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Reference intervals for estimated CBC parameters in cord blood: An Indian Scenario

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RESEARCH

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Introduction

Accurate reference intervals established in healthy subjects are essential for appropriate interpretation of laboratory test results and to assist clinicians in diagnosis, monitoring, and treatment of disease. These values are affected by key covariates including age, sex, race, geographical location and dietary pattern[1]. Reference intervals are defined as "limiting values within which a specified percentage (usually 95 Per Cent) of apparently healthy individuals' results would fall" i.e. usually the 2.5th and 97.5th percentiles of the test result distribution in the reference (healthy) population[2].

Creating precise and dependable reference ranges is a complicated and intricate task, necessitating the inclusion of a substantial sample of healthy individuals. This complexity is especially pronounced when dealing with paediatric groups. Despite the seemingly simple idea of reference intervals and their usefulness, their establishment is a challenging endeavour. Newborn infants, older children, and adults display significant variations in their hematological profiles, making it unsuitable to apply adult reference ranges when assessing the health of neonates[3].

Modern automated haematological analysers have the capability to measure more parameters than the traditional complete blood cell count with a 5-Part White Cell Differential. Among these additional parameters, there has been a growing interest in the measurement of immature lymphocytes in peripheral blood. Due to their typically low presence in normal conditions accurately quantifying immature lymphocytes through manual smear examination is generally challenging. Nevertheless, flow cytometry-based analysers have the ability to count numerous cells per sample routinely, making them capable of accurately enumerating even extremely rare cells in peripheral blood.

A rise in atypical lymphocytes and immature lymphocytes indicates that the infection or inflammation present has induced a bone marrow response of increased WBC production followed by early release into blood even before they have fully matured[4]. Elevated LIC (Large Immature Cells) is also a feature seen in Myelodysplastic syndrome (MDS)[5], thus it becomes essential to establish the "normal" reference interval for the same.

The umbilical cord, serves as the connection between the developing foetus and the placenta during its time in the uterus and contains umbilical arteries and a vein. It is typically clamped and cut at after delivery. The process of umbilical cord blood collection (UCBC) is non-invasive and does not pose any risk to either the mother or the foetus. However hematological parameters are subject to alteration due to delayed cord clamping and the practice of umbilical cord milking[6]. Compared to using cannulas and other vascular catheters, cord blood collection is a safer approach for sample retrieval, as it reduces the risk of complications like thrombophlebitis, infections, and extravasation[7].

Umbilical cord blood is a valuable source of hematopoietic stem cells for transplantation. Due to its ease of collection, lower risk of tissue rejection, and reduced transmission of

infectious agents, cord blood transplantation is considered a viable alternative to bone marrow transplantation for addressing various genetic disorders and specific cancers. Consequently, there is a growing need for neonatologists and transplant physicians to compare cord blood test results with established reference values [8, 9].

Materials & Methods

Study Design and Setting

A cross-sectional research investigation took place at a tertiary care hospital affiliated with a medical college in the western region of Maharashtra, India, following approval from the Institutional Ethics Committee (ICEC). This study spanned from October to December 2022 and involved enrolling participants from expectant mothers seeking delivery services at the hospital.

Sample size determination and sampling Technique

A cross-sectional study was conducted on healthy and term neonates (from 37 to 42 weeks) with normal birth weight born to apparently healthy pregnant mothers who approached the institute to get delivery service. As per the Clinical Laboratory Standard Institute (CLSI) guideline C28-A3, about 120 samples per partition is required to determine the 95 Per Cent reference interval[10].

A priori selection method was employed, eligible volunteering mothers aged 18 to 45 years were recruited.

Mothers with the following conditions were excluded

Those with medical conditions like infectious (e.g. Hepatitis B, HIV, Syphilis), chronic illness (e.g. Insulin-dependent diabetes mellitus), obstetric (e.g. preeclampsia), psychological problems and social habits (e.g. smoking, heavily alcohol drinking). Various diagnostic tests (laboratory tests, ultrasound) and history obtained were used to exclude mothers with the listed conditions. On the other hand, mothers who had Hemoglobin (HGB) > = 12.0 g/dl [11]and inter–pregnancy interval of more than or exactly 18 months as per WHO recommendation [12] were included in the study. Not only this, but also the posteriori selection method was used to include eligible neonates of gestational age (37-42 weeks), having 5th minute Apgar score of >=7. Babies with respiratory distress, meconium staining, gross congenital anomalies, umbilical cord with true knot, and babies delivered by instrumental delivery were excluded.

