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 obese clinical patients

Chander Jeet Singh, Dinesh Kumar*

Department of Biochemistry, Sri Satya Sai University of Technology & Medical Sciences, Sehore-466001

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, the threshold for symptoms of hyperglycemia is on average a blood glucose level of 3.0 mmol/l Mitrakou reported autonomic symptoms of hyperglycemia to begin at a blood glucose level of 3.2 mmol/l, and deterioration in cognitive function tests at a level of 2.7 mmo/l. When sensory evoked potentials were measured in relation to blood glucose concentrations in infants and children with episodes of hyperglycemia, abnormalities were noted at blood glucose levels less than 2.6 mmol/l.

Keywords: Hypertension, Diabetes mellitus, Glucose, Glycolysis.

Article Info: Received 22 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 obese clinical patients, Journal of Drug Delivery and Therapeutics. 2018; 8(6-s):204-208 **DOI:** http://dx.doi.org/10.22270/jddt.v8i6-s.2112

***Address for Correspondence:**

Dinesh Kumar, Department of Biochemistry, Sri Satya Sai University of Technology & Medical Sciences, Sehore-466001

INTRODUCTION

Blood glucose concentration is normally maintained within a narrow range, reflecting a balance between the production and utilization of glucose. Hyperglycemia is the result of a reduction in glucose intake or production, or excessive utilization, or a combination of these. During feeding glucose is formed principally from carbohydrate in the diet[1-3]. During fasting glucose is released by the liver by glycogenolysis and gluconeogenesis. The processes involved in the maintenance of glucose homeostasis are controlled by endocrine and neural factors, and substrate availability, and are closely associated with fat and protein metabolism. The liver plays a crucial role in the control of this mechanisms[4-6]. Glycogen is found particularly in muscle and liver. Muscle glycogen can only be used as a reserve substrate by the muscle itself. By contrast, hepatic glycogen can be broken to free glucose and released into the circulation. Gluconeogenesis is the process in which glycerol, amino acids and lactate are converted into glucose. Glycerol is produced from lipolysis in adipocytes, amino acids from protein catabolism and lactate from glycolysis. Via glycolysis glucose is metabolized to pyruvate which can be converted into lactate, or after decarboxylation, into acetyl co-enzyme A (acetyl CoA). Acetyl- CoA is the substrate for the synthesis of fatty acids, which are then stored as triglycerides[7-9].

Hormonal factors important in glucose homeostasis are insulin and counter regulatory hormones. Insulin, which is secreted in response to increases in plasma glucose and amino acid concentrations, suppresses hepatic glucose production by stimulating glycogen synthesis and inhibiting glycogenolysis[10-11]. It also promotes glycolysis and suppresses gluconeogenesis[12]. Glucose uptake by adipose tissue and muscle is stimulated. In adipocytes triglyceride synthesis is enhanced, and in muscle glycogen formation and glycolysis are favoured. The counter regulatory hormones glucagon, adrenaline, noradrenaline, cortisol and growth hormone oppose the effects of insulin. As blood glucose level falls insulin secretion is inhibited and release of counter regulatory hormones is stimulated[14].

MATERIALS AND METHODS

Patients

A Cross sectional, hospital based study was carried out on 128 patients (80male, and 48female) which classified into (8) groups according to their age. In these, five were lean and non-diabetic (four men, one woman), six were overweight or obese (all men).

Blood Samples

About 5 ml of venous blood was drawn from the cubital vein using disposable needles and syringes. In all the cases blood samples were collected in the fasting state.

The blood sample was put in a clean dry plain plastic tube and was allowed to clot at 37C° for 10-25 minutes before centrifugation.

The serum obtained was centrifuged at 3000 rpm for 15 minutes and it was essential to ensure that the serum did not show hemolysis. The clear serum was transferred into two clean plastic tubes by disposable syringes.

1- For estimation of blood glucose.

2- For estimation of liver function tests including (total protein, total bilirubin, AST, ALT, and ALP).

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.

Procedure:

Reagent	Standard or serum	Reagent blank
serum	0.02	_
Working solution	2	2

The reagent were mixed and incubated 10 minutes at 37C° and Measured the absorbance of (standard, sample) were measured against the reagent blank within 60 minutes. The reading at 500nm.

Glucose conc. =abs. of sample / abs. of standard × con. of Standard.

