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Original Research Article

Histomorphometric analysis of Wharton's jelly size in pregnancies with normal and pre-eclamptic conditions, and its relation to baby weight

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ABSTRACT

Background: Multiple factors affect fetal birth weight (FBW) and fetal well-being. This study aims to evaluate how hypertension affects FBW and the area of Wharton's jelly (WJ). It also seeks to explore the protective properties of WJ by analyzing its relationship with FBW in normal and pre-eclamptic (PreE) pregnancies.

Materials and Methods: Eighty umbilical cords (UCs) were analyzed, with forty from normotensive pregnancies and forty from PreE pregnancies. The cross-sectional area of the UC and vessels was measured using a vernier scale and ocular micrometer. The WJ area was calculated by subtracting the vascular area from the UC area, and then compared with FBW.

Results: In this study, the normal group had an average FBW of 2547.25 ± 240.79 grams and WJ area of 35.28 ± 8.42 mm², while the PreE group had an average FBW of 2424.75 ± 234.36 grams and WJ area of 29.04 ± 8.09 mm². Both FBW and WJ area were significantly lower in the PreE group. There was a strong positive correlation between WJ area and FBW in both the normal group ($r = 0.895$, $p < 0.0001$) and the PreE group ($r = 0.850$, $p < 0.0001$).

Conclusion: The findings indicated that both FBW and WJ area were notably lower in the PreE group compared to the normal group. Furthermore, a strong positive correlation between WJ area and FBW was observed in both groups. These results emphasize the importance of WJ in fetal growth and highlight the significance of its quantity in determining birth weight.

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1. Introduction

The umbilical cord (UC) is formed from the connective stalk or body stalk, which is a strip of mesoblastic tissue that extends between the embryonic disc and the chorion. As the embryo develops further, the UC gradually becomes narrower while retaining its tubular structure, acting as the conduit between the developing fetus and the placenta. The UCs structure and function are essential for the well-being of the fetus, as it facilitates the establishment of blood flow between the fetus and the mother through its internal

vessels. Typically, the UC contains two arteries and one vein. These vessels are protected by a specialized type of connective tissue known as Wharton's jelly (WJ), which is formed through the degeneration of mucoid cells in the connecting stalk.¹

WJ is comprised of a spongy network of interwoven collagen fibers that envelop the umbilical vessels, housing myofibroblasts within an extracellular matrix primarily consisting of glycosaminoglycans (GAGs) and proteoglycans. The predominant GAG found in WJ is hyaluronic acid, which forms a gel-like substance around the collagen fibrils, preserving the tissue structure of the UC.^{2,3} Its primary function is to safeguard the umbilical

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blood vessels, although its composition can undergo changes. Alterations in the components of WJ have been noted in conditions such as fetal distress,⁴ maternal hypertension,⁵ and gestational diabetes.⁶ Pre-eclampsia (PreE) is linked to decreased gelatinous activity, insulin-like growth factor-1 binding protein expression and cathepsin D activity, resulting in reduced WJ areas in affected pregnancies.^{7,8}

The objective of this research endeavor was to assess the impact of hypertension on fetal birth weight (FBW) and the area of WJ. Additionally, the study aimed to explore the potential protective effects of WJ by investigating the correlation between the area of WJ and FBW in pregnancies characterized by both normal conditions and PreE.

2. Materials and Methods

The research was conducted at the Department of Anatomy, Gandhi Medical College, Bhopal, Madhya Pradesh, India. A total of eighty UCs were gathered from expectant mothers who gave birth at Hospital, affiliated with the Department of Obstetrics and Gynaecology, G.M.C. Bhopal. Prior to the collection, informed consent was obtained and necessary permissions were granted by the institutional ethics committee. The diagnosis of PreE was established in women exhibiting a systolic BP ≥ 140 mmHg and diastolic BP ≥ 90 mmHg on two or more occasions, with a minimum interval 4 hours. After the 20th week of gestation, in addition to the existence of protein in the urine. Proteinuria was deemed present when the urine dipstick value was at least 1+ (>30 mg/dl) on two separate occasions, with a minimum interval of 6 hours.⁹ Afterwards, the subjects were sorted into two distinct groups. Group I comprised UCs from normal pregnant women ($n=40$) with a gestational age of 37-40 weeks, while Group II included UCs from women with PreE ($n=40$). Patients with essential hypertension, diabetes mellitus, anaemia, renal disorders, and other pregnancy-related illnesses were excluded from the study.

In all instances, samples of the UC were collected at a distance of 2 cm from the placental side. These samples had a thickness of approximately 4-5 mm and were routinely processed for paraffin embedding, sectioning, and H & E staining. To measure the diameters of the blood vessels and the UC, two diameters were taken from each histological section at a right angle using a vernier scale on the microscope stage. The diameters of the blood vessels were measured using an ocular micrometer.

