CHAPTER – III

GLYCOSYLATED HEMOGLOBIN IN
COMPLICATIONS OF DIABETES

Introduction:

The Diabetes control and complications Trial demonstrated that the more severe and sustained the degree of hyperglycemia, as assessed by Glycosylated Hemoglobin, the more likely it is that the chronic complications of Diabetes will develop. Ideally, lowering Glycosylated Hemoglobin by intensive blood glucose control will help to prevent the devastating complications of neuropathy, retinopathy, nepropathy and vascular disease(136).

Mechanism of complications of Diabetes mellitus:

Chronic complications occur mainly in tissues that do not require insulin for the uptake of glucose and its metabolites reflect the ambient glucose concentration of the blood supply and are involved in the etiology of this complication(136). The chronic complications of Diabetes mellitus affect many organ systems and are responsible for the majority of morbidity and mortality associated with the disease.

Chronic complications can be divided into non-vascular and vascular complications.

- **Nonvascular complications** include problems such as gastroparesis, sexual dysfunction and skin changes. The risk of
chronic complications increases as a function of the duration of hyperglycemia.

- **The vascular complications of Diabetes mellitus are further subdivided into microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular complications, (coronary artery disease, peripheral vascular disease, cerebrovascular disease).**

The **Microvascular complications** of Diabetes mellitus result from chronic hyperglycemia. Randomized, prospective clinical trials involving large number of individuals with Diabetes have conclusively demonstrated that a reduction in chronic hyperglycemia prevents or reduces retinopathy, neuropathy and nephropathy.

**Mechanism of Microvascular Complications:**

Three major theories have been proposed to explain how **hyperglycemia** might lead to the chronic complications of Diabetes mellitus.

1. Nonenzymatic glycosylation.
2. Aldose reductase pathway.
3. Diacylglycerol Protein kinase C activation.

1. **Nonenzymatic glycosylation**:

   **One hypothesis is that increased intracellular glucose leads to the formation of Advanced Glycosylation End products (AGES)**
via the non enzymatic glycosylation of cellular proteins. Non enzymatic glycosylation results from the interaction of glucose with amino groups on proteins. AGES have been shown to crosslink proteins (e.g. collagen, extracellular matrix proteins), accelerate atherosclerosis, promote glomerular dysfunction, reduce nitric oxide synthesis, induce endothelial dysfunction; alter extracellular matrix composition and structure. The serum level of AGES correlates with the level of glycemia, and these products accumulate as glomerular filtration rate declines. There are three main mechanisms of the nonenzymatic glycosylation pathway, postulated for the development of the chronic complications of Diabetes.

I. Chemical and structural changes to intracellular and membrane associated proteins via non enzymatic glycosylation and formation of AGES modified by reducing sugars, their metabolites, or both.

II. Changes in structure and function of matrix proteins by nonenzymatic glycosylation.

III. The interaction of AGE receptors with AGE- modified proteins.

2. Aldose reductase pathway:

A second hypothesis proposed to explain how chronic hyperglycemia leads to complications of DM is based on the observation that hyperglycemia increases glucose metabolism via the sorbitol pathway. Intracellular glucose is predominantly
metabolized by phosphorylation and subsequent glycolysis, but when intracellular glucose is increased, some glucose is converted to sorbitol by the enzyme aldose reductase. Increased sorbitol concentrations affect several aspects of cellular physiology (decreased myoinositol, altered redox potential) and may lead to cellular dysfunction.

3. **Diacylglycerol Protein Kinase C activation**:

A third hypothesis proposes that hyperglycemia increases the formation of diacylglycerol leading to activation of certain isoforms of Protein kinase C (PKC), which, in turn, affect a variety of cellular events that lead to DM-related complications for example, PKC activation by glucose alters the transcription of genes for fibronectin type IV collagen, contractile proteins, and extracellular matrix proteins in endothelial cells and neurons *in vitro*.

Growth factors appear to play an important role in Diabetes mellitus related complications. Vascular endothelial growth factor (VEGF) is increased in diabetic proliferative retinopathy and decreases after laser photocoagulation. Transforming growth factor B (TGF-B) is increased in diabetic nephropathy and appears to stimulate basement membrane production of collagen and fibronectin by mesangial cells. Other growth factors, such as platelet-derived growth factor, epidermal growth factor, insulin like growth factor I, growth hormone,
basic fibroblast growth factor, and even insulin, have been suggested to play a role in DM – related complications.

Finally, oxidative stress and free radical generation, as a consequence of the hyperglycemia, may also promote the development of complications(8).

Possible molecular mechanisms of diabetes- related complications. AGES; advanced glycosylation end products; PKC protein kinase c; DAG; diacylglycerol; CPLA2, phospholipase A2; Na, K ATpase, sodium-potassium AT Pase.
Mechanism of Macrovascular Complications:

Evidence implicating on causative role for chronic hyperglycemia in the development of macrovascular complications is less conclusive. However some results suggest a role for chronic hyperglycemia in the development of macrovascular diseases eg: coronary heart disease events and mortality are two to four times greater in patients with Diabetes mellitus. These events correlate with fasting and postprandial plasma glucose levels as well as with the Glycosylated Hemoglobin. Other factors (dyslipidemia and hypertension) also play important roles in macrovascular complications.

Patients who have Diabetes are two to four times more likely to suffer macrovascular complications such as coronary heart disease, myocardial infarction, stroke and peripheral vascular disease. Endothelial dysfunction is now believed to be the driving force behind macrovascular complications associated with Diabetes mellitus and metabolic syndrome, as endothelial cells regulate platelet aggregation and vascular inflammation. Atherogenic glycosylation of large vessel endothelium and low-density lipoprotein cholesterol (LDL-C) results from chronic hyperglycemia(137). LDL-C particles become and remain smaller and denser, even with normalization of blood glucose, and initiate a cascade of inflammatory cytokines, including elevation of C-
reactive protein (CRP) and mobilization of free fatty acids (FFAs) that further compromise vascular endothelium.

Diabetics are more likely to develop acrovascular disease, such as atherosclerosis,

CRP is considered an index of cardiovascular inflammation and risk, primarily through its capacity to destabilize and rupture atherosclerotic plaque. Moreover, insulin resistance and hyperinsulinemia appear to raise blood pressure independently and increase platelet adhesion by impairing fibrinolysis via increased production of plasminogen activator inhibitor 1 (PAI-1) and fibrinogen, resulting in a state of hypercoagulability(138). The end result is acceleration of atherosclerosis and increased likelihood of macrovascular events(139).
Increased stores of visceral adipose tissue (VAT) modulate peripheral insulin resistance and excess insulin production typical of Diabetes mellitus. This occurs primarily through suppression of the adipokines adiponectin (which increases insulin sensitivity) and leptin (which decreases appetite), increased secretion of resistan (which decreases insulin sensitivity) and lipolytic mobilization of free fatty acids (which inhibit that action of adiponectin). This results in a vicious cycle of hyperglycemia, central obesity and dyslipidemia. VAT also elevates PAI-1, increasing the risk of thrombosis of ruptured atherosclerotic plaque (140).

Interestingly, Type I Diabetes mellitus patients have a three-to sixfold greater age-adjusted risk of cardiovascular mortality even though they often have favorable lipid profiles compared with Type II
Diabetes mellitus patients(141). Recent evidence points to cardiac autonomic neuropathy (CAN) and glycosylation of large vessel intima-media as probable etiologies(142).

**Types of Microvascular Complications of Diabetes**

**Diabetic Retinopathy**

Diabetic retinopathy has been one of the foremost causes of blindness in the developed countries. In India, it was the 17th cause of blindness 20 years ago. Today Diabetes related blindness has ascended to the 6th position. As per the WHO statistics the number of adults with Diabetes in the world is estimated to increase by 122% from 135 million in 1995 to 300 million in 2025. This increase is expected to be 42% in the developed countries and 170% in the developing countries. The greatest increase is expected in India: 195% from 18 million in 1995 to 54 million in 2025(143).

Diabetic retinopathy is the most frequent cause of new cases of blindness among adults aged 20-74 years. During the first two decades of disease, nearly all patients with Type I Diabetes and >60% of patients with Type II Diabetes have retinopathy. In the Wisconsin Epidemiologic study of Diabetic Retinopathy (WESDR), in the younger onset group, 86% of blindness was attributed to diabetic retinopathy. In the older onset group, in which other eye diseases were common, one third of the cases of legal blindness were due to diabetic retinopathy(144).
The major risk factors for diabetic retinopathy are poor glycemic control, hypertension and diabetic nephropathy.

The Diabetic Complications Control Trial (DCCT) has demonstrated that intensive glucose control reduces the incidence and the progression of diabetic retinopathy in patients with Type I Diabetes(145).

The U.K. Prospective Diabetes Study (UKPDS) demonstrated that improved blood glucose control reduced the risk of developing retinopathy and nephropathy and possibly reduces neuropathy. The overall rate of microvascular complications was decreased by 25% in patients receiving intensive therapy versus conventional therapy(144).

Systematic hypertension in diabetic nephropathy, correlates well with the presence of retinopathy. Independently, hypertension may also complicate Diabetes which may result in hypertensive retinal vascular changes superimposed on the preexisting diabetic retinopathy, further compromising retinal blood flow(146).

The nephropathy as evidenced by proteinuria and elevated BUN/creatinine levels is an excellent predictor of the presence of retinopathy. This probably is due to the fact that both conditions are caused by Diabetes mellitus related microangiopathies such that the presence and severity of one reflects that one of the other. Evidence suggests that aggressive treatment of nephropathy may have a
beneficial effect on the progression of diabetic retinopathy and neovascular glaucoma(145).

Non-enzymatic glycosylation of proteins formed as a result of persistent hyperglycemia are involved in the pathogenesis of diabetic retinopathy. Diabetic patients with retinopathy have higher rates of formation of AGE than patients without diabetic retinopathy. Glycosylation enhance the immunogenicity of collagen and other proteins. Albumin which is glycosylated is taken up by these cells and may affect the endothelial cell function. Thus hyperglycemia alters the key biochemical reactions contributing to thickening of basement membrane, function of pericytes and endothelial cells and the potency of retinal vessels(147).

