1. Review1#

This study is focused on the effectiveness and safety of human umbilical cord mesenchymal stem cell (hUC-MSC) infusion for treating type 2 diabetes. The results suggest that hUC-MSC infusion can improve glycemia, restore islet β-cell function, and reduce the dosage of hypoglycemic agents without serious short-term adverse events. 2. The quality of this manuscript is good. It addresses a new paradigm in the management of T2D. The conclusions drawn appropriately summarize the data that this study provided. It confirms data obtained previously from both animal and human studies. 3. The most serious limitation of the study is the long-term safety of the new treatment procedure. The manuscript dealt only with short-term adverse events. Authors avoided discussion of the malignant transformation potential of the hUC-MSC or future rejection of further treatment. They also did not dicuss folow-up plans or progress of the study in future.

Response: We are grateful for the suggestion. We have revised it in the manuscript as follow.

"Embryonic stem cells have the risk of teratoma formation, which limits their clinical application. While the MSCs have been documented as having therapeutic efficacy for inflammation-related diseases, the concerns of possible tumorigenic effects are undeniable; although some studies have shown that MSCs do not undergo malignant transformation. Guan *et al* observed no immediate or delayed toxicity associated with MSC administration (within the follow-up period). In the present study, we observed no significant alterations in tumor-associated antigens (alpha-fetoprotein, carcinoembryonic antigen, carbohydrate antigen 199) within the follow-up period. Because the follow-up time was short, we plan to follow up the participants for 3 years for further observations of possible transplant complications. As this was a preliminary exploratory study, our sample size was limited; we plan to recruit more participants and include a healthy control group in our future study to evaluate the clinical utility of this therapy for T2DM."

2. Review2#

Introduction:

1) Authors should discuss T2DM pathophysiology in detail.

Response: Thank you for your advice. We have revised it in the manuscript as follow.

"T2DM is regarded as a chronic, progressive disease that arises from an impairment in the insulin-sensing mechanisms and culminates in insulin resistance (IR). Initially, the IR is compensated by increased insulin production; however, as the T2DM progresses over time, the general pancreatic dysfunction leads to increasingly lower insulin production. As glucose continues to accumulate in the bloodstream, chronic hyperglycemia promotes a chronic vicious cycle of metabolic decline. In the first 10 years of T2DM, the β-cell function reduces by ~ 10%, but this is followed by a period of much more rapid decrease, of an additional ~ 10% every 2 years, until it eventually results in insulin-dependent diabetes."

2) A few more studies pertaining to the cell-based therapy in T2DM should be given

Response: We are grateful for the suggestion. We have revised it in the manuscript as follow.

"Liu *et al* showed that injection of UC-MSCs with a 5-day interval decreased HbA1c levels and required insulin dose in patients with T2DM. In a relatively small T2DM patient study (n = 18), Kong *et al* showed responsiveness to treatment of intravenous transfusion of UC-MSCs three times with 2-week intervals, administered over a 6-mo period. Finally, in another small-size T2DM patient study (n = 6). Guan *et al* showed that treatment with intravenous transfusion of UC-MSCs two times with 2-week intervals led to one-half of the patients becoming insulin-free between treatment months 25 and 43."

Material and Methods:

1) Study Design needs to be written in precise way with proper explanation. For example, patients were assessed 16 weeks prior to the intervention and original therapy was maintained for 2 months. Here, authors should mention in which 2 months they have maintained the original therapy? Likewise, the significance of

±3 needs to be explained.

Response: Thank you for your comments. We have revised the study design section in the revised manuscript as follow.

"Upon study enrollment, all participants were assessed for diabetes, complications, diet, and exercise in the Diabetic Out-patient Clinic over a period of 16 week prior to the initiation of intervention. The participants were recommended a daily diet that did not exceed 25-30 kcal/kg body weight and an exercise routine composed of walking or similar exercise for 30 min three times per week; these recommendations were provided throughout the study and follow-up periods. By the time of initiation of hUC-MSC therapy, all patients had already accepted treatments based upon diet, exercise, and prescribed medication (oral hypoglycemic agents and insulin injections); the latter had been administered as a baseline, at stable doses for at least 2 month (day -56 ±3 to day 0 ±3).

