Evaluation of the Role of Gonadotropins in Regulating Differentiation of Mesenchymal Stem Cells Into Testicular Leydig Cells

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Abstract

Background: Testicular Leydig cells are the cells who responsible or testosterone hormone production which in turn is the principle male sex hormone and is responsible for reproductive growth and development in male 2nd ry sexual characters.

Leydig cells depletion may result from different issues may be congenital, acquired as a result of trauma, radiation, chemotherapy and others which result in low testosterone level.

Transplantation of Leydig cells which obtained from Mesenchymal stem cells differentiation by effect of gonadotropins has become a main treatment modality in patients with low testosterone levels.

Aim of Study: In this study, Wharton’s jelly MSCs were isolated and induced to differentiate into leydig cells as promising tool for transplantation.

Material and Methods: The Leydig differentiation was induced using gonadotropins and evaluated by secretion of testosterone were determined using immunoassay and the results were compared between the experimental and control groups.

Results: Wharton’s jelly MSCs induced with gonadotropins successfully differentiated to leydig cells that secret testoster-

Conclusion: The present study showed that Wharton’s jelly MSCs can differentiated in vitro to leydig cells as future therapy of male infertility.

Key Words: Mesenchymal stem cells – Wharton’s jelly – Gonadotrophins – Testosterone.

Introduction

INFERTILITY has been recognized as a public health problem worldwide by the World Health Organization (WHO), [1]. Infertility has been associated in patients with low testosterone levels, and has serious effects on patients’ mental and social well-being [2]. Though numerous testosterone formulations have been developed, none are fully capable of replicating the physiological patterns of testosterone secretion from within the testes [3].

Testosterone is a necessary hormone that is required for normal male physiologic growth. It plays a role in the growth of genital organs in utero and initiates spermatogenesis, as well as the development of secondary sexual characteristics during puberty [4]. Low testosterone known as hypogo-

Leydig cells are the site of synthesis and secretion of testosterone [6]. Gonadotropic hormones are the primary managers of postnatal Leydig cell development. Human luteinizing hormone (LH) and chorionic gonadotropin (hCG) are glycoprotein hormones regulating development and reproductive functions by acting on the same receptor (luteiniz-

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Mesenchymal stem cells (MSCs) are multipo-

A large amount of these cells can be found in several regions of the human umbilical cord (HUC [10].
Many advantages of HUC over other stem cell tissue sources. First, HUC is seen as biological waste and discarded after birth. Its use [12]. Third, HUC cells have an increased proliferative capacity [13].

There are two current directions for stem cell therapy in male hypogonadism. The first method involves differentiating stem cells of various origins from bone marrow, adipose, or embryonic sources to adult Leydig cells. The second method involves isolating, identifying, and transplanting stem Leydig cells into testicular tissue [14].

In this study, hormone-induced method was used for differentiation of Wharton jelly mesenchymal stem cells to Leydig cells as promising therapy for hypogonadism.

**Subjects and Methods**

The study involved 25 pregnant females coming for delivery, for umbilical cords collection and for umbilical cord serum collection during the period from October 2016 to December 2017. Samples were aseptically collected from patients, after obtaining their consent, females with known history of hepatitis, infectious diseases, DM, severe hypertension, abortions or bad obstetric history were excluded.

The Umbilical cords were collected aseptically from full-term cesarean section patients. Cords were collected in sterile 0.9% Nacl solution and were transported immediately to Clinical Pathology lab at room temperature.

Blood samples collected from patients were left at room temperature for clotting then centrifuged for 15min at 3000rpm, and then sera were collected in a single sterile falcon 50ml for heat inactivation by incubation at 56ºc for 20min then frozen for future use.

Wharton's jelly was cut into small pieces of about 1.5-2.5mm. Tissue culture plastic flasks 25cm² (Corning®) were prepared for culture by adding 5ml DMEM-LG with L-glutamine, cord blood serum (100 µg/mL), Penicillin streptomycin (100U/ml penicillin and 100 µg/ml streptomycin) (10 µg/mL), Fungizone (0.25 µg/mL) Wharton's jelly pieces were divided and distributed on tissue culture plastic flasks, the amount was added in each flask to be covered completely with media. The flasks were incubated in a horizontal position in a humidified incubator at 37ºc and 5% CO2. At day 7, the tissue removed by changing the medium. The flasks were washed extensively twice with warm media, and the adherent cells (MSCs) were kept in culture and were fed with fresh complete nutrient medium for 1 weeks later. These cells were kept until the outgrowth of fibroblast-like cells. At day 14, cells were examined microscopically to ensure 60-70% confluence.

Then, the Leydig differentiation was induced by Gonadotropins for 21 days. The Leydig cells were evaluated by post induction level of testosterone in the supernatant.

**Testosterone assay:**

The level of testosterone in the supernatant was measured in the experimental group as well as the control group by Immunoassay (Day 7 and 21). Results of induced testosterone production in vitro were compared between the both groups under study.