**Diabetic retinopathy is the result of microvascular retinal changes.** Hyperglycemia-induced pericyte death and thickening of the basement membrane lead to incompetence of the vascular walls. These damages change the formation of the blood retinal barrier and also make the retinal blood vessels become more permeable. Small blood vessels – such as those in the eye are especially vulnerable to poor blood sugar control. Over accumulation of glucose or fructose damages the tiny blood vessels in the retina(147).

**Clinical features of diabetic retinopathy are:**

1. Microaneurysms (outpouching of capillaries),
2. Abnormalities of the retinal veins,
3. Hemorrhages (tiny hemorrhages in the retina itself),
4. Exudates (retinal deposits occurring as a result of leaky vessels),
5. neovascularization (new, abnormal vessel growth),
6. glial proliferation,
7. Vitreous hemorrhage and
8. Retinal detachments(146).

Diabetic retinopathy is divided into two major groups:

1) Background retinopathy or Non-proliferative retinopathy.
2) Proliferative retinopathy.

**Background retinopathy or Non-proliferative retinopathy** :

Patients with microaneurysms, retinal hemorrhages or exudates are classified as having Background or Non-proliferative retinopathy.

**Proliferative Diabetic Retinopathy** :

Patients with preretinal hemorrhage, new vessel formation or glial proliferation are said to have Proliferative Diabetic Retinopathy.

**Diabetic retinopathy**

**Vascular lesion in Diabetic Retinopathy**

<table>
<thead>
<tr>
<th>Vascular lesions</th>
<th>Extravascular Lesions</th>
<th>Proliferative retinopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary</td>
<td>Cottonwool spot</td>
<td>New vessels</td>
</tr>
<tr>
<td>Arterial</td>
<td>Haemorrhage</td>
<td>Pre-retinal</td>
</tr>
<tr>
<td>Venous</td>
<td>Hard exudates</td>
<td>Glial proliferation</td>
</tr>
</tbody>
</table>
Nonproliferative Diabetic Retinopathy can develop into Proliferative Diabetic Retinopathy and/or diabetic maculopathy accompanied by visual reduction.

<table>
<thead>
<tr>
<th>Retinopathy</th>
<th>Inside the retina (Background retinopathy)</th>
<th>In front of the retina (Neovascularization)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-vision threatening</td>
<td>Simple</td>
<td></td>
</tr>
<tr>
<td>Vision threatening</td>
<td>Maculopathy</td>
<td>Proliferative retinopathy</td>
</tr>
</tbody>
</table>

The disease stages and common pathological change:

<table>
<thead>
<tr>
<th>Disease stage</th>
<th>Common Pathological change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preclinical stages</td>
<td>Alterations in retinal blood flow, loss of retinal pericytes. Thickening of basement membranes.</td>
</tr>
<tr>
<td>Early stages</td>
<td>Retinal vascular microaneurysms and blot hemorrhages. Increased retinal vascular permeability. Cotton wool spots.</td>
</tr>
<tr>
<td>Mild NPDR</td>
<td></td>
</tr>
<tr>
<td>Middle stages</td>
<td>Venous caliber changes or beading. IRMA’s Retinal capillary loss. Retinal ischemia. Extensive intraretinal hemorrhages and microaneurysms.</td>
</tr>
<tr>
<td>Moderate NPDR, Severe NPDR,</td>
<td></td>
</tr>
<tr>
<td>Very Severe NPDR</td>
<td></td>
</tr>
<tr>
<td>PDR</td>
<td></td>
</tr>
</tbody>
</table>
Results and Discussion

*Diabetic retinopathy* is one of the major microvascular complications of Diabetes. This microvascular complication is the leading cause of blindness (148).

In the present study the Glycosylated Hemoglobin profile in retinopathy was evaluated. The study population consisted of 40 patients. The mean age of the patients was $46.75 \pm 15.34$ years and the mean duration of Diabetes mellitus was $14.67 \pm 7.8$ years.

All the selected patients were screened for evidence of diabetic retinopathy by fundoscopy, i.e. having microaneurysm with exudates, hemorrhages and proliferative changes. Blood samples were analyzed for fasting plasma glucose, postprandial plasma glucose, Glycosylated Hemoglobin, lipid profile and renal function tests.

The results of the statistical analysis of the biochemical parameters in diabetic retinopathy are summarized in Table No. I, II and III.

*Table No.I show the results of glycemic control i.e. Glycosylated Hemoglobin, fasting plasma glucose and postprandial plasma glucose.* Analyzed data shows that Glycosylated Hemoglobin was significantly higher ($8.4 \pm 1.8\%$) in the patients as compared to the control (Table I). This is in confirmation to previous reports (54, 63). The mean Glycosylated Hemoglobin level was higher than the control and was statistically significant. The
higher levels of Glycosylated Hemoglobin indicate the risk for development of diabetic retinopathy. The evidence for the link between poor glucose control and greater progression of diabetic retinopathy was also reported(149). Raised levels of blood glucose were associated with microvascular complications like diabetic retinopathy(150).

The level of glycemic control appears to be the major predictor of the early development of proliferative retinopathy. Those with poorest glycemic control during the first 15 years of Diabetes had the highest risk of developing proliferative retinopathy. A strong relationship between the development of proliferative retinopathy and the level of glycemic control was found in Wisconsin study(4).

The relationship between long-term glycemic control and the proportion of patients developing proliferative diabetic retinopathy (PDR) in cases of mild proliferative diabetic retinopathy (PPDR) has been reported(151). The frequency and severity of retinopathy are related to Glycosylated Hemoglobin values after, but not at the diagnosis of Type II Diabetes(152).

In present results the mean fasting plasma glucose (157.77±73.84 mg/dl) and postprandial plasma glucose (217.45±69.86 mg/dl) levels were significantly on higher side compared to the control (Table I).
Table No. II shows the levels of lipid parameters in Diabetes and control group. Many factors related with the severity of diabetic retinopathy such as Glycosylated Hemoglobin and serum lipid. The objective was to determine the correlation between Glycosylated Hemoglobin and serum lipid with the severity of diabetic retinopathy.

Most workers have reported that elevated lipid levels are associated with macular exudates and moderate visual loss and partial regression of hard exudates(153-157).

The mean values of cholesterol, Triglycerides and LDL were significantly higher as compared to the control (Table II) and shows statistically significant correlation with Glycosylated Hemoglobin. Similar correlation is reported earlier(72,158). The results of this study indicate that there was significant correlation between Glycosylated Hemoglobin and serum lipids with the severity of diabetic retinopathy.

Two of the baseline characteristic identified as risk factor for high risk PDR in eyes assigned to deferral of photocoagulation in the ETDRS, were increased higher glycosylated hemoglobin and elevated triglycerides(159-162).

No such association was found between serum HDL and degrees of retinopathy (Table II). Kordonouri O et al reported that glycemic control and HDL cholesterol to be the most important variables related to the development of retinal lesions(163).
Table No III show the results of renal function in diabetic retinopathy patients. Diabetic patients with renal impairment are known to be particularly susceptible to cardiovascular complications as a result of AGES. The objective was to examine the impact of glycemic control on renal dysfunction in diabetic retinopathy patients.

Glycosylated Hemoglobin, a measure of long-term glycemic control was associated with increased levels of blood urea and serum creatinine levels with mean values of 46.64 ± 32.80 mg/dl and 1.39 ± 1.05 mg/dl (Table III).

The results suggest that diabetic patients with renal impairment as determined by serum creatinine have an increased risk for the development of cardiovascular disease. This may be because advanced glycosylation end products are involved in atherogenesis(79).
### VARIOUS PARAMETERS IN DIABETIC RETINOPATHY

#### TABLE – I

**Plasma Glucose and Glycosylated Hemoglobin**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Retinopathy</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Plasma Glucose (mg/dl)</td>
<td>157.77±73.84*</td>
<td>89.57±9.31</td>
</tr>
<tr>
<td>Postmeal Plasma Glucose (mg/dl)</td>
<td>217.45±69.86*</td>
<td>129.2±10.6</td>
</tr>
<tr>
<td>Glycosylated Hemoglobin (%)</td>
<td>8.4±1.8*</td>
<td>4.6±0.82</td>
</tr>
</tbody>
</table>

#### TABLE – II

**Serum Lipid Profile**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Retinopathy</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>192.42±29.99*</td>
<td>169.4±15.8</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>44.97±9.5</td>
<td>44.6±6.78</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>163.62±51.88*</td>
<td>139.85±10.0</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>116.09±24.42*</td>
<td>97.59±16.45</td>
</tr>
</tbody>
</table>

#### TABLE – III

**Kidney Function Test**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Retinopathy</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg /dl )</td>
<td>46.64±32.80*</td>
<td>29.35±5.66</td>
</tr>
<tr>
<td>Creatinine (mg /dl )</td>
<td>1.39±1.05*</td>
<td>0.74±0.225</td>
</tr>
</tbody>
</table>

All values are mean with ±standard deviation

* P < 0.01
Diabetic Cataract

Diabetes mellitus has been reported as the most critical factor causing visual loss. Among the various complications of Diabetes mellitus in the eyes, diabetic retinopathy has been regarded as the most common cause of visual loss (164-166). Diabetes mellitus is also known as an important risk factor for cataracts. In epidemiologic studies, factors such as a long duration of diabetic disease, advanced age at the time of clinical diagnosis, advanced retinopathy, treatment with diuretics and poor control of blood sugar level are reported as risk factor for cataract in diabetics (167-174).

Cataracts occur at a younger age in diabetics than non-diabetics. The incidence of cataracts increases proportionally with the degree and the period of the Diabetes mellitus (167-174). It has been reported that most patients having lenticular opacity had Diabetes mellitus for more than five years and that various types of opacities of the lens were developed in of diabetic patients under treatment (175). Carid et al reported that the cataract extraction rate in Diabetes mellitus cases was four to six times higher than the cases without Diabetes mellitus (176).

Many early workers reported that the retinopathy was an influential factor in the degree of cataract (171, 172, 175).
Results and Discussion

Cataract is a common complication of Diabetes indeed it has been estimated that upto 15% of cataract surgery is performed on diabetics.

