thalassemia reports

MDPI

Article

Comparing Spectrophotometric Hemoglobin Concentrations with Conventional Laboratory Cell Analyzers in Transfusion-Dependent Beta-Thalassemia Patients

Khaled Yassen [1,*], Nawal Omar [2], Abdulaziz Bushehab [3], Renad AlSubaie [4], Lina AlMudayris [4], Sara A. Albunyan [4], Shaima AlAkroush [4], Sherif Saleh [5], Dur I. Shahwar [1] and Ossama Zakaria [5]

[1] Anesthesia Unit, Surgery Department, College of Medicine, King Faisal University, Hofuf 31983, Al Ahsa, Saudi Arabia; dbhutta@kfu.edu.sa
[2] Hereditary Blood Disease Center, Hofuf 36422, Al Ahsa, Saudi Arabia; gelas3@hotmail.com
[3] Nursing Services, Hereditary Blood Disease Center, Hofuf 36422, Al Ahsa, Saudi Arabia; abdulazizbushehab@gmail.com
[4] College of Medicine, King Faisal University, Hofuf 31983, Al Ahsa, Saudi Arabia; 220010363@student.kfu.edu.sa or subaierenad@gmail.com (R.A.); leenaamudaires@gmail.com or linaalmudayris@gmail.com (L.A.); susuhmb@gmail.com (S.A.A.); shaimaaaliii18@gmail.com (S.A.)
[5] Surgery Department, College of Medicine, King Faisal University, Hofuf 31983, Al Ahsa, Saudi Arabia; ssaleh@kfu.edu.sa (S.S.); ozakaria@kfu.edu.sa or ossamaz2004@gmail.com (O.Z.)
* Correspondence: kyassen@kfu.edu.sa

Abstract

Background/Objectives: Thalassemias, a hereditary condition commonly linked to chronic anemia, require regular blood transfusions and repeated blood draws for assessments of hemoglobin (Hb) content, which can be uncomfortable. A promising substitute for laboratory hemoglobin testing is non-invasive spectrophotometric hemoglobin (SpHb) monitoring; however, its applicability particularly among blood transfusion-dependent thalassaemic patients needs to be investigated. This study's primary goal was to investigate the relationships and agreements between SpHb, g/dL, and an automated hematology analyzer (Hb, g/dL) in this particular patient population. The secondary goal was to track how blood transfusions affect SpHb, g/dL, laboratory Hb, and pleth variability index (PVI, %). Methods: In this study, sixty patients were included. A Masimo Radical-7 pulse CO-oximeter was used to measure the SpHb, and a Sysmex XN-1000 hematological analyzer measured the laboratory Hb. Results: The results revealed a significant correlation between SpHb and laboratory Hb (n = 108, r = 0.587, $p < 0.001$) but also demonstrated that SpHb consistently overestimated laboratory Hb levels, with a mean bias of −1.18 g/dL (95% CI: −1.4344 to −0.9267). The Bland–Altman analysis showed a good degree of reliability between this bias (SpHb–Hb) and laboratory Hb (g/dL), with an Intra Class Correlation (ICC) of 0.613 but with a wide 95% CI ranging from 0.557 to 0.736 (t = 3.817, $p < 0.001$). The 95% limits of agreement ranged from −3.7893 to +1.4228 g/dL. Conclusions: This significant bias restricted the application of SpHb as a trustworthy method for assessing hemoglobin levels in patients with blood transfusion-dependent thalassemia. Nonetheless, the capability to monitor SpHb and PVI variations during blood transfusions offered a real-time assessment of the impact of transfusions on patients' hemoglobin levels and volume status.

Keywords: thalassemia; spectrophotometric hemoglobin; laboratory hemoglobin; blood transfusion

check for updates

Academic Editor: Anastasios G. Kriebardis

Received: 13 March 2025
Revised: 14 August 2025
Accepted: 3 September 2025
Published: 10 September 2025

Citation: Yassen, K.; Omar, N.; Bushehab, A.; AlSubaie, R.; AlMudayris, L.; Albunyan, S.A.; AlAkroush, S.; Saleh, S.; Shahwar, D.I.; Zakaria, O. Comparing Spectrophotometric Hemoglobin Concentrations with Conventional Laboratory Cell Analyzers in Transfusion-Dependent Beta-Thalassemia Patients. *Thalass. Rep.* **2025**, *15*, 9. https://doi.org/10.3390/thalassrep15030009

