Biochemical studies on blood sample of diabetes mellitus patients

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ABSTRACT

Diabetes mellitus is a metabolic disorder of the endocrine system. People suffering from diabetes are not able to produce or properly use insulin in the body. The aim of our study is to find out the biochemical changes on blood sample of patients with diabetes mellitus. The level of glucose, urea, creatinine, total cholesterol, triglycerides, and low density lipoprotein were increased and high density lipoprotein was decreased in diabetic patients when compared with normal subjects. In Standard drug (Sulfonyl Urea) treated diabetic patients, all the above biochemical parameters were retrieved to normal. From this study, it was stated that the diabetes mellitus was controlled with oral hypo glycaemia agents which help patients improve their health and reduce their morbidity rate.

Key words: Diabetes mellitus, Hypoglycaemia agents, Sulfonyl urea, Glucose

INTRODUCTION

Diabetes mellitus (DM) is one of the most common endocrine diseases in all populations and age groups. It is a syndrome of disturbed intermediary metabolism caused by inadequate insulin secretion, or impaired insulin action, or both.

Globally, as of 2010, an estimated 285 million people had diabetes, with type 2 making up about 90% of the cases. Its incidence is increasing rapidly, and by 2030, this number is estimated to almost double [1]. Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in the more developed countries.

India has more diabetics than any other country in the world, according to the international diabetes foundation [2]. The disease affects more than 50 million adults- and kills about 1 million Indians a year.

There are three main types of diabetes mellitus (DM),
- Type 1 DM “insulin-dependent diabetes mellitus” (IDDM) or “juvenile diabetes” results from the body’s failure to produce insulin,
- Type 2 DM results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency and it is referred to as noninsulin-dependent diabetes mellitus (NIDDM) or “adult-onset diabetes”.
- The third main from, gestational diabetes occurs when pregnant women a previous diagnosis of diabetes develop a high blood glucose level. It may precede development of type 2 DM

The classic symptoms of untreated diabetes are
1. Loss of weight
2. Polyuria (frequent urination)
3. Polydipsia (increased thirst)
4. Polyphagia (increased hunger) [3].
Despite the great strides that have been made in understanding and management in this disease, serious problems like diabetic retinopathy, diabetic nephropathy [4] and lower extremity amputation.

### Diabetes diagnostic criteria [5]

<table>
<thead>
<tr>
<th>Condition</th>
<th>2hour Glucose Mmol/L(Mg/D)</th>
<th>Fasting Glucose Mmol/L(Mg/Dl)</th>
<th>Hba1c %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;7.8(&lt;140)</td>
<td>&lt;6.1(&lt;110)</td>
<td>&lt;6.0</td>
</tr>
<tr>
<td>Impaired fasting glycaemia</td>
<td>&lt;7.8(&lt;140)</td>
<td>&gt;6.1(&gt;110)&amp;&lt;7.0(&lt;12)</td>
<td>6.0-6.4</td>
</tr>
<tr>
<td>Impaired glucose tolerance</td>
<td>&gt;7.8(&gt;140)</td>
<td>&lt;7.0(&lt;126)</td>
<td>6.0-6.4</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>&gt;11.1(&gt;200)</td>
<td>&gt;7.0(&gt;126)</td>
<td>&gt;6.5</td>
</tr>
</tbody>
</table>

Hyperglycaemia is diagnosed by any one of the following:

i) Fasting plasma glucose level > 7.0mm/l (126mg/dl)

ii) Plasma glucose > 11.1 mmol/l (200mg/dl) two hours after a 75 g oral glucose load as in a glucose tolerance test

iii) Symptoms of hyperglycaemia and casual plasma glucose > 11.1 mmol/l (200 mg/dl)

iv) Glycated haemoglobin (HbA\textsubscript{1C}) > 6.5%

The major components of the treatment of diabetes are:

1. Diet (combined with exercise if possible).
2. Oral hypoglycaemic therapy.

The aim of the treatment is primarily to save life and alleviate symptoms. Secondary aims are to prevent long-term diabetic complications and, by eliminating various risk factors, to increase longevity.

