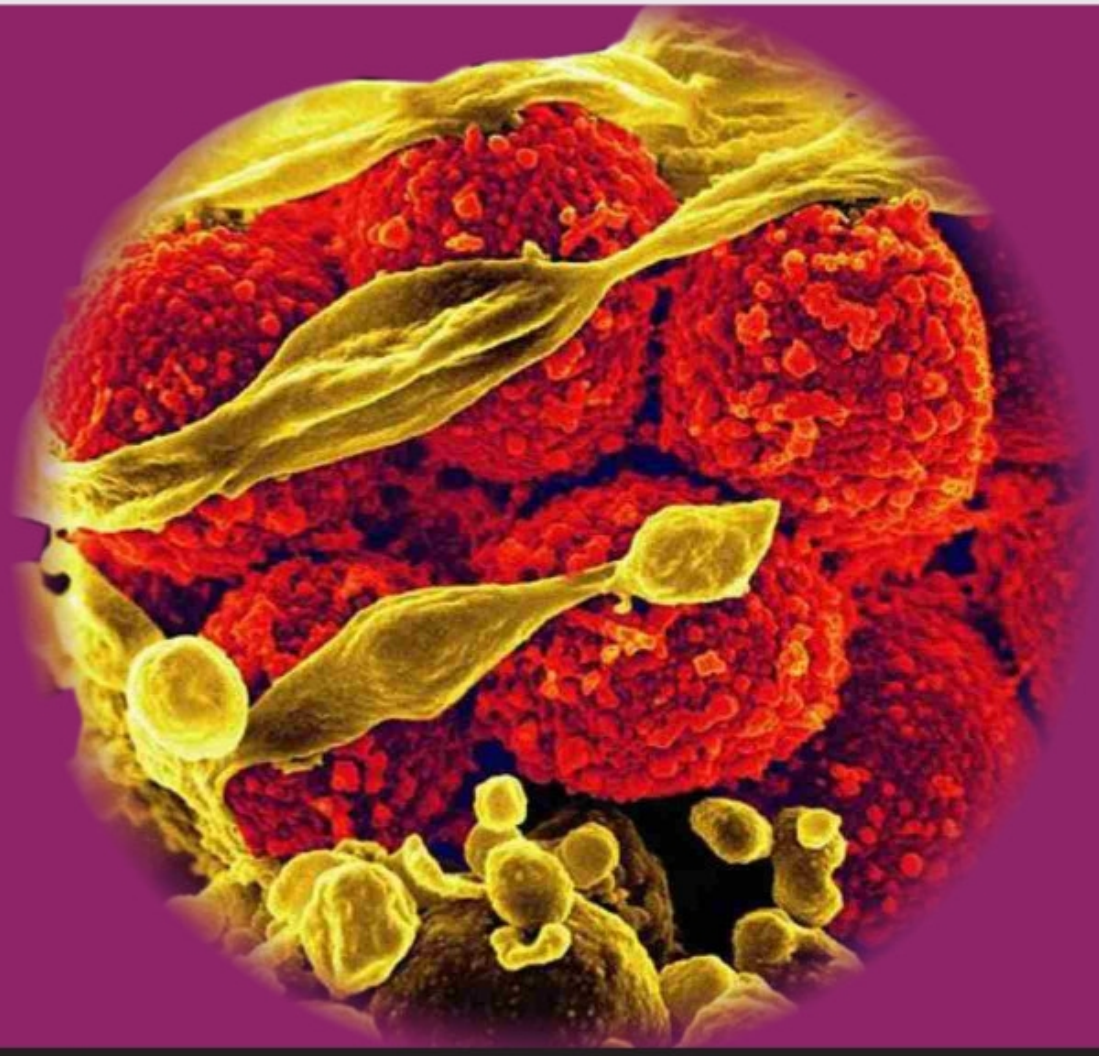
N S H B T

NIGERIAN JOURNAL OF HAEMATOLOGY

Journal of the Nigerian Society for Haematology & Blood Transfusion



ISSN: 2635-3024

VOL. 2 NO. 2, MARCH 2020

ORIGINAL RESEARCH ARTICLE

Haematological Reference Ranges in Healthy Term Neonates in a Tertiary Health Care Centre Northeastern Nigeria

