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Clinical biochemistry approaches of cardiac failure among diabetic patients

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ABSTRACT
70 - 80% of diabetic patients die of cardiovascular complications such as ischemic heart disease, atherosclerosis, hypertension, arrhythmias and congestive heart failure. All these complications seem to arise from the occurrence of oxidative stress and the development of calcium-handling abnormalities. In particular, the genetic machinery in both cardiac and vascular cells is altered in chronic diabetes. Therefore, a novel therapy needs to be designed for the treatment of diabetes-induced cardiovascular abnormalities. The present clinical study has shown that the abnormalities in cardiac functions. The insulin level was slightly elevated in diabetic patients compared to normal level, which is mainly originated from the pancreas. Glycosylated hemoglobin, triglyceride, total cholesterol and urinary glucose levels are moderately elevated in both male and female diabetic patients compared to non-diabetics. The major driving force for coronary atherosclerosis in patients with diabetes appears to reside in the lipid triad of low HDL, high triglycerides. To characterize clusters of correlated parameters in the lipid profiles of our patients. The HDL-related factor, but not the LDL-related factor was correlated to the glycemic status. These results may suggest a possible mechanism for dyslipidemia: resistance to the antilipolytic effect of insulin leads to an exaggerated flux of free fatty acids into the liver and the results are reported in this paper.

Keywords: Blood glucose, glycosylated hemoglobin, triglycerides, serum cholesterol, HDL-cholesterol and insulin.

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INTRODUCTION

Heart attacks in people with diabetes are more serious and more likely to result in death. High blood glucose levels over time can lead to increased deposits of fatty materials on the insides of the blood vessel walls. These deposits may affect blood flow, increasing the chance of clogging

and hardening of blood vessels (atherosclerosis). Diabetes itself is a risk factor for heart disease and stroke. Also, many people with diabetes have other conditions that increase their chance of developing heart disease and stroke. These conditions are called risk factors. One risk factor for heart disease and stroke is having a family history of heart disease. Risk of heart disease is higher because abdominal fat can increase the production of LDL (bad) cholesterol, the type of blood fat that can be deposited on the inside of blood vessel walls. LDL cholesterol can build up inside your blood vessels, leading to narrowing and hardening of arteries—the blood vessels that carry blood from the heart to the rest of the body. Arteries can then become blocked. Therefore, high levels of LDL cholesterol raise the risk of getting heart disease. Triglycerides are another type of blood fat that can raise the risk of heart disease when the levels are high. HDL (good) cholesterol removes deposits from inside the blood vessels and takes them to the liver for removal. Low levels of HDL cholesterol increase the risk for heart disease. Diabetics have greater prevalence of heart disease such as ischemic heart disease, hypertensive heart disease, cardiomyopathy and congestive heart failure[1,2,3]. The diabetic state shows many metabolic abnormalities, which adversely influence atherosclerosis causing coronary artery disease and peripheral vascular disease. On coronary angiography, diabetics often have multivessel CAD when compared to age and sex matched non-diabetics[4].

Ischemic heart disease in diabetics occurs at the same age as in non-diabetics. ECG abnormalities are as frequent in diabetic females as in males, unlike in non-diabetics. The occurrence of CAD is not related to the duration and severity of diabetes mellitus. Diabetics have a greater prevalence of painless sudden death particularly during sleep[4,5,6]. Anterior wall infarction is more common in diabetics and that explains the overall bad prognosis[6]. Silent infarctions are more common in diabetics (39%) when compared to non-diabetics(22%)[4]. [7]compared diabetic and non-diabetic patients in whom acute myocardial infarction was associated with atypical symptoms. Acute myocardial infarction in presence of pre-existing diabetic CMP will lead to a morbid course. Clinically, heart is enlarged and usually third and fourth sounds are audible. Basal crepitations may be present[5,6]. Loss of parasympathetic activity may be more than loss of sympathetic activity. Clinically, this presents as orthostatic hypotension, painless myocardial infarction and cardio respiratory arrests[7,8]. Sympathetic fibres get damaged at least 5 years after parasympathetic fibres and this causes postural hypotension. Patients having autonomic neuropathy may have sudden painless death. Some of these may be due to arrhythmias secondary to silent myocardial infarction[8,9].

