

# Cord, venous and capillary hemoglobin values in healthy neonates

Zainab W. Al-Maarooft<sup>1</sup> Jasim M. Al marzoki<sup>2</sup> Haider Hassan Rasheed<sup>3</sup>

<sup>1</sup>Department of pathology, Faculty of Medicine, University of Babylon, Hilla, Iraq

<sup>2</sup>University of Al-Ameed, Karbala, Ira ,

<sup>3</sup>Babylon Teaching Hospital for Gynecology and Children, Hilla, Iraq

## Abstract

**Background :** Determination of a reference range for the healthy neonates clinically important. The blood hemoglobin concentration are among the most commonly performed of all clinical laboratory tests, the gestational and chronological age of the infant, the infant's sex, timing of cord clamping, and the site of blood sampling are important factors to be considered.

**Objective:** To evaluate hemoglobin concentration in the first postnatal day at different maturity and from different sites of healthy neonatal blood sample.

**Methods:** A cross-sectional study conducted at the Babylon Teaching Hospital for Gynecology and children, in Hilla / Iraq from February 2016 to December 2016. Blood samples were collected from cord , venous and capillary sites in the first postnatal day and hemoglobin value measured by a Diagon® D-Cell 60 automated hematologic analyzer (Diagon Ltd, Budapest, Hungary).

**Results:** The mean  $\pm$ SD of hemoglobin (g/dl) value for preterm neonates were (14.29 $\pm$ 0.65) , (16.02 $\pm$ 0.94) and (18.14 $\pm$ 0.78) for cord , venous & capillary samples respectively. The mean  $\pm$ SD of hemoglobin (g/dl) value for term neonates were (15.78 $\pm$ 1.21) , (17.35 $\pm$ 1.22) and (19.78 $\pm$ 1.59) for cord , venous & capillary samples respectively. The mean  $\pm$ SD of hemoglobin (g/dl) value for post-term neonates were (15.56 $\pm$ 1.34) , (17.06 $\pm$ 1.54) and (19.25 $\pm$ 1.79) for cord , venous & capillary samples respectively. The capillary sample is significantly higher than the venous and cord sample. There is significant difference in results between preterm and (term & post-term) hemoglobin values.

**Conclusion:** The values of this study can be used as local reference values for Hilla / Iraq.

**Key words:** reference range, hemoglobin, cord , venous ,capillary, neonates, Iraq

## INTRODUCTION

It has been internationally agreed that almost all the previously accepted reference values are affected to some extent by age, race, diet, medicines, analytical method etc. This highlights the importance of establishing standard reference values for the local population.<sup>[1]</sup> The use of term "normal range" has lost its importance now. The latest concept is to use terms like "reference values/ranges".<sup>[2]</sup> The blood hemoglobin concentration are among the most commonly performed of all clinical laboratory tests. Many publications have sought to establish reference ranges for hemoglobin concentration among neonates.<sup>[3]</sup> Interpretation of laboratory data in the neonate requires consideration of a number of factors, like gestational and chronological age of the infant, the infant's sex, timing of cord clamping, and the site of blood sampling.<sup>[4]</sup> Hemoglobin concentration rises gradually throughout gestation and peaks shortly after birth. Hemoglobin concentration is relatively stable the last 6 to 8 weeks of gestation, with a mean concentration 16 to 17 g/dl at term. Hemoglobin may increase by 1 to 2 g/dL at birth as a result of placental transfusion. Decreased plasma volume leads to a peak in hemoglobin between 2 to 6 hours of life, with levels stabilizing by 8 to 12 hours of age.<sup>[5]</sup> The timing of cord clamping significantly influences hemoglobin values in the newborn. Around the time of birth, placental blood is rapidly transferred to the infant. Approximately a quarter of this transfusion occurs within 15 seconds of birth and half occurs by the end of the first minute after birth. Delayed cord clamping typically increases the infant's blood volume by 30%, because the placental vessels contain between 75 to 125 mL of blood at birth. Holding the infant above the level of