[1]Idi HT, [2]Awwalu S, [2]Babadoko AA, [2]Mamman AI, [3]Abjah U, [4]Mohammed IL

[1]Department of Haematology, Federal Medical Centre, Nguru, Yobe State. [2]Department of Haematology Ahmadu Bello University Teaching Hospital, Zaria. [3]Department of Haematology University of Maiduguri Teaching Hospital, Maiduguri. [4]Department of Paediatrics Federal Medical Centre Nguru, Yobe State.

Corresponding Author:
Idi Hadiza T.,
Department of Haematology,
Federal Medical Centre, Nguru, Yobe State, Nigeria.
Email: hadizatikau@gmail.com

ABSTRACT

Background:
The formation of blood in neonates needs extensive study because of dependence on maternal nutrition. Although haematological parameters for different age groups are important in clinical decision making, there is a dearth of locally-relevant data especially in Northeastern Nigeria.

Aim:
To determine the reference ranges of some haematological parameters of neonatal cord blood in Northeastern Nigeria.

Materials and Methods:
Cord blood samples of 174 apparently healthy neonates delivered in Federal Medical Centre (FMC), Nguru, Yobe State, irrespective of mode of delivery in July to October 2015 were sampled. Health and Research Ethics Committee approval was obtained. All neonates had complete blood counts (CBC) determined by automated haematology analyzer. Reference Value Advisor v2.1 was used to compute 95% Reference Intervals (RIs) using Robust Methods. The precision of the upper and lower limits was assessed by computing 90% Confidence Intervals (CIs) for each limit.

Results:
The mean ± standard deviation of the haematological parameters at birth were haematocrit: 45.8±8.5%, reticulocytes: 4.9±1.4%, mean corpuscular volume (MCV): 106.4±8.7fL, mean corpuscular haemoglobin (MCH): 34.4±2.3pg, mean corpuscular haemoglobin concentration (MCHC): 32.5±1.8g/L, white blood cell count (WBC): 13.2±5.2 x 10^9/L, Neutrophils: 50.8±13.8%, Lymphocytes: 42.7±11.6% and platelets: 355.1±152.0 x 10^9/L. The RI for haematocrit, WBC and platelet counts were 28.6% to 62.4%, 5.4 to 26.0 x 10^9/L and 139.0 to 758.0 x 10^9/L respectively. Other RI were reticulocytes 2.5 to 8.0%, MCV 87.0 to 124.7fL, MCH 29.4 to 38.3pg, MCHC 28.9 to 36.0g/L, Neutrophils 34.9 to 82.0% and Lymphocytes 18.5 to 59.6%.
Conclusion: The values obtained from this study are useful as neonatal reference ranges. We suggest validation in a different cohort of neonates.

Keywords:
Reference range, neonates, cord blood, haematological parameters

INTRODUCTION

Haemopoiesis is a complex process which occurs at different sites of the body depending on the age of the individual. In the foetus, it occurs in the yolksac, liver and preterm marrow.[1] However, immediately after birth the process occurs in the bone marrow with little assistance of the liver.[1,2] The newborn red cells are mainly macrocytic with mean corpuscular volumes (MCV) in excess of 110fl.[1] The haematocrit of neonates ie babies that are less than 28 days are high comparable

to that of adults.[3] Absolute neutrophils in preterm and term neonates have been reported to be greater than that found in older children however, the platelets are same as that of adult.[1]

Newborn haematocrit and haemoglobin concentrations can be used in diagnoses, assessment and follow up of different clinical conditions. Similarly, the white blood cell (WBC) count is essential in evaluating septicaemia in neonates, of which leucopenia is seen in most of the neonates with sepsis.[4]

Locally-relevant, age group-specific reference ranges of haematological parameters are very important in the clinical management of patients. This is important because of possible genetic differences as well as environmental peculiarities such as diets, traditional practices, and infections among others. Hence researchers have demonstrated that differences exist in the haematological and biochemical parameters between Caucasians and Africans.[5] The neonatal development, mode of delivery and Apgar score can affect neonatal parameters.[5] Therefore, reference ranges will be valuable for each geographical region.

