Original Article

Histomorphometric Analysis of the Human Umbilical Cord and Its Vessels in Pregnant Women with Anemia: A Case-Control Study

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Received: 26-Sep-2024; **Revision:** 09-Feb-2025; **Accepted:** 10-Feb-2025; **Published:** 11-Apr-2025

Abstract

Background: Anemia during pregnancy is a significant public health concern as it is associated with adverse maternal and fetal consequences. **Aim:** To assess the histomorphometric changes in the umbilical cord as an indicator of maternal physiological dysfunction and to evaluate the impact of maternal anemia on fetal development. **Methods:** A case-control study was conducted to explore the histomorphometric changes in UC and its vessels in women with anemia in Central Sudan. The following parameters were studied using the ImageJ software: vessel area (VA), wall area (WA), lumen area (LA), mean wall thickness boundary (MWTB), mean wall thickness skeleton (MWTS), and external diameter skeleton (EDS). **Results:** UCs were studied in 73 women with anemia and 102 women without anemia. Only one woman had severe anemia (hemoglobin level, <7 g/dl). Maternal age, parity, and gestational age showed no significant differences between women with and without anemia. However, the median (interquartile range) birth weight was significantly lower in women with anemia than in those without anemia [3.29 (2.91–3.58) g vs 3.42 (3.09–3.77) g, $P = 0.043$]. None of the investigated variables (VVA, WA, LA), MWTB, MWTS, and EDS) did not differ between women with and without anemia. No significant correlations were found between maternal hemoglobin levels and UC parameters. **Conclusion:** The current study showed no difference in UC parameters between women with and without anemia. The nonsevere form of anemia may explain the results of this study. Therefore, further research is required in this regard. **Data Access Statement:** The data supporting the findings of this study are available from the corresponding author upon reasonable request. Due to ethical and privacy concerns, some data may be restricted in accordance with institutional and regulatory guidelines.

Keywords: *Age, anemia, hemoglobin, pregnancy, umbilical cord*

Introduction

The human umbilical cord (UC) consists of two umbilical arteries and one umbilical vein embedded

Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/njcp.njcp_648_24

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How to cite this article: Ahmed I, Elmugabil A, Salih MM, Ali LE, Siniyeh AA, Khalifa EH, *et al*. Histomorphometric analysis of the human umbilical cord and its vessels in pregnant women with anemia: A case-control study. Niger J Clin Pract 2025;28:367-72.

in Wharton's jelly, a specialized connective tissue that provides structural support and protection against mechanical compression.[1] Maintenance of the structural integrity of the UC is critical for fetal circulation, and histological alterations in its components can significantly affect pregnancy outcomes, particularly in cases of maternal anemia.[2] Anemia during pregnancy can induce adaptive changes in the vascular structure and extracellular matrix, potentially compromising UC function and fetal development.[3] Investigating these histological changes is essential for understanding how maternal anemia influences fetal growth and overall pregnancy outcomes. Pregnant women are more susceptible to anemia than their nonpregnant counterparts, and anemia is associated with a range of adverse maternal and perinatal outcomes.[4] The World Health Organization (WHO) defines anemia during pregnancy as a hemoglobin level <11 g/dL.[5] Anemia during pregnancy is a major global health concern, with an estimated pooled worldwide prevalence of 36.8% among pregnant women.[6] Certain regions, such as Southeast Asia and sub-Saharan Africa (SSA), have even higher prevalence rates, with 57.0% of pregnant women affected.[7] Despite ongoing health interventions, anemia during pregnancy remains a persistent issue, with recent studies highlighting its high prevalence in several sub-Saharan African countries.[8–11] Several perinatal adverse effects such as preterm birth, intrauterine growth restriction (IUGR), and low birth weight (LBW) are associated with maternal anemia.[12–17] The exact mechanisms implicated in the development of IUGR and low birth weight in maternal anemia are not fully understood. However, placental changes such as increased numbers and dilatation of chorionic villi capillaries and wider intervillous space in placentas in women with anemia have been reported.[18] Moreover, an increase in placental volume, volume of the intervillous space, and number of chorionic villi was observed in the placentas of women with anemia.[19,20] Several studies have examined histomorphometric changes in the UC in women with preeclampsia, a condition associated with an increased risk of IUGR and LBW;[21–23] a few researchers have investigated the changes in UC in women with anemia during pregnancy.[24,25] Hence, more research is needed on changes in UC in women with anemia as this could explain the different adverse effects of anemia during pregnancy.

