https://doi.org/10.15263/jlmqa.2018.40.1.46

BRIEF COMMUNICATION

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Journal of LABORATORY MEDICINE and QUALITY ASSURANCE

Usefulness of $P_{50,std}$ for the Diagnostic Work-up of Patients with Erythrocytosis

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pISSN: 2384-2458
eISSN: 2288-7261

High oxygen-affinity hemoglobin (Hb) variants and a 2,3-diphosphoglycerate (2,3-DPG) deficiency could cause congenital (familial) erythrocytosis. High oxygen-affinity Hb variants and a 2,3-DPG deficiency might result in low tissue oxygen tension left-shifted oxygen dissociation curves and reduction in the standard P_{50} value ($P_{50,std}$, oxygen tension at which haemoglobin is 50% saturated). Hence, the $P_{50,std}$ value is considered while formulating diagnostic strategies for erythrocytosis. In this study, we established a reference range for $P_{50,std}$ using an International Federation of Clinical Chemistry and Laboratory Medicine-approved equation (Hill's equation) for individual single venous/arterial blood samples. Blood gas analysis results of 243 samples with oxygen saturation ranging from 40%–90% (Hb<16 mg/dL) were selected. The reference range of $P_{50,std}$ was in the 2.5th–97.5th percentile, and was 25.9–27.3 mm Hg. Hill's equation is a simple approved method for evaluating the $P_{50,std}$ values. Only a single sample of venous or arterial blood and a blood gas analyser are required to obtain the $P_{50,std}$. Our study provides a useful tool for the diagnostic work-up of patients with erythrocytosis.
(J Lab Med Qual Assur 2018;40:46-49)

Key Words: Hill's equation, P_{50}, Polycythemia

Received December 18, 2017, **Revision received** January 18, 2018, **Accepted** January 22, 2018

High oxygen-affinity hemoglobin (Hb) variants and a 2,3-diphosphoglycerate (2,3-DPG) deficiency could result in low tissue oxygen tension left-shifted oxygen dissociation curves and reduction in the P_{50} value (the oxygen tension at which haemoglobin is 50% saturated) [1-3]. The P_{50} value has been used as a diagnostic screening tool for erythrocytosis [4]. It can be calculated from a single blood gas analysis result. Hill's equation is derived from a mathematical model for the haemoglobin-oxygen dissociation curve [5]. The standard P_{50} ($P_{50,std}$) has been proposed by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) to minimise errors in the determination of the P_{50} value [6,7]. $P_{50,std}$ is defined as the P_{50} obtained when pH=7.40, PCO_2=5.33 kPa (40 mm Hg), and temperature=37.0℃ [6,7]. P_{50} and $P_{50,std}$ are calculated using the following equations [7]:

$$\log P_{50} = \log PO_2 - \log[SO_2/(1-SO_2)]/n_{Hill}$$

where n_{Hill}=2.7 (Hill's equation constant), PO_2 is the oxygen tension (mm Hg), and SO_2 is oxygen saturation.

$$\log P_{50,std} = \log P_{50} + \Phi H(7.4-pH) + \Phi H(\log 40 - \log PCO_2),$$

in which ΦH: proton *Bohr* factor, −0.43 and ΦC: carbamate *Bohr* factor, 0.05

In this study, we have established the reference range of $P_{50,std}$ using the IFCC approved equation (Hill's equation) for the results of a single venous/arterial blood gas analysis [2,7]. The results of blood gas analysis for samples (venous or arterial sample) obtained between 1st October 2016 and 31st December 2016 were selected. Results for samples showing oxygen saturation between 40% and 90% and Hb levels ≤16 mg/dL (no erythrocytosis) were included in this study [5,7]. An oxygen saturation range of 40%–90% was selected because the relationship between PO_2 and SO_2 in this range was better, as indicated by the calculations made using Hill's equation. Blood gas