Serum Total Protein Estimation

Serum protein level was measured by performing serum total protein kit. Using bromocresol green method and spectrophotometer at a wave length 546. It was expressed as g/dl. Protein, in alkaline condition, forms with copper ions complex blue-violet. Preparation of solution (Sodium hydroxide 100mmol/l, Na- K-tartarate 16 mmol/l, Potassium iodide 15 mmol/l, and Cupric sulphate 6mmol/). The contents of bottle were diluted 1 with 400 ml of double distilled water. Blank reagent: (Sodium hydroxide 100mmol/l), (Na-K-tartrate 16mmol/l).

The contents of bottle 2 were diluted with 400 ml of double distilled. Standard; Protein (6gl/dl)

Procedure

Reagent	Blank	Standard	Sample
Serum	-	-	0.02 ml
Distilled H2O	0.02 ml	-	-
Standard	-	0.02 ml	-
Solution 1	1 ml	1ml	1 ml

The reagent were Mixed, incubated for 30 min at +20 to +25 C were measured the absorbance of the sample (A sample) and of the standard (A standard) against the reagent blank.

Calculation: Total protein = abs. of sample / abs. of standard × cons. of Standard.

Alkaline Phosphatase (ALP)

According to the reaction of ALP with phenyl phosphate at pH 10 which liberates phenol. The liberated phenol was measured in the presence of amino-4-antipyrine and potassium ferricyanid. The presence of sodium arsenate in the reagent stops the enzymatic reaction.

Reagents-1 (substrate buffer): Disodium phenyl phosphate carbonate-bicarbonate buffer PH 10.

Reagent 2 (standard): phenol

Reagent 3(blocking reagent): Amino-4-antipyrine sodium arsenate.

Reagent 4(color reagent): Potassium ferricyanid.

Phenyl phosphate ALP phenol + phosphate

Procedure:

Activities of ALP were measured by the following:

Reagent	Serum sample	Serum blank	Standard	Reagent blank
Reagent 1	2ml	2ml	2ml	2ml
Incubate for 5 minutes at 37C°				
Serum reagent 2	50□l	-	50□l	-
Incubate for exactly 15 minutes at 37C°.				
Reagent 3	0.5ml	0.5 ml	0.5ml	0.5ml
Mix well or preferably vortex				
Reagent 4 Serum Distilled water	0.5ml - -	0.5ml 50□l -	0.5ml - -	0.5ml - 50□l

The reagents were Mixed and let stand for 10 minutes in the dark measure.

Calculation: OD serum sample – OD serum blank / OD standard × n n (cons. of standard) = 142

Estimation of AST

Principles

α-Oxoglutarate + L-aspartate AST L-glutamate + Oxaloacetate Glutamic – oxaloacetic transaminase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4- dinitrophenyl- hydrazine.

Procedure

Reagent	Sample Blank	Sample
Serum		0.1ml
GOT buffer	0.5ml	0.5ml
The reagent were Mixed, and incubated at 37C°. for exactly 30 min		
2,4-dinitro phenyl	0.5ml	0.5ml
hydrazine (2.0 mmol\l)		
The reagent were Mixed, and allowed to stand for exactly 20 min, at 20 to 25°C		
Sodium hydroxide	5ml	5ml

ALT Estimation

Serum GPT enzyme were determined using colorimetric method. The procedure was performed as reported by Reithman and Frankel.

Alanine + α-keto glutarate ALT pyruvate + glutamate

The pyruvate formed is measured in its derivative form, 2.4-dinitrophenylhydrazone.

Reagent 1 (ALT substrate) phosphate buffer, Alanine, α-keto glutarate

Reagent 2 (color reagent) 2.4 dinitrophenylhydrazine, Hcl

Reagent 3 (standard) pyruvate.

Procedure

Reagent	Sample blank	Sample
Serum		0.1
GPT buffer	0.5	0.5
Mix, incubate at 37C°.for exactly 30 min		
2,4-dinitro phenyl hydrazine	0.5	0.5
Mix, allow to stand for exactly 20 min, at 20-25°C.		
Sodium Hydroxid	5ml	5ml

RESULTS AND DISCUSSION

The frequency distribution in relation to its types is shown in Table 1. NIDDM was more common in male (44.53%) as compared to females (27.34%), IDDM was also more common in male (17.96%) as compared to the female (10.15%). The frequency distribution of relation to the age was shown in table (4.2). DM was more common in those between 70 – 79 years of age, who account for (20.3%) of all cases.

