



# Evaluation the Levels of Stem Cells Markers in the Cultured Cells Isolated from Human Chorionic Plate of the Fetal Side

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**Abstract :** Stem cells are exclusive population of cells located in all stages of lifetime that holds capability to self-renewal plus specialize to group of cell lineages of multiple organs. Stem cells within human chorionic tissues consider have potential applications because they serve as a source of multipotency for multiple cells regeneration. These cells express certain surface markers for their detection includes *CD90*, *CD105*, *CD73*, *CD34* and *CD14*, Samples were collected from newly baby born placenta. Stem cells enrichment was conducted using enzymatic method of collagenase type I and culturing using DMEM-low glucose for several days. The results showed that the collected cells after culture demonstrated increased level of gene expression for targeted markers mentioned above by RT-PCR in comparison with the cells.

In conclusion: the isolated cells demonstrated increase in the expression of stem cells markers after culture, which indicate stem cells self-enrichment after day six of culture.

**Keywords:** Mesenchymal stem cells, Hematopoietic stem cells, *CD90*, *CD105*, *CD90*, *CD34*, *CD14*, placenta, collagenase I

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## Introduction

Stem cells are unspecialized self-renewal undifferentiated with ability to convert to any cell type under various external signaling stimuli responses (Alomar and Erbas, 2024) bone marrow is the first source known as reservoir of numerous stem cells (1) at the embryonic level, stem cells are important for generating all organs and growth of the fetus while at the adult level they are responsible for maintenance and homeostasis of all tissues (2) The main source of hematopoietic stem cells (HSC) presented within bone marrow in human bones as a reservoir for all the

categories of myeloid and lymphoid cells (3) HSC present mainly in bone marrow and considered as adult stem cells (4) the first HSC presented in umbilical cord of the placenta and able to reconstitute hematopoietic system (5) however HSC can be presented in other tissues such as the chorionic part of the human placenta started to show earlier in the yolk sac and it is the region where erythroid production are created and their progenitors (6) bone marrow contain HSC and another stem cells called mesenchymal stem cells (7) mesenchymal stem cells (MSC) indeed are adult stem cells with general features of self-renewal ability as well

as multipotent power plus of that it shows immunomodulation efficacy, hormones and exosomes (8) Mesenchymal cells can be found among various organs and within several tissues comprised as a part of the cellular matrix and are located inside dental pulp, liver, even skin (9) due to their variety differentiation capability of ectodermal, mesodermal and endodermal layers, MSC has enlightened to be one of the most essential type of stem cells to be used as regenerative medicine approaches for wide range of organs repair (10)

### **Material and Method**

#### **Sample Collection**

The tissue of human placenta was obtained from newly born Iraqi baby from Al-yarmouk hospital in Baghdad governorate, instantly washed with distal water packed into ice bag and moved directly to Iraqi center for cancer research to continue the procedure, after the delivery into the facility, the tissue placed in a tray and prepared for dissection of 1gm for the mincing process Without forgetting to take small piece handled within Trizol (Geneaid) for later molecular test.

#### **Sample Preparation**

After 1gm of fetal side of placenta tissue were dissected from the fetal part, it was washed with phosphate buffer saline about three times then placed in glass petri dish submerged also with PBS (Capricorn scientific inc.) for mincing it into several small entities gently using needle holder to hold the small pieces and scissors cutting into tiny particles, Then collecting these tiny tissue particles from the submerged PBS within petri dish into centrifuge tube containing 5ml of free-serum DMEM (Capricorn scientific inc.) mixed with 1mg of lyophilized collagenase type I (Worthington inc.) and then placed about 45 minute inside

water-bath of above 39°C for the enzyme to activate notifying that each 10 minute the tube were held out of the water-bath for shaking gently to mix the components then replaced into the water-bath and so on for the separation process of the mesenchymal stem cells from the placental tiny tissue particles, the procedure were applied in similarity of de laorden *et al.*, (11).

