RESEARCH ARTICLE

Cord and peripheral blood mononuclear cell analysis by scanning electron microscopy and flow cytometry

Sayak Manna[1], Tapan K Naskar[2], Sujata Law [1,3*]

ABSTRACT

Background: Umbilical cord blood is a rich source of hematopoietic cells, which vary in phenotypic expressions from that of adult peripheral blood. Our study focused on differences in fresh and fixed cell populations isolated from umbilical cord and adult peripheral blood using flow cytometric analysis and high-resolution scanning electron microscopy (SEM). *Methods:* Adult peripheral blood (PB) and cord blood (CB) was collected from individuals from Kolkata. Our study emphasised on the differences in fresh and fixed cord and peripheral blood samples through forward and side scattering of flow cytometer and without any surface antigens. We also studied the morphological variations of both the cord and peripheral MNC using SEM images. *Results:* The flow cytometric analysis of whole cord blood and isolated cord MNC showed significant differences in counts when compared to adults in both fresh and fixed samples. Our SEM images indicated similar morphological features in cord leucocytes when compared to adult leucocytes. It was also observed presence of reticulocytes in SEM analysis only in cord MNC as compared to adult. *Conclusion:* Our study demonstrates the effectiveness of flow cytometry for analysing cellular populations in cord and adult peripheral blood, without requiring antigen markers. Additionally, our SEM analysis provides some insights on the morphology of mononuclear cells from CB and PB sources, enhancing our understanding of normal cellular dynamics in the regenerative medicine domain.

Keywords: Cord blood, Peripheral blood, Mononuclear cells, Scanning electron microscopy, Flow cytometry.

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INTRODUCTION

Human umbilical cord blood is rich in hematopoietic cells. Its usage has gained interest in the medical and research field, after the discovery of its effective use in stem cell transplantation[1,2] amongst adult and young recipients. There is an advancement in the rapid use of cord blood for the treatment of several hematopathological conditions and disorders like Fanconi's anaemia,[3] severe combined immune deficiency (SCID),[4] Krabbe's disease,[5] etc. Since cord blood (CB) has been in use as an alternate source of blood transplantation[6] for almost more than two decades now, having a full picture of the cord blood cellular population is of utmost importance.

In the past much work has been done on the characterisation of cord blood nucleated cells using surface antigens[7,8] but the purpose of our study was to establish differences in values of normal cord blood as well as adult peripheral blood nucleated cells in fresh and fixed conditions using flow cytometry, without any use of antibody markers. Studying nucleated cell populations using forward and side scatter was done to ensure cost-effective cell analysis. Focus was given on population changes, cell count, and distribution pattern post-fixation. Flow cytometry measures and analyses single-cell suspensions' optical properties as they pass through a laser beam following hydrodynamic focussing. Forward scatter indicates cell size, while side scatter reflects cell complexity and granularity.

Previous studies revealed the ultrastructure of adult peripheral blood using high-resolution scanning electron microscopy (SEM).[9,10] SEM analysis was also done in case of diseased conditions like leukaemia and other erythrocyte disorders in peripheral blood.[11] In 2002, a study was conducted to understand the ultrastructure of primary and cultured leucocytes obtained from cord blood, but the work focussed on the use of transmission electron microscopy (TEM) to analyse cord blood cells.[12] Building on our previous study, which analysed the differences in erythrocyte populations between cord blood (CB) and adult peripheral blood (PB),[13] we now tried to focus on examining the leukocyte populations in these blood sources. As little to no study exists on the three-dimensional structural analysis of cord mononuclear cells (MNC) using SEM, we aimed to draw a difference in the cell morphological structure of both cord blood and adult peripheral blood leucocytes using scanning electron microscopy.

[1]Department of Biochemistry and Medical Biotechnology, Stem Cell Research and Application Unit, Calcutta School of Tropical Medicine. 108, C.R. Avenue, Kolkata, West Bengal, India.

[2]Department of Obstetrics and Gynaecology, Medical College Kolkata, 88, College St, College Square, Kolkata, West Bengal, India.

[3]Department of Biotechnology, Brainware University, 398, Ramkrishnapur Road, Kolkata, West Bengal, India.

***Corresponding author:** Sujata Law, Department of Biotechnology, Brainware University, 398, Ramkrishnapur Road, Kolkata, West Bengal, India., Email: msuj2002@yahoo.co.in

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In this study, we compared the cellular components of cord blood (CB) and peripheral blood (PB) in both fresh and fixed states, alongside a detailed morphological examination using high resolution scanning electron microscopy. Understanding the differences in cell percentages by flowcytometry and their structural variations by SEM can be crucial for translational research. The purpose of the study was to gain knowledge that may help us grasp the cellular dynamics and potential clinical uses of CB and PB, aiding in the development of better treatments and improving the effectiveness of regenerative medicine and hematopoietic therapies.

MATERIALS AND METHODS

Procurement of Adult Peripheral Blood and Cord Blood

Peripheral blood was collected from consenting adult women, primarily postgraduate students or healthy individuals undergoing routine check-ups at Eden Hospital, Medical College, with an average age of 23.5 years. Approximately 10 mL of blood was drawn by venipuncture into sterile EDTA vials. Umbilical cord blood was collected from consenting mothers who delivered full-term newborns (average maternal age: 26.1 years; gestation period: 38–40 weeks) at the same hospital. Around 30 to 35 mL of cord blood was collected in sterile heparinized Falcon tubes (Tarson, India) immediately after delivery. The study followed ethical guidelines and received approval from the Clinical Research Ethics Committee (CREC) of Calcutta School of Tropical Medicine and Medical College, Kolkata (Ethical Approval Number: CREC-STM/52). Participants with hypertension, diabetes, hepatitis, respiratory issues, blood infections, genitourinary diseases, eclampsia, or a history of miscarriages were excluded. Blood samples were promptly transported to the Department of Biochemistry and Medical Biotechnology Laboratory, School of Tropical Medicine, within 10 to 15 minutes in 4 to 8°C ice containers. Written informed consent was obtained from all participants.[14]

