Diabetes mellitus (DM) is a metabolic disorder of the endocrine system. People suffering from diabetes are not able to produce or properly use insulin in the body, so they have a high level of blood glucose. The present aim of our study is to find out the biochemical studies on blood sample patient’s with diabetes mellitus. We measured the glucose level, Thioctic Acid Reactive Substance (TBARS), Superoxide dismutase (SOD), Catalase (CAT). Estimation of reduced glutathione (GSH), Estimation of Glutathione peroxides (GPx), vitamin-E and vitamin-C on 15 days and 30 days treatment of 10 patients for each.

**Keywords:** Diabetes mellitus, blood, Lipid peroxidation, enzymatic anti-oxidants, non-enzymatic antioxidants.

**INTRODUCTION**

Diabetes mellitus is characterized by altered carbohydrate, protein and fat metabolism caused by the complete or relative insufficiency of insulin secretion or insulin action. The disease occurs worldwide and its incidence is increasing rapidly in most parts of the world. Diabetes is becoming the third ‘killer’ of mankind, after cancer and cardiovascular diseases, because of its high prevalence, morbidity and mortality. Recent studies on geographical and ethical influences have shown that people of Indian origin are highly prone to diabetes. In 2000, according to the World Health Organization, at least 171 million people worldwide suffer from diabetes or 2.8% of the population; the American Diabetes Association reported in 2009 that there are 23.6 million children and adults in the United States - 7.8% of the population, living with diabetes. While an estimated 17.9 million in the US alone have been diagnosed with diabetes, nearly one in four (5.7 million) diabetics are unaware that they have this disease. Approximately 4% populations were affected by diabetes mellitus and are expected to increase by 5.4% in 2025. The worldwide survey reported that the diabetes mellitus is affecting nearly 10% of the population. Its incidence is increasing rapidly, and it is estimated that by the year 2030, this number will almost double, Diabetes are classified as type 1 diabetes (Insulin dependent diabetes mellitus-IDDDM), type 2 diabetes (Non–Insulin dependent diabetes mellitus-NIDDM), Gestational diabetes mellitus (GDM). There are three types of Diabétés such as Type 1 Diabetes (Insulin dependent diabetes mellitus): It is characterized by beta cell destruction caused by an autoimmune response, usually leading to insulin deficiency. It can occur in an older individual due to destruction of pancreas by alcohol, disease or removal of surgery. It also results from progressive failure of the pancreatic beta cells, which produce insulin and people with type 1 diabetes require daily insulin treatment to sustain life. Type 2 Diabetés (Non–Insulin dependent diabétés mellítés): It is a heterogeneous disorder. Most patients with type 2 diabetes mellitus have insulin resistance, and their beta cells lack the ability to overcome this resistance. This form of diabetes was previously uncommon in children but in some countries, more than 20% patients with diabetes in childhood only. It is usually controlled with diet, exercise and oral medications. More than half of all people require insulin to control their blood sugar levels at some point in the course of their illness. Gestational Diabetes mellitus (GDM): It is defined as a form of glucose intolerance which is first recognized during pregnancy. Gestational diabetes is caused by the hormones of pregnancy or by a shortage of insulin. Women are requiring insulin to avoid 50% risk of developing diabetes within the next five years. Women with gestational diabetes are more likely to have large babies.

**MATERIAL AND METHODS**

**PATIENTS**

In the present study blood samples collected from ten normal subject patients and ten diabetic patients with type 2 diabetes mellitus selected for this study were from the rural areas of Chidambaram and Kattumannar Koil, Tamil Nadu and were between 35 to 55 years, with 10 mild diabetic and 10 healthy normal subjects served as controls and compared with 10 standard drug treated patients. Patients attending the outpatient units of Kamala Computer Diagnostic Centre and Hospital were blood sugar was measured by physicians between 8 to 9 A.M. Normal subjects were chosen from the same age group and had normal blood sugar, free from diabetes mellitus and other chronic illness. Occupationally, they were indulging in almost similar physical activities. Their economic statuses were also similar.

**BLOOD COLLECTION**

Blood samples were collected at the beginning and end, after an overnight fasting by venipuncture in heparinized tubes. The plasma was separated by centrifuging at 3000 rpm for 15 minutes and stored at 4 °C until analysis.