To calculate various areas, the following formulas were used:

1. The area (A) was calculated using the formula $A = D_1 D_2 / 4$, where A represents the area and D represents the diameter.

2. The total vessel area was determined by adding up the total areas of artery 1, artery 2, and the vein in the cross section.
3. The WJ area was calculated by abating the total vessels area from the total UC area in the cross section.

These calculations were performed to obtain important measurements and information about the UC and its blood vessels.

2.1. Standardizations of the methods

All measurements were taken utilizing a 4X magnification of the objective lens. The measurements taken from the vernier scale on the microscope stage provide the actual size of the sample, as they are not influenced by the magnification of the microscope.

On the other hand, measurements taken with the ocular micrometer represent the magnified value of the objective lens. The reticle, which is mounted in the eyepiece, is magnified by the objective lens. For instance, a real particle with a diameter of 1 mm on the microscope stage will appear as a 4 mm diameter on the reticle when viewed with a 4X objective lens. Therefore, these magnifications must be taken into consideration when calculating the actual measurements.

The formula for determining the real size of the sample is as follows: Sample 'Real Size' = Size of sample displayed on reticle divided by the 'Magnification of Objective lens'.

2.2. Statistical analysis

Utilizing Statistical Package for Social Sciences (SPSS) version 15.0 (Chicago, IL), statistical analysis of the data was performed. Mean values $\pm$ standard deviation was used to display continuous variables. Pearson's correlation coefficient was applied to investigate the correlation between the parameters. Student's t-test was employed to assess differences between group parameters, with significance set at $p < 0.05$.

3. Results

Analysis of the demographic and clinical parameters demonstrated that teenage pregnancy and nulliparity were associated with an increased risk of PreE. Moreover, the PreE group exhibited lower maternal age and gestational age in comparison to the normal group. The mean FBW was 2547.25 grams for the normal group and 2424.75 grams for the PreE group, highlighting a significant difference in FBW between the two groups. Histomorphometric analysis of the UC demonstrated that the mean WJ area was 35.28 mm^2 in the normal group and 29.04 mm^2 in the PreE group, with the WJ area being significantly reduced in the PreE group compared to the normal group (Table 1).

A significant and strong positive correlation (Pearson correlation coefficient $r = 0.895$, $p < 0.0001$) was found in the normal group between FBW and WJ area. Analysis of figure 1 reveals a direct relationship where an increase in WJ area corresponds to an increase in FBW. This linear relationship can be mathematically represented by the following equation.

$$y = 25.60(x) + 1643$$

The relationship between the WJ area (x) in mm^2 and the FBW (y) in grams can be estimated using the given values. The slope of the regression line is estimated to be $+25.60 \text{ gm}/\text{mm}^2$, indicating that for every 10 mm^2 increase in WJ area in the normal group, there is an average increase in FBW of 256 gm .

In the group of individuals with PreE, a strong significant positive correlation ($r = 0.850$, $p < 0.0001$) was observed between FBW and WJ area. The relationship between FBW and WJ area in the PreE group [figure-2] can be represented by the following equation:

$$y = 24.62(x) + 1709$$

where x represents the WJ area in square millimeters and y represents the FBW in grams. The estimated slope of the regression line is $+24.62 \text{ gm}/\text{mm}^2$, while the estimated intercept is $+1709 \text{ gm}$. The interpretation of the slope indicates that, on average, a 10 mm^2 increase in WJ area in the PreE group is associated with a 246.2 gm increase in FBW. It is worth noting that the test for linearity of the relationship between FBW and WJ area is statistically significant in both the normal and PreE groups.

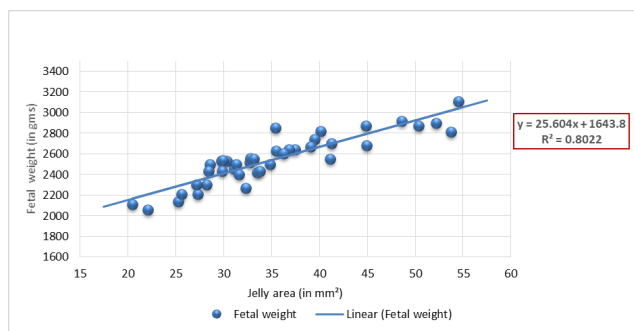


Figure 1: Correlation between fetal birth weight and jelly area in normal pregnancy

4. Discussion

The UC is essential for linking the fetus to the placenta, enduring the same pressures and challenges as the fetus in the womb. When the mother and fetus are affected by a disease, it also significantly impacts the UC.¹⁰ Complex metabolic diseases can diminish the efficiency and functionality of the placenta. One example is PreE, where the feto-placental unit may face adverse effects due to placental insufficiency, leading to hindered fetal growth.¹¹



