Original Paper

Methaemoglobin Analysis in Acute Dapsone Poisoning

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ABSTRACT

A case of acute dapsone poisoning in a 21 year-old female is being reported. Dapsone is well known for inducing serious methaemoglobinaemia and haemolysis in overdose situations. Estimating the methaemoglobin (MetHb) concentration in the blood is critical for effective management. Quantification of MetHb by spectrophotometer is generally advocated along with complete haematological and liver function investigations.

In this paper, we highlight the importance of quantifying MetHb by spectrophotometer, which is very simple, and demonstrates high sensitivity.

Key Words: Dapsone overdose; Methaemoglobin (MetHb); Spectrophotometry, Liver function tests

Introduction

Methaemoglobin is a form of the oxygen-carrying protein haemoglobin, in which the iron in the haeme group is converted from ferrous ion to ferric ion of the haemoglobin.[1-4] Methaemoglobin reduces oxygen carrying capacity of blood; the presence of oxidized iron changes the haeme tetramer and reduces oxygen release in the tissues, thus shifting the haemoglobin oxygen dissociation curve to the left, as in alkalosis. This results in a chocolate brown colour of blood.

Apart from acquired methaemoglobinaemia, there are various other causes for methaemoglobinaemia, including inorganic agents such as fertilizers (nitrates), contaminated well water, preservatives, industrial products, chlorates, copper sulfate, fungicides, organic nitrites/nitrates, amyl nitrite, isobutyl nitrite, certain local anaesthetics, antimalarials, analgesics, antibiotics (sulfonamides, nitrofurans), p-amino-salicylic acid, dapsone, and several industrial and household agents.

Dapsone was introduced in 1943 as an effective chemotherapeutic agent for leprosy and still is an important drug for treatment of this disease. Other uses of dapsone include dermatitis herpetiformis, maduromycosis, paniculitis due to alpha-1 antitrypsin deficiency and pneumocystis carinii pneumonia in HIV patients.

The objective of the present study was to determine the concentration of methaemoglobin in acute dapsone poisoning. Pulse co-oximetry and semiquatitative methods using comparison charts are available for methaemoglobin estimation.[5] We employed spectrophotometric method for quantitative analysis.

Materials and Methods

A 21-year-old female with methaemoglobinaemia due to acute dapsone poisoning was admitted to intensive care. Methylene blue 100mg in 300ml normal saline was administered over 30 minutes, and activated charcoal with magnesium sulphate was given.

Three milliliters of blood was drawn with appropriate anticoagulant, and routine test for methaemoglobin (MetHb), liver function (LFT), and bilirubin estimation was carried out on admission, and subsequently at 24 hours, 48 hours, and 72 hours.

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Determination of Methaemoglobin[6]: The principle involved is determination of MetHb before and after adding cyanide. The cyanmethaemoglobin formed does not absorb at that particular wavelength, and the difference in reading is proportional to the amount of methaemoglobin present. Potassium ferricyanide is added to the second portion of the blood to convert oxyhaemoglobin to methaemoglobin. Then the solution is read before and after adding cyanide, and this gives the total methaemoglobin, from which the percentage of total methaemoglobin was calculated.

Reagent required: Stock Phosphate Buffer M/15 PH 6.6 - Dissolve 95g of anhydrous disodium hydrogen phosphate and 1.36g of anhydrous potassium dihydrogen phosphate in water and make upto 250ml.

Phosphate Buffer M/60 pH 6.6 - Dilute the stock solution 1:4 and dissolve 0.02% saponin in it.

Neutralised Sodium Cyanide5% - Prepare fresh for use by mixing 10% aqueous sodium cyanide with an equal volume of 2% V/V glacial acetic acid.

Procedure: Dilute 0.5ml blood (heparinised is suitable) to 12.5ml with M/60 phosphate buffer. Divide into two parts, and small quantity of one portion is read at 630nm with the buffer as blank (R1). Pour this back into the remainder, add one drop of neutralized cyanide solution, mix and read again at 630nm (R2).

Add a drop of 5% ferricyanide to the other portion and read it as (R3), and after adding cyanide as (R4).

Calculation: Percentage methaemoglobin = [(R1-R2/R3-R4)x100]/2.

Results

We observed 28.4% MetHb level on admission, and a decrease in MetHb after 24 hours, 48 hours, and 72 hours as 14.80%, 6.23%, and 2% respectively. Blood Hb level on admission was 8g and increased gradually over 24 hours, 48 hours, and 72 hours to 9.8g, 10.2g, 10.3g respectively. No significant change was observed on total bilirubin on admission until discharge.