**ESTIMATION OF BLOOD GLUCOSE**

Glucose was estimated by the method of Sasaki using reagent Kit. Glucose is oxidized by the enzyme glucose oxidase (GOD) to give D-gluconic acid and hydrogen peroxide. Hydrogen peroxide in presence of enzyme peroxidase (POD) oxidizes phenol which combines with 4-aminoantipyrine to produce a red colored quinoneimine dye. The intensity of the colour developed is proportional to glucose concentration in the sample.

\[ \text{Glucose} + \text{H}_2\text{O} + \text{O}_2 \xrightarrow{\text{D-gluconic acid} + \text{H}_2\text{O}_2} \]

\[ \text{H}_2\text{O}_2 + 4\text{-aminoantipyrine} + \text{phenol} \xrightarrow{\text{red quinoneimine dye} + \text{H}_2\text{O}} \]
Procedure
0.01 mL of plasma, standard and distilled water (blank) in to three separate tubes, 1.0 mL of the enzyme reagent was added, mixed and kept at 37 °C for 15 minutes. The colour developed was read at 510 nm against reagent blank.

LIPEX PEROXIDATION PRODUCTS

Estimation of Thiobarbituric acid reactive substances (TBARS)
The concentration of TBARS in the plasma was estimated by the method of Niehaus and Samuelson³. In this method, malondialdehyde and other thiobarbituric acid reactive substances (TBARS) react with thiobarbituric acid in an acidic condition to generate a pink colour chromophore which was read at 535 nm.

Procedure
0.5 mL of sample was diluted to 0.5 mL with double distilled water and mixed well, and then 2.0 mL of TBA-TCA-HCl reagent was added. The mixture was kept in a boiling water bath for 15 min, after cooling, the tubes were centrifuged at 1000 g for 10 min and the supernatant was estimated. A series of standard solution in the concentration of 2-10 nmol was treated in a similar manner. The absorbance of the chromophore was read at 535 nm against reagent blank.

ENZYMATIC ANTIOXIDANTS

Assay of superoxide dismutase (SOD)
The assay is based on the inhibition of the formation of NADH-phenazinemethosulphate, nitroblue tetrazolium formazan. The reaction was initiated by the addition of NADH. After incubation for 90 sec, adding glacial acetic acid stops the reaction. The color developed at the end of the reaction was extracted into n-butanol layer and measured in a Spectronic 20 at 520 nm.

Procedure
0.5 mL of erythrocyte was diluted to 1.0 mL with water followed by addition of 2.5 mL of ethanol and 1.5 mL of chloroform (chilled reagents were added). This mixture was shaken for 90 sec at 4 °C and then centrifuged. The enzyme activity in the supernatant was determined as follows. The enzyme activity in the supernatant was determined as follows. The assay mixture contained 1.2 mL of sodium pyrophosphate buffer, 0.1 mL of phenazine methosulphate, and 0.3 mL of nitroblue tetrazolium and appropriately diluted enzyme preparation in a total volume of 3 mL. The reaction was started by the addition of 0.2 mL NADH. After incubation at 30 °C for 90 s, the reaction was stopped by the addition of 1 mL glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4mL n-butanol. The mixture was allowed to stand for 10 min; centrifuged and n-butanol layer was separated. The color density of the chromogen in n-butanol was measured in at 510 nm. A system devoid of enzyme served as control. The enzyme concentration required to inhibit the chromogen produced by 50% in one minute under standard conditions was taken as one unit.

Estimation of catalase (CAT)
The activity of Catalase in the erythrocytes erythrocys was determined by the method of Sinha⁴. Dichromate in acetic acid was converted to perchromic acid and then to chromic acetate, when heated in the presence of H₂O₂. The chromic acetate formed was measured at 620 nm. The catalase preparation was allowed to split H₂O₂ for various periods of time. The reaction was stopped at different time intervals by the addition of dichromate-acetic acid mixture and the remaining H₂O₂ as chromic acetate was determined colorimetrically.

Procedure
0.1 mL of hemolysate and 0.4 mL of hydrogen peroxide were added. The reaction was arrested after 15, 30, 45 and 60s by adding 2.0 mL of dichromate-acetic acid mixture. The tubes were kept in a boiling water bath for 10 min, cooled and the color developed was read at 620 nm. Standards in the concentration range of 20-100 µmol were taken and proceeded as for the test. The specific activity was expressed as µmol of H₂O₂ consumed /min/mg of Hb for erythrocytes

Estimation of glutathione peroxides (GPx)
The activity of GPx in the erythrocytes was measured by the method⁵. A known amount of enzyme preparation was allowed to react with H₂O₂ in the presence of GSH for a specified time period. Then the remaining GSH content was measured.

Procedure
0.2 mL of tris buffer, 0.2 mL of EDTA, 0.1 mL of sodium oxide, hemolysate were added. To the mixture, 0.2 mL of GSH followed by 0.1mL of H₂O₂ was added. The contents were mixed well and incubated at 37 °C for 10 min, along with a control containing all reagents except homogenate. After 10 min, the reaction was arrested by the addition of 0.5 mL of 10% TCA. The tubes were centrifuged and the supernatant was assayed for GSH by the method of Ellman (1959).