The present study was performed to quantitatively evaluate the level of Glycosylated Hemoglobin and other biochemical parameters in diabetic patients having cataract. A total of 40 patients (mean age 47.57 ± 15.18 years, mean duration 8.45 ± 4.34 years) underwent ophthalmologic evaluation were studied retrospectively. Diabetic patients were classified into a cataract group and control group (i.e. a group without cataracts). Risk factors like fasting plasma glucose, postmeal plasma glucose, Glycosylated Hemoglobin, fasting lipid profile, blood urea and serum creatinine were analyzed for comparison between patients with and without cataracts.

Biochemical analyses of the two groups are shown in Table No.IV, V and VI.

Table No.IV summarises the results of metabolic control in diabetic cataract patients. Significant differences were found between diabetic and control subjects for plasma glucose and Glycosylated Hemoglobin levels which were high in all diabetic cataract patients (Table IV).
Patterson reported that there was lenticular opacity when the blood sugar was more than 225 mg%(177). The fasting and postmeal blood sugar was higher in the cataract group than in the control group and shows positive correlation with Glycosylated Hemoglobin as reported earlier(56, 57). Previous studies reported that a direct relationship between glycosylated hemoglobin and cataract was not found in the prevalence data; however, cataracts were associated with the duration of Diabetes. In the present study poor blood sugar control reflected by higher Glycosylated Hemoglobin (figure-4(B)) have been found as a risk factor for cataract formation, confirming the findings of Mahsen Janghorbani et al(178).

Table No.V summarises the results of lipid profile in diabetic cataract subjects. Blood lipids showed a significantly higher concentration of total cholesterol, triglyceride and low density lipoprotein cholesterol (Table V and Figure-5) and shows positive correlation with Glycosylated hemoglobin. The level of HDL cholesterol was lowered in the cataract group as compared to the control group which is in consistent with the previous finding(179). The results of this study indicate that blood lipids along with elevated Glycosylated Hemoglobin level are associated with severity of diabetic cataract.
Table No. VI Summarises the results of renal function in diabetic cataract subjects. Raised blood glucose levels and related microvascular disease are associated with progressive damage to the kidneys as found in the present study by elevated levels of serum creatinine. The levels of serum creatinine were significantly higher as compared to the control group (Table VI). But no significant change in blood urea level (Table VI) was seen as compared to the control. Intensive glucose control in Diabetes patients can significantly reduce the risk of kidney damage(180).

Thus, poor blood sugar level as reflected by Glycosylated Hemoglobin may be a marker of dyslipidemia and renal dysfunction in diabetic cataract patients.
VARIOUS PARAMETERS IN DIABETIC CATARACT

TABLE – IV

Plasma Glucose and Glycosylated Hemoglobin

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cataract</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Plasma Glucose (mg/dl)</td>
<td>155.77±50.0*</td>
<td>89.57±9.31</td>
</tr>
<tr>
<td>Postmeal Plasma Glucose (mg/dl)</td>
<td>237.35±69.20*</td>
<td>129.2±10.6</td>
</tr>
<tr>
<td>Glycosylated Hemoglobin (%)</td>
<td>9.12±1.6*</td>
<td>4.6±0.82</td>
</tr>
</tbody>
</table>

TABLE – V

Serum Lipid Profile

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cataract</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>211.25±40.74*</td>
<td>169.4±15.8</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>40.02±6.75*</td>
<td>44.6±6.78</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>178.45±58.96*</td>
<td>139.85±10.0</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>137.25±41.73*</td>
<td>97.59±16.45</td>
</tr>
</tbody>
</table>

TABLE – VI

Kidney Function Test

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cataract</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg /dl)</td>
<td>31.75±7.13</td>
<td>29.35±5.66</td>
</tr>
<tr>
<td>Creatinine (mg /dl)</td>
<td>1.05±0.1582**</td>
<td>0.74±0.225</td>
</tr>
</tbody>
</table>

All values are mean with ±standard deviation  
* P < 0.01  **P < 0.05
Diabetic Nephropathy

**Diabetic nephropathy** is a clinical syndrome characterized by persistent albuminuria (>300 mg/dl or >200 mg/min), a decline in the glomerular filtration rate (GFR), hypertension and a high risk of cardiovascular morbidity and mortality(181).

It has been estimated that by the year 2030, 366 million people will have Diabetes worldwide. Over 5% of newly diagnosed patients with Type II Diabetes will already have diabetic kidney disease and or further 30-40% will develop diabetic nephropathy, mostly within 10 years of diagnosis(182).

The Diabetic patients with microalbuminuria have an increased risk of progression to overt proteinuria, and after sometime, renal failure(183).

The pathophysiology of diabetic nephropathy is complex and multifactorial. Two major factors have been characterized: *hyperglycemia* and hemodynamic disturbances. Hyperglycemia causes protein glycosylation, formation of advanced glycosylated end products (AGES), glomerular hypertrophy, increased mesangial hypertrophy, increased mesangial matrix deposition, glomerulosclerosis and eventually obliteration of glomerular capillaries and ESRD. Hyperglycemia also causes increased secretion of vasodilatory prostaglandins, hyperfiltration, abnormal response to angiotensin-II, increased secretion of arterial natriuretic peptide, an
abnormal endothelin/NO ratio, increase in growth factors secretion, and hyperinsulinemia.

Hemodynamic disturbances cause an increase in glomerular filtration rate (GFR) particularly in younger patients. This increase in intraglomerular capillary pressure resulting in endothelial damage and eventual glomerulosclerosis(184).
Glomerulosclerosis describes the pathological change in the kidney that occurs with Diabetes and other disease processes. Chronic hyperglycemia leads to irreversible non-enzymatic glycosylation of proteins that result in protein cross linking. Accumulation of cross linked protein in the extracellular matrix is thought to cause ischaemia resulting in fibrosis.

The natural course of diabetic renal disease may be summarized as follows(185):

<table>
<thead>
<tr>
<th>Stages</th>
<th>Laboratory Features</th>
</tr>
</thead>
</table>
| Stages 1: | • Normal urinary albumin excretion rate  
            • Normal serum creatinine |
| Stages 2: | • Increased urinary albumin excretion rate  
             (microalbuminuria)  
            • Dipstick Negative for proteinuria  
            • Normal serum creatinine |
| Stages 3: | • Dipstick positive proteinuria  
            • Serum creatinine normal or minimally elevated |
| Stages 4: | • Progressive decline in renal function  
            • Rising serum creatinine |
| Stages 5: | • End stage renal failure |
Results and Discussion

*Diabetic nephropathy* is the single leading cause of end stage renal disease and there has been dramatic increase in the number of patients entering renal replacement therapy in the last few years (186). A total of 40 patients (mean age: 48 years, mean duration of Diabetes 14.17 ± 6.51 years) were selected for studying the profile of Glycosylated Hemoglobin in diabetic nephropathy which is a microvascular complication of Diabetes mellitus. Those patients who had alterations in renal function test were considered to have nephropathy. Biochemical parameters such as fasting plasma glucose, postprandial plasma glucose, Glycosylated Hemoglobin, fasting lipid profile and renal function test were determined in these subjects. The results of the statistical analysis of the biochemical parameters are summarized in Table No. VII, VIII and IX.

Table No. VII shows the results of markers of glycemic control. A significant increase in Glycosylated Hemoglobin concentration was found in all the diabetic nephropathy subjects. The mean value of Glycosylated Hemoglobin was 8.33±1.98% (Table No. VII). The present results also demonstrate that along with Glycosylated Hemoglobin, fasting plasma glucose and postmeal plasma glucose were significantly elevated as compared to the control (Table No. VII) and shows statistically significant correlation with Glycosylated Hemoglobin value (56, 65). As reported recently,
undiagnosed chronic kidney disease is common in Diabetes. It is of importance because almost a half of the patients die before reaching end stage renal failure and diabetic nephropathy has now become the single most common cause of end-stage renal disease. Early identification of kidney disease may allow for timely treatments that could arrest or delay the progression of renal damage(187). Important factors contributing to the high prevalence of diabetic nephropathy in the population of patients with Diabetes are delayed diagnosis of Diabetes and poor glycemic control(188,189). Poor glycemic control as reflected by Glycosylated Hemoglobin concentration is related to the development of this microvascular disease. Advanced glycosylation end products formed as a result of persistent hyperglycemia are shown to be important pathogenetic factors, causing renal damage by increasing the production of mesangial cell transforming factor beta and platelet derived growth factor. AGES increase the synthesis of basement membrane, collagen and mesangial matrix, and enhance vascular permeability(190). The exact cause of rise in Glycosylated Hemoglobin concentration in chronic renal failure is still not known. Some studies suggest that this rise in Glycosylated Hemoglobin level may be due to formation of carbomylated hemoglobin(191), impaired glucose metabolism(192) or presence of glucose in the dialyzed fluid(193,194). In contrast, the level of Glycosylated Hemoglobin decreases in chronic renal failure due to anemia and shorter life span of RBCs has been reported(195).
An association between microalbuminuria and nephropathy are retrospective in nature. Association between glucose control and nephropathy may be more complex than that with retinopathy. The occurrence of nephropathy in no more than 40% of patients with Type I Diabetes and 25% of patients with Type II Diabetes suggest that variable other than glycemia are operant(4).

Although scanty data exist on the relation between Glycosylated Hemoglobin and risk of microalbuminuria and on the relation between Glycosylated Hemoglobin and the risk of nephropathy progression, several cohort studies and clinical trials, the Diabetes control and complication trial (DCCT) and the UK prospective Diabetes study (UKPD), support strong and significant positive association in individuals with Diabetes. Both studies showed that tight glycemic control was associated with a reduction in the progression of diabetic nephropathy. In the latter trial, any decrease of Glycosylated Hemoglobin by 1% was accompanied by a 37% decrease in the incidence rate of nephropathy and renal complications. The American Diabetic Association recommends an average Glycosylated Hemoglobin value should be less than 7%.

