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COMPARISON OF THE IMMUNOMODULATORY PROPERTIES OF THE CORD BLOOD AND WHARTON'S JELLY DERIVED MESENCHYMAL STEM CELLS

KORDON KANI VE WHARTON JELİ KAYNAKLI MEZENKİMAL KÖK HÜCRELERİN İMMUNOMODÜLATÖR ÖZELLİKLERİNİN KARŞILAŞTIRILMASI

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ABSTRACT

Objective: Mesenchymal stem cells (MSCs) are promising as a treatment option for many immune-related diseases due to their immune regulatory properties. Wharton's Jelly (WJ-MSC) and cord blood (CB-MSC) have recently received more attention than the other MSC sources. In this study, it was aimed to investigate the difference in the mechanisms of the immunological effects of WJ-MSCs and CB-MSCs.
Material and Methods: The intracellular cytokine levels of peripheral blood mononuclear cells (PBMC) and CD4+ T cells before and after MSC co-culture (Interleukin-4 (IL-4), Interferon-γ (IFN-γ), and Interleukin-17 (IL-17)) were determined by flow cytometry. At the same time, tumor growth factor (TGF)-β, IL-4, IL-17, IFN-γ supernatant cytokine levels were measured by ELISA. In the study, incubation times of 24 hours and 72 hours were applied with co-culture MSC/PBMC ratios of 1/5 and 1/10.
Results: Our data showed that WJ-MSCs and CB-MSCs have different morphological features, proliferation capacities, proliferation times and immunomodulating abilities. One of the cytokines of IFN-γ decreased significantly at both 1/5 and 1/10 ratios in the cell at 24 hours and increased significantly at 72 hours after CB-MSC/PBMC co-culture compared to the level after WJ-MSC/PBMC co-culture ($p<0.05$). Unlike for IL-17 cytokine, the intracellular level decreased significantly in the CB-MSC group only at 72 hours compared to the WJ-MSC group. For IL-4, the 1/10 ratio in the CB-MSC group decreased significantly at 24 hours, while the intracellular level was increased in all other groups. After CB-MSC/PBMC co-culture, TGF-β supernatant level decreased by 1/5 in 24 hours and increased in 72 hours. On the contrary, TGF-β supernatant level increased in the CB-MSC group at 24 hours and decreased significantly at 72 hours in 1/10 ratios ($p<0.05$).
Conclusion: CB-MSCs and WJ-MSCs show different immunomodulatory properties. Based on these findings, it can be said that the use of WJ-MSCs is more advantageous in terms of cell therapies due to the different isolation, proliferation capacity and immunomodulatory properties of CB-MSCs.
Keywords: Cord blood, Cord tissue, MSCs, Immunomodulation