Isolation of Mononuclear Cells (MNC)

The isolation of mononuclear cells (MNC) from both peripheral and cord blood was done using density-gradient centrifugation within 30 minutes of procurement. The centrifugation of blood samples was conducted in a sterile environment within a laminar air flow hood. Blood samples stored at 4°C were diluted with an equal volume of cold, sterile 0.9% sodium chloride solution (NaCl) or 1X phosphate buffered saline solution (PBS, pH 7.4). Each sample (5 mL) was layered over 3 ml of HiSep LSM 1077 solution (Himedia) at room temperature in a 15 mL centrifuge tube. Care was taken to avoid mixing the HiSep solution with the diluted blood. The tubes were then centrifuged at 500 g for 25 minutes in a REMI (R8C) centrifuge machine.[15]

Post centrifugation, the MNC layer was carefully isolated from the plasma and HiSep interface. The MNC layers from both peripheral and cord blood were washed twice with chilled 1X PBS by centrifugation at 200g for 5 minutes. The supernatants were discarded, and the MNC pellets were resuspended in FACS fluid (Becton and Dickinson) for further analysis.

Flow Cytometry Analysis

Flow cytometric analysis was performed on fresh (Fr) and fixed (Fx) samples from peripheral blood (PB) and cord blood (CB). MNCs and whole blood (WB) were fixed in 1.5% paraformaldehyde (PFA) for 30 minutes in darkness, washed with 0.9% NaCl, and resuspended in sheath fluid at $1x10^6$ cells/mL. Fresh samples were similarly prepared without fixation. Forward scatter (FSC) and side scatter (SSC) of PB and CB samples were analysed using a Beckton and Dickenson FACS Calibur with Cell Quest Pro software.

Gating of blood cellular components was done to compare the differences in adult peripheral blood cells and umbilical cord blood cells in both fresh (Fr) and fixed (Fx) conditions. The gating strategies focused on grouping cellular components, highlighting the percentages of lymphocytes, monocytes, and granulocytes in corresponding positions on dot plots for both PB and CB samples. The analysis considered the percentage of cells, mean values, and variations in cellular distribution patterns when comparing different haematological parameters between fresh and fixed samples of PB and CB. The SSC and FSC settings were done with the logarithmic amplification scale for the dot plot analysis. The dot plots were done for WCB (Fr) lymphocytes and WPB (Fr) lymphocytes; WCB (Fr) monocytes and WPB (Fr) monocytes (Figure 1A) and Table 1A; CB MNC (Fr) and PB MNC (Fr) (Figure 1B) and Table 1B; CB MNC (Fr) Quadrant and PB MNC (Fr) Quadrant (Figure 1C) and Table 1C; CB MNC (Fx) lymphocytes and PB MNC (Fx) lymphocytes; CB MNC (Fx) monocytes and PB MNC (Fx) monocytes (Figure 1D) and Table 1D; CB Buffy (Fr) lymphocytes and CB Buffy (Fx) lymphocytes; CB Buffy (Fr) monocytes and CB Buffy (Fx) monocytes (Figure 1E) and Table 1E; WCB (Fr) lymphocytes and WCB (Fx) lymphocyte; WCB (Fr) monocytes and WCB (Fx) monocytes (Figure 1F) and Table 1F and quadrant of CB MNC (Fr) and CB MNC (Fx) (Figure 1G) and Table 1G.

Morphological Studies of MNC Under High Resolution SEM

The isolated mononuclear cells from adult and cord blood were fixed with 2.5% glutaraldehyde in PBS for 1 hour. The samples were again washed with PBS for 5 minutes × three times followed by serial dehydration, with 30, 50, 70, 90% and three times with 100% ethanol. The cells were dried, mounted, and coated with platinum, and observed under a scanning electron microscope (Zeiss EVO18 special edition SEM, Jena, Germany). The micrographs were taken at 15 kV accelerating voltage.

Statistical Analysis

Flow cytometric comparison of CB and PB cellular components in both fresh (Fr) and fixed (Fx) samples was performed using Independent Student's t-test with normal distribution of different variables in SPSS version 16. Statistical significance was considered at $p < 0.01$.

