Original Article

Comparative measuring between fresh and stored Drabkin's reagent preparations on hemoglobin estimation

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Abstract

This pilot case control hospital base study was conducted to determine the effect of using stored Drabkin's reagent on hemoglobin estimation compared with freshly prepared Drabkin's reagent. Freshly 50 ethylenediaminetetraacetic acid venous blood samples were collected from volunteers from Omdurman Military Hospital, Sudan, the Hb estimation performed using manual method (Hemiglobincyanide or cyanmethemoglobin method), Drabkin's used was prepared in a manner (fresh, 4 days, 8 days and 12 days stored Drabkin's). Then the collected data were analyzed by SPSS computer program. The result shows that the mean concentration of Hb (g/dL) when estimated by prepared Drabkin's reagent for several interval, the fresh reagent result was 13.0 g/dL, 4 days was reagent 11.9 g/dL, 8 days reagent was 11.4 g/dL and 12 days reagent was 11.3 g/dL. The study reveals that the mean of Hb when measured by 4 days stored Drabkin's reagent were statically insignificant and significant when measured by 8 days and 12 days stored Drabkin's reagent ($P = 0.05$).

Key words: Drabkin's reagent, hemoglobin estimation, venous blood

INTRODUCTION

Hemoglobin is the most common routine hematological parameter that measured to determine pathological condition of the patients, specifically diagnosis and follow-up of anemia. Hemoglobin is the oxygen carrying pigment of erythrocytes, formed by developing erythrocytes in the bone marrow.[1]

Each molecule of hemoglobin contains four linked polypeptide (globin) chains that in an adult consist of two alpha chains amino acids and two beta chains. About 96–98% of normal adult hemoglobin is HbA. Up to 3.5% is HbA2 and <1% is HbF (fetal).

The objectives of this study is to estimate Hb level using freshly prepared Drabkin's, to estimate Hb level using stored Drabkin's which are prepared at different interval (4, 8 and 12 days) and to compare the result of cyanomethemoglobin method to the automated method.

Hemoglobin is the oxygen carrying pigment of erythrocytes, formed by developing erythrocytes in the bone marrow.[1]

Each molecule of hemoglobin contains four linked polypeptide (globin) chains that in an adult consist of two alpha chains amino acids and two beta chains. 96–98% of normal adult hemoglobin is HbA, Up to 3.5% is HbA2 and <1% is HbF (fetal). Each polypeptide chain is combined with an iron containing porphyrins pigment called heme that is the oxygen carrying part of the hemoglobin molecule. Oxygen binds reversibly to the ferrous ion (Fe+2) contained in each heme group.

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Hemoglobin takes up oxygen when the partial pressure of oxygen in the blood is high and releases it when the partial pressure of oxygen is low. When the blood is in the lungs, therefore, it rapidly combines with hemoglobin, forming oxyhemoglobin. The blood becomes about 97% saturated with oxygen (every gram of hemoglobin combining with 1.34 ml of oxygen). When the blood passes through the tissues where the partial pressure of oxygen is low, oxygen dissociates from the hemoglobin. Under resting conditions, the blood normally loses about 30% of its oxygen content in the tissues.

Oxygenation and deoxygenating of hemoglobin involve interactions between the globin chains and the red cell metabolite, 2,3diphosphoglycerate (2, 3-DPG). As the oxygen is bound, the chains move close together. As the oxygen is released, the two chains move apart slightly, allowing the entry a binding of 2, 3-DPG. This lowers oxygen affinity, facilitating the release of oxygen from the hemoglobin.[2]

Hemoglobin performs three functions, each of which is essential for live:

Transport of molecular O_2 from the lungs to the tissues. Transport of carbon dioxide from the tissue to the lungs.

Buffering of the blood to prevent change in PH that may be incompatible with life. The structure and composition of Hb make it eminently suited for such purposes.[3]

After senscent red blood cells are phagocytes by the macrophage, the red blood cells is broken-down by heme oxygenize (HMOXI) to release Iron as ferrous Iron, it can be either enter as ferritin (where it is oxidized to ferric Iron by the ferritin protein) or be released into plasma (via ferroprotein I) where its binding to transferring.[4]

When red cells are destroyed in the vascular compartment the hemoglobin escaping into the plasma is bound to haptoglobin. A dimeric glycoprotein, each molecule of haptoglobin can bind two hemoglobin dimmers. The haptoglobin hemoglobin complex is cleared from the plasma within 10–30 min. After the complex is carried to the liver parenchyma, the heme of the hemoglobin is converted to iron and biliverdins by HMOXI and the biliverdin is further catabolized to bilirubin. CO is released in the course of cleavage of heme by HMOXI. Free haptoglobin, in contrast to the hemoglobin, haptoglobin complex has at half of 5 days, and when large amounts of the rapidly turned over haptoglobin hemoglobin complex are formed, the haptoglobin content of the plasma is depleted. The haptoglobin content of the plasma is diminished not only in the plasma of patients undergoing frank intravascular hemolysis, but also from the plasma of patients who, like those with sickle cell disease, have accelerated red cell destruction occurring primarily within macrophages. Presumably, there is either enough intravascular hemolysis in such hemolytic disorders to lower the plasma haptoglobin level or sufficient leakage from the phagocyte cells into the plasma to bind to haptoglobin. Thus, the measurement of plasma haptoglobin levels has some usefulness in diagnosing the presence of hemolysis.[5]

MATERIALS AND METHODS

Study population

This study was conducted in Omdurman Military Hospital, Omdurman, Sudan during the period from October 2011 to April 2012.

