The world has been facing a new viral disease, COVID-19 pandemic since 2019, which has left a great stress among oncologists and stem cell transplant specialists. The novelty of the COVID-19 disease, lack of knowledge, and absence of antiviral agent has aggravated the condition. Based on the reports, every part of the body is influenced by the disease, but no clear report has been found on the effects of COVID-19 on stem cells’ characteristics.

Mesenchymal stem cells are cells with a small cell body and long, thin appendages derived from mesoderm tissue. These cells are found in bone marrow, umbilical cord, amniotic fluid, and adipose tissue. Mesenchymal stem cells have a differentiating capacity and are involved in regulating the immune system (CICIARELLO et al., 2019).

To study these cells and their use in clinical treatments, it is very important to study the characteristics of these cells and their differentiation potential after isolation. In this regard, the study of biological features including viability, morphological features, and membrane surface markers is of particular importance. Mesenchymal stem cells have their own membrane surface markers that are used to identify them. Cell surface markers are known as CDs. The system has been developed to classify many monoclonal antibodies by laboratories around the world against epitopes in cell surface molecules and is used as a protocol for cell identification and testing (HAYBAR ET AL., 2020).

The world has been facing a new viral disease, COVID-19 pandemic since 2019, which has left a great stress among oncologists and stem cell transplant specialists. The novelty of the COVID-19 disease, lack of knowledge, and absence of antiviral agent has aggravated the condition. Based on the reports, every part of the body is influenced by the disease, but no clear report has been found on the effects of COVID-19 on stem cells’ characteristics.

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been developed to classify many monoclonal antibodies by laboratories around the world against epitopes in cell surface molecules and is used as a protocol for cell identification and testing (Haybar et al., 2020).

On the other hand, it is important to study cell survival from an oxidative point of view as well as the potential for cell death. In fact, the survival of cells, especially mesenchymal stem cells, depends to a large extent on their apoptosis. The process of apoptosis or programmed cell death as a conserved method is controlled by genes used to remove unwanted or unnecessary cells in living organisms (Cannavo et al., 2019). Stem cells are classified into four types, which include embryonic stem cells, embryonic stem cells, umbilical cord stem cells, and adult stem cells (Nadig, 2009) and (Yousefi et al., 2016) and (Billing et al., 2016).

One of the types of stem cells is mesenchymal cells that can be isolated from different tissue sources. The most important means of identification and confirmation of mesenchymal stem cells are biomarkers and among the biomarkers, biomarkers CD44, CD90, CD105, CD106, and CD166 are the most specialized positive identifiers of mesenchymal stem cells (Stoltz et al., 2015) and (Li & Hua, 2017).

Stem cells, especially mesenchymal stem cells, remain hidden from the immune system and can therefore be used as a biological technique to repair tissue damage or transplant tissue and organs (Le Blanc & Davies, 2015) and (Vladimirovna et al., 2016) and (Li et al., 2017) and (Fullle et al., 2012) and (Choudhery et al., 2012).

Among the sources of mesenchymal stem cells, the human umbilical cord is one of the most important sources. In fact, this source is readily available and contains numerous stem cells (Ding et al., 2015). On the other hand, umbilical cord mesenchymal stem cells are considered the most important stem cells in the field of cell therapy due to their unique properties such as low stimulatory power of the immune system, non-invasive use, and the possibility of easy culture and proliferation (Li et al., 2015).

Of course, some reports claim that the injection of umbilical cord mesenchymal stem cells does not stimulate the immune system in the first place, but gradually increases the immune response with increasing frequency (Cho et al., 2008), and in this regard can cause problems. It has also been reported in clinical trials that repeated injections of mesenchymal cells cause relative resistance (Gaò et al., 2016). On the other hand, research shows that mesenchymal stem cells can differentiate into different cells such as bone cells (Zajdel et al., 2015), liver cells (Khosravi et al., 2018), and epithelial cells (Sierra-Sánchez et al., 2018). In fact, the study of mesenchymal stem cell differentiation into adult cells is an important area of research today.

