EVALUATION OF SOME CHARACTERISTICS OF PLATELET-RICH PLASMA FROM UMBILICAL CORD BLOOD

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SUMMARY

The study aims to evaluate some characteristics of platelet-rich plasma (PRP) from umbilical cord blood.

Research on 6 umbilical cord blood samples compared with 6 donor venous blood samples, using the GeneWorld kit. The results show that PRP from umbilical cord blood had fewer red blood cells (0.105 (0.07 - 0.17) T/L, 0.32 (0.12 - 0.55), p < 0.01), higher platelet count (701.17 ± 124.75 G/L vs. 457.83 ± 88.95 G/L, p < 0.01) and higher platelet count concentration ratio (2.3 vs. 1.82; p < 0.05). The concentration of EGF in umbilical cord blood plasma was 85.478 ± 28.195 pg/ml, that of healthy donors was 74.973 ± 29.475 (p > 0.05) with VEGF concentrations of 88,090 (67,536 - 135.32) and 10,504 (4,094 - 16,029) pg/ml, respectively, p < 0.05. The EGF concentration in activated PRP from umbilical cord blood and adult blood was 115.72 (50.98 -178.96) pg/mL and 94.91 (50.92 - 159.28) pg/ml (p < 0.05), with VEGF being 281.48 (141.59 - 324.12) pg/mL and 32.6 (11.7 - 53.4) pg/ml, respectively, p < 0.01.

In conclusion, umbilical cord blood had normal hematological indices. PRP from umbilical cord blood had fewer red blood cells, higher platelet counts, platelet aggregation ratio, and VEGF concentration than PRP from adult blood. The VEGF concentration in activated PRP from umbilical cord blood was higher than that in adult blood.

Keywords: *Characteristics, platelet-rich plasma, umbilical cord blood*

1. PROBLEM STATEMENT

Platelet-rich plasma (PRP) is a plasma concentrated with a platelet content many times higher than normal. Activated platelets will produce growth factors that stimulate the wound healing stages. Autologous PRP has been used to treat difficult-to-heal wounds, ensuring many advantages but encountering some difficulties. The source from umbilic al cord blood has a lot of potential, is an abundant blood source, creating a readily available

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PRP product, ensuring economic and medical ethics, especially being able to bring a good quality product for wound treatment, with no safety risks for donors, an easily accessible source, a very low risk of infectious diseases and low immunogenicity [1 - 2].

In the world, stored umbilical cord blood has been applied for autologous transfusion to patients with blood diseases. Umbilical cord blood is applied in the form of allogeneic for cases of patients with neurological diseases, lung diseases, systemic skin diseases, etc. In Vietnam, the application of umbilical cord blood is mainly to store stem cells to treat blood diseases or to store stem cell banks to reserve for autologous treatment in the future. To continue to have stronger evidence in the application of PRP umbilical cord blood in clinical wound treatment, we conducted this study to *evaluate some characteristics of umbilical cord blood and platelet-rich plasma from umbilical cord blood*.

2. RESEARCH OBJECTS AND METHODS

2.1. Research subjects healthy adult donors

Selection criteria: Pregnant women and voluntary blood donors with no accompanying diseases: congenital diseases, tuberculosis, cancer, autoimmune diseases, mental illness, screened for HIV, HBV, HCV with negative results, age 20 - 45; pregnant women have no history of obstetric diseases, no obstetric complications; No fever during labor (< 38^0C). Gestational age from more than 36 weeks; Born within 24 hours from the time the membranes break; Newborn weight ≥ 2800g.

Exclusions: 1) Violation of one or more of the above criteria; 2) Postpartum: Multiple pregnancies (for collection for community cord blood banks); Infants with congenital malformations at birth; Umbilical cords that are too short, severed, crushed, or damaged; Placenta suspected of infection at collection (collection after delivery).

2.2. Raw materials and equipment

New-PRP Pro kit PRP separation kit.

K4500 hematology analyzer with 18 parameters manufactured by Sysmex (Japan). Blood biochemistry analyzer (AU480. Centrifuge. Freezer -80^0C. Sample storage refrigerator: from 2 to 8^0C.

2.3. Research location

Umbilical cord blood was collected at the Department of Obstetrics and Gynecology - Military Hospital 103. Blood was collected from healthy adults, PRP extraction was performed, hematology, biochemistry, and bacterial culture were performed at the Department of Paraclinical Medicine - Le Huu Trac National Burn Hospital.

EGF and VEGF tests were performed at the Military Medical Research Institute/Military Medical Academy. The research period was from May 2023 to September 2024.

2.4. Method of implementation

The umbilical cord was immediately disinfected after birth, using a sterile 10ml syringe to collect blood into a test tube in the kit. Peripheral blood from a volunteer donor was collected using a negative-pressure needle inserted directly into the test tube of the kit. PRP was separated according to the kit's procedure. A portion

of whole blood collected in the same process and a portion of PRP after activation were tested, evaluated and compared.