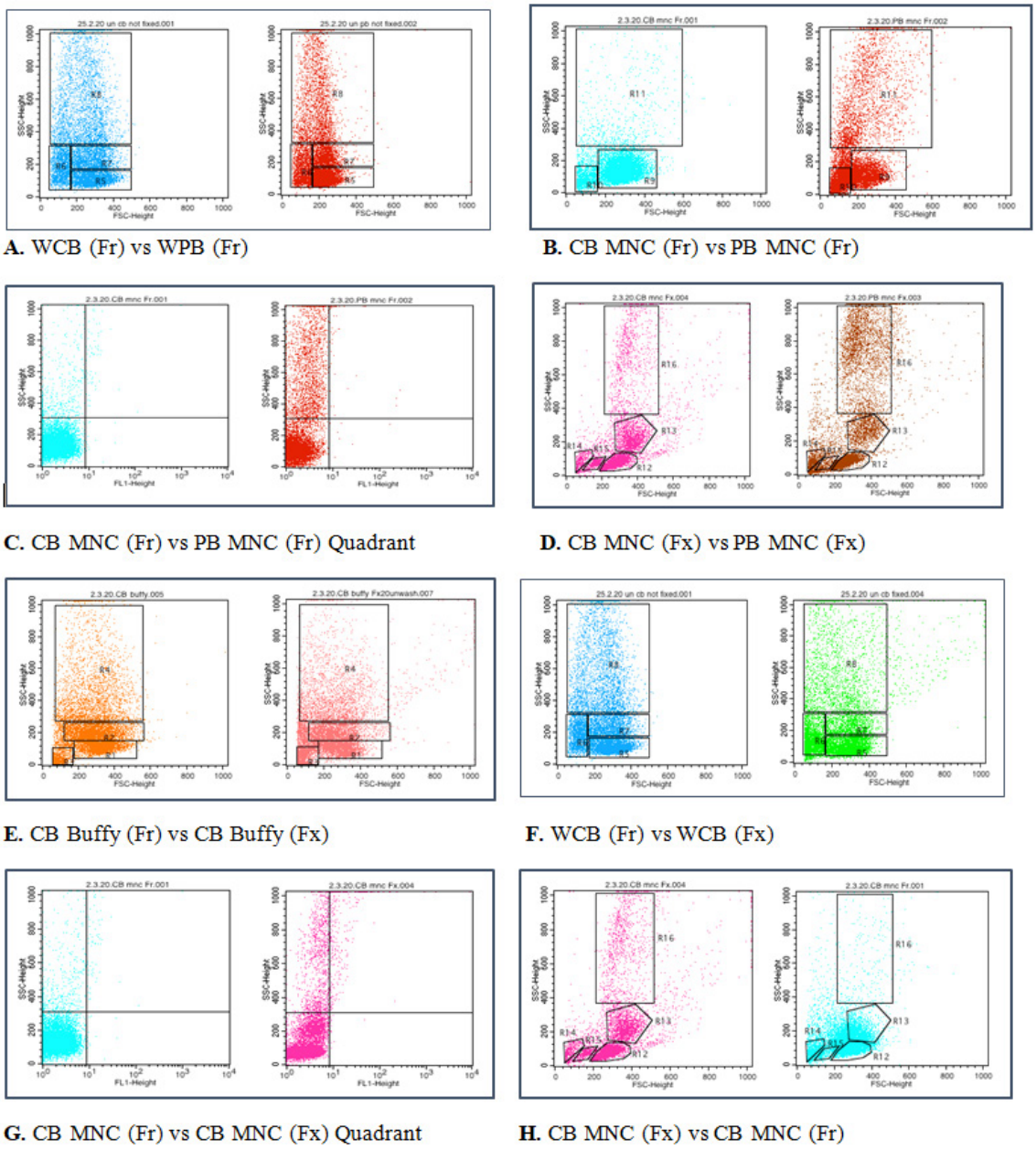

Figure 1: Flowcytometric dot plot analysis of cord and adult peripheral blood cells in fresh and fixed states A. Forward scatter (FSC) and Side scatter (SSC) of fresh whole cord blood cells (WCB Fr) and fresh whole peripheral blood cells (WPB Fr); B. FSC and SSC of fresh cord blood mononuclear (MNC) cells (CB MNC Fr) and fresh peripheral blood MNC (PB MNC Fr); C. Quadrant graph of fresh cord blood mononuclear (MNC) cells (CB MNC Fr Q) and fresh peripheral whole blood MNC (PB MNC Fr Q); D. FSC and SSC of fixed cord blood MNC cells (CB MNC Fx) and fixed peripheral blood MNC (PB MNC Fx); E. FSC and SSC of cord blood buffy coat cells fresh (CB Buffy Fr) and fixed (CB Buffy Fx); F. FSC and SSC of fresh whole cord blood cells (WCB Fr) and fixed whole cord blood (WCB Fx); G. Quadrant graph of fresh cord blood MNC (CB MNC Fr) and fixed cord blood MNC (CB MNC Fx); H. FSC and SSC of fixed cord blood MNC (CB MNC Fx) and fresh cord blood MNC (CB MNC Fr)

RESULTS

Flowcytometric Analysis

The analysis of cellular components from cord blood (CB) and peripheral blood (PB) revealed significant variations in their fresh (Fr) and fixed (Fx) states. In fresh whole cord blood (WCB (Fr)) the lymphocyte population (32.4 ± 0.7) was 0.87-fold lower than fresh whole peripheral blood (WPB (Fr)) (37.2 ± 0.2) (Figure 2A), while in fixed samples, the lymphocyte value of WCB (Fx) (25.5 ± 0.5) was 1.09-fold higher than WPB (Fx) (23.5 ± 0.3) (Figure 2C). Conversely, the monocyte population in WCB (Fr) (17.2 ± 0.2) was 1.19-fold higher than in WPB (Fr)

Table 1: Cellular percentage differences in cord and peripheral blood samples in fresh and fixed conditions using flow cytometer

Cell population	*Region/ gate*	*Gate%*		*Total events*
		WCB (Fr)	*WPB (Fr)*	
Lymphocytes	R5	32.8	37.6	10000
Monocytes	R7	17.3	14.6	10000

A. Gated percentage of lymphocytes and monocytes of fresh whole cord blood (WCB Fr) and fresh whole peripheral blood (WPB Fr)

Cell population	*Region/ gate*	*Gate%*		*Total events*
		CB MNC (Fr)	*PB MNC (Fr)*	
MNC	R9	79.7	35.2	10000

B. Gated percentage of mononuclear cells (MNC) of fresh cord blood MNC (CB MNC Fr) and fresh peripheral blood MNC (PB MNC Fr)

Cell population	*Quadrant*	*Gate%*		*Total events*
		CB MNC (Fr)Q	*PB MNC (Fr)Q*	
MNC	Lower Left	95.2	78.2	10000

C. Quadrant of MNC of fresh cord blood MNC (CB MNC Fr Q) and fresh peripheral blood MNC (PB MNC Fr Q)

Cell population	*Region/ gate*	*Gate%*		*Total events*
		CB MNC (Fx)	*PB MNC (Fx)*	
Lymphocytes	R12	58.1	34.1	10000
Monocytes	R13	16.3	11.2	10000