Discussion

Dapsone is a synthetic sulfone increasingly used in the treatment of a variety of dermatological disorders in tropical countries. The toxicity is directly related to the methaemoglobin levels in the blood.[7] Dapsone is a dihydrofolate reductase inhibitor. Methaemoglobinaemia and haemolysis are two important complications of acute intoxication. Earlier studies indicated a decrease of Hb in acute dapsone poisoning because of haemolysis. The released Hb is metabolized in the liver and correspondingly increases the bilirubin levels. The principal metabolite is N-acetyl dapsone, which binds to plasmaprotein and is responsible for inducing methaemoglobinaemia. A second pathway involves N-oxidation of dapsone to 4-amino 4-hydroxamine diphenyl sulphone, which is responsible for haematological toxicity.

In our study, we observed a gradual increase in Hb, and normal bilirubin level on second, third and fourth days, indicating only methaemoglobinaemia, and not haemolysis, which was probably due to lesser toxicity. We observed the falsely elevated MetHb levels on delayed analysis. Though we did have access to spectroscopy, a semi-quantitative method for measurement of MetHb in blood, the results obtained by such methods can be considered only as a screening test for methaemoglobin levels in blood. Also, we required repeated analysis of MetHb levels at various intervals so as to adjust the dose of the antidote (methylene blue).

Table 1 Summary of Results

	MetHb %	Hb g/dl	Total Bilirubin mg/dl
On admission	28.40	8	0.7
24 hours	14.80	9.8	0.7
48 hours	6.23	10.2	0.9
72 hours	2.00	10.3	0.7

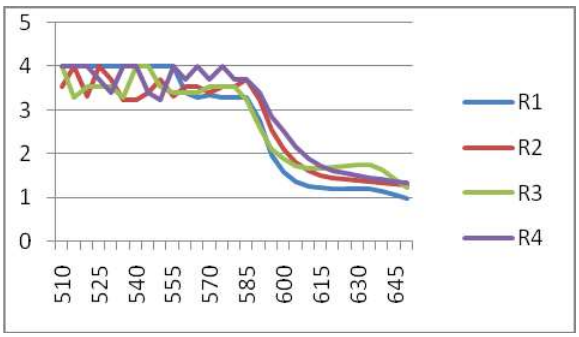

Fig 1: MetHb on admission

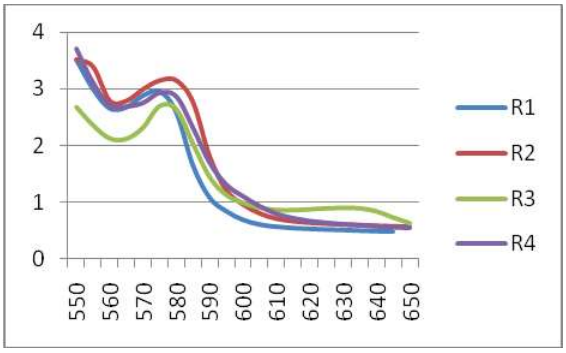

Fig 2: MetHb at 24 Hrs

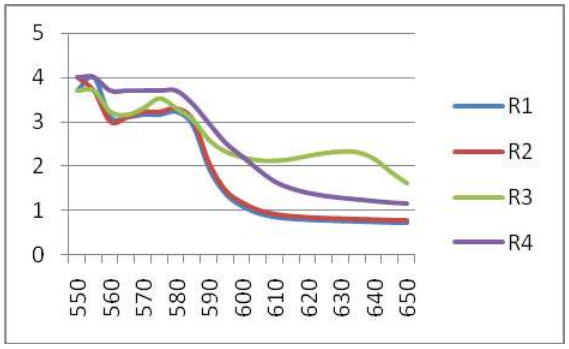

Fig 4: MetHb at 72 Hrs

The present method of quantifying MetHb by spectrophotometer is very simple with high sensitivity. Our observations with regard to mild dapsone toxicity is that quantification of MetHb will be useful, and in moderate and severe toxicity, a complete haematological and liver function investigation must be performed.

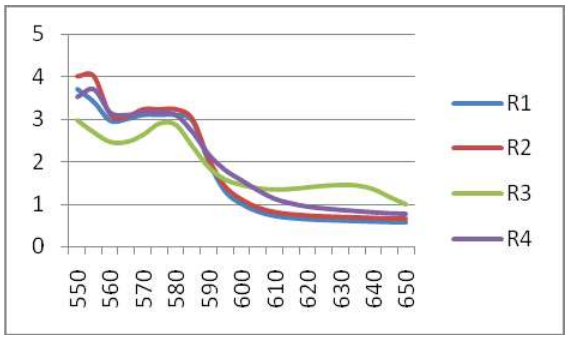

Fig 3: MetHb at 48 Hrs

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