the placenta prevents placental transfusion and may even lead to neonatal transfusion into the placenta, resulting in neonatal anemia.<sup>[4]</sup> Significant variation in hemoglobin levels may exist, depending on the site of blood sampling. Hemoglobin levels from capillary blood samples typically are higher than those obtained from indwelling venous or arterial catheters. capillary samples may lead to overestimation of the hemoglobin , particularly when the samples are obtained from a poorly perfused extremity. The largest differences between capillary and venous hematocrits exist in very preterm infants, particularly those with acidosis, hypotension, and anemia. In infants younger than 30 weeks' gestation, capillary hematocrit values are 20% higher than venous hematocrit values, compared with capillary values that are 12% higher than venous hematocrit levels at term. A capillary sample by pre warming the extremity may improve the correlation between capillary and venous hematocrit levels. Finally, values of samples from similar sources, such as arterial, venous, and capillary samples, correlate independent of the site of sampling. For example, umbilical, radial, and femoral arterial hemoglobin levels show little variation from sample to sample.<sup>[6]</sup> In the current study, we try to evaluate hemoglobin concentration in the first postnatal day at different maturity ( term , preterm & post term ),and from different sites of healthy neonatal blood (umbilical cord , venous and capillary samples).

## METHODS

A total of 500 healthy neonates were enrolled in this cross-sectional study conducted at the Babylon Teaching Hospital for Gynecology and Children, in Hilla / Iraq from February 2016 to December 2016. They were

subcategorized by gestational age (preterm ,term & post term ) , gender and weight to account for the effects on hemoglobin levels. Exclusion criteria for mothers were abruptio placenta, placenta previa, multiple pregnancy, hypertension, diabetes mellitus, heart, kidney, or lung disease, hematologic disease, and emergency cesarean section .Exclusion criteria for neonates were anemia (a venous hemoglobin value below13.0 g/dl or a capillary hemoglobin value below 14.5 g/dlin term neonates. and in preterm 1 g/dl below those in term) <sup>[4,7]</sup>, polycythemia (central hematocrit >65%).<sup>[7]</sup> (IUGR), Rh & ABO incompatibilities, birth trauma ,perinatal blood loss, birth asphyxia, and obvious congenital abnormality. Written consent had been taken from each mother. Gestational age was determined by abdominal ultrasonography , last menstrual period and neonatal examination. After a routine neonatal care and assessment, a general physical examination then conducted by researcher with special emphasis on skin color, signs of respiratory distress, organomegally, signs of birth trauma and obvious congenital anomalies. After that all newborns were weighted. As soon as the newborn had been delivered, the umbilical cord was clamped, and 2 mL of cord blood was taken from the umbilical vein and collected into a tube containing (K<sub>3</sub>EDTA) as the anticoagulant with gentle inversion several times to avoid sample clotting. After a tourniquet was applied above the cubital fossa with proper cleansing of skin with 70% alcohol, 2 ml of venous blood were withdrawn slowly from the median cubital vein using 5 ml syringe with a gauge 23" needle, The withdrawn blood was dropped in (K<sub>3</sub>EDTA) tube with gentle inversion several times. Capillary blood was drawn by skin prick, from the heel or toe using a sterile lancet followed by gentle squeezing collecting byVitrex<sup>®</sup> Micro hematocrit tube contains uniform sodium heparinisation. Then the name, ID number and site of blood collection

were labeled and transferred to laboratory for analysis. The hemoglobin concentration had been measured from the 3 blood samples using a Diagon<sup>®</sup> D-Cell 60 automated hematologic analyzer (Diagon Ltd, Budapest, Hungary), and the result had been recorded in a formula sheet for data analysis.

**Statistical analysis:** The data were collected, organized, and tabulated using the SPSS software version 24 . The results are expressed in the form of numbers, ranges, and the mean  $\pm$  standard deviation. Independent *t*-test used to analyze the difference in means between two groups. *P* value <0.05 was considered to be statistically significant.

### RESULTS:

This was a cross sectional study ,of 500 neonates, 235 (47%) were males and 265 (53%) females. Term newborns were 441 (88.2%) and 44 (8.8%) were preterm(mean  $\pm$ SD gestational age was 33.4  $\pm$  2.07 , range from 30-36 week ) and 15 (3% ) were post term ( >42 week ). The mean  $\pm$ SD of body weight among preterm neonates was 1.9  $\pm$  0.24, range (1.5-2.3kg) while in term was 3.2  $\pm$ 0.46, range (2.6-4kg) and in post-term neonates was3.2  $\pm$ 0.42 , range (2.7-3.8kg). The hemoglobin values in the 3 sites of blood sampling are summarized in Table1 for male and female, and show no significant gender differences and the mean hemoglobin concentration that obtained from capillary sample was higher than venous and cord sample and in both sex.