Oral hypoglycaemic agents include sulphonylureas, biguanides, alpha glucosidase inhibitors and thiazolidinediones. The main objective of these drugs is to correct the underlying metabolic disorder such as insulin resistance and inadequate insulin secretion. They should be prescribed in combination with an appropriate diet and lifestyle changes. Diet and lifestyles are to reduce weight, improve glycaemic control and reduce the risk of cardiovascular complications.

3. Insulin.
Type 1 diabetes is typically treated with combinations of regular and NPH insulin, or synthetic insulin analogs. When insulin is used in type 2 diabetes, a long-acting formulation is usually added initially, while continuing oral medications. Doses of insulin are increased to effect [6].

More than hundred million people are affected by diabetes worldwide and in the next ten years it may affect about five times more people than it does now. In India, the prevalence rate of diabetes estimated to be 1.5% complication is the major cause of morbidity and mortality in diabetes mellitus.

Diabetes is best controlled either by diet alone and exercise or diet with oral hypoglycaemic agents or insulin. Oral hypoglycaemic agents such as sulfonylurea, biguanides are to correct the underlying metabolic disorder. The aim of our study is to measure the biochemical parameters in diabetes and non-diabetic samples.

### EXPERIMENTAL SECTION

**Patients**
In the present study, blood samples were collected from ten normal subject, ten diabetic patients with type 2 diabetes mellitus with treatment and 10 diabetes without treatment selected and were from the rural areas of Pattukottai, Tamil Nadu and were age between 35 to 65 years.

**Weight and Height**
Healthy normal subjects were served as controls and compared with diabetic patients. Weight was recorded to the nearest kilogram (kg) with the subject standing on the weight machine without shoes and using minimum of clothing. The same weights machine was used for all the patients and the machine was tested with a known set of weight for any error [7]. Height was recorded with the subject erect, bare footed, feet together, back and heels against the upright bar of height scale, head upright in Frankfort horizontal plane ‘look straight ahead’. The height measuring equipment consisted of a vertical bar with a steel tape attached. Attached perpendicularly to the vertical bar was a horizontal bar which was brought down snugly on the examinee’s head [8].

Weight thus recorded (in kgs) was compared with the average weight/height tables for Indian males/females [9].
Body Mass Index (BMI)
Body mass index was calculated from the formula;

\[
\text{BMI} = \frac{\text{weight in kilograms}}{(\text{height in meters})^2}
\]

Age, Sex, height and weight of study population.

<table>
<thead>
<tr>
<th>Age</th>
<th>35 to 65</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Males 5 (50%)</td>
</tr>
<tr>
<td></td>
<td>Females 5 (50%)</td>
</tr>
<tr>
<td>Height</td>
<td>150 to 180</td>
</tr>
<tr>
<td>Weight</td>
<td>53 to 92</td>
</tr>
</tbody>
</table>

Collection of blood
The blood samples were collected from normal and diabetes mellitus patients by venous puncture in a heparinised tube. Serum and plasma were separated by centrifugation at 3000 rpm for 15 minutes. Separated serum and plasma were analysed by using autoanalyzer for various biochemical parameters like glucose [11], Urea [12], creatinine [13], triglycerides [14], cholesterol [11], HDL-cholesterol [15], LDL-cholesterol at PG and Research Department of Biochemistry, S.T.E.T Women’s College, Sundarakkottai, Mannargudi.

Statistical Analysis
All the values of present investigation are expressed as mean ± S.D (n=10).

RESULTS AND DISCUSSION
The present number of diabetics worldwide is over 150 million and this likely to increase to 300 million or more by the year 2025 [16,17]. Reasons for this increased include increased in sedentary lifestyle, consumption of energy-rich diet, obesity and life span.

The present study was carried out to analyse the various biochemical parameter in normal and diabetic subject. This study was conducted on ten diabetes patients (type 2) without treatment and 10 diabetes patients treated with drug (sulfonyl urea) and 10 normal subjects.