**Statistical analysis:** Analysis was performed using IBM SPSS Statistics version 20. Data was expressed into descriptive which include mean value, and standard Deviation (SD). Analytic which include Student's t-test and the Mann-Whitney test, Wilcoxon signed ranked test and F-test (one way ANOVA test).

**Results**

**Morphological identification of MSCs:**

From Umbilical cord Wharton's jelly: Attached cells were observed at 10-14 days after the initial plating. These cells then gradually reached 60%-70% confluence at about 14 days (Fig. 1).

Detection of testosterone after MSCs induction to Leydig cells:

The cells in the experimental group give higher levels of testosterone in comparison to the control group; the highest value was obtained on the 7th and 21st day after induction, and the experimental group showed an increase in testosterone level with an increase in time, while the control group did not show any significant change.

<table>
<thead>
<tr>
<th>Variable (%)</th>
<th>Experimental group (ng/million cell)</th>
<th>Control group (ng/million cell)</th>
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<tbody>
<tr>
<td></td>
<td>Min-Max.</td>
<td>Mean±SD.</td>
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<tr>
<td>Testosterone D7</td>
<td>0.5–1.60</td>
<td>1.01±0.35</td>
</tr>
<tr>
<td>Testosterone D21</td>
<td>4.20–5.70</td>
<td>5.00±0.45</td>
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Discussion

Infertility affects 10-15% of couples, in human it accounts for 40-50% of infertility it affect approximately 7% of all men. Male infertility is commonly due to deficiencies in semen count and quality [15].

Leydig cells are located between the seminiferous tubules of the testis, synthesize and secrete the hormone testosterone. Testosterone plays an important role in spermatogenesis when production. While numerous testosterone formulations have been developed, none are yet fully capable of replicating the physiological patterns of testosterone secretion [16].

Multiple stem cell therapies to restore androgenic function of the testes are under investigation. Leydig cells derived from bone marrow, adipose tissue, umbilical cord, and the testes have shown promise for future therapy for hypogonadism [14].

In this study, 25 umbilical cord samples were used for isolation of MSCs and induction of their differentiation to leydig cells. 25 umbilical cord samples were used in order to isolate MSCs and to obtain cord blood serum. The success rate in isolating and culturing of MSCs were 64% (16/25). Failure was due to contamination.

Umbilical cord was used as source of MSCs as it easily obtained, no ethically concept and give large numbers of MSCs [17]. UC-MSCs have lower immunogenicity than BM-MSCs, have immuno-suppressive functions and can inhibit proliferation of T cells.

The explant method that we used was agreed by a lot [18], however it was reported that there are limitations for the isolation of UC-MSCs for clinical use. For cord lining MSCs, the isolation methods are extremely time-consuming [10].

We used low glucose DMEM and this was associated with good isolation of MSCs [19,20], it was reported that use of low glucose DMEM is better than high glucose for isolation of MSCs [20]. Also DMEM with low glucose concentration (DMEM-LG) supported MSCs growth and maintained population doubling time up to 10 passages [21].

We used human cord blood serum (CBS) and it gave better isolation results, as it is rich in growth factors needed for the cells and was able to increase numbers of MSC from different sources like bone marrow and cord blood as well as from different species. Also CBS free of zoonotic pathogens and proteins [10].

UC-MSCs cell exhibit fibroblast-like morphology after ten days. These cells gradually reached 50%-60% confluence at about 14 days [22], freshly isolated WJ-MSCs normally demonstrate fibroblast-like appearance during the first culture period 10-15 days and require 2-4 week until fibroblast-like adherent cells reach 80%-90% confluence [3].

In this study WJ-MSCs induced with Gonadotropins to differentiate to leydig cells [1,25].

After 14 days of culturing in vitro, the MSCs exhibited the characteristics of Leydig cells and displayed the ability to secrete testosterone [7,1,25].

The level of testosterone secretion was measured by immunoassay, the cells in the experimental group give higher levels of testosterone in comparison to the control group; the highest value was
obtained on the 21 day [17] differentiated UC-MSCs secreted significantly more steroidogenic hormones including testosterone and cortisol. Also it was revealed that testosterone in the experimental group increased with time, while the control group did not change significantly [1].

On the basis of our results, we conclude that: (1) Wharton jelly mesenchymal stem cells can be easily isolated and cultured in vitro and, (2) Gonadotropin induce the differentiation of Leydig cells and establish the cellular model. MSCs may be therapeutic potential for the clinical treatment of patients who have complete loss of endogenous Leydig cell due to chemotherapy or radiation. They may find long-term success with the transplantation of cells with more regenerative capacity.

References
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تقييم دور الجونادوتروبين في تمايز الخلايا الجذعية الوسيطة إلى خلايا Leydig بالخصوصية