NON ENZYMATIC ANTIOXIDANTS

Estimation of reduced glutathione (GSH)
Reduced glutathione in the erythrocytes was estimated by the method of Ellman (1959). This method was based on the formation of 2-nitro-5-thiobenzoic acid (a yellow colour compound) when 5, 5'-dithio-bis (2-nitrobenzoic acid) (DTNB) was added to compounds containing sulphhydryl groups.

Procedure
Plasma was pipetted out and precipitated with 2.0 mL of 5% TCA. 2.0 mL of supernatant was taken after centrifugation and 1.0 mL of Elman’s reagent and 4.0 mL of 0.3M disodium hydrogen phosphate were added. The yellow color developed was read in a Spectronic 20 at 412 nm. A series of standards (20–100 µg) was treated in a similar manner along with a blank containing 1.0 mL of buffer.

Estimation of ascorbic acid (vitamin C)
Ascorbic acid in the erythrocytes was estimated by the method of⁶. The ascorbic acid was converted to dehydroascorbic acid by mixing with norit and then coupled with 2, 4 dinitrophenylhydrazine (DNPH) in the presence of thiourea, a mild reducing agent. The coupled dinitrophenylhydrazine was converted into a red colored compound when treated with sulphuric acid.

Procedure
0.5 mL of sample, 1.5 mL of 4% TCA was added and allowed to stand for 5 min and centrifuged. To the supernatant, 0.3 g of acid washed norit was added, shaken vigorously and filtered. This converts ascorbic acid to dehydroascorbic acid. 0.5 mL of the filtrate was taken and 0.5 mL of DNPH was added, stoppered and placed in a water bath at 37 °C for exactly 3 h. Removed, placed in ice-cold water and added 2.5 mL of 85% sulphuric acid drop by drop. The contents of the tubes were mixed well and allowed to stand at room temperature for 30 min. A set of standards containing 20-100 µg of ascorbic acid were taken and
processed similarly along with a blank containing 2.0 mL of 4% TCA. The color developed was read at 540 nm.

**Estimation of α-tocopherol (vitamin E)**

α-Tocopherol in the plasma was estimated by the method of Ref. The method involves the reduction of ferric ions to ferrous ions by α-tocopherol and the formation of a red colored complex with 2', 2' dipyridyl. Absorbance of the chromophore was measured at 520 nm.

**Procedure**

To 0.5 mL of sample, 1.5 mL of ethanol was added, mixed and centrifuged. The supernatant was evaporated and to the precipitate, 3.0 mL of petroleum ether, 0.2 mL of 2', 2' dipyridyl solution and 0.2 mL of ferric chloride solution were added. Mixed well and kept in dark for 5 min. An intense red color was developed. 4.0 mL of n-butanol was added to all the tubes and mixed well. Standard tocopherol in the range of 10-100 µg was taken and treated similarly along with a blank containing only the reagent. The color in the n-butanol layer was read at 520 nm.

**Statistical Analysis**

Statistical analysis was performed using SPSS software program, version 16. The results were expressed as mean ± standard deviations. The data were analyzed by analysis of variance (ANOVA). A Probability level (p-value) of less than 0.05 was considered statistically significant using Duncan’s Multiple Range Test (DMRT).

**RESULTS AND DISCUSSION**

Diabetes mellitus is the most common serious metabolic disorder and it is considered to be one of the five leading causes of death in the world Ref. It is characterized by absolute or relative deficiency in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid, and protein metabolism. As a consequence of the metabolic derangements in diabetes, various complications develop including both macro and micro-vascular dysfunctions Ref.

**TABLE-1: TO MEASURE THE BLOOD GLUCOSE LEVELS IN STANDARD DRUG TREATMENT AND DIABETES SUBJECT PATIENTS**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control subjects (n=10)</th>
<th>Diabetes patients (n=10)</th>
<th>Diabetes + standard drug 15 days (n=10)</th>
<th>Diabetes + standard drug 30 days (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose mg/dL</td>
<td>F: 110.23</td>
<td>+ 170.26</td>
<td>+ 130.45</td>
<td>+ 121.26</td>
</tr>
<tr>
<td></td>
<td>PP: 128.95</td>
<td>± 210.14</td>
<td>± 163.24</td>
<td>± 155.24</td>
</tr>
<tr>
<td></td>
<td>± 11.35</td>
<td>± 15.28*</td>
<td>± 16.55**</td>
<td>± 12.27**</td>
</tr>
<tr>
<td></td>
<td>± 10.21</td>
<td>± 16.55***</td>
<td>± 15.2**</td>
<td>± 14.25***</td>
</tr>
</tbody>
</table>

Values are means ± SD for each group

*p < 0.05 diabetic patients compared with normal subjects
**p < 0.05 diabetic treatment patients compared with fasting normal subjects
***p < 0.05 diabetic treatment patients compared with Post-prandial normal subjects

**Table-1** shows the effect of standard drugs treatment in blood glucose levels control subject and diabetic treatment patients. Blood glucose levels in fasting and postprandial levels were significantly increased (p<0.05) in diabetic patients. Standard drug treatment subject patients with 15 and 30 days significantly attenuated (p<0.05) in blood sugar levels.