Oral hypoglycaemic drugs (both sulphonylureas and biguanides) accelerate CAD, adversely affect myocardial function and facilitate heart disease in diabetics[4]. Patient suffers from claudication, nocturnal pain, rest pain in limbs, repeated infections of foot, skin atrophy, ulcerations and patchy areas of gangrene. Nocturnal pain is a form of ischemic neuritis that precedes rest pain[10]. Obesity is an important contributor to the risk of developing insulin resistance, diabetes and heart disease. Both hypothalamic and cardiac studies have demonstrated that control of malonyl Co-A levels has an important impact on obesity and heart disease. Targeting enzymes that control malonyl Co-A levels may be an important therapeutic approach to treating heart disease and obesity[11]. Increased urinary albumin excretion (UAE) has been shown to be associated with increased cardiovascular mortality in patients with type 2 diabetes[12]. The development of experimental type-2 diabetes mellitus in rats was accompanied by dysfunction of inhibitory and stimulatory heterotrimeric G-proteins, components of hormone-

sensitive adenylate cyclase signal system. These changes reflect abnormal coupling between receptors and G-proteins in tissues of diabetic patients[13].

EXPERIMENTAL SECTION

The study included 10 patients in the age group between 45–65yrs, treated at the Sooriyan Clinical Laboratories, Avadi in Chennai. All subjects had glucose level greater than 150mg/dl. The patients were monitor during a period of one month. All subjects gave informed consent to participate in this study. The venous blood samples were drawn for all the estimation. Blood samples from fasting subjects were collected. The Serum was separated and the following biochemical parameters were analyzed. Parameters analyzed include estimation of glucose, glycosylated hemoglobin, triglycerides, serum cholesterol, HDL-cholesterol and insulin.

2.1 Blood Glucose

Glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. In a subsequent peroxidase catalyzed reaction, the oxygen liberated is accepted by the chromogen system to give a red colored quinoemine compound. The red color so developed is measured at 510nm and is directly proportional to glucose concentration. Taken 3 set of test tubes and marked as blank, standard and test. 20µl of serum sample was taken the test tube, and then added 20 µl of glucose standard reagent was added to the blank, Test and standard tubes. These solutions are mixed well and incubated at 37°C for 10 minutes or room temperature for 30 minutes. Then added 1.5ml of deionized water to all the tubes and mixed well. Read the absorbance at 490 – 550 nm against a reagent blank. The amount of blood sugar level was calculated.

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 100$$

2.2 Glycosylated Hemoglobin

Pipette out 0.25 ml of lysing reagent in a test tube. Mixed well the sample and allow standing at room temperature for 5 minutes. Bring a resin tube to assay temperature by incubating the tube in a water bath. Add 0.1 ml of hemolysate to it. Contents are mixed on vortex mixer continuously for 5 minutes. Allowed push down the resin separator in the tube until the resin was firmly packed. Poured the supernatant directly into a cuvette and measured the absorbance against deionized water. Pipette out 5 ml of deionized water into a test tube. Added to it 0.02 ml of hemolysate. Mixed well and read absorbance against deionized water.

$$\text{GHb \%} = \frac{\text{A of GHb}}{\text{A of THb}} \times 10 \times \text{temperature factor } (T_f)$$

For assay at 23°C $T_f = 1.0$: at 30°C $T_f = 0.9$

2.3 Triglyceride

Triglycerides are extracted into isopropanol and after saponification with potassium hydroxide the Hantzsch reaction is applied. Measure 0.1 ml of serum, standard and water into screw-capped

tubes. Add 4 ml isopropanal to each, mix well, then add 400 mg of washed alumina and place in a mechanical rotator for 15 min, then centrifuge and transfer 2 ml supernatants in to 13 x 100 mm tubes. Add 0.6 ml potassium hydroxide, stopper, and incubate at 60 to 70 C for 15 min. Cool, add 1 ml metaperiodate solution, mix, add 0.5 ml acetyl acetone reagent, mix and stopper and incubate at 50 C for 30 min. cool and read 405 nm against the reagent blank.

$$\text{Serum triglyceride (mmol/l)} = \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 4$$

