Bone Marrow–Derived Mesenchymal Stem Cells and Their Therapeutic Role in Type 1 Diabetes Mellitus

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Abstract

Diabetes Mellitus (DM) is the most widespread endocrine disease characterized by hyperglycemia, and impaired carbohydrate, lipid, and protein metabolism. Recently, numerous research focused on the potential application of adult mesenchymal stem cells (MSCs) as an alternative therapy for diabetes treatment owing to the diverse properties of these cells with hopeful results. Thus, the present study aimed to investigate the potential of type 1 diabetes mellitus (T1DM) recovery through the engraftment of undifferentiated MSCs, as well as the impact on the pancreatic β cells. The experiment was carried out on thirty adult male and female albino rats weighing (180-200 gm each). Twenty-five female rats used in the experiments were divided into 3 groups; the control group (5 rats), the diabetic group injected intra-peritoneal at a dose (60 mg/kg, i.p.) streptozotocin (STZ) (10 rats), and the diabetic group treated with 3×106 of undifferentiated male bone marrow MSCs) BM-MSCs) I/V at a single dose (10 rats). BM-MSCs were isolated (from 5 male donor rats), cultured, and characterized by flow cytometry. The pancreatic tissues from the experimental groups were dissected and prepared for histological, histochemical, immunohistochemical, molecular as well as morphometric studies. The obtained results showed that the diabetic untreated animals had vacuolations, reduction in the size of the pancreatic islets, and congestion of the blood vessels. Moreover, a positive PCR product of the Sry gene, a highly significant increase in insulin immunoreactivity, and restoration of the normal architecture of pancreatic islets in the treated diabetic group with BM-MSCs were detected.

Key words: Blood vessels, Diabetes Mellitus (DM), Mesenchymal stem cells (MSCs), Pancreas, STZ.

Introduction

Diabetes mellitus (DM), is a chronic metabolic disorder characterized with progressive destruction of pancreatic β cell resulting in constant hyperglycemia, ***(Chhabra and Brayman, 2013 ; El-Sawah et al., 2021).*** DM is considered one of the top ten reasons of mortality in adults, with an estimated four million deaths worldwide in 2017, additionally DM-related global health expenditure was estimated to be United States dollar (USD) 727 billion in 2017 ***(IDF, 2017)***.

The traditional method of treatment of T1DM is exogenous insulin injections ***(Amer et al., 2018).*** The fact is, exogenous insulin can't maintain the optimum physiological level of glucose and is often accompanied by hypoglycemic coma ***(Lilly et al., 2016).*** Pancreas/ islet cell transplantation is the alternative treatment method for β cell replacement therapy but there is an obstacle of donor deficiency, non-availability, and surgical as well as post-surgical complications ***(Bouwens et al., 2013).*** Besides, possibility of immune rejection is recorded ***(Dang et al., 2017).*** Such challenges have prompted researchers to look for other sources of glucose-responsive insulin-producing cells. Strategies based on different types of stem cells appear to be the most promising to date ***(McCall et al., 2009).*** Cell therapy has become prevalent in the recent years, owing to its paracrine action, which produces immunomodulation as well as anti-inflammatory and anti-apoptotic effects ***(da Silva et al., 2022).***

Stem cells (SCs) can obtained from variety of sources, involving embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), germ cell derived stem cells, and mesenchymal stem cells (MSCs) ***(Lilly et al., 2016).*** MSCs are non-hematopoietic SCs, have multipotency differentiation capacity, as their capability to specialize into several types of adult cells. *Plus,* their self-regeneration ability ***(Wang et al., 2018),*** promoting homeostasis and repair, making them the promising regenerative medicinal tools ***(Naji et al., 2019).*** MSCs can derived from variety of tissues, including adipose, muscle, umbilical cord blood, peripheral blood, liver, placenta, skin, amniotic fluid, breast milk, synovial membrane, and tooth root, in addition to bone marrow ***(Xie et al., 2015).*** Wherefore, bone marrow stem cells (BMSCs) is considered the most common SCs type used in diabetes treatments, as a result of its great potential, easily isolation, copious source and negligible ethical issues ***(Dang et al., 2017).*** Numerous studies have shown that MSCs can delay the onset of T1DM and ameliorate the hyperglycemia by enhancing the pancreatic cell regeneration, insulin producing cells

(IPCs) differentiation, insulin resistance improving, increasing insulin secretion, and promoting α pancreating cells transformation to β cells. Thus, this thereby is restricting the long-term complications, improving the quality life, and reducing immunosuppression-related impacts ***(Zang et al., 2017).***

Hence, this study will evaluate the potential effects of the BM-MSCs for T1DM treatment and their roles in the pancreatic β cell regeneration.

Materials and Methods

Animals and housing

Thirty adult male and female albino rats weighting (180-200 gm each) (twenty-five females and five males) were purchased from the animal house, Faculty of pharmacy, Suez Canal University, Ismailia, Egypt. All rats were housed in commodious wire mesh cages and were maintained under laboratory conditions at 22°C on 12-hrs. dark & light cycles, received food and water ad libitum. All experimental procedures were approved according to the guidelines for the care and use of laboratory animals which announced by The National Institute of Health, (approval no. 201901). Experimental and laboratory work were performed in the Center of Excellence in Molecular and Cellular Medicine (CEMCM), Faculty of Medicine, Suez Canal University.

Animal grouping

One week after acclimation, five male rats were used for MSCs donation. The 25 female rats were divided into three groups as following: group I; negative control group (five female rats), group II; diabetic group (ten female rats) and group III; diabetic group treated with BM-MSCs (ten female rats).

Induction of diabetes mellitus

After fasting the rats for 16 hrs., diabetic groups (II & III) were injected with streptozotocin (STZ). STZ was purchased in a powder form from Loba Chemie, India, and it was dissolved in freshly prepared 0.1 mM sodium citrate buffer; that was obtained from Biochemazone, Canada, USA (pH 4-4.5). The freshly prepared STZ solution was injected intra-peritoneal at a single dose (60mg/kg, i.p) ***(Mali et al., 2017)***. Blood samples were collected from tail vein under general anesthesia, 7 days after diabetic induction. The glucose level measured using AGM-2200 Gluco Dr. Super sensor. Hyperglycemia was confirmed 1 week after injection of STZ, the fasting glucose level was ≥200 mg/dl ***(Qinna and Badwan, 2015).*** From the beginning of diabetic induction until one month the glucose level was monitored periodically. At the same time isolation of BM MSCs and culturing procedures were began.