#### **Cell Culturing and Additive factors**

After the process of making HSC free from the placental tissue, factors-serum media first incubated to reach optimum 37°C then poured into two cell culture flask about 7ml each and then 4ml from the centrifuge tube containing collagenase-free serum DMEM with Mesenchymal stem cells and hematopoietic stem cells from the fetal side of the placenta were collected from the supernatant and inoculated into each flask and kept inside non CO<sub>2</sub> incubator overnight for two days and under inverted microscope was showed as spherical cells for the first 24hours.

#### **Gene Expression**

The Quantitative Real Time PCR (qRT-PCR) was carried out using the QIAGEN Rotor gene Q Real-time PCR System, by evaluating levels of *CD90*, *CD105*, *CD73*, *CD14* and *CD34* with *GAPDH* housekeeping gene as a control gene, The primers were modeled using Primer-Bank website and double-checked using reference sequences in the National Center for Biotechnology Information (NCBI) database. Primers were designed by SIGMA inc.

The study's designed primers:

| Primer         | Sequence (5'→3' direction) | primer size bp | Product size bp | Tm °C |
|----------------|----------------------------|----------------|-----------------|-------|
| CD105          |                            |                |                 |       |
| Forward        | GCATCCTTCGTGGAGCTACC       | 20             | 103             | 62.4  |
| Reverse        | GAGGAGTGGTCTGGATCGG        | 19             |                 | 60.8  |
| Primer Bank ID | 168693646c3                |                |                 |       |
| CD90           |                            |                |                 |       |
| Forward        | ATCGCTCTCCTGCTAACAGTC      | 21             | 135             | 61.3  |
| Reverse        | CTCGTACTGGATGGGTGAACT      | 21             |                 | 60.9  |
| Primer Bank ID | 221136764c1                |                |                 |       |
| CD73           |                            |                |                 |       |
| Forward        | CCAGTACCAGGGCACTATCTG      | 21             | 136             | 61.3  |
| Reverse        | TGGCTCGATCAGTCCTTCCA       | 20             |                 | 62.5  |
| Primer Bank ID | 325651882c2                |                |                 |       |
| GAPDH          |                            |                |                 |       |
| Forward        | ACAAC TTTGGTATCGTGGAAGG    | 22             | 101             | 60.2  |
| Reverse        | GCCATCACGCCACAGTTTC        | 19             |                 | 61.7  |
| Primer Bank ID | 378404907c2                |                |                 |       |
| CD14           |                            |                |                 |       |
| Forward        | ACGCCAGAACCTTGTGAGC        | 19             | 122             | 62.5  |
| Reverse        | GCATGGATCTCCACCTCTACTG     | 22             |                 | 61.6  |
| Primer Bank ID | 291575162c1                |                |                 |       |
| CD34           |                            |                |                 |       |
| Forward        | ACCAGAGCTATTCCCAAAAGACC    | 23             | 99              | 61.9  |
| Reverse        | TGCGGCGATTTCATCAGGAAAT     | 21             |                 | 62.5  |
| Primer Bank ID | 68342037c3                 |                |                 |       |

### RNA extraction from placental tissue and enriched culture

Total RNA was extracted from all samples using the *TransZol Up* Plus RNA Kit Reagent according to the manufacturer's instructions. As follows:

1. One ml of the entire sample was centrifuged (1 minute/12,000 rpm), the supernatant was discarded, and the pellet was resuspended in 1000  $\mu$ l *TransZol Up*. Overnight, the samples were kept at at-23°C.
2. For each ml of *TransZol Up* Reagent, add 200  $\mu$ l of chloroform. the tube was vortexed gently for 30 seconds and incubated for 3 minutes at room temperature.
3. The tube was Centrifuged at 10,000 rpm for 15 minutes at 2-8 °C. The mixture was separated into a lower pink organic phase, an interphase, and a colorless upper aqueous phase containing the RNA. The aqueous upper phase accounts for 50–60% of the total volume of *TransZol Up*.
4. The RNA-containing colorless upper phase is transferred to a new RNase-free tube (to avoid DNA contamination from interphase, a portion of the aqueous phase can be left). Add an equivalent volume of 96–100% ethanol (precipitates may be seen in this phase). Mixed gently by inverting the tube.

