

Reference values of reticulocyte counts in five age groups of healthy infants at Rawalpindi, Pakistan

Kiran Tauseef Bukhari,¹ Humaira Zafar²

Abstract

Objectives: To determine the reference range values of reticulocyte counts among various infantile age groups at tertiary care hospitals.

Methods: The descriptive cross-sectional study was carried out at the Haematology Department of the Armed Forces Institute of Pathology (AFIP), Rawalpindi, from March 2010 to March 2011. The sample size was 2000 which was equally distributed between males and females across five age groups. <27 days Group I; 03 months Group II; 06 months Group III; 09 months Group IV; and 1 year Group V. Each group had 400 patients. Reticulocyte staining was used to calculate the percentage of reticulocyte in all the groups. SPSS 15 was used for statistical analysis.

Results: Gender had no effect on reticulocyte count in all the five groups. The average range of reticulocyte count was 5.1 to 7.5 in Group I, observed in 272 (68%) infants, with a mean value of 1.58 ± 0.65 . For all the remaining groups, 7.6 to 10 was the average range. The mean reticulocyte count value in Group II was 0.73 ± 0.37 , while the values were 0.83 ± 0.44 , 0.82 ± 0.44 and 0.80 ± 0.43 in Group III, Group IV and Group V respectively. A significant difference was seen between Group I and the remaining four ($p < 0.05$).

Conclusion: Reference values of reticulocyte counts currently used in our region were somewhat different from those currently used.

Keywords: Reference values, Reticulocyte counts, Healthy infants, Rawalpindi. (JPMA 63: 1108; 2013)

Introduction

The ranges for specific laboratories for different age groups should always be considered for reference. The World Health Organisation (WHO) has strongly suggested the necessity for implementing accurate values because these values vary amongst growing ages amid specific regional factors.^{1,2}

In Pakistan and other developing countries, most of the hospital laboratories are using the reference values and ranges recommended by Western studies. There are no elaborate studies available locally which could help in formulating reference ranges for haematological parameters. The goal of the present study was to establish the reference values for reticulocyte counts in infants of Rawalpindi/Islamabad. This will help the clinicians to compare the laboratory test results with locally generated reference ranges.³

Reticulocytes are the immature red cells and contain the remnants of ribosomes and ribonucleic acids (RNA).⁴ The normal value of reticulocyte count in newborns is 2.5% to 6.5% and this value drops within 2 weeks to 0.5% to 2.0%.^{5,6}

The exact counting of reticulocytes is actually the estimation of reticulocytes percentage in the blood. This test will be helpful to assess the rate of red blood cells (RBC) production by the bone marrow. A higher than normal percentage of reticulocytes may indicate the underlying bleeding disorder, Erythroblastosis fetalis, Haemolytic anaemia, and any renal disorder resulting in increased erythropoietin production. Lower percentage of reticulocytes is usually suggestive of bone marrow failure (for example, from drug toxicity, tumour, or infection), cirrhosis of the liver, folate deficiency, iron deficiency, kidney disease with decreased erythropoietin production, radiation therapy and vitamin B12 deficiency.⁷

This all can only be judged if compared with accurate reference range values of reticulocyte count. The current study was, therefore, planned to assess the reference range values of reticulocyte counts among various infantile age groups of Rawalpindi, Pakistan.

Subjects and Methods

The descriptive cross-sectional study was carried out at the Department of Haematology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, between March 2010 and March 2011 after formal approval of the institutional review committee.

Non-probability convenience sampling was adopted for

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¹Department of Microbiology, ²Department of Haematology, Al Nafees Medical College, Islamabad.

Correspondence: Kiran Tauseef Bukhari. Email: kiran.tauseef@yahoo.com

the study and to assess the difference between males/females, different age groups <1 year and a comparison with Western data. Sampling was done from the infants visiting the immunisation centres of Military Hospital (MH), Combined Military Hospital (CMH), Holy family Hospital (HFH), and Benazir Bhutto Hospital of Rawalpindi. The study was carried out on blood samples of 2000 healthy infants with equal gender distribution. The study population was divided into five groups i.e 400 subjects each for <27 days, 3 months, 6 months, 9 months and 1 year of age infants.

Healthy neonates up to 1 year of age of both gender with no obvious sign of illness were included in the study. Infants whose guardians were not willing to participate, pre-mature infants, those with any congenital diseases like thalassaemia, sickle cell disease, leukaemia and blood dyscrasia, history of recent blood loss, drug intake, presence of any systemic illness like kidney disease, bone disorder and malignancy whether primary or secondary, anywhere in the body, were excluded.