Despite the utility of reference ranges there is a dearth of information regarding the reference ranges of neonates in Northeastern Nigeria. Hence this study aimed to determine the reference ranges of haematological parameters of cord blood of neonates at Federal Medical Centre, Nguru Yobe State, Nigeria.

MATERIALS AND METHODS

This was a cross-sectional study conducted on cord blood samples of 174 apparently healthy neonates delivered in FMC Nguru, Yobe State, Nigeria, irrespective of mode of delivery in 2015 from July to October. Institutional Ethical and Scientific approval was obtained. FMC Nguru is located at latitude 12.8°N and longitude 10.5°N respectively. Yobe State shares boundaries with Jigawa and Bauchi States to the Southwest, Borno State to the East and the Niger Republic to the North. The hospital is a tertiary Health Centre, which provides services to all local governments in the states and the Southern part of Niger republic. The hospital provides specialty care to victims affected by insurgency in the region.

The following sample size formula for determination of reference ranges was used:

$$n \geq \frac{Z^2_{(1-\alpha/2)}\,(D+\frac{Z^2_P}{2})}{Z^2_{(1-\frac{\beta}{2})}\left(\frac{\Delta}{100}\right)^2} \quad [6]$$

Where n = minimum sample size, D = constant 1, margin of error = 10%, Z_p = 0.84, $Z_{(1-\alpha/2)}$ = 1.96 and $Z_{(1-\beta/2)}$ = 1.64. Alpha (α) and β were set at 0.05 and 0.20 respectively. The calculated minimum sample size was 181. However, seven were removed because of small for gestational age; only 174 termed normal weight neonates were included in the study.

Following parental consent, all term neonates (37-40 weeks) having normal weights (2.5-4.0kg) and Apgar scores of >8 were enrolled for the study. Neonates born to mothers with hypertensive heart disease, chronic cough, diabetics, sickle cell anaemia, twins, renal disease, malaria, Newborns with birth asphyxia and obvious congenital deformities were excluded.

For each neonate, 5 mls of cord blood were collected immediately at delivery (after cord clamping) into an anticoagulated (Ethylene diamine tetraacetic acid) bottle. Complete blood count (CBC) determined using a 3-parts Shenzhen Mindray B-3200 Haematology autoanalyzer within two hours of sample collection. Reticulocytes count was assessed manually as described by Dacie and Lewis.[7]

Qualitative variables were reported as percentages and charts. Reference Value Advisor v2.1 was used to compute 95% Reference Intervals (RIs) using Robust Methods. The precision of the upper and lower limits was assessed by computing 90% Confidence Intervals (CIs) for each limit. The Anderson-Darling/Symmetry test was used to assess symmetry about the median for the Robust test.

RESULTS

The mean ± standard deviation of the gestational age and birth weight of the neonates were 38.5 ± 1.1 weeks and 3.0 ± 0.5 kg respectively. The mothers' ages ranged from 16 years to 45 years with a mean of 26.5 ± 6.2 years. The mean parity was 3.8 ± 2.8 and with a range of 1-11.