Considering the destructive effects of COVID-19 on almost every part of the body and possibly stem cells, potential applications of mesenchymal stem cells in research and clinical fields and also considering the relatively easy access to mesenchymal stem cells in human umbilical-cord tissue and the unique properties of these cells in the field of cell therapy, the present study examines the biological properties and the differentiation potential of mesenchymal stem cells derived from human umbilical-cord tissue in COVID-19 positive patients. Naturally, the results of this study can be considered in the field of cell therapy.

We present this article in accordance with the “STARD-2015-checklist”.

**METHODOLOGY**

During this experimental laboratory study, a complete sample of 30 COVID-19 positive donors was obtained among women aged 17 to 40 years undergoing cesarean section. Oral and written consent was received prior to delivery. The mean gestational age was 39 weeks (37-42 weeks). The sample vessels were transferred to the laboratory by the sterile method and its various components were isolated for culturing its cells.

**Method of preparation of mesenchymal cells**

The umbilical cord tissue was completely crushed with scissors and then centrifuged (1750 RPM and 7 minutes) and the supernatant was drained. 25 cc of collagenase (1 mg/ml) was added to 10 cc of the tissue sample in the Falcon tube and incubated for 2 hours. The enzyme activity was then neutralized by adding an equal volume of 10% culture medium. The contents of the
Falcon tube are passed through a funnel containing gas as a filter and all cell contents were collected. The contents were centrifuged (1750RPM and 7 min) and the supernatant was drained and added to a 1 cc cell pellet of DMEM medium.

**Microscopic examination of cells**
After isolation, the cells were evaluated and photographed daily under an inverted microscope (Olympus IX70), infected and problematic specimens were removed, and healthy specimens were kept changing the environment and passage. A total of three passages were performed on the cells.

**Cell count and determination of percentage of living cells**
Cells were counted by slide hemocytometer and trypan blue solution. For this purpose, 0.4 g of trypan blue dye was dissolved in 100 ml of physiological serum and then, for counting, 50 μl of cell suspension was mixed with the same amount of trypan blue dye and placed in the space between the lamellar and the neobar slide. The microscope was counted at X10 magnification and the percentage of living cells was calculated.

**Evaluation of apoptosis and necrosis in cells**
This study was performed based on cell analysis using Annexin V kit. In this regard, the cells in flask 25 (passage 3, density 80%, and 10^6 cells) were sent to the flow cytometry laboratory to evaluate the extent of apoptosis and necrosis. In the sample preparation process, first, the Annexin-binding buffer was prepared and then the cells were washed and centrifuged once after separation and then the supernatant was discarded and 100 microliters of Annexin binding buffer was added to the cells. Annexin V and phosphatidylcholine (PI) were then added and placed at room temperature, kept in a dark place for 15 minutes. Finally, apoptosis was assessed using a flow cytometer.

**Examination of surface markers**
To check CD markers, the sample was sent to the flow cytometry laboratory. To check for the presence or absence of CD markers of fluorescently conjugated antibodies including CD34-PE antibody, CD45-FITC antibody, CD44-FITC antibody, CD73-PE antibody, CD29-antibody PE and CD166-PE antibodies were used. After antibodies were added, the samples were refrigerated for 30 minutes, and then the markers were examined.

**Differentiation into fat cells**
In order to differentiate mesenchymal cells from adipocytes, the cells were cultured in two groups of control and experimental (under differentiation) and when they reached a density of 40-60%, they were placed in adipose tissue for 15 days. The differential culture medium was changed every 48 hours and on the 16th day a cis-formalin was poured on the cells and placed at room temperature for 1 hour. The "Oil-Red" dye was then poured on the cells and after 15 minutes the sample was washed with a suitable buffer. Finally, the cells were evaluated and photographed with an inverted microscope.

**Differentiation into bone cells**
In order to differentiate mesenchymal cells from bone cells, the cells were cultured in two groups of control and experimental (under differentiation) and when they reached a density of 40-60%, they were placed in bone differentiation medium for 15 days. The differential culture medium was changed every 48 hours and on the 16th day a cis of methanol was poured on the cells and placed at room temperature for 10 minutes. Then Alizarin-Red dye was poured on the cells for 2-5 minutes and 3 were washed with a suitable buffer. Finally, the cells were evaluated and photographed with an inverted microscope.