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Thalassemias are a genetic disorder characterized by the insufficient production of alpha or beta hemoglobin chains leading to anemia and associated complications. Beta-thalassemia requires regular blood transfusions to maintain adequate hemoglobin levels and prevent severe anemia [1]; however, chronic blood transfusions carry significant risks, including iron overload, which can lead to long-term complications such as organ damage and endocrine dysfunction [2]. The accurate monitoring of hemoglobin (Hb) levels is therefore critical to optimize transfusion therapy, ensure adequate oxygen delivery, and minimize associated morbidities [3]. The traditional methods of hemoglobin measurement, including laboratory-based automated hematology analyzers, remain the gold standard for these measurements' precision and reliability. However, these methods have several disadvantages including patient discomfort from repeated blood sampling and venous access complications [4]. The repetitive nature of invasive testing, particularly in critical ill, can also cause significant stress, further diminishing the quality of life [5]. Real-time non-invasive hemoglobin monitoring methods such pulse CO-oximetry-based spectrophotometric hemoglobin (SpHb) via the skin have shown promising results. These gadgets are especially desirable because of their mobility, simplicity of use, and quick results without requiring blood samples [4]. Despite their promise, SpHb monitoring's accuracy and clinical usefulness are still not well understood, especially in patients with transfusion-dependent beta-thalassemia whose hemoglobin profiles are frequently complicated by hemolysis, chronic anemia, and frequent transfusions. In stable patients, non-invasive hemoglobin assessment has been demonstrated in numerous trials to be generally reliable. This may lessen the need for frequent invasive blood collection particularly in groups who require frequent transfusions [5,6]. By reducing the need for recurrent invasive procedures, non-invasive hemoglobin assessment has the potential to improve the quality of care for patients [6]. The clinical application of non-invasive hemoglobin testing in thalassemia patients is still being investigated despite its potential. Even though pulse CO-oximetry has shown consistent results in a variety of clinical contexts, the accuracy can be impacted by variables such as patient movement, skin pigmentation, and perfusion [5]. This study primarily aimed to compare spectrophotometric hemoglobin (SpHb, g/dL) measured by a point-of-care pulse CO-oximetry device with standard laboratory hematology analyzers (Hb, g/dL) in transfusion-dependent beta-thalassemia patients. The secondary aim was to monitor the changes in SpHb (g/dL), laboratory Hb (g/dL), and pleth variability index (PVI, %) with blood transfusion.

2. Materials and Methods

The Al-Ahsa Health Cluster in Hofuf City, Saudi Arabia, authorized this diagnostic test accuracy study (IRB KFHH No.: H-05-HS-065; IRB Log No.: 04-EP-2025) on the 1 January 2025.

2.1. Patients

This study was performed at the Hereditary Blood Disease Center in Hofuf, Al-Ahsa, Kingdom of Saudi Arabia. Before the study began, all subjects gave their informed consent. Clinical and demographic information was taken from their medical records. Patients of all age groups with transfusion-dependent beta-thalassemia who had a stable clinical status and presenting for regular, planned blood transfusions met the inclusion criteria. Patients who were hemodynamically unstable, in need of an immediate blood transfusion, or who refused consent were excluded.

2.2. SpHb, Hb, and Pleth Variability Index (PVI)

The Sysmex XN 100i (Sysmex Europe SE, Norderstedt, Germany) was used to assess the conventional laboratory hemoglobin, while the Radical-7 pulse CO-oximeter (Masimo Corporation, Irvine, CA, USA) was used to measure non-invasive hemoglobin concentrations (SpHb). The Radical-7 pulse CO-oximeter (Masimo Corporation, Irvine, CA, USA) was used to determine the PVI (%). A fingertip probe fitted with rainbow signal extraction technology, which measures hemoglobin concentrations non-invasively utilizing a range of wavelengths (575–1100 nm), was used to measure the SpHb. To reduce movement interference, SpHb was solely monitored while patients were at rest. To prevent light interference, an optical shield was placed over the finger sensors. The fluctuation in plethysmographic signals throughout respiratory cycles is known as the PVI, and it is a measure of fluid responsiveness. It typically ranges between 9 and 13%. Both a trend graph and a percentage (numerical value) representation of the PVI were shown. There is less variation in the Perfusion Index (PI) across a respiratory cycle when the PVI value is lower. The patient is more likely to react to fluid infusion by increasing cardiac output if the variability is higher. The Perfusion Index (PI), which is determined by dividing the pulsatile blood flow by the static blood flow, indicates the intensity of the pulse at the measurement site. Patients were excluded if their PI was less than 0.3, which is necessary for reliable SpHb values. Patients were instructed to stay still for five minutes prior to measurement in order to reduce interference. Using the Masimo Radical-7, the oxygen saturation percentage (%) was calculated and recorded in the findings. According to a recent study by Gomaa et al., the accuracy of SpHb is impacted by oxygen saturation [7].

2.3. Measurement of Hemoglobin in the Laboratory

The Sysmex XN-1000 hematological analyzer (Sysmex Europe SE, Norderstedt, Germany) was used to measure the blood Hb concentration in the laboratory. Venipuncture was performed to obtain the blood samples, and the cyanide-free sodium lauryl sulfate (SLS), which creates a detectable SLS-HGB complex, was used in the measurements. After being carefully combined in a K2EDTA test tube (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA), two milliliters of blood were sent immediately to the central laboratory for hematology analyzer analysis. All blood samples and SpHb tests were carried out by qualified phlebotomy professionals. Prior to and during blood transfusion, SpHb and venous laboratory Hb measurements were simultaneously recorded.

2.4. Sample Size and Methodology

The study by Okazaki et al. [8], who evaluated the precision of non-invasive hemoglobin monitors, served as the basis for determining the sample size. Assuming a statistical power ($1 - \beta$) of 80% and a significance level of 5% ($\alpha = 0.05$), a minimum of 59 patients was determined to be adequate for this agreement study. To keep the sample size at the necessary level, any patient withdrawals were substituted. Participants were chosen from clinical cases that were available at the Hereditary Blood Disease Center in Hofuf, Alahsa, Saudi Arabia, during the study period using convenience sampling.