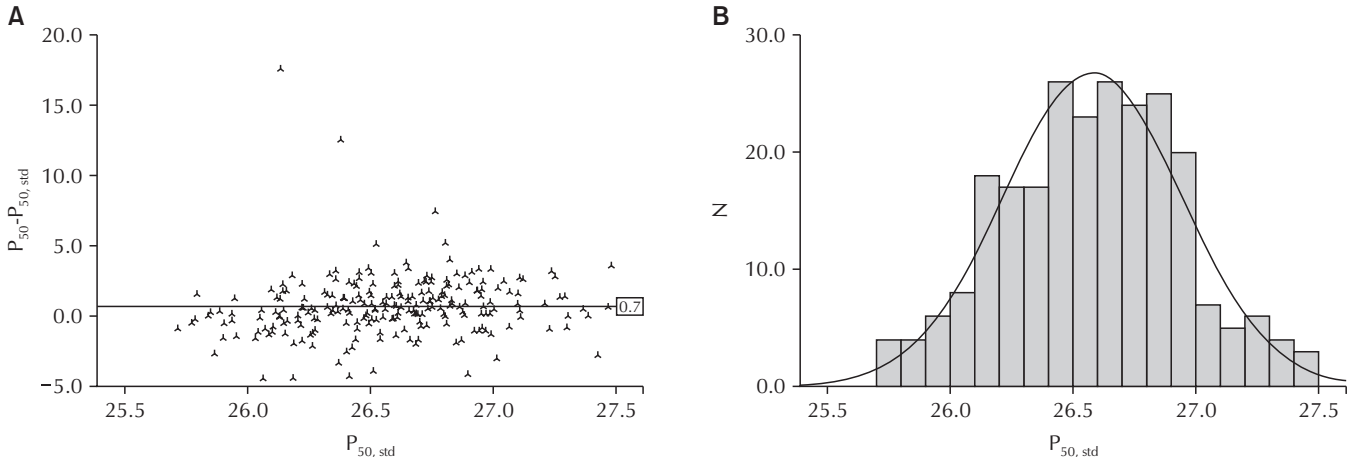

Fig. 1. (A) Bland-Altman plot of the P_{50} and $P_{50,std}$ values, (B) Distribution of the $P_{50,std}$. P_{50} value is slightly higher than the $P_{50,std}$ value (A). A normal distribution (Kolmogorov-Smirnov value=0.458, *P*=0.985) is observed for the $P_{50,std}$. The reference range for the $P_{50,std}$ is in the 2.5th–97.5th percentile (25.9–27.3 mm Hg) (B). Abbreviation: $P_{50,std}$, standard P_{50}.

analysis was performed using ABL 80 Flex (Radiometer, Westlake, OH, USA). All tests were performed at 37℃± 0.2℃, according to the manufacturer's instructions.

Results from 243 individuals (155 males and 88 females) were included in this study. According to the Clinical and Laboratory Standards Institute guidelines, the reference range of $P_{50,std}$ was in the 2.5–97.5 percentile (25.9–27.3 mm Hg) [8]. There were no significant differences between the $P_{50,std}$ values for males and females (26.6 mm Hg in male versus 26.5 mm Hg in female, *P*=0.101). The P_{50} value was slightly higher than the $P_{50,std}$ value (Fig. 1A). Using the Kolmogorov–Smirnov (K–S) test, it was observed that $P_{50,std}$ showed a normal distribution (K–S value=0.458, *P*=0.985) (Fig. 1B).

During the initial diagnostic work-up of patients with erythrocytosis, individuals with acquired primary erythrocytosis (polycythaemia vera with *JAK2* mutation and low serum erythropoietin [EPO] level) and acquired secondary erythrocytosis (pulmonary, renal, cardiac, and others) must be excluded based on their medical histories, results of the *JAK2* mutation study, and serum EPO levels. A low serum EPO level with absence of *JAK2* mutation is suggestive of primary familial congenital polycythaemia associated with an *EPOR* mutation. In individuals with a normal or high serum EPO level, determination of the $P_{50,std}$ value is recommended to rule out the presence of high oxygen-affinity Hb variants or 2,3-DPG deficiency [4]. In a previous report, 4 out of 102 patients with isolated erythrocytosis exhibited low P_{50} values (three Hb variant patients and one 2,3-DPG deficiency patient) [9]. Based on this diagnostic strategy, there has been a report on a Korean patient with a high oxygen-affinity Hb variant (Hb Heathrow; isolated erythrocytosis, normal EPO level, and low P_{50}) [10].

Hb variants are frequently reported in Korea, while 2,3 DPG deficiency is relatively rare [11–13]. Two patients with Hb variants have been identified among 38 Korean patients with isolated erythrocytosis [12]. Therefore, the $P_{50,std}$ value could be useful in the diagnostic work-up of patients with idiopathic erythrocytosis.

ACKNOWLEDGEMENTS

This research was supported by a grant (NRF-2017R1D1A1B04028149) provided by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Republic of Korea.