Sudan is the third largest country in Africa by area, and anemia during pregnancy remains a significant public health concern.[26,27] Maternal anemia has been associated with LBW[28,29] and stillbirth.[30] This study aimed to investigate the histomorphometric changes in the UC and its vessels associated with maternal anemia in Central Sudan.

Method

This case-control study was conducted at Rabak Maternity Hospital, White Nile, Central Sudan, from September to December 2022. The hospital is the largest tertiary hospital located in the White Nile in Rabak City, one of Sudan's largest cities.[31]

Cases were UCs of live newborns whose mothers had anemia (hemoglobin <11 g/dl), while the controls were umbilical cords of live newborns whose mothers had no anemia. Mothers younger than 18 years, those with multiple pregnancies, and those with pre-existing medical conditions such as hypertension, diabetes mellitus, or thyroid disorders were excluded from both the case and control groups. Additionally, newborns with congenital malformations were not included in the study. Only full-term neonates were considered, with preterm births (<37 weeks of gestation) explicitly listed as an exclusion criterion.

Data collection

After obtaining written informed consent, pregnant women were interviewed during routine antenatal visits in the third trimester (gestational weeks 32–36). A structured questionnaire was used to collect sociodemographic, clinical, and obstetric data, including maternal age and parity. Blood samples were collected at the time of hospital admission for delivery, prior to the onset of labor. Specifically, 2 mL of venous blood was drawn from each participant via venipuncture and collected in an ethylenediaminetetraacetic acid (EDTA) tube for further analysis. To determine the hemoglobin levels, the samples were analyzed using an automated hematology analyzer (Sysmex KX-21, Japan). Maternal anemia was defined as a hemoglobin level of less than 11 g/dl, and a severe form of anemia was defined as a hemoglobin level of less than 7 g/dl.[2] Neonatal birth weight was measured within the first 10 min of life using a digital floor scale (Seca, Hamburg, Germany) following standard procedures. Weight was recorded to the nearest two decimals. UC samples from full-term neonates were collected immediately after the delivery. To ensure consistency and minimize anatomical variability, the sections were excised 3–5 cm from the placental insertion site. A 2–3 cm segment of the UC was obtained, rinsed in normal saline to remove residual blood, and further sectioned into 1.5 cm-long parts. The cross-sections were fixed in 10% buffered formalin for 48 h at room temperature, followed by dehydration, clearing, and paraffin embedding using standard histological procedures. Five-micron-thick sections were prepared, dewaxed, and stained with hematoxylin and eosin (H and E). Microscopic examination was conducted using a stereomicroscope (BRESSER Analyst

STR). The normal is 50–60 cm in extent and two centimeters in width, with a fit for 40 helical turns.[32] Therefore, the stereo microscope is perfect for viewing the whole surface of the UC tissue section in order to observe its boundary and different blood vessels. Slides were observed (×20) and imaged using a digital camera iPhone XS Max (Dual 12MP wide-angle). The images were used to calculate the geomorphology of the UC arteries and veins using H and E staining. The study noted qualitative medial vessel thickening and measured these vessels in the lumen and wall areas. Additional procedures for calculating the wall thickness are the boundary and skeleton processes. We used the Vascular Medicine Institute (VMI) calculator, which determines the wall thickness using these variables, as well as vessel element areas. From these images, the parameters of the UC arteries and veins were measured with the VMI calculator software.[33] Vessel area (VA), wall area (WA), lumen area (LA), mean wall thickness boundary (MWTB), mean wall thickness skeleton (MWTS), and external diameter skeleton (EDS) were the other parameters. ImageJ software was used to calculate the total UC area (TUCA). All tissue sections were examined by a single investigator who was blinded to the patient data. To assess intraobserver variability, a randomly selected subset of slides (n = 35) was reanalyzed by the same investigator at two separate time points, with an interval of [3 days/week] between evaluations.