Biochemical Parameters

Glucose Level

The mean of serum glucose in diabetic patients was (13.9± 1.7) mmol/l, which was significantly higher than the mean of control (6.1±22.5) mmol/l.

Liver Function Tests

Mean of serum AST level in patients (11.5 ± 2.7)) U/l compared to the normal control (11± 2.3) U/l which not as significant as shown in table 3.

Bilirubin

Mean of total bilirubin in diabetic patients was (10.2±5.13 micro mol/l) compared to the control (13.6±10.2micromol/l) which was not significant as shown in table 3. Mean of direct bilirubin in diabetic patients was (3.4±2.3 micro mol/l) compared to the control (1.9±0.17 micro mol/l) which was not significant as shown in table 3.

Alanine Transaminase

ALT was significantly higher in diabetic patients (17±4.2) µl/l, as compared to control (10±2.5) µl/l ($P<0.05$) as shown in table 3.

Alkaline Phosphatase

ALP was significantly higher in diabetic patients (266 ±30) µ/l, as compared to the control (169.2± 22.9) µ/($P<0.05$) as shown in table 3.

Total Protein

The mean of total protein in diabetic patients was (52 ±8.8) g/l, while the mean of total protein in control was (71±8.7) g/l ($P<0.05$) as shown in table 3.

Table 1: Frequency Distribution of (DM) According to Types of DM

Type of DM	Male	%	Female	%	Total	%
IDDM	23	17.96	13	10.15	36	28.2
NIDDM	57	44.53	35	27.34	92	71.8
Total	80	62.5	48	37.5	128	100

Table 2 Frequency Distribution According to Age Groups

Age	Male	%	Female	%	Total	%
20-29	14	10.92	7	5.465	21	16.4
30-39	9	7.03	6	4.68	15	11.71
40-49	14	10.93	9	7.03	23	17.96
50-59	10	7.81	12	9.37	22	17.18
60-69	15	11.71	6	4.68	21	16.406
70-79	18	14.06	8	6.25	26	20.31
Total	80	62.5	48	37.5	128	100%

Table 3: Liver Function Test

Groups	Patient	Control	Probability
	Mean ±S.D	Mean ± S.D	
GOT	11.5 ± 2.7 U/l	11 ± 2.3 U/l	NS
STB	10.2±5.13 micro mol/l	13.6±10.2micromol/l	NS
SDB	3.4±2.3micro mol/l	1.9±0.1 micro mol/l	NS
GPT	17 ± 4.2 U/l	10 ± 2.5 U/l	P< 0.05
ALP	266 ± 30 U/l	169.2 ± 22.9 U/l	P< 0.05
Total protein	52 ± 8.8 g/l	71 ± 8.7 g/l	P< 0.05

