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A Comparative Study of Morphological Changes in Mesenchymal Stem Cells Between Early Term and Postdate Human Umbilical Cord

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Abstract

Background The stem cells have been reported in umbilical cord blood (UCB), the Wharton's jelly, and in other perivascular mesenchymal areas within the cord. Two types of stem cells that have been identified in UCB are haemopoietic stem cells (HSCs), which are blood-forming, and mesenchymal stem cells (MSCs), which are supposedly pluripotent. Stem cells within the umbilical cord other than those in cord blood have been broadly termed umbilical cord matrix stem cells (UCMSCs).

Objective To analyze the histological and histomorphometric changes in early term and postdate human umbilical cord (HUC).

Methods Fifty samples of the HUC were collected from normal vaginal delivery and were divided into 25 UC as normal early term and 25 UC as normal postdate labor. The age of pregnant women was (18-30 years), whose mother had normal pregnancy and normal vaginal delivery. The UC were fixed in 10% formaldehyde for paraffin blocks preparation. Transverse sections were placed on standard glass slides for hematoxylin and eosin staining.

Results The mean±SD of the length in postdate UC was 58.76±4.72 cm, significantly more than in early term UC (46.12±2.65 cm, P <0.001). The diameter of postdate UC was 0.61±0.12 cm, more than in early term UC (0.55±0.04 cm, P <0.02). The artery diameter in postdate UC was 504.45±44.97 pixel/µm², compared to 422.23±52.82 pixel/µm² in early term UC (P <0.001). The vein diameter in postdate UC was 612.20±62.54 pixel/µm², less than in early term UC (712.84±64.11 pixel/µm², P <0.001). The MSCs thickness in postdate UC was 40.18±15.22 pixel/µm², more than in early term UC (29.05±7.10 pixel/µm², P <0.002).

Conclusion In early gestation, oxidative stress likely increases vessel diameters, such as the umbilical vein, to enhance blood return to the fetus. As the gestational period progresses, the growth of smooth muscle walls results in thicker arterial walls and larger diameters in postdate compared to early term.

Keywords Stem cells, oxidative stress, term of gestation, blood of human umbilical cord

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List of abbreviations: ECM = Extracellular matrix, HUC = Human umbilical cord, MSCs = Mesenchymal stem cells, ROS = Reactive oxygen species, SD = Standard deviation, UC = Umbilical cord, UCB = Umbilical cord blood, WJ = Wharton jelly

Introduction

The human umbilical cord (HUC) consists of two arteries and a vein surrounded by an embryonic mucoid connective tissue gelatinous substance, known as Wharton's jelly (WJ), which is covered by amniotic epithelium that contains a very interesting number of mesenchymal stem cells (MSCs) in an abundant extracellular matrix (ECM). The UC is the structure that connects the fetus to the placenta. It is an important source to get MSCs, because its use would not generate ethical conflicts, it is easily obtained MSCs have been obtained from UCB and from WJ [1-2].

The oxidative stress is characterized by deregulated production and/or scavenging of reactive oxygen and nitrogen species (ROS and RNS, respectively), in general, increased ROS inhibit MSCs proliferation, increase senescence, enhance adipogenic but reduce osteogenic differentiation, and inhibit MSCs [3]. The objectives of this study was to analyze the histological and histomorphometric changes in early term and postdate human umbilical cord (HUC).

Methods

Samples of the HUC were collected from the labor rooms in (Al-Imamein Al-Kadhimein Medical City and Gynecology and Obstetrics Teaching Hospital in Karbala). The total of the samples collected from 50 UC of normal vaginal delivery were divided into 25 UC as normal early term and 25 UC as normal postdate labor. In both early term human umbilical cords baby was born between 37 weeks, 0 days and 38 weeks, 6 days. While in post-date human umbilical cords delivery extends beyond 40 weeks plus one to 13 days (anytime past the estimated due date of delivery). The age of pregnant women was (18-30 years), whose mother was normal pregnancy and normal vaginal delivery.

Microscopic examination

Arterial diameter

The UC artery diameter measurement was done by applying the measuring scale of the Image J software program and was assessed in µm, this was done randomly for more than a diagonal line of these blood vessels that were seen within Wharton gel at 10X magnification power.

Vein diameter

the UC vein diameter measurement was done by applying the measuring scale of the Image J software program and was assessed in µm, this was done randomly for different directions of these blood vessels that were seen within Wharton gel at 10X magnification power.