Data Collection Procedure

All professionals who participated in the collection of the data were oriented about the aim of the study, selection of participants, data confidentiality, safety precautions during collection, transportation, and storage of cord blood samples before the collection of data was initiated. A predesigned questionnaire was utilised in order to assimilate the demographic information and a brief history from all those mothers who had consented for the research and fit into the eligibility criteria. The process of delivery being an exhaustive event, the attending midwives obtained a written informed consent in accordance with the Helsinki Declaration and assisted all the participating mothers to answer the questionnaire before entering the labor room. It is only after the complete separation and expulsion of the placenta from the mother and cutting the umbilical cord post clamping, the cord blood sampling is carried out. The umbilical cord was clamped within 1 min after birth and 2–3 ml of cord blood was collected by operation room (OR) nurse into EDTA vacutainers (for CBC). The sample was well mixed and immediately transported to hematology laboratory for further investigation.

Screening Tests

As part of the routine antenatal care (ANC) follow-up, all participants underwent screening for HIV using antibody tests, as well as for Hepatitis B surface antigen and syphilis. Additionally, ultrasonography (USG) was performed to detect any potential congenital anomalies in the developing foetus.

Quality Assurance

Experienced OR nurses conducted the collection of cord blood samples in accordance with the cord blood sample collection guidelines. They provided a detailed and educational briefing on the correct procedures for collecting and managing the samples. All actions taken adhered to the Standard Operating Procedures (SOPs).

Hematological Analysis

Specific hematological parameters namely Absolute and Percentage of Atypical Lymphocytes (ALY#, ALY Per Cent) and Large Immature Cells (LIC#, LIC Per Cent), Neutrophil to Lymphocyte Ratio (NLR), Red Cell and Platelet Distribution Width – Standard Deviation and Coefficient of Variation (RDW-SD, RDW-CV, PDW-SD, PDW-CV), Mean Platelet Volume (MPV), Plateletcrit (PCT), Platelet-Large Cell Ratio (P-LCR), Platelet Large Cell Count (P-LCC) were analysed using an automated hematology analyser. In order to investigate the RBC morphology, WBC and PLT abnormalities, peripheral blood smears from cord blood samples were prepared and stained using Wright's stain.

Statistical analysis

All the data from the questionnaire and results received from the laboratory were checked for completeness. The

collected data was entered in MS Excel and further analysis was done on IBM® SPSS® Statistics. Following the CLSI guide, 2.5th and 97.5th percentiles for hematological and parameters were calculated for 127 neonates of both genders. To compare the distribution of the parameters between sex of infants the non-parametric Mann-Whitney U test was used. Additionally, statistics were expressed in terms of percentage, ranges, charts and graphs. P value less than 0.05 was considered to declare statistical significance.
Normality of the data distribution was checked with Shapiro and Wilk Test.

Results

In the study, we included 127 new-borns who were born healthy and at full term. Among these, 68 were males, accounting for 53.5 Per Cent of the total, while 59 were females, constituting the remaining 46.5 Per Cent.
Approximately 81.3 Per Cent of the mothers fell within the age range of 18 to 30 years. Around 12 Per Cent of the mothers were from areas outside Mumbai. All the mothers were married, and out of the total sample, 35 infants, or 27.56 Per Cent, were born to primiparous mothers. A detailed overview of the demographics is provided in Table 1 for reference.
The analysed data showed a p value above 0.05 for all parameters. This resulted in establishing that these haematological parameters are independent of the sex of the infant.
A comparison between mean values of cord blood RDW of the current study (India) and other similar studies conducted in nations worldwide (Iraq, Nigeria, Greece)[13,14] is shown in Figure 1.

Discussion

Establishing reference intervals for the selected parameters of the complete blood work-up, providing a comparison between these findings and peripheral venous blood ranges as well as published reports was the main aim of this study. It was concluded after applying the non-parametric test that these parameters do not vary on the basis of the sex of the infant since their p values were above 0.05, implying no statistical significance.
The mean values of RDW in Nigeria and Iraq were found to be higher, while that of Greece were lower than that in India. This can be associated with higher prevalence of anemia or other blood disorders in countries like Nigeria and Iraq while lower incidence in Greece as compared to that in India.
The RI of atypical lymphocytes (ALY), large irregular cells (LIC), MPV, P-LCC and P-LCR were found to be narrow as compared to that in PVB sample. In contrast, the RI of RDW, PDW, PCT were found to be elevated when compared to the PVB sample.
A higher plateletocrit can be associated with a maternal transfer of platelets to the infant in the intro-uterine life. A higher PDW can be associated with the developmental phase of the bone marrow, which may lead to a variation in the sizes of the platelets. Infants have higher HbF concentration, and the cells with this type of haemoglobin are larger in size. Due to this variation in size, the RDW is also seen to be elevated in infants.
Variations among populations can be attributed to a combination of physiological, socio-economic, geographical, nutritional, ethnic, and maternal stress factors. Some of the disparities observed in research outcomes may be linked to the stress experienced during the birth process. During birth, the foetus undergoes inflammatory stress, triggering alterations in the blood composition. Establishing hematological reference ranges specific to Indian cord blood can play a vital role in enhancing neonatal care and its effective management. Additionally, it has the potential to enhance the functioning of Umbilical Cord Blood Banks throughout India and promote the utilisation of Stem Cell Transplantation, which is not commonly practiced. Therefore, it is advisable for laboratories, stem cell transplant units, surgical units, and all healthcare facilities involved in neonatal care to incorporate cord blood analysis as a routine test. This recommendation aligns with the overarching goal of improving neonatal care and should also be reflected in relevant manuals and handbooks.