D. Gated percentage of Lymphocytes and Monocytes of fixed cord blood MNC (CB MNC Fx) and fixed peripheral blood MNC (PB MNC Fx)

Cell population	*Region/ gate*	*Gate%*		*Total events*
		CB Buffy (Fr)	*CB Buffy (Fx)*	
Lymphocytes	R12	27.8	25.7	10000
Monocytes	R13	32.2	24.1	10000

E. Gated percentage of lymphocytes and monocytes of fresh cord blood buffy (CB Buffy Fr) and fixed cord buffy coat (CB Buffy Fx)

Cell population	*Region/ gate*	*Gate %*		*Total events*
		WCB (Fr)	*WCB (Fx)*	
Lymphocytes	R5	32.8	30.6	10000
Monocytes	R7	17.3	11.2	10000

F. Gated percentage of lymphocytes and monocytes of fresh whole cord blood (WCB Fr) and fixed whole cord blood (WCB Fx)

Cell population	*Quadrant*	*Gate%*		*Total events*
		CB MNC (Fr)Q	*CB MNC (Fx)Q*	
MNC	Lower left	95.2	86.5	10000

G. Quadrant of MNC of fresh cord blood MNC (CB MNC Fr Q) and fixed cord blood MNC (CB MNC Fx Q)

(14.4 ± 0.3) (Figure 2B), while the monocyte population in WCB (Fx) (9.6 ± 0.3) was 0.86-fold lower than WPB (Fx) (11.2 ± 0.7) (Figure 2D).

The mean of CB mononuclear cells (MNC (Fr)) (79.6 ± 0.1) was significantly higher at 2.26-fold compared to PB MNC (Fr) (35.2 ± 0.1) (Figure 2E), and the confirmation quadrant analysis showed similar results where the CB MNC (Fr) (95.0 ± 0.4) to be 1.21 fold higher than PB MNC (Fr) (78.4 ± 0.7) (Figure 2F). In fixed states, CB MNC (Fx) lymphocyte (58.1 ± 0.3) and monocyte (16.3 ± 0.3) populations were 1.70-fold and 1.46-fold higher than PB MNC (Fx) lymphocyte (34.2 ± 0.2) and monocyte (11.2 ± 0.2) populations, respectively (Figure 2G and H).

Cord blood buffy coat analysis revealed CB Buffy (Fr) lymphocyte (27.8 ± 0.1) and CB Buffy (Fr) monocyte (32.6 ± 0.6) populations to be 1.09-fold and 1.35-fold higher than those in CB Buffy (Fx) lymphocyte (25.5 ± 0.2) and CB Buffy (Fx) monocyte (24.2 ± 0.3) populations, respectively (Figure 2I, 2J). We also compared fresh and fixed states of whole cord blood (WCB), where, the lymphocyte (32.4 ± 0.6 vs. 30.4 ± 0.8) and monocyte (17.2 ± 0.8 vs. 11.2 ± 1.0) values of WCB (Fr) were 1.07-fold and 1.54-fold of WCB (Fx), respectively (Figure 2K, 2L). The quadrant value of CB MNC (Fr) (95.2 ± 0.6) was 1.10-fold higher than CB MNC (Fx) (86.3 ± 0.6) (Figure 2M). These observations highlight the distinctive cellular distribution and differences between cord and peripheral blood under different conditions.

Scanning Electron Microscopy of Cord and Peripheral Blood Mononuclear Cells

To study the morphological and cellular difference in cord blood and peripheral blood mononuclear cells (MNC), SEM analysis was carried out and three dimensional topographical aspects were noticed in cord leucocytes and adult peripheral blood. It was observed, the cord lymphocytes showed a mixed population of cells with pleomorphic microvilli. Some showed microvilli fewer in numbers, more spaced and shorter in length (stubby) (Figure 3A), whereas most of them had short yet dense microvilli population on the surface of lymphocytes thus making them fully villous in appearance (Figure 3B). The early myeloid cells both in cord and peripheral showed close similarity with short transverse ridges and undeveloped ruffles, so no clear differences in topographical structures were observed (Figure 3C and 3D). Both cord blood and peripheral blood monocytes have distinct transverse ridges and typical developed ruffled membranes (Figure 3E and 3F). There was no difference noticed in monocytes. SEM images of cord blood mononuclear cells showed reticulocytes whereas no reticulocytes were seen in peripheral mononuclear cells (Figure 3G and 3H).

DISCUSSION

We have compared the cord blood and peripheral blood parameters by flow cytometric FSC and SSC analysis. The comparisons were drawn on the mean cell percentage