**TABLE-2: EFFECT ON LIPID PEROXIDATION MARKERS IN STANDARD DRUG TREATMENT AND DIABETIC SUBJECT PATIENTS**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control subjects (n=10)</th>
<th>Diabetic Patients (n=10)</th>
<th>Diabetic + standard drug 15 days (n=10)</th>
<th>Diabetic + standard drug 30 days (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol/mL)</td>
<td>2.95 ± 0.021</td>
<td>5.85 ± 0.55*</td>
<td>4.70 ± 0.65**</td>
<td>4.69 ± 0.44**</td>
</tr>
</tbody>
</table>

Values are means ± SD for each group

*p < 0.05 diabetic patients compared with normal subjects
**p < 0.05 diabetic treatment patients compared with normal subjects

**Table-2** shows the levels of TBARS (a marker of lipid peroxidation), in normal subjects and patients with diabetic and drug intake subjects. There were observed an increased level of TBARS as compared to normal subjects. It is well known that diabetic condition is associated with increased content of lipid hydroperoxide Ref. Standard drug treated patients 15; 30 days to measure the TBARS levels were significantly decreased compared to control subjects. Hyperglycemia is one of the major causes of enhanced free radical accumulation Moreover, an earlier few reported that intestinal absorption of glucose as well as lipid is increased in diabetic patients thus oxidative stress is one of the typical characteristics of type 2 diabetes mellitus. However, it is still unclear whether the elevated level of LPO plays any role in steps leading to diabetes, such as impairment of insulin secretion Ref. Diabetes mellitus is associated with increased formation of free radicals and decrease in antioxidant potential. Due to these events, the balance normally present in cells between radical formation and protection against them is disturbed. This leads to oxidative damage of cell components such as proteins, lipids, and nucleic acids. In both insulin dependent (type 1) and non-insulin-dependent diabetes (type 2) there is increased oxidative stress Ref. Free radicals have the potential to cause damage to critical cellular targets, such as DNA, proteins, and lipids as a consequence of increased mitochondrial oxygen (O2) flux Ref. Oxidative damage occurs as an outcome of an imbalance between the formation and inactivation of oxygen free radicals. This process leads to the destruction of membrane lipids and production of lipid peroxides and their products. Since the reactive oxygen species (ROS) removal rate is mostly controlled by a variety of low molecular weight antioxidants, there is a great interest in determining their levels and the way they are related to pathological states Ref. In our study diabetic treated patients with standard drugs decreased the levels of blood glucose and lipid peroxidation.
Diabetes is known to affect large number of metabolic pathways, including lipid metabolism, by altering the activities of various enzymes involved in these pathways. The Elevated levels of HDL cholesterol and decreased levels of phospholipids are important risk factors for atherosclerosis and coronary heart disease (CHD)\(^{20}\). The concentration of low density lipoprotein cholesterol (LDL-C) is one of the most important predictors of atherosclerosis and coronary heart disease (CHD)\(^{24}\) and reduction in its level reduces the morbidity and mortality in patients with CHD. In diabetic patients, the rise in TC and TG is associated with the increase in LDL-C and VLDL-C and decrease in HDL-C. 30 day treatment with standard drugs significantly decreased the above lipid levels Phospholipids are vital components of biomembrane and play an important role in the transport of triglycerides\(^{25}\). The elevated phospholipids levels in the serum are a consequence of elevated lipoproteins. FFA and TC\(^{30}\) also promote the synthesis of phospholipids. Good glycemic control and elevated levels of HDL cholesterol and decreased levels of triglycerides are correlated with the phospholipids levels\(^{27}\). The serum cholesterol/phospholipids ratio is one of the important markers of development of diabetic and atherosclerosis.

**CONCLUSION**

In the present study, we examined the biochemical parameters of diabetes mellitus patients. From this study we know the TBARS, SOD, CAT, GPx, GSH and Glucose levels.
of diabetes patients and the concentration of low density lipoprotein cholesterol (LDL-C) is one of the most important predictors of atherosclerosis and coronary heart disease for diabetes patients. We concluded that the 30 day treatment with standard drugs significantly maintain the lipid levels.

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REFERENCES