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Hemoglobin Estimation by Invasive and Non-Invasive Methods: AReview

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Abstract: -- **Hemoglobin is an imperative element in the human blood, the deficiency of which may lead to Anemia. Thus identifying the level of Hemoglobin in patients' blood is a vital investigation. In order to evade such a condition, there is a requirement for a regular blood test to detect the presence of Anemia in the blood. There are multiple techniques with different applications for estimation of hemoglobin; however, it is broadly categorized into two types namely: Invasive method and Non-Invasive method. In an Invasive method, there is a requisite of patients' blood sample whereas in Non- invasive technique there is no need to draw blood from the patients'. This review article focuses on an assortment of methods for estimating Hemoglobin. Direct cyanmethemoglobin method has been the gold standard for Hemoglobin estimation but other methods like Color scale, Sahli's technique; Tallqvist, Hemocue, etc. are also available. The study, in addition, explores the frontier involving latest methods such as image processing techniques, pulse oximetry and occlusion spectroscopy which embraces the enhanced scope for the detection of Hemoglobin.**

Keywords: -- **Hemoglobin, Anemia, Invasive and Non- Invasive methods, Image Processing Techniques.**

Abbreviations: Hemoglobin (Hb), WHO (World Health Organization),National Family Health Survey (NFHS),The Haemoglobincolour scale (HCS), The Cyanmethemoglobin method (HiCN),Fetal haemoglobin (HbF),arterial oxygen saturation (SaO2),Methemoglobin (MetHb), Carboxyhemoglobin (COHb), Near Infrared(NIR), plethysmo-graphic (ppg)

I. INTRODUCTION

Hemoglobin is a significant respiratory protein in Red corpuscles have a molecular weight of approximately 64,500 and consists of four polypeptide chains linked together by the non- covalent bonds each with electroactive iron heme group [1]. The iron contained in hemoglobin is responsible for the red color of blood. [2]Hb is frequently measured by complete blood count method and is expressed in grams (gm) per deciliter (dl) of whole blood.The volumetric determination of Hb in red corpuscles in blood is defined as Hematocrit. [3]The normal ranges of Hb and hematocrit depend on the age, beginning in adolescence, the gender of the person which is mentioned in Table 1. [4]:

TABLE 1: Showing the normal Ranges of Hb and Hematocrit

Population	Hemoglobin	Hematocrit
Infants	10.5 - 19.5 g/dl	32 - 60%
Children (1-9)	11.0 - 14.0 g/dl	33 - 40%
Children (10-12)	11.5 - 15.0 g/dl	35 - 45%
Men (adults)	13.0 - 18.0 g/dl	40 - 50 %
Women (adults)	12.0 - 16.0 g/dl	36 - 44%
Pregnant Women	11.0 - 14.0 g/dl	33 - 42%

Anemia is one of the most serious global public health problem and world's second-leading disability which is generally defined as a reduced hemoglobin concentration in red corpuscles in the blood, which is associated with augmented perinatal mortality, increased child mortality, impaired immune competence and reduced performance at work. [5]In developing countries of Southeast Asia an alarming 600 million populace are suffering from iron deficiency anemia, mainly disturbing adolescent girls, women of reproductive age and young children. [6]The situation is predominant amongst pregnant women in the area ranging from 13.4 % in Thailand to 87% in India. Approximately 74% of pregnant women in Bangladesh, 63%in Nepal, 58% in Sri Lanka and Myanmar, and 51% in Indonesia suffer from Anemia. [7]According to the National Family Health Survey (NFHS)-3rd in 2005- 2006, more than half of women in India 55% & 24% of men have Hemoglobin level lower than the cutoff. According to the studies made an increasing trend in prevalence of anemia from 49.7% (NFHS-2, 1998-1999) to 50.5% (NFHS-3, 2005-2006) has been reported. [8]There is wide range of clinical methods accepted by World Health Organization (WHO) to identify abnormal level of hemoglobin in the blood in the pathology laboratory. [9]

Invasive method: A medical process pervades the body

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cavity i.e., it requires a puncture, an prick, a catheterization, etc. into the body can be termed as invasive technique. [10]Direct cyanmethemoglobin technique has been the gold standard designed for hemoglobin estimation and is economical but time consuming. A number of additional methods with their specific working principle are available such as hemoglobin color scale, Sahli technique, Tallqvist technique, copper-sulfate method and HemoCue. Every technique has a different functioning principle and its own advantages and disadvantages. Simple techniques to measure Hb exist but they are relatively expensive and require commercial reagents and good technical skills to interpret. [11]Non invasive methods are generally defined as the type of hemoglobin estimation methods that has been introduced with the aim of preventing ache to blood donor. Other than avoiding venipuncture, this technique in addition minimizes the risk of infection for health care personnel, eliminates the production of biohazardous squander, reduces the need for trained workers, cuts down on consumables and is sampling error proof. [12]

II. METHODS

Hemoglobin estimation has conventionally relied on the services of a well-resourced clinical laboratory. A number of methods with their specific working principle, advantages and disadvantages are present.

