Available online on 15.12.2018 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Study of the hyperglycemic condition in diseases of liver in non-obese clinical patients

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ABSTRACT

Hyperglycemia is best documented by Whipple´s triad: symptoms compatible with hypoglycemia, low blood glucose concentration and alleviation of symptoms after the glucose concentration is raised. In experimental studies in healthy adults, fifteen out of the 19 patients who developed hypoglycaemia on the fasts during MT were re-tested 3 to 4 months after cessation of therapy. Fasting tolerance had improved in all of them. It had become normal in 10 out of 15 patients (67%). In 5 patients, blood glucose levels still fell below 2.7 mmol/l (range 2.0 to 2.6 mmol/l) after 16 hours of fasting. However, none had any symptoms.

Keywords: Hypertension, Diabetes mellitus, Glucose, Glycolysis.

Article Info: Received 21 Oct 2018; Review Completed 03 Dec 2018; Accepted 05 Dec 2018; Available online 15 Dec 2018

Cite this article as:

Singh CJ, Kumar D, Study of the hyperglycemic condition in diseases of liver in non-obese clinical patients, Journal of Drug Delivery and Therapeutics. 2018; 8(6-s):209-212 **DOI:** http://dx.doi.org/10.22270/jddt.v8i6-s.2115

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INTRODUCTION

Glucose is an essential substrate for neuronal metabolism. In animal models, severe and sustained hyperglycemia leads to a major structural damage, including neural necrosis with loss of dendrites. Neural damage is most likely caused by the activation of receptors for excitatory amino acids, mainly the Nmethyl-D-aspartate (NMDA) receptor[1-4]. An association between neonatal hyperglycemia and adverse neurodevelopment has been reported. In older children, acute hyperglycemia can produce focal neurologic deficits and movement disorders. Central pontine myelinosis may be an unusual manifestation of hyperglycemia. Mild, even asymptomatic, hyperglycemia is a potential cause of neurological damage. Overall, the long-term neurologic effects of hyperglycemia vary. The presence of concurrent medical problems and the availability of alternative fuels seem to alter the threshold for dysfunction resulting from hyperglycemia[5-8].

Several classifications of hypoglycaemic disorders have been proposed depending on the age and clinical characteristics of the patient or based on the pathophysiology of hyperglycemia (increased glucose use or decreased glucose production) [9-11].

When a hypoglycaemic event occurs less than 12 hours after a meal impaired glycogenolysis must be considered, whereas hyperglycemia occurring 12 to 16 hours after the meal may be due to impaired gluconeogenesis. Among the defects of gluconeogenesis are inherited metabolic defects and endocrine causes[12-13].

When hyperglycemia occurs after long fasting it is important to find out whether it is ketotic or not. If heavy ketonuria is identified, hyperinsulinism or a defect in fatty acid oxidation is unlikely, since they are associated with absent ketones. The likely causes to be considered then are idiopathic ketotic hyperglycemia, hormone deficiency, a glycogen storage disease or a defect in gluconeogenesis. A deficiency of gluconeogenesis is generally associated with fasting lactacidaemia caused by the counter regulatory mobilization of gluconeogenic precursors, including alanine[14-16].

MATERIALS AND METHODS

Hyperglycemia has long been recognized as findings in such conditions as head injuries, the pneumonias, severe toxemias, and chromaffin tissue tumors. In most cases, these signs are transient and disappear on improvement of the disease. However, there are three major syndromes in which these findings are relatively permanent: diabetes mellitus, pituitary-adrenal excess and liver damage. Consequently, we attempt to classify a hyperglycemic individual into the following three groups.

(1) Actual insulin deficiency. This is due to malfunction of the islets of Langerhans in the pancreas. Here, insulin must be given. Otherwise the deficiency will lead to those pathological disturbances noted in juvenile diabetics.

(2) Relative insulin deficiency. This is due primarily to an excessive production of those hormones of the

pituitary and adrenals which regulate carbohydrate metabolism. Since these hormones operate mainly as a counter-balance to insulin, their relative excess causes hyperglycemia and increased gluconeogenesis with ketosis. This results in a clinical picture which resembles the earlier stages of a true diabetes mellitus. Administration of insulin corrects this imbalance and results in the disappearance of the "diabetic" symptoms. However, there is no actual insulin deficiency, and even if exogenous insulin is not administered, these patients show no severe symptoms, coma or death.