PRP collection process (according to GeneWorld's instructions):

- Phase 1 (collecting unactivated PRP): Collect venous/umbilical cord blood into a test tube, each tube with a total of 10 ml (including 1.5 ml of available anticoagulant). Centrifuge the first blood tube at 2,000 rpm x 10 minutes to separate plasma, red blood cells and platelets. Gently aspirate the yellow Plasma layer on top into a centrifuge tube labeled PLASMA (total volume recovered from 3 tubes is about 12 - 14 ml). Centrifuge the PLASMA tube a second time at 3,500 rpm x 5 minutes, to obtain a 2-layered solution. Gently aspirate the upper part and discard, leaving 6ml of PRP at the bottom of the tube. This is an unactivated PRP.

- Phase 2 (activate PRP with $CaCl_2$): Continue to draw all remaining 6 ml of PRP into the tube containing the $CaCl_2$ activator, add and mix well for 2 - 5 minutes until a solid mass appears. Use a pipette to gently rotate until the solid mass separates from the tube wall. Wait until the solid mass shrinks completely (5 - 15 minutes), then draw out the solid mass. The final product contains 4 - 5 ml of activated PRP solution with a transparent yellow color.

2.5. Research indicators

Epidemiological and clinical characteristics, blood test indexes of pregnant women and voluntary blood donors; fetal age, infant weight, placental weight, umbilical cord length.

Characteristics and test indexes of EGF, VEGF of PRP from umbilical cord blood and peripheral blood of volunteer donors: Hematological indexes such as red blood cell count, white blood cell count, platelet count, hemoglobin level, hematocrit index; biochemical indexes such as quantification of ALT, AST, Protein, Albumin, Cholesterol; immunohistochemistry: quantification of Epidermal growth factor (EGF) and Vascular Endothelial Growth Factor (VEGF) of plasma and activated platelet-rich plasma; microorganisms.

The data obtained are calculated as the average in the form of X ± SD, compared by the T-test or Q2 (Q1 - Q3) algorithm, compared by the Mann-Whitney U-test. Use SPSS 22.0 software to analyze the data, compare the differences with statistical significance when $p < 0.05$.

3. RESULTS

Table 3.1. Some characteristics of pregnant women and volunteer donors

Characteristic	Maternity (n = 6)	Volunteer (n = 6)
Year Old	31.83 ± 5.38	30.00 ± 5.22
Height (cm)	157.83 ± 7.55	164.00 ± 5.44
Weight (kg)	63.67 ± 10.41	65.15 ± 7.10
Systolic blood pressure (mmHg)	120.00 ± 10.95	114.67 ± 10.17
Diastolic blood pressure (mmHg)	74.50 ± 7.31	67.83 ± 9.81
Body temperature (degrees C)	36.68 ± 0.13	36.65 ± 0.43

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Pregnant women and voluntary blood donors must be healthy, of average physical condition, and free of infectious diseases.

Table 3.2. Some characteristics of the fetus and umbilical cord

Characteristic	Average index
Gestational age (weeks)	38.50 ± 1.22
Child weight (grams)	3155.00 ± 356.41
Placenta weight (grams)	553.17 ± 84.12
Umbilical cord length (cm)	50.00 ± 8.51

Table 3.3. Some characteristics of umbilical cord blood compared with blood from volunteer donors

Characteristic	Umbilical cord (n = 6)	Blood donor (n = 6)	p
Red blood cells (T/L)	4.64 ± 0.31	5.22 ± 0.63	< 0.05
Hemoglobin (g/L)	144.33 ±11.99	154.33 ±15.03	> 0.05
Hematocrit (%)	43.48 ± 2.92	45.63 ± 4.26	> 0.05
White blood cells (G/L)	16.25 ± 7.26	6.98 ± 1.16	0.02
Platelets (G/L)	302.50 ± 60.95	248.50 ± 56.31	> 0.05
Glucose (mmol/L)	4.95 (3.4 - 9.2)	5.5 (4.9 - 5.9)	> 0.05*
Urea (mmol/L)	3.28 ± 0.62	5.30 ± 2.75	> 0.05
Creatinine (µmol/L)	66.67 ± 11.80	79.75 ± 13.59	> 0.05
AST (U/L)	29.40 ± 9.40	20.05 (19.00 - 29.40)	> 0.05*
ALT (U/L)	43.7 (7.1 - 45.3)	25.55 (16.10 - 42.20)	> 0.05*
Protein (g/L)	52.92 ± 3.58	78.40 ± 2.93	< 0.001
Albumin (g/L)	33.78 ± 1.49	45.93 ± 2.32	< 0.001
Cholesterol	1.40 ± 0.45	5.22 ± 0.62	< 0.001

** Mann Whitney U test*

Umbilical cord blood has a high white blood cell count, higher than adults ($p < 0.05$), red blood cell, hemoglobin, platelet within limits, not statistically different from adults ($p > 0.05$). Umbilical cord blood has lower protein, albumin, cholesterol than adults ($p < 0.05$).