Isolation of BM-MSCs

Under restricted hygienic and sterilized conditions, the bone marrow specimens were obtained from five adult male albino rats

(180-200 gm each), age was about 4 months, via flushing their femur and tibia with prewarmed Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 1% penicillin streptomycin (volume / volume) (Lonza, Belgium). The isolation, monolayer culture and expansion to passage 2 (P2) were processed based on method described by ***Soleimani and Nadri,(2009).***

Characterization of BM-MSCs by flow cytometry

The analysis of rat BM-MSCs surface marker CD44 which represents BM-MSCs marker and CD45 indicative of the hematopoietic markers, flow cytometry was performed according to method described in previous study ***(Zou et al., 2020).***

Treatment stage

One month after diabetic induction, the animals of group III were injected I / V by 3×10^6 of cultured BM-MSCs suspended in 0.5 ml phosphate buffer saline (PBS) at a single dose in the tail vein. One week later, blood glucose level was measured using AGM-2200 Gluco Dr. Super sensor and was measured again 3 times / week, from the 2^{nd} week of the treatment till the 4^{th} one.

Tissue collection

One month after BM-MSCs treatment, rats were sacrificed by cervical dislocation under light anesthesia with ketamine-xylazine mixture(0.15 ml/100 g / i.p.). After dissection, pancreatic tissues of control group I, diabetic untreated group II, and diabetic-treated group III were immediately removed, then were fixed in 10% neutral formalin for one week for histological examination. Concerning, histochemical examination, small specimens of pancreatic tissue were fixed in Bouin's solution for 24 hrs. Also, pancreatic samples were fixed in 4% paraformaldehyde for 2 days for immunohistochemical investigation.

Histological and histochemical procedures

After trimming, the preserved specimens were briefly washed in 70% ethanol, then were subjected to ascending series of ethanol (75%, 80%, 90%,95%, absolute alcohol I, II and III) for dehydration, then were cleared in xylene (I and II) two hours each, then were embedded in paraffin wax (melting point 56-58 °C). By using rotatory microtome, paraffin sections of 5-7 um thickness were obtained. Standard staining steps of the paraffin sections were done using Hematoxylin and counter stained with eosin (H&E), then the slides were mounted in DPX, and ready for histological examination ***(Suvarna et al., 2018).*** Histochemical procedures were used to differentiate pancreatic beta cells from other pancreatic islet cells, by using Chromium-Hematoxylin-Phloxin stain, which is specific stain for cytoplasmic granules of islet B cells ***(Gomori, 1941).*** Meanwhile trichrome stain either Masson trichrome or its modifications was used for visualization the connective

tissue specifically collagen fibers ***(Suvarna et al., 2018).***

Immunohistochemical analysis

After fixation the pancreatic tissue samples from control, diabetic non treated as well as diabetic group treated with BM-MSCs were processed; and then 5-7 µm thickness paraffin sections were prepared to detect anti-insulin antibody using streptavidin–biotin–peroxidase staining method ***(Cemek et al., 2008)*** via using rat anti-insulin antibody provided by Bio Genex (Cat. No. AR. 295-R).

PCR detection of male derived MSCs (Sry gene):

To confirm homing of MSCs obtained from male rats into recipient female rats, the presence or absence of the sex determination region on the Y chromosome (*Sry*) gene in recipient females was assessed by conventional PCR. Genomic DNA was isolated from tissues of the rats in MSCs-treated groups using Gene JET genomic DNA extraction kit, following the manufacturer protocol (Thermo Scientific, #K0721). Amplification of *Sry* gene was performed using forward primers: 5'-CATCGAAGGGTTAAAGTGCCA-3' and reverse primer 5'-ATAGTGTGTAG-GTTGTTGTCC-3' were obtained from published sequences ***(An et al., 1997).***

Quantitative parameters applied for histological, histochemical and immunohistochemical analysis:

The evaluation of images of targeted parts were done by utilizing image J software established by The National Institute of Health (Betheda, Maryland. USA).

Morphometric examination:

Morphometric analysis of the diameter of pancreatic islets using H&E sections:

We were investigated six pancreatic sections in each group at a scale bar 100µm for measuring the diameter of the pancreatic islets (non-overlapping islets).

Morphometric analysis of the mean area% of collagen fibers in pancreatic tissue using Masson trichrome sections

The mean area% of collagen fibers surrounded either the stromal blood vessels or among pancreatic acini was measured according to pervious study ***(Sorour et al., 2019)***. This was applied on six tissue sections at scale bar 100 µm in each group.

Measurement of immunostaining intensity of β cells

Mean % area of β cells immunoreactivity for insulin in pancreatic tissue

For the revealing of the percentage of the immunostained area of the insulin hormone in β cells; six images of chosen parts from each group were examined using the image J software at scale bar 50 µm.

Statistical analysis

Data were presented as mean ± SE for statistical analysis. One-way Analysis of variance (ANOVA) followed by least significant difference (LSD) and Duncan tests

were employed for comparison between groups. P value >0.05 was judged statistically non-significant, while if P value ≤ 0.05 and P ≤ 0.01 was considered statistically significant and highly significant, respectively.

Results

MSCs characterization

The isolated cultured bone marrow cells appeared floating and rounded during passage 0 (P0) at the time of seeding (Fig. 1 A). During the 2nd and 3rd day post seeding the cultured cells segregated into 2 different cell populations: rounded non-adherent cells, mostly of hematopoietic origin and were discarded with the 1st exchange of medium at the 3rd day of culture. The 2nd population of cells exhibited plastic adherent affinity to the substratum of the tissue culture flasks. The adherent cells took different morphological features during P0 ranging from polygonal, spindle shaped, star shaped, sperm shaped and fibroblast-like. The cells displayed granular cytoplasm, many cytoplasmic processes, and a single large vesicular nucleus with multiple nucleoli. These cells referred to BM-MSCs (Fig. 1 B). By the end of the first week, BM-MSCs began to form colonies (Fig. 1 C). At the end of the 2nd week the cells reached 80% confluency (Fig. 1 D) after which they were subjected to subculture. During the 1st and the 2nd passage (P1 & P2, respectively); the cells appeared homogenously fibroblast-like instead of their heterogenous morphology during P0 (Fig. 2 A & B).

