

# To Re-Emphasize the Need for Fluoride in Blood Samples for Glucose-Level Testing

Pages with reference to book, From 113 To 114

Irtaza I. Khan, Hasan A. Usmani ( Medical Student, Faculty of Health Sciences, The Aga Khan University, Karachi. )  
Fawzia H. Mughal ( Department Pathology, Shaikh Zayed Hospital, Lahore. )

## Abstract

Blood samples were collected from 30 subjects. To a portion from each sample was added Fluoride/Ethylene Diamine Tetra Acetate (E.D.T.A) to inhibit glycolysis and clotting. The remaining portions were allowed to clot without any inhibitor. On subjecting to glucose concentration testing, the portions without the inhibitor showed a decline in the glucose level of 8 mg/dl (0.44 mmol/l) in the first hour and of 7 mg/dl (0.39 mmol/l) per hour in the next two hours. It is re-emphasised that a glycolysis inhibitor should always be added to blood samples drawn for glucose level testing. Otherwise, the reported results could be misleading (JPMA 48:113,1998).

## Introduction

Blood glucose level testing is one of the common clinical investigations these days. It is used to screen out glucose intolerance and regulate blood glucose levels in diabetes. The blood samples sent to laboratories for chemical analysis are usually clotted and some time lapse before they are tested. During this time, glucose in the blood is being utilized by the erythrocytes through glycolysis. Therefore, the reported blood glucose levels are misleading. This is especially true for cases with glucose levels at the margin of normal values. Thus for proper determination of blood glucose levels samples have to be mixed with fluoride which inhibits the glycolysis enzyme enolase by binding to its active site as magnesium fluoro-phosphate<sup>1</sup>.

Unfortunately most of the laboratories do not insist upon collecting samples in fluoride-EDTA tubes. In order to reemphasize the essentiality of this inhibitor study of glucose utilization by the erythrocytes was carried out at the Department of Biochemistry, Shaikh Zayed Hospital<sup>1</sup>, Lahore.

## Materials, Methods and Results

Collection tubes were prepared containing 1 mg sodium fluoride and 3 mg Ethylene Diamine tetra acetate (EDTA). A total number of thirty samples were collected randomly from different wards and the Outpatient Department, Diabetes Clinic of Shaikh Zayed Hospital. Specimens were properly labelled and the age, sex and registration numbers of the subjects noted. From each subject 4 ml blood was drawn, out of which 1.5 ml was placed in EDTA - fluoride tubes, while the rest was allowed to clot without the EDTA and fluoride inhibitors. From the tubes with the inhibitors, the first samples were analyzed for blood glucose levels (giving the initial glucose concentration), while serum samples were taken from the clotted samples and their glucose levels were determined at one hour interval, to note any change in glucose concentration.

Both the samples were analyzed manually by glucose-oxidase-PAP method using Randox™ kit no. GL2623.

Precautionary measures were taken into care to ensure the quality of the results. These included avoiding pipetting error and accurate time of incubation. The results were tabulated and the rate of utilization of glucose was determined.

The samples were split into three groups according to their initial glucose concentrations:

A) Upto 150 mg/dl.

B) 151-250mg/dl.

C) 251-350 mg/dl.

(Tables I and II)

**Table I. Mean glucose levels ( $\pm$ One Standard Deviation) (mg/dl) at one-hour intervals.**

Initial Levels	Hour Zero	Hour One	Hour Two	Hour Three
Upto 150	105 $\pm$ 22.4	98 $\pm$ 21.9	90 $\pm$ 22.5	83 $\pm$ 22.3
151-250	193 $\pm$ 35.7	181 $\pm$ 36.8	177 $\pm$ 36.9	169 $\pm$ 37.2
251-350	307 $\pm$ 5.8	300 $\pm$ 5.3	289 $\pm$ 7.9	283 $\pm$ 7.8

**Table II. Mean falls in the glucose ( $\pm$ One Standard Deviation) (mg/dl).**

Groups	First hour	Second hour	Third hour
A: (Upto 150)	7 $\pm$ 2.1	8 $\pm$ 3.0	7 $\pm$ 2.0
B: (151-250)	13 $\pm$ 5.9	9 $\pm$ 1.1	8 $\pm$ 0.7
C: (251-350)	7 $\pm$ 1.2	8 $\pm$ 2.9	6 $\pm$ 0.6

There is said to be a decrease of 8-10 mg/dl (0.44-0.56 mmol/l) per hour in the blood glucose concentrations if fluoride is not used as a glycolysis inhibitor<sup>2</sup>.

Analysis of the results split in three groups showed that there is a mean decrease of 8 mg/dl (0.44 mmol/l) in the first hour and 7 mg/dl (0.39 mmol/l) in the second and third hours respectively (Tables I and II). This gradual decrease in the glucose level is due to the utilization of glucose by the erythrocytes that use it for glycolysis<sup>3</sup>.

When the cases were analyzed according to initial glucose levels, there was no difference in the glucose utilization of the three groups A, B and C. This shows that the utilization of glucose is independent of the initial glucose concentration and the erythrocytes take up similar amounts of glucose from the blood at all the tested glucose concentration levels. Increasing the initial concentration has no effect on the uptake of glucose.

When the samples with fluoride-EDTA were tested for blood glucose levels after one hour, there was no decrease in the glucose concentrations in the blood. This indicated that the inhibitor was working adequately and not letting the glucose levels fall.

### Comments

These findings reinforce the fact that blood glucose levels do decrease if the proper inhibitor is not employed. Testing for and reporting these erroneous values may misguide health care providers. This can have serious implications on the health of the diabetic patient, especially if he is on insulin therapy. Therefore, health care professionals must insist on adding the glycolysis inhibitor to the blood samples

drawn for glucose testing. Without that, the authenticity of the reports will remain questionable. Strict guidelines should be issued by governing bodies and medical associations in this regard.

### **Acknowledgements**

The authors wish to acknowledge the help and guidance of Professor S. Shah Jahan, Department of Biochemistry, Shaikh Zayed Hospital, Lahore. His co-operation is greatly appreciated.

### **References**

1. Zubay, G. Biochemistry. In: Zubay, G. (ed). 2nd ed. New York, Macmillan Publishing Company, 1988, p. 449.
2. Gowenlock, All. Varley's Practical Clinical Biochemistry, In: Gowenlock, All. (ed) 6th ed, London, Heinemann Medical Books, 1988, p. 321.
3. Lehninger, AL., Nelson, DL., Cox, MM. Principles of Biochemistry. In: Nelson, DL., Cox, M.M. (ed). 2nd ed. USA, Worth Publishers Inc., 1993, pp. 400-439.