Three ml of venous blood samples were drawn aseptically from the selected infants. The samples were ultimately transferred for processing to the Haematology Department of AFIP, Rawalpindi.

Then 1.0g of brilliant cresylblue (CI 52030) was dissolved in 100 ml of iso-osmotic phosphate buffer pH 6.5. Two to three drops of the dye solution were delivered to a 75×10-mm plastic tube by means of a plastic Pasteur pipette. About 2-4 volumes of the patient's EDTA anti-coagulated blood were added to the dye solution and mixed well and kept at 37°C for 15-20 min. The red cells were re-suspended by gentle mixing, and films were made on glass slides in the usual way. When dry, the films were examined without fixing or counter-staining.

The reticulocytes were counted out of at least 1000 red cells and then percentage was calculated as follows:⁸

$$\text{Retic (\%)} = \text{Retics counted} / \text{RBC counted}$$

Following precautions for reticulocyte staining were observed: The exact volume of blood that must be added to the dye solution for optimal staining depended on the RBC number. A larger proportion of anaemic blood, and a smaller proportion of polycythemia blood was to be added compared to the positive control sample; In a successful preparation, the reticulofilamentous material was stained deep blue and the non-reticulated cells were stained diffused shades of pale greenish blue.

The results were analysed using SPSS 15. The mean SD along with frequencies and percentages were assessed among different age groups for statistical inference. A p value <0.05 was calculated using analysis of variance (ANOVA) with post hoc Tukey test to observe the difference of reticulocyte count among the groups.

Results

Out of the total 2000 infants, 1870 (93.5%) were the residents of Rawalpindi, Punjab; 47 (2.35%) were from Peshawar, Khyber Pakhtunkhwa; 45 (2.25%) were from Karachi and Hyderabad, Sindh; and 38 (1.9%) were the residents of Quetta, Baluchistan.

In Group I, the overall mean value of the reticulocyte was observed to be $1.58 \pm 0.65\%$. This value was statistically ($p < 0.05$) higher than all the age groups. The mean reticulocyte count of $1.57 \pm 0.63\%$ in females was comparable ($p > 0.05$) to $1.59 \pm 0.67\%$ in males. Qualitative analysis showed that 272 (68%) presented with the average range of 3-4% (Table-1).

In Group II, the mean reticulocyte count was $0.73 \pm 0.37\%$

Table-1: Distribution data and statistics of reticulocyte.

SR. No.	Variables Used	Statistical Analysis	Gender	< 27 Days	03 Months	06 Months	09 Months	01 Year
1.	Quantitative Variables	Mean $\pm$ Standard Deviation of Reticulocyte %	Female	1.57 ± 0.63	0.81 ± 0.41	0.77 ± 0.42	0.79 ± 0.43	0.80 ± 0.43
			Male	1.59 ± 0.67	0.65 ± 0.30	0.88 ± 0.45	0.85 ± 0.46	0.80 ± 0.42
			Average	1.58 ± 0.65	0.73 ± 0.37	0.83 ± 0.44	0.82 ± 0.44	0.80 ± 0.43
2.	Qualitative Variables	Range 0.1-1	Frequency (total n=400)	7	53	44	58	23
			Percentage	1.75	13.25	11	14.5	5.75
		1.1-1.9	Frequency (total n=400)	7	166	152	140	168
			Percentage	1.75	41	38	35	42
		2-2.9	Frequency (total n=400)	50	140	126	135	126
			Percentage	12.50	35	31.5	33.8	31.5
		3-4	Frequency (total n=400)	272	41	75	67	83
			Percentage	68.0	10.25	18.75	16.75	20.75
		4-5	Frequency (total n=400)	64	-	-	-	-
			Percentage	1.0	-	-	-	-

Table-2: Reticulocyte statistics for comparison between pairs of different age groups.

Parametre	Pair of age group compared		P value
Reticulocyte	New Born	3 Month	0.00*
	New Born	6 Month	0.00*
	New Born	9 Month	0.00*
	New Born	1 Year	0.00*
	3 Month	6 Month	0.03*
	3 Month	9 Month	0.06ns
	3 Month	1 Year	0.25ns
	6 Month	9 Month	1.00ns
	6 Month	1 Year	0.89ns
	9 Month	1 Year	0.97ns

*=Significant difference. ns=Non-significant different.

Table-3: Comparative haematological parameters in the current study and that reported in western populations.