5. Transfer the precipitated material along with the generated solution into a spin column. Subject the sample to centrifugation at 12,000 rpm for 30 seconds at room temperature. Discard the flow-through. If the lysate volume exceeds the spin column's maximum capacity, repeat this step accordingly.
6. Introduce 500  $\mu$ l of CB9 buffer into the spin column, followed by centrifugation at 12,000 rpm for 30 seconds at room temperature. Discard the flow-through.
7. Perform Step 6 once more to ensure thorough processing.
8. Add 500  $\mu$ l of WB9 buffer (confirm that ethanol has been pre-added) to the spin column. Centrifuge at 12,000 rpm for 30 seconds at room temperature, then discard the flow-through. Repeat this step to enhance purification.
9. Centrifuge the column matrix at 12,000 rpm for 2 minutes at room temperature to facilitate the removal of residual ethanol. Following centrifugation, allow the column to air-dry for several minutes.
10. Place the spin column into a clean 1.5 mL RNase-free tube. Introduce 50-200  $\mu$ l of RNase-free water, then allow incubation at room temperature for 1 minute.

11. Proceed with RNA elution by centrifuging at 12,000 rpm for 1 minute. For optimal RNA yield, performing a second elution is recommended.
12. Store the purified RNA at -20°C for long-term preservation.

#### Synthesis the cDNA from mRNA:

Using the EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix Kit, total RNA was reverse-transcribed to complementary DNA (cDNA). According to the manufacturer's instructions. The operation was performed in a reaction volume of 20  $\mu$ l. (4 $\mu$ l) of total RNA had to be reversely transcribed.

