Hemoglobin Estimation Methods:
A Review of Clinical, Sensor and Image Processing Methods

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Abstract

Hemoglobin is an important substance in the human blood which leads to anemia. Anemic condition in the women and children across the world is the serious concern. This makes identifying Hb level of patient’s blood is preliminary but important investigation. In order to avoid such condition there is requirement of regular blood test to detect the anemia in the blood. This review article focuses on various methods for estimation of hemoglobin. There are mainly two categories namely, invasive methods and non-invasive methods. In invasive method needs blood sample of patients. In Non invasive technique there is no need to draw blood from patient. A brief review of methods used in microbiology or pathology laboratory also carried out. This paper will give the brief review of various invasive and non-invasive methods to estimate the hemoglobin in the blood using various techniques.

1. Introduction

Hemoglobin (Hb) is a blood substance containing iron and protein. Hb is responsible for carrying oxygen from lungs to the various parts of the body through blood. It is also responsible for carrying carbon-di-oxide from various parts of the body to the lungs. The normal level for hemoglobin in the blood is as given below [1].

1) Women 12 - 16 g/100 ml blood
2) Men 14 - 18 g/100 ml blood
3) Newborn 14 - 20 g/100 ml blood

Anemia is an unhealthy condition that develops in human beings when Hb level in their blood is below the normal level of the blood. This drop in the Hb level in blood can be due to the deficiency of iron, vitaminB12 or folic acid. The problem of anemia gets aggravated specifically during menstruation, pregnancy, and athletic and sports activities. The problem of anemia during pregnancy not only affects the person suffering from that condition, but also extends to situations such as stillbirths or babies with low birth weight and prenatal and maternal mortality. Anemia afflicts about 2 billion people worldwide, mainly women and children as per the report by InFocus [2]. Anemia is not very prevalent in developed Countries, but is observed in alarming proportions in the developing countries [2]. Almost all the laboratory setup provides facility for hemoglobin test.

In order to detect abnormal level of Hb in the blood there is requirement of regular blood test. Various methods of estimation of Hemoglobin are discussed here. The invasive technique includes estimation of hemoglobin in the blood using color image processing along with the artificial neural network. This article gives the overview of non invasive technique which uses the sensor and electrodes to record the reading and hemoglobin values are calculated using various regression models. This paper also explains the methods which are used in pathology laboratory.

2. Hemoglobin Measurement Methods

There are two broad categories of measurement. The Invasive technique involves collecting blood sample of the subject. There are various clinical methods available depending on laboratory setup. Now with the advent of image processing, neural network microscopic photograph can be processed for parameters required. This is currently wide open research area. Few authors have reported work based on regression methods and artificial neural network model. The non-invasive methods are sensor based. The work in this category involves design of sensor signal conditioning circuits, processor or controller for analyzing the observation.

2.1 Pathology laboratory Methods

There are various clinical methods approved by world health organization (WHO) to estimate the hemoglobin in the blood in pathology laboratory. Some of these are Sahli’s method of hemoglobin estimation, portable hemoglobinometer or HemoCue method, colorimetry-hemiglobincyanide method or cyanmethemoglobin method etc [2]. In Sahli’s method of hemoglobin estimation, to carry out the test requires dilution of blood and visual color match.
The Sahli’s method is based on principle of converting hemoglobin to acid hematin and then visually matching its color against a solid glass standard. The procedure involves dilute hydrochloric acid which is added to a graduated cylinder containing a blood sample until the color of the diluted blood sample matches that of the glass standard. The quantity of dilute acid added will be determined by the hemoglobin level of the blood sample. The key requirements for this method are Sahli hemoglobinometer and Sahli blood pipette. The readings are based on the judgment of human eye. There is always room for incorrect color matching and readings may go wrong.

Another method for hemoglobin estimation is HemoCue method. It is also known as portable hemoglobinometer. This method requires the lysis (breaking down of a cell) of the blood. Here, lysis refers to the breaking down of a cell, often by viral, enzymic, or osmotic mechanisms that compromise its integrity. The HemoCue uses a disposable cuvette that is treated with chemicals that rupture the red blood cell wall and combine with the hemoglobin to form a compound that can be measured photometrically. The result is displayed in digital form on the face of the instrument. The HemoCue is an example of a rugged, portable, and accurate hemoglobinometer readily available for use.

The next method is the hemiglobincyanide or cyanmethemoglobin method is the most accurate method of measuring hemoglobin and is considered the “reference standard”. The equipment is also the most complex among the above described tests. In this method, a measured sample of whole blood is added to Drabkin’s diluting fluid. The red cells are hemolyzed and the hemoglobin is converted into a stable compound, hemiglobincyanide. The sample is placed in a colorimeter and the amount of light that is absorbed by the blood sample at a certain wavelength is proportional to the amount of hemoglobin in the blood. A reference solution of a known hemoglobin level is used to standardize the method.