2.5. Analysis of Statistics

SPSS (Statistical Package for Social Science, version 25) was used to analyze the data [9]. The Shapiro–Wilk test indicated that the continuous variables were normally distributed [10]. Then, parametric statistics were used [11]. The paired samples *t* test was used for comparisons [12]. The Bland–Altman assessment, intra-class correlation (ICC), and parametric Pearson's correlation were used to test correlations and agreements [13–15].

3. Results

A total of 63 transfusion-dependent thalassaemia patients consented to the trial, but 3 were disqualified because their PI signals were too weak (<0.3). Males (n = 30, 50.0%) and females (n = 30, 50.0%) made up the remaining 60 patients. Table 1 displays the body mass index (BMI), sex, and age (years) of the individuals. Overall, 16 of the 60 patients included were under 18 years old. According to the blood group analysis, the largest percentage of patients had O+ (26, 43.33%), followed by A+ (12, 20.0%), B− (11, 18.33%), B+ (5, 8.33%), O− (4, 6.67%), and AB+ (2, 3.33%) (Table 1).

Table 1. Demographics, body mass index (BMI), and blood group type.

Age (years)	
Min–Max	0.42–52.17
Mean ± S.D.	23.67 ± 12.60
95% CI of the mean	20.41–26.92
Sex	
Male	30 (50.00%)
Female	30 (50.00%)
BMI	
Min–Max	14.42–31.88
Mean ± Std. Deviation	20.60 ± 3.91
95% CI of the mean	19.59–21.61
Blood Group	
A+	12 (20.00%)
B+	5 (8.33%)
B−	11 (18.33%)
O+	26 (43.33%)
O−	4 (6.67%)
AB+	2 (3.33%)

Min–Max: Minimum–Maximum. S.D.: Standard Deviation. CI: Confidence Interval.

SpHb consistently overestimated the laboratory Hb levels, with a mean bias of −1.18 g/dL (95% CI: −1.4344 to −0.9267)—Figure 1. A positive correlation existed between laboratory Hb and SpHb, with a Pearson's correlation (r) (n = 108) of r = 0.587, $p < 0.001$—Figure 2.

A simple scatter diagram representing this is shown in Figure 2, with a regression line showing a moderately positive correlation between the spectrophotometric hemoglobin (SpHb, g/dL) and laboratory hemoglobin (Hb, g/dL).

A Bland–Altman plot was used to evaluate the agreement between SpHb and laboratory Hb values (Figure 3). With a 95% CI ranging from −1.434 to −0.926 and a limit of agreement between −3.78 and 1.4 at ±1.96 SD, the mean difference or bias of Hb-SpHb was −1.18 (SD 1.76) g/dL. Nevertheless, this wide 95% bias range (=−5.2 g/dL) suggests that SpHb has poor precision and that it cannot be used to accurately predict laboratory hemoglobin levels in thalassemic patients. A good degree of reliability was observed between the bias (Hb-SpHb) and laboratory Hb (g/dL), with an Intra Class Correlation (ICC) of 0.613 and a 95% CI from 0.557 to 0.736 (t = 3.817, $p < 0.001$)—Figure 3. Table 2 demonstrates a significant ($p < 0.001$)) increase in post transfusion hemoglobin concentrations as measured by both Masimo Radical-7 pulse CO-oximeter (Masimo Corporation, Irvine, CA, USA) (SpHb) and Sysmex XN 100i (Sysmex Europe SE, Norderstedt, Germany).

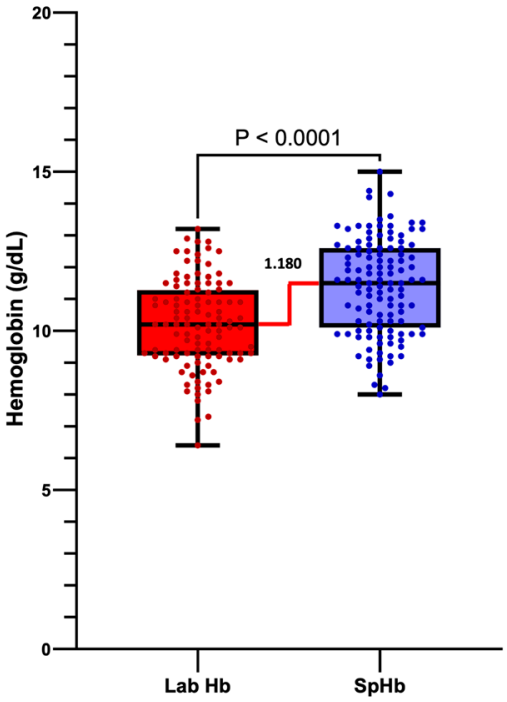


Figure 1. Dot plots with connecting lines for mean and 25th to 75th percentiles of both spectrophotometric hemoglobin (SpHb, g/dL) and laboratory hemoglobin (Hb, g/dL). SpHb overestimated (mean difference = 1.180) laboratory Hb concentration values. Paired samples *t* test was performed.