Perivascular Wharton jelly thickness

The perivascular Wharton Jelly thickness measurement was done by applying the measure scale of image j software program and was assessed in µm and this was done randomly for different direction of these spaces that were seen around the blood vessel of UC at 40X magnification power.

MSCs thickness

The measurement of the clumps of MSCs thickness was done by applying the measure scale of image j software program and was assessed by µm and this was done randomly for these clumps of MSCs cells that were seen disperse within Wharton jelly at 40X magnification power.

Intercellular space thickness

The intercellular space thickness measurement was done by applying the measure scale of image j software program and was assessed in µm and this was done randomly for different direction of these spaces that were seen in between MSCs clumps within Wharton gel at 400x magnification power.

Statistical analysis

For all statistical analyses, the statistical package for social sciences (SPSS) software program (version 21) was used to interpret the data. The information is given in the form of a mean ± standard deviation (SD). Unpaired ttest (Independent ttest) was used to compare between tested mean data. P values >0.05

were considered statically non-significant while P ≤0.05 considered significant results.

Results

1. Macroscopic examination of human Umbilical cord in early term and postdate

A. The length of umbilical cord

The mean±SD of the length in postdate UC recorded 58.76±4.72 cm, which was significantly more in value than in the early term UC, which was 46.12±2.65 cm (P <0.001) (Figures 1 and 2).

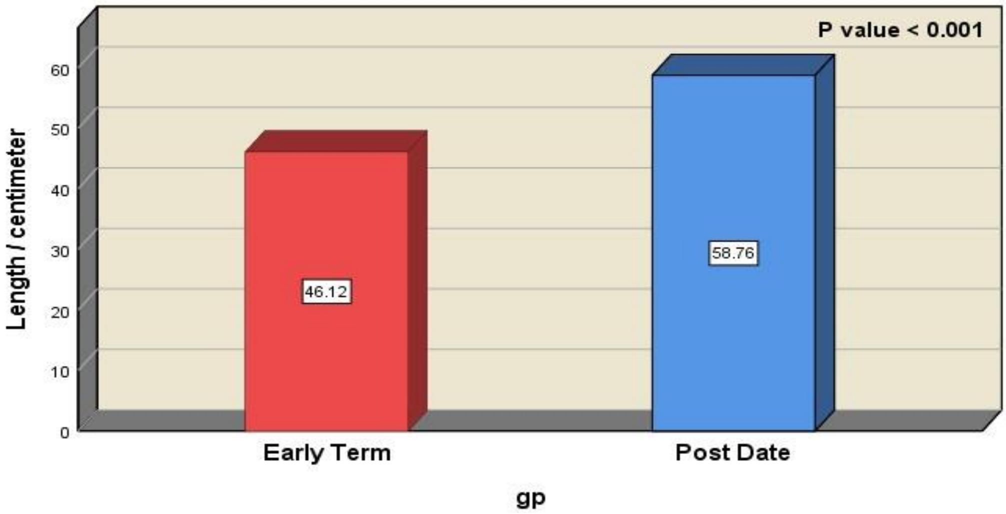

Figure 1. Mean and standard deviations the length of UC in early term and postdate umbilical cord in centimeter

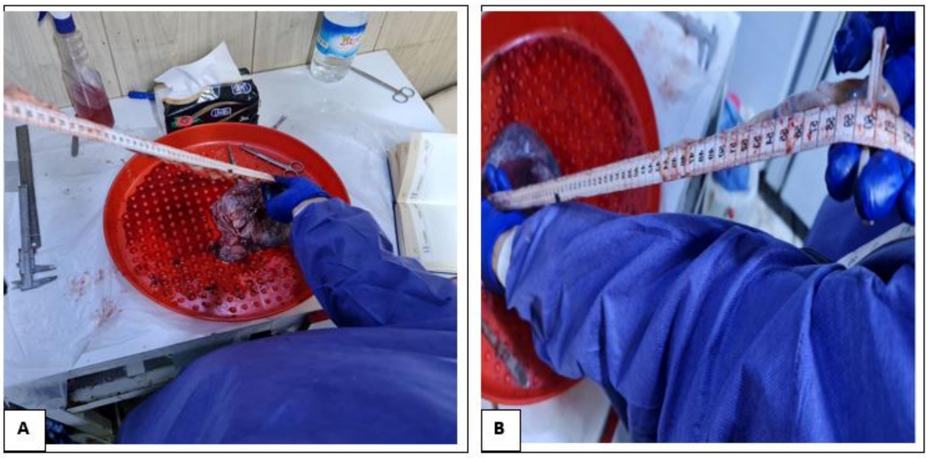

Figure 2. The measurement the length of umbilical cord (cm) in early term (A) and postdate (B)

B. Thickness of umbilical cord

The mean±SD of the UC diameter in postdate umbilical cord recorded 0.61±0.12 cm, which was significantly more than in the early term UC which was 0.55±0.04 cm (P value <0.02) (Figures 3 and 4).