ÖZ

Amaç: Mezenkimal kök hücreler (MKH'ler), immün düzenleyici özellikleri nedeniyle bir çok immün sistem ilişkili hastalık için tedavi seçeneği olarak umut vaad etmektedir. Wharton Jeli (WJ-MKH) ve kordon kanı (KK-MKH), diğer MKH kaynaklarına göre son dönemlerde daha çok ilgi görmektedir. Çalışmada, WJ-MKH'ler ve KK-MKH'lerin immünolojik etkilerinin mekanizmalarındaki farkı araştırmak amaçlanmıştır.
Gereç ve Yöntem: Periferik kan mononükler hücreler (PKMH) ve MKH ko-kültür öncesi ve sonrası CD4+ T hücrelerin hücre içi sitokin seviyeleri (Interlökin-4 (IL-4), Interferon-γ (IFN-γ), and Interlökin-17 (IL-17)) flow sitometri ile tespit edildi. Aynı zamanda Tümör büyüme faktörü (TGF)-β, IL-4, IL-17, IFN-γ süpernatant sitokin seviyeleri ELISA ile ölçüldü. Çalışmada, ko-kültür MKH/PKMH 1/5 ve 1/10 oranlarında, 24 saat ve 72 saat inkübasyon süreleri uygulandı.
Bulgular: Verilerimiz WJ-MKH'lerin ve KK-MKH'lerin farklı morfolojik özellikleri, çoğalma kapasiteleri, çoğalma süreleri ve immünomodülasyon yetenekleri olduğunu gösterdi. IFN-γ, KK-MKH/PKMH ko-kültür sonrası WJ-MKH/PKMH ko-kültürü sonrasına göre hücre içerisinde hem 1/5 hem 1/10 oranlarında 24. saatlerde anlamlı derecede azalırken, 72. saatlerde anlamlı derecede arttı ($p<0,05$). IL-17 sitokinin için farklı olarak sadece 72. saatte KK-MKH grubunda WJ-MKH grubuna göre anlamlı derecede hücre içi seviyesi düştü. IL-4 için KK-MKH grubunda 1/10 oran 24 saatte hücre içi seviyesi anlamlı derecede düşerken diğer tüm gruplarda artış gösterdi. KK-MKH/PKMH ko-kültür sonrası TGF-β supernatant seviyesi 1/5 oranında 24 saatte azalırken 72 saatte artış gösterdi. 1/10 oranlarında ise tam tersi 24. saatte KK-MKH grubunda artarken 72 saatlerde anlamlı derecede azaldı ($p<0,05$).
Sonuç: KK-MKH'ler ve WJ-MKH'ler, farklı immünmodülatör özellikler göstermektedir. Bu bulgular, KK-MKH'lerin izolasyonun, çoğalma kapasitesinin ve immünmodülatör özelliklerinin farklı olması sebebiyle hücre tedavileri açısından WJ-MKH'lerin kullanılmasının daha avantajlı olduğu söylenebilir.
Anahtar Kelimeler: Kordon kanı, Kordon dokusu, mezenkimal kök hücre, PKMH, ko-kültür, immunmodülasyon

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INTRODUCTION

Mesenchymal stem cells (MSCs) are the multipotent stromal cells that have the ability to differentiate into different cell types such as osteoblasts, chondrocytes, and adipocytes (1).

MSCs have the ability to trans-differentiate into various germ layers such as ectoderm, mesoderm and endoderm and are the undifferentiated cells with high proliferation and differentiation potential that are found in various tissues such as bone marrow, adipose tissue, cord blood, wharton's jelly. Conventionally, MSCs have been isolated from bone marrow, and bone marrow-derived MSCs (BM- MSCs) have been identified as an important candidate for cell therapy (2). However, their clinical application is limited because the invasive procedures in obtaining, and the decrease of their proliferation and differentiation capacities with age, their use in treatment of patients with hereditary diseases are difficult (3, 4). Therefore, other adult and fetal tissues including the adipose tissue, umbilical cord blood, umbilical cords, amniotic fluid and placental tissue have been investigated as the alternative stem cell sources (5-7).

Mesenchymal stem cells are the cells of interest due to their proliferation, differentiation and immunomodulatory properties. MSCs showing low expression of major histocompatibility complex (MHC) class II and costimulatory molecules can be considered as immune privileged cells. MSCs can alter immune system cell function by inhibiting the T-cell, B-cell, and natural killer (NK) cell proliferation and through directing monocytes and dendritic cells to an immature state (8, 9).

Although the exact mechanisms underlying the immunomodulation of the mesenchymal stem cells are still not fully understood, a number of soluble factors involved in the process have been identified. Its immunosuppressive effects are mainly revealed with the production of the soluble factors such as indolamine 2,3-dioxygenase (IDO), converting growth factor-β, human leukocyte antigen (HLA) -G and hepatocyte growth factor (10-13). While the soluble factors are the primary mechanism by which MSCs exert immunosuppressive effects, the direct cell-cell contact is also a contributing factor (14). Cell-to-cell contact between CD3+ T cells and MSCs can induce the CD4+CD25+ regulatory T cells. Researchers reported that it significantly reduces the inducing capacity of CD3+ T cell proliferation and secretion of the inflammatory factor, and has the ability to induce the Th2 responses (15).

Various studies emphasize that the cord-derived MSCs are preferable over other MSC sources due to their immunomodulatory properties. In particular, the potential of the cord-derived MSCs was emphasized to be higher as an alternative to other MSC sources. However, the cord-derived MSCs were not compared in detail, both for their proliferation capacity and their potential to affect the immune system, as cord blood and cord tissues. Therefore, we compared the similarities and differences between WJ-MSCs and CB-MSCs in our study. In particular, we aimed to compare the cord blood and cord tissue MSC isolation and culture conditions, and the immunomodulatory effects of these two MSC sources through measuring the cytokine levels secreted after co-cultures with PBMC (1, 2).

