# Efficiency of differentiated human umbilical cord mesenchymal stem cells in to neurons for treating Alzheimer disease

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**Keywords**: Wharton's cells, mesenchymal stem cells, choline acetyl transferase (CHAT), Alzheimer disease,

**Abstract**

After isolation of mesenchymal stem cells from Wharton's jelly of umbilical cord. Using flow cytometry analysis showed positive in differently markers of cells (Cluster of differentiation (CD) 146, 90 and 105), and negative in (Cluster of differentiation (CD 45). Their differentiation under definite conditions indicated that they consumed multilineage variation potential. From the results we obtained which built cells of human umbilical cord involved, we inaugurated that after treatment with Neurobasal –A media for 12 days, hUCMSCs showed moderately MAP2 and highly choline acetytransferase using Immunocytochemistry staining this action should be enhanced when cells are in planted with Neurobasal® –A media, thus MSCs hold great promise for different neurodegenerative diseases. This work aimed to the efficiency of WJMSCs to differentiate into neurons and use these cells in the treatment of (AD).

**Introduction**:

Stem cells can be defined as unspecialized cells that have the ability to renew itself and differentiate into a specialized cell type in order to be used to develop an embryo (embryonic stem cell, ESC) or replacing tissue in the adult organism (adult stem cell, ASC). The unique peculiarity of self-renewal affirms of a stem cell pool. Cells that appear in a developing embryo post fertilization are totipotent or "totally potent" and have the ability to divide into one of over two hundred different cell types that make up the human body .(2). Stem cells have the capacity to give rise to progenies with certain functional and morphological traits and, in recent years, remarkable scientific advances have commenced a new age of confidence for clinical regenerative strategies and tissue engineering applications. We concentrate on the available benefits of human umbilical cord derived stem cell therapy to use these stem cells in corneal epithelial, Stromal, and endothelial disorders (3).

**Subjects and Methods:**

The study was carried out in Mansoura Research Center for Cord Stem Cells in faculty of medicine Mansoura university-Egypt, (MARC - CSC) and the samples were collected from the women's section and Obstetrics Mansoura University Hospital.

**Isolation of mesenchymal stem cells (MSCs):**

The mesenchymal stem cells from Wharton's jelly were isolated by using mixed three enzymes. Collagenase/hyaluronidase /trypsin (CHT). These methods affected the quantity and the quality of the isolated cells of human umbilical cord-derived mesenchymal (hUCM) cells. Culturing of MSCs was done according to (6).

**Flow cytometry of human umbilical cord mesenchymal stem cells (hUCMSCs):**

Detection of cell of MSCs was done according to Human Mesenchymal Stem Cell Multi-Color Flow Cytomery Kit. Catalog Number: FMC002.This kit contains four conjugated antibodies and four corresponding isotype controls that can be used for single –step staining of human mesenchymal stem cells (MSCs).

**Differentiation of hUCMSCs to neurons:**

Differentiation of MSCs were done according to (9).Neurobasal- A Media (Neurobasal- A Medium. Catalogue number: 10888022 (Gibco)

**Immunocytochemistry staining of differentiated neuron cells:**

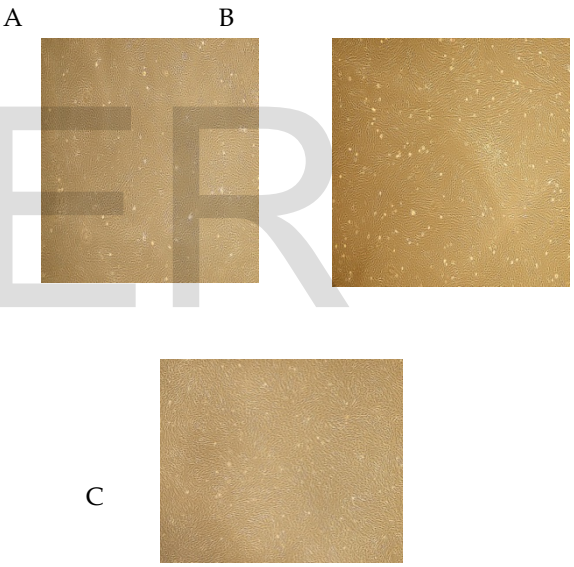
After Trypsinization then the Cells were fixed in 4% paraformaldehyde for about15 minutes. Staining according to Power –stain ™ 1.0 Poly HRO DAB Kit for Mouse + Rabbit .Cat No.52-0017 (Genemed Biotechnologies, Inc.).Primary Antibody Antibodies of MAP2. Catalogue number: YPA1359. . (1). (Biospes). ,a neuron – specific marker . Primary Antibody Antibodies of Choline acetyl transferase. Catalogue number: BBP1057. (7) (Biospes).

**Flow cytometry of tumor necrosis factor (CD95) in differentiated neural cell:**

We were make phenotypic flow cytometry for differentiated neural stem cells: (BD Accri Flow Cytometry). According to (4).