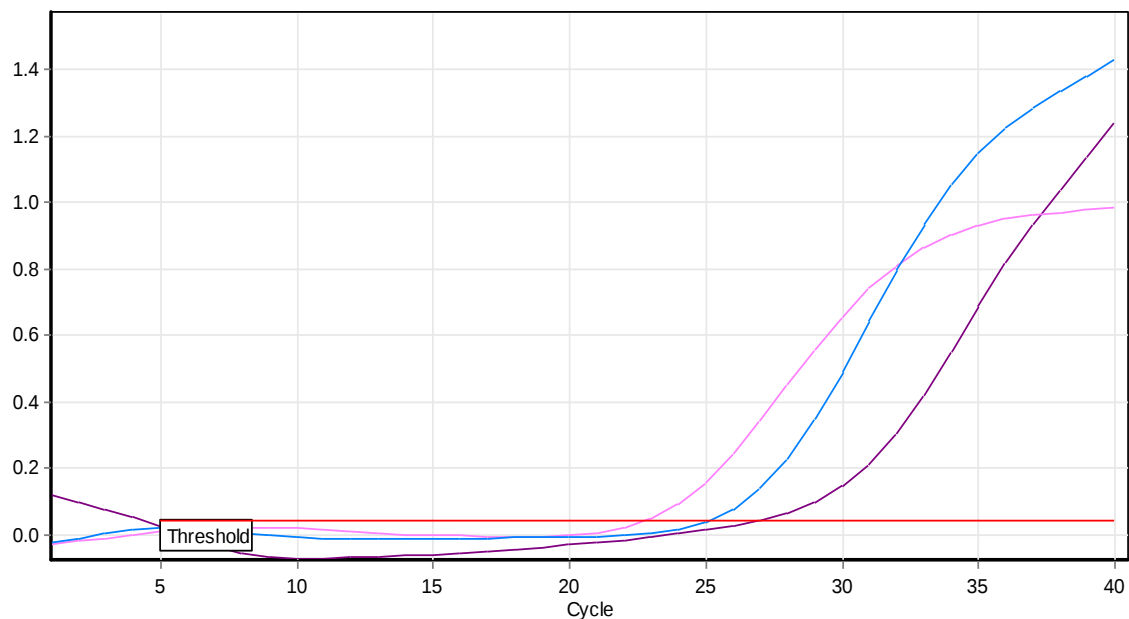
#### Results and discussion

The results of the current study revealed valuable mesenchymal and hematopoietic gene expression located both inward the chorionic plate of the placental tissue as well as after isolation from the tissue of an Iraqi baby sample, the biologic activity of HSC among different tissue parts of the placenta may indicate their strength and stemness, According to the results recorded from the experiment done HSC within chorionic plate of surface markers such as CD90, CD105, CD73, CD34 and CD14 folding expression which are marked as indicator of MSC showed positive in the tissue itself plus the isolated cells from the same tissue, using RT-PCR to catch higher folding value of those markers.

| Marker of interest | Chorionic plate |                 |                  | Tissue culture |                 |      |
|--------------------|-----------------|-----------------|------------------|----------------|-----------------|------|
|                    | $\Delta$ ct     | $2^{-\Delta$ ct | fold             | $\Delta$ ct    | $2^{-\Delta$ ct | fold |
| CD90               | 5.7             | 0.01            | 1.000 $\pm$ 0.04 | 2.97           | 0.12            | 6.6  |
| CD73               | 1.75            | 0.29            | 1.000 $\pm$ 0.24 | 1.05           | 0.48            | 1.6  |
| CD105              | 1.91            | 0.260           | 1.000 $\pm$ 0.21 | -0.38          | 1.30            | 4.89 |
| CD34               | 3.19            | 0.109           | 1.000 $\pm$ 0.04 | -2.19          | 4.5             | 41.6 |
| CD14               | -1.97           | 0.255           | 1.000 $\pm$ 0.01 | -1.9           | 3.7             | 14.6 |
| GAPDH              | 19.54           |                 |                  | 19.88          |                 |      |

Table below describes folds and Ct value of stemness gene expressions

The threshold curve cycles chart of CD90, CD105, CD73, CD34 and CD14 done by RT-PCR are shown below:



Chart(1): shows PCR threshold of CD90 marker.