Sample size calculation

OpenEpi Menu was used to calculate the desired sample size.[34] The sample size of the 73 cases and 102 controls was calculated at a ratio of 1:1.5. We assumed a difference of 0.5 in the mean UC parameters (VVA, WA, LA, MWTB, MWTS, and EDS) between the cases and controls. This assumption was based on a previous report on the difference between UC parameters in women with hypertensive disorders of pregnancy.[35]

Ethical approval

This study was approved by the Ethical Committee of the Faculty of Medicine, El Imam El Mahdi University, Kosti, Sudan (Approval No. 2021/09). Written informed consent was obtained from all participants prior to enrolment. This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

Statistics

The data were analyzed using SPSS for Windows version 22 (IBM, Armonk, NY, USA). They were checked for normality using the Shapiro–Wilk test and were not normally distributed; therefore, they were described by the median [interquartile range (IQR)] and compared between the two groups using a nonparametric test (Whitney–Mann test). Spearman correlations were performed between maternal hemoglobin levels and UC parameters. A P value less significance was set at $P < 0.05$.

Results

UC samples were analyzed in women with and without anemia. Among the anemic group, only one woman had severe anemia (hemoglobin level < 7 g/dL). Maternal age, parity, and gestational age did not differ significantly between the two groups. However, the median (IQR) birth weight was significantly lower in neonates born to mothers with anemia than in those born to mothers without anemia (3.29 [2.91–3.58] g vs 3.42 [3.09–3.77] g, $P = 0.043$, Table 1).

Analysis of umbilical cord parameters, including vessel wall area (VWA), Wharton's jelly area (WA), lumen area (LA), media thickness of both arteries (MWTB) and veins (MWTS), and extracellular density score (EDS), showed no significant differences between women with and without anemia [Table 2]. Furthermore, no significant correlations were observed between maternal hemoglobin levels and umbilical cord parameters [Table 3].

Discussion

The current study found no significant differences in the UC parameters between women with and without anemia. In contrast, a previous study reported a smaller UC diameter, fewer placental cotyledons, and reduced total UC area in women with anemia.[24] Moreover, a significant negative correlation between maternal hemoglobin level and the absolute volume or surface area

Table 1: Histomorphometric parameters of the UC and its vessels in the anemia and control groups

Variables	Group with anemia (number=73)	Control group (number=102)	P
Maternal age, years	24.0 (21.0–29.0)	25.0 (21.0–30.0)	0.510
Parity	3 (2–4)	2 (1–4)	0.430
Gestational age, weeks	38.0 (37.0–40.0)	38.5 (37.0–40.0)	0.974
Birth weight, gm	3.29 (2.91–3.58)	3.42 (3.09–3.77)	0.043
Cord length, cm	42.0 (39.0–49.0)	41.0 (36.0–47.0)	0.204
Cord diameter, mm	10.10 (8.80–11.20)	10.15 (8.40–11.50)	0.748

Table 2: Histomorphometric parameters of the UC and its vessels in the anemia and control groups

Variables	Umbilical cord vessels	Group with anemia (number=73)	Control group (number=102)	*P*
Artery 1	Vessel area, mm^2	0.487 (0.199–7.2)	0.710 (0.239–11.8)	0.237
	Lumen area, mm^2	0.041 (0.015–0.322)	0.061 (0.015–1.0)	0.247
	Wall area, mm^2	0.389 (0.172–6.6)	0.669 (0.213–9.5)	0.271
	Wall thickness, boundary, mm	0.246 (0. 171–0. 975)	0. 339 (0. 172–1. 0)	0.540
	Wall thickness, skeleton, mm	0. 236 (0. 168–0. 994)	0. 300 (0. 170–1. 0)	0.514
Artery 2	Vessel area mm^2	0. 384 (0. 244–10. 8)	1. 2 (0. 260–12.5)	0.317
	Lumen area, mm^2	0. 065 (0. 020–0. 546)	0. 117 (0. 023–1.0)	0.152
	Wall area, mm^2	0. 355 (0. 216–10.3)	1.1 (0.233–11.1)	0.379
	Wall thickness, boundary, mm	0. 239 (0. 158–1. 1)	0. 382 (0. 169–1. 1)	0.615
	Wall thickness, skeleton, mm	0. 228 (0. 155–1. 0)	0. 372 (0. 167–1. 1)	0.624
Vein	Wall area, mm^2	0. 556 (0. 305–9. 5)	1. 5 (0. 329–14.5)	0.312
	Lumen area, mm^2	0. 170 (0. 051–1. 8)	0. 302 (0. 049–8.2)	0.444
	Wall area, mm^2	0. 451 (0. 243–7.6)	1.0 (0. 264–12.1)	0.254
	Wall thickness, boundary, mm	0. 219 (0. 156–0. 906)	0. 335 (0. 174–1. 0)	0.367
	Wall thickness, skeleton, mm	0. 211 (0. 151–0. 892)	0. 327 (0. 169–0. 953)	0.277

