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CLINICAL & BASIC RESEARCH

Non-Invasive Haemoglobin Estimation in Patients with Thalassaemia Major

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تقدير مستوى تركيز الهيموجلوبين بطريقة غير تداخلية في مرضى الثلاسيميا الكبرى

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ABSTRACT: ***Objectives:*** This study aimed to validate pulse CO-oximetry-based haemoglobin (Hb) estimation in children and adults with thalassaemia major (TM) and to determine the impact of different baseline variables on the accuracy of the estimation. ***Methods:*** This observational study was conducted over a five-week period from March to April 2012. A total of 108 patients with TM attending the daycare thalassaemia centre of a tertiary care hospital in Muscat, Oman, were enrolled. Spot (Sp) Hb measurements were estimated using a Pronto-7® pulse CO-oximetry device (Masimo Corp., Irvine, California, USA). These were compared to venous samples of Hb using the CELL-DYN Sapphire Hematology Analyzer (Abbott Diagnostics, Abbott Park, Illinois, USA) to determine the reference (Ref) Hb levels. A multivariable linear regression model was used to assess the impact of baseline variables such as age, gender, weight, height, Ref Hb and blood pressure on the Hb estimations. ***Results:*** Of the 108 enrolled patients, there were 54 males and 54 females with a mean age of 21.6 years (standard deviation [SD] = 7.3 years; range: 2.5–38 years). The mean Ref Hb and Sp Hb were 9.4 g/dL (SD = 0.9 g/dL; range: 7.5–12.3 g/dL) and 11.1 g/dL (SD = 1.2 g/dL; range: 7.5–14.7 g/dL), respectively. The coefficient of determination (R^2) was 21% with a mean difference of 1.7 g/dL (SD = 1.1 g/dL; range: −0.9–4.3 g/dL). In the multivariable model, the Ref Hb level (P = 0.001) was the only statistically significant predictor. ***Conclusion:*** The Pronto-7® pulse CO-oximetry device was found to overestimate Hb levels in patients with TM and therefore cannot be recommended. Further larger studies are needed to confirm these results.

Keywords: beta Thalassemia; Validation Studies; Hemoglobin; Pulse Oximetry.

الملخص: الهدف: تهدف هذه الدراسة إلى التحقق من صحة تقدير مستوى تركيز الهيموجلوبين بطريقة قياس التأكسج النبضي لدى الأطفال والبالغين المصابين بمرض الثلاسيميا الكبرى. **الطريقة:** أجريت هذه الدراسة الوصفية على مدى خمسة أسابيع خلال الفترة من مارس إلى أبريل 2012. وشملت الدراسة 108 مرضى بالثلاسيميا الكبرى المراجعين للعيادة النهارية بمركز الثلاسيميا في مستشفى مرجعي بمسقط، سلطنة عمان. تم قياس مستوى تركيز الهيموجلوبين لكل مريض بطريقة التأكسج النبضي بواسطة جهاز Pronto-7® (شركة ماسيمو، إرفين، كاليفورنيا، الولايات المتحدة الأمريكية) ثم مقارنتها بقياس الهيموجلوبين في عينة دم وريدي بواسطة جهاز CELL-DYN Sapphire Hematology Analyzer (شركة أبوت، أبوت بارك، إلينويس، الولايات المتحدة الأمريكية) لتحديد الهيموجلوبين المرجعي. اشتمل نموذج الانحدار الخطي متعدد المتغيرات لتوقع تقدير الاختلافات على المتغيرات التالية: العمر والجنس والوزن والطول وقياس ضغط الدم والهيموجلوبين المرجعي. **النتائج:** شملت الدراسة 108 مرضى نصفهم من الذكور والنصف الآخر من الإناث وكان متوسط العمر 21.6 سنة (الانحراف المعياري 7.3 سنة؛ النطاق 38–2.5 سنة). وكان متوسط تركيزالهيموجلوبين المرجعي 9.4 غرام/ديسيليتر (الانحراف المعياري 0.9 غرام/ديسيليتر؛ النطاق 12.3–7.5 غرام/ديسيليتر) أما متوسط تركيزالهيموجلوبين بطريقة التأكسج النبضي فكان 11.1 غرام/ديسيليتر (الانحراف المعياري 1.2 غرام/ديسيليتر؛ النطاق 14.7–7.5 غرام/ديسيليتر). وأما معامل التعيين (R^2) فكان 21% مع متوسط فارق وقدره 1.7 غرام/ديسيليتر (الانحراف المعياري 1.1 غرام/ديسيليتر؛ النطاق 4.3–0.9 غرام/ديسيليتر). وفي نموذج متعدد المتغيرات كان مستوى تركيز الهيموجلوبين (P = 0.001) هو العامل المتنبئ الوحيد ذو دلالة إحصائية معنوية. **الخلاصة:** يبالغ جهاز التأكسج النبضي Pronto-7® في قياس مستوى تركيز الهيموجلوبين لمرضى الثلاسيميا الكبرى. ولذا لا ننصح باستخدامه في هذه الشريحة من المرضى. وننصح بإجراء دراسات أكبر للتثبت من هذه النتائج.