Increasing levels of Glycosylated Hemoglobin reported to be associated with a decline in GFR(196). Clinical trial data examining the GFR outcomes on the relation between Glycosylated Hemoglobin and GFR in individuals with Type II Diabetes is not reported.
Previously, most of the studies examined the impact of glycemic control status at initiation of hemodialysis on prognosis following this initiation (197-201). Patients with better Glycosylated Hemoglobin values had longer survival and average blood glucose level before initiation of hemodialysis was reported to be a predictor of survival (197-198). Wu et al reported in a 10-year follow-up study of diabetic patients on hemodialysis that poor glycemic control with Glycosylated Hemoglobin >10% before initiation of hemodialysis was a predictor of cardiovascular morbidity and long-term survival (199).

Romano Nosadini and Giancarlo Tonolo reported relationship between blood glucose control, pathogenesis and progression of diabetic nephropathy (202). They found that the risk of a rapid decline of glomerular function abruptly increases when Glycosylated Hemoglobin and postprandial blood glucose is higher. Their findings suggest that Glycosylated Hemoglobin levels >7.5 to 8% are closely associated with a rapid deterioration of renal function in Diabetes. Also postprandial plasma glucose values were closely linked to a rapid deterioration of GFR. Further fasting plasma glucose values were significantly related to the changes of GFR.

Table No.VIII shows the results of parameters of lipid profile in diabetic nephropathy subjects. Atherosclerosis is the main cause of mortality in diabetic patients and therefore a better understanding of lipid abnormalities and their pathophysiology in
Diabetes is a prerequisite for successful prevention of coronary artery disease (CAD) and other complications of Diabetes mellitus.

Diabetes mellitus patients develop overt diabetic nephropathy which additionally impairs lipidic metabolism. In early and advanced stages of Diabetes Nephropathy lipid disorders may be present. Lipidic metabolism in Diabetes mellitus may also be altered when renal replacement therapy is instituted.

The risk of death from coronary heart disease is substantially increased in diabetic nephropathy patients compared with normal subjects or patients with Diabetes without nephropathy.

The present study was carried out to assess the link between poor glycemic control and lipid profile in diabetic nephropathy subjects. Serum triglyceride and serum cholesterol levels were significant elevated as compared to the control (Table VIII). The increase in triglyceride rich lipoproteins probably induces a mild elevation in total serum cholesterol. In the case of poor glycemic control, total cholesterol is increased due to an accumulation of LDL(203).

Dyslipidemia and hypertension, as well as longer disease duration, elevated Glycosylated Hemoglobin level, are all significant risk factor for diabetic nephropathy in patients with Diabetes(204).

Increased levels of triglyceride rich lipoproteins have been reported in diabetic patients and this atherogenic profile becomes
more apparent when diabetic nephropathy is present. These abnormal plasma lipoproteins may contribute to the increased coronary heart disease, peripheral arterial disease and cerebrovascular disease risk in diabetic nephropathy.

Tsutomu Hirano in his study on lipoprotein abnormalities in diabetic nephropathy reported that diabetic nephropathy, including the subclinical stage, plays a critical role in the hypertriglyceridemia associated with Diabetes mellitus (205). Alberto Martínez Castealo et al. in their study on physiology of lipid metabolism reported that the degree of glycemic control is an important determinant of serum lipoprotein concentration in Diabetes mellitus (206).

Uusutipa et al. demonstrated that in addition to hypertriglyceridemia, compositional abnormalities of lipoproteins are related to CAD mortality (207).

**In the present study serum LDL level was significantly higher as compared to the control (Table VIII).** This may be due to the prevalence of small, dense LDL, glycosylation of LDL and oxidative modification. Increase LDL may promote nephropathy and atherosclerosis. Diabetes mellitus with good or reasonable glycemic control exhibit LDL cholesterol concentrations similar to non-diabetic subjects.

Epidemiological studies suggest that a predominance of the smaller and less dense low-density lipoprotein (LDL particles) is a new
risk factor for CAD. They found that LDL particle diameter was
significantly smaller in diabetic patients with nephropathy compare
with diabetics without nephropathy or non-diabetic controls.
Glycosylation and AGE formation lead to a modification of lipoproteins
which impair receptor specific uptake. This leads to accumulation
and further modification of lipoprotein closing a vicious circle. Besides
advanced glycosylation endproducts, an advanced lipooxidation
endproducts also play important role in the pathogenesis of diabetic
nephropathy. AGEs and ALEs accumulate in plasma proteins.
Glycosylated LDL and specially more dense LDL are even more
susceptible to oxidative modification. Oxidize modified lipoproteins
could be direct mediators of glomerular injury and might promote
diabetic nephropathy. Further lipid modification and peroxidation are
important promoters of atherosclerosis.

The level of serum HDL-C was lower as compared to the control
(Table VIII). Laakso et al found a correlation between low HDL and
CAD mortality(208). Lehto et al demonstrated that low HDL,
hypertriglyceridemia and poor glycemic control were strong predictors
of CAD in diabeteic patients(209).

The result of the present study shows that atherogenic lipid
profile associated with poor glycemic control accelerates the
development of diabetic nephropathy which in turn accelerates
vascular damage inducing CAD morbidity and mortality in Diabetes.
Table No. IX shows the results of renal function in diabetic nephropathy subjects. Raised blood glucose levels and related microvascular disease are associated with progressive damage to the kidneys. The present study thus focuses the impact of glycemic control on renal function in diabetic nephropathy patients.

The levels of blood urea and serum creatinine were significantly higher as compared to the control (Table IX). This may be due to dyslipidemia as evidence in (Table VIII). Dyslipidemia has been involved in the development of direct renal injury in animal models. The treatment of hyperlipidemia has led to an improvement of glomerular injury in both diabetic and non-diabetic renal disease(206). The altered serum lipoproteins interact with structures of the glomerulus. Glycooxidated modified LDL exhibited enhanced binding to glycosaminoglycanes of the glomerular basement membrane inducing an increased permeability of these membrane. The depositions of modified LDL particles in the mesangium induce chemotactic signals for macrophages and stimulate mesangial cell proliferation. The scavenger receptor uptake of these modified LDL particles by monocyte and macrophages is responsible for the formation of glomerular and mesangial foam cells. The mesangial expansion could be induced by other mechanisms: the accumulation of apoB and apoE leading to or reduction in the glomemacular filtration area, an alteration in renal cortical tissue lipids or in the membrane
fluidity and function due to disturbances in fatty acids concentrations and alterations in glomerular haemodynamics (206).

Hyperlipidaemia has been identified as a risk factor for developing a more rapid decline in GFR and increased mortality in diabetic nephropathy patients. High triglycerides and low HDL cholesterol has been associated with more rapid progression of microalbuminuria in Diabetes with well controlled blood pressure. Hypertriglyceridaemia and hypertension seems to have a synergistic effect on the decline of GFR.

Evaluation of renal function is important in order to select the appropriate strategy to reduce the progression of renal damage. There is a need for early diagnosis and optimal management of diabetic patients with renal dysfunction.
**VARIOUS PARAMETERS IN DIABETIC NEPHROPATHY**

**TABLE – VII**

Plasma Glucose and Glycosylated Hemoglobin

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<tr>
<th>Parameters</th>
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<th>Control</th>
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<td>Fasting Plasma Glucose (mg/dl)</td>
<td>135.02±39.95*</td>
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<td>Postmeal Plasma Glucose (mg/dl)</td>
<td>220.25±83.85*</td>
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<td>Glycosylated Hemoglobin (%)</td>
<td>8.33±1.98*</td>
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**TABLE – VIII**

Serum Lipid Profile

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<td>HDL (mg/dl)</td>
<td>43.6±9.45</td>
<td>44.6±6.78</td>
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<td>Triglycerides (mg/dl)</td>
<td>181.6±55.02*</td>
<td>139.85±10.0</td>
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<tr>
<td>LDL (mg/dl)</td>
<td>157.8±30.22*</td>
<td>97.59±16.45</td>
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**TABLE – IX**

Kidney Function Test

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<th>Control</th>
</tr>
</thead>
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<td>Urea (mg /dl )</td>
<td>53.43±40.36*</td>
<td>29.35±5.66</td>
</tr>
<tr>
<td>Creatinine (mg /dl)</td>
<td>1.702±1.26*</td>
<td>0.74±2.25</td>
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</tbody>
</table>

All values are mean with ±standard deviation  
* P < 0.01
Diabetic Neuropathy

**Diabetic neuropathy** is a complication of Diabetes that affects the nerve. It is the most common and troublesome complication of Diabetes mellitus, leading to great morbidity and an increase in the economic burden of public health. Diabetes is a heterogeneous disorder that encompasses a wide ranges of abnormalities affecting proximal, distal, and peripheral sensory-motor, as well as the autonomic nervous system (210).

The most common type of diabetic neuropathy is called peripheral neuropathy and affects the peripheral nerves. Peripheral nerves are the nerves that go out from the brain and spinal cord to the muscles, skin, internal organs and glands. Peripheral neuropathy impairs proper functioning of these sensory and motor nerves. The most common symptoms of neuropathy include tingling, numbness, burning sensation and ultimately loss of sensation usually in the feet and hands (211).

Diabetic neuropathy has been reported to affect more than 50% of patients with a history of Diabetes of more than 25 years duration making it one of the most common diseases affecting the nervous system. The most important risk factors for the development of neuropathy are long duration and increased severity of hyperglycemia. The presence of neuropathy significantly increases the risk of foot
ulcerations and infections and amputations. Diabetic foot is the leading cause of foot amputation in India(212).

The factors leading to the development of peripheral neuropathy in Diabetes are not understood completely and multiple hypotheses have been advanced. It is generally accepted to be a multifactorial process. Both basic science research and large prospective clinical studies, such as the Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) have shown that tight control and euglycemia (or near euglycemia) can prevent the onset or slow progression of diabetic neuropathy.

Currently, the factors recognized in the pathogenesis of diabetic neuropathy are metabolism, vascular insufficiency, loss of growth factor trophism, and autoimmune destruction of small unmyelinated nerves (c fibers) in a visceral and cutaneous distribution. The 2 main features that explain symptoms and complications of diabetic neuropathy are believed to be the degeneration of nerve fibers and grossly diseased blood vessels that supply those nerve fibres. Proper circulation determines whether or not nerve fibers repair themselves or proceed to total degeneration.