MATERIAL and METHODS

This study was approved by Istanbul Faculty of Medicine Ethics Committee (Date:11.11.2022, No: 20). The human cord blood and tissue samples used in the study were provided with the official permission after the informed consent forms were obtained from pregnant women who had no chronic disease and a healthy pregnancy process from the T.C. Ministry of Health Istanbul Bakırköy Dr.Sadi Konuk Training and Research Hospital, Obstetrics Clinic.

Mesenchymal stem cell isolation and culture

After the cesarean delivery, the blood transfer from the umbilical cord to the heparin tube was performed by an authorized clinician. For each sample, an average of 70-80 cc (8 cc heparin tubes) of blood samples were obtained by the physician in charge of the maternity ward. The blood and tissue brought to the laboratory promptly were processed in a sterile cabin. Cord blood samples were isolated by the Ficoll paque gradient method under the sterile cabin within 24 hours. Cells were planted on 25 cm^2 flasks, and 1% pen/strep complex, 10% FBS, DMEM medium was incubated at 37^0 C, 5% CO2. 24 hours after being planted, the medium was refreshed to remove other mononuclear cells in the cord blood. The cells were removed by trypsinization when the cells were 80-90% confluent, and were prepared for characterization.

The WJ-MSCs were isolated using mechanical fragmentation method in sterile cabine in the laboratory within 24 hours. Tissue pieces were washed with the transfer and shaking process into 4 separate 50 cc falcons into pre-prepared antibiotic complex (Streptomycin, Penicillin, Amphotericin B), and the last falcon was incubated for 1 hour at 37degrees on the shaker. The tissue pieces were placed carefully and regularly into 6 wells. 4 ml DMEM containing 10% FBS was incubated at 37^o C,5% CO2. Sufficiently proliferated cells were passaged on 75 cm^2 flask. The cells were trypsinizated when the cells were 80-90% confluent, and were prepared for characterization.

Characterization of mesenchymal stem cells

The cells were trypsinizated and washed once with PBS (centrifuge at 2000 rpm for 10 min). They were tested in accordance with the Human MSC Characterization Kit (BD- AB_2869404) protocol and analyzed by reading on a flow cytometry device.

Peripheral bood mononuclear cells isolation

Peripheral blood taken from a healthy person in approximately 20-30 cc into heparin tubes was processed. The peripheral blood mononuclear cells were isolated with Ficoll paque gradient method.

The appropriate dose and period of the phytohemaglotunine (PHA) demonstrated for cytokine release from PBMCs were identified as 10µg/ml PHA, and 72 hours for IL-4, IFNγ and IL-17 cytokine release using the flow cytometry methods for intracellular cytokine identification, and ELISA for cytokine release

from supernatant after the preliminary study.

Co-Culture of Mesenchymal Stem Cells, Wharton's Jelly Mesenchymal Stem Cells and Peripheral Blood Mononuclear Cells

The mesenchymal stem cells were characterizated by flow cytometry up to the 3rd passage. The PBMCs were co-cultured in different doses (1/5, and 1/10) for 24 h and 72h with both MSCs sources (Figure 1).

The intracellular cytokine was performed using the Human Th1/Th2/Th17 Phenotype Kit (BD - AB_2869360) flow cytometry, and the supernatant was performed using the Human IFN-gamma ELISA Kit (Diaclone-950.000.192); Human TGF-β ELISA Kit (Abbkine-Ket6030); Human IL-4 ELISA Kit (Diaclone-950.020.192); Human IL-17A ELISA Kit (Diaclone-850.940.192)) was used to perform the Elisa method. The intracellular cytokine levels were evaluated as the % values. The supernatant cytokine levels were calculated and evaluated in pg/ml unit by taking absorbance values from the ELISA plate reader.

Statistical analysis

The mean, standard deviation, median lowest, highest, frequency and ratio values were used in the descriptive statistics of the data. The distribution of the variables was measured by the Kolmogorov-Smirnov test. The Mann-Whitney U test was used for the analysis of quantitative independent data. The Statistical Package for the Social Sciences (SPSS) 28.0 program was used in the analyses. $p<0.05$ was considered statistically significant.