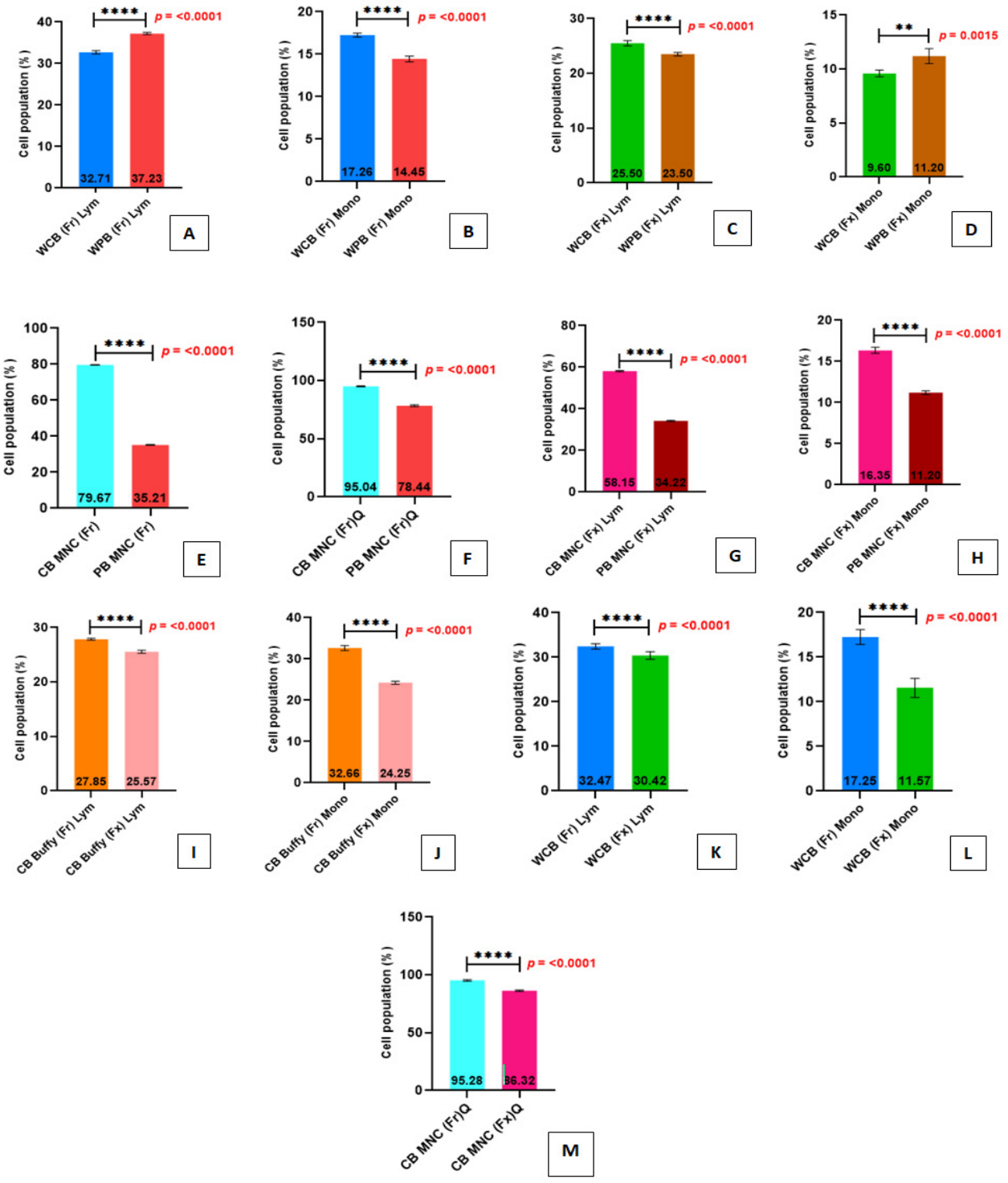

Figure 2: Graphical representations of cellular percentage differences from cord blood (CB) and peripheral blood (PB) samples in fresh and fixed states. A. Mean difference in Lymphocyte population of fresh whole CB and PB; B. Mean difference in Monocyte population of fresh whole CB and PB; C. Mean difference in Lymphocyte population of fixed whole CB and PB; D. Mean difference in Monocyte population of fresh whole CB and PB; E. Mean difference in mononuclear cell population of fresh CB (CB MNC Fr) and fresh PB (PB MNC Fr); F. Mean Quadrant difference in mononuclear cell population of fresh CB (CB MNC Fr) and fresh PB (PB MNC Fr); G. Mean difference in Lymphocyte cell population of fixed CB (CB MNC Fx) and fixed PB (PB MNC Fx); H. Mean difference in Monocyte cell population of fixed CB (CB MNC Fx) and fixed PB (PB MNC Fx); I. Mean difference in Lymphocyte cell population of fresh CB Buffy (Fr) and fixed CB Buffy (Fx); J. Mean difference in Monocyte cell population of fresh CB Buffy (Fr) and fixed CB Buffy (Fx); K. Mean difference in Lymphocyte cell population of fresh whole CB and fixed whole CB; L. Mean difference in Monocyte cell population of fixed whole CB and fixed whole CB; M: Mean Quadrant difference in mononuclear cell population of fresh CB (CB MNC Fr) and fixed CB (CB MNC Fx)