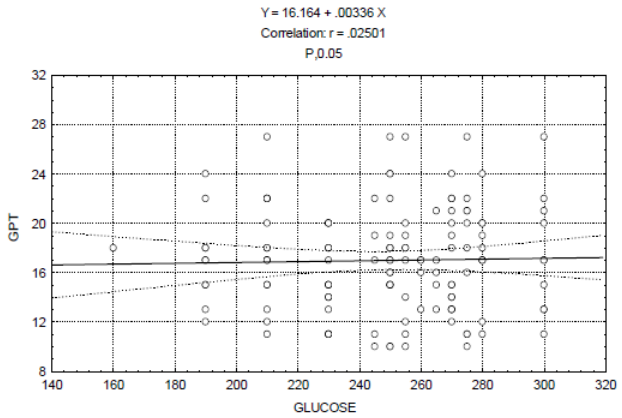

Figure 1: Correlation between glucose and GPT

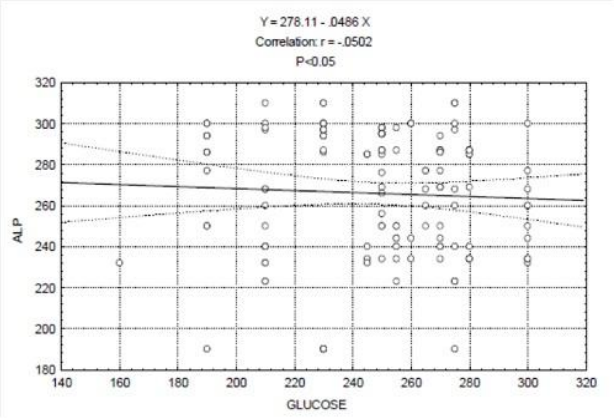

Figure 2: Correlations between glucose and ALP

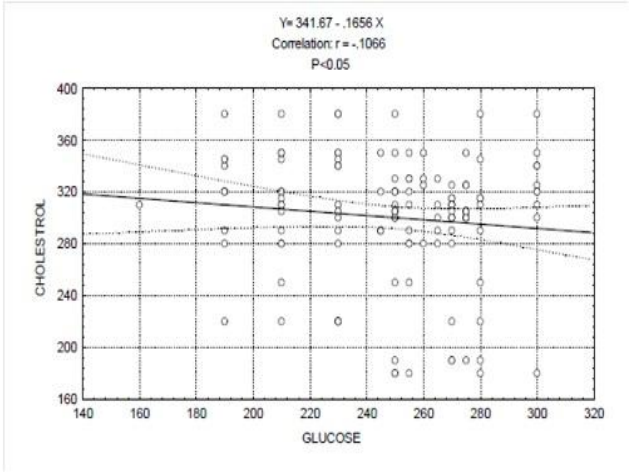

Figure 3: Correlations between glucose and cholesterol

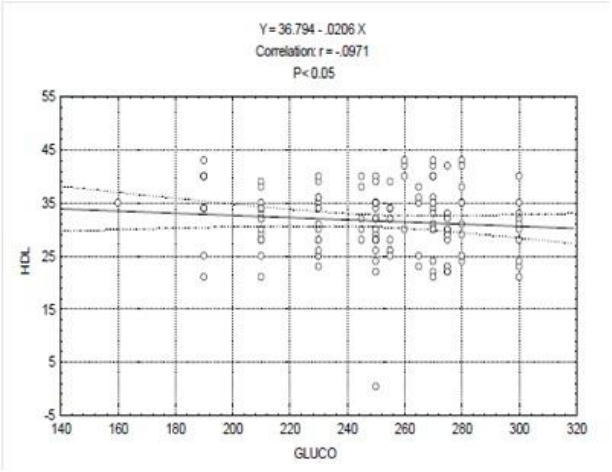

Figure 4: Correlations between glucose and HDL

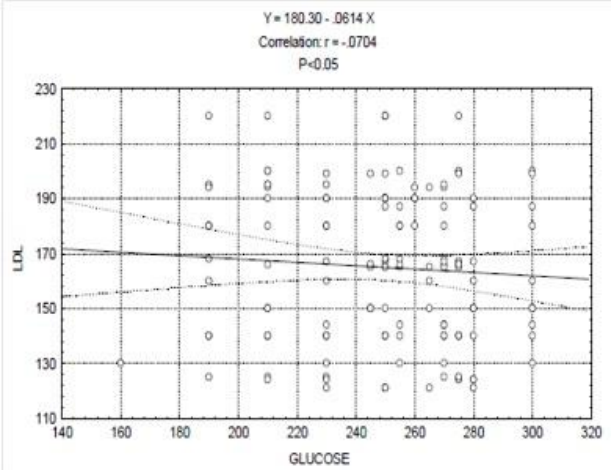

Figure 5: Correlations between glucose and LDL

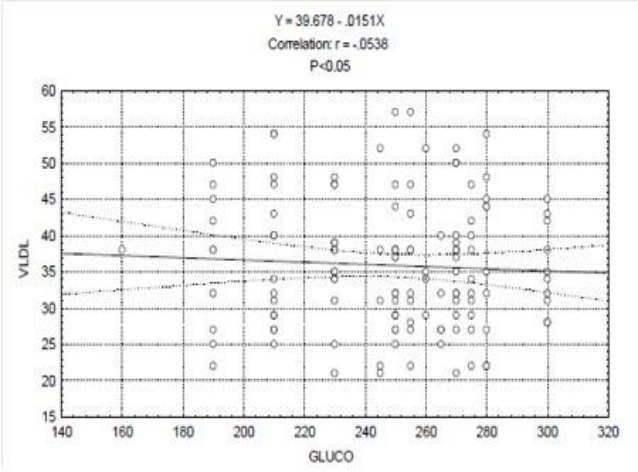

Figure 6: Correlations between glucose and VLDL

DISCUSSION

The study showed that fasting hypoglycaemia is a potential, yet reversible, adverse effect of MT in children being treated according to the Nordic regimens for SR or IR ALL. Children under six years of age are especially prone to fasting hypoglycaemia; in this study 74% of the children with hypoglycaemia were younger. Moreover, only 26% of the children under the age of 16 years remained normoglycaemic during fasting. Over half of all study patients reached hypoglycaemia within 11 to 16 hours of fasting. This is a pathological finding, since the normal lower limit for a blood glucose level (2.5th percentile) in healthy children aged 3 to 15 years after a 14 .hour fast is reported to be 3.7 mmol/L (Lamers et al. 1985). In almost a fifth of all patients (6/35) hypoglycaemia was detected after 11 to 12.5 hours of fasting. This indicates that in the course of overnight sleep, children with ALL are at risk of becoming hypoglycaemic, with or without symptoms. No differences in sex, height (SD score), BMI, or ALL risk group between the patients with hypoglycaemia and those with normoglycaemia were detected, and did not explain the tendency to hypoglycaemia.

Hypoglycaemia was clearly ketotic and free fatty acids were increased in the patients with hypoglycaemia, pointing respectively to intact fatty acid oxidation and lipid mobilization. There was no evidence of hyperinsulinism or hypocortisolism. Deficiency of growth hormone was not suspected in anyone, even though the fasting and hypoglycaemia did not stimulate growth hormone secretion. Hyperlactic acidaemia, an indicator of defective gluconeogenesis (Lee and Leonard 1995), was not detected. Serum levels of free carnitine were low, but levels of total carnitine within the normal range, suggesting high acylcarnitine formation in the liver rather than deficiency of carnitine because of low biosynthesis or intake in these patients. High urinary excretion of dicarboxylic acids in the hypoglycaemic patients was thought to be in accordance with abundant oxidation of fatty acids to ketoacids.