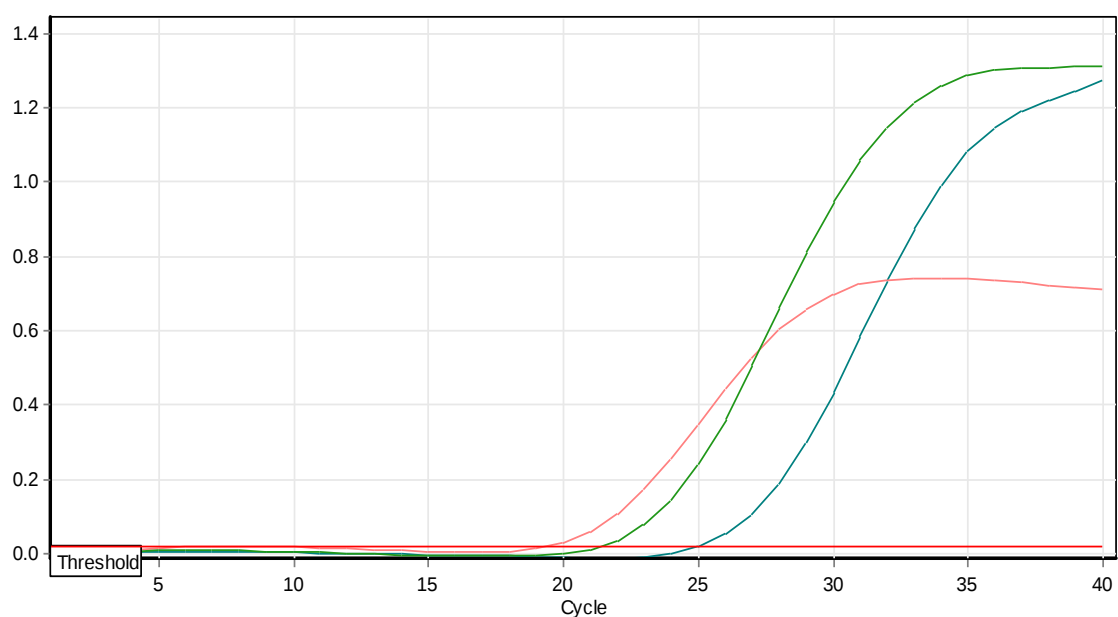
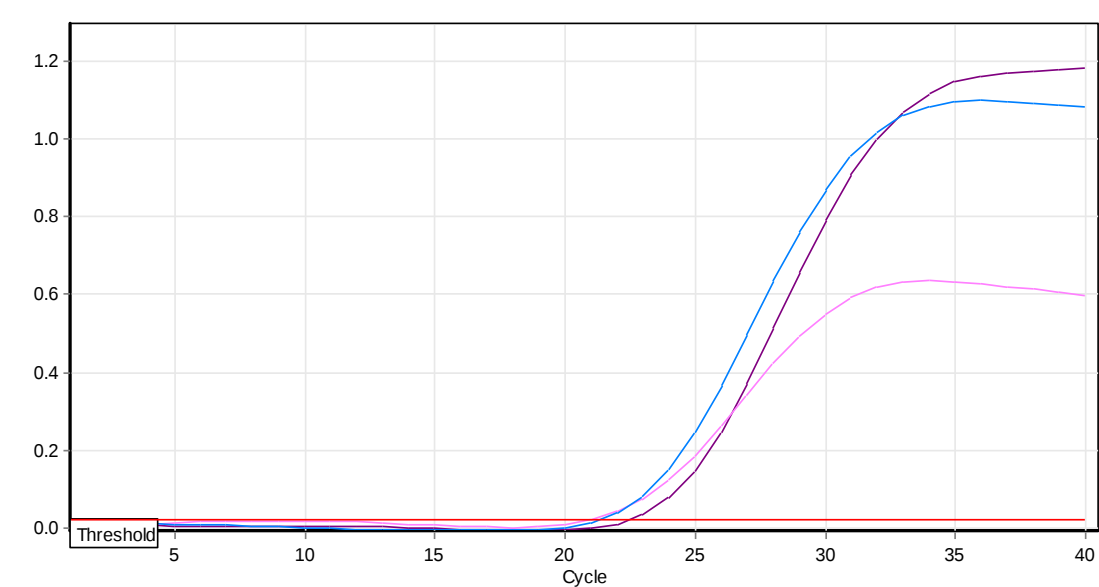
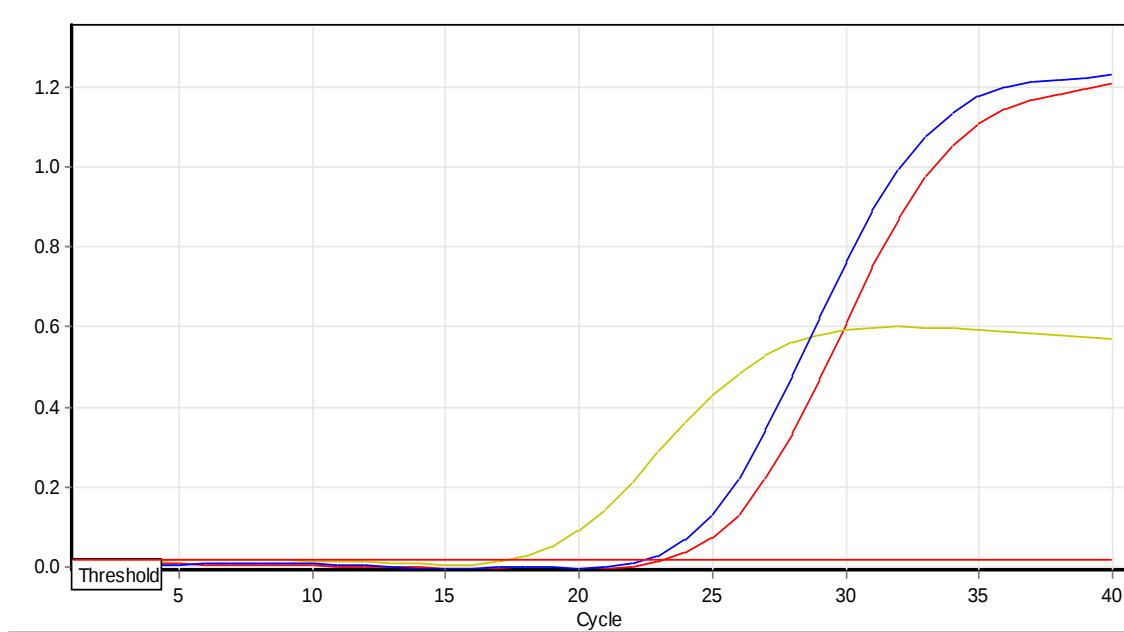