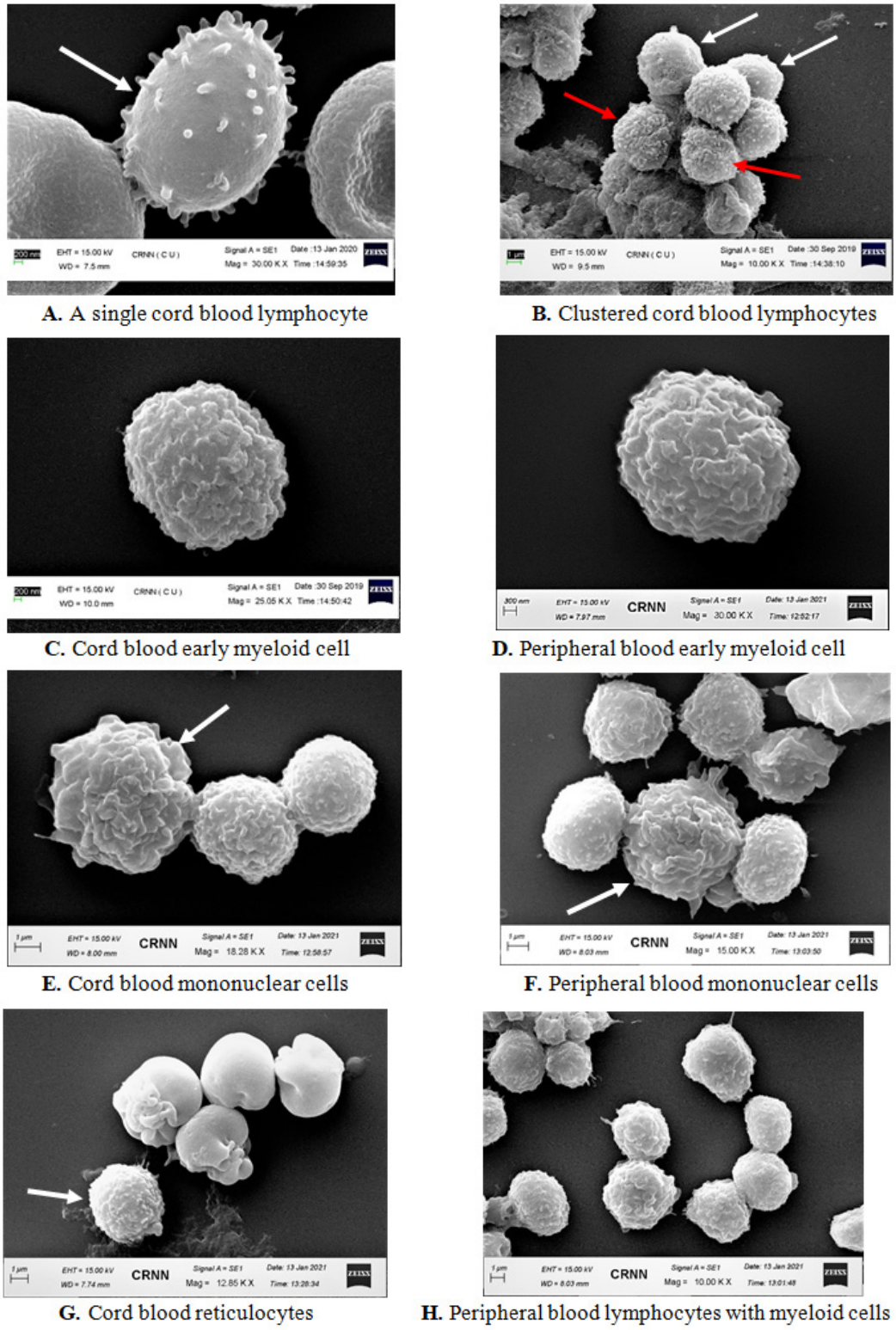

Figure 3: Morphological study of cord and peripheral blood mononuclear cells under SEM. A. Cord blood lymphocyte showing fewer distantly placed, tiny microvilli (white arrow), Magnification in 30000X; B. Cord blood lymphocytes in cluster, showing dense, stubby microvilli giving fully villous appearance (red arrows), with few round early progenitor cells having few, scanty microvilli (white arrows), Magnification in 10000X; C. Cord blood early myeloid cells, Magnification in 25000X; D. Peripheral blood early myeloid cells, Magnification in 30000X; E. Cord blood monocyte (white arrow), Magnification in 18000X; F. Peripheral blood monocyte (white arrow), Magnification in 15000X; G. Cord blood lymphocyte (white arrow) and four reticulocytes, Magnification in 12000X; H. Peripheral blood cells showings lymphocytes and myeloid population, Magnification in 10000X

obtained from the dot plot analysis of different hematopoietic cells in both fresh and fixed samples. The obtained data is expected to contribute in understanding the basic differences in cellular components of blood without any antigenic markers, when procured and studied from cord and peripheral sources. Though studying the differences of cell population on the basis of only FSC and SSC analysis is limited, we still tried to establish a simple procedure that would be cost effective and could give us an idea about the percentage of cells according to size, granularity, types and distribution in unfixed and fixed conditions.

The analysis of cellular components in cord blood (CB) and peripheral blood (PB) revealed distinct differences in fresh and fixed states. Our study to investigate the difference in the cell population on the basis of fixation was inspired by the study[16] conducted by Pinto *et al,* where the observation highlights 'fixation' of hematopoietic cells pre and post staining is a reliable method without compromising the cell percentage and MFI by flow cytometry. The observed result of FSC and SSC also gave us an idea that isolation and distribution of blood cellular components was better when the samples were fixed (Figure 1H). The fresh samples did show isolations and the cell populations were merged in appearance, thus gating procedure while flow cytometric analysis caused minor discrepancy in some of the samples. In case of fixed samples the lymphocyte percentage was higher in WCB (Fx) than in WPB (Fx), this was in sync with previous reports, where undiluted whole cord blood's absolute lymphocyte mean value was more than the adult peripheral blood's lymphocyte count[17-19] but we cannot decipher why the mean lymphocyte of fresh WCB (Fr) was less than the WPB (Fr). In our study, the fresh CB samples showed higher monocyte counts compared to PB, indicating CB's richer monocyte content. This characteristic nature may enhance CB's therapeutic potential in regenerative medicine.

Fixed samples demonstrated a notable decrease in cell counts, particularly in monocytes, underscoring the importance of sample handling. The less mean value in fixed samples could be because of the repeated washing of the isolated MNCs after fixation with 1.5% PFA solution that may lead to loss of some cells for the removal of residual PFA, unwanted RBC, debris and other granulocytes. Fresh CB mononuclear cells (MNCs) were significantly higher than those in PB, as confirmed by quadrant analysis. This suggests CB's robust cellular profile, making it suitable for stem cell therapies and hematopoietic transplants. Our monocyte values in fresh samples were similar to the previous studies which showed high percentage of monocytes (classical) in CB when compared to APB.[20] Since our comparison did not involve neonates with related mothers but non-related volunteers, so there was a slight difference in the report with another paper, which showed less differences in lymphocytes population between neonates' CB and related mothers' PB.[7] The differences seen in fresh blood cellular counts with that of the fixed samples were probably because of less distribution and isolation of the subsets in FCS and SSC dot plots. This can be a new observation when fresh and fixed whole blood samples from cord and peripheral is compared. The buffy coat separated from the whole cord blood without using any density-gradient chemical also showed significantly high lymphocyte and monocyte values in fresh CB when compared to fixed CB similar to the whole cord blood. The estimated results were statistically significant and are reported in Table 2.