The distribution of haematocrit, WBC and platelet counts are as shown in Figure 1. The mean ± standard deviation of the haematological parameters at birth were haematocrit: 45.8±8.5%, reticulocytes: 4.9±1.4%, mean corpuscular volume (MCV): 106.4±8.7fL, mean corpuscular haemoglobin (MCH): 34.4±2.3pg, mean corpuscular haemoglobin concentration (MCHC): 32.5±1.8g/L, white blood cell count (WBC): 13.2±5.2 x 10^9/L, Neutrophils: 50.8±13.8%, Lymphocytes: 42.7±11.6% and platelets: 355.1±152.0 x 10^9/L. The RI for haematocrit, WBC and platelet counts were 28.6% to 62.4%, 5.4 to 26.0 x 10^9/L and 139.0 to 758.0 x 10^9/L respectively. Other RI were reticulocytes 2.5 to 8.0%, MCV 87.0 to 124.7fL, MCH 29.4 to 38.3pg, MCHC 28.9 to 36.0g/L, Neutrophils 34.9 to 82.0% and Lymphocytes 18.5 to 59.6%. The 90% CI for each lower and upper limit are as shown in Table 1.

DISCUSSION

The reference interval is a yardstick indicating upper or lower limits of laboratory results based on a group of otherwise healthy individuals. The lower limit for haematocrit in this study is lower than the findings of Al-Marzoki *et al* (2012) in Iraq and that of Pasha *et al* (2015) in Pakistan.[8,9] Additionally, the upper limit of the cord blood reference haematocrit in this study is higher than the finding of Basnet *et al* (2016) in Nepal and that of Alharbi *et al* (2017) in Saudi Arabia.[10.11] This buttresses the variability in RI and the need for generating locally relevant data. It is however important to note that RIs are different from clinical decision limits (CDLs). The use of clinical decision limits is very important in the interpretation of the data obtained in this study, because it gives a limit to decide a level of risk as either normal or diseased.[12] The differences in the data may be as a result of differences in the geographical location.

Table 1: Cord Blood Haematological Reference Ranges (n = 174)

Haematological Parameter	**Lower Limit of Reference Interval** (90% CI for Lower Limit)	**Mean ± SD**	**Upper Limit of Reference Interval** (90% CI for Upper Limit)
Haematocrit (%)	**28.6** (26.5, 31.2)	**45.8±8.5**	**62.4** (60.1, 64.4)
Reticulocytes (%)	**2.5** (1.2, 2.6)	**4.9±1.4**	**8.0** (7.2, 9.0)
MCV (fL)	**87.0** (77.8, 91.6)	**106.4±8.7**	**124.7** (121.4, 129.4)
MCH (pg)	**29.4** (26.7, 30.9)	**34.4±2.3**	**39.3** (38.1, 41.0)
MCHC (g/L)	**28.9** (28.6, 29.3)	**32.5±1.8**	**36.0** (35.6, 36.3)
WBC (x 10^9/L)	**5.4** (4.9, 6.0)	**13.2±5.2**	**26.0** (23.7, 28.5)
Neutrophils (%)	**34.9** (34.9, 34.9)	**50.8±13.8**	**82.0** (82.0, 82.0)
Lymphocytes (%)	**18.5** (18.5,18.5)	**42.7±11.6**	**59.6** (59.6, 59.6)
Platelets (x 10^9/L)	**139.0** (104.0, 168.0)	**355.1±152.0**	**758.0** (608, 1134.0)

Key: MCV - mean corpuscular volume; MCH – mean corpuscular haemoglobin; MCHC - mean corpuscular haemoglobin concentration