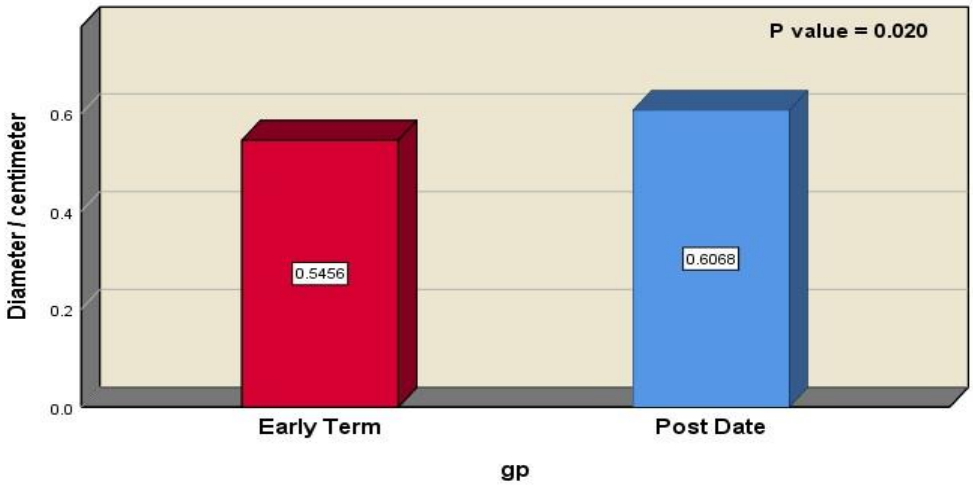

Figure 3. Mean and standard deviations of the umbilical cord Diameter in early term and postdate umbilical cord in centimeter

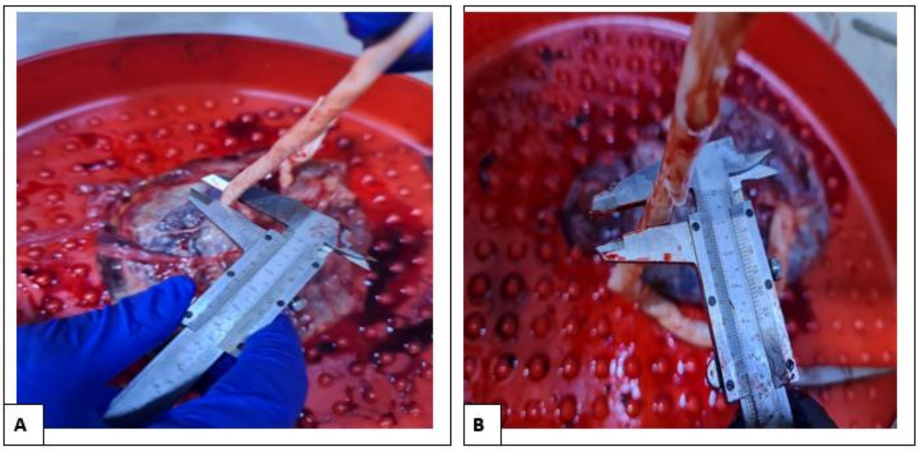

Figure 4. The measurement the diameter of umbilical cord (cm) in early term (A) and postdate (B)

C. Number of coils of umbilical cord

The mean±SD for the number of coils in early term and postdate UC was evaluated. The postdate UC recorded 16.0±1.0, which was significantly more than that of the early term, which 13.0±1.0 with significant (P value <0.001) (Figures 5 and 6).