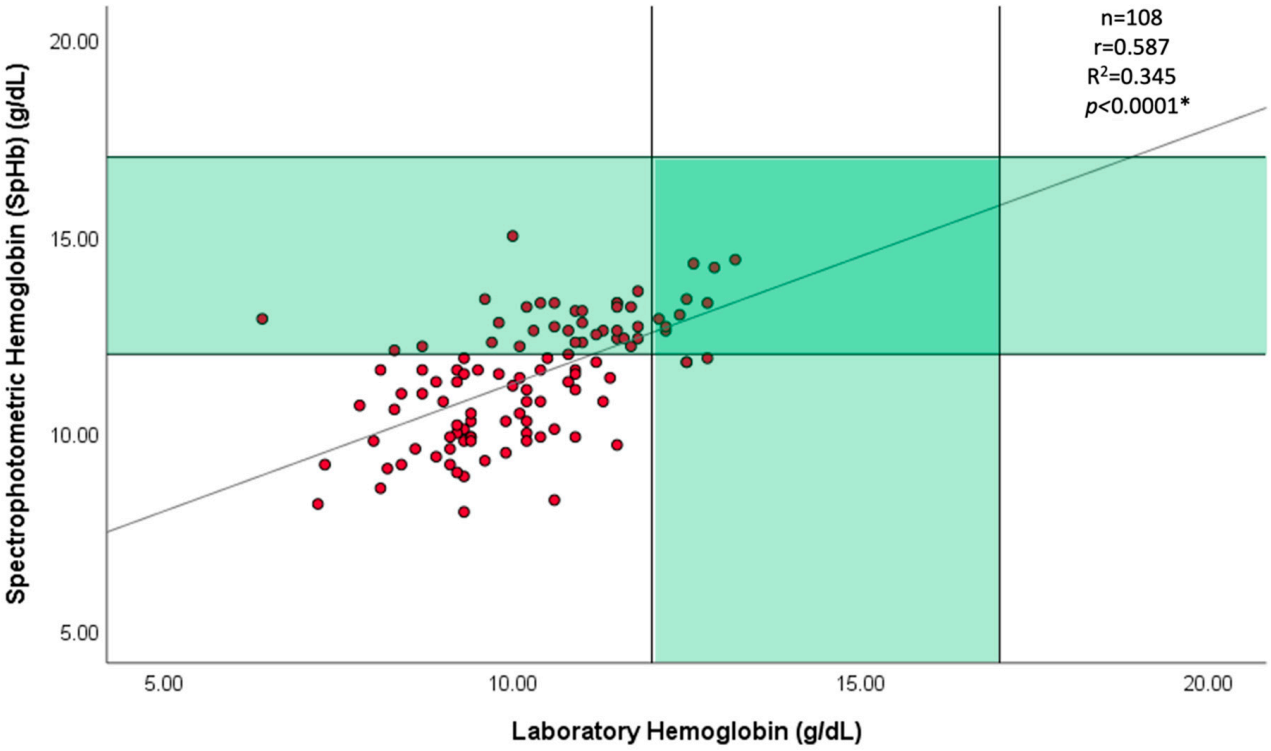


Figure 2. Simple scatter plot with regression (best-fit) line showing moderate positive correlation between laboratory hemoglobin (g/dL) and spectrophotometric hemoglobin (g/dL). The mean difference or bias of SpHb–Hb was 1.18 (SD 1.76) g/dL, with a 95% confidence interval (CI) from −1.434 to −0.926 and a limit of agreement from −3.8 to 1.4 at ±1.96 SD. * is statistical significance = $p < 0.0001$.