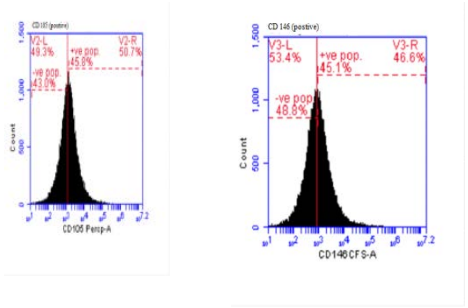
**Result:**

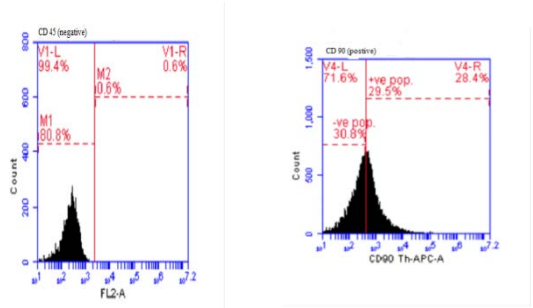
1. **Culturing and Expansion of WJ-MSCs:**




(Figure 1). The confluence of WJ-MSCs in the 1st, 2nd and 3th passages. (A)Adherent WJ-MSCs in the first passage. (B) Adherent WJ-MSCs in the second passage. (C) Adherent WJ-MSCs in the third passage.

2. **Characterization of WJ-MSCs by flow cytometry analysis**

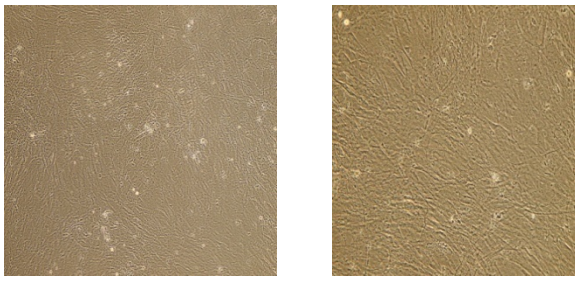



**(Figure 2).** Verification of Human Mesenchymal Stem /Stromal Cell Identity for analysis of MSC Marker by flow cytometry.

3. **Differentiation of WJ-MSCs into Neurons**

**A** **B**



**C**

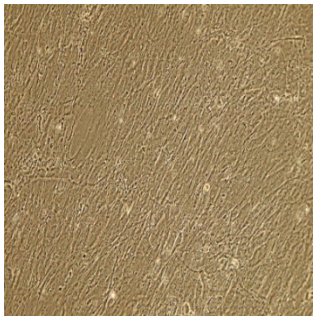
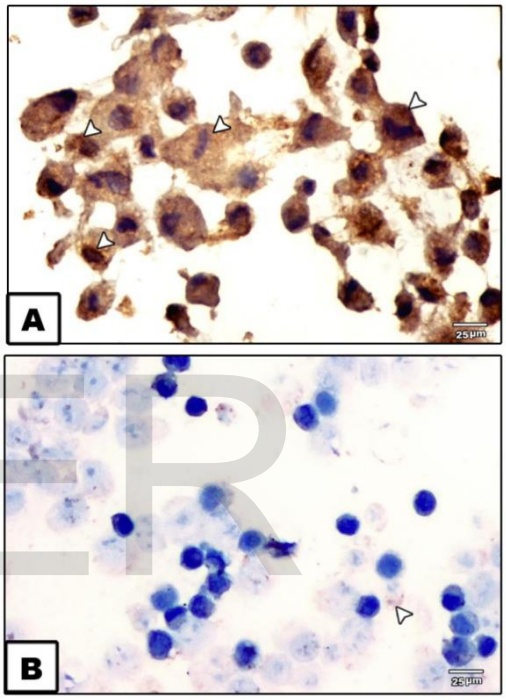


**Figure 3.** (A, B): the cells became thinner with longer extension after 6 days from culturing with Neurobasal media. (C):The cells overlabbed and touching each other with shorter extension after 12 days from culturing with neurobasal media