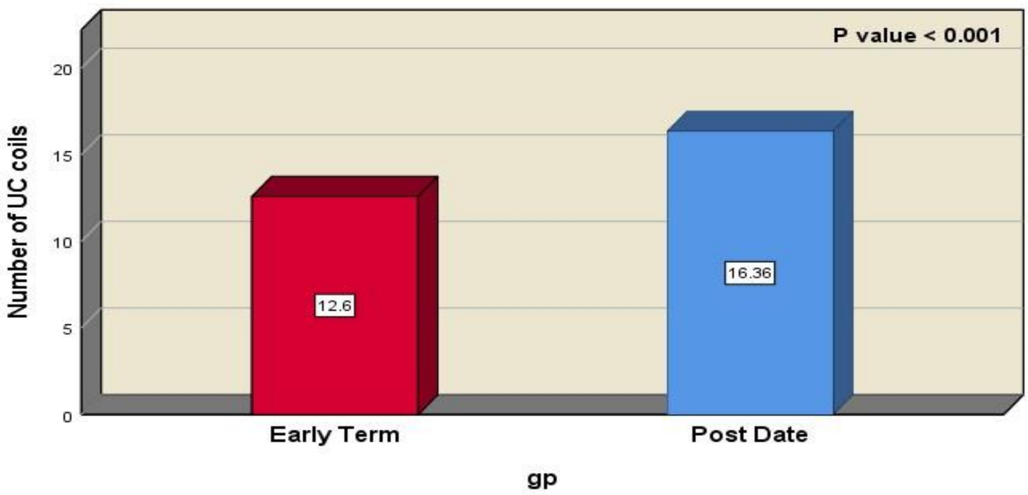

Figure 5. Mean and standard deviations of the number of coils in early term and postdate umbilical cord

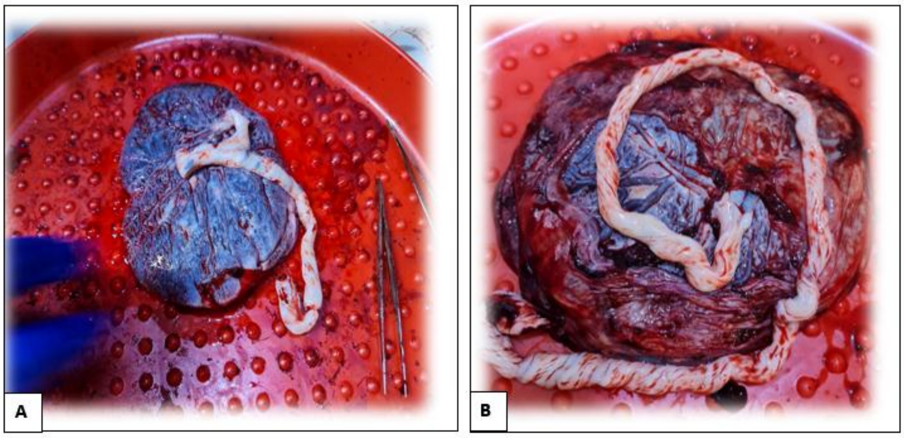

Figure 6. The measurement the number of coils in umbilical cord (cm) in early term (A) and postdate (B)

2. Microscopic examination

A. Arterial Diameter

The mean±SD of the artery diameter in postdate UC recorded 504.45±44.97 µm, which was significantly more than in the early term UC, which was 422.23±52.82 µm (P value <0.001) (Figures 7 and 8).

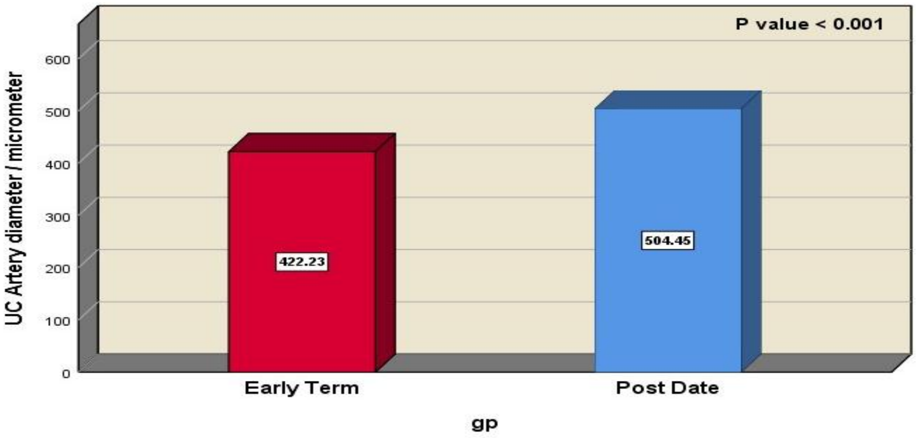

Figure 7. Mean and standard deviations of the artery diameter in early term and postdate umbilical cord in micrometer

