REDUCED GLUTATHIONE : IMPORTANCE OF SPECIMEN COLLECTION.

Sarita Chavan, Laxmayya Sava, Vishal Saxena, Sandhya Pillai, Alka Sontakke and Digamber Ingole.

Department of Biochemistry, Pad. Dr. D.Y. Patil Medical College, Pune-4110 018.

ABSTRACT

Glutathione, the dominant intracellular thiol, plays an important protective role against oxidative stress. The accidental findings of increased reduced glutathione level postprandially as compared to post absorptive level prompted the design of present study. Reduced Glutathione levels were estimated in 50 healthy individuals in post absorptive and postprandial phase by taking whole blood in ACD bulb. Mean postprandial reduced Glutathione ($9.60 \pm 3.39 \mu\text{mole / gm of Hb}$) is significantly increased than mean postabsorptive level ($5.53 \pm 0.88 \mu\text{mole / gm of Hb}$; $p < 0.001$). It also shows positive correlation ($r = 0.65$) between these two GSH levels. So present study suggests that post absorptive specimen collection is preferable over random or postprandial as the former reflects the true basal level of reduced glutathione.

KEY WORDS

Reduced Glutathione, Non enzymatic Antioxidant

INTRODUCTION

The discovery of glutathione dependent enzymes and their precise role in detoxification mechanism has made glutathione as 'inevitable glutathione' since its discovery by De Rey-Pailhade over hundred years ago. Reduced Glutathione (GSH) is the smallest intracellular thiol (-SH) molecule. Its high electron donating capacity (high negative redox potential) combined with high intracellular concentration (mmol) generate great reducing power. This characteristic underlies its potent antioxidant action and enzyme cofactor properties and supports a complex thiol exchange system which hierarchically regulates cell activity (1).

Plasma concentration is in the micromolar range (appx. 4.5 mM). GSH is under tight homeostatic control both intracellularly and extracellularly. A dynamic balance is maintained between GSH synthesis, its recycling from GSSG / oxidized glutathione, and its utilization (2) The primary biological function of glutathione is to act as a non-enzymatic reducing agent to assist in keeping cysteine thiol side chains in a reduced state on the surface of proteins. GSH is involved in the synthesis and repair of DNA, assists the recycling of vitamins C and E, blocks free radical damage, enhances the antioxidant activity of vitamin

C, facilitates the transport of amino acids, and play critical role in detoxification (3).

GSH is a well-established, metabolic regulator that may qualify as a putative index of health (4). In humans GSH depletion is linked to a number of disease states so today there is a growing awareness about this molecule's role in the pathophysiology of many diseases as an non-enzymatic antioxidant. The accidental finding of increased reduced glutathione levels in the postprandial phase, prompted the present study to be carried out, to confirm whether there was a significant difference in the reduced glutathione levels in postabsorptive and postprandial phases.

MATERIALS AND METHODS

The present study was carried out at Pad. Dr. D.Y. Patil Medical College, Hospital and Research Centre, between the period of October 2002 and September 2003. 50 healthy individuals of either sex reported to the biochemistry department, were enrolled in the study. They gave informed consent for the study.

The individuals in the age group of 20-56 years were not suffering from any disease and were not on any medication before and during the study period.

The post absorptive sample was collected 12 -14 hours after the meals and then postprandial blood sample was collected exactly 2 hours after heavy breakfast. Both samples were collected in Acid Citrate Dextrose (ACD) bulbs and processed within three hours of sample collection.

Author for Correspondence

Miss Sarita N. Chavan

Dept. Of Biochemistry,

Pad. Dr. D. Y. Patil Medical College,

Pimpri, Pune 411018.

Email Address :- snc_unc@yahoo.com

ACD Reagent Preparation

Trisodium Citrate 2.2gm, Citric Acid 0.8gm and Dextrose 2.45gm dissolved in 100ml distilled water.

1ml of whole blood was collected in a plain sterile bulb containing 100µl of ACD reagent.

The level of erythrocyte reduced glutathione was assayed by Beutler et al method (5).

A) Preparation of Erythrocytes

Mix 0.2ml of whole blood and 1.8ml of distilled water into the test tube to prepare a hemolysate. Promptly added 3.0ml of precipitating solution (glacial meta phosphoric acid 1.67gm, disodium EDTA 0.20gm & 30gm NaCl dissolved in 100ml distilled water) & filtered through coarse grade filter paper after 5 min.

B) Assay – Marked two capped cuvettes as 'Assay' & 'Blank'. In Assay cuvette 2.0ml of filtrate, 0.8ml of Na_2HPO_4 reagent (0.3 mol/l) and 1.0ml of 5' Dithiobis-2 nitrobenzoic acid

(DTNB) reagent was taken. In Blank cuvette 1.2ml of precipitating solution, 0.8ml of distilled water was taken. Then added 8.0ml of Na_2HPO_4 & 1.0ml of DTNB reagent in both the cuvettes. Both the cuvettes were capped and inverted three times. Read absorbance at 412nm within 4mins.