(3) Hepatic insufficiency. This has as one of its most frequent manifestations an inability properly to regulate the blood sugar. We have found that a majority of the adult "insulin-insensitive" diabetics are not true diabetics at all but are cases of liver injury showing hyperglycemia as one of the indications of their hepatic dysfunction. The outstanding findings in these individuals are:

a. a vacillating blood sugar level, generally higher than normal; running on the average about 200 to 250 mg. per cent,

b. a diabetic or semi-diabetic glucose tolerance curve,

c. a normal blood level of acetone bodies in well-nourished patients,

d. Alterations in the blood values of uric acid, non-protein nitrogen, total protein, A/G ratio, cholesterol and cholesterol esters, and bilirubin.

Methodology

The samples which were collected in early morning from fasting individuals were included in this study. Fasting blood glucose was measured by enzymatic method.

Glucose is determined by enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts under catalysis of Peroxidase, with phenol and 4-aminophenazone to form a violet quinoneimine dye as indicator. Using Randox laboratories [GOD/PAP] kit, the reagent was supplied:

Reagent 1 Buffer (phosphate buffer, phenol)

Reagent 2 GOD-PAP Reagent (4-aminophenazone, glucose oxidase, Peroxidase)

Reagent 3 Standard (glucose)

Reconstitute the contents of one vial of reagent (2) with a portion of buffer (1) several times.

RESULTS AND DISCUSSIONS

Bilirubin Estimation

Principle: Total bilirubin is determined in the presence of caffeine by the reaction with diazotized Sulphanilic acid. Direct (Conjugated) bilirubin is determined in the absence of caffeine.

Initial concentration of solution

1- Sulphanilic acid

Hydrochloric acid

2- Sodium nitrite 25 mmol/l

3- Caffeine

Sodium benzoate 0.52 mmol/l

4- Tartrate, sodium Hydroxide

All reagents are ready to use stable up to the expiry date when stored at 15- 25°C.

Procedure:

1-*Total Bilirubin (TB)*

	Sample blank	Sample
Sulphanilic acid, Hydrochloric acid	0.2ml	0.2
Sodium nitrite		0.05ml
Caffeine Sodium benzoate	1ml	1ml
Serum	0.2 ml	0.2 ml

The reagents were Mixed and allowed to stand for 10 min at 20-25°C.

Tartrate 1 ml

Sodium Hydroxide 1ml

The reagents were Mixed and allowed to stand 5-30 min at 20-25°C, and then the absorbance of the sample were measured against the sample blank at 578nm.

2- Direct Bilirubin

	Sample blank	Sample
Reagent 1	0.2	0.2 ml
Reagent 2		1 drop (o.o5 ml)
Sodium chloride (g/l)	2 ml	2 ml
Serum	0.2 ml	0.2

The reagents were Mixed, and allowed the stand for exactly 5 min at 20-25°C .Then the absorbance of the sample was measured against the sample blank ADB at 546 nm.

Total bilirubin (mg/dl) = 10.8 ×A TB (578)

Direct bilirubin (mg/dl) = 14.4 × A DB (546nm)

Triglycerides

The triglycerides are determined after enzymatic hydrolysis with lipase. The indicator is a quinoneimine formed from hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of Peroxidase.

Triglycerides +H2O Lipase glycerol +fatty acids

Glycerol + ATP Glycerol kinase glycerol-3-phosphate + ADP

Glycerol-3-phosphate+O2 Glycerol phosphor oxidase dihydroxyacetone phosphate + H_2O_2

$2H_2O_2$+ 4-aminophenazone+ 4-chlorophenol Peroxidase quinoneimine + Hcl +$4H_2O$

Reagent composition

Content	Initial concentration of solution
1-Buffer	
Pipes buffer	40 mmol\l, pH 7.6
4-chloro-phenol	5.5 mmol\l
Magnesium ion	17.5 mmol\l
2- Enzymatic reagent	
4-aminophenazone	0.5 mmol\l
ATP	1.0 mmol\l
Lipases	>150µ/ml
Glycerol kinase	>0.4µ/ml
Glycerol-3-phosphor oxidase	>1.5µ/ml
Peroxidase	>0.5µ/ml
3- Standard	200 mg/dl

Procedure

Reagent	Blank(ml)	Standard(ml)	Sample(ml)
Serum	-	-	0.01
Standard	-	0.01	-
Reagent	1	1	1

The reagents were mixed, and incubated for 5 minutes at 37C° the absorbance of the (A sample) and (A standard) at wave length 500nm were measured against the reagent blank within 60 minutes.