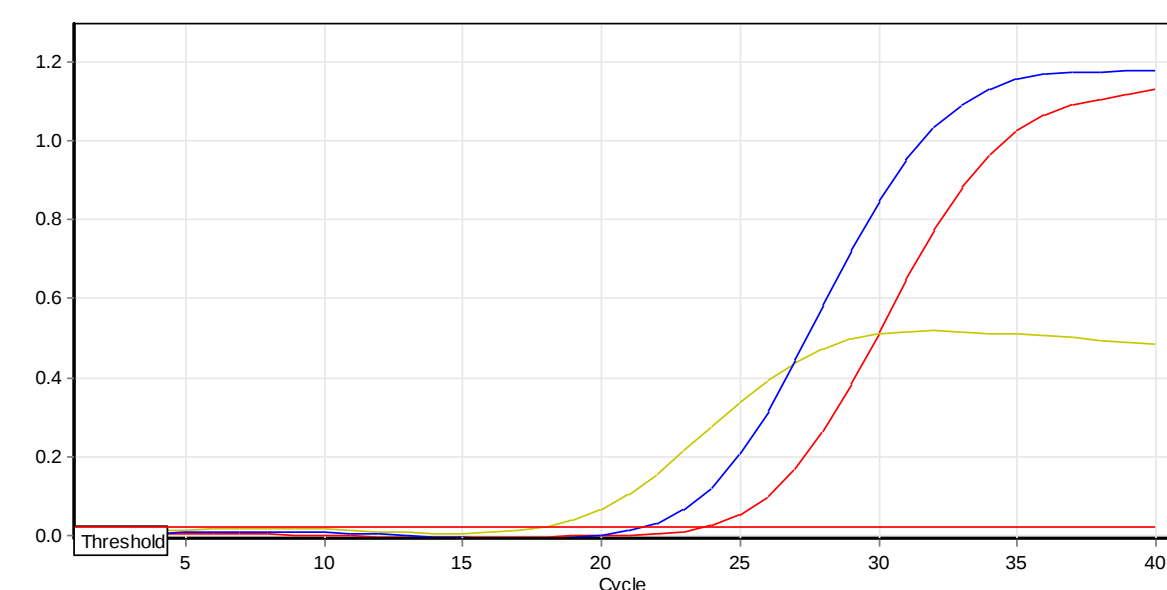
Chart (2): shows PCR threshold of CD105 marker.