Invasive Technique

Haldane technique designed for hemoglobin (Hb) estimation is the oldest method, mainly based on carbon monoxide (CO) carrying ability of blood and comparison by using a standard. Nevertheless, commercially accessible standards are faded quickly & unreliable. [11]The Haemoglobincolour scale (HCS) is an easy, rapid and inexpensive method which has been developed where there is no laboratory for estimating the concentration of hemoglobin by means of a finger prick. The process relies on comparing the color of a plunge of blood absorbed onto a clean filter paper with standard colours on a laminated card, varying from pink to dark red. These colours correspond to haemoglobin levels of 4, 6, 8, 10, 12, and 14 g/dl. Intermediate shades can be identified, allowing haemoglobin levels to be judged to 1 g/dl. [13]Dare method uses a diminutive glass chamber that is entirely filled with blood by capillary deed. The chamber is illuminated via a battery- lit bulb and the color of blood is matched with a standard following by viewing all the way through an eyepiece .[14]The Tallqvist technique is a type of filter paper process which does not require lysis or blood dilution. The development of method took place in 1995. A plunge of blood is obtained by puncturing either the earlobe or fingertip with a sterilized lancet. Wipe up the primary drop of blood. Subsequently collect next drop onto the filter paper and remain until the blood spot has almost dried. The concentration of Hb is interpolated by comparing the color of the blood with a set of color standards on paper. [15]The Sahli's technique is commonly defined as a process based on the theory of converting Hb to acid hematin and then visually matching its color adjacent to a solid glass standard.Thehaemometer is a supreme measuring system by graduation with g/dl reading.. The key requirements designed for this method are Sahli blood pipette and Sahlihemoglobinometer. The measuring graduated tube is filled up to the base graduation line with hydrochloric acid. A capacity of 20 µl of blood is blown into the tube and water is added until the solution's color matches the colour of the test rods. The result can be read by gentle daylight, 3 min after adding up the 20 µl of blood to hydrochloric acid.The amount of dilute acid added will be determined by the hemoglobin level of the blood samples. The readings are based on the judgment of human eye. [16]

Hemocue is yet another method extensively used for as a point- of – care device for Hb estimation in health facilities. It is also known as portable hemoglobinometer which is highly sensitive and specific in nature. [17]The portable hemoglobinometer mainly requires only a small sample of capillary/venous blood, is relatively inexpensive and simple to use. Thus giving immediate, digitally displayed results. [18]This technique requires the lysis (breaking down of a cell) of the blood frequently by viral, enzymic, or osmotic mechanisms that compromise its integrity. HemoCue primarily uses a disposable cuvette that is treated with chemicals that rip apart the red blood cell wall and coalesce with the hemoglobin to figure a compound that can be deliberate photometrically.The absorbance is measured at two wavelengths (570 nm and 880 nm) in order to compensate for turbidity in the sample. The end result is displayed in digital form on the face of the instrument. [11]
Reference Range for Adult male's 130 – 170 g/L
Adult females 120 – 150 g/L Infants, after neonatal period 110 – 140 g/L Children, two years to teenage: gradual increase to adult normal. [19]
The Cyanmethemoglobin method or modified Drabkin's method (HiCN) is used for the measurement of hemoglobin and has been in wide applicability worldwide; its pros are available as steady and internationally acknowledged cite standard calibrator.[20] The cyanmethemoglobin method entirety is on the explanation of modification of hemoglobin to cyanmethemoglobin by the summation of potassium