2.2 Image Processing and Neural Network Approach Based Approach

H. Ranganathan and N. Gunasekaran put forward a concept of invasive estimation of hemoglobin in blood using color analysis and artificial neural network [3]. Authors have tried to estimate the hemoglobin in the blood by color measurement technique. According to authors, this method is very useful in remote areas with the low resource setting. In order to avoid error which is due to human interpretation, an Artificial Neural Network (ANN) approach is utilized by the author for the estimation of Hb in the blood. ANN uses the color coded values of the sample as input and gives the Hb values as the output, which are then compared with the standard Cyanmethemoglobin method by the researchers. The method used by the author includes the smearing of the blood on the glass slide. This glass slide was then photographed with high resolution camera to obtain the digitized information of pixel for processing. The authors have used the ANN approach for this problem which is capable of providing the acceptable solution to the problems that do not establish the empirical formula between the input and output parameters of the problem. The authors have recommended the Back-Propagation network architecture for problem where input and output are not related explicitly.

Authors have worked on 2007 samples taken from different cross sections of people. They have carried out normalization with respect to color and Hb value 1500 sample were used for training and 507 samples are used for testing. From experiment carried out, the authors have concluded that back propagation network configuration with three hidden layers containing five neurons each offers the best results. Also, pointed that results of BPN method proposed to estimate Hb in blood are within 5% of the results of standard but complex cyanmethemoglobin method in 85% of the samples tested. The authors have standardizing the procedure of smear, the angle of acquiring photograph, distance between camera and the slide, the ambient light, and the time of taking the photograph after preparing the slide. Finally authors have concluded that proposed ANN method is future substitute for reference method.

Saif Zahir, Rejaul Chowdhury, and Geoffrey W. Payne reveal the technique to estimate red blood cell count, hemoglobin level and mean corpuscular hemoglobin (MCH) in the blood [5]. Authors have employed an artificial neural network approach together with image processing to automate the assessment of above mentioned parameters. In
this scheme, they have trained the neural network for Hb level and RBC sequentially and observed strong correlation of Hb level and RBC with the color of the blood sample. They have built two trained networks: one for Hb level and the other for RBC count.

For this purpose, the authors have captured digital pictures of 1000 blood samples. The images are captured using a high-resolution digital camera mounted on a microscope. Eight hundred (800) samples of which were used for training and the remaining 200 samples were used for testing purpose.

The following are the main steps:

Step1: The blood samples were smeared on the slides by the automated system. They captured high resolution microscopic color pictures of the blood samples using a digital camera mounted on a microscope.

Step2: Researchers selected a segment of the image of (256x256) pixels for further processing.

Step3: The calculations are made to extract the color information for each image using MATLAB. Then, they have calculated the average intensity for each of the R, G and B plane, which eventually gave the average value of red, green and blue color for each image. These three values were stored in a matrix. The size of the generated matrix became 3xN, where N is the number of images.

Step4: Researchers implement a program for training the Neural Network.

Authors carry out training of back-propagation network separately for Hb and RBC and tested the trained network against 200 samples for both parameter values. They have achieved very promising results for more than 90% of the samples with results tolerance of less than 5% compared to the laboratory results. For the remaining 10%, which are outside the specified range, the authors have claimed that the tolerance was between 5% and 15%, which is still better than the published results. In addition, a fast training time of less than 15 minute was achieved by the author.

### 2.3 Regression Model Based Methods

Saif Al Zahir and Han Donker published a paper wherein they have proposed regression based model for anemia detection [4]. In this paper authors have presented an efficient method to find hemoglobin value in the blood and to detect anemia using color image data. The authors have developed a logit regression model using 1000 blood sample. The researchers have compared the output of proposed method with the standard lab result. The model proposed by the author mainly works in following steps:

- Blood sample collection
- Blood color image analysis
- Building the model
- Detecting the anemia

Database used by researchers collects 1000 blood sample from which are randomly chosen from the public. After smearing it on the glass slide microscopic color images of the sample are captured using a digital camera mounted on microscope with 10x magnification.

Due to distortion created in the image because of staining process and nature of blood smearing, authors have selected a segment that is uncorrupted portion of image of window size 256x256 from each image. For purpose of building the model authors have plotted a histogram of blood sample in order to analyse their statistics and properties. In this experiment they have used Eview-5 software to produce the logit regression model for samples. Using E-view software and values of R, G, and B of the sample result can be produced using following model.

\[
Hb = \frac{e^{-1.922 + 0.206R - 0.241G + 0.012B}}{e^{-1.922 + 0.206R - 0.241G + 0.012B}}
\]

To test the performance and effectiveness of the Logit Regression Model (LRM) model, the authors have tested one thousand samples using this Logit model and compared them with the results of the hospital results and they found to be almost identical.