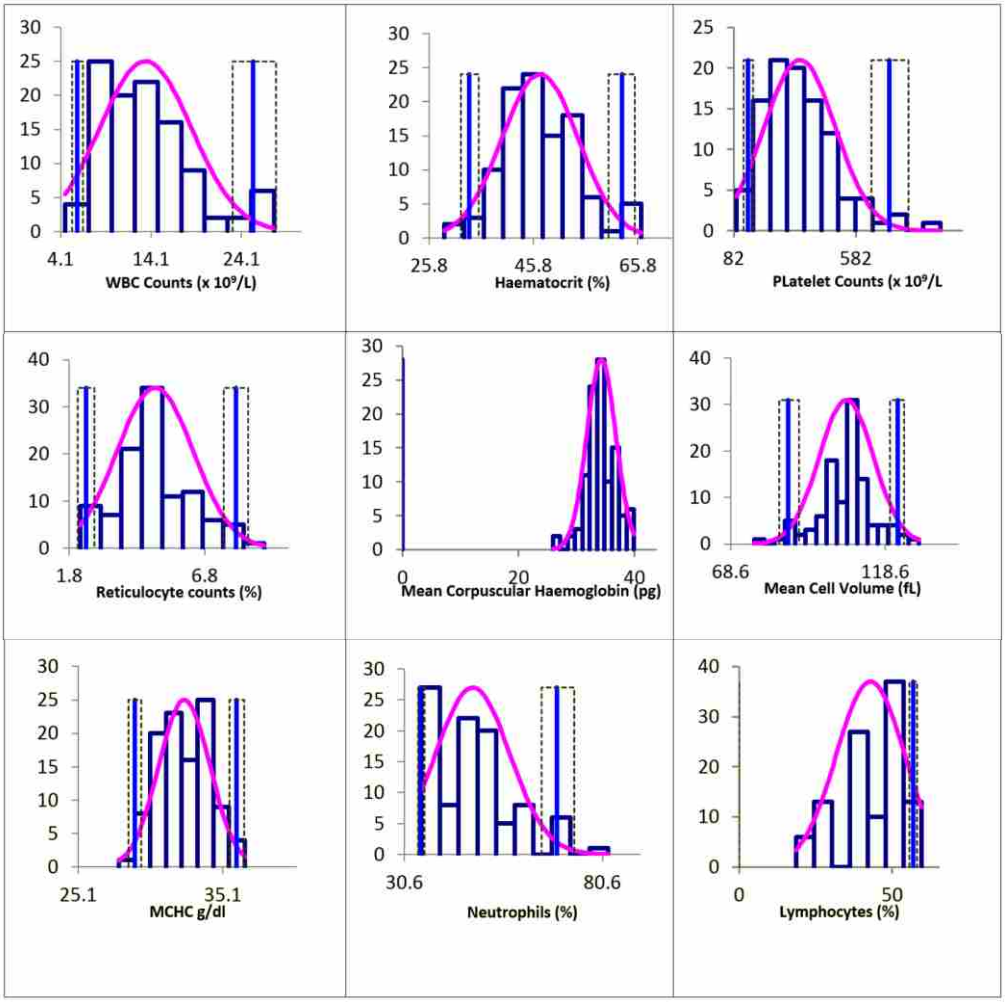


Figure 1: Distribution of neonatal haematological parameters using cord blood (vertical axis represents frequencies; vertical blue lines are upper and lower reference limits; broken lines represent 90% confidence intervals around upper and lower reference limits).

Data obtained in this study indicates that while the lower limit of the reticulocyte RI is lower than what was reviewed by Elgari *et al* (2013) in Sudan the upper limit is higher than the finding of Abdulqadir *et al* (2017) in Kano.[13,14] Reticulocyte production depends on the rate of red cell production in the bone marrow, therefore, neonates have high reticulocyte counts since their bone marrow is 100% red marrow at birth. This shows that irrespective of geographical location, the production of reticulocytes in normal term neonates should be the same, unless there is an underlying disorder.