RESULTS

Mesenchymal stem cells express CD73, CD90, CD105 and CD44 from plastic adherent and specific surface markers and can differentiate into adipocytes, chondrocytes, osteoblasts, in vitro environment (1).

In our study, the adherent properties of the mesenchymal stem cells were examined under a microscope after mesenchymal stem cells were isolated and cultured (Figure 2). CB-MSCs began to adhere and reproduce in the first 7 days. Wharton's Jelly mesenchymal stem cells, on the other hand, separated from the tissue in a mean of 10 days and began to adhere and reproduce. Although cord blood-derived MSCs adhered and reproduced faster, we found in our study that Wharton's jelly MSCs preserved their morphology up to P7 and have an increased capacity to become confluent compared to CB-MSCs. The comparison for the surface markers showed that the percentages of CD73, CD90, CD105 and CD44 in MSCs obtained from both sources were over 95%.

With the comparison of the intracellular IFN-γ and IL-17 levels in terms of WJ-MSC and CB-MSC, a significant increase was detected at 24h both in 1/5 and 1/10 ratios in the group which was included WJ-MSC. However, at 72h, IFN-γ was significantly less, while IL-17 levels were higher. The comparison of the supernatant IFN-γ levels showed, on the contrary, that the level was significantly smaller at 24h in both 1/5 and 1/10 ratios in the group which was included WJ-MSC compared with the level in CB-MSC group. However, the level was higher at 72h ($p<0.0001$). The IL-17 supernatant level changes at the 1/5 ratio which was added WJ-MSC were significantly lower both at 24h and 72h. The level was significantly lower at 1/10 ratios ($p<0.0001$).

The comparison of the intracellular IL-4 levels in terms of WJ-MSC and CB-MSC showed that there was a significant decrease in the 1/5 ratio at 24h and 72h, and at the ratio of 1/10 at 72h in the group which was included WJ-MSC. However, the ratio of 1/10 was significantly higher at 24h. The comparison of the supernatant IL-4 levels showed that the 1/5 ratio at 24h, and the ratio of 1/10 at 72 hours decreased in the group with WJ-MSC and the ratio of 1/5 at 72h, and the ratio of 1/10 at 24h significantly increased ($p<0.0001$). The TGF-β supernatant level in the group which was added WJ-MSC, the ratio of 1/5 at 24h, and the ratio of 1/10 at 72h had significantly increased, however it had significantly decreased in other groups ($p<0.0001$) (Table 1-4).

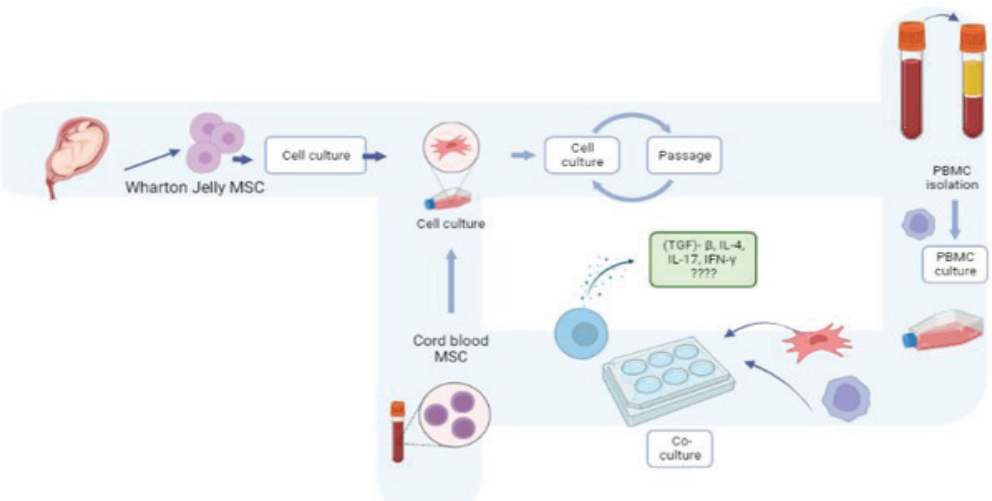

Figure 1: The experimental plan flow schema of the cord derived MSC and PBSC isolation and cultures