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cyanide and ferricyanide whose absorbance is calculated at 540 nm in a photoelectric colorimeter against a standard solution. [11]When Blood is mixed with a solution of Drabkin's solution (potassium cyanide, potassium ferricyanideand potassiumdihydrogen phosphate)[21], the erythrocytes are lysed by producing evenly disturbed hemoglobin solution. Potassium ferricyanide change hemoglobin to methemoglobin, and methemoglobin mix with potassium cyanide to produce hemiglobincyanide (cyanmethemoglobin). And then all forms of hemoglobin present in blood are completely changed to a single conjugate cyanmethemoglobin. When the reaction gets completed, absorbance of the solution is deliberate in a spectrophotometer at 540 nanometer. Hemoglobincyanide has a wide absorbance peak at this wavelength. The absorbance is compared with that of the standard hemiglobincyanide solution by using a formula to obtain the amount of hemoglobin.[22]

Where,
= concentration of hemoglobin gram per liter of blood
= absorbance of the solution at λ =540 nm;
M= relative molecular mass of haemoglobin,derived from 64 458/4;
F = dilution factor used (for example, 251);
= milimolarabsorptivity = 11.0;
1=light path in cm; and 1000=conversion factor mg to g. (23)

Alkaline hematin method measure total pigment with a reasonable degree of accuracy and yet it is simple enough. to be employed in a laboratory where many determinations are performed daily. [24]This procedure consist of a solution of crystalline haemin in sodium hydroxide.[25]In the alkaline hematin D-575 (AHD-575) method of haemoglobin estimation, blood is diluted using an alkaline solvent sodium hydroxide comprising of a non-ionic detergent Triton X100. All haemoglobin derivatives are converted into a steady end-product, alkaline hematin D-575, whose absorption maximum is at X = 575 nm.(26,27) Alkaline hematin methods in which blood is diluted using alkaline solutions without detergent have been described; however, in these methods, certain forms of haemoglobin are resistant to alkali denaturation, notably fetal haemoglobin (HbF).(24)

EXPECTED RESULTS can be calculated by the following formula

The absorbances obtained were converted into haemoglobin concentrations (g/dl) using the following relationship:

Haemoglobin concentration of test sample = At/ As * concentration of standard

Where,

At = absorbance of the test sample and

As = absorbance of the standard. (26)

NON- INVASIVE METHOD

Pulse oximetry is a form of Spectrophotometric method which is ubiquitously used for monitoring oxygenation in critical care setting.Pulseoximetry measures oxygen saturation by illuminating the skin and measuring changes in light absorption of oxygenated and deoxygenated blood using two light emitting diodes that absorbs light at two different wavelengths: 660 nm (red) and 940nm (infrared). At the defined wavelengths the ratio of absorbance is calculated and calibrated against direct measurements of arterial oxygen saturation (SaO2) to establish the pulse oximeter's measure of arterial saturation (SpO2).[28]The light passing as of the LED through the finger is calculated by a photo detector positioned contradictory to LED. The software determines the disparity between absorption during diastole and systole at both wavelengths. [29] In general pulsatile signal's amplitude is approximately 1% of the d.c. level.The measurement of methemoglobin (MetHb), Carboxyhemoglobin (COHb) & total Hemoglobin can be done non- invasively using multi-wavelength pulse oximetry technique that follows theory of "The new Rainbow Technology" developed by Masimo Corp.[29]

Occlusion spectroscopy

A peculiar apparatus named NBM200 by Orsense Ltd., Nez Ziona, Israel has been designed and assembled for in vivo occlusion spectroscopy (Fig.1) .It comprise of three major units: the pressurizing assembly (occluder), the measuring unit, and the control unit.[30] This new method is used to estimate Haemoglobin levels has been developed, using the principle of occlusion spectroscopy, which let off the donor finger stick and makes the process more easy,[31]The system consist a ring-shaped re-usable sensor/probe, arranged for preference around the thumb, and a small portable monitor with a graphical display, comprise a microprocessor that compute the result and shows it on the screen. The probe is composed of eight diodes generating bands of light in the red (610 nm) and infrared (935 nm) fields, in addition to that a photocell receiving the light after it has passed through the patient's skin and circulation. At those wavelengths Hb absorbs light. The photocell records the light penetrating the thumb and converts the intensity of the light spectrum into an electronic signal which is transmitted to the device to be processed and the result

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displayed. A measuring is taken on five consecutive occlusion cycles. [32]

PHOTOPLETHYSMOGRAPHY (IMAGE PROCESSING)

