package for 10 minutes to obtain the immune label. Lastly, the nuclei were stained by Harry’s Haematoxylin stain and a light microscope was used to examine the binding antibody [2].

**Morphometrical studies**

Using a light microscopic examination of the x400 images of the Mallory trichrome stain and anti-insulin antibody, the mean area percentage of collagen fibres and the number of insulin-positive β-cells of all the animal groups were counted. By light microscopic inspection of the X 400 Hx&E, the diameter of the Langerhans islets was determined. Light microscope analyses of the Toluidine blue stained section of x400 were used to count the number of zymogen granules. In the Department of Histology, Faculty of Medicine, Menoufia University (Leica Q 500 MC programme, Wechsler, Germany), all measurements were taken using the image analyzer. Examinations in 10 high-power fields (HPF) in each sample were carried out.

**Statistical studies**

The statistical analysis was conducted in the mean±SEM format using SPSS version 17 software (IBM Corporation, Somers, New York, USA). Using a one-way analysis of variance with post hoc analysis, the average of each group was compared with that of the other groups. In terms of chance, less than 0.01 at the P-value was the least significant quantity used.

**RESULTS**

**General observation**

Rats displayed normal behavior in all groups, with the exception of excessive urination in diabetic rats, two days after the injection of STZ.

**Biochemical results**

**Fasting blood glucose, serum lipase and amylase.** In the control group, there was no significant difference between the rats (subgroups Ia, Ib, Ic and Id). In the diabetic group, administration of STZ significantly increased blood glucose levels and decreased serum amylase and lipase levels relative to control rats (P < 0.001). Important rises in glucose levels
and decreases in amylase and lipase levels in the Diabetic + PRP group were also observed relative to the control group (P < 0.001). PRP triggered a significant decrease in glucose and a rise in the amount of amylase and lipase, relative to the diabetic group (P < 0.001). The Diabetic + BMSCs group displayed negligible variations in contrast to the control group (Table I- Fig. 2a,b,c).

**Histological results**

The histological and immunohistochemical findings of all group I (Control Group) subgroups were similar. So, Group I (Control group) applied to them.

**Hematoxylin and eosin**

All control subgroups displayed regular pancreatic architecture; the gland is covered by a capsule of thin connective tissue that sends septae separating it into lobes and lobules. The pancreatic lobules were formed of crowded acini separated by thin connective tissue septa. Acinar cell cytoplasm revealed both basal basophilia and apical acidophilia. Langerhans islets appeared among pancreatic acini as lightly stained areas (Figs. 3a-4a).

Diabetic group showed loss of normal lobular architecture with congested dilated blood vessels. Some acinar cells showed vacuolation with pyknosis of their nuclei. Islets of Langerhans showed multiple empty spaces, congested blood vessels and degenerative changes in some cells in the form of pyknosis of their nuclei and vacuolation of their cytoplasm (Figs. 3b-4b).

Diabetic + PRP group pancreatic lobular architecture appeared almost identical to the control group. However, there were still congested blood vessels and vacuolation of some acinar cells (Figs. 3c-4c).

The group of Diabetic+ BMSCs displayed almost normal lobular pancreatic architecture. Acinar cells appeared in the nuclei of the vesicles. Normal Langerhans islet was found surrounded by normal acinar pancreatic tissue (Figs. 3d-4d).

**Mallory's trichrome stain**
Sections of control group showed thin connective tissue around blood vessels, in septae, in between pancreatic lobules and in islets of Langerhans (Fig. 5a).

Diabetic group showed excessive deposition of collagen fibers around thickened hypertrophied congested dilated blood vessels. Furthermore, islets of Langerhans showed massive deposition of collagen fibers (Fig. 5b).

The group treated with PRP showed moderate collagen fibers deposition between the pancreatic lobules (Fig. 5c). On the other hand, the injection of BMSCs showed a marked decrease in the value of collagen deposition (Fig. 5d).

**Toluidine reaction**

Sections of control group showed abundant dark zymogen granules in the apical part of pancreatic acinar cells with normal features of their cells (Fig. 6a), while diabetic group showed few apical zymogen granules in pancreatic acinar cells (Fig. 6b).

Meanwhile, PRP administration to diabetic rats (Diabetic + PRP group) induced an increase in number of zymogen granules in pancreatic acinar cells (Fig. 6c). Diabetic + BMSCs group showed normal distribution of zymogen granules in pancreatic acinar cells (Fig. 6d).