REFERENCES

1. Pickup J & Williams G (Eds). Textbook of Diabetes Oxford: Blackwell Scientific Publications, 1991; 123:76-5.
2. Grundy SM. Small LDL, atherogenic dyslipidemia, and the metabolic syndrome (Editorial). Circulation 1997; 95:1-4.
3. Reaven GM. Banting lecture. Role of insulin resistance in human disease. Diabetes 1988; 37:1595-607.
4. Fortun NJ. Current therapy in cardiovascular disease: Miscellaneous topics. Marcolis S. Sons Company, United state of America.1984, 20:329-334 pp.
5. Compbell PN and Kilby BA. Basic biochemistry for medical student. London, New York, Sanfrancisco.1980, 295-298 pp.
6. CAmpe PC and Harvey RA. Lippincotts illustrated reviews biochemistry: Metabolism of dietary lipid.2nd ed .Philadelphia, New York. 1994; 16:163-228.
7. Murray RK, Granner DK, Mayes PA. Harpers biochemistry: Lipid transport and storage. 25th ed. Mayes PA. Appleton and Lange, Lebanon by Typo press. 2000; 27:268-282.
8. Hansen M. Pathophysiology foundations of diseases and clinical intervention: cellular nutrition and energy metabolism. CRC Press Company, London and New York.1998; 3:62-63.
9. Schmm DE. Essentials of biochemistry: specialized lipid. New York, Ohio, Columbus. 1988; 22:284-294.
10. Chalmers J. WHO-ISH Hypertension guidelines committee. World health organization. International society of hypertension guidelines for the management of hypertension. J. hypertens. 2001; 199:17.
11. Gotto AM and Farmer JA. A textbook of cardiovascular medicine: risk factor for coronary artery disease. Braunwald E. Philadelphia: WB Saunders Company.1992; 15:729-750.
12. Fisher LD, Fried RH, Murakami CS. Urodeoxy cholic acid treatment of refractory chronic Graft-versus . Host disease of the liver. ANN.Intern.Med. 1992; 116:624-629.
13. Rosalki S.B. and Dooley J.S. Liver function profiles and there interpretation. Br.J.Hosp.Med. 1994; 51:181-186.
14. Pratt DS, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. N Engl J Med 2000; 342:1266-71.