Metabolic failure can affect several pathways, greatly contributing to diabetic neuropathy. Hyperglycemia causes several biological changes, including an increase in the production of advanced glycosylated end products, a defect in the polyol pathway
and involvement of aldose reductase enzyme, and impaired resistance to oxidative stress. All the above biological changes are closely related and work together to bring on the neuropathic complications(213).

**Results and discussion**

*Diabetic neuropathy* is a common cause of morbidity and death among patients with Diabetes, generating a huge economic burden. Apart from tight glycemic control, no other evidence based treatments are known to ameliorate or prevent neuropathy(214).

The study population consisted of 40 patients of Diabetes having neuropathy with mean age 56 years. The mean duration of Diabetes was $10.62 \pm 4.28$ years. Patients were screened for the presence of diabetic neuropathy on the basis of ankle jerking reflex. Fasting sample were analyzed for plasma glucose, Glycosylated Hemoglobin, lipid profile, and blood urea and serum creatinine. Postmeal plasma glucose was also recorded.

The results of various biochemical parameters in diabetic neuropathy subjects are summarized in Table No. X XI and XII.

Age and Diabetes duration are non-controllable risk factors for diabetic neuropathy. Controllable risk factors include hyperglycemia, hypertension, cholesterol, triglycerides and smoking. Hyperglycemia is generally considered the strongest risk factor for diabetic neuropathy(215-217). But some studies have shown hypertension is the strongest risk factor(218). The risk factors for diabetic neuropathy
are interrelated with the other diabetic microvascular complications, diabetic retinopathy and diabetic nephropathy(219,220).

**Table No. X shows the results of diabetic control in diabetic neuropathy subjects.** Elevated levels of Glycosylated Hemoglobin were found as compared to the control as shown in (Table X and figure 10(B)). This indicates that hyperglycemia induced formation of advanced glycosylation end products (AGES) may be the causative factor(221). AGE modified peripheral nerve myelin is susceptible to phagocytosis by macrophages and contributes to segmental demyelination, modification of major axonal cytoskeletal proteins such as tubulin, neurofilament, and actin by AGES results in axonal atrophy, degeneration and impaired axonal transport and glycosylation of extracellular matrix protein laminin leads to impaired regenerative activity in diabetic neuropathy. Recently, the receptor for AGES (RAGE) has been found to localize with AGES in diabetic peripheral nerves. This suggests that, in diabetic neuropathy, AGES and AGE/RAGE interactions induce oxidative stress, result in upregulation of nuclear factor (NF) - Kappa B and various NF - Kappa B mediated proinflammatory genes and exaggerate neurological dysfunction, including altered pain sensation. Additionally, AGE/RAGE induced oxidative stress further accelerates formation of glycooxidation products such as Nepsilon (carboxymethyl) lysine and pentosidine.
Persistent elevations in blood sugar and therefore, Glycosylated Hemoglobin increase the risk for the long term vascular complications of Diabetes(222). Glycosylated Hemoglobin was a significant risk determinant in diabetic peripheral neuropathy(223-225). The data on the association between Glycosylated Hemoglobin and the risk of autonomic neuropathy is scanty.

As shown in Table X and figure 10(A), fasting and postmeal plasma glucose were significantly elevated as compared to the control and shows statistically significant correlation with Glycosylated Hemoglobin(63,65).

Table No. XI shows the results of lipid profile in diabetic neuropathy patients. The levels of serum cholesterol with a mean of 201.82 ± 48.0mg/dl, triglycerides with a mean of 201.37±62.4mg/dl and LDL with a mean of 121.45±49.66mg/dl were significantly increased as compared to the control whereas the level of HDL cholesterol decreased as compared to the control (Table XI).

This may be due to the association of poor glycemic control with increased lipid peroxidation. This is in agreement with the studies of Jyoti M Sawant et al. who evaluated association of poor glycemic control with increased lipid peroxidation and reduced antioxidant vitamin status in diabetic neuropathy(226). The increased tendency of LDL to undergo lipid peroxidation in diabetic patients contributes to
increased levels of blood Glycosylated Hemoglobin, mainly in those with Glycosylated Hemoglobin <7.2(227).

Hyperglycemia causes the autoxidation of glucose, the glycosylation of proteins and the activation of polyol metabolism(228-230). These changes accelerate the generation of free radicals and result in an increase in the oxidative modification of lipids, DNA and proteins in various tissues. An imbalance between the generation and scavenging of these free radicals leads to oxidative stress which may be associated with the pathogenesis of the complications of Diabetes mellitus including nerve damage leading to diabetic neuropathy.

The findings of Diabetes Control and Complications Trial (DCCT) suggest that neuropathy can develop, despite intensive control of the glucose level(231). Significant correlations were observed between the presence of diabetic peripheral neuropathy with age, duration of Diabetes, quality of metabolic control, the presence of background or proliferative diabetic retinopathy, cigarette smoking, high density lipoprotein cholesterol and the presence of cardiovascular disease thus confirming previous associations(232). New associations have been identified from this study namely with elevated diastolic blood pressure, the presence of severe ketoacidosis, an increase in the levels of fasting triglyceride and the presence of microalbuminuria. This study has identified previously known and new potential risk factor for the development of diabetic peripheral neuropathy.
Solomon Tesfaye et al. reported that after adjustment for Glycosylated Hemoglobin value and the duration of Diabetes, higher levels of total and low-density lipoprotein cholesterol and triglycerides, a higher body mass index; higher von willebrand factor levels and urinary albumin excretion rate, hypertension and smoking were all significantly associated with the cumulative incidence of neuropathy(233). Cardiovascular disease was associated with double the risk of neuropathy, independent of cardiovascular risk factors.

The incidence of neuropathy is associated with potentially modifiable cardiovascular risk factors such as increased triglyceride, total cholesterol and LDL levels whereas decreased HDL levels (Table XI and Figure 11).

Table No. XII shows the results of renal function in diabetic neuropathy subjects. No significant change in levels of blood urea was found as compared to the control. The levels of serum creatinine shows marked increased as compared to the control (Table XII). The probable cause is Diabetes mellitus may be associated with increased lipid peroxidation caused by oxidative stress. Lipid peroxidation occurs in the plasma membrane and damages the membrane structure and permeability. Recently, a relationship between diabetic nephropathy and neuropathy and oxidative stress reported suggesting that oxidative stress affects the progress of diabetic complications. Therefore antioxidants could ameliorate these complications(234,235).
Diabetic nephropathy is a serious microvascular complication of Diabetes mellitus. The natural history of diabetic nephropathy is well known, i.e. dipstick positive protenuria and the development of renal failure follow the appearance of microalbuminuria(236).

The production of peroxynitrite increase in the proximal tubules of patients with diabetic nephropathy suggesting that oxidant injury of the proximal tubules play an important role in the pathogenesis of diabetic nephropathy(237).

Diabetic neuropathy is associated with a decrease in nerve conduction velocity(238). Diabetes induced oxidative stress and the generation of superoxides may be responsible in part for the development of vascular and renal complications(239).

H.Yokoyama et al. reported that diabetic neuropathy is closely associated with arterial stiffening and thickness, was significantly associated with age, duration, Glycosylated Hemoglobin, systolic blood pressure, diastolic blood pressure, pulse pressure, hypertension, retinopathy, urinary albumin excretion rate, neuropathy stages, pulse wave velocity and intimamedia thickness(240).
### VARIOUS PARAMETERS IN DIABETIC NEUROPATHY

**TABLE – X**

**Plasma Glucose and Glycosylated Hemoglobin**

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Plasma Glucose (mg/dl)</td>
<td>152.40±40.11*</td>
<td>89.57±9.31</td>
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<tr>
<td>Postmeal Plasma Glucose (mg/dl)</td>
<td>252.44±69.09*</td>
<td>129.2±10.6</td>
</tr>
<tr>
<td>Glycosylated Hemoglobin (%)</td>
<td>9.38±1.84*</td>
<td>4.6±0.82</td>
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</tbody>
</table>

**TABLE – XI**

**Serum Lipid Profile**

<table>
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<th>Control</th>
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<tr>
<td>Total Cholesterol (mg/dl)</td>
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<td>169.4±15.8</td>
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<tr>
<td>HDL (mg/dl)</td>
<td>40.82±6.47*</td>
<td>44.6±6.78</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>201.37±62.4*</td>
<td>139.85±10.0</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>121.45±49.66*</td>
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**TABLE – XII**

**Kidney Function Test**

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<th>Parameters</th>
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<td>Creatinine (mg/dl)</td>
<td>1.07±0.20*</td>
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</tbody>
</table>

All values are mean with _standard deviation_  * P < 0.01
Diabetes and Hypertension

**Hypertension**, defined as a blood pressure $\geq 140/90$ mmHg is an extremely common comorbid condition in diabetics, affecting more than 20-60% of patients with Diabetes, depending on obesity, ethnicity and age(241).

Blood pressure (BP) increases when arteries are narrowed, due to atherosclerosis or to chronically high blood glucose levels and blood flow is restricted. Also, the risk for developing Diabetes is almost doubled by the presence of hypertension, even among non-obese people with blood pressure $>130/85$ mmHg(242).

Coexistent Diabetes and hypertension affects an estimated 2.5 million persons in the United States. Hypertension occurs approximately twice as frequently in persons with Diabetes as without and contributes to most of the chronic complications of Diabetes, including coronary artery disease, stroke, lower extremity amputations, and renal failure and perhaps to diabetic retinopathy and blindness.
Annual incidence and total prevalence of complication of Diabetes, United States, 1984:

<table>
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<tr>
<th>Complications</th>
<th>Incidence</th>
<th>Prevalence</th>
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<tbody>
<tr>
<td>Stroke</td>
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<td>392,000</td>
</tr>
<tr>
<td>Coronary Artery Disease</td>
<td>101,000</td>
<td>781,000</td>
</tr>
<tr>
<td>Peripheral Vascular Disease</td>
<td>50,000</td>
<td>573,000</td>
</tr>
<tr>
<td>Blindness</td>
<td>6,900</td>
<td>47,000</td>
</tr>
<tr>
<td>End Stage Renal Disease</td>
<td>5,900</td>
<td>13,000</td>
</tr>
<tr>
<td>Amputation</td>
<td>47,000</td>
<td>86,000</td>
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</table>

Subjects with hypertension have significantly more hyperglycemia than those with normal BP and hypertensive subjects may be increasingly more susceptible to develop Diabetes mellitus(147).