Sr. No.	Group Distribution of Infants	Current Study Values	Comparison with Western Studies
1.	Group I (<27 Days)	1.58±0.65	5.7±6-7 ²⁰
2.	Group II (3 Months)	0.73±0.37	2.00±1.56 ²¹
3.	Group III (6 Months)	0.83±0.44	3.5±0.45 ²²
4.	Group IV (9 Months)	0.82±0.44	7.6±0.45 ²³
5.	Group V (1 Year)	0.80±0.43	0.3 - 2.5 ³

which was the lowest amongst all the groups and was statistically ($p < 0.05$) different from Groups I and Group III. Within the group, gender revealed no difference ($p > 0.05$); $0.81 \pm 0.41\%$ in female and $0.65 \pm 0.30\%$ in males. Qualitative analysis showed 1.1-1.9% average range and was observed in 166 (41.5%).

In Group III, the mean reticulocyte count was $0.83 \pm 0.44\%$. This was higher than Group II, IV and V, but lower than that observed in Group I. A statistically significant ($p < 0.05$) difference, however, was observed only with Group I. The value was statistically ($p < 0.05$) lower in females ($0.77 \pm 0.42\%$) compared to the males ($0.88 \pm 0.45\%$). Qualitative analysis showed 1.1-1.9% an average range observed in 152 (38%) subjects.

The mean reticulocyte count in Group IV was $0.82 \pm 0.44\%$ which was lower than Group I. The mean value in females was $0.79 \pm 0.43\%$ which was statistically ($p < 0.05$) lower than $0.85 \pm 0.46\%$ in males. Qualitative analysis showed 1.1-1.9% in 140 (35%) subjects.

The mean reticulocyte count in Group V was $0.80 \pm 0.43\%$ which was statistically ($p < 0.05$) lower than Group I. Within the group, the females and males were similar: $0.80 \pm 0.43\%$ vs $0.80 \pm 0.42\%$. Qualitative analysis showed that 1.1-1.9% was the frequently observed

range in 168 (42%) subjects.

All groups were then compared among themselves (Table-2).

Discussion

Literature review of different areas of the world has shown a great variation in haematological parameter and indices due to various factors. The highlighted amongst those factors are; maternal age, socioeconomic status, maternal habits like smoking or alcoholism, hypertension, haemolytic disorder, mode of delivery, complications due to low birthweight and during delivery, nutritional status, sampling site, high altitude, seasonal variation etc. They all affect haematological parameters.⁹⁻¹³

The accurate measurement of reticulocyte counts based upon the local reference ranges provides useful information in diagnosing the underlying pathology¹⁴ and the assessment of bone marrow function.¹⁵

The mean reticulocyte counts in the current study in all age groups, except in the 9-month group, were within the range given for Western population³ (Table-3). In the 9-month age group, the range for Western population has not been reported.³ In the current study, the gender had an impact in reticulocyte count in 3-month and 6-month age groups, whereby the female presented higher counts in the former and lower in the latter group. The findings are in line with a British study that the reticulocyte count value decreases with growing age of an infant.³

The reference range must be obtained, reviewed and established for specific localities due to significant differences and variations depending upon various racial, environmental and maternal factors.¹⁶ They are useful tools in interpreting the result for accurate diagnosis.¹⁷ The infantile reticulocyte count varies with growing age of an infant. Care must be taken when interpreting reticulocyte count results because the percentage reticulocyte count should always be interpreted in conjunction with the haemoglobin levels and red cell count and the nature of blood sample whether capillary or venous.^{18,19}

An overall comparison with different international studies (British, Poland, Thailand, and American) showed variation in reference values of reticulocyte count when compared with the current study results.^{3,20-23}

Early and accurate diagnosis can improve disease management plans. Moreover, timely provision of treatment can reduce infant morbidity and mortality rates. Therefore, the reference values of all the haematological parameters and indices must be reviewed

in specific regions in order to extract the accurate value and its simultaneous comparison to diagnose a disease.

Conclusion

The reticulocyte count in infantile age groups did not show a significant gender-based difference. The values extracted from the current study results are different from the Western studies. Therefore, the reference haematological values must be reviewed and then implemented accurately in order to diagnose the pathology. It will facilitate early management of disease and reduction in infant morbidity and mortality rates.