2.4 Serum Cholesterol

Cholesterol esters are hydrolyzed and the cholesterol liberated from the lipoprotein complexes by ethanolic potassium hydroxide. The cholesterol is then extracted into light petroleum a portion of which is evaporated and the Libermann –Burchard reaction carried out on the residue. For the test add 5 ml ethanolic potash to 0.5 ml serum in 25 ml stoppered centrifuge tube, shake well and warm to 37°C to 40°C for an hour. For the standard take 5 ml of the above standard and as reagent blank 5 ml ethanol to each of which add 0.5 ml ethanolic potash and heat as for the test. Then cool each tube to room temperature, add 10 ml light petroleum and mix well. Add 5 ml of water and shake vigorously, then centrifuge until two clear layers are obtained. Transfer 4 ml of the upper light petroleum layer to clean dry test tubes and evaporate to dryness in a bath at 37°C using a gentle stream of air. Cool to room temperature. Place the tubes in a water bath at 25°C for 5 min before adding 6 ml libermann- Burchard reagent. Mix well and read exactly 30 min later at 620 nm against the reagent blank.

$$\text{Serum cholesterol (mmol / l)} = \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 10$$

2.5 HDL-Cholesterol

LDL, VLDL and chylomicrons are precipitated by polyions in the presence of metal ions to leave HDL solution. The cholesterol content of the supernatant fluid is then determined. Place 1 ml serum in a centrifuge tube, add 0.1 ml phosphotungstate reagent and mix on a vortex shaker for 10 sec. add 50 micro l magnesium chloride reagents and re- mix similarly. Centrifuge at ambient temperature for 30 min at 1500 g ensuring that the temperature does not increase appreciably. Carefully remove the clear supernatant for analysis avoiding any surface deposit. Analyze for cholesterol by enzymatic method bearing in mind dilution factor of 1.125.

HDL cholesterol (mmol/l) = cholesterol result (mmol/l) X dilution factor

2.6 Insulin

100µl of sample diluents was added to all wells except first well meant for blank. Then 100µl of test sample was added to test and control wells of the micro well strips. The micro well strips were covered with adhesive tapes and incubated at room temperature for 1 hour. The adhesive strip's cover was carefully removed and the content were carefully aspirated and discarded. 50µl of conjugate stabilizer followed by 100µl of conjugate was added to each well except blank well. The micro well strips were incubated at room temperature for 30 minutes. The micro well strips

were washed five times with 350 µl with washing buffer providing a soak time of 30 seconds in between each wash. 100µl of color reagent was added each well and incubated at room temperature for 30 minutes. 100µl of stopping solution was added to each well and the micro well strips were read at 450nm and also using 630nm for qualitative method. The cut off value was found out by taking mean value of OD of three negative controls added with the normal range. Another dichromatic 405nm and 630nm mode of filters were also used for finding the OD value for those similar samples for comparison and for finding the concentration of PSA (antigen) for quantitative method.

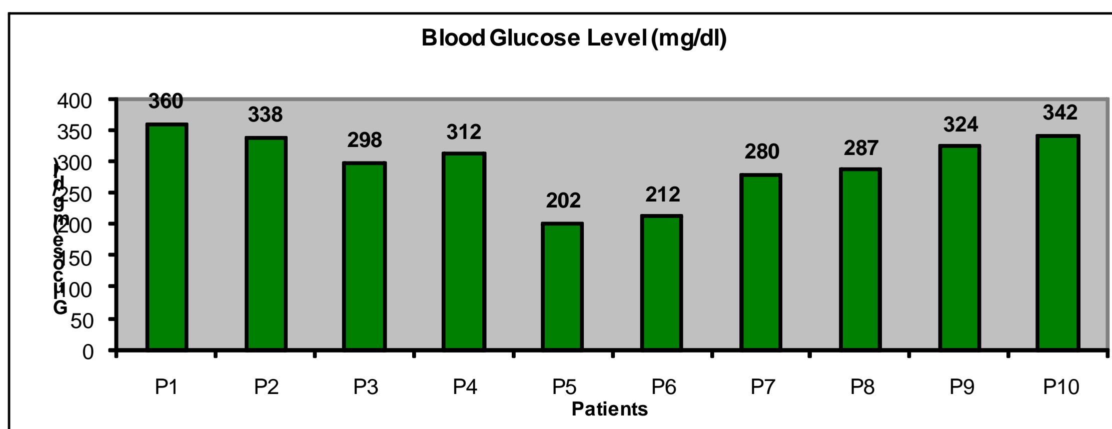
RESULTS

The results obtained from the above mentioned parameters analyzed are listed below:

Table-1 Blood Glucose Level (mg/dl)