A Non invasive estimation of Hb in the blood using color analysis utilized the approach to find out an option for Hb estimation using color analysis where there is no need to extract blood by invasion [6]. Authors have tried out examining the option where color of the blood can be determined without invasion. In the paper, authors have states that the anemia affects more than 60% population in the developing countries. In the study carried out by the authors, model retrieves the color information
from the photograph of the fingertip under the standard condition and later the occlusion of the blood flow to the fingertip using some standard condition. This standard condition mainly includes specific illumination, specific distance between finger and camera, the angle of taking photograph and standard pressure on fingertip. The method used by the authors creates two images for each person one without applying any pressure in the fingertip and other with applying pressure on the fingertip with the help of rubber band which leads to the deposition of blood at the fingertip. After this they have tabulated the changes in the R, G, and B values of the two pictures. The authors have multivariate regression analysis to find if there is a correlation between the difference in R, G and B values before and after applying pressure and the Hb value of the blood of the person. The software used by them is from NCSS, Utah, USA. The analysis of data was over after about 550 iterations. The analysis by them resulted in defining a relationship between Hb value and the primary colors that we have taken. The multivariate regression analysis carried out by the authors gave the equation in terms of R, G and B values for Hb.

\[ \text{Hb} = \frac{N_r}{D_r}, \]

Where Authors have defined the formulae;

\[ N_r = 11.5 - 3.7R - 1.4G - 0.1B + 0.08r^2 + 0.03RG + 0.02G^2 + 0.1RB + 0.04GB + 0.01B^2 \]

\[ D_r = 1 - 0.3R - 0.2G + 0.02B + 0.07R^2 + 0.004RG + 0.003G^2 + 0.02RB + 0.005GB - 0.0005B^2 \]

To analyse the result the authors have carry out the experimentation on near about 200 people. The readings are compared with the standard Cyanmethemoglobin method which is recognized by the WHO.

**2.4 Sensor Based Approach**

Herlina, Fatimah, and Nasir Put forward a non invasive system to identify Hb in the blood during Dengue fever [7]. In this paper authors have used bioelectric impedance analysis (BIA) for predicting hemoglobin dengue patients. Since, single frequency BIA is an inexpensive quick and painless means of estimating body components as suggested by the author. To carry out the experiments researchers collect the BIA data from 210 samples comprising of 119 males and 91 females serologically confirms serologically confirmed dengue fever (DF) and dengue hemorrhagic fever (DHF) patients, hospitalized at the Hospital University Kebangsaan Malaysia (HUKM). To measure the BIA parameter, they placed two electrodes on the patient’s right hand and another two electrodes were placed on patient’s right foot. A constant current less than 1 mA and single frequency of 50 kHz was produced by a biodynamic Model 450 bio impedance analyzer (Biodynamic Corporation, USA) and injected to-the base of the knuckles and base of the toes and the signal was picked up by the other two sensor electrodes. After collecting the BIA parameters, authors carry out correlation Correlations between variables were analyzed using Spearman’s correlation coefficient. Linear regression was used to identify the most significant variable among the bioelectrical impedance parameters.

Finally authors concluded that the developed model based on BIA parameter can be used for calculation of Hb in the dengue patients and confirms its validity by checking against previous results.

U. Timm, D. McGrath, E. Lewis, J. Kraitl and H. Ewald proposed a Sensor System for Non Invasive Optical Hemoglobin Determination. In this paper authors have used the non-invasive approach to estimate the hemoglobin in the blood in order to avoid the delay between blood collection and analysis which does not allows the real time analysis. The method described by the author allows pain free online patient monitoring with minimum risk of infection and allows immediate clinical reaction to the measured data. In the measuring method, the system suggested by the author is based on the multispectral measurement method. For which the researchers have transilluminated the area of skin on the finger by monochromatic light of suitable wavelength which is in the range of 600nm-1400nm. This light allows interacting with the blood in arteries and vein during both systolic and diastolic phase. Upon the interaction with the tissues, researchers have applied a photo diode to detect the transmitted light. The authors have selected the suitable wavelength for the analysis of relative hemoglobin concentration change and SpO2 measurement. Using the Beer-Lambert’s law, the
authors have carried a series of equation for calculation of incident light intensity (I), total absorption (A₀) of medium. These parameters used by the authors to calculate the relative attenuation co-efficient R and H. The sensor system being developed by authors consists of hardware modules including appropriate light sources and receivers, a microcontroller, MSP430 and a wireless interface. With application software programmed in LabVIEW. After performing the series of evaluation of samples, publishers came to the conclusion that the newly developed sensor device is able to measure the SpO₂ and the hemoglobin concentration.

3. Conclusion

This review article gives the brief overview of various invasive, non-invasive as well as pathological laboratory methods used for estimation of hemoglobin in the blood by implementing techniques based on various approaches. The diagnostic value of a particular test depends greatly on the accuracy and reliability. Accuracy can be estimated by the comparison of the results obtained by method with the results of a standard method. The invasive methods are more accurate as compared to the non-invasive methods since; color of blood directly gives the indication of hemoglobin in blood. Also for non-invasive techniques, more accurate mathematical analysis is required to calibrate the data from the sensors which may leads to false reading. But looking at today’s demand, there is requirement of non-invasive online monitoring system in which patients reading can be monitored continuously which results in better treatment.

References