Saif Al Zahir and Han Donker disclose the non invasive method to estimate hemoglobin level in the blood which utilizes the approach to estimate hemoglobin using colouranalysis.[33] The proposed method of haemoglobin detection is to get signal image scanning using photo-plethysmo-graphic technique, when extremity is placed near proximity to Near Infrared(NIR).[34] The model fetches the color information from the image of the fingertip under the standard condition. The method in use creates two images for each person one without applying pressure on the fingertip in the assistance of rubber band which directs the deposition of blood at the fingertip.[13]

The block diagram of the system for the measurement of haemoglobin, from signal to image scanning using photo-plethysmo-graphic (ppg), is shown in Figure 1. The clip-on sensor to be affixed on an extremity (say the finger) of a patient being tested concerns NIR on one side and a photo diode on the other side. NIR emit infrared rays which are captured by photonic detector. The signal from the device is recorded with Sigview software and then the recorded signal was processed using MatLabcoding.Then signal to image conversion was done finally. A regressive logical thinking will be taken for the received values to determine the haemoglobin amount. In order to determine the haemoglobin content, variance and mean values are compared and output was plotted in PPG graph.[34]

III. DISCUSSION

For investigating various anemic or other blood related cases hemoglobin estimation plays a vital role. In rural areas for hemoglobin levels a method should be used which is less expensive and most reliable. Different invasive and non-invasive methods were analyzed and was interpretated that Hemocue and cyanmethemoglobin methods are widely used for invasive methods.[11] Previously it was believed that Hemoglobin Color Scale has a sensitivity and specificity of 95% and 99.5% respectively.[35] But a recent study in 2014 by WHO considered it to be less reliable method.[36]Sahli's method is still routinely used in hospital settings but has inbuilt disadvantages mentioned in below table.[37]Hemocue is yet another method for hemoglobin estimation whose sensitivity and specificity is 94.1% and 95.2%.[38]Cyanmethemoglobin is referred to as gold standard invasive method and in India approximately 70% of laboratories still use direct cyanmethemoglobin method (HiCN)for hemoglobin estimation especially in rural areas.[20] The non invasive methods such as pulse oximetry, occlusion spectroscopy and photoplethysmography have come in to picture for hemoglobin estimation recently which overcomes the pain faced by patients during tests. The study for the non invasive is yet to go through many challenges which includes the expenses initially where these methods cannot be utilized for rural areas.The advantages and disadvantages has been shown in the Table 2.

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TABLE 2: ADVANTAGES AND DISADVANTAGES OF VARIOUS METHODS

METHODS	ADVANTAGES	DISADVANTAGES
INVASIVE METHODS		
1. Hemoglobin Colour Scale	Portable, No requisite of electricity&Inexpensive	Inter-observer variability
2. Sahli's method	Easy & Economical	Imprecise, Inter-observer unpredictability, Exercise of manual pipetting prone to inaccuracy, Color produced is unstable & No international standard
3. Hemocue method	Quick- immediate result, Convenient, Accurate, Non toxic, Battery Operated, Reliable &Easy to employ in poor settings where skills as well as resources are inadequate	Use disposable cuvette which makes it expensive
4.Tallquist method	Inexpensive, Rapid and Simple, Portable, Electricity not required & No reagents required	Results influenced by lighting, Temperature, Humidity & Size and thickness of blood spot
5.CYANMETHEMOGLOBIN METHOD	error due to subjective visual matching is avoided as spectrophotometer is used, provides accurate and precise results, precise and reliable &all hemoglobin can be estimated (except sulfhemoglobin).	Potassium cyanide is photosensitive and toxic, expensive, Turbidity can deviate the Estimate
6. alkaline hematin method AHD-575	AHD-triton reagent is not toxic or photosensitive; Reaction is very fast in non-cyanide methods & cheap.	method does not work when non-ionic reagents replaced by cationic reagents
NON INVASIVE METHODS		
7. Pulse oximetry method	No need to withdraw blood from patient body & Quick and easy to measure the reading	Affected by light interference & Error can occur in oxygen saturation reading
8. NBM-200	Donor Comfort, No Pain, No Blood, Easy & Safe to Use, Infection Free, Hands Free, Immediate Results batteries required No Disposables	Expensive, Screening Should Occur With Meticulous Attention

IV. CONCLUSION

The study is done to provide the information regarding various Hemoglobin estimation techniques and rising advances in it. Also data generated for large number of anemic patients' in most of the developing countriesfor determining Hemoglobin level and to overcome Hemoglobin complications. Future research can be done on developing more rapidand advance economic method for hemoglobin estimation.

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