**Immunohistochemical results**

Strong positively stained insulin secretory granules of the B-cells comprising the most common cell population of the islets were seen in sections of the control group (Fig. 7a). STZ administration in diabetic group resulted in a decrease in the intensity of stained secretory granules of B-cells, as well as a decrease in the number of cells and in the diameter of Langerhans islets (Fig. 7b). PRP administration to diabetic rats caused the positive stained secretory granules of B-cells to increase moderately, primarily in the central core. There were also a few groups of unstained cells still present in the periphery of the islets (Fig. 7c). The group of Diabetic + BMSCs displayed normal numbers of strong positively stained B-cell secretory granules predominantly in the central core (Fig. 7d).

**Electron microscopic results**
With respect to electron microscopic results, Subgroup Ia, Ib, Ic & Id showed no significant difference, so they were represented as control group.

Pancreatic acinar cells with basal rounded vesicular nuclei and prominent nucleoli were shown by electron microscopic analysis of the control rat's pancreas. Acinar cells displayed packed cisterns of the rough endoplasmic reticulum (RER) and variable mitochondria in their cytoplasm. Apically, electron dense zymogen granules of variable sizes were located (Fig. 8a).

There were primarily β-cells in the Langerhans islets. Many electron dense secretory granules surrounded by large lucent halo, mitochondria, and euchromatic nucleus accommodate their cytoplasm. Alpha-cell granules were small and electron dense (Fig 8b).

Ultrathin sections of Diabetic group (II) showed pancreatic acini with irregular electron dense nuclei, dilated RER, degenerated (balloon-shaped) mitochondria and phagosomes, some acinar cells were degenerated (Figs. 9a-9b). Langerhans islets primarily contain β-cells with few secretory granules displaying distorted Golgi, degenerated mitochondria and pyknotic nucleus (Fig. 9c).

PRP-treated rat pancreas (Group III) with acinar cells displayed euchromatic nuclei surrounded by disrupted RER focal area and normal mitochondria (Fig.10a). There are many electron dense secretory granules in the cytoplasm of Islets β-cells surrounded by large lucent halos, mitochondria, euchromatic nucleus, and mild dilated Golgi (Fig. 10b).

As shown by the presence of regular pancreatic acinar cells, the pancreatic acini of the Diabetic + BMSCs group showed marked improvement. Cytoplasm of acinar cells revealed regularly organized cisternae of RER, typical mitochondria and variable sized electron dense granules (Fig. 11a). The nuclei appeared vesicular and rounded. Cytoplasm β-cells containing several dense electron secretory granules surrounded by wide lucent halos, mitochondria, and euchromatic nucleus were shown (Fig. 11b).

**Morphometric results**

**Mean area percentage of collagen fiber deposition in pancreatic tissues.** The diabetic group (Group II) reported a marked increase in the mean area percentage of pancreatic collagen deposition, which was significantly higher compared to the mean value of
the control subgroups (Ia, Ib, Ic and Id). The mean area percentage of collagen fibers displaying an increased value was seen in group III, which was statistically significant compared to the control group (P>0.05). Group IV, on the other hand, displayed normal value with no noticeable difference relative to control group.

**Number of B-Cells/Islet, diameter of islets and number of zymogen granules.** In the control subgroups (Ia, Ib, Ic and Id), there were no substantial variations between the rats in the number of cells of the Langerhans islets, the diameter of the Langerhans islets and the number of zymogen granules. The administration of STZ in the diabetic group resulted in a substantial decrease in the number of cells of the Langerhans islets, in the diameter of the Langerhans islets, as well as in the number of granules of zymogen. Meanwhile, the administration of PRP to diabetic rats (Diabetic + PRP group) has led to a significant decrease in the number of Langerhans islet cells, the diameter of Langerhans islets and the number of zymogen granules compared to the control group, and a significant increase in the number of Langerhans islet cells, the diameter of Langerhans islets and the number of zymogen granules compared to diabetic rats (P < 0.001).

The Diabetic + BMSCs group showed a noticeable increase in the number of Langerhans islet cells, the diameter of Langerhans islets and the number of zymogen granules (P < 0.001) compared to Diabetic and Diabetic + PRP groups, and showed an insignificant difference compared to the control group (Table II and Figs. 12a, b, c, d).