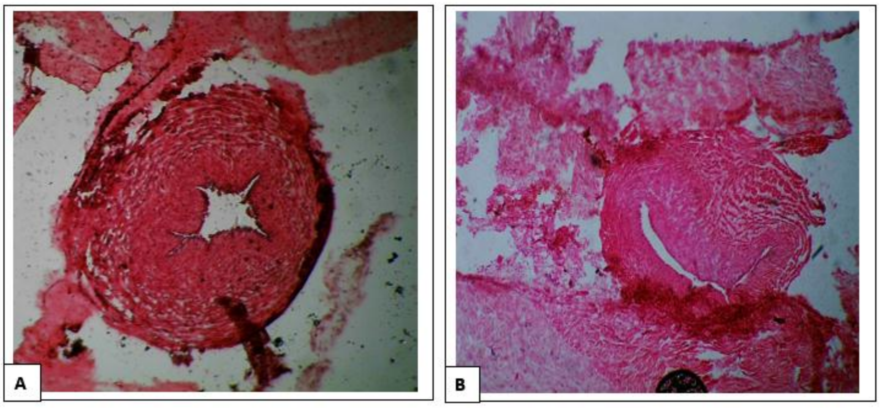

Figure 8. Measurement the artery diameter in early term (A) and postdate (B) of umbilical cord (40X)

B. Vein diameter

The mean±SD of the vein diameter in postdate umbilical cord recorded 612.2±62.54 µm, which was significantly less than in the early term UC, which was 712.84±64.11 µm (P value <0.001) (Figures 9 and 10).

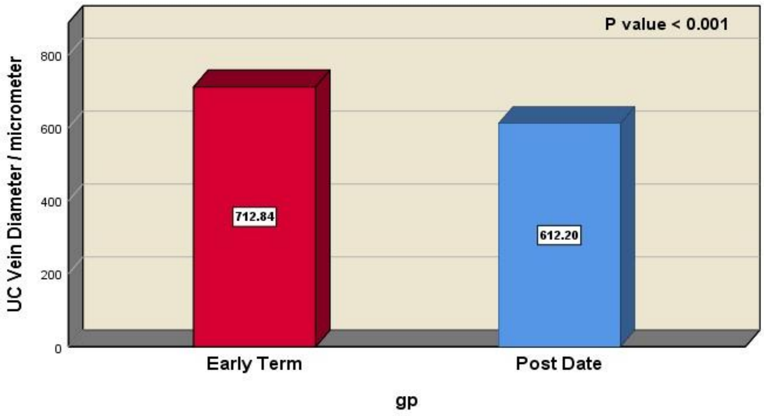

Figure 9. Mean and standard deviations of the Vein Diameter in early term and postdate umbilical cord in micrometer

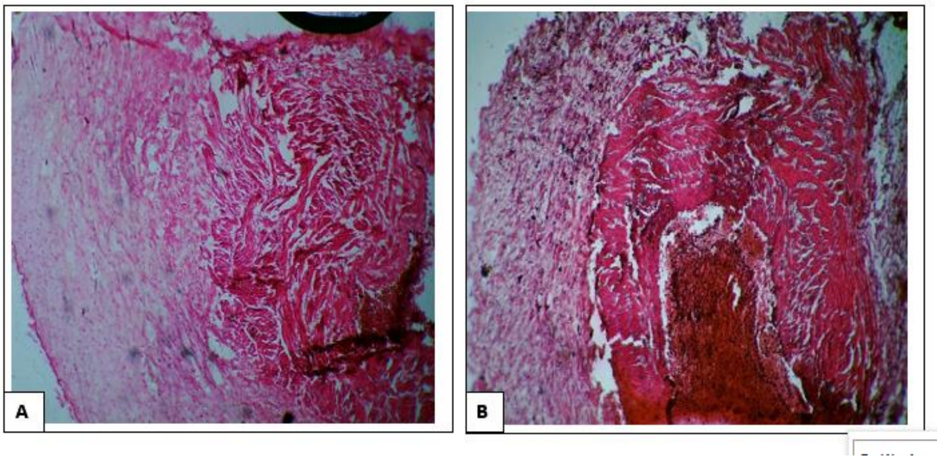

Figure 10. Measurement the Vein Diameter in early term (A) and postdate (B) of umbilical cord (40X)

C. Perivascular Wharton Jelly thickness

The mean±SD of the perivascular Wharton jelly thickness in postdate UC recorded 358.86±75.70 pixel/µm^2 which was significantly less than in the early term UC, which was 620.82±173.2 pixel/µm^2 (P value <0.001 (Figures 11 and 12).