S. No.	Sex	Age	Body Weight	Glucose (mg/dl)
P1	FM	47	68	360
P2	M	52	72	338
P3	M	54	64	298
P4	FM	48	68	312
P5	M	50	72	202
P6	M	62	72	212
P7	FM	68	74	280
P8	FM	68	64	287
P9	M	54	68	324
P10	M	63	72	342

Figure-1 Blood Glucose Level (mg/dl)



A female at the age of 47 whose body weight is 68 has the glucose level of 360mg/dl. A male at the age of 52 whose body weight is 72 has the glucose level of 338mg/dl. As such experiment has been made to 10 patients and the result was also the same.

Table-2 Glycosylated Hemoglobin Level (in %)

S. No.	Sex	Age	Body Weight	Glycosylated Hemoglobin (%)
P1	FM	47	68	10.0
P2	M	52	72	10.5
P3	M	54	64	9.50
P4	FM	48	68	9.00
P5	M	50	72	8.20
P6	M	62	72	7.50
P7	FM	68	74	10.0
P8	FM	68	64	10.2
P9	M	54	68	9.30
P10	M	63	72	8.00

Figure-2 Glycosylated Hemoglobin Level (in %)

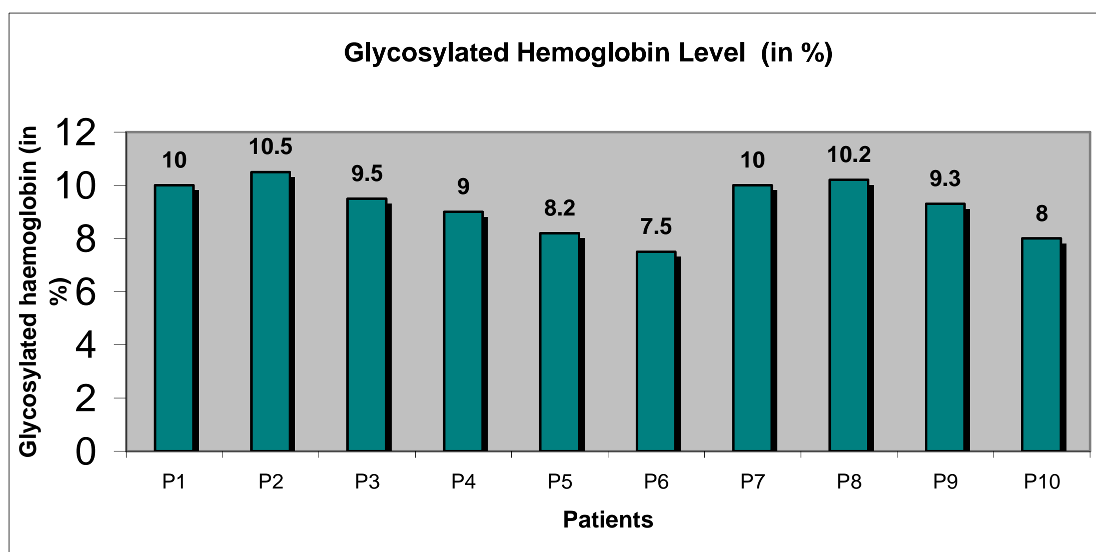


Table-3 Triglyceride Level (mg/dl)

S. No.	Sex	Age	Body Weight	Triglyceride (mg/dl)
P1	FM	47	68	166.5
P2	M	52	72	156.2
P3	M	54	64	145.5
P4	FM	48	68	153.4
P5	M	50	72	140.3
P6	M	62	72	141.8
P7	FM	68	74	149.5
P8	FM	68	64	150.2
P9	M	54	68	160.2
P10	M	63	72	165.7

Figure-3 Triglyceride Level (mg/dl)