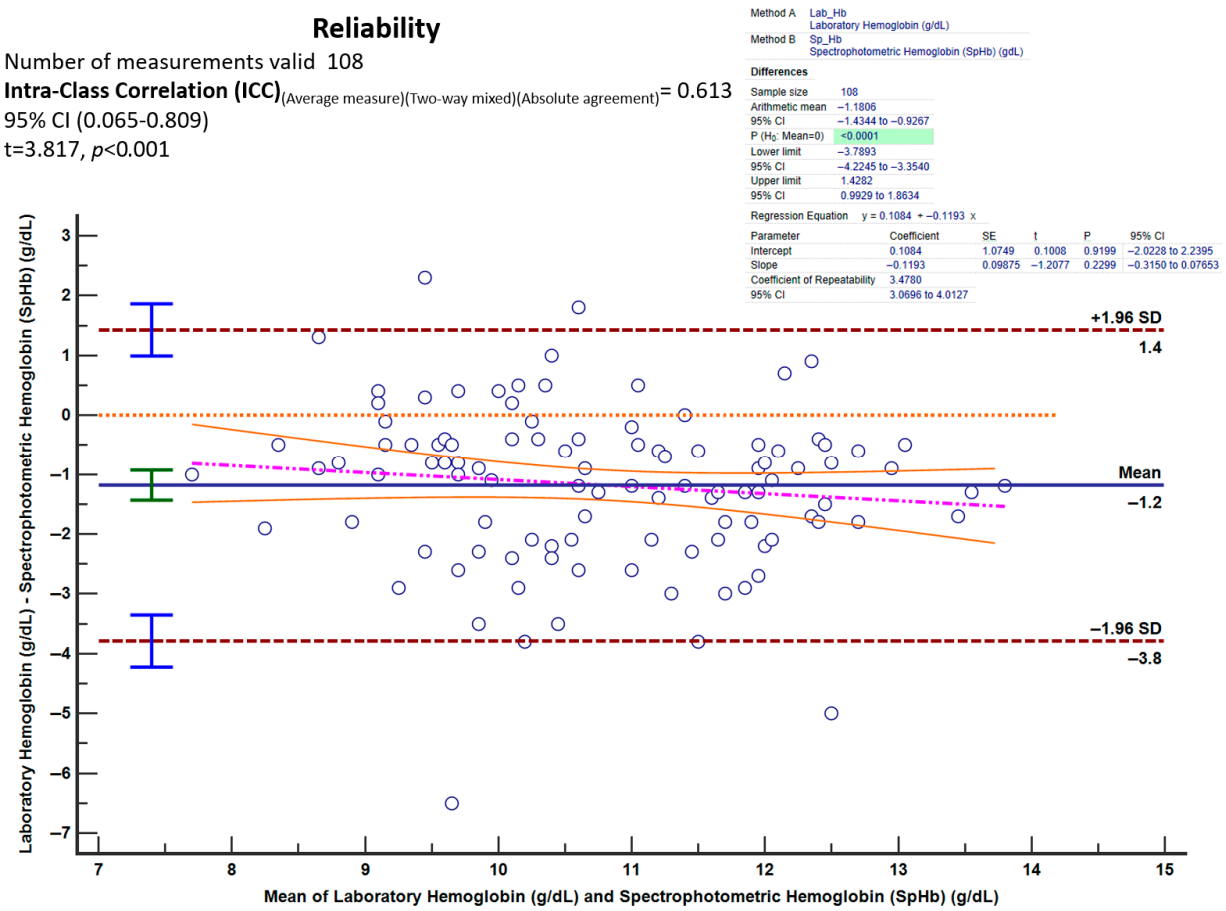


Figure 3. Bland–Altman plot demonstrating the agreement between SpHb and laboratory Hb concentrations. The 95% limits of agreement ranged from −3.7893 to +1.4228 g/dL, reflecting moderate variability in the measurements.

Table 2. The effect of blood transfusion on the laboratory hemoglobin (Hb, g/dL) and spectrophotometric hemoglobin concentrations (g/dL) before and after blood transfusion.

	Hb (g/dL)	**SpHb (g/dL)**	**Paired Sample *t* Test *p* Value**
Pre Blood Transfusion			
n	60	60	$t_{(df=59)} = 9.047$
Min–Max	6.40–12.20	8.00–15.00	$p < 0.001$ *
Mean ± S.D.	9.48 ± 1.10	10.72 ± 1.40	
95% CI of the mean	9.19–9.76	10.35–11.08	
Post Blood Transfusion			
n	48	59	$t_{(df=47)} = 7.553$
Min–Max	8.40–13.20	9.00–14.40	$p < 0.001$ *
Mean ± Std. Deviation	11.14 ± 1.14	12.08 ± 1.29	
95% CI of the mean	10.81–11.47	11.74–12.41	
Paired sample *t* test	$t_{(df=47)} = 14.061$	$t_{(df=58)} = 7.744$	
p value	$p < 0.001$ *	$p < 0.001$ *	
Percentage change (%)			
n	48	59	$t_{(df=47)} = 1.450$
Min–Max	0.00–44.19	−29.33–43.43	$p = 0.154$ NS
Mean ± Std. Deviation	19.29 ± 9.81	14.11 ± 13.22	
95% CI of the mean	16.44–22.14	10.67–17.56	

Min–Max: Minimum–Maximum. S.D.: Standard Deviation. CI: Confidence Interval. df = degree of freedom. *: Statistically significant ($p < 0.05$). NS: Statistically not significant ($p \geq 0.05$).

Table 3 shows the oxygen saturation (%) and pleth variability index (%) before and after blood transfusions. It also shows that the study group of thalassemia patients had significantly high blood levels of serum bilirubin (pre) (µmol/L) due to the repeated blood transfusions they received. It is anticipated that the spectrophotometric measurements of hemoglobin (SpHb) will be affected by these elevated blood levels that precipitate in the skin.

Table 3. Pleth variability index (%) and oxygen saturation (%) before and after blood transfusion. High serum bilirubin levels before the blood transfusion affected SpHb values, as it precipitates in the skin.

	Pleth Variability Index (PVI) (%)	**Oxygen Saturation (%)**	**Serum Bilirubin (µmol/L)**
Pre Blood Transfusion			
n	60	60	60
Min–Max	7.00–21.00	96.00–100.00	1.00–95.50
Mean ± S.D.	14.50 ± 2.93	98.45 ± 1.14	27.33 ± 19.53
95% CI of the mean	13.74–15.26	98.16–98.74	22.28–32.37
Post Blood Transfusion			
n	60	60	
Min–Max	7.00–25.00	98.00–100.00	
Mean ± Std. Deviation	13.77 ± 3.06	99.53 ± 0.65	
95% CI of the mean	12.98–14.56	99.37–99.70	
Paired sample *t* test	$t_{(df=59)} = 1.469$	$t_{(df=59)} = 6.129$	
p value	$p = 0.147$ NS	$p < 0.001$ *	
Percentage change (%)			
n	60	60	
Min–Max	−53.33–92.31	−2.00–4.17	
Mean ± Std. Deviation	−1.31 ± 29.26	1.11 ± 1.41	
95% CI of the mean	−8.87–6.25	0.75–1.48	

Min–Max: Minimum–Maximum. S.D.: Standard Deviation. CI: Confidence Interval. df = degree of freedom. *: Statistically significant ($p < 0.05$). NS: Statistically not significant ($p \geq 0.05$).