**DISCUSSION**

In this study, 48 hours after STZ administration, TIDM was induced when blood glucose levels exceeded 250 mg/dl [59]. This correlates with previous studies demonstrating that a single 60 mg/kg dose of STZ can minimize the secretion of insulin and destruct pancreatic β-cells in rats [3].

In this study, hematoxylin and eosin in the diabetic group of exocrine pancreatic acini as well as the Langerhans islets showed loss of normal lobular architecture, congested dilated blood vessels, necrosis, vacuolation and apoptotic pyknotic nuclei.

These changes are close to the observations of the congested blood vessels and darkly stained pyknotic nuclei of some diabetic rat acinar cells [46, 42] that observed nearly all
Acinar cells in diabetic rats were swollen with small vacuoles. These results are consistent with Nurdana et al. [42] that revealed histological destruction of the pancreatic islets of diabetic rats. Our findings coincide with Omar and Aboulkhair [46] who documented cell necrosis with congested blood capillaries, apoptosis in β-cell of Langerhans and vacuolations in their cytoplasm. Ismail et al. [26] documented damage to the β-cell of Langerhans due to STZ injection. Vacuolation has been described as one of the membrane permeability disorders that led to the transport of water and electrolytes into the cell. Significant cellular membrane damage resulting from lipid peroxidation mediating reactive oxygen species can be referred to as a permeability disorder [22].

The effect of STZ on the pancreas was explained by a decrease in cellular NAD+ and ATP and an increase in the release of reactive oxygen species secondary to the passage of STZ through the cell membranes, causing alkylation and DNA damage, as well as the release of nitric oxide inside the cells which inhibits the action of aconitase, causing further DNA damage [3]. In addition, chronic diabetes hyperglycemia contributes to increased development of oxygen-free radicals through glucose autoxidation, lipid peroxidation, and protein glycosylation, resulting in disruption of the oxidative/anti-oxidative balance, resulting in oxidative stress and cellular harm in turn. These effects could result in the degeneration after STZ injection of islet β-cells and pancreatic acinar cells [53].

The previous histological results of the destruction of islet β-cells in the present study could explain the substantial increase in blood glucose levels and the apparent decrease in the number of Langerhans islet cells and the diameter of Langerhans islet cells in the diabetic group compared to the control group. This interpretation is agreed by others [41] who have explained that the drop in plasma insulin levels caused by selective pancreatic beta cell necrosis is the cause of hyperglycemia.

There was a decrease in serum amylase and lipase in the diabetic group in this study. These results are in agreement with Sano et al. [52] who documented damage to β-cells with decreased synthesis of amylase and release from the exocrine pancreas of diabetic rats, pointing to the close connection between the endocrine and exocrine sections of the pancreas of the vascular network. In addition, marked decrease in amylase enzyme have been documented in STZ-diabetic mouse pancreatic acinar cells [4]. These findings suggest that the
presence of insulin receptors on acinar cells is involved in the control of insulin function. In order to control cytosolic Ca$^2+$ signals in acinar cells, adenosine triphosphate (ATP) is well recognized and ATP production in acinar cells was inhibited by glucose metabolism in DM. Therefore, decreased cytosolic Ca$^2+$ signals secondary to DM may be linked in reducing the secretion of digestive enzymes [30]. However, elevations in serum pancreatic amylase and lipase were reported for paediatric diabetic ketoacidosis [50].

Excessive deposition of collagen fibres was detected by Mallory’s trichrome stain inspection of the diabetic group. These results coincide with El-Desouki et al. [18] and Sorour et al. [56] that revealed increased collagen fibres around the islands of Langerhans, the pancreatic acini, the blood vessels and intercalated ducts in the diabetic group. Pancreatic fibrosis may be caused by pancreatic stellate cells (PSCs). Hyperglycemia leads to the activation of PSCs in diabetes, resulting in fibrotic changes. In addition, stellate cell invasions of the pancreatic islets contribute to the destruction of fibrotic islets, resulting in a decline in beta-cell proliferation and a rush to apoptosis in diabetic patients [29]. Localized PSCs have begun to proliferate, morphologically turn into myofibroblast-like cells, and secrete components of the extracellular matrix once activated [8]. Moreover, collagen fibres were found allowing inadequate oxygen to enter the tissue, leading to degenerative changes and necrosis [3]. Furthermore, severe tissue damage following cytotoxic STZ-induced diabetes has caused lymphocytic depletion to increase reticular fibers [1].