Each -10 mmHg decrease in mean systolic blood pressure was associated with reduction in risk of 12% for any complication related to Diabetes.

At least 90% of hypertension in the general population is essential hypertension. Essential hypertension accounts for most of the cases in the diabetic population(243).

The pathophysiology of diabetic hypertension involves three theories: Hemodynamic, metabolic and hormonal.
Abnormal haemodynamics in patients with Diabetes may increase the risk of tissue damage attributable to hypertension. For any level of systemic blood pressure, patients with Diabetes are more susceptible to tissue damage. Postulated mechanisms include:

Autoregulation: when this is interrupted (through hyperglycemia) in vulnerable vascular beds such as those of the retina and renal glomeruli, systemic blood pressure is transmitted directly to the microvasculature. Decreased vascular compliance of major vessels such as the aorta, perhaps resulting from non-enzymatic glycosylation may lead to the transmission of higher pressures to distal vascular beds. This abnormality may also contribute to isolated systolic hypertension in patients with Diabetes.

Metabolic abnormalities that are often present in diabetic hypertensive patients accelerate atherosclerosis. Both hypertension and Diabetes are well-identified risk factors for atherosclerosis. Several mechanisms acting together mediate the damage to the vasculature in the diabetic hypertensive patient. Plasma levels of lipoprotein have been noted to be elevated in diabetic individuals. Particularly those with poor glycemic control. Augmented oxidation of low density lipoprotein cholesterol and formation of glycosylated low density lipoprotein, which enhances foam cell formation, have been observed in diabetic states.
Diabetes mellitus and hypertension are also associated with hematologic abnormalities that encourage thrombosis. Enhanced platelet adhesion and aggression as well as higher levels of some coagulation factors contribute to the procoagulation state in diabetic hypertensive patients.

Hormonal theory shows the role of blood insulin in hypertension. The increase in blood insulin in both types of Diabetes promotes hypertension by affecting key checkpoints in the body. For example, increased blood insulin makes the body vessels widen (vasodilate) and this widening of the blood vessels affects the sympathetic nervous system that increases blood pressure, directly or indirectly, by making kidney retain salt. Another way the increase in blood insulin can lead to increased blood pressure is by promoting atherosclerosis, which hardens the blood vessels. It is also believed that those diabetics who live with untreated high blood sugar for a long time are more likely to have hypertension due to early atherosclerosis. The hypertension accompanying diabetic nephropathy with the histological picture of diffuse glomerulosclerosis is termed “diabetic hypertension”. The biochemical hallmark of this type of hypertension are diminished Plasma Renin Activity (PRA) and hence plasma aldosterone with progressive renal disease, the reduced free water clearance tends to raise the circulating fluid volume, which in turn results in low PRA.
The following three factors are believed to operate in a diabetic developing hypertension:

1. Moderate hyperglycemia (in the absence of osmotic diuresis).

2. Diabetes accentuating atherosclerosis with a decreased vascular resilience diabetic glomerulopathy and

3. Recurrent attacks of polynephritis (silent at times) also contribute to hypertension in a diabetic through ill defined mechanism.
Irrespective of whether or not hypertension is involved in the pathogenesis of nephropathy, it is clear that nephropathy accelerates hypertension and hypertension accelerates nephropathy. Hence kidney becomes the “victim and the culprit”(147).

**Results and Discussion**

*Hypertension* in all populations including those with Diabetes is one of the strongest risk factors for cardiovascular disease. This has been confirmed in the Framingham, Bedford, Whitchall and Multiple Risk factor International Trial (MRFIT) studies(244).

**The present study was carried out to assess the association of glycemic control and hypertension to chronic complications in diabetic subjects.**

Forty patients were analyzed for this study. Mean age of the patient was 45.82 ± 14.59 years and mean duration of Diabetes was 14.7 ± 6.55 years. Various blood samples were collected from all the subjects after at least 8 hour fasting for the analysis of fasting plasma glucose, Glycosylated Hemoglobin, lipid profile and renal function, whereas the blood samples from the non-fasted subjects were collected for the analysis of postmeal blood glucose. The diagnosis of hypertension was based on blood pressure >140/90 mmHg.

The results of biochemical parameters have been shown in **Table No. XIII, XIV and XV.**
Table No. XIII depicts the results of glycemic profile in diabetic hypertensive patients. Diabetes causes persistent hyperglycemia and it is a major health problem. The measurement of Glycosylated Hemoglobin was carried out to assess the diabetic control of the patient. Concentration of Glycosylated Hemoglobin was significantly higher in diabetic hypertensive patients as compared to the control (Table XIII), Confirming the findings of Abdual Basit et al and La Rocca et al (245-246). The present study provides information which is in direct contrast to that by others who found blood pressure reduction due to hemoglobin glycosylation in diabetic patients. Treatment for hypertension may be associated with a level of endothelial dysfunction that interferes with the antihypertensive effect of Glycosylated Hemoglobin (247).

In addition to Glycosylated Hemoglobin, fasting and postmeal blood glucose levels show significant rise as compared to the control (Figure 13 (A)) and confirming previous results (57, 65), shows positive correlation with Glycosylated Hemoglobin. Postprandial plasma glucose besides being a marker for the onset of Diabetes appears to be associated with the development of both the macrovascular and microvascular complications of Diabetes, independently of Glycosylated Hemoglobin and fasting plasma glucose levels.

Table No. XIV depicts the results of lipid profile in diabetic hypertensive patients. The present experiment was carried out to
evaluate the influence of lipid profile and hypertension on the development of microvascular complications of Diabetes. A Significant change in serum cholesterol, triglycerides and LDL levels were observed in diabetic hypertensive patients as compared to the control. No significant change in HDL cholesterol was found as compared to the control (Table XIV and Figure-14). This shows the influence of lipid profile and blood pressure, in addition to glycemic control on the development of microvascular complications of Diabetes. Many previous studies have shown similar findings. The lipid profile and blood pressure are the risk factor for the development of early background retinopathy and incipient nephropathy in children with insulin-dependent Diabetes mellitus(163). Dyslipidemia and hypertension as well as longer disease duration, elevated Glycosylated Hemoglobin levels are all significant risk factors for diabetic nephropathy(204). Further, A Elis et al. evaluated the association between glycemic, lipid and blood pressure control among Israeli diabetic patients and found that non-compliance with treatment and sub-optimal follow-up by family physicians are associated with increased risk of failure to control major risk factor among diabetic patients(248). Another study by Hamid Nasri et al. has shown association of serum lipoprotein (a) with hypertension in diabetic patients(249). This study suggests that kidney function is an independent determinant of lipoprotein (a) and hypertension in diabetic patients.
Table No.XV summarises the results of renal function in diabetic hypertensive patients. Diabetes mellitus and arterial hypertension are the leading causes of the end stage renal disease. The combined presence of hypertension and Diabetes concomitantly accelerates the decrease in renal function, the development of diabetic retinopathy and the development of cerebral disease. The present study was carried out to describe the relationship between blood glucose and lipid abnormalities and the occurrence and progression of renal damage in Diabetes mellitus.

The levels of serum creatinine were significantly higher in diabetic hypertensive patient as compared to the control while no significant change in blood urea level was found as compared to the control (Table XV). This may be due to hyperglycemia, arterial hypertension and dyslipidemia causing disorders of albumin excretion rate and extracellular release of oxygen radical species at glomerular level. The risk of a rapid decline of glomerular function abruptly increases when Glycosylated Hemoglobin is steadily higher and postprandial blood glucose is above 200 mg/dl(250). The WHO multinational study of vascular disease in Diabetes confirmed the role of proteinuria and retinopathy as markers of renal failure and the importance of hyperglycemia in renal failure in Diabetes. Plasma triglyceremia seem to be an important predictor of renal failure in Diabetes(251). In patients with nephropathy total cholesterol and low density lipoprotein cholesterol were correlated with GFR(252).
## VARIOUS PARAMETERS IN DIABETIC HYPERTENSION

### TABLE – XIII

**Plasma Glucose and Glycosylated Hemoglobin**

<table>
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<th>Parameters</th>
<th>Hypertension</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Plasma Glucose (mg/dl)</td>
<td>131.45±30.3*</td>
<td>89.57±9.31</td>
</tr>
<tr>
<td>Postmeal Plasma Glucose (mg/dl)</td>
<td>215.7±75.22*</td>
<td>129.2±10.6</td>
</tr>
<tr>
<td>Glycosylated Hemoglobin (%)</td>
<td>8.3±1.68*</td>
<td>4.6±0.82</td>
</tr>
</tbody>
</table>

### TABLE – XIV

**Serum Lipid Profile**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hypertension</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>200.0±26.80*</td>
<td>169.4±15.8</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>42.15±7.6</td>
<td>44.6±6.78</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>178.72±46.06*</td>
<td>139.85±10.0</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>122.33±30.35*</td>
<td>97.59±16.45</td>
</tr>
</tbody>
</table>

### TABLE – XV

**Kidney Function Test**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hypertension</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg /dl )</td>
<td>30.35±8.55</td>
<td>29.35±5.66</td>
</tr>
<tr>
<td>Creatinine (mg /dl )</td>
<td>0.933±0.27*</td>
<td>0.74±0.225</td>
</tr>
</tbody>
</table>

All values are mean with ±standard deviation  
* P < 0.01

125
Diabetic Dyslipidemia

Patients with Diabetes mellitus have a high prevalence of coronary artery disease (253). An increase of 1 mmol/L LDL-c is associated with a two-fold increase in the risk of coronary heart disease in people with Diabetes mellitus (254). The major risk factors in DM are hyperglycemia, dyslipidemia and hypertension. Individuals with DM may have several forms of dyslipidemia. The most common pattern of dyslipidemia is hypertriglyceridemia and reduced HDL cholesterol levels, often referred to as the lipid triad (255). DM itself does not increase levels of LDL, but the small dense LDL particles found in Type-II DM are more atherogenic because they are more easily glycosylated and susceptible to oxidation (8). Glycosylation, oxidation and triglyceride enrichment of lipoprotein contribute to the observed increase in atherogenicity. Glycosylation of LDL increases its half-life, causes it to be the more atherogenic small dense variety, interfere with the clearance of LDL by the LDL receptor, prolong its residence time in the circulation and make it more likely to be oxidized and taken up by macrophages to form foam cells (256). Glycosylation of HDL cholesterol shortens its half-life and causes the less protective HDL₃ to predominate over the more protective HDL₂ form of the lipoprotein (257). Triglyceride enrichment leads to increased production of the small dense form of LDL cholesterol and to depletion of HDL cholesterol. The ability of HDL to transport
cholesterol from peripheral tissues back to the liver may be decreased when HDL is triglyceride enriched(258). Lipid abnormalities in diabetic patients are likely to play an important role in the development of atherogenesis and so are called atherogenic dyslipidemia(259). An issue of considerable interest is the relative contribution of each component of atherogenic dyslipidemia to coronary artery disease (CAD) risk. Growing evidence suggests that all the components of lipid triad are independently atherogenic(260). The present study was an effort to provide an insight into some of the risk factors in DM.