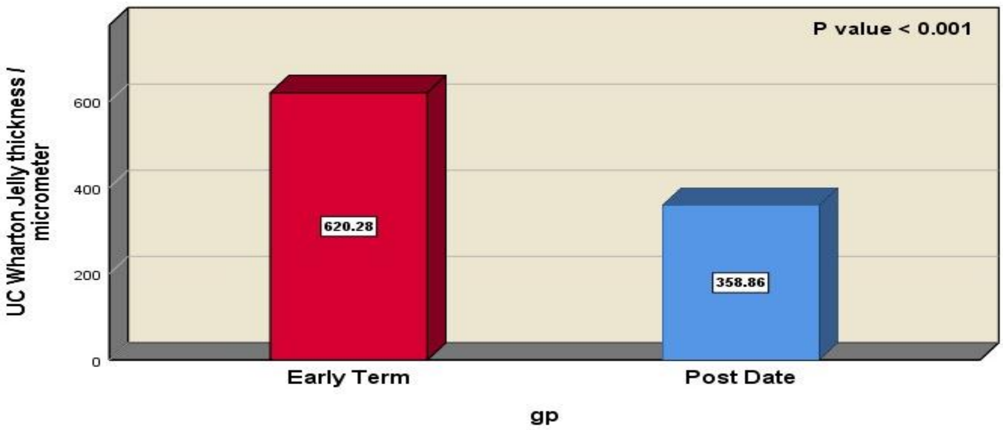

Figure 11. Mean and standard deviations of the perivascular Wharton jelly thickness in early term and postdate umbilical cord in micrometer

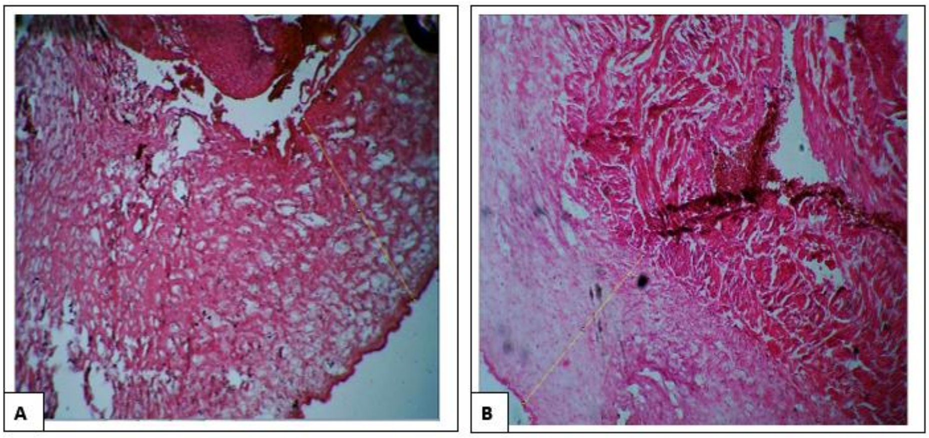

Figure 12. Measurement the perivascular Wharton Jelly thickness in early term (A) and postdate (B) of umbilical cord at (40X)

D. Mesenchymal stem cells thickness

The mean±SD of the MSCs thickness in postdate UC recorded 40.18±15.22 pixel/μm^2, which was significantly more than in the early term UC, which was 29.05±7.10 pixel/μm^2 (P <0.002) (Figures 13 and 14).

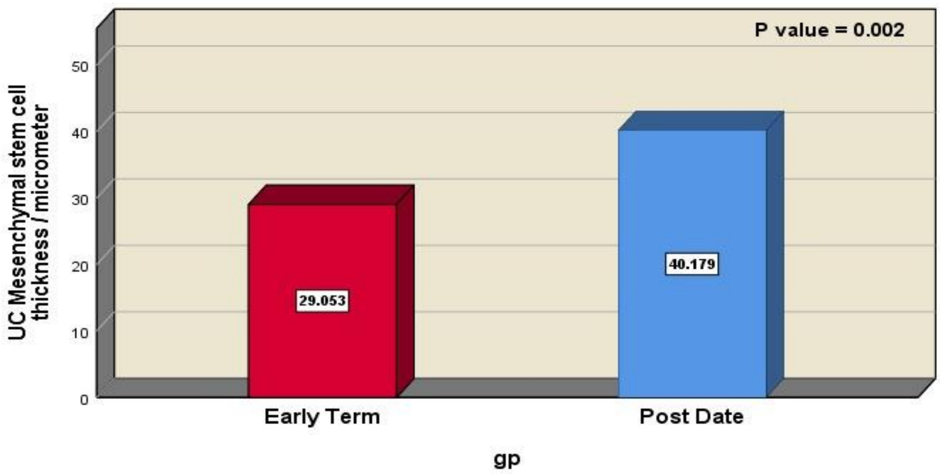

Figure 13. Mean and standard deviations of the mesenchymal stem cell thickness in early term and postdate umbilical cord