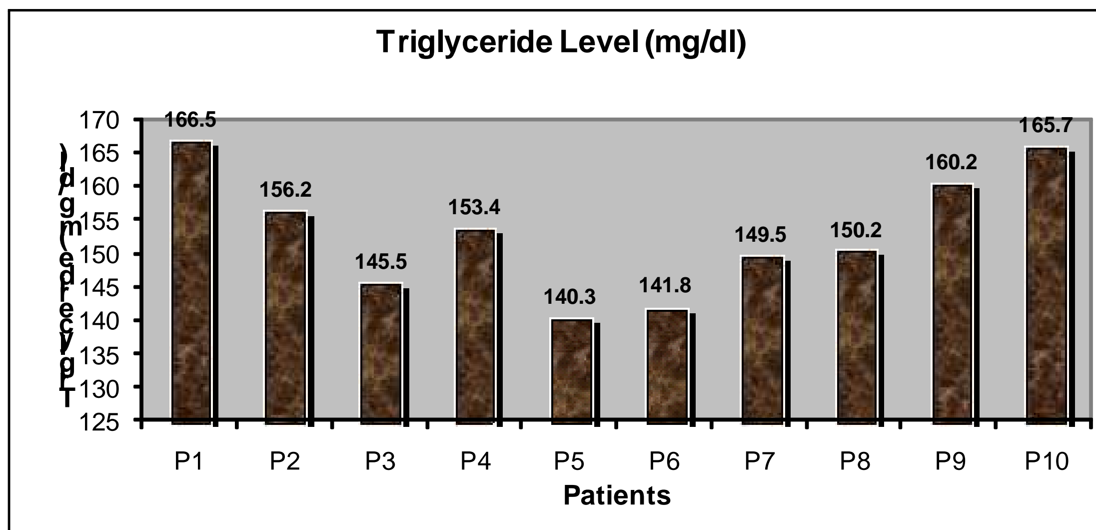


Table-4 Serum Cholesterol Level (mg/dl)

S. No.	Sex	Age	Body Weight	Cholesterol (mg/dl)
P1	FM	47	68	226.5
P2	M	52	72	196.2
P3	M	54	64	185.5
P4	FM	48	68	193.4
P5	M	50	72	180.3
P6	M	62	72	181.8
P7	FM	68	74	189.5
P8	FM	68	64	190.2
P9	M	54	68	200.2
P10	M	63	72	205.7

Figure-4Serum Cholesterol Level (mg/dl)

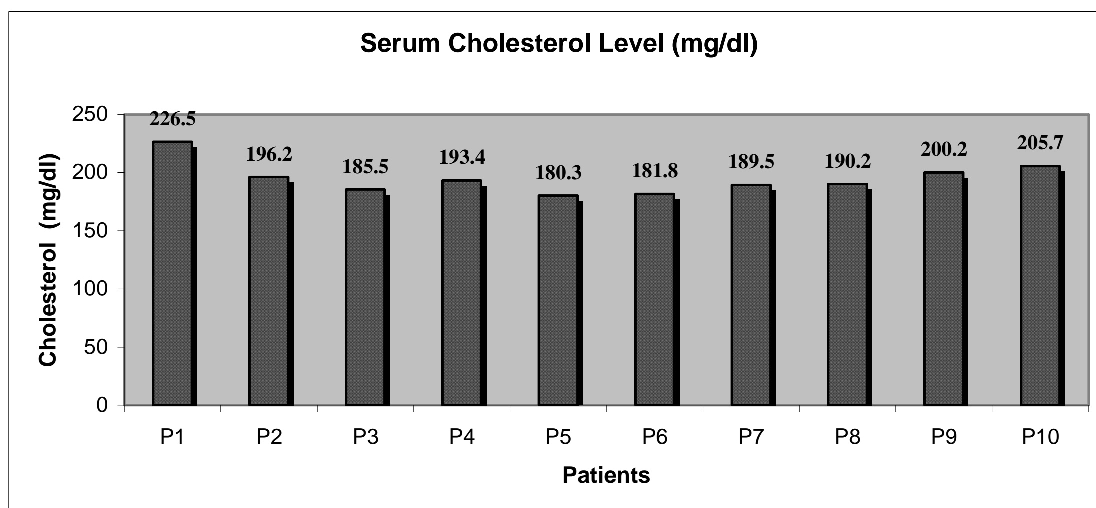


Table-5 HDL-Cholesterol Level (mg/dl)

S. No.	Sex	Age	Body Weight	HDL-Cholesterol (mg/dl)
P1	FM	47	68	43.3
P2	M	52	72	49.5
P3	M	54	64	46.2
P4	FM	48	68	50.9
P5	M	50	72	48.5
P6	M	62	72	48.1
P7	FM	68	74	46.6
P8	FM	68	64	46.9
P9	M	54	68	43.4
P10	M	63	72	42.5

Figure-5 HDL-Cholesterol Level (mg/dl)