**Lipoproteins: Particles to transport Lipids**

![Diagram of Lipoproteins]

- **Lipids**: Mainly Cholesterol.
- **Normal (Plasma) level**: 175 mg/100ml
- **Hypercholesterolemia**: Upto 300mg/100ml
Cholesterol nodules (xanthomas) deposits in arterial plaques results into:

- Diabetes mellitus
- Heart Attacks
- Strokes
- Peripheral Vascular Disease

**Pathophysiology of Diabetic Dyslipidemia:**

Four key features of diabetic dyslipidemia are –

1. Hypertriglyceridemia.
2. A high proportion of small dense low-density lipoprotein cholesterol.
3. Low high-density lipoprotein cholesterol.
4. Postprandial lipemia.

Plasma LDL levels per se are not usually higher than those of non-diabetic patients. A cascade of pathogenic steps, resulting from insulin resistance together with dysfunction of the enzyme lipoprotein lipase (LPL), could account for most of these abnormalities. Insulin resistance in adipocytes allows exuberant lipolysis stimulated by hormone-sensitive lipase, resulting in excessive free fatty acid (FFA) release into the blood. The excess delivery of FFA to the liver (together with hepatic insulin resistance) results in upregulation of
apolipoprotein B (apoB), by preventing its degradation. Therefore, the liver produces and exports an increased amount of triglyceride (TG) rich/apoB rich very low-density lipoproteins (VLDL). Normally, VLDL would interact with LPL in the vessel wall of adipocyte and muscle cells, and LPL would clear TGs for storage in adipocytes and convert VLDL to LDL. As a result of LPL activity, HDL would also be increased.

In Diabetes, LPL activity is blunted, which accounts in part for the elevated plasma VLDL-TG and the diminished HDL as well as the relatively low LDL. A second factor contributing to these circulating abnormalities is the circulating plasma enzyme **Cholesterol Ester Transfer Protein (CETP)**. When plasma VLDL – TG is elevated, CETP causes TG to move to HDL and LDL, and conversely, causes cholesterol ester to move from HDL and LDL to VLDL-TG. Hence diabetics with high plasma VLDL concomitantly have low measured plasma HDL because the cholesterol ester is being transferred out of the HDL fraction, as well as a diminution in the size of the LDL particle because LDL become TG enriched, and this is converted by hepatic lipase to small dense LDL. This explains, to a large extent, 3 of the 4 dyslipidemic features.

The fourth feature is postprandial lipemia. Dietary fat is absorbed in the form of chylomicrons, and in diabetic patients, the defect in LPL leads to a persistent and prolonged elevation of
chylomicron - TG in the blood. Furthermore, there is a second defect in the normal processing of “chylomicron remnant” particles, the product of LPL-mediated lipolysis of chylomicrons. Chylomicron remnants are normally cleared by a special pathway in the liver via attachment to specific heparin sulfate proteoglycans. The proteoglycan trapping mechanism is known to be deficient in Diabetes (at least, in diabetic mice); hence, people with Diabetes may accumulate chylomicron remnants in the blood as well(261).

**Results and Discussion**

*Diabetic dyslipidemia*, a risk factor for cardiovascular disease is a profile of lipid abnormalities typically seen in people with Diabetes(262).

To assess Glycosylated Hemoglobin and its correlation with other biochemical parameters in patients with diabetic dyslipidemia, fasting and postmeal blood samples were collected from 40 patients with mean age 47 years and mean duration of Diabetes 16 years. The blood samples were analyzed for blood glucose, Glycosylated Hemoglobin, lipid profile and renal function. A diagnosis of dyslipidemia was made by person’s lipid profile.
The results of biochemical parameters in diabetic dyslipidemia patients have been shown in Table No. XVI, XVII and XVIII.

**Table No.XVI indicate the results of markers of glycemic status in diabetic dyslipidemia patients.** Raised levels of Glycosylated Hemoglobin with mean value of $7.48\pm1.32\%$ found in the present study indicates poor glycemic control of the patient (Table XVI). Poor glycemic control increases dyslipidemia. Improved glycemic control in Diabetes improves dyslipidemia. Glucose promotes hepatic lipogenesis since the flux of glucose through the liver is increased in DM, glucose alone can increase lipid content and promote dyslipidemia. Glucose can also modify lipoproteins like hemoglobin which becomes glycosylated to form a marker for chronic hyperglycemia; components of lipoproteins such as apoB can become glycosylated. Advanced glycosylation end products are also found in lipoproteins from people with Diabetes(210). Richard Bucala et al also reported advanced glycosylation of LDL in the dyslipidemia of Diabetes and ESRD(257).

**Table XVI and figure 16(A)** indicates that the other two markers of glycemic status, fasting and postmeal plasma glucose were significantly elevated as compared to the control and confirming previous observation showing association of fasting blood sugar and postmeal blood sugar with Glycosylated Hemoglobin(263).
Table No.XVII indicates the results of circulating lipids in diabetic dyslipidemia patients. Impaired lipid metabolism resulting from uncontrolled hyperglycemia has been implicated in cardiovascular complications in diabetic patients. The present study has examined the impact of glycemic control on the lipid profile of diabetic patients. The poor control of Diabetes was associated with significant increases in total cholesterol, triglycerides and LDL-c as compared with the control group. HDL-c amounts were lower as compared to the control group and not related to Glycosylated Hemoglobin (Table No XVII).

Khan, H et al(263), Ana Marice Ladeia et al(264), Virtanen et al(265), Beylot M et al(266), Akanij AO et al(267) and Ezarra et al(268) have also shown association of glycemia control with lipid profile in diabetic patients.

Correlation of Glycosylated Hemoglobin with cholesterol in Type I and no correlation of Glycosylated Hemoglobin with triglycerides were observed in either group of diabetics by Atabani et al(58). Positive correlation between Glycosylated Hemoglobin and triglycerides and no significant correlation was found between HbA1 and total cholesterol by Odetti et al(76) while in contrast to these studies Bener et al. found that obesity, consanguinity, total cholesterol, reduced HDL cholesterol and triglyceride were more prevalent in diabetic patients and fail to show any association of Lp(a) levels with glycemic control in Type II
diabetic patients(269). There is a difference in the mean levels of Lp(a) between the study groups, although Glycosylated Hemoglobin did not correlate significantly with Lp(a) in subjects with or without Type II Diabetes.

**Table No.XVIII and figure 18 show the results of renal function in diabetic dyslipidemia patients.** The present study was designed to assess the correlation of poor metabolic control with kidney dysfunction.

The mean values of blood urea (41.85 ± 32.34 mg/dl) and serum creatinine (1.43 ± 1.48 mg/dl) were significantly higher (p<0.01) as compared to the control (Table XVIII).

Poor glycemic control, as measured currently by the Glycosylated Hemoglobin, has been associated with an increased risk for both microvascular and macrovascular disease. As the level of Glycosylated Hemoglobin rises, there is an increased risk of cardiovascular disease as well as increased risk for renal disease and retinopathy(115, 270).

These findings suggest that control of Diabetes should decrease the risk of proteinuria thus decreasing ESRD and its associated mortality(271).
VARIOUS PARAMETERS IN DIABETIC DYSLIPIDEMIA

**TABLE – XVI**

Plasma Glucose and Glycosylated Hemoglobin

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dyslipidemia</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Plasma Glucose (mg/dl)</td>
<td>138.075±63.91*</td>
<td>89.57±9.31</td>
</tr>
<tr>
<td>Postmeal Plasma Glucose (mg/dl)</td>
<td>219.3±84.18*</td>
<td>129.2±10.6</td>
</tr>
<tr>
<td>Glycosylated Hemoglobin (%)</td>
<td>7.48±1.32*</td>
<td>4.6±0.82</td>
</tr>
</tbody>
</table>

**TABLE – XVII**

Serum Lipid Profile

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dyslipidemia</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>230.625±18.59*</td>
<td>169.4±15.8</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>34.75±7.80*</td>
<td>44.6±6.78</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>227.6±22.09*</td>
<td>139.85±10.0</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>151.16±20.24*</td>
<td>97.59±16.45</td>
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</table>

**TABLE – XVIII**

Kidney Function Test

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dyslipidemia</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg /dl )</td>
<td>41.85±32.34**</td>
<td>29.35±5.66</td>
</tr>
<tr>
<td>Creatinine (mg /dl)</td>
<td>1.43±1.48*</td>
<td>0.74±0.225</td>
</tr>
</tbody>
</table>

All values are mean with standard deviation  
* P < 0.01  **P < 0.05
Ischemic Heart Disease (IHD)

Ischaemia refers to a lack of oxygen due to inadequate perfusion, which results from an imbalance between oxygen supply and demand. The most common cause of myocardial ischaemia is atherosclerosis disease of epicardial coronary arteries. Ischemic Heart Disease (IHD) is the common, serious, chronic, life-threatening illness in the United States, where more than 11 million persons have IHD. This condition causes more deaths and disability and incurs greater economic costs than any other illness in the developed world(8).