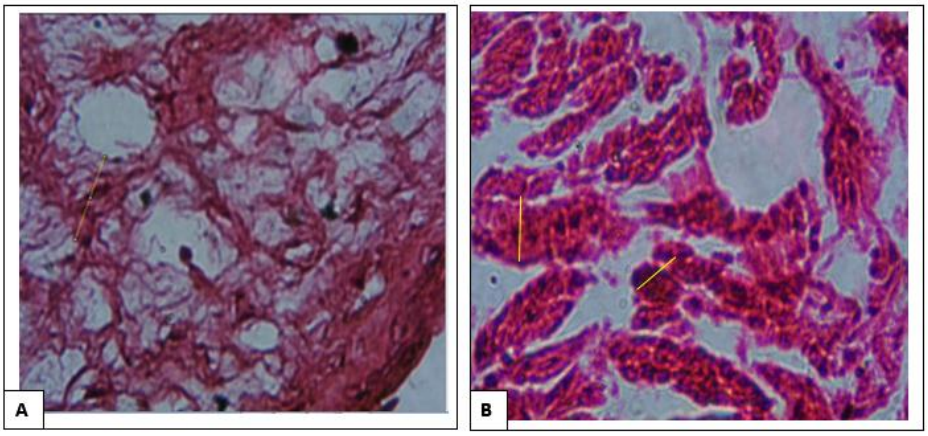

Figure 14. Measurement the mesenchymal stem cells thickness (μ) in early term (A) and postdate (B) of umbilical cord (40X), hematoxylin and eosin stain

E. Intercellular space thickness

The mean±SD of the intercellular space thickness in postdate UC recorded 39.04±13.28 µm, which was significantly more than in the early term UC, which was 22.75±9.96 µm (P <0.001) (Figures 15 and 16).

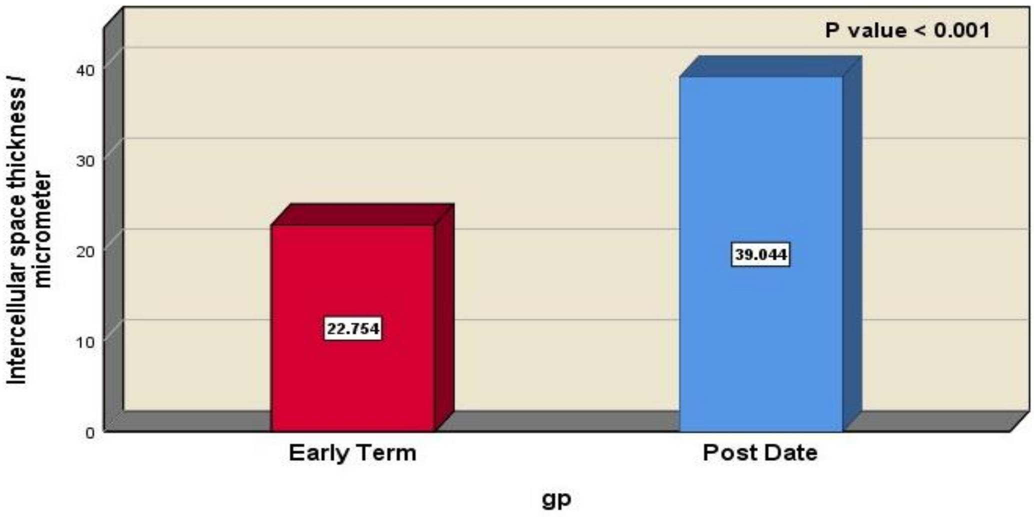

Figure 15. Mean and standard deviations of the intercellular space thickness in early term and postdate umbilical cord in micrometer