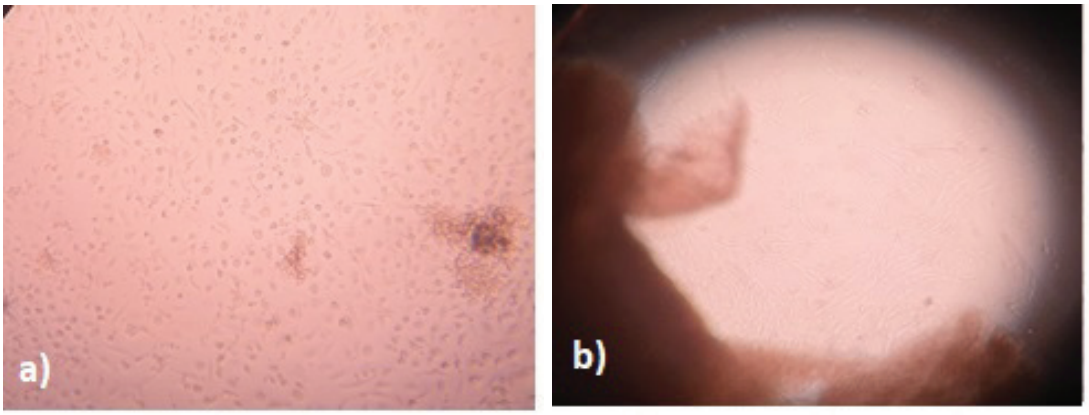

Figure 2: The microscopic images of mesenchymal stem cell in the first 7 days a) Cord blood derived MSC b) Wharton's Jelly derived MSC (Images were taken with 40x magnification on Motic AE21 inverted microscope)

Table 1: Comparison of the cytokine changes of groups which were added 1/5 ratio WJ-MSC, and CB-MSC for 24 hours

		WJ-MSC/STI-PBMC 1/5-24h		CB-MSC/STI-PBMC 1/5-24h		*p*
		avg.±sd	**Median**	**avg.±sd**	**Median**	
Intracellular	**IL-17**	5.47±0.06	5.48	2.32±0.15	2.24	***0.000***
	IFN-γ	11.62±0.02	11.62	7.96±0.06	7.99	***0.000***
	IL-4	8.94±0.01	8.94	10.22±0.01	10.22	***0.000***
Supernatant	**TGF-β**	167.86±0.11	167.80	12.03±0.06	12.02	***0.000***
	IFN-γ	104.20±0.21	104.28	136.18±0.16	136.24	***0.000***
	IL-4	0.02±0.00	0.02	2.35±0.03	2.36	***0.000***
	IL-17	11.02±0.02	11.02	17.04±0.02	17.04	***0.000***

IL-4: Interleukin-4, IFN-γ: Interferon-γ, IL-17: Interleukin-17, TGF-β: Tumor growth factor, MSC: Mesenchymal stem cells, WJ: Wharton's Jelly, STI: Stimule, PBMC: Peripheral blood mononuclear cells, CB: Cord blood, avg.: Average, SD: Standard deviation, h: Hour

Table 2: Comparison of the cytokine changes of groups which were added 1/5 ratio WJ-MSC, and CB-MSC for 72 hours

		WJ-MSC/STI-PBMC 1/5-72h		CB-MSC/STI-PBMC 1/5-72h		*p*
		avg.±sd	**Median**	**avg.±sd**	**Median**	
Intracellular	**IL-17**	2.05±0.02	2.05	14.54±0.03	14.53	***0.000***
	IFN-γ	1.32±0.03	1.31	31.05±0.01	31.04	***0.000***
	IL-4	0.94±0.02	0.95	7.13±0.03	7.13	***0.000***
Supernatant	**TGF-β**	0.69±0.01	0.69	6.62±0.43	6.51	***0.000***
	IFN-γ	90.88±0.30	90.96	63.35±46.75	90.31	***0.000***
	IL-4	0.31±0.00	0.31	0.18±0.00	0.18	***0.000***
	IL-17	11.04±0.02	11.04	19.30±0.04	19.31	***0.000***