Phenotypic analysis of BM-MSCs by flowcytometry:

Flowcytometric analysis of BM-MSCs revealed that all the isolated cells expressed the mesenchymal stem cell marker CD 44 while few percentages of them (24.3%) expressed the hematopoietic stem cell marker CD 45 (Fig. 2 C).

Histological and histochemical findings

Within control group I, histological examination of pancreatic tissue revealed normal architecture of the exocrine and endocrine pancreatic portions (Fig. 3 A). The exocrine portion was represented as pancreatic acini, and the connective tissue stuck in between with blood vessels. On the other side, lightly stained oval or rounded masses were represented the endocrine pancreatic portion (islets of Langerhans) which distributed among pancreatic acini. Every individual islet included several types of endocrine cells which were organized in anastomosing cellular cords that separated by numerous blood capillaries, and their nuclei were spherical and centrally located with abundant acidophilic cytoplasm. Alpha (α) and beta (β) cells were the most significant islets cells that were difficult to be distinguished with H & E stain (Fig. 3 A). While staining with Phloxin B stain could differentiate between α and β cells, which stained red and blue, respectively. The center of the islets

contained β cells while the peripheral part of islets was occupied by α cells (Fig. 4 A). On the other side, there were several histological changes observed in the pancreatic tissue of the diabetic untreated group II, includes vacuole formation of pancreatic acini; besides congestion of the blood vessels with increase in its thickness (Fig. 3 B & C). While reduction in size of islets of Langerhans as well as irregularity in their shape was observed (Fig. 3 C & 4 B). Using Masson trichrome stain; The collagen fibers around the pancreatic acini, as well as blood vessels revealed delicate fibers in the control group (Fig. 5 A & B), whereas, became dense in the diabetic non treated group, in addition to congested blood capillaries in islets of Langerhans (Fig. 5 C). Also, highly congested stromal blood vessels, besides hypertrophy as well as increase the thickness in the tunica media with cytoplasmic vacuolation (Fig. 5 E & F).

On the opposite side, there was restoration in the pancreatic tissue in diabetic group treated with BM-MSCs which was evident by increase of the size of islets of Langerhans. As well as it appeared in the normal architecture with normal blood capillaries in-between (Fig. 3 D & 4 C). Some vacuoles may be seen (Fig. 4 C) Additionally, by using Masson trichrome stain, little collagen fibers were observed around pancreatic acini and blood vessels. Besides some blood vessels were slightly congested in comparison with diabetic non treated group (Fig. 5 D).

Immunohistochemical expression of insulin hormone

Very strong immunoreactivity for insulin hormone in cytoplasm of β cells was observed in the control group (Fig. 6 A), while the reaction was very weak in the diabetic non treated group that confirmed β-cell damage with reduction in their number (Fig. 6 B). In the Diabetic treated one, the immunoreactivity was mild positive for insulin hormone indicated restructuring of pancreatic islets in addition to incompletely renovation of β-cells (Fig. 6 C).

Molecular results

The male control rats and female diabetic treated rats with BM-MSCs were positive for Sry gene in PCR product (lane 1, 104 bp) and (lane 3, 104 bp) respectively in the pancreatic tissue sample (Fig. 7). While negative PCR products for Sry gene in female control and female diabetic non treated rats in pancreatic tissue sample (Fig. 7).

Quantitative analysis

Morphometric analysis

Morphometric analysis of pancreatic tissue using H&E sections

Morphometric analysis of pancreatic islets' diameter in H&E-stained sections showed that there was a highly significant decrease ($P<0.01$) in diabetic non treated group when compared to control one (Fig. 8 A). As well as the diameter in diabetic

group treated with BM-MSCs was highly significant increase (P<0.01) compared with diabetic non treated group (Fig. 8 A). However, no significant difference between control and diabetic BM-MSCs treated group (Fig. 8 A).

Morphometric analysis of mean area% of collagen fibers in pancreatic tissue using Masson trichrome sections

There was a highly significant increase (P<0.01) in the mean area % of collagen fibers in the pancreatic tissue in diabetic non treated group when compared to the control group (Fig. 8 B). While highly significant decrease (P<0.01) of area occupied by collagen fibers in the diabetic BM-MSCs treated group versus the diabetic non treated group (Fig. 8 B). By comparing between the diabetic BM-MSCs treated group and the control one, the mean area % of collagen fibers statistically had no significant differences (Fig. 8 B).

Measurement of immunostaining intensity of β cells

Mean % area of β cells immunoreactivity was showed highly significant difference (P<0.01) between the groups, as it was observed that the most reduced intensity was observed in the diabetic non treated group (Fig. 8 C), while the highest intensity was recorded in the control group (Fig. 8 C). Additionally, the diabetic BM-MSCs treated group was showed moderate intensity (Fig. 8 C). The control and the diabetic BM-MSCs treated group had no significant difference (Fig. 8 C).

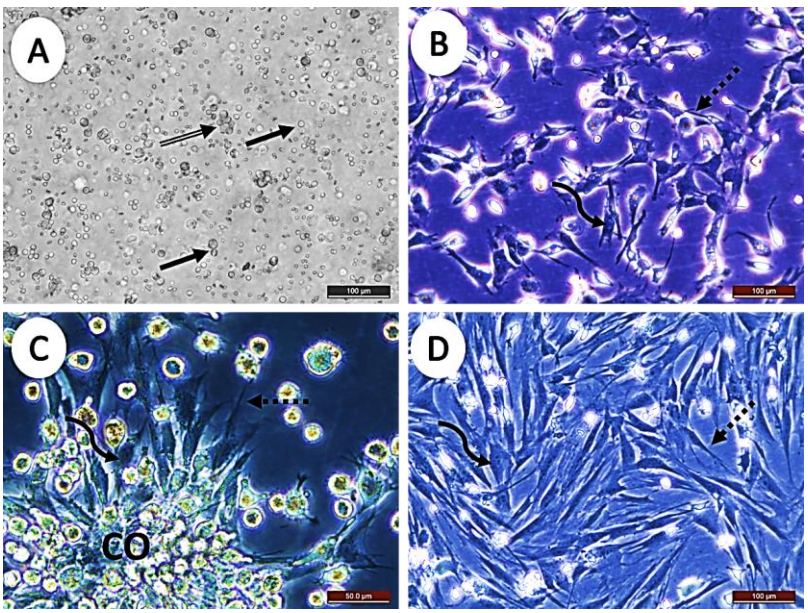

Fig. (1) Phase contrast photomicrographs of BM-MSCs isolation showing: (A) Bone marrow cells at the time of seeding. All the cells are floating and rounded

(arrow). Some of them float in clusters (double arrow). (B) BM-MSCs during P0 with different morphological character. (C) Evidence of colonies formation (CO) by the end of the 1st week. (D) The seeded stromal cells reached 80 % confluency around the end of 2nd week. Dashed arrow: cytoplasmic process, curved arrow: large nucleus with multiple nucleoli.