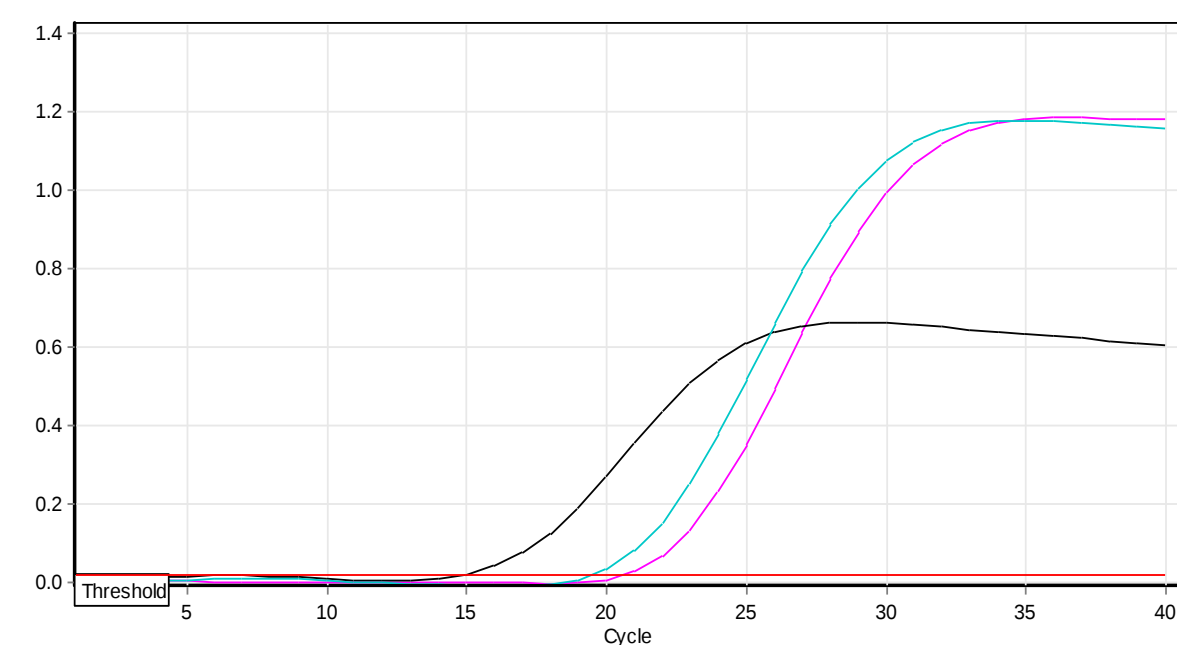
**Chart (3): shows PCR threshold of CD73 marker**



**Chart(4): shows PCR threshold of CD34 marker.**



**Chart (5): shows PCR threshold of CD14 marker.**



**Chart (8): shows PCR threshold of GAPDH marker.**

The whole experiment is to detect the viability and site of hematopoietic like stem cells as well as Mesenchymal stem cells within the chorionic plate of the placental fetal part with high stemness expression showing isolated hematopoietic stem cells clearly and actively positive inside the chorionic plate site of the placenta as well isolated one which could be also considered as a source for naïve hematopoietic stem

cells regardless of their main site such as bone marrow later in life that might be useful for HSC transplant as a fresh hematopoietic packed stem cells for allogenic individuals lacking relatively intact donor with hematopoietic stem cells.

Hematopoietic stem cells and their progenitor ability of reconstitution to entirely blood and immunity cells for further transplantation for

immunodeficient patients having immunocompromised situations, the current study shows that enriched HSC collected from placental tissue has high gene expression activity as an available source for application of numerous approaches more than other tissues. At the same time lineage of HSC to produce positively CD14 cells as immune cells like monocytes freshly unexhausted for targeting foreign antigens such as tumors or even autoimmune disease balance.

Using placenta as a source of Hematopoietic stem cells and mesenchymal stem cells for propagation and making it valuable for stem cell therapy to those with thalassemia and even patients with leukemia without the need to first class relative donor as a part of repairing the damaged bone marrow, immune boosting for tumors fighting and reviving exhausted immunity cells since placenta tissue contains various stem cells types of fresh state as mentioned by Rhodes *et al.*, 2023 (12)

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