Inclusion criteria

Fifty military individuals apparently healthy aged 18–60 years old.

Method of blood sample collection

Volunteer was sat up at the right position for the blood sample collection. The skin was cleaned with 70% alcohol and allowed to dry, to avoid stinging when the skin is penetrated, a tourniquet was applied to the arm, tight sufficiently to distend the vein, but not so tightly to cause discomfort and then the needle was inserted and 2.5 ml of blood were collected.

The principle

The basis of this method is dilution of blood in a solution containing potassium cyanide and potassium ferricyanide.

Hemoglobin, Hi and HbCO (but not SHb) are converted to HiCN. The absorbance of the solution is then measured in a spectrophotometer at a wavelength of 540 nm or photoelectric colorimeter with a yellow-green filte.[6]

Diluents

This is based on Drabkin's cyanide-ferricyanide solution. It consists of:

- Potassium ferricyanide: 200 mg
- Potassium cyanide: 50 mg
- Potassium dihydrogen phosphate: 140 mg
- Distal water: 1 L.[6]

Stock Drabkin's reagent

- Potassium ferricyanide: 200 mg
- Potassium cyanide: 50 mg
- Potassium dihydrogen phosphate: 140 mg
- Distal water: 50 ml
- Stock solution was stored in the dark bottle and kept in the refrigerator at temperature 4°C.

Working Drabkin's solution

Working Drabkin's solution was prepared in a manner 4 days interval (12 days, 8 days and 4 days) before the collection of blood samples.

Then lastly freshly working Drabkin's solution was prepared on the day of sample collection.

The working solution was prepared by taking 5 ml from stock solution and 195 ml from distal water for preparation of 200 ml for working.

Working solution was clear not turbid, pale yellow in color, and when measured against water as blank in a spectrophotometer at a wavelength of 540 nm, absorbance was zero.

Requirements

- Drabkin's reagent
- Ethylenediaminetetraacetic acid (EDTA) venous blood
- Plain container
- Graduated pipette (5 ml)
- 0.02 ml pipette
- Cotton
- Cuvette
- Spectrophotometer
- Racks.

Methods

A volume of 0.02 ml of EDTA blood was added to 5 ml of Drabkin's reagent. Stopper the tube containing the solution and invert it several times, then allowed to stand at room temperature for a sufficient period to ensure the completion of the reaction (15 min). The absorbance was measured against the reagent blank, in the spectrophotometer at 540 nm.

Concentration of Hb was obtained from Hb chart (g/dL).

Each sample was estimated for Hb concentration by all the four prepared working Drabkin's reagent following the above-described method.

Ethical consideration

All enrolled subjects were informed about the targets of this study, and their consent was taken, before the beginning of the sample collection. Approval letter was obtained from the competent authority of the institute where the study was carried out.

Data analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS) software version 12. Mean values of normally distributed continuous data were compared using students' test. $P \geq 0.00$ was considered significant at 95% confidence limit.

DISCUSSION

This study involved thirty volunteers from which blood sample were collected to estimate the Hb using Drabkin's reagent that had been prepared in each 4 days interval.

Freshly prepared working Drabkin's reagent was considered to be as a standard (clear not turbid, pale yellow in color and give zero absorbance when measured against water as blank).[6]

Each stored working Drabkin's reagent was compared with freshly prepared Drabkin's reagent to establish if there is significant statistical variation.

The study reveals that the mean of Hb (g/dL) when estimated by fresh Drabkin's reagent was 13.0 g/dL and 11.9 g/dL when estimated by 4 days stored Drabkin's reagent when compared with the mean of Hb estimated by freshly prepared working Drabkin's reagent; Statistically there is insignificant variation ($P = 0.1$).

The result also shows that the mean of Hb when estimated using stored Drabkin's reagents (8 days and 12 days) were 11.4 g/dL and 11.3 g/dL respectively. And statistically there is significant variation ($P = 0.02$ and 0.01) respectively.

This means that storage of Drabkin's reagent (Working reagent) for time >4 days from preparation can affect the result of hemoglobin concentration. After completion of the study, the following were recommended:

Prepared working Drabkin's solution can be used for Hb estimation up to 4 days when stored in a suitable condition (brown bottle, dark place and at room temperature).

Sample size must be increased in further study, and also storage condition of stored Drabkin's reagent must be changed (two stored Drabkin's reagent one stored in room temperature and the other stored in the refrigerator).

CONCLUSION

The fundamental purpose of this study is to exhibit precise and accurate measurement of Hb by manual methods.

The results concluded that the use of Drabkin's reagent that is stored >4 days will insignificantly decrease the result of Hb estimation when compared with freshly prepared Drabkin's reagent. And there is no significant change on Hb

measurement when estimated by 4 days stored Drabkin's reagent.

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