The lower limits of MCV, MCH and MCHC in this study were lower than the findings of Imoru *et al* (2016) in Sokoto.[15] Similarly, the upper RI limits among the neonates in this study are lower than the findings of Mukuibi *et al* (1995) in Malawi, but higher than the findings of Waggiallah *et al* (2014) in Sudan.[16-17] Values below the lower limit may suggest ineffective erythropoiesis and the value above the upper limit may suggest increased foetal erythropoietic activity.[18]

The WBC, neutrophil and lymphocyte reference counts lower limits in this study are lower than the findings of Imoru *et al* (2016) in Sokoto and that of Basnet *et al* (2016) in Nepal.[10,15] Our upper reference value is higher than the values obtained by Keramati *et al* (2011) in Iran and that of Basnet *et al* (2016) in Nepal.[10,19] White blood cell count is one of the methods used in diagnosis and

management of sepsis. As reported by some researchers, low WBC, low neutrophil count and immature white cells are associated with early onset of sepsis among Caucasian neonate.[20,21] However, Chacha *et al* (2014) in Tanzania have demonstrated that leukocytosis equal to or greater than 13×10^9 /l had sensitivity and specificity of 64.5% and 66.7% respectively in sepsis.[22] White blood cell counts may be relatively feasible and objective tools for diagnosing neonatal sepsis especially in resource-constrained settings such as ours. The differences in the results may be as a result of environmental and genetic differences. Changes in the single nucleotide polymorphism (SNP) rs2814778 has been demonstrated to affect WBC and neutrophil counts.[23] .

The reference lower limit of platelet counts in our study is slightly higher than what was reported by Chrristensen *et al* (2012) in USA.[24] Also, the upper limit in this study is higher than the values obtained by Wiedmeier *et al* (2009) in USA and that of Katsares *et al* (2010) in Greece.[25,26] Monitoring of platelets counts in neonates is very important especially those that are sick because of the possibility of neonatal thrombocytopenia as reported by Jeremiah *et al* (2010) in Port Harcourt.[27] The differences in the results may be as a result of differences in geographical location. Genetic differences have been demonstrated to determine ethnic group low platelet counts. These include SNPs affecting the Thrombopoietin (THPO), Glycoprotein IX (GPIX) and Glycoprotein VI (GPVI) genes.[28,29] Additionally, studies have reported relatively lower limits for platelets among adult Blacks.[30]

It is pertinent for pediatricians' and neonatologists to note that the haematological parameters of neonates changes after 72 hours of birth.[3,31] It is important to note that we studied cord blood samples collected at birth hence changes may be expected beyond this time frame. These changes include declining in PCV, WBC and platelet counts.[31] Therefore, this results is applicable to neonates that are less than or equal to 72 hours of age.

CONCLUSION

The first set of reference intervals for selected haematological parameters for neonates in Northeastern Nigeria have been provided. The 90% CI for both upper and lower limits were computed, thus providing neonatologists with additional data to interpret the variability of each of these limits. Studies to validate these RIs and also longitudinal studies that include the whole of the neonatal period in future are advocated.

Acknowledgment:

We thank the following individuals and laboratory scientists at the study site Mal. Zannah Baba Ali, Mal. Abdullahi Usman Malammadori, Mal. Mohammed Yasidi Bularafa, Mr. Yakubu Dauda Shehu for their roles in patient laboratory analysis.

Conflict of Interest:

There are no conflicts of interest.

Author's Contributions:

Conceptualisation of the study by IHT; study design by IHT, AS, BAA AU and MIL. Literature review by IHT, MAI, AU and MIL; Data was collected by IHT, AS, BAA, MAI. Data analysis was by IHT, AS, BAA. Statistical analysis was done by IHT, AS, BAA, MAI and AU. Manuscript was prepared by IHT, AS, MAI, AU and MIL; and edited/reviewed by all.