During the follow-up period, the participants performed self-monitoring of their fasting plasma glucose (FPG) and 2-h postprandial plasma glucose (P2PG) ≥ 7 times per week. The dosages of oral hypoglycemic agents and insulin were adjusted according to the patient's blood glucose to keep the level stable, at FPG range of 79.2-126 mg/dL and P2PG range of 79.2-180 mg/dL. If the total daily insulin dose was ≤ 0.2 U/kg at any time during the study period, the administration of exogenous insulin was withdrawn; if the level of blood glucose was stable with the lowest dose of a single oral hypoglycemic drug, the oral hypoglycemic drug was withdrawn.

All patients were assessed again after 16 week and were administered hUC-MSC infusions. The infusion was administered at a dosage of 1 × 106 cells/kg per week for 3 wk. Considering the possible accidental episodes in the real-life that may interrupt the patients' follow-up visits plan in due time, we set a flexible time range (±3 day) at the patient's discretion but which would not affect the safety and effectiveness of the study. This flexible schedule was structured for in-clinic evaluations to occur on day 14 ± 3, day 21 ± 3, day 28 ± 3 and day 84 ± 3 after the first dosage (Figure 1)."

By the time of initiation of hUC-MSC therapy, all patients had already accepted treatments based upon diet, exercise, and prescribed medication (oral hypoglycemic agents and insulin injections); the latter had been administered as a baseline, at stable doses for at least 2 months (day -56 ± 3 to day 0 ± 3). Considering the possible

accidental episodes in the real-life that may interrupt the patients' follow-up visits plan in due time, we set a flexible time range (± 3 day) at the patient's discretion but which would not affect the safety and effectiveness of the study.

2) The authors should explain why they have observed patients at day28 and then directly at day84?

Response: Thank you for your comments. The effectiveness assessments were performed on day 14 ± 3, day 21 ± 3, day 28 ± 3, and day 84 ± 3, including FBG, 2-h postprandial blood glucose (P2BG), HbA1c, fasting CP (FCP), 2-h postprandial CP (P2CP), IR index, islet β-cell function, and hypoglycemic agent dosage. In our previous study, it showed that the hUC-MSC improved glucose control in the 4th week after hUC-MSC infusions intervention (data not published), so our patients were evaluated on day 28 ± 3. As HbA1c is a measurement of average glycemia during 60-90 days, our patients were tested on days 84 ± 3.

Results:

1) Authors have mentioned that the third infusion was given at day 21 while first assessment was made at day 28 which is mentioned as 4th week by the authors. However, it should be considered as day 7 or 1st week after intervention.

   Response: Thank you for your valuable advice, yes, it should be considered as day 7 or 1st week after intervention. We have revised week to day as the time point in our manuscript to avoid any confusion.

2) Page 8: Safety assessment: "It did not recur after reducing the dosage of insulin in the following period". Is insulin given to these patients? Or is it written by mistake, because it is not mentioned anywhere else in the manuscript?

   Response: Thank you for the comment. It is not written by mistake. We didn't mention the previous treatment of participants in detail. During the intervention and follow-up period, all patients also had been treated with some other oral hypoglycemic agents or insulin. We have added the description in the revised study design section (page 7).

Characteristics:

1) Total number of patients included is 16 in which 12 were males while 4 were

females. Is there any relation between gender and the incidence of diabetes? On what criteria patients have been selected?

Response: Thank you for the question. According to the data from the International Diabetes Federation (2021), there is no significant difference in the diabetic incidence (10.8% in men and 10.2% in women). In our present study, the gender was not considered as a major parameter of the inclusion criteria or the exclusion criteria. More females will be added to evaluate the influence of gender on the hUC-MSC therapy in our future study.

2) The difference between the disease group is long (i.e., 10.06±5.74). What other parameters have been included in the patient selection beside duration of diabetes?

Response: Thank you for your question. The inclusion criteria of this trial were the age and HbA1c. The major exclusion criteria were T1D diagnosis or other situations not applicable for the hUC-MSC therapy. The duration of diabetes was not included in either of the criteria. We will assess this parameter in our future study inclusion.