Table 2: Statistical difference in the distribution of cord and peripheral blood cellular components

	t-value	*df*	*p-value*
WCB Mono (Fr)/WPB Mono (Fr)	16.795	10	< 0.01
CB MNC (Fr)/PB MNC (Fr)	548.001	10	< 0.01
CB MNC (Fr)Q/PB MNC (Fr)Q	47.13	10	< 0.01
CB MNC Lym (Fx)/PB MNC Lym (Fx)	134.243	10	< 0.01
CB MNC Mono (Fx)/PB MNC Mono (Fx)	28.544	10	< 0.01
CB Buffy (Fr) Lym/CB Buffy (Fx) Lym	16.747	10	< 0.01
CB Buffy (Fr) Mono/CB Buffy (Fx) Mono	29.154	10	< 0.01
CB MNC Lym (Fr)/CB MNC Lym (Fx)	4.796	10	<0.01
CB MNC Mono (Fr)/CB MNC Mono (Fx)	10.25	10	<0.01
CB MNC (Fr)Q/CB MNC (Fx)Q	23.21	10	<0.01

The major purpose of our study was to understand the morphology of cord blood leucocytes using scanning electron microscopy, as most of existing literature, highlights the morphology of adult peripheral leucocytes in both normal[9,21] and hematopathological conditions.[22-24] Cord blood have higher number of primitive, immature hematopoietic cells compared to adult peripheral blood[8] so we intend to study the difference in between adult and cord whole blood and MNC population. The observed cord blood lymphocytes which are primitive in nature,[25,26] exhibit similar morphology as the peripheral lymphocytes as studied by Hoffmann *et al*.[27] Majority of the cord blood lymphocytes have short or stubby, dense villous surface and few cells have distantly spaced, scanty microvilli.[9] These distantly spaced, less-numbered microvilli, round cells were probably the early progenitor cells, which are naïve and have high proliferative capacity (Figure 3B). These almost featureless and scanty or distantly spaced microvilli cells are commonly observed in pre-B and T lymphoblastic leukemic bone marrow cells.[24] The lengthening of microvilli projections occurs in the late developed stages or terminally differentiated stages in the haematopoietic lineage differentiation. No morphological differences in B and T lymphocytes were observed in cord or peripheral lymphocyte population, as both the cell populations from two groups showed villous appearances. There were no smooth surfaced cells observed as mentioned by Polliack *et al* and Renau-Piqueras.[21] Early myeloid cells in both cord and peripheral showed short-transverse ridges and undeveloped ruffles unlike their matured monocytic stage which showed typical large flowy ruffles and distinct transverse ridges.[11] Reticulocyte cells were noticed in cord blood mononuclear

cells whereas little to no reticulocytes were observed in adult peripheral blood. The hypoxic environment in cord blood induces the production of erythropoietin, one of the hematopoietic growth factors, which favours the production of cord erythrocytes. As erythrocyte production is high in cord blood it is expected that there will be higher reticulocyte count in cord as compared to the adult peripheral blood for the production of red blood cells.

CONCLUSION

Our objective was to investigate the differences in leukocyte populations between cord blood and peripheral blood. Building on our previous findings of significant variations in erythrocyte populations, we anticipated notable differences in the white blood cell (WBC) study. This study was motivated by the premise that cord blood contains primarily naive, low immunogenic cells, while peripheral blood comprises more mature, highly immunopotent cells. In our study we focussed on the flow cytometry analysis of fresh and fixed cellular populations of whole blood cells and isolated mononuclear cells from both cord and adult peripheral blood samples. The mean cell counts of different cellular population showed significant differences and was in sync with other studies which did not highlight flow cytometric analysis without any antigen markers. This simple yet cost effective advanced technique can be one of the procedures to study cellular differences in samples along with other sophisticated methods. We also emphasised on the three-dimensional topographical aspects in mononuclear cells isolated from cord blood and adult peripheral blood using high resolution scanning electron microscope (SEM). For several decade focus was given in understanding the morphological differences in peripheral blood leucocyte types, bone marrow cell population in both normal and hematopathological conditions but SEM analysis considering cord blood mononuclear cells was hardly studied.