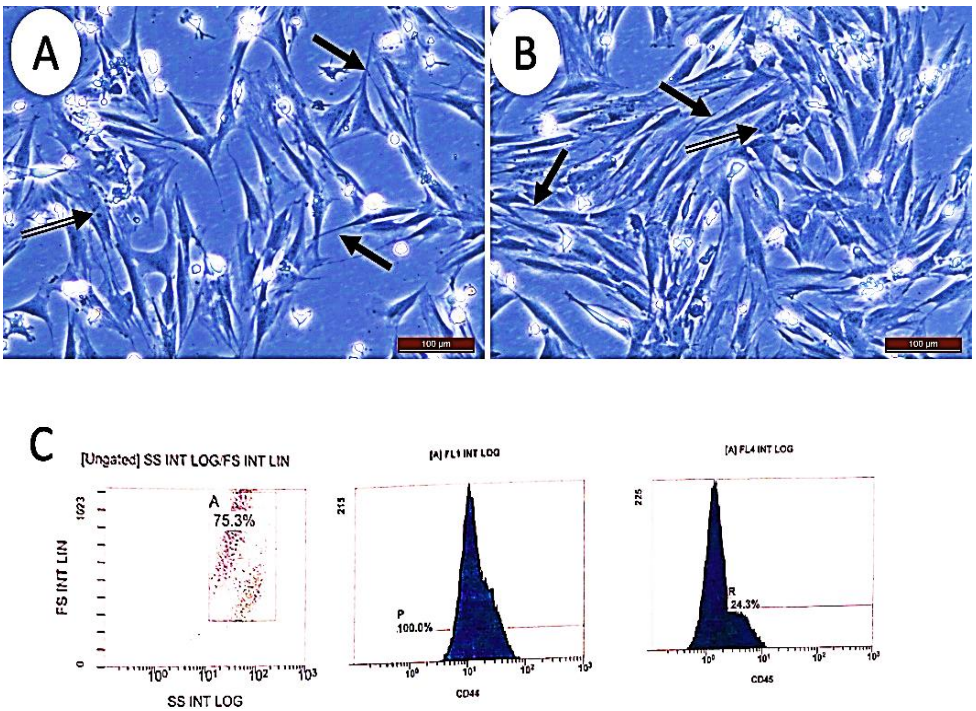

Fig. (2): Phase contrast photomicrographs of BM-MSCs during P1 (A) and P2 (B). The cells were homogenously fibroblast-like with long cytoplasmic processes (Arrow), large vesicular nucleus with multiple nucleoli (Double arrow). (C): flowcytometric analysis of CD 44 and CD 45 in BM-MSCs during P2.

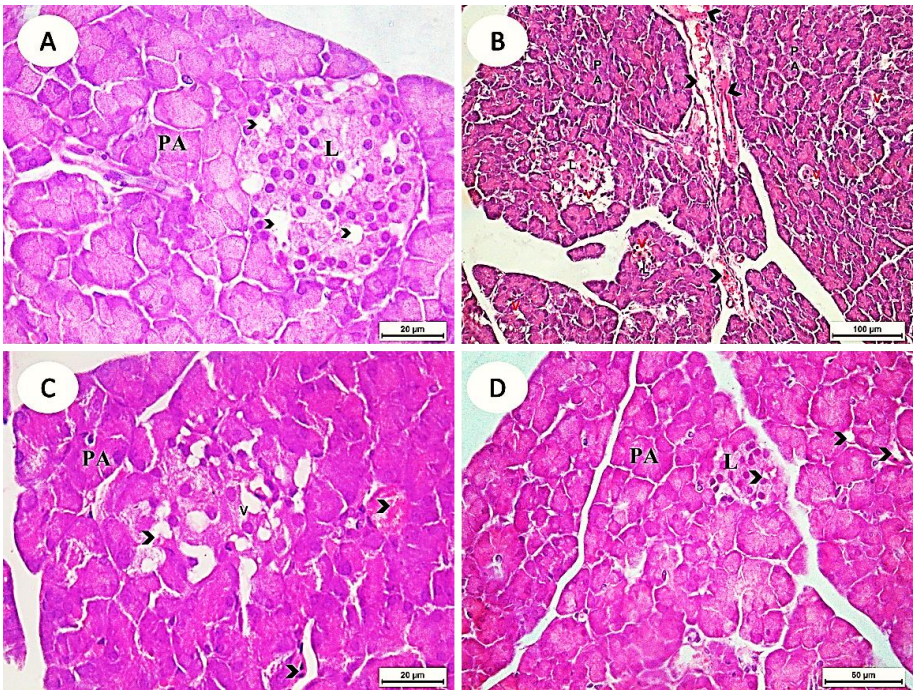

Fig. (3) A photomicrograph of a section in the Langerhans islet (L) among pancreatic acini (PA). (A)The control group of adult female albino rat showing normal blood capillaries (arrowhead) among Langerhans cells (L) as well as among pancreatic acini (PA). (B & C) In diabetic untreated group had vacuole formation (V) in some of PA as well as in L. Congestion in blood vessels and increase in its thickness (arrowhead) among PA. (D) The treated diabetic group with BM-MSCs showing restoration of L cells. Normal blood capillaries (arrowhead) among L as well as among PA. H&E stain.