Table 3.4. Characteristics of platelet-rich plasma

Characteristic	Cord blood source (n = 6)	Blood from healthy donors (n = 6)	p
Plasma volume (ml) after first centrifugation	14.50 ± 1.18	15.92 ± 0.58	< 0.05
White blood cell count (G/L)	8.40 ± 4.76	5.27 ± 2.80	> 0.05
Red blood cell count (T/L)	0.105 (0.07 - 0.17)	0.32 (0.12 - 0.55)	< 0.05*
Platelet count (G/L)	701.17 ± 124.75	457.83 ± 88.95	< 0.01
PRP Platelet/Whole Blood Ratio	2.3 (1.95 - 2.59)	1.82 (1.7 - 1.94)	< 0.05*
Pre-activation PRP culture (T0)	Negative	Negative	
PRP culture after activation (T1)	Negative	Negative	
Color	100% pure gold	100% pure gold	

** Mann-Whitney U test*

Table 3.5. Concentrations of growth factors EGF and VEGF in blood and PRP after activation from umbilical cord blood compared with adults

EGF, VEGF concentrations	Cord blood (n = 6)	Adults (n = 6)	p
EGF (pg/ml) plasma	85.478 ± 28.195	74.973 ± 29.475	> 0.05
VEGF (pg/ml) plasma	88.090 (67.536 - 135.32)	10.504 (4.094 - 16.029)	< 0.01*
EGF (pg/ml) PRP after activation	115.72 (50.98 - 178.96)	94.91 (50.92 - 159.28)	> 0.05*
VEGF (pg/ml) PRP after activation	281.48 (141.59 - 324.12)	32.6 (11.7 - 53.4)	< 0.01*

** Mann-Whitney U test*

The concentrations of EGF in plasma and activated PRP from umbilical cord blood were higher than those from healthy human blood, with no statistical significance ($p > 0.05$). The concentrations of VEGF in plasma and activated PRP from umbilical cord blood were significantly higher than those from plasma and activated PRP from healthy human blood, with statistical significance ($p < 0.01$).

4. DISCUSSION

4.1. Characteristics of umbilical cord blood

The umbilical cord contains three blood vessels, one vein and two arteries, which coil around the vein in a spiral configuration. The umbilical vein supplies the fetus with oxygen-rich blood and nutrients. The arteries return deoxygenated

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blood to the placenta. The umbilical vein is large and easy to see. When taking umbilical cord blood, blood is taken from the vein, which ensures easy collection and quality blood, rich in nutrients transported from the placenta to the fetus.

The fetus in our study also had the following indicators within the limits: gestational age 38.50 ± 1.22 weeks, weight 3155 ± 356.41 grams, placental mass 553.17 ± 84.12 grams, umbilical cord length 50.00 ± 8.51 cm. This result is similar to the research results of Dang Thi Thu Hang with a gestational age 39.3 ± 0.9 weeks, fetus weight 3.257 ± 394 g, placental weight 505 ± 41.4 g, umbilical cord length 58.1 ± 5.8 cm. The hematological and biochemical indices of umbilical cord blood were similar to those in the study of Dang Thi Thu Hang with blood samples collected from the community with white blood cells 13.5 ± 4.0 G/L, hemoglobin 154.3 ± 14.0 g/L, hematocrit 48.2 ± 4.5 L/L, platelets 303 ± 56.6 G/L [3]. Thus, the report also found that the hematological indices of normal umbilical cord blood, platelets in umbilical cord blood were higher than in pregnant women ($p < 0.05$) and higher than in healthy women ($p > 0.05$), promising better quality PRP.

Studies have shown that autologous PRP has wide clinical applications. However, it may also contain inflammatory cytokines and its quality depends on many patient-related factors such as platelet quantity and quality, concomitant therapies, and patient age [4]. Studies have shown that umbilical cord PRP contains more growth factors and anti-inflammatory molecules than peripheral blood-derived PRP [5].

In this study, a GenWould V10 kit was used to extract PRP from 30 ml of anticoagulated blood (1.5 ml of ACD-acid anticoagulant*).* Citrate dextrose in each tube, a total of 14.50 ± 1.18 ml of plasma from umbilical cord blood was lower than that of adults at 15.92 ± 0.58 ml ($p < 0.01$). Although PRP was extracted using the same kit and procedure, the amount of red blood cells and platelets was lower and the platelet count was higher in PRP from umbilical cord blood than in adult blood, and the platelet and thrombocytosis ratio of PRP from umbilical cord blood were higher than in adult blood (701.38 ± 124.75 vs. 457.83 ± 88.95 and 2.3 (1.95 - 2.59) vs. 1.82 (1.7 - 1.94) with $p < 0.01$), from which we can believe that the quality of PRP from umbilical cord blood is better as published observations [4 - 5].