**Investigation of physical properties of cells**
Absorption spectroscopy was performed to confirm the mesenchymal properties of cells for cell adsorption. In this regard, an empty sample of the cell was used to calibrate the device and a sample containing the cell was used to read the absorption and reflection of the waves.

**Statistical Method**
Descriptive statistics have been used to analyze the data.
RESULTS
Mesenchymal cells were successfully isolated and cultured from the human umbilical cord. In the early cultures, the visible mesenchymal cells mainly had the endothelial cell morphology, but after 10 days, the endothelial-like cells gradually disappeared and were replaced by fibroblast-like mesenchymal cells. In different passages, the newly formed colonies were different. In other words, they were larger in the second passage than the first passage, but in the third and first passage, the growth of the colonies was almost the same. In this study, the cells of passage 3 were used to study the cellular characteristics (Figure 1).

Figure 1. Figures A, B, and C show cells in passage 1, 2, and 3, respectively (20X)

Source: Search data.

Examination of apoptosis in umbilical cord-derived mesenchymal stem cells showed that 83% of the cells were alive and only about 14% of them had apoptosis (Figure 2).

Figure 2. Evaluation of apoptosis in umbilical cord mesenchymal stem cells

Source: Search data.

Flow cytometric results showed that these cells express CD44, CD73, CD90, and CD105 markers and are negative in terms of CD34 and CD45 markers (Figure 3).
Figure 3. Evaluation of expression of specialized markers in umbilical cord-derived mesenchymal stem cells

This proved the mesenchymal nature of umbilical cord-derived cells. Absorption spectroscopy performed on umbilical cord-derived cells also showed that the cultured cells were mesenchymal cells (Figure 4).

Figure 4. 4A. shows the absorption region and 4B. Represents the reflection region

Examination of the differentiation of umbilical cord mesenchymal stem cells into fat and bone cells showed that these cells have the ability to differentiate into bone and fat cells (Figure 5).
DISCUSSION

The COVID-19 pandemic has put human beings in a great crisis, from numerous points of view, specifically the health issues, through which tens of millions of people have died who have had comorbidities of COVID-19 positive and other diseases, yet the consequences of COVID-19 on stem cells has not been established.

In the present study, mesenchymal stem cells were isolated from human umbilical cord tissue of 30 women whose COVID-19 positive status had been confirmed by PCR test during their pregnancy. The results of this study showed that mesenchymal stem cells isolated from the umbilical cord could be cultured and propagated in a suitable culture medium after proving the nature of mesenchymal and viability and proper reproduction. These cells could be differentiated into mature fat and bone cells, and in line with the findings of this study, other studies show that mesenchymal cells could be isolated from different parts of the umbilical cord and differentiated into adult cells (SIERRA-SANCHEZ et al., 2018) and (NAGAMURA-INOU & HE, 2014) and (PIRES et al., 2016).

Mesenchymal stem cells are present in the bone marrow, but these cells can also be extracted from other tissues, such as adipose tissue and the umbilical cord. Mesenchymal stem cells have a specific morphology, they also need to express CD73, CD90 and CD105 markers and at the same time they do not have CD34 and CD45 markers (KOBOLAK et al., 2016). In the present study, flow cytometry was used to evaluate the basal mesenchymal nature of cells isolated from the umbilical cord, especially in terms of specialized markers. Mesenchymal cells were isolated from the umbilical cord. Previous studies on the specific biomarkers of mesenchymal cells were consistent with the results of the present study (Ding et al., 2015) and (KARGOZAR al., 2018).