REFERENCES

1. Palis M, Sagel GB. Haematology of the Fetus and Newborn: In Williams Hematology. Lichtman MA, Buetler E, Kipps JI, Seligsohn U, Kaushansky K, Prchal JT. eds, 6th Edition. McGraw-Hill Medical Publishing USA; 2008; 99-116.
2. Chang HK. Homeostatic and Pathogenic Extramedullary Hematopoiesis. *J Blood Med*. 2010; 1:13-19.
3. Esan AJ. Haematological Differences in Newborn and Aging: a Review Study. *Hematol Transfus Int J*. 2016; 3(3):178-190.
4. Das B, Das A, Saikia M. A Study of Haematological Parameters in Neonatal Septicaemia. *Indian J Bas Apl Med Res*. 2016; 5(2):491-500.
5. Bain BJ. Ethnic and Sex Differences in the Total and Differential White Cell Count and Platelet Count. *J Clin Pathol*. 1996; 49:664–666.
6. Momeni A, Pincus M, Libien J. Calculating Sample Size for Reference Interval Estimation. In Introduction to Statistical Methods in Pathology.1st edn. Springer International Publishing AG Switzerland 2018. Page 17-18.
7. Bain BJ, Lewis SM, Bates I. Basic Haematological Techniques in Dacie and Lewis Practical Haematology. 10th ed. Bain BJ, Lewis SM, Bate I, eds. Philadelphia: Churchill livingstone, Elsevier; 2006. Page 25-57.
8. Al-Marzoki JM, Al-Maaroof ZW, Kadhum AH. Determination of Reference Ranges for Full Blood Count Parameters in Neonatal Cord Plasma in Hilla, Babil, Iraq. *J Blood Med*. 2012; 3:113-118.
9. Pasha W, Ali W, Ahmed N, Khattak A, Idris M, Nayyer ZA. Reference Haematological Values for Full Term Healthy Newborns from Rural Sindh, Pakistan. *J Ayub Med Coll Abbottabad.* 2015; 27(2):375-377.
10. Basnet S, Singh SK, Sathian B, Mishra R. Reference Ranges for Haematological Values in Umbilical Cord Blood in Pokhara, *Nepal. J Paediatr.soc*. 2016; 36:160-164.
11. Alharbi S, Alkhotani A. Haematological Reference Values for Full-Term, Healthy Newborns of Jeddah, Saudi Arabia. *J Clin Neonatol*. 2017; 6:19-22.
12. Ceriotti F, Henry J. Are my Laboratory Result Normal? "Considerations to be Made Concerning Reference Intervals and Decision Limits. *Ejifcc*. 2008; 19(2):106-114.
13. Elgari AM, Mohammed BA, Hamza KM, Ibraheem SA. Evaluation of Haematological Values of Newborns Umbilical Cord Blood in Khartoum State [Ph D thesis]. Sudan University of Science; 2013. (cited 20 Jan 2020). Available from university of Sudan Library E-Reserve.
14. Abdulqadir I, Sagir GA, Kuliya AG, Tukur J, Yusuf AA, Ndakotsu MA. The Haematological Parameters of Normal Pregnant Women and their Newborns in Aminu Kano Teaching Hospital, Kano, Nigeria. *SSJM*. 2017; 4(3):75-78.
15. Imoru M, Buhari H, Nuhu A. Haematological Reference Values for Full Term Healthy Neonates Delivered within 24 Hours in Sokoto, Northern Nigeria. *Int J Hematol Ther*. 2016; 2(2):1-4
16. Mukuibi JM, Mtimavalye LA, Bradhead R,Mzula E,Dzinyemba WE,Merrick R, *et al*. Some Haematological Parameters in Malawian Neonates. *East Afr Med J*. 1995; 72(1):10-14.
17. Waggagiallah H, Elgari MM. Cord Blood Haematological Profile of Sudanese Neonate at Birth in Khartoum State. *NJIRM*. 2014; 5(5):22-25
18. Patidar S, Shrivstava J, Agrawal A, Dwivedj R. Assessment of Iron Status and Red Cell Parameters in Full Term Small for Gestational Age Neonates at Birth. *J Clin Neonatol*. 2013; 2(30:121-124
19. Keramati MR, Mohammadzadeh A, Farhat AS, Sadeghi R. Determination of Haematological Values of Neonates in Mashhad-Iran. *Inter J Hematol and Oncol*. 2011; 21(2):101-105
20. Thomas BN, Karen PK, Soora WI, David D, Gabriel JE. Interpreting Complete Blood Counts Soon After Birth in Newborns AT Risk for Sepsis. *Paediatr J*. 2010; 126(5):903-909
21. Hornik CP, Benjamin DK, Becker KC, Benjamin DK Jr, Li J, Clark RH, *et al*. Use of the Complete Blood Cell Counts in Early-Onset Neonatal Sepsis. *Paediatr Infect Dis J*. 2012; 31(8):799-802
22. Chacha F, Mirambo MM, Mushi MF, Kayange N, Zuechner A, Kidenya BR, *et al*. Utilization of Qualitative C-reactive Protein Assay and White Blood Cells Counts in the Diagnosis of Neonatal Septicemia at Budango Medical Centre, Tanzania. *BMC Paedr J.* 2014; 14:248.
23. Reich D, Nalls MA, Kao WH, Akylbekova EL,