To account for the effect of body weight on hemoglobin values, we divided the neonates into 3 groups , the first group involve neonates < 2.5 kg , while the second group involve those between 2.5 and 3.5 kg and third group involve neonates whose weight > 3.5 kg. As shown in the table 2.

**Table 1. Distribution of hemoglobin concentration (g/dl) in three sites of blood sampling according to gender**

Gender	Male (235)	Female (265)	p.value
sample	Mean $\pm$ SD	Mean $\pm$ SD	
Cord	15.56 $\pm$ 1.23	15.72 $\pm$ 1.26	0.43
Venous	17.14 $\pm$ 1.25	17.3 $\pm$ 1.28	0.67
Capillary	19.52 $\pm$ 1.62	19.7 $\pm$ 1.59	0.43

**Table 2. Distribution of hemoglobin concentration (g/dl) in three sites of blood sampling according to weight.**

sample	Weight (kg)	Hb. Mean $\pm$ SD	p.value		
			<2.5 kg	2.5-3.5 kg	>3.5 kg
cord	<2.5 (n=44)	14.29 $\pm$ 0.65		0.03	0.03
	2.5-3.5 (n=350)	15.82 $\pm$ 1.18	0.03		0.24
	>3.5 (n=106)	15.63 $\pm$ 1.32	0.03	0.24	
venous	<2.5 (n=44)	16.02 $\pm$ 0.94		0.04	0.03
	2.5-3.5 (n=350)	17.43 $\pm$ 1.21	0.04		0.22
	>3.5 (n=106)	17.05 $\pm$ 1.27	0.03	0.22	
capillary	<2.5 (n=44)	18.14 $\pm$ 0.78		0.02	0.02
	2.5-3.5 (n=350)	19.83 $\pm$ 1.53	0.02		0.19
	>3.5 (n=106)	19.54 $\pm$ 1.78	0.02	0.19	

**Table 3. Distribution of hemoglobin concentration (g/dl) in three sites of blood sampling according to gestational age.**

sample	G.A	Hb. Mean±SD	Range	p.value		
				Preterm	Term	Post-term
cord	Preterm (44)	14.25±0.65	13.1-15.9		0.03	0.04
	Term (441)	15.78±1.21	13.8-17.8	0.03		0.47
	Post-term (15)	15.56±1.34	13.7-18.1	0.04	0.47	
venous	Preterm (44)	16.02±0.94	14.3-17.6		0.02	0.02
	Term (441)	17.35±1.22	14.8-19.4	0.02		0.45
	Post-term (15)	17.06±1.54	15.2-19.8	0.02	0.45	
capillary	Preterm (44)	18.14±0.78	16.5-20.1		0.01	0.01
	Term (441)	19.78±1.59	17.2-22.5	0.01		0.36
	Post-term (15)	19.25±1.79	17.1-22.6	0.01	0.36	

**Table 4. Correlation among cord, venous and capillary Hb values at different gestational age**

G.A	sample	Hb. Mean±SD	Range	p.value		
				Cord	Venous	capillary
Preterm n=44	Cord	14.25±0.65	13.1-15.9		0.001	0.001
	Venous	16.02±0.94	14.3-17.6	0.001		0.001
	Capillary	18.14±0.78	16.5-20.1	0.001	0.001	
Term n=441	Cord	15.78±1.21	13.8-17.8		0.001	0.001
	Venous	17.35±1.22	14.8-19.4	0.001		0.001
	Capillary	19.78±1.59	17.2-22.5	0.001	0.001	
Post-term n=15	Cord	15.56±1.34	13.7-18.1		0.001	0.001
	Venous	17.06±1.54	15.2-19.8	0.001		0.001
	capillary	19.25±1.79	17.1-22.6	0.001	0.001	