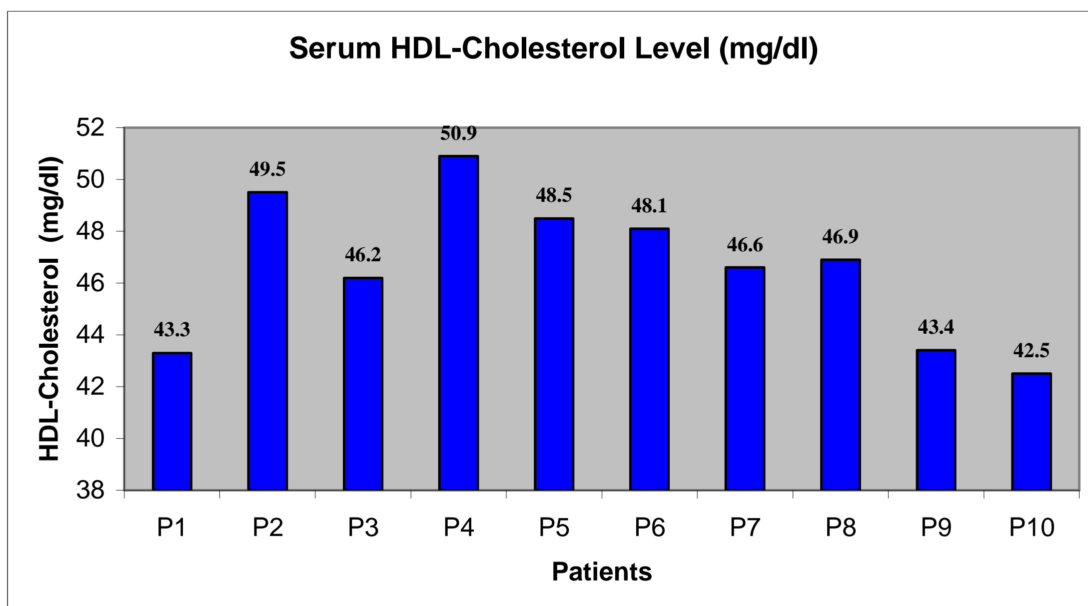
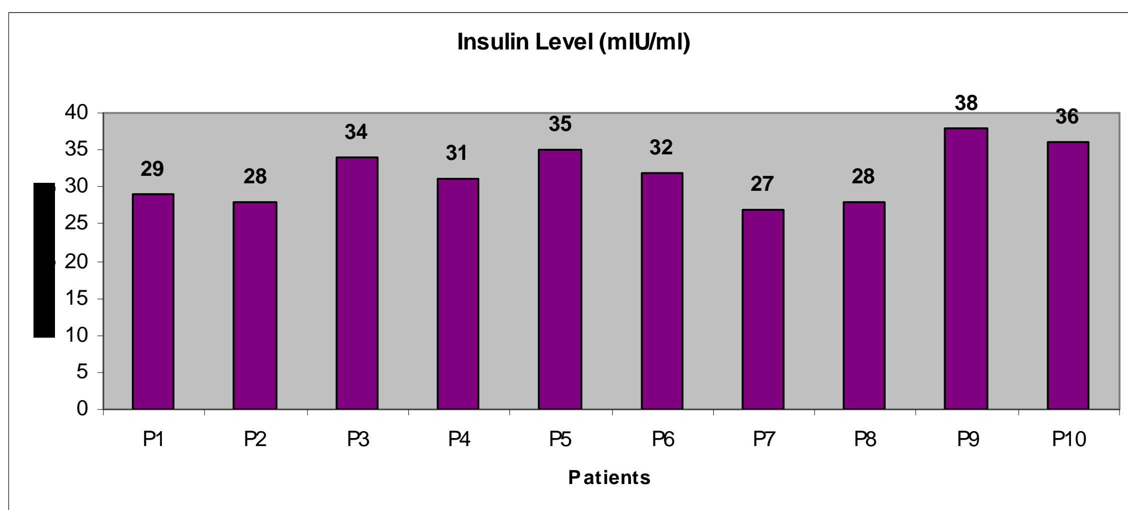


Table-6 Insulin Level (mIU/ml)

S. No.	Sex	Age	Body weight	Insulin (mIU/ml)
P1	FM	47	68	29
P2	M	52	72	28
P3	M	54	64	34
P4	FM	48	68	31
P5	M	50	72	35
P6	M	62	72	32
P7	FM	68	74	27
P8	FM	68	64	28
P9	M	54	68	38
P10	M	63	72	36

Figure-6 Insulin Level (mIU/ml)



3.1 Benedicts's Test

5ml Benedict's reagent was taken in a test tube. 9 drops of urine sample was added and heated till boiling in the Bunsen flame. Cool the tube. Color change was noted and results were recorded.