Table 1 shows the BMI of normal and diabetic subjects. The body mass index was calculated from their height and weight under of all subjects. BMI for diabetes patients was (3.48±0.15) increased compared with that of control subjects (2.39±0.11) and standard drug treated patients (2.63±0.07). These variations in BMI reflected alterations in subcutaneous and visceral fat which showed that it was associated with elevated risk factors because of its relation with visceral fat accumulation due to exposure of liver to fatty acids. Variations were found as subjects with diabetes mellitus had more of sedentary lifestyle and less of physical activity. Inactivity is associated with increase in intra abdominal adiposity in type 2 diabetes mellitus and is strongly related with impaired insulin sensitivity.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body mass index (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.39±0.11</td>
</tr>
<tr>
<td>Diabetics without treatment</td>
<td>3.48±0.15</td>
</tr>
<tr>
<td>Diabetics with treatment</td>
<td>2.63±0.07</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=10)

Table 2 shows the levels of glucose of fasting and post prandial in control, diabetes patients, and diabetic patients treated with standard drug. Blood glucose levels in fasting (175.4±1.46) and post prandial (189.7±1.74) levels were increased in diabetic patients than control of fasting (88±4.04) and post prandial (108.1±3.65). In diabetics treated patients fasting blood sugar (121.8±1.80) and post prandial (118.6±2.34) retrieved to normal than without treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sugar (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasting</td>
</tr>
<tr>
<td>Normal</td>
<td>88±4.044</td>
</tr>
<tr>
<td>Diabetics without treatment</td>
<td>175.4±1.46</td>
</tr>
<tr>
<td>Diabetics with treatment</td>
<td>121.8±1.80</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=10)
Normally, Diabetes is detected by measuring blood glucose levels. However, due to wide deviations in the circulating glucose concentrations, a randomized glucose measurement which is a normally used laboratory test for measuring long-term diabetic control [18]. In addition, diabetic patients have reduced glucose tolerance. Additional burden of glucose is found to weaken the tolerance further. In present study, there was an elevation in blood glucose both fasting and postprandial in diabetes as compared with normal healthy individuals.

From the result obtained, it was evident that fasting blood glucose levels intensely rose together with corresponding deteriorating oral glucose tolerance in diabetic patients. In diabetes without treated patients there is an increased glycation of a number of proteins including haemoglobin and α-crystalline of lens [19]. Glycated haemoglobin (HbA1c) was found to increase in patients with diabetes mellitus and the amount of increase is directly proportional to the fasting blood glucose level [20]. The level of glycated haemoglobin is measured as one of the markers of degree of oxidative stress in diabetes mellitus.

In sulfonylurea treated diabetic patients sugar level decreased than without treatment diabetic patients. Sulfonylurea causes hypoglycaemia by stimulating insulin release from pancreatic β-cells. They bind to sulfonylurea (SUR) receptors on the β-cell membrane, causing closure of adenosine tri phosphate (ATP)-sensitive potassium channels, leading to depolarization of the cell membrane. This in turn opens voltage-gated channels, allowing influx of calcium ions and subsequent secretion of preformed insulin granules. Acute administration of sulfonylurea to type 2 DM patients increases insulin release from the pancreas and also may further increases insulin levels by reducing hepatic clearance of the hormone. Initial studies showed that a functional pancreas was necessary for the hypoglycaemic action of sulfonylurea[21].

Table 3 shows the levels of lipid profile in normal and diabetic patients. There was an increased levels of total cholesterol (175.1±5.81), LDL-C (164.8±3.26), triglycerides (174.4±1.98) and decreased HDL-C (56.5±1.27) concentration in diabetic patients compared with control subjects. In control, the level of cholesterol, triglycerides, LDL-C, and HDL-C were 175.1±5.81, 95.9±13.42, 99.12±6.14, 56.8±3.77, respectively. In patients treated with standard drug, the level of total cholesterol (233.2±1.96), triglycerides (175.5±1.20) and LDL-C (109±7.13) were reduced and HDL-C (83.7±3.64) increased than without standard drug treated patients.

Table 4 shows the levels of urea and creatinine in normal subjects and diabetes patients. Plasma urea (30.1±3.20) and creatinine (0.95±0.06) were observed to be higher in diabetic patients than normal subjects. The levels of urea (50.6±0.82), and creatinine (2.07±0.07) were reduced. When diabetic patients treated with sulfonyl urea which was nearer to normal subjects.