Figure 2: Correlation between fetal birth weight and jelly area in pre-eclampsia pregnancy

This study observed significantly lower mean FBW in the PreE group [Table-1], aligning with the research done by Nakimuli A et al¹² and Blanco MV et al.¹³

WJ, a substance with a gel-like consistency that encases the vessels of the UC, is essential for shielding them against different types of forces like stretching, bending, twisting, and compression. Despite having a low cell count, this tissue produces a significant amount of extracellular matrix, including collagen, hyaluronate, and proteoglycans, which bind peptide growth factors.¹⁴ The high hyaluronate content ensures excellent hydration, while the abundant collagen content provides resistance against the movements and contractions experienced during pregnancy. However, in cases of PreE, there is a notable decrease in hyaluronate and an increase in sulphated GAGs/proteoglycans content in WJ, leading to reduced hydration.¹⁵ This decline in WJ size, as observed in the current study (Table 1), aligns with previous research findings.^{5,16} A thin UC, resulting from a deficiency of WJ, increases the vulnerability of the cord vessels to compression, potentially contributing to intrauterine growth restriction and abnormal fetal heart rate tracings during labor.^{17,18} The loss of protection provided by WJ can ultimately compromise fetoplacental circulation and lead to fetal death.¹⁹

WJ, during the second and third trimester of pregnancy, serves as the primary constituent of the UC. According to Barbieri C et al.'s research, the size of WJ progressively expands until the 32nd week of gestation, after which it remains stable until the conclusion of pregnancy.²⁰ Likewise, Ghezzi et al. documented a significant correlation between the WJ area and fetal biometric measurements before reaching 32 weeks of gestation. However, once the gestational age surpassed 32 weeks, no associations were detected between the WJ area and fetal anthropometric parameters.²¹ Our study highlights a robust positive correlation between the area of WJ and FBW in normotensive and PreE pregnancies, as depicted in figures 1 and 2. The research implies that a larger WJ area is associated with increased FBW, supporting findings from

Table 1: Relationship of various parameters between normal and pre-eclamptic pregnancies

S. No.	Parameters	Normal (n=40)	Pre-eclamptic (n=40)	P value ^a
1.	Fetal birth weight (grams)*	2547.25 ± 240.79	2424.75 ± 234.36	0.032 ^b
2.	Wharton's jelly area (mm ²)*	35.28 ± 8.42	29.04 ± 8.09	0.0039 ^b

*Parameters were expressed as mean value ± standard deviation; a. p-values were calculated by using Student's t- test. p ≤ 0.05 was considered significant; b. statistically significant.

earlier studies.^{22,23} In a study by Debebe SK et al., it was revealed that the WJ area had a significant association with the estimated fetal weight centile at the time of the ultrasound scan, as well as a positive correlation with placental length, width, and surface area. A decrease in WJ area was found to be significantly related to placental pathology, particularly maternal-vascular malperfusion, which often resulted in anoxia and fetal demise.²⁴

5. Limitations of the Study

One possible constraint of our research is that the measurement of WJ area was conducted at a single gestational age postpartum for each participant. Implementing a longitudinal study approach could have bolstered the study's statistical strength and facilitated exploration of the relationship between the rate of WJ area change throughout gestation and placental pathology.

6. Conclusion

An important finding from the current study highlights the significant decrease in FBW and WJ area in the PreE group. Furthermore, there was a notable correlation between the WJ area and FBW in both groups. WJ plays a crucial role in safeguarding the fetal blood vessels against external influences by providing a cushion-like protection. With an increase in this protective layer, the resistance within the vessels decreases, leading to improved blood flow and ultimately benefiting fetal nutrition and growth. Consequently, an increase in the amount of WJ should correspondingly result in an increase in FBW. Our research findings support this phenomenon and suggest that the occurrence of intrauterine growth restriction and fetal distress in PreE cases may be partially attributed to a reduced amount of WJ. Therefore, understanding this correlation can greatly aid in the early assessment of WJ quantity and the overall well-being of the fetus.

7. Abbreviations

UC: Umbilical cord; WJ:- Wharton's jelly; GAG: Glycosaminoglycan; PreE: Pre-eclampsia; FBW: Fetal birth weight

8. Author's Contribution

RJ: Study design, Interpreted the results, prepared first draft of manuscript; **AJ:** Concept and design of the study, data

collection, statistical analysis and interpretation of data, finalisation of the draft manuscript; **AC:** Interpreted the results, reviewed the literature and manuscript preparation.

9. Source of Funding

None.

10. Conflicting Interest

None.

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