Finally, this study revealed a significant prevalence of SpHb sensor failure. The following is the stated number of trials required to obtain accurate measurements: 1 trial in 30 patients (50%), 2 trials in 21 patients (35%), and 3 in 9 patients (15%).

4. Discussion

This study is among the few to evaluate the accuracy and reliability of non-invasive spectrophotometric hemoglobin (SpHb) readings in comparison to laboratory hemoglobin (Hb) assays for patients with transfusion-dependent major beta-thalassemia. The results showed a mean bias (Hb-SpHb) of −1.18 g/dL, indicating that SpHb consistently overestimated Hb concentrations. In comparison to the standard laboratory tests, Yassen, K. et al. [16] documented similar SpHb overestimations in other hemoglobin disorders, such as sickle cell anemia. SpHb has a propensity to overread hemoglobin blood levels, which can lead to clinical issues, especially in cases when transfusion therapy requires an accurate measurement, as in patients with blood transfusion-dependent thalassemia. The Bland–Altman analysis's limits of agreement further emphasize the disparity and significant variability between SpHb and LabHb data.

A well known problem in diagnostic research is spectrum bias, which frequently causes the sensitivity and specificity of new diagnostic tests to be overestimated. Nonetheless, earlier research by Al-Khabori et al. has shown that the Pronto-7 pulse CO-oximetry

equipment is useful for measuring SpHb in sickle cell disease (SCD) patients as well as for healthy blood donors [17,18]. Radical 7 (Masimo), which employs the same technology and is the focus of our current research, overestimates hemoglobin (Hb) levels in patients with transfusion-dependent beta-thalassemia by a clinically significant amount, in contrast to the results of above studies by Al-Khabori et al. This may be explained by the elevated levels of hemosiderin and bilirubin in thalassemic individuals as result of the disease pathology, which causes them to precipitate in the skin and interfere with their ability to absorb light.

Another reason for this discrepancy in results could be the generally lower hemoglobin blood levels (anemia) seen in patients with transfusion-dependent beta-thalassemia. Our current study's findings, however, are in line with the third study by Al Khabori et al., which also discovered that individuals with beta-thalassemia exhibited a significant bias in Hb values measured by pulse CO-oximetry [19]. The idea that pulse CO-oximetry tends to overestimate hemoglobin values in this thalassemia population is further supported by another study by Bıcılıoğlu et al. [20], which discovered a similar overestimation of hemoglobin levels. SpHb values cannot be used exclusively as an accurate or primary technique for hemoglobin trend monitoring in patients with transfusion-dependent thalassemia due to these observed bias and variabilities. SpHb can provide a broad approximation of the hemoglobin levels in this specific population.

As was already indicated, the elevated blood bilirubin may have contributed to the overestimation of SpHb in this study of patients with blood transfusion-dependent thalassaemia. Its presence in thalassemia patients' skin changes how light is absorbed. Prolonged hemolysis can produce bilirubin, which might distort results when measuring SpHb with spectrophotometry. High ferritin levels, often caused by iron overload after repeated blood transfusions, can also alter the optical properties of the skin tissue. This is consistent with the results of related studies [19,21,22]. The bias seen in the current investigation is most likely a result of the aforementioned causes.

Another significant study limitation was sensor failure; in 30 instances, the SpHb device was unable to provide reliable readings from the initial trial application. There could be several reasons for these failures. The low perfusion and anemia may have made it difficult for the sensor to measure hemoglobin levels effectively. For SpHb to receive accurate sensor readings, there must be sufficient blood flow. Patient movements, improper sensor placement, or issues with the sensor's fit on the patient's fingertip could all be additional contributing causes. Al-Khabori and colleagues observed similar difficulties and irregularities in determining the SpHb values [19].

In contrast, Yassen et al.'s study of sickle cell anemia patients revealed a lower failure rate of only 1.25 percent. In future studies, short sub-section or clarification on sensor performance stratified by patient parameters need is recommended, as this could help in developing this technology.

SpHb can still be utilized to instantly track the effects of blood transfusions, even with its broad bias and sensor malfunctions. The SpHb sensor could be a helpful tool for monitoring the effect of administering blood transfusions. The trend changes in hemoglobin levels, even though it is not accurate enough for precise clinical decision-making, will help to optimize the blood unit volumes infused. Furthermore, the elimination of frequent blood draws by this non-invasive monitoring method may lessen patient pain and the hazards involved with invasive blood sampling. However, before using SpHb in patients with transfusion-dependent thalassemia, further validation and improvements in technology to overcome the challenges are needed, and the limitations must be noted.

Hiscock et al. [23], in a systematic review and meta analysis assessed the agreement in hemoglobin measurement between Masimo pulse co-oximeters (Rad-7™ and Pronto-7™) with standard laboratory tests. They studied 39 relevant studies and came to the conclusion

that Masimo devices have lower precision. They recommended that these limits should be considered before basing blood transfusion or other clinical management decisions on them.