IL-4: Interleukin-4, IFN-γ: Interferon-γ, IL-17: Interleukin-17, TGF-β: Tumor growth factor, MSC: Mesenchymal stem cells, WJ: Wharton's Jelly, STI: Stimule, PBMC: Peripheral blood mononuclear cells, CB: Cord blood, AVG: Average, SD: Standard deviation, h: Hour

Table 3: Comparison of the cytokine changes of groups which were added 1/10 ratio WJ-MSC, and CB-MSC for 24 hours

		WJ-MSC/STI-PBMC 1/10-24h		CB-MSC/STI-PBMC 1/10-24h		*p*
		avg.±sd	Median	avg.±sd	Median	
Intracellular	IL-17	16.93±0.02	16.93	6.17±0.21	6.10	***0.000***
	IFN-γ	10.24±0.02	10.24	5.33±0.03	5.34	***0.000***
	IL-4	6.96±0.01	6.96	5.80±0.02	5.80	***0.000***
Supernatant	TGF-β	1.36±0.36	1.30	23.40±0.35	23.37	***0.000***
	IFN-γ	94.02±0.11	94.04	126.77±0.22	126.79	***0.000***
	IL-4	2.24±0.03	2.25	0.65±0.03	0.64	***0.000***
	IL-17	19.94±0.14	19.87	17.92±0.07	17.92	***0.000***

IL-4: Interleukin-4, IFN-γ: Interferon-γ, IL-17: Interleukin-17, TGF-β: Tumor Growth Factor, MSC: Mesenchymal stem cells, WJ: Wharton's Jelly, STI: Stimule, PBMC: Peripheral blood mononuclear cells, CB: Cord blood, avg.: Average, SD: Standard deviation, h: Hour

Table 4: Comparison of the cytokine changes of groups which were added 1/10 ratio WJ-MSC, and CB-MSC for 72 hours

		WJ-MSC/STI-PBMC 1/10-72h		CB-MSC/STI-PBMC 1/10-72h		*p*
		avg.±sd	Median	avg.±sd	Median	
Intracellular	IL-17	12.37±0.03	12.36	8.67±0.02	8.68	***0.000***
	IFN-γ	13.4±0.06	13.50	27.15±0.05	27.16	***0.000***
	IL-4	2.15±0.05	2.15	6.46±0.04	6.46	***0.000***
Supernatant	TGF-β	5.28±0.03	5.28	0.93±0.12	1.00	***0.000***
	IFN-γ	103.28±0.48	103.38	89.19±0.04	89.21	***0.000***
	IL-4	0.27±0.00	0.27	0.76±0.01	0.77	***0.000***
	IL-17	16.50±0.00	16.50	13.86±0.01	13.87	***0.000***

IL-4: Interleukin-4, IFN-γ: Interferon-γ, IL-17: Interleukin-17, TGF-β: Tumor growth factor, MSC: Mesenchymal stem cells, WJ: Wharton's Jelly, STI: Stimule, PBMC: Peripheral blood mononuclear cells, CB: Cord blood, avg.: Average, SD: Standard deviation, h: Hour

DISCUSSION

Mesenchymal stem cells can be isolated from various sources including peripheral blood, adipose tissue, bone marrow, umbilical cord blood, and umbilical cord tissue. Various studies have shown that adipose-derived MSCs have the most colony-forming ability, and that bone marrow and adipose tissue are more successful than cord compared to those that can be best isolated. Although the bone marrow is a suitable source for obtaining MSC, the fact that it is invasive and that the differentiation potentials decrease with age, raises the issue of obtaining MSC from different sources. Cord blood and cord tissue are considered as the suitable cells for cell replacement therapy due to their non-invasive nature, having the same morphological, characteristics features, low immunogenicity, high proliferation and differentiation potential (5). MSCs are candidates as a suitable treatment option for immune system-related diseases such as autoimmune diseases, especially due to their immunomodulatory effects. It acts with some factors secreted by the immune system cells in the regulation of the immune response by MSCs (15).

In light of these data, we aimed to compare two different sources of MSCs by investigating the effects of MSCs derived from cord blood and cord tissue on isolation, culture stages and immune system in our project.