C) GSH calibrators curve was plotted by assaying different GSH calibrators (50, 10, 5, 4, 2 mg/dl) to

determine GSH concentration of blood sample from the calibrator curve.

GSH (mg/dl of RBC) =

$$\frac{\text{GSH concentration (calibration curve)}}{\text{Hb (gm/dl)}}$$

The GSH concentration unit is then calculated as micromole per gram of hemoglobin by estimating hemoglobin concentration of original whole blood.

The results were analyzed using paired ' t ' test and Pearson's correlation coefficient.

RESULTS

Postprandial erythrocyte reduced glutathione level is significantly increased ($p < 0.001$) than post absorptive level. It also showed a positive correlation ($r = 0.65$) between these two reduced glutathione levels. (Table No. 1)

DISCUSSION

Glutathione , the tripeptide γ -glutamylcysteinylglycine, has several important functions. It is a reductant, conjugated to drugs to make them more water soluble, involved in transport of amino acids across cell membranes, part of some leukotriene structures, a cofactor for some enzymatic reactions, and an aid in the rearrangement of protein disulfide bonds. Erythrocytes require a plentiful supply of reduced glutathione. The usual steady – state ratio of GSH to

Fig No. 1. Glutathione Metabolism

Table 1. Erythrocyte reduced glutathione levels

n = 51	Reduced Glutathione μmol / gm of Hb	P value and Pearson' correlation coefficient
Postabsorptive level	5.53 ± 0.88	P < 0.001
Postprandial level	9.60 ± 3.39	r = 0.65

GSSG in erythrocytes is 100:1 (6).

A major function of GSH in the erythrocyte is to reductively eliminate H₂O₂ and organic peroxides, which are reactive oxygen metabolites that can irreversibly damage hemoglobin and cleave the C-C bonds in the phospholipids tails of cell membranes (7).

Glucose used by the Hexose Monophosphate pathway (HMP) in red blood cells provides NADPH to keep glutathione in the reduced state. Glutathione reductase links the pathway to the HMP through the reversible oxidation and reduction of NADP as shown in fig no.1 (8).

Free radicals are produced mostly by biochemical redox reaction involving oxygen, which occur as part of normal cell metabolism by series of univalent reduction. In postprandial phase insulin secretion is responsible for increased oxidation of glucose by HMP in RBC's. This lead to increased production of NADPH leading to regeneration of GSH, which can combat free radicals produced during oxidative catabolism (9).

Secondly, raised reduced glutathione level in postprandial phase may also be due to availability of sufficient nutrients in form of amino acid and dietary antioxidants in form of vitamin C which plays an important role in regeneration of GSH (10).

The present study clearly demonstrates that levels of GSH are increased in postprandial phase as compared to post absorptive phase. Therefore it is suggested that for determination of GSH levels in post absorptive or fasting sample is always better, which reflects the true basal GSH levels as opposed to the raised GSH in the postprandial as well as random phase.

SUMMARY

Determination of erythrocyte reduced glutathione levels in post absorptive or fasting specimen is always better,

which reflects the true basal reduced glutathione levels as opposed to the raised GSH in the postprandial as well as random phase.

ACKNOWLEDGMENT

We are very grateful to ICMR for providing the funds without which the study would not have been possible. We would also like to acknowledge the kind cooperation extended by the staff of Biochemistry Department of Pad. Dr. D.Y. Patil Medical College, Hospital and Research Centre in completing this research projects.

REFERENCES

1. Kosower NS, Kosower EM. (1978) The Glutathione status of cells. *Intl Rev Cytol* 54, p.109 – 157.
2. Kidd PM (1997) Glutathione : systemic protectant against oxidative and free radical damage. *Altern Med Rev* 1, p. 155 – 176.
3. William D. Misner (2003) Does Glutathione enhances exercise performance? A case study. *Townsend Letter For Doctors and Patients*, July.
4. Meister A, Anderson ME (1983) Glutathione. *Ann Rev Biochem.* 52, p.711-60.
5. Beutler E, Duron, O Kelly B.M. (1963) Improved method for the determination of blood. Glutathione. *J. Lab. Clin. Med.* 61, p.882 – 888.
6. Thomas Devlin, *Textbook of Clinical Biochemistry with clinical correlations* (2002) 5th. Edition Wiley –Liss New York p. 819
7. Donald voet, Judith G.Voet, *Fundamentals Biochemistry* (1999) 1st Edition John Wiley & sons New York p.422-423.
8. Carl A. Burtis, Edward R. Ashwood, *Tietz Textbook of Clinical Chemistry* (1999) 3rd. Edition W.B. Saunders company, Philadelphia p.1643-46
9. Murray R. K., Granner D.K, Mays P.A. & Rodwell V. W. *Harper's Biochemistry.* (2000) , 25th Edition p. 221 & 648.
10. CS Johnston, CG Meyer and JC Srilakshmi. (1993). Vitamin C elevates red blood cell Glutathione in healthy adults. *American Journal of Clin. Nutrition* , 58, p.103-105