4.2. Growth factors EGF and VEGF

Epidermal growth factor (EGF) is secreted by fibroblasts, platelets, and macrophages and is present throughout the epidermis, especially in the basal layer. EGF facilitates re-epithelialization by stimulating keratinocyte proliferation and migration, enhancing the tensile strength of new skin. In chronic wounds, a decrease in growth factors, including EGF, affects the wound-healing process [10].

Vascular Endothelial Growth Factor (VEGF) is produced not only by platelets but also by many types of cells such as endothelial cells, fibroblasts, macrophages, smooth muscle cells, and neutrophils. VEGF participates in the wound healing process with a major role in promoting neovascularization and enhancing capillary permeability [6], [11].

In normal wounds, VEGF increases starting from day 1, increases significantly

from 3 - 5 days, and returns from day 7 to day 14 after injury. In chronic wounds, VEGF decreases abnormally [6]. In chronic wounds, impaired angiogenesis leads to more tissue damage due to chronic hypoxia and compromised micronutrient supply. This phenomenon leads to a decrease in many cytokines, chemokines, and growth factors necessary for wound healing, including VEGF [10].

The use of exogenous VEGF in the treatment of chronic wounds has been mentioned in many studies. VEGF has a high concentration in PRP, which is a good source of VEGF in the treatment of chronic wounds [6], [11].

High concentrations of EGF and VEGF in PRP, when applied, will have a better effect on the wound healing process. Studies also show that when using EGF or VEGF alone at different concentrations, has a good effect on wound healing [6].

In our study, the concentration of EGF in umbilical cord blood plasma and adult blood was 85,479 ± 28,196 pg/ml and 74,974 ± 29,477 pg/ml, not significantly different ($p > 0.05$), the concentration of VEGF in umbilical cord blood was 88,090 (67,536 - 135,32) pg/ml, higher than in adult blood 10,504 (4,094 - 16,029) pg/ml ($p < 0.05$). With umbilical cord blood, PRP had a platelet to unconcentrated blood ratio of 2.3, higher than that of adult blood, which was 1.82. The EGF concentration in activated umbilical cord blood PRP and activated adult blood PRP was 115.72 (50.98 - 178.96) pg/ml and 94.91 (50.92 - 159.28) pg/ml, the difference was not significant with $p > 0.05$, the corresponding VEGF concentration was 281.48 (141.59 - 324.12) pg/ml and 32.6 (11.7 - 53.4) pg/ml ($p < 0.01$). This result of EGF is consistent with the studies of Fréchette et al., by his concentration, he obtained PRP with a platelet concentration ratio of 5.5 times, the concentration of EGF in PRP after activation was 470 ± 130 pg/ml [7].

The concentration of EGF in activated PRP is many times higher than in plasma. Using PRP in wound treatment, the concentration of EGF at the wound site will be compensated and increased compared to the deficient concentration. The concentration of VEGF in umbilical cord blood PRP is much higher than that in PRP from adult blood ($p < 0.01$). On the other hand, we see that this concentration in PRP is much higher than in whole blood. Studies have shown that when treating chronic wounds by injecting PRP at the wound site, the increased concentration of EGF and VEGF in the wound tissue has a good biological effect on the wound-healing process.

Our results are similar to the results of the study by Ju Tian et al. (2023) when comparing growth factors of umbilical cord blood with growth factors from the blood of adult females in 3 age groups (18 - 25, 26 - 45, 46 - 65) and found that VEGF from umbilical cord blood was higher and EGF in the 18 - 25 age group was higher than the other groups [8]. The study by ME Rhéaume et al. showed that EGF and VEGF in umbilical cord blood serum had higher concentrations than in adult blood. He also showed that EGF in umbilical cord blood serum was higher than in activated serum [9]. Thus, the concentrations of EGF and VEGF in umbilical cord blood were higher than in adult blood, VEGF in activated umbilical cord blood PRP was higher than in activated adult blood PRP.

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5. CONCLUSION

Umbilical cord blood had normal hematological parameters. Platelet-rich plasma from umbilical cord blood had fewer red blood cells, higher platelet counts, and higher platelet aggregation ratios than platelet-rich plasma from adult blood. VEGF concentrations were higher in umbilical cord blood than in adult blood. VEGF concentrations in activated platelet-rich plasma from umbilical cord blood were also higher than in adult blood.

PROPOSAL

Continue to evaluate the effects of PRP from umbilical cord blood experimentally and clinically for application in the treatment of wounds and burns.

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