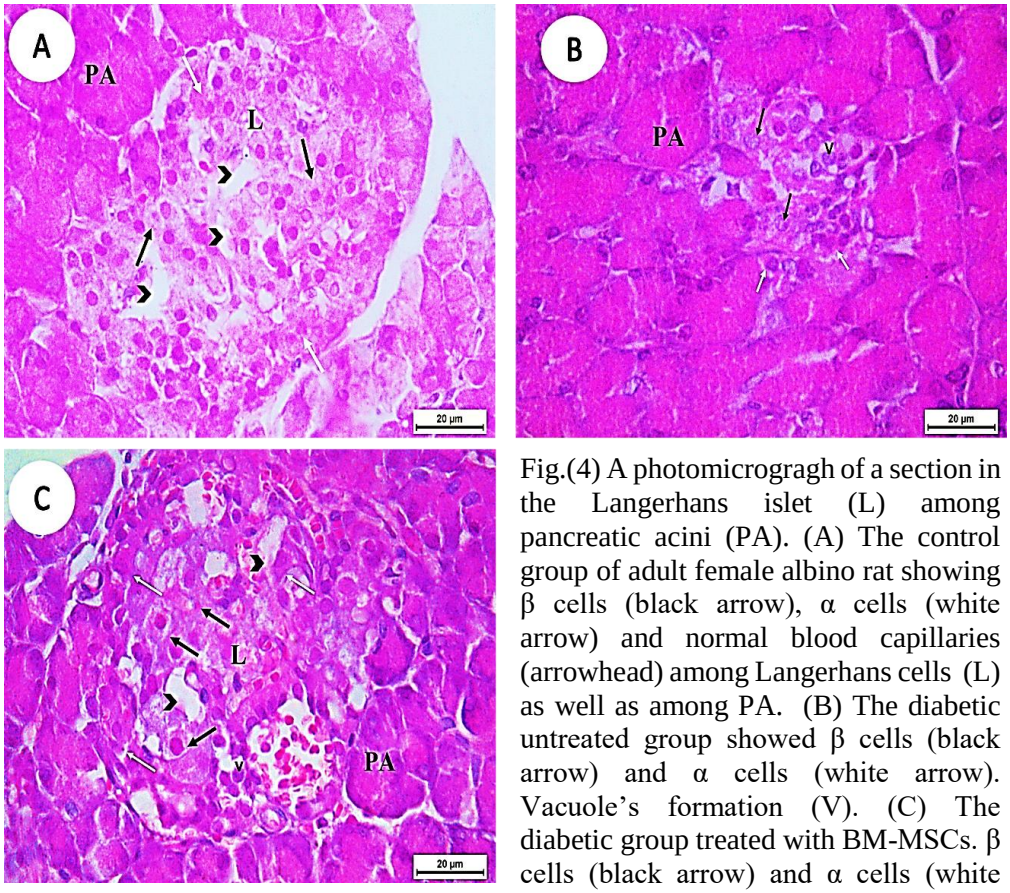

Fig.(4) A photomicrogragh of a section in the Langerhans islet (L) among pancreatic acini (PA). (A) The control group of adult female albino rat showing β cells (black arrow), α cells (white arrow) and normal blood capillaries (arrowhead) among Langerhans cells (L) as well as among PA. (B) The diabetic untreated group showed β cells (black arrow) and α cells (white arrow). Vacuole's formation (V). (C) The diabetic group treated with BM-MSCs. β cells (black arrow) and α cells (white arrow). Vacuole's formation (V). Normal blood capillaries (arrowhead) among Langerhans cells (L). Phloxin B stain.