مفتاح الكلمات: الثلاسيميا الكبرى؛ دراسات التحقق؛ هيموجلوبين؛ التأكسج النبضي.

Advances in Knowledge

- *This study found that the investigated CO-oximetry device (Pronto-7® Pulse, Masimo Corp., Irvine, California, USA) overestimates haemoglobin levels in patients with thalassaemia major (TM).*

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Application to Patient Care

- *The results of this study indicate that this pulse CO-oximetry device should not be used for the purpose of haemoglobin estimation in TM patients as it was found to overestimate haemoglobin levels among this patient population.*

Thalassemia major (TM) is a relatively common inherited blood disorder that is prevalent among the population of Oman. It results from the homozygous loss of beta-globin gene expression and presents in childhood with impaired growth and a failure to thrive.[1,2] Those affected need regular, adequate blood transfusions in order to maintain normal growth and to prevent extramedullary haematopoiesis which leads to the skeletal abnormalities typically seen in TM-affected individuals.[3–5]

Patients with TM are able to tolerate low haemoglobin (Hb) levels without showing the typical symptoms of anaemia and, as a result, the parents of young TM patients do not always realise the importance of maintaining adequate Hb levels. It was hypothesised that regular non-invasive spot (Sp) Hb measurements would improve transfusion planning for TM patients and optimise the prevention of extramedullary haematopoiesis, along with the related skeletal abnormalities of this condition.

The portable Pronto-7® device (Masimo Corp., Irvine, California, USA) has been previously evaluated as a non-invasive method for assessing Hb levels via pulse CO-oximetry Hb estimation.[6–9] Additionally, a former study validated the use of Sp Hb measurements in patients with sickle cell disease (SCD) and in normal blood donors.[10,11] Research has also shown that the Pronto-7® device, which is based on spectrophotometry,[12] could be used effectively as a continuous Hb monitor to assess trends in Hb levels among patients in intensive care settings.[8] However, the use of this device in TM patients with lower Hb levels has not previously been examined and the ability of the device to accurately estimate Sp Hb levels in this patient group has yet to be validated. The aim of this study, therefore, was to validate the pulse CO-oximetry-based method of Hb estimation in patients with TM and to assess the impact of different baseline variables on the accuracy of the estimation.

Methods

This observational study involved 108 patients with TM from the thalassaemia daycare unit of the Sultan Qaboos University Hospital (SQUH) in Muscat, Oman. The study was conducted over a five-week period from March to April 2012 and included participants of all ages and both genders. The diagnosis of TM was based on the absence of Hb A via high-performance liquid chromatography in patients requiring regular blood transfusions.