Diabetic group showed few apical zymogen granules, which documented in agreement with other findings, that related decreased zymogen granules significantly to decrease serum amylase and acinar cell atrophy in diabetic rats [52]. Likewise, ultrastructural changes of acinar cells as electron dense nuclei, dilated RER, degenerated mitochondria, and phagosomes were detected. These findings agreed with others [18] that recorded dilatation and vesiculation of exocrine acinar cells with rER cisternae and mitochondrial disruption in diabetic rats due to disorganization of cytoskeleton and integral proteins on the RER and the accumulation of iso-osmotic fluid in the injured cell.

Langerhans islets β-cells revealed degenerative changes such as distorted golgi, degenerated mitochondria and pyknotic nucleus with few secretory granules ultrastructurally. Similar findings have been documented in pancreatic beta cells of diabetic rats with alloxan or
Moreover, El-Desouki et al [18] showed that pancreatic beta cells were degenerated with a pronounced decreased and breakdown of their granules. STZ caused damage to both the exocrine and endocrine portions of the pancreas. The most promising methods for the treatment of DM have recently been targeting the pancreatic β-cell [61].

Current findings of the Diabetic + PRP group have shown an improvement in the histological picture of pancreatic islet cells and acini. These improvements have been confirmed by a decrease in blood glucose, increase in serum amylase and lipase levels with a modest increase in the amount of positively stained B-cell secretory granules. These findings coincided with El-Tahawy et al. [19] who found improvement in the morphology of the pancreas, several healthy islets with an increase in the number of β-cells, and a substantial decrease in blood glucose levels in the Diabetic + PRP treated group. The biological characteristics of PRP depend on the platelets concentration; convenient PRP preparations can provide high concentrations of many growth factors (GFs), including Insulin-like growth factor, epidermal growth factor, and β-transforming growth factor. PRP should contain at least one million platelets per microliter to be value for tissue repair [21]. GFs originated from PRP may be used as prospective therapies for diabetes as it can induce in vivo pancreatic β-cell proliferation, regeneration and insulin production [36]. Moreover, a substantial elevation in GFs molecules, like vascular endothelial growth factor (VEGF), was noticed after treatment with a single dose of leukocyte-rich PRP [7]. In addition, Bonner-Weir [12] clarified the production of new islet cells from progenitors in postnatal rodents via the neogenesis process. Some cells derived from hormone progenitors present near the ducts in the adult pancreas and are transformed in adult mice into hormone-positive islet cells [64]. In both rodents and humans, endocrine cells can also be discovered in exocrine cells. Similarly, the long-term low dose administration of EGF induced β-cell neogenesis in diabetic mice and resulted in ductal cell differentiation into β-cells [65].

From the current study, it could be inferred that BMSCs could alleviate the degeneration of β-cells and acinar cells induced by STZ. These findings were confirmed by the results of Omar and Aboulkhair [46] that showed normal histological structure of the Langerhans islets and the pancreatic acini with a substantial decrease in the mean random
blood sugar level value, abundant positive insulin immunoreaction, with a significant increase in the mean area of islets β-cells in the treated BMSCs group compared to the diabetic group.

These assumptions in the histological and biochemical improvement of the Diabetic + BMSCs group in the current study were coincided with the findings that BMSCs have a crucial role in repairing injured tissues. They may differentiate, to replace the dead cells and to stimulate surrounding cells in the microenvironment, by secreting stimulant factors to boost the tissue repair process [62]. Consequently, BMSCs may be applied to treat tissue dysfunction in chronic hyperglycemia. By replacing T1DM with either in vitro or in vivo differentiation, local microenvironment modulation by cytokines, chemokines and factors that promote endogenous regeneration, and by reducing or preventing autoimmunity of beta cells, MSC transplantation may increase the mass of beta cells [20]. Similarly, others [24] found that the stem cells survived and differentiated into β-cells resulting in an increase in the number and size of islets of Langerhans. Similarly, by observing normal blood glucose levels and neovascular formation after co-transplantation of pancreatic MSCs and islet mass, Sordi et al. [55] suggested the role of MSCs as helper cells. In comparison, other authors [16, 23] have found that the hyperglycemic environment of diabetic patients reduces the proliferative capacity of MSCs, thus increasing cell apoptosis.

CONCLUSIONS

The present study reveals a model by STZ for TIDM. Promising treatments for diabetics are BMSCs and PRP. Their results are clearly visible. Due to their improved histological and ultrastructural protection of the pancreas and their ability to increase the number of cells in the islets of Langerhans, to decrease the amount of glucose and to increase serum amylase and lipase enzymes, the use of BMSCs may be more successful than PRP. PRP, however, is much less expensive than BMSC.

