Validity of Estimation of Haemoglobin Content in Dried Blood Spot Samples

Aakriti Gupta¹ · Umesh Kapil¹ · Lakshmy Ramakrishnan² · Preetika Khenduja¹ · Chandra Prakash Yadav³ · Nighat Yaseen Sofi¹ · Ritika Khandelwal¹

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Abstract The ability to use indirect cyanmethemoglobin method using 20 µl of dried blood spots (DBSs) on filter paper for the analysis of haemoglobin (Hb) levels could be an important diagnostic tool for areas that have limited access to laboratory facilities. We assessed the validity of a method for Hb estimation in which a single drop of whole blood was directly taken on the filter paper. We collected 124 DBSs containing 20 µl of blood (filter paper A) and single drop of whole blood (filter paper B) from subjects living in Nainital, Uttarakhand. Estimation of Hb was done by indirect cyanmethemoglobin method in both the filter papers. A correction factor for predicting value of Hb from DBSs of single drop of whole blood was established. The Bland–Altman plot suggested that the difference in Hb values obtained by the single drop of blood and 20 µl of DBSs was within the 1.5 SD limits, suggesting high validity of the correction factor. The estimation of Hb using single drop of whole blood on filter paper after applying the correction factor provides results similar to indirect cyanmethemoglobin method using 20 µl of blood. Hence, single drop of whole blood on filter paper can be used as an alternate method for estimation of Hb in large scale community surveys.

Keywords Indirect cyanmethemoglobin method · Dried blood spot method · Hemoglobin estimation · Filter paper method

Introduction

Anaemia as defined by low haemoglobin (Hb) concentration in blood, is a major public health problem in India affecting all age groups. It has adverse negative effects on the health, as well as on the social and economic development in the country.

Estimation of Hb by cyanmethemoglobin method is considered as the gold standard method. In large scale epidemiological surveys using direct cyanmethemoglobin method for estimation of Hb has several challenges in developing countries like India. Storage and transportation of the blood samples from field is a constraint, especially in regions which lack modern laboratory facilities. Hence, indirect cyanmethemoglobin method using dried blood spot sample (DBSs) on filter paper provide a viable alternative for estimation of Hb as it eliminates the need to store and transfer samples.

Several community based studies have utilized Hb estimation through indirect cyanmethemoglobin method using 20 µl of DBSs [1–3]. These studies have shown high correlation between Hb values assessed by direct and indirect cyanmethemoglobin method.

However, indirect cyanmethemoglobin method requires skill and training of the field staff in collecting 20 µl of blood and transferring it on the filter paper. In the present study we have assessed the validity of a method for Hb estimation in which a single drop of whole blood was directly taken on the filter paper. This method has an advantage as the research field staff does not require the

✉ Umesh Kapil
umeshkapil@gmail.com

¹ Department of Human Nutrition, All India Institute of Medical Sciences, New Delhi, India

² Department of Cardiac Biochemistry, All India Institute of Medical Sciences, New Delhi, India

³ Department of Biostatistics, All India Institute of Medical Sciences, New Delhi, India

use of micropipette and other supportive services for collection of 20 µl of blood.

Methodology

A total of 124 subjects were randomly selected in a community based study conducted in district Nainital, Uttarakhand, India. Informed consent was obtained from all the subjects after explaining the objectives of the research study. Estimation of Hb was done by indirect cyanmethemoglobin method. Finger tip was cleaned and punctured with a sterilized disposable lancet. First drop of the free flowing blood was wiped off. The second drop of blood was utilized for collecting 20 µl of blood using a micropipette on the Whatman no. 3 filter paper (Filter paper A). The third drop was directly transferred to the filter paper (Filter paper B). We obtained 124 samples of filter paper A and filter paper B of each subject.

The filter papers A and B were labeled and kept on a nonabsorbent surface (thermacol) and left at room temperature (16–20 °C) for drying. After drying, the filter papers were kept in sealed plastic bags to protect them from dust and moisture. Filter paper A and B were analyzed at the central laboratory facilities at AIIMS within 10 days of collection.

We utilized the standard procedure for estimation of Hb by indirect cyanmethemoglobin method in Filter paper A and B.

Filter paper A The 20 µl of DBSs was cut and transferred in a labeled test tube containing 5 ml of Drabkin solution (cyanmethemoglobin solution).

Filter paper B The DBSs containing one drop of blood was punched using a standard paper hole punch size of 5 mm in diameter. DBSs of 5 mm in diameter contains 5 µl of blood [4, 5]. This DBSs of 5 mm in diameter was transferred in a labeled test tube containing 1.25 ml of Drabkin solution.

The tubes were kept stationary for 2 h at room temperature (20°–25°) and then stirred on a Vortex mixer for 5 min. After full elution, Drabkin's solution in which blood spot had been eluted was transferred to a cuvette and measured at optical density of 500 nm (OD₅₀₀) on a spectrophotometer (Spectronic instruments Inc. New York, USA) using whole blood zero standard as blank.

Ethical clearance for the study was obtained from institutional ethics committee of All India Institute of Medical Sciences, New Delhi.

Calculation of the Correction Factor

Hb values of filter paper B were compared with the Hb values of filter paper A for establishing the correction

factor for predicting Hb values obtained from single drop of whole blood spot (filter paper B). For this purpose filter paper A and B of 80 subjects were randomly selected.

The following correction factor was established:

$$\text{Predicted value of Hb} = 2.3 + 0.53 \times \text{Hb value in DBSs of 5 mm in diameter (filter paper B)}$$

Results

Mean Hb values obtained from filter paper A (indirect cyanmethemoglobin method) was 7.1 ± 1.0 g/dl. Mean Hb values in whole blood spot (filter paper B) was 8.8 ± 1.1 g/dl. Mean difference in Hb values of filter paper A and B was 1.8 ± 1.0 g/dl.

Bland–Altman plot was prepared to validate the corrected Hb values obtained from whole blood spot (filter paper B) with Hb values in filter paper A (indirect cyanmethemoglobin method) for remaining 44 subjects. The Bland–Altman plot in Fig. 1 suggested that the difference in values obtained by the two methods was within the 1.5 SD limits for most of the samples. Less than 5% of the values were outside the 1.5 SD limits, suggesting high validity of the correction factor.

Conclusion

The findings of the present study revealed that estimation of Hb using single drop of blood on filter paper after applying the correction factor provides results similar to indirect cyanmethemoglobin method using 20 µl of blood.

Fig. 1 Bland–Altman plot of differences in hemoglobin values between filter paper A and B

Estimation of Hb in one drop of blood taken directly on the filter paper can be used as an alternate method for 20 µl of blood spot in extensive community surveys since it requires minimum training of the field staff and support facilities.

In addition, cleaning the micropipette after every blood sample taken requires a tedious process, which may not be possible in areas with inadequate laboratory facilities leading to errors in estimation of Hb. Other sources of error in collection of 20 µl of blood using a micropipette are: (1) inadequate removal of excess blood from the pipette (2) blood in the pipette not fully blown out (3) improper wiping of the blood outside the pipette before depositing the blood on the filter paper and (4) air bubbles in the blood column in the pipette. Therefore, this method may be more convenient and effective in reducing errors in blood collection in case of untrained staff.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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