**Table 5. Comparison of neonatal cord blood hemoglobin concentrations(g/dl)in current study with the other studies.**

study	Current study Babil / Iraq		Yazd <sup>[8]</sup> Iran2012		Buenos Aires <sup>[15]</sup> Argentina 1999		Baghdad <sup>[16]</sup> Iraq 2000	Lagos, <sup>[36]</sup> Nigeria 2014	Karachi <sup>[17]</sup> Pakistan 2009
	Term n=441	Preterm n=44	Term n=1558	Preterm n=42	Term n=438	Preterm n=26	Term n=300	Term n=130	Term n=404
<b>Hb. Mean ±SD</b>	15.78 ±1.12	14.29 ±0.65	15.4 ±5.07	14.77 ±1.69	15.5 ±1.1	14.7 ±1.9	15.3 ±0.88	13.29 ±1.5	14.99 ±1.47

**Table 6. Comparison of neonatal venous blood hemoglobin concentrations (g/dl) in current study with the other studies.**

Study	Current study Babil/Iraq			Benin Nigeria <sup>[21]</sup> 1985			Yazd Iran <sup>[8]</sup> 2012		Tübingen Germany <sup>[19]</sup> 2016		Genoa Italy <sup>[20]</sup> 2011		Ankara turkey <sup>[18]</sup> 2006
	Preterm n=44	Term n=441	Postterm n=15	Preterm n=51	Term n=304	Postterm n=47	Preterm n=42	Term n=1558	Preterm early=241 Late =55	Term n=217	Preterm n=164	Term n=656	Term n=45
<b>Hb. Mean ±SD</b>	16.02 ±0.94	17.35 ±1.22	17.06 ±1.54	15.06 ±1.92	15.58 ±2.03	15.73 ±2.17	16.15 ±1.76	17.01 ±7.81	17.2 ±2.0 16.0 ±2.5	17.0 ±2.3	18	18.1	17.0 ± 0.4

**Table 7. Comparison of neonatal capillary blood hemoglobin concentrations (g/dl) in current study with the others.**

Study	Current study Babil / Iraq		Yazd/Iran <sup>[8]</sup> 2012		Ankara Turkey <sup>[11]</sup> 2003	Kenya <sup>[22]</sup> 1999
	Term n=441	Preterm n=44	Termn=1558	Preterm n=42	Term n=95	Term n=942
<b>Hb mean ±SD</b>	19.78 ±1.59	18.14 ±0.78	19.63 ±5.76	18.85 ±1.79	19.36 ±2.32	18.3

There is significant difference in mean hemoglobin values in all samples between newborns weight < 2.5 kg. and ≥ 2.5kg. and this may be related to gestational age factor as most of newborns weight < 2.5 kg. were preterm, and there is no significant difference in mean hemoglobin values between newborns weight 2.5 – 3.5 kg. and > 3.5kg.

As shown in the table 3. the data were compared among preterm, term and post-term groups, the finding showed significant difference in the mean Hb levels between preterm neonates and both term & post-term neonates in 3 sites of blood sampling, while there were no significant differences in Hb levels between term and post-term newborns.

There were statistically significant differences among the mean values of cord, capillary and venous Hb in all gestational age. In all these groups, the capillary blood samples had consistently higher Hb level than venous sample which is higher than cord blood sample, as shown in table 4

### DISCUSSION

This current study shows no significant gender differences in hemoglobin values from the 3 sites of neonatal blood sampling (table 1), and this agrees with Eslami *Z et al.*<sup>[8]</sup>, Adewumi *A et al.*<sup>[9]</sup>, Tauseef *K et al.*<sup>[10]</sup> and Kayiran *SM et al.*<sup>[11]</sup> studies.