The future evolution and development of other non-invasive, pain-free, and accessible anemia screening tools tailored specifically for pediatric patients and depending on artificial intelligence programs were recently explored by Gordon et al. (2024) [24]. They designed a clinical study to measure hemoglobin by an optical artificial intelligence method and reported an initial ability to diagnose anemia with 87% sensitivity and 84% specificity.

In conclusion, the substantial wide bias (SpHb-Hb) severely restricts the reliability of SpHb as a hemoglobin monitoring tool in blood transfusion-dependent thalassemia patients, even if a moderate correlation was found between SpHb and laboratory Hb values. Although SpHb exhibits considerable agreement with laboratory Hb readings, its accuracy is still low for routine clinical usage in this specific population. SpHb can be helpful for tracking broad patterns, but it cannot be trusted for the accurate hemoglobin values needed for clinical decision-making. SpHb is not precise enough to be utilized as a primary tool for transfusion management, but it might be useful as an additional tool for monitoring the overall changes in hemoglobin levels prior to and following transfusions. SpHb is not suitable for standalone diagnostic decision-making in thalassemia management but may serve as a supportive adjunct during transfusion sessions.

SpHb can be utilized for trend monitoring to reduce the discomfort that patients endure from recurrent blood sampling.

Author Contributions: Conceptualization, K.Y. and N.O.; methodology, K.Y., N.O., A.B.; software, A.B.; validation, R.A., S.A.A. and S.A.; formal analysis, K.Y.; investigation, A.B.; resources, K.Y.; data curation, D.I.S.; writing—original draft preparation, K.Y.; writing—review and editing, K.Y., N.O., S.S., O.Z., L.A.; visualization, K.Y., N.O.; supervision, K.Y., N.O.; project administration, K.Y., L.A.; funding acquisition, K.Y., N.O. All authors have read and agreed to the published version of the manuscript.

Funding: Deanship of Research King Faisal University, Alahsa, Saudi Arabia, Grant number: 242449.

Institutional Review Board Statement: The Al-Ahsa Health Cluster in Hofuf City, Saudi Arabia, authorized this diagnostic test accuracy study (IRB KFHH No.: H-05-HS-065; IRB Log No.: 04-EP-2025). Date of approval 1st of January 2025.

Informed Consent Statement: Written informed consent was obtained from all patients involved in the study.

Data Availability Statement: Data are available from the authors upon reasonable request.

Acknowledgments: We acknowledge the help from the students participating in the Future Scientist Program sponsored by the Deanship of Research, King Faisal University, Hofuf, Al Ahsa, Saudi Arabia (Grant 242449), and the Abdulmonem Alrashed Humanitarian Foundation, Ministry of Education Al Ahsa. Names of students: Zahra AlAli, Budur Al-majed, Bayan Alalawi, Ruqaya Alobaidan, Fatimah Al-Mubarak, and Zahra Al-Hudaibi. We acknowledge also the support and help provided by Adil Alshoaibi, Central Laboratories, King Faisal University, Hofuf, Al Ahsa, Saudi Arabia.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Cappellini, M.D.; Cohen, A.; Eleftheriou, A.; Piga, A.; Porter, J.; Taher, A. *Guidelines for the Clinical Management of Thalassaemia*, 2nd ed.; Thalassaemia International Federation: Nicosia, Cyprus, 2008.
2. Taher, A.T.; Weatherall, D.J.; Cappellini, M.D. Thalassaemia. *Lancet* **2010**, *376*, 226–237. [CrossRef] [PubMed]
3. Borgna-Pignatti, C.; Rugolotto, S.; De Stefano, P.; Zhao, H.; Cappellini, M.D.; Del Vecchio, G.C.; Romeo, M.A.; Forni, G.L.; Gamberini, M.R.; Ghilardi, R.; et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. *Haematologica.* **2004**, *89*, 1187–1193. [PubMed]