Table-7 Benedicts's Test Results

S. No.	Sex	Age	Body Weight	Result
P1	FM	47	68	Present ++++
P2	M	52	72	Present ++++
P3	M	54	64	Present +++
P4	FM	48	68	Present ++++
P5	M	50	72	Present ++
P6	M	62	72	Present ++
P7	FM	68	74	Present +++
P8	FM	68	64	Present +++
P9	M	54	68	Present ++++
P10	M	63	72	Present ++++

DISCUSSION

Hyperglycemia occurs commonly in acutely and critically ill patients and has been associated with adverse clinical consequences. An emerging body of literature describes the beneficial effects of intensive glycemic monitoring and treatment[14]. Free fatty acids are the preferred substrate for the myocardium. However, under conditions of ischemia, glucose becomes the primary myocardial energy source. Its metabolism avoids the toxic end-products of free fatty acids, which include oxygen free radicals[15]. Diabetes is an established major factor of poor prognosis after an acute coronary syndrome. Recent findings also argue for a direct deleterious effect of hyperglycemia on myocardium[16]. iabetes mellitus is a metabolic disorder in the body characterized by hyperglycemia altered metabolism of lipid, carbohydrate and protein with an

increased risk of complications of vascular disease[17]. The renin-angiotensin system inhibition decreases cardiovascular and renal morbidity and mortality and the incidence of new onset type 2 diabetes[18]. The activation of the renin-angiotensin system plays an important role in diabetes-induced changes in SL and SR membranes as well as cardiac function[19]. The use of echocardiography as a screening tool in the asymptomatic diabetic population is problematic. Biomarkers of cardiac dysfunction have been proposed for diagnosis. The role of biomarkers in the diagnosis of this condition and proposed a diagnostic algorithm that may be useful for the assessment of asymptomatic patients with diabetes[20]. The ethnomedical use of the *Bauhinia monandra* in the management of diabetes and stimulating insulin release is one of the modes of action of the butanol fraction and some of its subfractions[21]. Navneet *et al.* concluded that aqueous extracts of *Momordica balsamina* seeds is having significant antihyperglycemic potential and can be further fractionated in order to get a responsible molecule for this vary action[22]. A global epidemic of type-2 diabetes exists and in the near future it may be closely associated with an epidemic of cardiovascular disease. Overwhelming evidence exists for the linear association between worsening glycemic control and increased risk for coronary heart disease. Brief episodes of cardiac ischemia render the heart more resistant to subsequent ischemic events; this phenomenon is called ischemic preconditioning[23]. A significant relationship between receiving care calls and reduction in elevated LDL-C levels was observed; members who received calls achieved up to a 32.5% relative reduction in elevated LDL-C values compared to members who did not receive calls. In conclusion, these findings demonstrate the ability of DM interventions to assist a large, geographically diverse member population in reducing a clinical laboratory value[24]. Strict control of arterial pressure and glycemia may prevent or even ameliorate heart disease in patients with hypertension and diabetes[25]. Parasympathetic cardiac nerve dysfunction, expressed as increased resting heart rate and decreased respiratory variation in heart rate, is more frequent than the sympathetic cardiac nerve dysfunction expressed as a decrease in the heart rate rise during standing[26].

Systolic and diastolic time intervals were used to examine left ventricular performance diabetic men with no apparent clinical heart disease. It provides no definite evidence of a cause, the abnormalities found may reflect a subclinical diabetic cardiopathy[27]. Diabetic patients had significantly more frequent intraventricular conduction disorders than non diabetic myocardial infarction patients[28]. The metabolic changes in the heart increased glycogen, triglycerides, and cyclic AMP, and decreased ATP and creatine phosphate indicate that diabetes is a generalized disorder of cellular metabolism. Cognizance of three metabolic events are relevant to the treatment of the diabetic patient during acute cardiac events such as myocardial infarction[29].

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