In present study we found that the mean value of capillary sample is higher than the venous which in turn higher than cord sample and these findings agree with Eslami *Z et al.*<sup>[8]</sup>, Özbek *Net al.*<sup>[12]</sup>, Kayiran *SM et al.*<sup>[11]</sup> and Thurlbeck *SM & McIntosh N*<sup>[13]</sup> studies. It is believed that the capillary sample contains a mixture of undetermined proportion of blood from both venous/arterial and interstitial fluids. In addition, the degree of perfusion and other factors may further affect the composition of the capillary blood. Microcirculatory disturbances, in particular, can significantly increase capillary hemoglobin compared with venous hemoglobin in neonates.<sup>[14]</sup>

In our study that enrolled 500 newborns, the mean ±SD of Hb values of cord blood were (15.78±1.21) (14.29±0.65) & (15.56±1.34) for term (n= 441), preterm (n=44) and post-term neonates (n=15) respectively. Comparison of Hb values in neonatal cord blood for the current study with reference values cited by other studies (table 5) shows that hemoglobin values comparable with those reported in Yazd/ Iran<sup>[8]</sup>, Buenos Aires/Argentina,<sup>[15]</sup> Baghdad/ Iraq<sup>[16]</sup> and Karachi/ Pakistan.<sup>[17]</sup> but were higher than those from Lagos/ Nigeria.<sup>[9]</sup> Possibly due to varying numbers of neonates, low socioeconomic status, poor nutrition, maternal factors such as low iron, high gravidity and time between birth and clamping of the cord.<sup>[17]</sup>

Our study values of Hb from venous blood samples were (17.35 ±1.22), (16.02 ±0.94) and (17.06 ±1.54) for term, preterm and post-term newborns respectively and in comparison with the other studies that summarized in table 6. the mean Hb concentration is approximately similar to results provided by Eslami *Z et al* in Yazd/Iran 2012<sup>[8]</sup> regarding term and preterm neonates, and to Özyürek *E et al* in Ankara/Turkey 2006<sup>[18]</sup> regarding term neonates, A

study conducted in Tübingen/ Germany 2016<sup>[19]</sup> showed results similar to the present study concerning term and late preterm but its higher in early preterm newborns, on the other hand Melioli *G et al.*<sup>[20]</sup> in Genoa/Italy 2011 revealed a mean Hb values higher than ours in term and preterm newborn and no significant difference between them which is disagree with our results. A study reported by Lorenz *Let al.*<sup>[21]</sup> in Benin/ Nigeria 1985 which is included term, preterm and post-term newborns shows a results of mean Hb lower than ours regarding all groups (table 6).

We found that the mean Hb value of capillary blood samples were (19.78 ±1.59), (18.14 ±0.78) and (19.25±1.79) for term, preterm and post-term respectively, these results agree with Eslami *Z et al* in Yazd/Iran 2012<sup>[8]</sup> and Kayiran *SM et al* in Ankara/ turkey 2003.<sup>[11]</sup> but its higher than the studies provided by McElroy *PD et al* in Kenya 1999.<sup>[22]</sup> (table 7).

### CONCLUSION

The capillary sample is significantly higher than the venous which is higher than cord sample, also there is significant difference in results between preterm and (term & post-term) newborns and these results are comparable to reports from other studies in Asia, Europe and Latin America but its higher than African.

### Recommendation

The hemoglobin reference value for Iraqi neonates warrant a further studies included larger numbers of blood samples and obtaining samples from different areas of Iraq. The values of this study can be used as local reference values for Babil / Iraq.

*Ethical Clearance: Taken from Ethical Committee of Babylon Health Directorate.*

*Source of funding: our selves.*

*Conflict of interest: No conflict*

### REFERENCES

1. Pasha W, Ali W, Ahmed N, Khattak AL, Idris M, Nayyer ZA. Reference haematological values for full term healthy newborns from rural sindh, pakistan. *Journal of Ayub Medical College Abbottabad.* 2015 Jun 20;27(2):375-7.
2. Solberg HE. Establishment and use of reference values. In: Burtis CA, Ashwood ER, eds. *Tietz textbook of clinical chemistry*, 4th ed. St. Louis: Saunders, 2006:425-48.
3. Jopling J, Henry E, Wiedmeier SE, Christensen RD. Reference ranges for hematocrit and blood hemoglobin concentration during the neonatal period: data from a multihospital health care system. *Pediatrics.* 2009 Feb 1;123(2):e333-7.
4. Patrick G. The Neonatal Erythrocyte and its Disorders. In: Orkin, Stuart H. *Hematology and oncology of infancy and childhood*. 8th ed. Philadelphia, US: Elsevier Health Sciences; 2015: 52-75
5. Ohls RK. Evaluation and treatment of anemia in the neonate. *Hematologic problems of the neonate.* Philadelphia: WB Saunders. 2000:137-69.
6. Christensen RD. Expected hematologic values for term and preterm neonates. *Hematologic Problems of the neonate.* Philadelphia, PA: WB Saunders. 2000:120-2.
7. Akhil Maheshwari and Waldemar A. Carlo. The Fetus and the Neonatal Infant, *Blood Disorders In: Robert M. Kliegman, Nelson Textbook of Pediatrics.* 20th ed. Philadelphia, PA: Elsevier Health Sciences; 2016: 880-889.