Table 3: Spearman correlations between parameters of the UC and its vessels and maternal hemoglobin level

Variables	Umbilical cord vessels	Coefficient (*r*)	*P*
Artery 1	Vessel area, mm^2	0.081	0.284
	Lumen area, mm^2	0.103	0.173
	Wall area, mm^2	0.077	0.309
	Wall thickness, boundary, mm	0.047	0.533
	Wall thickness, skeleton, mm	0.093	0.222
Artery 2	Vessel area mm^2	0.048	0.524
	Lumen area, mm^2	0.067	0.379
	Wall area, mm^2	0.045	0.552
	Wall thickness, boundary, mm	0.047	0.535
	Wall thickness, skeleton, mm	0.046	0.550
Vein	Wall area, mm^2	0.072	0.344
	Lumen area, mm^2	0.518	0.175
	Wall area, mm^2	0.310	0.175
	Wall thickness, boundary, mm	0.434	0.175
	Wall thickness, skeleton, mm	0.333	0.175

of the intervillous space or villi was previously observed in women with anemia without a significant difference in the volume fraction or surface density of placental structures.[19] Likewise, the placentas of pregnant women with anemia showed significantly increased numbers and dilatation of chorionic villi capillaries and wider intervillous spaces.[18] Previous studies have shown an increase in placental volume, volume of the intervillous space, and number of chorionic villi in the placentas of women with anemia.[19,20] It is possible that the UC parameters are associated with UC hemoglobin (which we did not assess) rather than maternal hemoglobin. A previous study showed that placental and birth weights were associated with UC blood hemoglobin rather than maternal hemoglobin.[35] Another explanation for our results (no difference in UC parameters between women with and without anemia) is the degree of anemia itself. Except for one case of severe anemia, all enrolled women in the current study had nonsevere anemia. Previous studies have shown that poor maternal and perinatal outcomes are associated with severe anemia.[17,30] Moreover, an increased number of syncytial knots and capillaries per villus was observed, along with increasing severity of anemia.[36]

Numerous studies have reported significant alterations in UC parameters in hypertensive disorders of pregnancy, particularly pre-eclampsia. These alterations include a reduction in the umbilical cord diameter, vessel lumen area, and Wharton's jelly volume, along with increased arterial wall thickness and vascular resistance. Such histomorphometric changes indicate impaired placental perfusion and endothelial dysfunction, which may negatively affect fetal development. Consistent with these findings, previous studies have demonstrated significant differences in UC parameters among women with hypertensive disorders of pregnancy and pre-eclampsia.[21–23] Both anemia and hypertensive disorders of pregnancy can share common perinatal adverse effects, such as IUFR and LBW. This point (difference in the UC parameters between women with and without hypertensive disorder of pregnancy and preeclampsia) is behind our hypothesis of the difference in these parameters in women with anemia. The hypothesized changes in UC, which were not detected in our study, would be a predictor of LBW, IUGR, and small for gestational age, which are expected in maternal anemia. Several maternal, placental, and fetal physiological adaptations have been observed in maternal anemia, allowing easier oxygen unloading to the placenta and increased oxygen transfer to the fetus.[36] The development of UC depends on hemodynamic factors, such as blood flow rate, oxidative stress, and oxygen tension.[37] The placentas of anemic mothers have been shown to express an increased number of angiogenic factors, including proteins, which is probably an adaptive response leading to changes in placental vessels.[38]

Limitations

This study was limited by the omission of umbilical cord blood assessment, which could have provided further insight into the fetal impact of maternal anemia. In addition, severe anemia was not included, placental changes were not assessed, and inflammatory and angiogenic factors were not investigated.

Conclusion

The current study showed no difference in UC parameters between women with and without anemia. The nonsevere form of anemia may explain the results of this study. Therefore, further research is required in this regard.

Authors' contributions

Conceptualization: Itedal Ahmed, Ishag Adam

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Writing - review and editing: [Magdi M Salih, Itedal Ahmed, Abdelmageed Elmugabil, Ishag Adam]

Acknowledgments

The authors acknowledge the Deanship of Graduate Studies and Scientific Research, Taif University, for funding this work and all participants who participated in this study.

Take-home message

Histomorphometry study of umbilical cords showed no significant differences in vessel parameters between anemic and non-anemic pregnant women in central Sudan.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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