Limitations to the study

The study exclusively focused on new-borns delivered at a single hospital within a specific locality. To enhance the study's reliability, it would be beneficial to expand the research to encompass multiple locations. Ideally, reference intervals should be established with consideration for various factors like ethnicity, gestational age, mode of delivery, among others. However, this particular study provides general reference intervals without taking these factors into account.

Conclusion

This research employed a priori method to investigate Reference Intervals (RIs) in term infants. The study focused on specific parameters that are scarcely explored in the

existing medical literature in umbilical cord blood. The study featured well-defined inclusion and exclusion criteria, standardized sample processing procedures, and innovative data analysis techniques. While this study was conducted within a more limited population, its inclusion remains relevant due to the diversity in the participants' backgrounds. Nonetheless, it is imperative to corroborate the results through larger sample sizes from diverse geographical regions within the country to ensure broader applicability and generalizability. Subsequently, the proposed hematological ranges could be used as a guide for further UCB analyses and interpretation.

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Tables & Figures

Table 1: Sociodemographic & clinical characteristics of study participants (n = 127)

Information		Percentage
Resident of	Mumbai	88.19
	Outside Mumbai	11.81
Marital status	Married	100
	Other (unmarried, widowed, etc.)	0
Obstetrics history (I)	Non–primi	72.44
	Primi	27.56
Obstetric history (II)	Abortions	18.9
	No abortions	81.1
Delivery mode	VD	18.12
	CS	81.88
Sex of neonate	Male	53.5
	Female	46.5

Table 2: statistical data in comparison to peripheral venous blood (PVB) sample.

Parameter	Current Study			PVB Sample
	Median	Mean	95 Per Cent RI	RI
ALY#	0.03	0.032	0 - 0.1022	0 - 0.2
ALY Per Cent	0.2	0.282	0 - 0.7	0 - 2.0
LIC#	0	0.015	0 - 0.07	0 - 0.2
LIC Per Cent	0	0.065	0 - 0.3225	0 - 2.5
NLR	1.34	1.642	0.41 - 3.956	-
RDW	16.5	16.853	14.3 - 21.88	11.0 - 16.0
MPV	9.6	9.656	8.3 - 11.53	6.5 - 12.0
PDW	14.95	14.995	7.2 - 17.82	9.0 - 17.0
PCT	0.229	0.204	0.016 - 0.346	0.108 - 0.282
P-LCR	23.2	24.251	15.13 - 37.32	11.0 - 45.0
P-LCC	65	53.354	10 - 93.6	30 - 90

Table 3: Reference Intervals for umbilical cord hematological parameters by Sex.

Parameter	Sex	N	Mean	Median	2.5	97.5	P-value
	M	68	0.033667	0.03	0	0.081	
	F	59	0.030366	0.02 0	0	0.10725	
ALY#	Combined	127	0.032134	0.03	0	0.1023	0.13104
	M	68	0.306667	0.2	0.0475	0.7	
	F	59	0.252606	0.2	0.0275	0.5725	
Aly Per Cent	Combined	127	0.281567	0.2	0.037	0.7	0.71884
	M	68	0.012667	0	0	0.06525	
	F	59	0.018562	0	0	0.0645	
LIC#	Combined	127	0.015404	0	0	0.07	0.35238
	M	68	0.08	0	0	0.181	
	F	59	0.04894	0	0	0.272	
LIC Per Cent	Combined	127	0.065579	0	0	0.32	0.4593
	M	68	1.618167	1.38	0.52225	3.579	
	F	59	1.668637	1.26	0.40275	4.368	
NLR	Combined	127	1.641599	1.34	0.4077	3.9557	0.3843
	M	68	16.59167	16.45	14.5425	19.01	
	F	59	17.14919	16.6	14.23	24.3	
RDW-CV	Combined	127	16.85316	16.5	14.3	21.88	0.4712
RDW- SD	M	68	61.72833	62.8	45.73	72.2175	

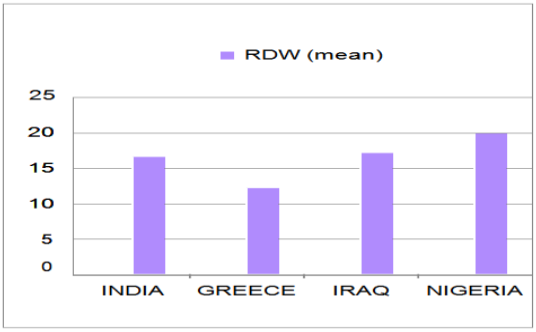

Figure1: Comparison of RDW (mean) between India, Nigeria, Greece.