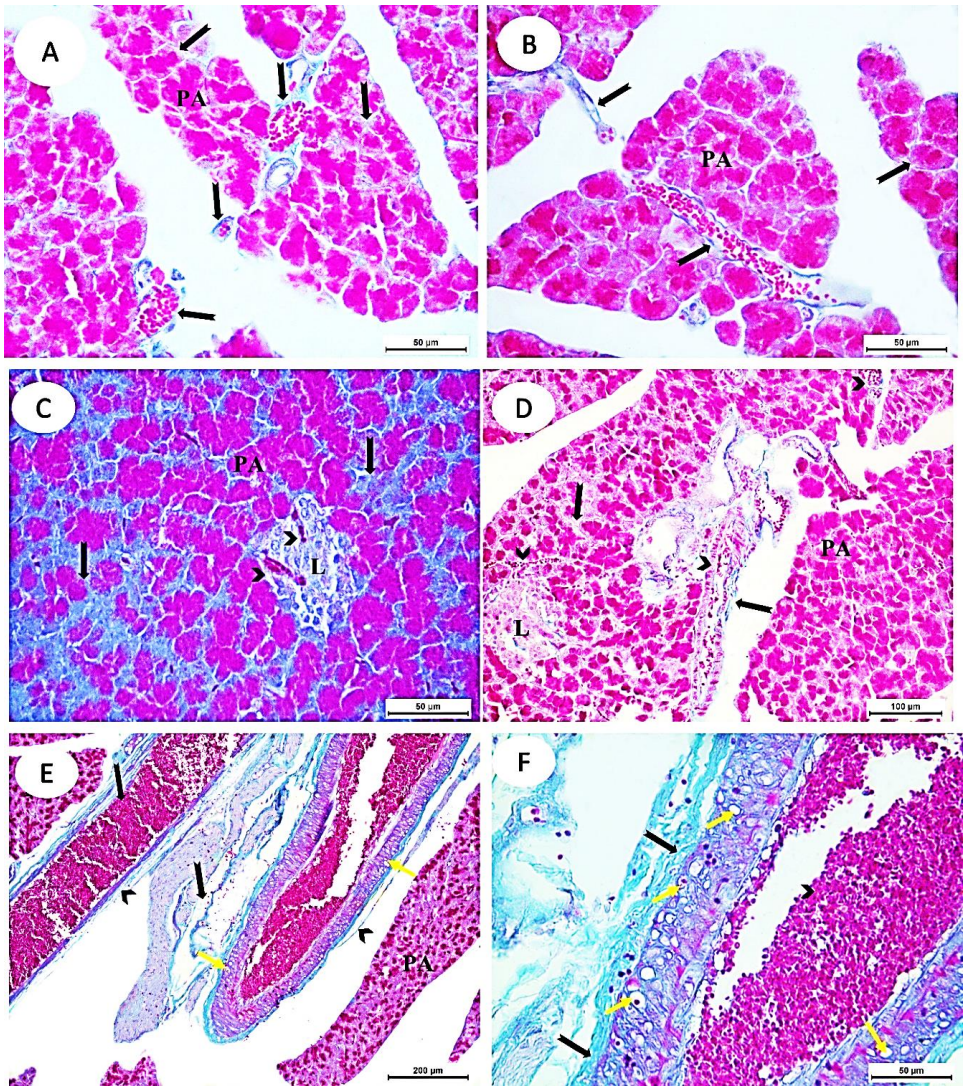

Fig.(5) A photomicrograph of cross & longitudinal sections in the pancreatic acini (PA). (A&B) The control group of adult female albino rat had delicate connective tissue among pancreatic acini (PA) as well as around blood vessels. (C) The diabetic untreated group showing thick connective tissue septa (notched arrow) among pancreatic acini (PA). Highly congested blood capillaries (arrowhead) within Langerhans cells (L). (D) In the diabetic group treated with BM-MSCs revealed decrease the thickness of connective tissue septa (notched arrow), Slightly congested blood vessels (arrowhead). (E&F) In the diabetic untreated highly congested blood vessels (arrowhead). Vacuoles (yellow arrow) in tunica media. Thick connective tissue (notched arrow) around blood capillaries among pancreatic acini (PA). Masson trichrome stain.

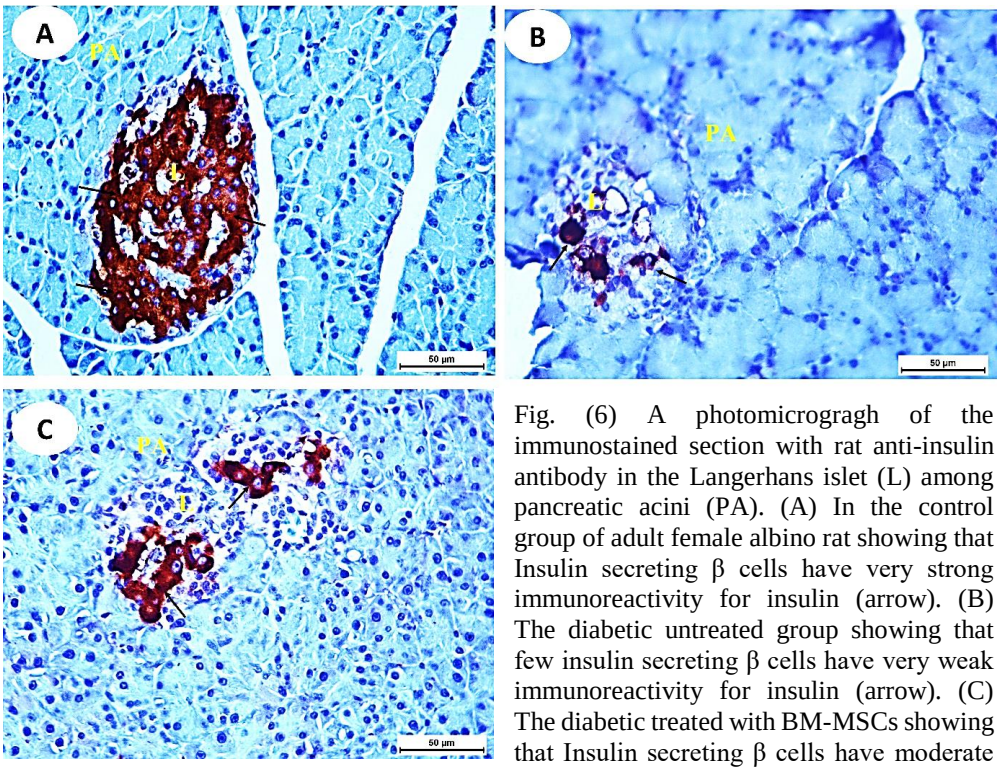

Fig. (6) A photomicrogragh of the immunostained section with rat anti-insulin antibody in the Langerhans islet (L) among pancreatic acini (PA). (A) In the control group of adult female albino rat showing that Insulin secreting β cells have very strong immunoreactivity for insulin (arrow). (B) The diabetic untreated group showing that few insulin secreting β cells have very weak immunoreactivity for insulin (arrow). (C) The diabetic treated with BM-MSCs showing that Insulin secreting β cells have moderate immunoreactivity for insulin (arrow). The reaction was appeared as brown granules occupying the whole cytoplasm of β cell.

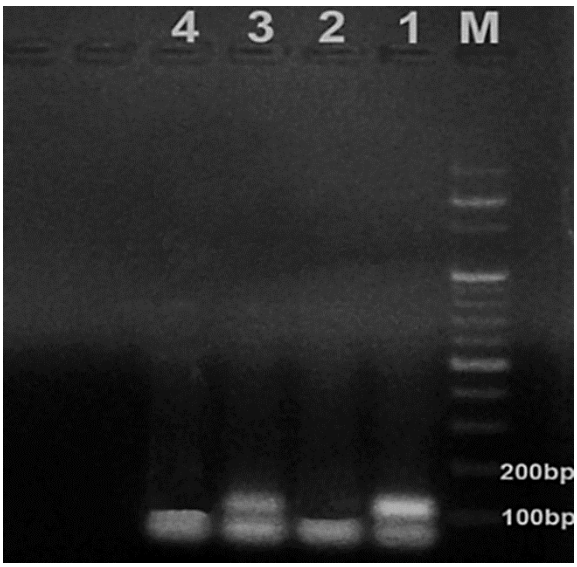

Fig. (7) 2% agarose gel electrophoresis stained with ethidium bromide of PCR product of Sry gene in the pancreatic tissue from all studied groups
M- DNA Ladder, 1- Male control rats, 2- Female diabetic untreated rats, 3- Female diabetic treated rats with BM-MSCs, 4- Female control rats