Previous studies, exactly in line with the findings of this study, have shown that mesenchymal cells express their specific biomarkers such as CD44, CD73, CD90, and CD105, while other markers such as CD34 and CD45 are not expressed (HOFFMANN et al., 2017) and (NISHIKAWA et al., 2017). Another indicator of mesenchymal cells is their ability to differentiate into other adult cells, such as bone and fat cells. The results of this study showed that umbilical cord-derived mesenchymal cells could be successfully transformed into bone and fat cells in the third passage and in a specific culture medium. Studies have shown that mesenchymal stem cells can differentiate into different adult cells such as osteocytes, hepatocytes, and epithelial cells (ZAJDEL et al., 2017) and (KHOSRAVI et al., 2018) and (SIERRA-SANCHEZ et al., 2018).
Mesenchymal stem cells can also differentiate into cartilage cells in a suitable culture medium. Previous studies on the umbilical cord have also shown that umbilical cord-derived mesenchymal stem cells have the ability to differentiate into bone and fat cells (Hassan et al., 2018; FUJII et al., 2017; TSAGIAS et al., 2011).

Based on previous researches, the differentiation of mesenchymal stem cells into bone cells occurs in several stages. First, cell proliferation occurs and in the next stage, the initial differentiation occurs which is accompanied by the expression of specific proteins. Osteoblasts are then formed and eventually the expression of osteocalcin protein and the position of calcium and phosphate on bone cells are all occurred in succession (KRAUSE et al., 2011) and (HUANG et al., 2007).

Moreover, the differentiation of mesenchymal stem cells into adipocytes also occurs in two general stages. In this regard, first mesenchymal cells become specialized and committed to precursor cells, then these cells enter the differentiation pathway and become distinct adipose fat cells (CHEN et al., 2016). Finally, it should be noted that the present study was conducted to investigate the biological characteristics of human umbilical cord tissue-derived mesenchymal cells of 30 COVID-19 positive pregnant women and due to the limited financial support and facilities available to the researchers of this study, it was not possible to investigate the issue at a deeper cellular and molecular level, therefore it is highly suggested to future researchers to study more biomarkers and evaluate the expression of specialized genes effective in differentiating mesenchymal stem cells into bone and fat cells, as well as electron microscopy analysis of the project.

CONCLUSION
Firstly, the results of this study showed that mesenchymal stem cells derived from placenta of COVID-19 positive patients could have regenerative capabilities in spite of infectious condition of COVID-19, which is considered as the main finding of this project. Secondly, the results of this study showed that by using this paper’s methods, mesenchymal stem cells could be isolated from the human umbilical cord. In addition, these cells express their specific markers by proliferation in a suitable culture medium, which makes it easy to prove their mesenchymal nature. Moreover, umbilical cord-derived mesenchymal stem cells can differentiate into mature fat and bone cells in a specific culture medium. Based on this, the use of umbilical cord-derived mesenchymal cells can be considered in cell therapy, especially in the field of repair of bone and tissue injuries.

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AVAILABILITY OF DATA AND MATERIALS
The data used in this study are available from the corresponding author on request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for using human Umbilical Cord tissue. In addition, informed consent was obtained for experimentation with human Umbilical Cord tissue.

CONSENT FOR PUBLICATION
By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

COMPETING INTERESTS
The authors declare that they have no competing interests.

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Resumen
El presente estudio investigó las propiedades biológicas de las células madre mesenquimales derivadas del cordón umbilical y su capacidad para diferenciarse en pacientes COVID-19 positivos. En este estudio experimental de laboratorio, se obtuvieron muestras de placenta completa de 30 mujeres COVID-19 positivas sometidas a cesárea, con edad de 20 a 40 años, y mantenidas en condiciones estandarizadas. Las células mesenquimales se aislaron por método enzimático y sus características morfológicas se examinaron mediante microscopía y espectroscopía de absorción y sus propiedades biológicas, en particular la expresión de marcadores de CD, se determinaron mediante citometría de flujo. Finalmente, las células madre mesenquimales se cultivaron en medios específicos para diferenciarse en osteocitos y adipocitos. Los datos fueron analizados mediante estadística descriptiva. Los exámenes morfológicos y físicos por microscopio y espectroscopía de absorción, así como la presentación de marcadores CD44, CD73, CD90 y CD105 y la falta de marcadores CD34 y CD45 demostraron la entidad mesenquimal de las células madre. Las células madre mesenquimales se diferenciaron con éxito en osteocitos y adipocitos.

Palabras-clave: COVID-19. Células madre mesenquimales. Cordón umbilical. Osteocito. Adipocito.