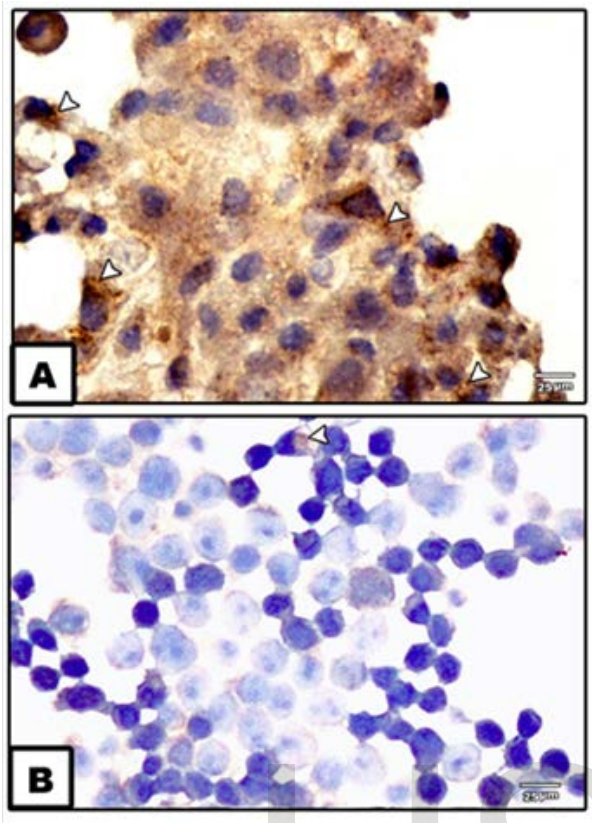
**4. Characterization of neurons derived fromWJMSCs(Immunocytochemistry staining):**




**(Figure4).** Immunocytochemistry of MAP2 antibody.

A: Cells treated with Neurobasal media only were moderately expressed MAP-2.

B: Control negative cells cultured in serum free media showed undifferentiated cells stained negative for MAP-2.




**(Figure4).** Immunocytochemistry of CHAT antibody.
A: Cells treated with Neurobasal media only were highly expressed CHAT

B: Control negative cells cultured in serum free media showed undifferentiated cells stained negative for CHAT.

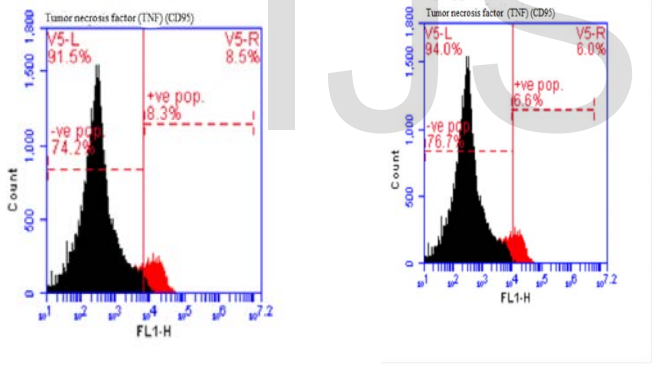
| | MAP2 | Acetyl choline |
|---|---|---|
| Negative | 0.495±0.104 | 1.408±0.64 |
| Positive | 18.631±0.712** | 27.48±1.86** |

**Table (2):**
Correlation of Immunocytochemistry between negative and positive antibodies markers [(18.631±0.712%) and negative MAP2 antibody (0.495±0.104) and positive CHAT antibody (27.48± 1.86%), negative CHAT (1.408 ± 0.64%)].

**Data are expressed as mean±S.D.**

** Significantly different from negative group at *p< 0.01 level.*

**Flow cytometric analysis of (CD95) tumor necrosis factor:**




**(Figure 5).**Verification of differentiated neural Stem /Stromal Cell Identity for analysis of (CD95) TNF Marker by flow cytometry. Results showed that by flow cytometric analysis of surface marker expression, the cells demonstrate a ($CD95^{-}$) TNF.

**Discussion**:

The alternative sources of stem cells in the human and animal body, the umbilical cord appeared to be a promising reservoir of fetal cells that could be easily used as multipotent stem cells. (5).

This study focuses on the therapeutic potential of stem cells harvested from the Wharton's Jelly of the human umbilical cord. Moreover, investigators have found that a potent stem cell population exists within the Wharton's Jelly (8). In our studies we used Neurobasal- A Media, cells induced with Neurobasal- A Media.

**Conflict of interest statement:**
This study was funded by the science and Technology Development fund "STDF"

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# كفاءة الخلايا الجزعية المستخرجة من الحبل السرى للإنسان وتمايزها إلى خلايا عصبية فى علاج مرض الزهايمر

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**الملخص**

لقد قمنا بعمل فصل خلايا مزنكيميه من الحبل السرى للإنسان ,وعند عمل التدفق الخلوى للخلايا تأكدنا من أنها خلايا مزنكيمية وذلك لأن كتلة التمايز ايجابية فى (146,90,105) وسالبة فى (45) ,تمايز الخلايا المزنكيمية إلى خلايا عصبية فى المرحله الثالثة باستخدام وسط عصبى واثبتت الدراسات التى قمنا بعملها أن هذه أن الخلايا التي حصلنا عليها من هلام وارتون من الحبل السري البشري يحتوي على الخلايا العصبية باستخدام فى وجود الوسط العصبى لمدة 12 يوما , وبتحليل كيمياء الهيستولوجيا المناعية ، وجدنا ان الخلايا التى تم تمايزها إلى خلايا عصبية تحتوى على البروتين MAP2 وكولين اسيتيل ترانسفيراز , مما يجعلنا من امكانية استخدام هذة الخلايا فى علاج مرض الزهايمر.