Tandon A, Patterson N. Reduced neutrophil count in people of African descent is due to a regulatory variant in the Duffy antigen receptor for chemokines gene. *PLoS Genet*. 2009 Jan;5(1):e1000360. doi: 10.1371/journal. pgen.1000360. Epub 2009 Jan 30. PMID: 19180233; PMCID: PMC2628742.

24. Chrristensen RD, Vecchio AD, Henry E. Expected Erythrocyte. Platelet and Neutrophil Value for Term and Preterm Neonates. *J Mater-feta and Neonatol Med*.2012; 25(55):77-79.
25. Wiedmeier SE, Henry E, Sola-Visner MC, Christensen RD. Platelet Reference Ranges for Neonates, Defined using Data From Over 47000 Patients in a Multinational Healthcare System. *J Perinatol*. 2009; 29:130-136.
26. Katsares V, Paparidis Z, Nikolaidou E, Karvounida I, Ardelean K, Drossas N, *et al*. Reference Ranges for Umbilical Cord Blood Hematological Values. *Labmed*. 2009; 40(7):437-439.
27. Jeremiah Z, Oburu JE. Pattern and Prevalence of Neonatal Thrombocytopenia in Port Harcourt, Nigeria. *Pathol and lab Intern*. 2010; 2:27-3.
28. Garner C, Best S, Menzel S, Rooks H, Spector TD, Thein SL. Two candidate genes for low platelet count identified in an Asian Indian kindred by genome-wide linkage analysis: glycoprotein IX and thrombopoietin. *Eur J Hum Genet* 14**,** 101–108 (2006). https://doi.org/10.1038/sj.ejhg.5201499
29. Evans DM, Zhu G, Duffy DL, Montgomery GW, Frazer IH, Martin NG. Multivariate QTL linkage analysis suggests a QTL for platelet count on chromosome 19q. *Eur J Hum Genet*. 2004; 12(10):835–842. doi:10.1038/sj.ejhg.5201248
30. Zezulka AV, Gill JS, Beevers DG. 'Neutropenia' in black West Indians. *Postgrad Med J*. 1987; 63(738):257–261. doi:10.1136/pgmj. 63.738.257
31. Ogundeyi MM, Olarewaju DM, Njokanwa OF, Ogunlesi TA. Haematological Profile of Apparently Healthy Term Babies aged One Day, Three Days and Six Weeks Delivered in Sagamu, Nigeria. *Nig J Paediatr*. 2011; 38(3)125-130.

NSHBT

Nigerian Society For Haematology and Blood Transfusion

ANNUAL SCIENTIFIC CONFERENCE

PORT HARCOURT
2020

17-21 August 2020

Hago Heights
Plot 29 Dr. Peter Odili Road,
Trans Amadi, Port Harcourt

Prepare to share your haematology-related scientific research because it's going to be multidisciplinary. Early bird registration starts in **January 2020.**

For further enquiries send an email to
nshbtloc.phc2020@gmail.com

See You there!