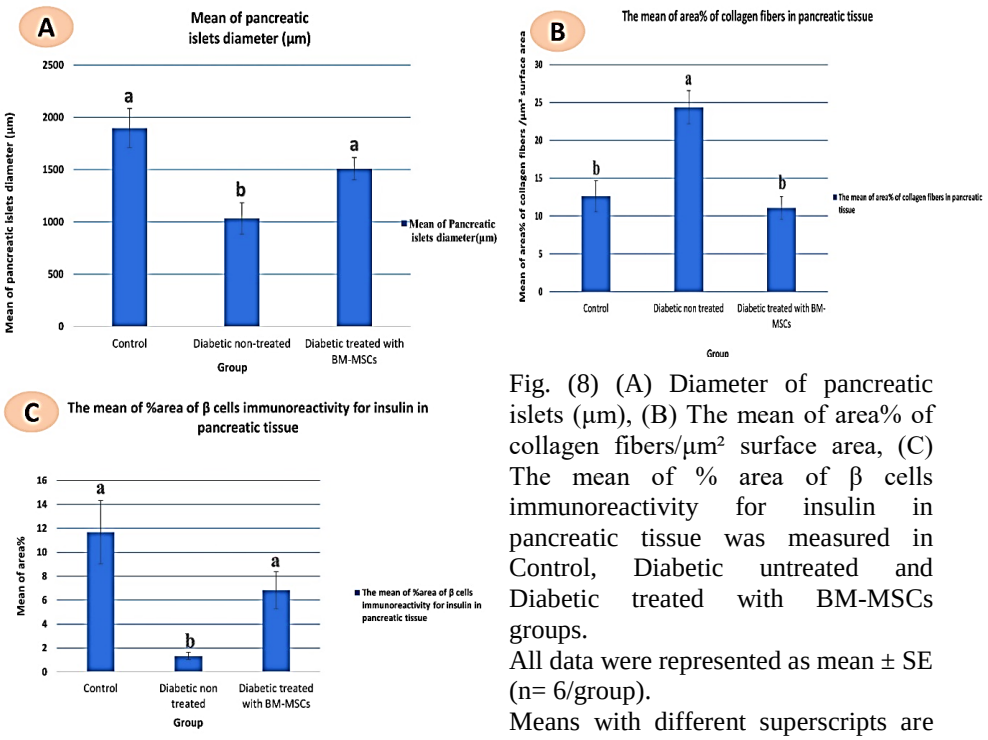

Fig. (8) (A) Diameter of pancreatic islets (µm), (B) The mean of area% of collagen fibers/µm² surface area, (C) The mean of % area of β cells immunoreactivity for insulin in pancreatic tissue was measured in Control, Diabetic untreated and Diabetic treated with BM-MSCs groups.
All data were represented as mean ± SE (n= 6/group).
Means with different superscripts are statistically significant (P ≤0.01).

Discussion

Diabetes mellitus (DM) is widespread metabolic disorder mainly in glucose metabolism that influences about 5% of the world people, resulting in long lasting complications ***(El-Fiky et al., 2016).*** In type 1 diabetes mellitus (T1DM), an autoimmune reaction attacks β cells, damage them, subsequent stoppage of insulin secretion and therefore hyperglycemia occurred resulting in advanced glycated end-products ***(***AGEs) production associated with sever oxidative stress ***(Atkinson et al., 2014 ; Laddha and Kulkarni, 2019). Rogal et al., (2019)*** added that the long term of DM leads to complications in different organs varying in their severity**.** Thus, external insulin supply is vital for management of T1DM ***(Devendra et al., 2004).***
According to ***Jamshidi et al. (2018),*** the conventional treatments of T1DM were aimed to regulation the insulin level which bring blood glucose near normal level without improving the diabetic complications. While currently, for acceleration the restoration of the pancreatic β-cells and other tissues' cells; the therapeutic conventions were engrossed on their antioxidant defense ***(El-Sawah et al., 2021).*** Therefore, the cellular therapy specifically stem cells was a hopeful therapeutic application for diabetic

complications as they have the capacity of self-renewal and differentiation into different cell types ***(Aminzadeh et al., 2020).*** Recently, there is a great interest for applying mesenchymal stem cells (MSCs) in DM therapy ***(El-Kholy et al., 2018).***

Therefore, in this study, T1DM was induced, and we observed its effect on pancreatic β cells in albino rats, then evaluated the pancreatic β cells regeneration using rat bone marrow MSCs (BM-MSCs).

The induction of diabetes was done using STZ which mainly acts on pancreatic β cells that induces oxidative stress causing production excess amount of ROS, subsequently lipid peroxidation (LPO) production and DNA damage, resulting in inflammatory reaction, pancreatic islet necrosis and diabetes, this was agreed with ***(Jamshidi et al., 2018).*** As well as ***Sheweita et al.(2016)*** recorded that DNA damage resulting from STZ induced ROS, stimulates DNA repair enzyme poly ADP-ribose polymerase-1 (PARP-1). Consequently, exhaustion of ATP because of the decrease in the intracellular NAD and finally β cells death. Additionally, DNA damage was enhanced through the nitric oxide (NO) that produced in a lethal quantity by STZ which in-turn causes destruction in β cells and pancreatic acinar cells ***(Omar and Aboulkhair, 2017).***

Our histological results were revealed changes in the normal architecture of the pancreatic tissue in the diabetic non treated group in which decrease the size of pancreatic islets, loss of its regular cellular pattern, damage of most β- cells, cytoplasmic vacuolations, and congestion in the blood capillaries. As well as pancreatic acini would have some vacuolations besides congestion in stromal blood vessels. This was matched with ***Omar and Aboulkhair (2017)*** who observed a necrotic and degenerative changes in pancreatic acini and islets, vacuolations in the cytoplasm, pyknosis in the nucleus and congestion in the blood capillaries. These findings also were in compliance with ***Akbarzadeh et al. (2007)*** who stated that the destruction of β- cells in rats caused by STZ injection.

The histological findings of the pancreatic tissue by Masson trichrome was showed a significant increase in the amount of collagen fibers around blood vessels and in-between pancreatic acini in diabetic non treated group in comparison with the control group, this result was coordinated with ***Gawad et al. (2016)*** who discovered that the presence of collagen fibers around blood vessels and between the pancreatic acini as well as the islets prevented enough oxygen from reaching the tissue, leading to necrosis and degenerative changes.

As we found a highly significant reduction ($P<0.01$) in mean area% of β- cells immunoreactivity for insulin hormone in diabetic non-treated

group compared with the control group. This was in agreement with ***Ahmadi et al. (2010)*** who observed that there was a reduction in insulin immunoreactive β- cells' number of the diabetic rats, besides disruption in their structure.