4. Frasca, D.; Dahyot-Fizelier, C.; Catherine, N.; Levrat, A.; Debaene, B.; Mimoz, O. Accuracy of a continuous noninvasive hemoglobin monitor in critically ill patients. *Intensive Care Med.* **2011**, *37*, 283–289. [CrossRef]
5. Teboul, J.L.; Saugel, B.; Cecconi, M.; De Backer, D.; Hofer, C.K.; Monnet, X.; Perel, A.; Pinsky, M.R.; Reuter, D.A.; Rhodes, A.; et al. Less invasive hemodynamic monitoring in critically ill patients. *Intensive Care Med.* **2016**, *42*, 1350–1359. [CrossRef] [PubMed]
6. Cappellini, M.D.; Motta, I. Anemia in Clinical Practice-Definition and Classification: Does Hemoglobin Change with Aging? *Semin. Hematol.* **2015**, *52*, 261–269. [CrossRef] [PubMed]
7. Gomaa, D.; Rodriquez, D., Jr.; Petro, M.; Blakeman, T.C.; Branson, R.D. Impact of Oxygenation Status on the Noninvasive Measurement of Hemoglobin. *Mil. Med.* **2017**, *182*, 87–91. [CrossRef] [PubMed]
8. Okazaki, K.; Okazaki, K.; Uesugi, M.; Matsusima, T.; Hataya, H. Evaluation of the accuracy of a non-invasive hemoglobin-monitoring device in schoolchildren. *Pediatr. Neonatol.* **2022**, *63*, 19–24. [CrossRef] [PubMed]
9. IBM Corp. *IBM SPSS Statistics for Windows*, version 25.0; IBM Corp.: Armonk, NY, USA, 2017.
10. Shapiro, S. The Shapiro-Wilk and Related Test for Normality. Statistics 2015. Available online: https://www.statskingdom.com/shapiro-wilk-test-calculator.html (accessed on 2 September 2025).
11. Field, A. *Discovering Statistics Using IBM SPSS Statistics*, 4th ed.; SAGE Publications Ltd.: Thousand Oaks, CA, USA, 2013.
12. Ross, A.; Willson, V.L. Paired Samples T-Test. In *Basic and Advanced Statistical Tests: Writing Results Sections and Creating Tables and Figures*; Ross, A., Willson, V.L., Eds.; Sense Publishers: Rotterdam, The Netherlands, 2017; pp. 17–19. [CrossRef]
13. Koch, G.G. Intraclass correlation coefficient. In *Encyclopedia of Statistical Sciences*; Kotz, S., Johnson, N.L., Read, C.B., Eds.; John Wiley & Sons.: New York, NY, USA, 1982; pp. 213–217. Available online: https://books.google.com.eg/books?id=2JBFAAAAYAAJ (accessed on 2 September 2025).
14. Cicchetti, D.V. Guidelines, criteria, and rules of thumb for evaluating normed and standardized assessment instruments in psychology. *Psychol. Assess.* **1994**, *6*, 284. [CrossRef]
15. Bland, J.M.; Altman, D.G. Comparing methods of measurement: Why plotting difference against standard method is misleading. *Lancet* **1995**, *346*, 1085–1087. [CrossRef] [PubMed]
16. Yassen, K.; Omer, N.; Alsahaf, F.; Al Amer, F.; Alhamad, F.; Alherz, I.; Bushehab, A.; Alniniya, F.; Alwabari, M. Comparing Non-Invasive Spectrophotometry to Hematology Analysis for Hemoglobin Measurements in Sickle Cell Disease Patients. *J. Clin. Med.* **2023**, *12*, 7517. [CrossRef] [PubMed]
17. Al-Khabori, M.; Al-Riyami, A.Z.; Al-Farsi, K.; Al-Huneini, M.; Al-Hashim, A.; Al-Kemyani, N.; Daar, S. Validation of a non-invasive pulse CO-oximetry-based hemoglobin estimation in normal blood donors. *Transfus. Apher. Sci.* **2014**, *50*, 95–98. [CrossRef] [PubMed]
18. Al-Khabori, M.; Al-Hashim, A.; Jabeen, Z.; Al-Farsi, K.; Al-Huneini, M.; Al-Riyami, A.; Al-Kemyani, N.; Daar, S. Validation of a noninvasive pulse CO-oximetry-based hemoglobin estimation in patients with sickle cell disease. *Int. J. Lab. Hematol.* **2013**, *35*, e21–e23. [CrossRef] [PubMed]
19. Al Khabori, M.K.; Al-Riyami, A.Z.; Al-Farsi, K.; Al-Huneini, M.; Al-Hashim, A.; Al-Kemyani, N.; Al-Qarshoubi, I.; Khan, H.; Al-Amrani, K.; Daar, S. Non-invasive haemoglobin estimation in patients with thalassaemia major. *Sultan Qaboos Univ. Med. J.* **2014**, *14*, e468–e472. [CrossRef] [PubMed]
20. Bıcılıoğlu, Y.; Bal, A.; Demir Yenigürbüz, F.; Ergonul, E.; Geter, S.; Kazanasmaz, H.; Bal, U. Noninvasive Hemoglobin Measurement Reduce Invasive Procedures in Thalassemia Patients. *Hemoglobin* **2022**, *46*, 95–99. [CrossRef] [PubMed]
21. Al-Moshary, M.; Imtiaz, N.; Al-Mussaed, E.; Khan, A.; Ahmad, S.; Albqami, S. Clinical and Biochemical Assessment of Liver Function Test and Its Correlation with Serum Ferritin Levels in Transfusion-dependent Thalassemia Patients. *Cureus* **2020**, *12*, e7574. [CrossRef] [PubMed] [PubMed Central]
22. Tsuji, T. Experimental hemosiderosis: Relationship between skin pigmentation and hemosiderin. *Acta Derm. Venereol.* **1980**, *60*, 109–114. [CrossRef] [PubMed]
23. Hiscock, R.; Kumar, D.; Simmons, S.W. Systematic review and meta-analysis of method comparison studies of Masimo pulse co-oximeters (Radical-7™ or Pronto-7™) and HemoCue® absorption spectrometers (B-Hemoglobin or 201+) with laboratory haemoglobin estimation. *Anaesth. Intensive Care* **2015**, *43*, 341–350. [CrossRef] [PubMed]
24. Gordon, D.; Hoffman, J.; Gamrasni, K.; Barlev, Y.; Levine, A.; Landau, T.; Shpiegel, R.; Lahad, A.; Koren, A.; Levin, C.; et al. Artificial intelligence-enabled non-invasive ubiquitous anemia screening: The HEMO-AI pilot study on pediatric population. *Digit. Health* **2024**, *10*, 20552076241297057. [CrossRef] [PubMed] [PubMed Central]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.