Two spot Hb measurements (Sp1 Hb and Sp2 Hb) were taken 10 minutes apart using small, medium and large Rainbow® reusable spot check sensors (Masimo Corp.), for patients weighing 10 to 50 kg. These were connected to the Pronto-7® pulse CO-oximetry device according to the manufacturer's recommendations. In addition, a venous sample was taken in order to make a laboratory-based Hb estimation and determine the patients' reference (Ref) Hb levels. For this purpose, the CELL-DYN Sapphire Hematology Analyzer (Abbott Laboratories, Abbot Park, Illinois, USA), was used, as this is considered the gold standard for accurately recording Hb levels.[13]

Continuous variables were presented as means with standard deviations and ranges, while categorical variables were shown as frequencies and percentages. Scatter plots were used to compare the continuous variables (Ref Hb *versus* Sp1 Hb and Sp1 Hb *versus* Sp2 Hb). A linear regression model was used to estimate the intercept, the beta coefficients and the coefficient of determination (R^2). The R^2 value was used as an estimate of goodness-of-fit and the two methods of Hb estimation were compared using a Bland-Altman plot. Multivariable linear regression was used to assess the impact of selected baseline characteristics (age, gender, weight, height, Ref Hb and blood pressure) on the difference between the Sp1 Hb and Ref Hb levels. An alpha error threshold of 0.05 was considered for statistical significance. The statistical software Stata, Version 11 (StataCorp LP, College Station, Texas, USA) was used for all descriptive statistics, graphs and analytical tests.

The study protocol was reviewed and approved by the Medical Research & Ethics Committee at the College of Medicine & Health Sciences, Sultan Qaboos University (MREC#506). All patients gave informed consent after being briefed by a trained medical officer or nurse.

Results

A total of 108 patients (54 male and 54 female) with TM were enrolled in this study, with a mean age of 21.6 ± 7.3 years (range: 2.5–38 years). Only 10% of

Table 1: Baseline characteristics among patients with thalassaemia major (N = 108)

Variable	Mean value
Age in years	21.6 ± 7.3
Male: female ratio*	54:54
Ref Hb in g/dL	9.4 ± 0.9
Spot 1 Hb in g/dL	11.1 ± 1.2
Spot 2 Hb in g/dL	10.8 ± 1.2
Weight in kg	52.4 ± 16.4
Height in cm	154 ± 15
BP in mmHg	111/66 ± 13/9

Ref = reference; Hb = haemoglobin; BP = blood pressure.
**This value is not expressed as mean ± standard deviation.*

the patients were aged 11 years or younger. The mean Ref Hb, Sp1 Hb and Sp2 Hb levels were 9.4 ± 0.9 g/dL (range: 7.5–12.3 g/dL), 11.1 ± 1.2 g/dL (range: 7.5–14.7 g/dL) and 10.8 ± 1.2 g/dL (range: 7.5–14.2 g/dL), respectively. The mean weight and height of the participants were 52.4 ± 16.4 kg (range: 12.8–100.7 kg) and 154 ± 15 cm (range: 88–184 cm), respectively. The mean blood pressure of the patients was 111/66 ± 13/9 mmHg (range: 80/47–138/100 mmHg) [Table 1].

The scatter plot of Sp1 Hb *versus* Ref Hb is shown in Figure 1, with an R^2 of 21%. The mean difference between Sp1 Hb and Ref Hb was 1.7 ± 1.1 g/dL (range: –0.9–4.3 g/dL). A Bland-Altman plot of the differences in estimation between Sp Hb and Ref Hb levels indicated that most of the variations were greater than 1 g/dL [Figure 2], indicating that the Pronto-7® pulse CO-oximetry device overestimated the patients' Hb levels. The upper and lower limits of agreement were