REFERENCES


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<td>59.4±2.72</td>
<td>61.5±1.78</td>
<td>P1=0.45, P2=0.41, P3=0.65, P4=0.001**, P5=0.004**, P6=0.11</td>
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<td>Serum lipase</td>
<td>85.3±4.45</td>
<td>83±3.37</td>
<td>86.5±2.46</td>
<td>87.9±2.02</td>
<td>36±2.87</td>
<td>64.1±4.82</td>
<td>84.3±6.52</td>
<td>P1=0.067, P2=0.494, P3=0.119, P4=0.001**, P5=0.001**, P6=0.578</td>
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P1=-ve cont vs sodium citrate
P2=-ve cont vs PRP
P3=-ve cont vs BMSCs
P4=-ve cont vs STZ
P5=-ve cont vs STZ+ PRP
P6=-ve cont vs STZ+ BMSCs

* Significant difference P<0.01

** Highly significant difference P<0.001
Table II. Morphometric results: Number of B-Cells/Islet, Diameter of islets, Number of zymogen granules and Mean Area Percentage of Collagen Fibers

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<th>-ve cont</th>
<th>sodium citrate</th>
<th>PRP</th>
<th>BMSCs</th>
<th>STZ</th>
<th>STZ+ PRP</th>
<th>STZ+ BMSCs</th>
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<td>Mean Area Percentage Of</td>
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<td>Collagen Fibers</td>
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<td>8.5±0.419</td>
<td>8.52±0.48</td>
<td>8.41±0.55</td>
<td>29.4±2.27</td>
<td>20.6±2.07</td>
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P1= -ve cont vs sodium citrate
P2= -ve cont vs PRP
P3= -ve cont vs BMSCs
P4= -ve cont vs STZ
P5= -ve cont vs STZ+ PRP
P6= -ve cont vs STZ+ BMSCs

* Significant difference P< 0.01

** Highly significant difference P<0.001
**Figure 1.** (a) 10-day culture, inverted microscope micrograph of bone marrow mesenchymal stem cells (BMSCs). The cells are adherent spindle-shaped cells (b) An image of a fluorescent microscope showing BMSCs labelled with fluorescent PKH67 appearing inside acini and pancreas islets as bright dots. Scale bar = 25µm.

**Figure 2.** Fasting glucose, serum pancreatic amylase and lipase levels are illustrated in the graph.

**Figure 3.** (a) Stained pancreatic section of hematoxylin and eosin of the control group showing pancreatic acinar cells (arrow). In between pancreatic acini, formed by cords of pale stained cells with foamy, the islet of Langerhans (arrow head) is also seen. (b) Group II (Diabetic group) demonstrating congested dilated blood vessels (BV). Some acinar cells showed vacuolation (v) with their nuclei pyknosis. The Langerhans Islets showed empty spaces (arrow head) and degenerative changes in some cells in the form of pyknotic nucleus and cytoplasm vacuolation (star). (c) Group III (Diabetic + PRP group) showing congested blood vessels and certain acinar cells with pyknotic nucleus (arrow). The Langerhans Islets showed empty spaces (arrow head). (d) Group IV (Diabetic + BMSCs) reveals normal pancreatic lobular architecture. The acinar cells appear with vesicular nuclei (arrow). Normal pancreatic islet of Langerhans (arrow head) surrounded by normal pancreatic acini is seen. Scale bar = 40µm.

**Figure 4.** (a) Stained pancreatic section hematoxylin and eosin of the Control group exhibiting pancreatic acinar cells appearing pyramidal with basal rounded vesicular nuclei (arrow). Between pancreatic acini, formed of cords of pale stained cells with foamy cytoplasm, the islet of Langerhans (I) is also seen. (b) Group II (Diabetic group) showing dilated congested blood vessels (BV). Some acinar cells showed vacuolation (V) with their pyknotic nuclei. Langerhans islets showed empty spaces (arrow head) and degenerative changes in some cells in the form of cytoplasmic vacuolation (star) (c) Group III (diabetic + PRP group) showing vacuolation (v) of some acinar cells with pyknotic nucleus (arrow). The islets of Langerhans showed empty spaces in their cells (arrow head) and vacuolation (star). (d) Group IV (Diabetic + BMSCs group) showing normal pancreatic lobular architecture, normal pancreatic islet of Langerhans (I) surrounded by normal pancreatic acini (arrow). Scale bar = 25µm.