REFERENCES

1. Patnaik MM, Tefferi A. The complete evaluation of erythrocytosis: congenital and acquired. Leukemia 2009;23:834-44.

2. Doyle DJ. A simple method to calculate P50 from a single blood sample. Int J Clin Monit Comput 1997;14:109-11.
3. Thom CS, Dickson CF, Gell DA, Weiss MJ. Hemoglobin variants: biochemical properties and clinical correlates. Cold Spring Harb Perspect Med 2013;3:a011858.
4. Bento C, Almeida H, Maia TM, Relvas L, Oliveira AC, Rossi C, et al. Molecular study of congenital erythrocytosis in 70 unrelated patients revealed a potential causal mutation in less than half of the cases (Where is/are the missing gene(s)?). Eur J Haematol 2013;91:361-8.
5. Siggaard-Andersen O, Wimberley PD, Gothgen I, Siggaard-Andersen M. A mathematical model of the hemoglobin-oxygen dissociation curve of human blood and of the oxygen partial pressure as a function of temperature. Clin Chem 1984;30:1646-51.
6. Burnett RW. Minimizing error in the determination of P50. Clin Chem 2002;48:567-70.
7. Burnett RW, Covington AK, Fogh-Andersen N, Kulpmann WR, Maas AH, Muller-Plathe O, et al. International Federation of Clinical Chemistry (IFCC), Committee on pH, Blood Gases and Electrolytes: approved IFCC recommendation on definitions of quantities and conventions related to blood gases and pH. Eur J Clin Chem Clin Biochem 1995;33:399-404.
8. Clinical and Laboratory Standards Institute. Defining, establishing, and verifying reference intervals in the clinical laboratory: approved guideline. Wayne (PA): Clinical and Laboratory Standards Institute, 2010.
9. Rumi E, Passamonti F, Pagano L, Ammirabile M, Arcaini L, Elena C, et al. Blood p50 evaluation enhances diagnostic definition of isolated erythrocytosis. J Intern Med 2009;265:266-74.
10. Shin SY, Kim HY, Kim HJ, Kim HG. Hb Heathrow [β103(G5)Phe→Leu], a first report in an Asian patient with erythrocytosis. Yonsei Med J 2017;58:665-7.
11. Hoyer JD, Allen SL, Beutler E, Kubik K, West C, Fairbanks VF. Erythrocytosis due to bisphosphoglycerate mutase deficiency with concurrent glucose-6-phosphate dehydrogenase (G-6-PD) deficiency. Am J Hematol 2004; 75:205-8.
12. Jang JH, Seo JY, Jang J, Jung CW, Lee KO, Kim SH, et al. Hereditary gene mutations in Korean patients with isolated erythrocytosis. Ann Hematol 2014;93:931-5.
13. Lee ST, Kim MS, Choi DY, Kim SK, Ki CS. Incidence of variant hemoglobin (Hb) and increased fetal Hb concentrations and their effect on Hb A1c measurement in a Korean population. Clin Chem 2006;52:1445-6.

적혈구증가증 환자의 진단 워크업을 위한 $P_{50,std}$의 유용성

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High oxygen–affinity hemoglobin (Hb) variants와 2,3–diphosphoglycerate (2,3–DPG) 결핍은 선천성(가족성) 적혈구증가증의 원인이다. High oxygen–affinity Hb variants와 2,3–DPG 결핍은 조직의 산소분압을 낮추고 산소해리곡선을 좌방이동 시키며 standard P_{50} ($P_{50,std}$, 헤모글로빈을 50% 포화시키는 산소분압)을 낮춘다. 따라서 $P_{50,std}$은 적혈구증가증의 진단과정에 사용될 수 있다. 본 연구에서 저자들은 IFCC (International Federation of Clinical Chemistry and Laboratory Medicine)에서 승인한 공식(Hill's equation)을 사용하여 정맥/동맥 혈액가스분석 결과를 토대로 $P_{50,std}$ 참고치를 설정하였다. 산소포화도가 40%–90%인 243개의 혈액가스분석 결과를 선택하였다(Hb < 16 mg/dL). $P_{50,std}$의 참고치는 2.5–97.5 퍼센타일로, 25.9–27.3 mm Hg이었다. Hill's equation은 $P_{50,std}$ 계산을 위한 간편하고 승인된 공식이다. 한 번의 정맥혈 또는 동맥혈과 혈액가스 분석기만으로도 $P_{50,std}$을 구할 수 있다. 본 연구는 적혈구증가증 환자의 진단 워크업을 위한 유용한 진단도구를 제시한 연구라 할 수 있다.

(J Lab Med Qual Assur 2018;40:46–49)

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