Meanwhile, in the diabetic treated group with BM-MSCs, we found the histological sections of pancreatic tissue were some extent like the control group. This was matched with the observations documented by ***Ezquer et al. (2011)*** in which approximately normal pancreatic islets in the BM-MSCs transplantation group. In this current study, the diabetic BM-MSCs treated group had mild immunoreactivity for insulin hormone in pancreatic islets compared to diabetic non treated group results which referred to restoration of β-cells was happened. In addition to highly significant increase in islets diameter and highly significant decrease in mean area% of collagen fibers in the pancreatic tissue when compared to diabetic non treated group.

Davey et al. (2014) recorded that several preclinical animal studies were proved that transplanted MSCs had a therapeutic impact on glycemia via direct differentiation to IPCs (less commonly to occur), or indirectly via their immune-modulating properties that inhibit the autoimmunity attack of β cells. ***Volarevic et al. (2010)*** reported that the damaged tissues were restored by MSCs, even they did not home in these tissues via their trophic factors like; growth factors, anti-apoptotic, immunomodulatory in addition to angiogenic factors. MSCs can arrest β-cells destruction through the immunomodulatory mechanism that was involved decrease the immunoreactivity via prevention the autoantibodies production against β-cells ***(Scuteri and Monfrini, 2018).*** ***Ezquer et al. (2012)*** who found that in T cells isolated from diabetic rats injected with MSCs, the pro-inflammatory cytokines like tumor necrosis factor-α (TNF-α) and Interleukyn-2 (IL-2) were changed into anti-inflammatory pattern. Moreover, ***Maccario et al. (2005)*** said that MSCs were found to be able to increase the production of regulatory Fox3p positive T cells while suppressing the development of auto aggressive T cells. As immune-regulating cells, MSCs can reduce inflammation in injured tissue ***(Rasmusson, 2006),*** create a variety of trophic factors that prevent parenchymal cells from succumbing to apoptosis, and encourage the growth and differentiation of endogenous precursors ***(Caplan and Dennis, 2006).*** Additionally, ***Chen et al. (2014)*** was recorded that the trophic substances such IL-6, IL-11, Insulin-like Growth Factor-1(IGF-1), and Vascular Endothelial Growth Factor (VEGF) were produced by MSCs as well as by their microvesicles, that were being implicated in the maintenance of the endogenous pancreatic -cells and promoting their

renewal as well as the proangiogenic characteristics of MSCs ***(Hashemian et al., 2015).*** MSCs can preferentially migrate and engraft in injured tissue when delivered systemically ***(Chen et al., 2003)*** and can also develop into cells that produce insulin ***(Vija et al., 2009).*** On the other side, Sry gene, which is mainly located on Y chromosome, so its presence in the pancreatic tissue of the female rats confirmed the homing of injected BM-MSCs into the pancreatic islets that were responsible for the tissue regeneration. Thus, our molecular results showed that diabetic BM-MSCs treated group was positive for Sry gene in PCR product compared with diabetic non treated and control female groups. This was in accordance with ***El-Fiky et al. (2016).*** This homing capacity or the attraction of BM-MSCs to the targeted tissue mainly caused by several signaling molecules and chemical messengers like cytokines which were released by the damaged cells or caused by the changes occurred in cell surface receptors ***(Körbling et al., 2002).***

Conclusion

Systemic administration of BM-MSCs at a dose 3×10^6 can restore pancreatic β-cell structure and function, so we strongly recommend BM-MSCs for treating T1DM as a future treatment, however further investigations for their long-term behavior after their transplantation is recommended.

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الخلايا الجذعية المزنشيمية المشتقة من النخاع العظمي ودورها العلاجي في داء السكري من النوع الأول

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الملخص العربي

داء السكري هو أكثر أمراض الغدد الصماء انتشارًا ويتميز بارتفاع السكر في الدم وضعف التمثيل الغذائي للكربوهيدرات والدهون و البروتين. في الآونة الأخيرة ركزت العديد من الأبحاث على التطبيق المحتمل للخلايا الجذعية المزنشيمية المشتقة من النخاع العظمي للبالغين كعلاج بديل لداء السكري بسبب الخصائص المتنوعة لهذه الخلايا مع نتائج مبشرة. وبالتالي تهدف الدراسة الحالية إلى التحقق من إمكانية التعافي من داء السكري (النوع الأول) من خلال حقن الخلايا الجذعية المزنشيمية غير المتمايزة ، وكذلك التأثير على خلايا البنكرياس. وقد أجريت التجربة على عدد ثلاثين من ذكور وإناث

الجرذان البيضاء وزنها (180-200 جم لكل منهما). وتم تقسيم 25 أنثى من الجرذان المستخدمة في التجارب إلى ثلاث مجموعات. المجموعة الضابطة (5 جرذان) ، مجموعة مصابة بداء السكري تم حقنها داخل الغشاء البريتوني بجرعة واحدة من الستربتوزوتوسين 60 ملجم / كجم (10 جرذان) ، ومجموعة مصابة بـ داء السكري تم حقنهم بـ 3×10^6 من الخلايا الجذعية المزنشيمية المشتقه من النخاع العظمي غير المتمايز والتي تم عزلها من الجرذان الذكور وعددهم 5 ، ثم زراعتها، وتمييزها بقياس التدفق الخلوي ، وقد تم حقنها جرعة واحدة عن طريق الحقن الوريدي. تم الحصول علي أنسجة البنكرياس من جميع المجموعات التجريبية وتحضيرها لاجراء الدراسات النسيجية و النسيجية الكيميائية المناعية والجزيئية وكذلك المورفومترية. أظهرت النتائج أن الحيوانات المصابة بداء السكري والتي لم يتم علاجها بها انخفاض في حجم جزر البنكرياس وتكون فجوات بها ، واحتقان في الأوعية الدموية. علاوة على ذلك ، فإن منتج تفاعل البوليميراز المتسلسل الإيجابي لجين اس آر واي ، وزيادة ملحوظة في النشاط المناعي للأنسولين ، واستعادة الشكل الطبيعي لجزر البنكرياس في مجموعة مرضى السكري التي تم علاجها بالخلايا الجذعية المزنشيمية المشتقة من النخاع العظمي.

الكلمات الدالة: الأوعية الدموية، داء السكري، الخلايا الجذعية المزنشيمية، البنكرياس، الستربتوزوتوسين.