Our results showed that CB-MSCs and WJ-MSCs share similar phenotypic properties and immunomodulatory capacities. The comparison of the obtaining and isolation of cord blood and Wharton's Jelly showed that cord blood isolation and replication procedures were easier. At the same time, we observed that cord blood MSCs have a faster proliferation capacity (average 7 days). For the isolation of WJ-MSC, longer and more careful procedures to protect against any infection were required for isolation from the cord tissue which was taken into the container after childbirth. We found with the investigation of MSCs under microscopy after tissue fragmentation and subsequent culture procedures that MSCs started reproduction in a mean of 10 days. Although CB seems to be more advantageous than WJ MSCs in terms of isolation and proliferation capacities, WJ-MSCs reach up to 90% in terms of their confluent capacity, however, CB-MSCs reaching up to 80% in the 1st passage and

their capacity to become confluent were highly lower with the increase of the passage count. Similarly, as the number of passages increased, the CB-MSCs morphologically changed. The comparison of the two different sources in terms of characterization showed no significant difference between the percentage values of CD73, CD90, CD44 and CD105. Studies have shown that MSCs obtained from three different sources were negative for hematopoetic stem cell markers and positive to similar degrees in terms of expression of CD73, CD90, CD105 and CD44 markers (16, 17).

The immunomodulatory potential of the mesenchymal stem cells has been demonstrated on T cells, which plays a key role in the immune system and on the cytokines secreted from these cells (18-19). Studies have suggested that adipose-derived MSCs exhibit stronger immunomodulatory effects compared with the effects of bone marrow-derived MSCs, which suggests that adipose-derived MSCs would be a better alternative for immunomodulatory therapy. On the other hand, it has been suggested that CB-MSC and WJ-MSCs have a minimal risk of initiating an allogeneic immune response when administered in vivo. In addition to the ease of collection, this will make cord-derived MSCs as the suitable therapeutic candidates (20).

Mesenchymal stem cells do not always have an immunosuppressive effect, and they can also have a pro-inflammatory effect depending on the microenvironment in which they are located. Stimulation with low levels of IFNy and TNF, can confer immunostimulating properties on MSCs. Some clinical studies show that a certain level of inflammation is necessary to induce immunosuppressive properties in MSCs, and the lessons learned from these studies should be used to guide therapeutic methodologies (21, 22). The MSCs obtained in our study were not stimulated by a stimulating factor. We predict that more clear results can be obtained by adding stimuli to the MSC culture.

In general, the studies show relationship between MSCs and immune system from the adipose tissue and bone marrow sources. In this present study, the relationship between WJ and CB as the source of MSC and immune system was evaluated. It has given a different perspective to the literature. The effect of different MSC sources on the immune system in the study was evaluated in different combinations as 1/5 and 1/10, evaluated as 24 hours and 72 hours. The separate evaluation of these parameters, analysis and comparison have given a unique value to this study. The comparison of WJ-MSC and CB-MSC showed that the intracellular and supernatant cytokine levels differed in terms of affecting the immune system. The secretion of intracellular proinflammatory cytokines were suppressed in the WJ-MSC added experimental group particularly at 72h compared with the level in CB-MSC, and the level of anti-inflammatory cytokines were found to be significantly higher. In the studies conducted so far, bone marrow, adipose tissue and cord tissue have been compared as a source of mesenchymal stem cell. The comparison of cord blood and cord tissue provides new data to the literature together with this study. Considering the limitations of our study, we suggest that more clear and understandable results will be obtained with a more detailed study of intracellular cytokine expression pathways, with the addition of any stimulus when replicating MSC, and with the presence of cytokines secreted from MSC as well as with the inclusion of cytokines secreted from PBMC into the study.

Ethics Committee Approval: This study was approved by Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date:11.11.2022, No: 20).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- A.E., F.A.S., M.S., G.D., D.S.K.; Data Acquisition- A.E., F.A.S., M.S., G.D.; Data Analysis/Interpretation- A.E., F.A.S., M.S.; Drafting Manuscript- A.E., F.A.S., M.S., G.D., D.S.K., F.S.O.; Critical Revision of Manuscript- A.E., F.A.S., M.S., G.D., D.S.K., F.S.O.; Final Approval and Accountability- A.E., F.A.S., M.S.; Material and Technical Support- D.S.K., F.S.O.; Supervision- D.S.K., F.S.O.

Conflict of Interest: The authors have no conflict of interest to declare.

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