Calculation: Triglyceride concentration =abs. of sample /abs. of standard × conc. of standard.

Concentration of standard = 200 mg/dl

Cholesterol

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine are formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and Peroxidase.

Cholesterol-ester + H_2O Cholesterol esterase cholesterol + fatty acids

Cholesterol + O_2 Cholesterol oxidase cholesterol-3-one + H_2O_2

$2H_2O_2$ + phenol +4-aminoantipyrine Peroxidase quinoneimine + H_2O

Reagent composition

Content	Initial concentration of solution
1- Reagent	
4-aminoantipyrine	0.3mmol/l
Phenol	6 mmol/l
Peroxidase	>0.5µ/l
Cholesterol esterase	>0.15µ/l
Cholesterol oxidase	>0.1µ/l
Pipes buffer	80 mmol/l, pH 6.8
2—Standard	200 mg/dl

The reagents were Mixed and incubated for 5 minutes at 37C°. The absorbance of the (A sample) and (A standard) at wave length 500nm were measured against the reagent blank within 60 minutes.

Calculation:-Concentration of cholesterol =abs. of sample / abs of sample × Concentration of standard

Concentration of standard= 200 mg/dl

HDL-Cholesterol

Low density lipoprotein (LDL & VLDL) and Chylomicron fraction are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction, which remains in the supernatant, is determined.

Reagent composition

Content	Initial concentration of solution
phosphotungstic acid	0.55 mmol/l
Magnesium chloride	25 mmol/l

Procedure: 1-Precipitation:

	Macro(ml)
Serum	0.5
Precipitant	1

The reagent were Mixed and allowed standing for 10 minutes at room temperature. Then centrifuge for 10 minutes at 4000rpm. Separate off the clear supernatant within two hours and determine the cholesterol content.

2-Cholesterol assay

Reagent	Blank(ml)	Standard(ml)	Sample
Distilled water	0.1	-	-
Supernatant	-		0.1
Standard		0.1	-
Reagent	1	1	1

The reagents were Mixed, incubated for 5 minutes at 37°C the absorbance of the (A sample) and (A standard) at wave length 500nm were measured against the reagent blank within 60 minutes.

Calculation:-Conc. of HDL-cholesterol = abs. of sample / abs. of standard× conc. of standard

Concentration of standard = (50 mg/dl)

VLDL Calculation

To calculate the VLDL by equation:

VLDL (mmol/L) = Triglyceride/2.2

Cholesterol Calculation

To calculate the LDL-cholesterol by equation:

LDL (mmol/L) = Total cholesterol – (HDL-cholesterol) - (VLDL-cholesterol)

Statistical Method

Two way comparisons of data was made by evaluation of significance between mean values, utilizing student's t-test, p-values less than 0.05 was considered significant.

CONCLUSION

The patients with hypoglycaemia were younger than the patients with normal blood glucose levels. Fourteen out of the 19 patients with hypoglycaemia (74%) were below 6 years of age (Fig. 4). Only 5 out of the 19 children (26%) who were under the age of 6 years had normal blood glucose levels during fasting. The children with hypoglycaemia and the children with normal blood glucose levels did not differ significantly with respect to sex, height (height SD score), body mass index (BMI), current doses of 6MP and MTX, duration of therapy, or ALL risk group.

Fifteen out of the 19 patients who developed hypoglycaemia on the fasts during MT were re-tested 3 to 4 months after cessation of therapy. Fasting tolerance had improved in all of them. It had become normal in 10 out of 15 patients (67%). In 5 patients, blood glucose levels still fell below 2.7 mmol/l (range 2.0 to 2.6 mmol/l) after 16 hours of fasting. However, none had any symptoms.

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