References

- Harris RJ. Nutrition in the 21st century: what is going wrong. *Arch Dis Child* 2004; 89: 154-8.
- World Health Organization. Iron deficiency anaemia. Assessment, prevention and control: A guide for programme managers. (Online) (Cited 2012 May 22). Available from URL: http://www.who.int/nutrition/publications/en/ida_assessment_prevention_control.pdf.
- Mitchell SL. Reference ranges and normal values. In: Lewis SM, Bain BJ, Bates I, Dacie JV (eds.) *Dacie and Lewis Practical Haematology*. 8th ed. Edinburgh: Churchill Livingstone; 2006; pp 13-20.
- World Health Organization. Blood Safety and Clinical Technology. Guidelines on Standard Operating Procedures for Haematology. Chapter 12 - Manual Reticulocyte Count. (Online) (Cited 2012 May 15). Available from URL: http://www.searo.who.int/en/Section10/Section17/Section53/Section480_1733.htm.
- WebMD. Reticulocyte count. (Online) Page Last Updated: July 29, 2010. (Cited 2012 May 22). Available from URL: <http://www.webmd.com/a-to-z-guides/reticulocyte-count?page=2>.
- WebMD. Reticulocyte count. (Online) Page Last Updated: July 29, 2010. (Cited 2012 April 13). Available from URL: <http://www.webmd.com/a-to-z-guides/reticulocyte-count>.
- Medline Plus. Reticulocyte count. (Online) (Cited 2012 April 13). Available from URL: <http://www.nlm.nih.gov/medlineplus/ency/article/003637.htm>.
- Newborn Services Clinical guidelines. Reticulocyte count. (Online) (Cited 2012 April 13). Available from URL: <http://www.adhb.govt.nz/newborn/Guidelines/Blood/ReticulocyteCounts.htm>.
- Bae SY, Yoon SY, Lim CS, Kim KH, Choi BM. Hematological profile of Korean very low birth weight infants. *J Perinat Med* 2008; 36: 348-52.
- Chen W, Srinivasan SR, Berenson GS. Influence of birth weight on WBC in biracial (black-white) children, adolescents, and young adults. *Am J Epidemiol* 2009; 169: 214-8.
- Yadar S, Chaudhry D, Narayan KC, Mandal RK, Sharman A, Chauhan SS, et al. Adverse reproductive outcomes associated with teenage pregnancy. *McGill J Med* 2008; 11: 141-4.
- Gomo E, Vennervold BJ, Ndhlovu PD, Koestel P, Nyazema NZ, Friss H. Reference values and predictors of white blood cell subset counts. A cross sectional study among HIV seronegative pregnant women in Zimbabwe. *Eur J Obstet Gynaecol Reprod Biol* 2003; 107: 156-62.
- Villamor E, Fowzi WW. Effects of Vitamin A supplementation on immune responses and correlation with clinical outcomes. *Clin Microbiol Rev* 2005; 18: 446-64.
- Ullrich C, Wu A, Armsby C, Rieber S, Wingerter S, Brugnara C, et al. Screening healthy infants for iron deficiency using reticulocyte hemoglobin content. *JAMA* 2005; 294: 924-30.
- Monterrosa EC, Frongillo EA, Vásquez-Garibay EM, Romero-Velarde E, Casey LM, Willows ND. Predominant breast-feeding from birth to six months is associated with fewer gastrointestinal infections and increased risk for iron deficiency among infants. *J Nutr* 2008; 138: 1499-504.
- Keramati MR, Zadeh MA, Farhat AS. The determination of haematological reference values in neonates in Mashhad. *J Ofoghe Danesh J* 2010; 16: 64-71.
- Ahmed FE, AlBakrah MS. Neonatal familial Evans syndrome associated with joint hypermobility and mitral valve regurgitation in three siblings in a Saudi Arab family. *Ann Saudi Med* 2009; 29: 227-30.
- Lab tests online. Reticulocyte Count. (Online) (Cited 2012 April 13). Available from URL: <http://labtestsonline.org/understanding/analytes/reticulocyte/tab/test>.
- Daaea LN, Halvorsen S, Mathisen PM, Mironska K. A comparison between haematological parameters in 'capillary' and venous blood samples from healthy adults. *Scand J Clin Lab Invest* 1988; 48: 723-6.
- Sasanakul W, Singalavanija S, Hathirat P, Mahaphan W, Chuansumrit A. Hemogram in normal newborn babies with special reference to platelet count. *Southeast Asian J Trop Med Public Health* 1993; 24 (Suppl 1): 237-40.
- Turowski D, Wysocka J, Butkiewicz AM. Peripheral blood reticulocytes and their reference range values for percentage, absolute count, and immature fraction in children, measured with flow cytometry. *Folia Histochem Cytobiol* 2000; 38: 31-6.
- Brown AK, Sleeper LA, Miller ST, Pegelow CH, Gill FM, Waclawiw MA. Reference values and hematologic changes from birth to 5 years in patients with sickle cell disease. Cooperative Study of Sickle Cell Disease. *Arch Pediatr Adolesc Med* 1994; 148: 796-804.
- Wolff JA, Goodfellow AM. Hematopoiesis in premature infants with special consideration of the effect of iron and of animal-protein factor. *Pediatrics* 1955; 16: 753-62.