8. Eslami Z, Ghilian R, Abbasi F. Evaluation of Hemoglobin Concentration of Cord, Capillary and Venous sampling in Neonates. *Iranian journal of pediatric hematology and oncology*. 2012;2(4):159.
9. Adewumi A, Adeyemo TA, Akinsegun AA, Abidoye G, Ebele U, Sulaimon AA. Cord blood full blood count parameters in Lagos, Nigeria. *Pan African Medical Journal*. 2014;17(1).
10. Tauseef K, Ali N, Ahmed S, Zafar H, Anwar J. Variation in reference values of haematological parameters between Regional and International Literature amongst the neonates. *Isra Med J*. 2011 Jan;3(1):20-4.
11. Kayiran SM, Özbek N, Turan M, Gürakan B. Significant differences between capillary and venous complete blood counts in the neonatal period. *Clinical & Laboratory Haematology*. 2003 Feb 1;25(1):9-16.
12. Özbek N, Gürakan B, Kayiran SM. Complete blood cell counts in capillary and venous blood of healthy term newborns. *Actahaematologica*. 2000 Sep 18;103(4):226-8.
13. Thurlbeck SM, McIntosh N. Preterm blood counts vary with sampling site. *Archives of disease in childhood*. 1987 Jan 1;62(1):74-5.
14. Wong EC. Hematology analyzers: special considerations for pediatric patients. *Clinics in laboratory medicine*. 2015 Mar 31;35(1):165-81.
15. Noguera NI, Detarsio G, Perez SM, Bragos IM, Lanza O, Rodriguez JH, Acosta I, Davoli R, Milani AC. Hematologic study of newborn umbilical cord blood. *MEDICINA-BUENOS AIRES*. 1999 Jan 1;59:446-8.
16. Al-Mudallal SS, Al-Moeen MA. Evaluation of the effect of mode of delivery on hematological parameters of healthy full-term newborns. *Iraqi Journal of Medical Sciences*. 2000:29.
17. Qaiser DH, Sandila MP, Ahmed ST, Kazmi T. Haematological reference values for full term, healthy, newborns of Karachi, Pakistan. *JPMA. The Journal of the Pakistan Medical Association*. 2009 Sep;59(9):618.
18. Özyürek E, Cetintaş S, Ceylan T, ÖgÜş E, Haberal A, Gürakan B, Özbek N. Complete blood count parameters for healthy, small-for-gestational-age, full-term newborns. *International Journal of Laboratory Hematology*. 2006 Apr 1;28(2):97-104.
19. Lorenz L, Peter A, Arand J, Springer F, Poets CF, Franz AR. Reference Ranges of Reticulocyte Haemoglobin Content in Preterm and Term Infants: A Retrospective Analysis. *Neonatology*. 2016 Nov 15;111(3):189-94.
20. Melioli G, Risso FM, Sanna A, Serra G, Bologna R, Mussap M, Mangraviti S, Fortini P, Facco F, Reggiardo G, Buonocore G. Reference values of blood cell counts in the first days of life. *Front Biosci*. 2011 Jun 1;3:871-.
21. Scott-Emuakpor AB, Okolo AA, Omene JA, Ukpe SI. Normal hematological values of the African neonate. *Annals of hematology*. 1985 Jul 1;51(1):11-8.
22. McElroy PD, Lal AA, Hawley WA, Bloland PB, Kuile FO, Oloo AJ, Harlow SD, Lin X, Nahlen BL. Analysis of repeated hemoglobin measures in full-term, normal birth weight Kenyan children between birth and four years of age. III. The Asemobo Bay Cohort Project. *The American journal of tropical medicine and hygiene*. 1999 Dec 1;61(6):932-40.