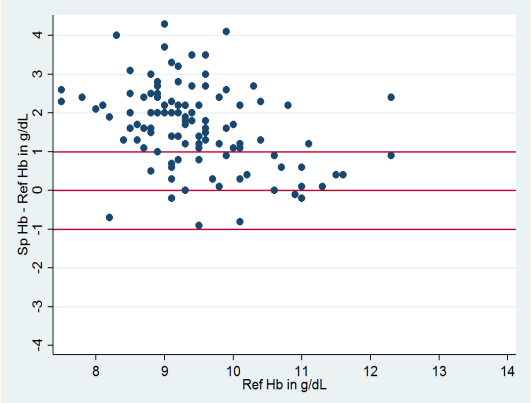

Figure 2: Distribution of the differences between spot (Sp) haemoglobin (Hb) levels estimated by a pulse CO-oximetry device and reference (Ref) Hb levels among patients with thalassaemia major (N = 108). The y-axis shows the difference between Sp Hb and Ref Hb (Sp Hb – Ref Hb) and the x-axis shows the value of the Ref Hb. The three continuous lines represent the differences at +1, 0 and -1 (starting from the top).
Ref Hb = reference haemoglobin; Sp Hb = spot haemoglobin.

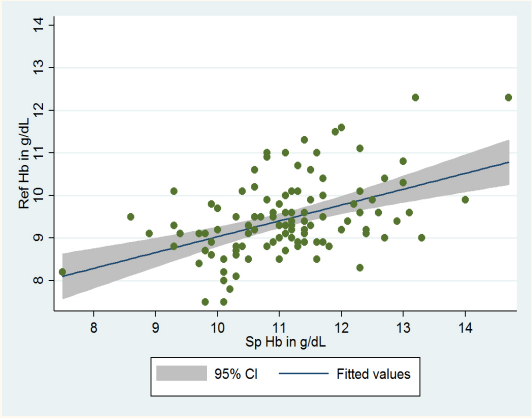

Figure 1: Scatter plot showing reference haemoglobin (Hb) *versus* spot Hb levels estimated by a pulse CO-oximetry device among patients with thalassaemia major (N = 108).
Ref Hb = reference haemoglobin; Sp Hb = spot haemoglobin; CI = confidence interval.

3.8 and –0.4 g/dL, respectively. In the multivariable model, Ref Hb level was the only statistically significant predictor of the difference (P = 0.001). The R^2 of the two CO-oximetry Hb measurements, which were taken 10 minutes apart, was 46% [Figure 3].

Discussion

The Pronto-7® pulse CO-oximetry device was found to overestimate the Hb level in TM patients by a mean of 1.7 g/dL, with clinically unacceptable limits of agreement. The use of this device in estimating Hb levels, therefore, cannot be recommended for this group of patients. The only significant predictor for this discrepancy in estimation was the Ref Hb level, which was assessed using a gold standard method. The reproducibility of the estimation remained high when

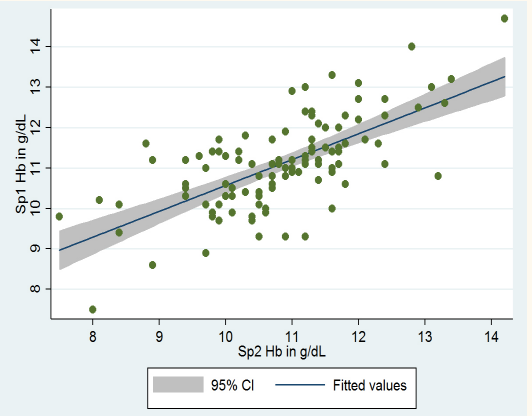

Figure 3: Scatter plot of two spot haemoglobin measurements by the CO-oximetry device among patients with thalassaemia major (N = 108).
Sp Hb = spot haemoglobin; CI = confidence interval.

the test was repeated after a short interval.