### Table 3: Level of lipid profile in control and diabetic patients

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol mg/dl</th>
<th>Triglycerides mg/dl</th>
<th>LDL mg/dl</th>
<th>HDL mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>175.1±5.81</td>
<td>95.9±13.42</td>
<td>99.12±6.14</td>
<td>56.8±3.77</td>
</tr>
<tr>
<td>Diabetics without treatment</td>
<td>257.7±4.47</td>
<td>174.4±1.98</td>
<td>164.8±3.26</td>
<td>56.5±1.27</td>
</tr>
<tr>
<td>Diabetics with treatment</td>
<td>233.2±1.96</td>
<td>175.5±1.20</td>
<td>109±7.13</td>
<td>83.7±3.64</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=10)

Diabetes is known to affect large number of metabolic pathways, including lipid metabolism, by altering the activities of various enzymes involved in these pathways. Since, there is a high incidence of mortality for type2 diabetes with their first myocardial infarction, aggressive therapy for treating diabetic dyslipidemia is recommended. The concentration of low density lipoprotein cholesterol (LDL-C) is one of the most important predictors of atherosclerosis and coronary heart disease (CHD) and reduction in its level reduces the morbidity and mortality in patients with CHD. In present study diabetic patients, rise in total cholesterol and triglycerides is associated with the increase in LDL-C and decreased in HDL-C when compared with normal treatment with standard drugs normalised the above lipid levels. This is in agreement with the result of Sharma, 1970 and Jain, 1980 [22, 23].

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### Table 4: Level of urea and creatinine in control and diabetic patients

<table>
<thead>
<tr>
<th>Group</th>
<th>Urea(mg/dl)</th>
<th>Creatinine(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>30.1±3.20</td>
<td>0.96±0.06</td>
</tr>
<tr>
<td>Diabetics without treatment</td>
<td>58.9±4.195</td>
<td>1.96±0.109</td>
</tr>
<tr>
<td>Diabetics with treatment</td>
<td>50.6±0.82</td>
<td>2.07±0.07</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=10)

Impairment of renal function due to type 2 diabetic mellitus was assessed by measurement of plasma concentrations of creatinine and urea. In present study plasma creatinine and urea concentration were observed to be higher in diabetic patients compared to non-diabetic control subjects. These finding reveal that there is strong relationship of
blood sugar level with urea level. As there is increase in blood sugar level on increase in urea level has been detected. The Hyperglycaemia is one of the major causes of progressive renal damage. An increase in urea level is seen when there is damage to the kidney or the kidney is not functioning properly [24]. From the above observation increment of blood urea levels with the increment of blood sugar level clearly indicates that the increase blood sugar level may cause damage to the kidney.

An increase in urea level is seen when there is damage to the kidney is not functioning properly. Increment of blood urea level with the increment of blood sugar level clearly indicates that the increase blood sugar level causes damage to the kidney. Research conducted by Anjaneyulu et al., 2004 had found that increase urea and serum creatinine in diabetic rats indicates progressive renal damage [25]. Standard drug treated diabetic patients the levels of urea and creatinine were decreased near to normal.

CONCLUSION

Diabetes mellitus is the most common series metabolic disorder and it is considered to be one of the five leading causes of death in the world. It is characterized by absolute or relative deficiency in insulin secretion and/or insulin action associated with chronic hyperglycaemia and disturbances of carbohydrate, lipid, and protein metabolism.

In our study, various biochemical parameters were investigated for control and diabetic patients. Diabetic patients showed increased level of fasting and post prandial glucose, total cholesterol, triglycerides, low density lipoproteins, urea and creatinine and decreased the level of high density lipoprotein than the normal healthy controls. When the diabetic patients treated with sulfonylurea, restored the level of all above parameters.

From this study, it was stated that the sulfonylurea have an important role in the management of type2 diabetes mellitus who cannot achieve proper control with changes in diet alone. When used appropriately, sulfonyleureas are safe, particularly the short acting ones.

REFERENCES