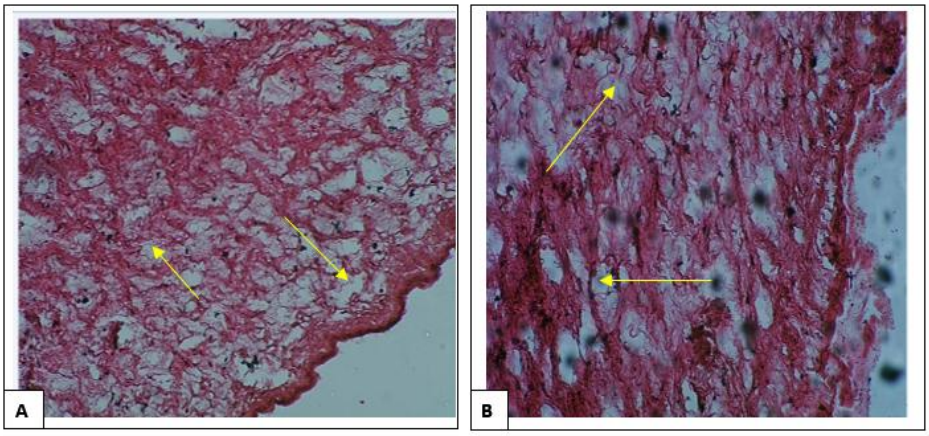

Figure 16. Measurement the intercellular space thickness (µm) in early term (A) and postdate (B) of umbilical cord

Discussion

UC vessels diameters

The present study recorded the postdate UC vein diameter to be less in value than that in early term, this result was obtained by applying image J analysis software (Image j version 1.4.3.67), and the reason for that may be was due to less amount of WJ and small intercellular spaces between MSCs cords in postdate UC that leading to less value of UC vein diameter. This in agreement with other researchers who found that morphological and structural parameters of UC veins including lumen diameter, wall thickness, tunica media thickness that were analyzed by using image analysis software (Image-pro Plus 6.0). Umbilical veins wall thickness calculation had been done [4]. Oxidative stress may be one of causes that might lead to early labor (early term) as in the present study, and this consequently leading to increment in diameter of the vessels as umbilical vein to increase the blood return to fetus and this cope with further researchers who had evaluated the lumen diameter of umbilical veins that gradually decreased, while wall thickness, tunica media thickness, and wall-luminal ratio gradually increased, from placental to fetal segments of umbilical veins [5]. As in the present study the arterial wall thickness and diameter is more in postdate than in early term and this due to increment in the smooth muscular wall with progress time of gestation and this agree with other researchers who found that in normal blood pressure, the umbilical vein tunica media was predominantly muscular and its wall thickness is smaller than that of the artery at corresponding misunderstanding. The apparent increase was due to the tunica media and the tunica intima. The media showed thicker muscle bundles with increased trabeculations and more prominent connective tissue setae. The tunica media smooth muscle often displayed different orientations as opposed to the predominantly circular orientation seen in the vein of normotensive women mentioned above [6,7].

MSCs in UC

In the present study, the early term labor reveals low mesenchymal stem cells in UC and this probably due to cells that were damaged by oxidative stress and this agree with other researchers who found that the reactive oxygen species (ROS) are essential regulators of various cellular processes, and, in a normal state, the number of free radicals and antioxidants are in balance while with excessive free radicals are produced, the antioxidant defense fails, and healthy cells are damaged by oxidative stress [8,9]. Oxidative stress leads to DNA damage [10,11], all of which deteriorates biological functions and finally trigger the aging process leading to cell death [12]. The oxidative stress in the present study reveals low cellularity of MSCs in UC in early term labor and this agree with other authors who study the effect of oxidative stress that brings detrimental consequences to the ovaries, uterus, oocytes, and embryos. In the ovaries, oxidative stress restricts follicle and oocyte maturation, which leads to a decreased number and competence of oocytes and an abnormal increase of the follicle-stimulating hormone and also had been seen in placentation failure [13-16].

Relation of numbers of coils of the UC with gestational age

The present study reveals low numbers of coils in the UC, which was seen more in early term labor and this agree with other researchers who recorded that there were intrauterine deaths and preterm labor that were accompanied by the low or non-coiled UC and poorly coiled UC that had been suggested the non-coiled UC are structurally less able to resist external forces [17].

In conclusion, in the early term probably due to oxidative stress that consequently leading to increment in diameter of the vessels as umbilical vein so as to increase the blood return to fetus.

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Author contribution

Abed and Dr. Jaafar: designed the study and contributed to the acquisition of data. Shaker: contributed to sample preparation and was the main person in writing the manuscript. Both authors provided critical feedback and the shape of the research, interpreted the data and read and approved the final manuscript. Dr. Al-Rawaf and the third author helped us collect the required samples.

Conflict of interest

No potential conflict of interest was reported by the authors.

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