A spectrum bias is a common problem in diagnostic studies, leading to a false increase in the sensitivity and specificity of new diagnostic tests.[14] Previous studies have demonstrated the validity of the Pronto-7® pulse CO-oximetry device for use in normal blood donors and in patients with SCD.[10,11] In contrast, the current study observed that the same device overestimates Hb levels in TM patients by a clinically significant margin. The difference in the validation results may potentially be due to the lower Hb levels commonly seen in patients with TM; these lower HB levels are not frequently observed among patients with SCD or in normal blood donors. The use of this device is therefore not recommended in patients with low Hb levels, for instance among those suspected of being anaemic.

Another reason why different validation results were observed in the current study may be due to variations within the chosen patient populations. The present study included paediatric patients, whereas the previous studies investigating blood donors and patients with SCD did not.[10,11] In the study investigating blood donors, differences in height may have been partially responsible for the different estimates.[10] This is because the three different sizes of sensor may not have been optimal in terms of fit for certain patients with different heights. In the two previous studies, as well as the current study, the same sized sensors were used.[10,11] However, if the fit of the sensor was not optimal on all patients, this could have affected the accuracy of the estimates produced, leading to a larger difference between estimates. In the current study, the age and height of the participants were not found to be statistically significant enough to explain the difference in estimates. It is possible that modelling the interaction between patients' heights or ages and differing Hb estimates may shed further light on the issue; this was not carried out in the present study due to the complexity of such modelling and the relatively small sample size.

Although different conclusions were drawn with regards to the validity of the pulse CO-oximetry device, it is interesting to note that similar findings were noted between the current study and the previous studies in the numerical estimates of the studied subjects, when comparing the R^2.[10,11] In validation studies, it is important that any conclusions drawn should not be based on a single parameter, but instead take into consideration all available parameters, the limitations of each and the fact that these complement each other. In the current study, the Bland-Altman plot showed the distribution of the difference between estimations and revealed the bias of overestimation in the Sp Hb levels compared with the Ref Hb levels. The standard methodology of the present study has many merits and is frequently utilised in diagnostic studies.[15]

In light of its limitations, the results of this study should be interpreted with caution. There was a limited supply of sensor sizes, although every attempt was made among individual patients to choose the finger with the best physical fit for the sensor used. The fact that only three sizes of sensors were available for use (small, medium or large), without taking into consideration the need for other sizes among the patients, likely biased the difference between estimations to be larger than the true value. Moreover, the inclusion of both children and adults in one study may have increased the variance and decreased the power, making it difficult to truly show the validity of the device. High levels of fetal Hb in children may have interfered with the measurement accuracy of the device.[16] Additionally, as the majority of the patients in the study were adults, the conclusions drawn from this study cannot be generalised to paediatric patients, although the authors would still caution the use of this device in children. Paediatric patients were included in this study so as to assess this device among the age group that may potentially benefit the most from its use. Furthermore, it should be noted that previous research has indicated that the Pronto-7®'s performance was satisfactory when used as a continuous monitor, rather than a single spot measurement as in the present study.[8] Finally, the study was also limited by its relatively small sample size, which decreased the power to detect significant factors to predict the discrepancy in Hb estimates.

The study has a number of strengths despite the above limitations. Foremost, this is the first and only study to specifically address the population of TM patients. This study included patients with Hb levels as low as 7.5 g/dL, although patients with TM can have levels lower than this. In addition, the biased estimate found in this evaluation of the Pronto-7® device calls for caution in the interpretation of diagnostic studies with potential spectrum bias.

Conclusion

The results of this study demonstrate that the Pronto-7® pulse CO-oximetry device overestimates the haemoglobin level in patients with TM, and therefore cannot be recommended for use with this group. The authors further caution the use of this device in children and in patients suspected of being anaemic. Studies with larger patient populations are needed to confirm these results.

CONFLICT OF INTEREST

The Pronto-77® pulse CO-oximetry device and the reusable sensors were provided free of charge by Muscat Pharmacy, Oman. Muscat Pharmacy had no access to the data and had no input in the analysis or the writing of the manuscript.

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