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REFERENCES

1. Tse W, Laughlin MJ. Umbilical cord blood transplantation: A new alternative option. Hematology / the Education Program of the American Society of Hematology. American Society of Hematology. *Edu Prog*. 2005; DOI: 10.1182/asheducation-2005.1.377.
2. Shearer WT, Lubin BH, Cairo MS, Notarangelo LD. Cord blood banking for potential future transplantation. *Obstet Gynecol Surv*. 2018;73(3): 135-6. DOI: 10.1097/01.ogx.0000531866.75606.5a.
3. Gluckman E, Rocha V, Ionescu I, *et al*. Results of unrelated cord blood transplant in Fanconi anemia patients: Risk factor analysis for engraftment and survival. *Biol Blood Marrow Transplant*. 2007;13(9): 1073–82. DOI: 10.1016/j.bbmt.2007.05.015.
4. Fernandes JF, Rocha V, Labopin M, *et al*. Transplantation in patients with SCID: Mismatched related stem cells or unrelated cord blood? *Blood*. 2012;119(12): 2949-55. DOI: 10.1182/blood-2011-06-363572.
5. Escolar ML, Poe MD, Provenzale JM, *et al*. Transplantation of umbilical cord blood in babies with infantile Krabbe's disease from the program for neurodevelopmental function in rare disorders, Clinical Center for the Study of Development and Learning. *N Engl J Med*. 2005;352:2069-81. DOI: 10.1056/NEJMoa042604.
6. Broxmeyer HE, Douglas GW, Hangoc G, *et al*. Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. *Proc Nat Acad Sci*. 1989;86(10): 3828-32. DOI: 10.1073/pnas.86.10.3828.
7. Chirumbolo S, Ortolani R, Veneri D, *et al*. Lymphocyte phenotypic subsets in umbilical cord blood compared to peripheral blood from related mothers. *Cytometry B Clin Cytom*. 2011;80 B(4): 248-53. DOI: 10.1002/cyto.b.20588.
8. Hordyjewska A, Popiołek Ł, Horecka A. Characteristics of hematopoietic stem cells of umbilical cord blood. *Cytotechnology*. 2015;67(3): 387-96. DOI: 10.1007/s10616-014-9796-y.
9. Newell DG, Roath S, Smith JL. The Scanning Electron Microscopy of Normal Human Peripheral Blood Lymphocytes. *Br J Hematol*. 1976;32(3): 309-16. DOI: 10.1111/j.1365-2141.1976.tb00934.x.
10. Buys A V., Van Rooy MJ, Soma P, Van Papendorp D, Lipinski B, Pretorius E. Changes in red blood cell membrane structure in type 2 diabetes: A scanning electron and atomic force microscopy study. *Cardiovasc Diabetol*. 2013;12:25. DOI: 10.1186/1475-2840-12-25.
11. Polliack A. The contribution of scanning electron microscopy in haematology: its role in defining leucocyte and erythrocyte disorders. *J Microsc*. 1981;123(2): 177-87. DOI: 10.1111/j.1365-2818.1981.tb01293.x.
12. Mikami T, Eguchi M, Kurosawa H, *et al*. Ultrastructural and cytochemical characterization of human cord blood cells. *Med Elect Microsc*. 2002;35:96-101. DOI: 10.1007/s007950200012.
13. Manna S, Dhar I, Naskar T, Law S. Cord blood haematological parameters reference range difference in urban and rural population of West Bengal as compared to global scenario. *J Biomed Res Environ Sci*. 2021;2(3):206-12. DOI: 10.37871/jbres1212.
14. Bieback K. Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood. *Stem Cells*. 2004;22(4):625-34. DOI: 10.1634/stemcells.22-4-625.
15. Pinto LA, Trivett MT, Wallace D, *et al*. Fixation and cryopreservation of whole blood and isolated mononuclear cells: Influence of different procedures on lymphocyte subset analysis by flow cytometry. *Cytometry B Clin Cytom*. 2005;63(1):47–55. DOI: 10.1002/cyto.b.20038.
16. D'Arena G, Musto P, Cascavilla N, *et al*. Flow cytometric characterization of human umbilical cord blood lymphocytes: Immunophenotypic features. *Haematologica*. 1998;83(3): 197–203. PMID: 9573672.

17. Beck R, Lam-Po-Tang PRL. Comparison of cord blood and adult blood lymphocyte normal ranges: A possible explanation for decreased severity of graft versus host disease after cord blood transplantation. *Immunol Cell Biol*. 1994;72(5):440-4. DOI: 10.1038/icb.1994.65.
18. Katsares V, Paparidis Z, Nikolaidou E, *et al*. Reference ranges for umbilical cord blood hematological values. *Lab Med*. 2009;40(7): 437–439. DOI: 10.1309/LMWCY2YGYCF9EEMQ.
19. Prabhu SB, Rathore DK, Nair D, et al. Comparison of human neonatal and adult blood leukocyte subset composition phenotypes. *PLoS ONE*. 2016;11(9): 1-17. DOI: 10.1371/journal.pone.0162242.
20. Renau-Piqueras J. morphological differences in human peripheral blood lymphocytes. *Acta Haematologica*. 1978;59(1):19-24. DOI: 10.1159/000207740.
21. Polliack A, de Harven E. Surface features of normal and leukemic lymphocytes as seen by scanning electron microscopy. *Clin Immunol Immunopathol*. 1975;3(3): 412-30. DOI: 10.1016/0090-1229(75)90029-X.
22. Soligo D, Quirici N, Caneva L, Baldini L, Cro L, Deliliers GL. Scanning and transmission electron microscopy of clonal peripheral blood lymphocytes in low grade non-Hodgkin's B-cell lymphomas with predominant splenomegaly. *Microsc Res Tech*. 1994;28(4): 345–355. DOI: 10.1002/jemt.1070280409.
23. Soligo D, De Harven EP, Quirici N, *et al*. Cell surface changes of hemopoietic cells during normal and leukemic differentiation: An immuno-scanning electron microscopy study. *Scan Microsc*. 1995;9(1): 175-84. PMID: 8553015.
24. Harris DT, Schumacher MJ, Locascio J, Besencon FJ, Olson GB, Deluca D, et al. Phenotypic and functional immaturity of human umbilical cord blood T lymphocytes. Proceedings of the National Academy of Sciences of the United States of America. 1992;89(21): 10006–10010. DOI: 10.1073/pnas.89.21.10006.
25. Veneri D, Ortolani R, Franchini M, Tridente G, Pizzolo G, Vella A. Expression of CD27 and CD23 on peripheral blood B lymphocytes in humans of different ages. *Blood Trans*. 2009;7(1):29-34. DOI: 10.2450/2008.0007-08.
26. Hoffmann CC, Moore KC, Shih CY, Blakley RL. Scanning electron microscopy of human lymphocytes during transformation and subsequent treatment with methotrexate. *J Cell Sci*. 1977;8: 151-65. DOI: 10.1242/jcs.28.1.151.
27. Jacob EA. Hematological differences in newborn and aging: A review study. *Hematol Trans Int J*. 2016; DOI: 10.15406/htij.2016.03.00067.

PEER-REVIEWED CERTIFICATION

During the review of this manuscript, a double-blind peer-review policy has been followed. The author(s) of this manuscript received review comments from a minimum of two peer-reviewers. Author(s) submitted revised manuscript as per the comments of the assigned reviewers. On the basis of revision(s) done by the author(s) and compliance to the Reviewers' comments on the manuscript, Editor(s) has approved the revised manuscript for final publication.