**Figure 5.** (a) Mallory's trichrome stained portion of the control group pancreas showing thin connective tissue septae (arrow) between pancreatic acini and lobules. (b) Group II (diabetic group) exhibiting dilated blood vessels (BV) with large deposition of collagen fibres around blood vessels (arrow) (c) Group III (diabetic group + PRP) exhibiting mild deposition of collagen fibres between pancreatic acini (arrow head) and around blood vessels.
(arrow). (d) Group IV (diabetic group + BMSCs) exhibiting thin collagen fibres between pancreatic acini (arrow) and cells of islets of Langerhans (arrow head). Scale bar = 25 µm.

**Figure 6.** (a) Toluidine blue stained pancreatic section of the control group displaying zymogen granules (ZG) in the apical part of the pancreatic acini-lined cells. (b) Group II (diabetic group) showing few apical zymogen granules (ZG) in most pancreatic acini with multiple vacuoles (v). (c) Group III (Diabetic + PRP) showing increase in number of apical zymogen granules (ZG) with several vacuolations (v) of their cytoplasm in most acinar cells. (d) Group IV (Diabetic + BMSCs) displaying zymogen granules (ZG) in the apical portion of the cells lining pancreatic acini.

**Figure 7.** (a) Pancreatic section stained anti-insulin antibodies of the control group exhibiting strong positively stained B-cells for anti-insulin antibodies constituted by the main population of the Langerhans islet, leaving few central unstained cells. (b) Group II (Diabetic group) with very few weak positive stained B-cells exhibiting anti-insulin antibodies located mainly in the central part of the islet. (c) Group III (Diabetic + PRP group) with a moderate positive reaction of stained B-cells to anti-insulin antibodies. The majority of unstained cell groups are located in the peripheral portion of the islet. (d) Group IV (Diabetic + BMSCs) showing strong positively stained B-cells for anti-insulin antibodies which constitute the largest population of the Langerhans islet leaving few unstained central cells.

**Figure 8.** (a) Group I (control group) demonstrating acinar cells with euchromatic nuclei (N) and many electron dense secretory granules (arrow) of variable size, more or less normal mitochondria (M) and rough endoplasmic reticulum (RER). (X 8000). (b) Group I (Control group) showing Langerhans islets incorporating β-cells (β) and alpha-cells (α). Many electron-dense secretory granules of β-cells surrounded by a large light halo (arrow), mitochondria (arrow head), and euchromatic nucleus (N), while alpha-cell granules were small and electron-dense. (X 17500).

**Figure 9.** (a) Group II (diabetic group) displaying a large central nucleolus (Nu) hyperchromatic nucleus (N), massive dilated RER (RER), balloon-shaped mitochondria (M) and phagosomes (arrow head). Note the variable-sized apical secretory granules (arrow). (X 8000). (b) Pancreatic acinar cells of Group II (diabetic group) with abnormal pancreatic acinar nucleus (N) surrounded by dilated RER (RER) with distorted mitochondria (M) and phagosomes (arrow head). (X 8000). (c) Group II (Diabetic group) Langerhans islets include distorted Golgi (circle), degenerated mitochondria (M) and non-clear and swollen nuclear membrane (N) of β-cells. (X 17500).
Figure 10. (a) Group III (Diabetic + PRP group) showing euchromatic nucleus (N), acinar cells surrounded by focal disrupted RER (arrow), most likely normal mitochondria (M). (X 8000). (b) Group III showing β-cell-containing islets of Langerhans with several dense electron secretory granules surrounded by a large light halo (arrow), mitochondria (arrow head), euchromatic nucleus (N), and mild dilated golgi (curved arrow). (x 17500).

Figure 11. (a) Group IV (Diabetic + BMSC group) displaying euchromatic nucleus (N) acinar cells and multiple electron dense secretory granules of variable size (arrow), mild degenerated mitochondria (M) and most likely regular RER (RER). (X 8000). (b) Group IV (Diabetic + BMSCs group) showing Langerhans Islets incorporate β-cells with several dense secretory granules surrounded by a large light halo (arrow), mitochondria (arrow head) and euchromatic nucleus (N), golgi (curved arrow). (X 17500).

Figure 12. View graphs (a) Mean area percentage of collagen (b) Number of B-cells / islet, (c) Islet diameter (d) Number of granules of zymogen.