There is an increased incidence of large-vessel atherosclerosis and myocardial infarction in patients with Diabetes mellitus. IHD is the most common cause of death in adults with Diabetes mellitus. Patients with Diabetes mellitus are more likely to have an abnormal or absent pain response to myocardial ischaemia probably as a result of generalized autonomic nervous system dysfunction. Ambulatory ECG monitoring has shown that upto 90% of episodes of ischaemia are silent in diabetic patients with IHD(8). The incidence rate of myocardial infarction was increased in diabetic patients, including those with and without prior myocardial infarction. These data suggest that cardiovascular risk factors in diabetic patients should be treated as aggressively as in nondiabetic patients with previous myocardial infarction(272).
**Hyperglycemia represents an independent risk factor for developing IHD.** The UK prospective Diabetes Survey (UKPDS) study has shown that the hazard ratio increases significantly in relation to the increasing Glycosylated Hemoglobin, so that for every 1% increase in Glycosylated Hemoglobin there is a 14% increase in fatal and non-fatal MI(244).

The incidence of IHD mortality and events increased with each tertile of Glycosylated Hemoglobin in elderly men Diabetes. The most important single risk factor associated with IHD death or event was Glycosylated Hemoglobin(273).

Hyperglycemia is a well established independent risk factor for CVD and intensive treatment of hyperglycemia has been shown to prevent or slow the progression of long-term microvascular complications of Diabetes. **However, whether tight glycemic control influence the development of macrovascular complications remains to be determined.**

**Results and Discussion**

The study was undertaken to evaluate association between Glycosylated Hemoglobin and cardiovascular disease in diabetic persons. The study group consisted of 40 patients with mean age 52.72 ± 14.41 years and mean duration of Diabetes 14.37±4.60 years. Blood samples were analyzed for Glycosylated Hemoglobin, fasting and postmeal plasma glucose, lipid profile and renal function. All
patients had either ischaemic heart disease on the basis of ECG changes indicating definite ischaemia/infarction, or a positive treadmill test or other stress test.

Table No.XIX, XX and XXI summarises the results of biochemical parameters in diabetic IHD patients.

Table No.XIX indicates the results of glycemic control in diabetic IHD patients. Chronic hyperglycemia has been hypothesized to contribute to IHD, but the extent to which Glycosylated Hemoglobin level, a marker of long-term glycemic control is independently related to IHD risk is uncertain. Therefore Glycosylated Hemoglobin was monitored(274).

The results of this study suggest an elevated level of Glycosylated Hemoglobin with mean value of 8.26 ± 1.074% in diabetic IHD patients (Table XIX). These findings are well correlated with the earlier studies. In persons with newly diagnosed Diabetes, A.C. Dale et al. study shows that poor long term glucose control assessed by elevated Glycosylated Hemoglobin strongly increased the risk of IHD mortality(275). It has been reported that DM patients with prior myocardial infarction and plasma glucose <130 mg/dl showed lower mortality rate, than those with PG >150 mg/dl. 30 mg/dl decrease in PG, 30-40 mg/dl decrease in FG and 1.5% drop in Glycosylated Hemoglobin levels result in 10% decline in mortality rate(276).
All diabetic patients show significant rise in fasting and postmeal plasma glucose as compared to the control (Table XIX). Fasting plasma glucose was correlated significantly with cardiovascular complications (277). A continuous relationship between fasting plasma glucose and Glycosylated Hemoglobin and cardiovascular risk was reported by David R. Jesudason (278). Recent studies suggest that PPG is more strongly correlated with Glycosylated Hemoglobin than is the fasting glucose level and is an independent risk factor for development of cardiovascular complications (279). Thus all aspects of glucose metabolism appear to be clinically relevant and should be monitored for effective Diabetes management.

Table No. XX indicates the results of lipid profile in diabetic IHD patients. The aim of the study was to investigate correlation between carbohydrate metabolism imbalance (using Glycosylated Hemoglobin level as criteria) and blood lipid spectrum changes in diabetic IHD patients. Mean values of serum cholesterol, triglyceride and LDL-c were significantly higher (p<0.01) as compared to the control. HDL-c level was lower as compared to the control (Table XX). Glycosylated Hemoglobin, marker of dyslipidemia accelerates diabetic dyslipidemia development and progression. High level of Glycosylated Hemoglobin indicates atherogenic changes in blood lipid spectrum. Similar results were obtained by Skybchyte VA et al (280) and Glycosylated Hemoglobin was shown to be a marker of diabetic
dyslipidemia progression and complications in patients with acute myocardial infarction.

Increase Glycosylated Hemoglobin level and deteriorated blood lipid spectrum indices may be accompanied by more frequent development of the myocardial infarction complications as well as worse course and prognosis of the disease.

Table No.XXI indicates the results of renal function in diabetic IHD patients. The aim of the work was to find out the relationship of hyperglycemia with renal function in diabetic IHD patients.

Uncontrolled hyperglycemia as reflected by Glycosylated Hemoglobin level is associated with raised levels of serum creatinine (p<0.01) as compared to the control. No significant change in blood urea level was found when compared with the control group (Table XXI). Renal dysfunction predicts attenuation of ischemic heart disease mortality risk from elevated glucose which is in conformation to earlier report(281).
### Various Parameters in Diabetic Ischemic Heart Disease

#### Table – XIX

**Plasma Glucose and Glycosylated Hemoglobin**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>IHD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Plasma Glucose (mg/dl)</td>
<td>141.25±38.22*</td>
<td>89.57±9.31</td>
</tr>
<tr>
<td>Postmeal Plasma Glucose (mg/dl)</td>
<td>186.95±81.67*</td>
<td>129.2±10.6</td>
</tr>
<tr>
<td>Glycosylated Hemoglobin (%)</td>
<td>8.26±1.074*</td>
<td>4.6±0.82</td>
</tr>
</tbody>
</table>

#### Table – XX

**Serum Lipid Profile**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>IHD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>204.7±39.29*</td>
<td>169.4±15.8</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>38.85±10.51*</td>
<td>44.6±6.78</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>193.02±62.19*</td>
<td>139.85±10.0</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>128.88±33.62*</td>
<td>97.59±16.45</td>
</tr>
</tbody>
</table>

#### Table – XXI

**Kidney Function Test**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>IHD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>28.30±8.67</td>
<td>29.35±5.66</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.95±0.28*</td>
<td>0.74±0.225</td>
</tr>
</tbody>
</table>

All values are mean with standard deviation * P < 0.01
Coronary Artery Disease (CAD)

Coronary artery disease is a condition that can be brought on by Diabetes. This disease occurs when the arteries that supply blood to the muscles of the heart become hardened and very narrow. The hardening and thickening of the coronary arteries occur as a result of the build up of a material called atheromatous plaque formed due to lipid peroxidation. This condition is known as atherosclerosis. The coronary arteries of a diabetic or non-diabetic individual will become more and more narrowed as the build up of this plaque material increase. This process of atherosclerosis is directly proportional to chronic hyperglycemia. This condition will lead to a reduction in blood and oxygen flow to the heart muscle(282).

The most common cause of death in adults with Diabetes is coronary heart disease. The prevalence of CAD was increased with age and with duration of Diabetes. After 20 years of duration of Diabetes upto 29% of childhood-onset Type-I diabetic patients with nephropathy will have CAD(283).

The relative risk for CAD in diabetic subjects is greater than non-diabetics. The prevalence of serious CAD increase from 9% in subjects with normal glucose tolerance to 17% in those with impaired glucose tolerance and 20% in those with Diabetes(284).

The pathogenesis of CAD associated with Diabetes is not yet fully understood. However, because atherosclerotic macrovascular
complications occur at an earlier age and with greater severity in people with Diabetes, it is likely that its pathogenesis is directly influenced by the diabetic state.

Long term exposure to elevated glucose levels alone can contribute to the endothelial cell dysfunction observed in Diabetes(285). Increasing evidence suggest that endothelial dysfunction may play a central role in the development of atherosclerosis(286). Endothelial dysfunction is characterized by inhibited vasodilation, increased vascular smooth-muscle proliferation, increases thrombogenesis and proatherogenic cellular processes(287). Abnormal endothelium-dependent vasodilation also occurs in the microcirculation of diabetic patients, where it may contribute to ischemia and its sequelae(288). In addition to accelerated atherosclerosis, endothelial dysfunction has been linked with increased thrombosis, hypertension and dyslipidemia, all of which contribute to the pathogenesis of vascular disease in Diabetes(285).

Hyperglycemia might contribute to atherosclerosis in Diabetes in a number of other ways. For example, hyperglycemia causes glycosylation of proteins in a process that induces cross linking of collagen and other extracellular matrix proteins in the arterial wall(289,290). The end products of glycosylation modify LDL cholesterol, prolonging its half life and producing change in the artery
rendering it more susceptible to atherosclerosis(257). Among other proposed biochemical pathways in the pathogenesis of diabetic macrovascular disease are glucose-induced activation of Protein kinase C isoforms and increased intracellular oxidative stress(291).

Macrovascular disease results in morbidity and mortality in Diabetes mellitus to a large degree, especially coronary artery disease (CAD)(292).

Hyperglycemia is a well-established independent risk factor for CAD(293,294). Glycemic control as reflected by Glycosylated Hemoglobin is strongly associated with microvascular disease in individuals with Diabetes, but its relation to macrovascular disease and atherosclerosis is less clear(295).

From the Diabetes Control and Complication Trial (DCCT) and the United Kingdom Prospective Diabetes study it is clear that the degree of metabolic control influences the development of complications, particularly microvascular complications. A similar protective effect on macrovascular complication has not been demonstrated(296).

**Results and Discussion**

The present study was carried out to examine the relationship between Glycosylated Hemoglobin and traditional cardiovascular risk factors in individuals with Diabetes. The study consisted of 40 patients with a mean age of 45.53 ± 12.93 years and mean duration of
Diabetes 18.29 ± 3.58 years. A fasting blood sample was taken from all the patients for the estimation of biochemical parameters. The diagnosis of CAD was made by clinical symptoms of angina pectoris, electrocardiogram or documented myocardial infarction.

The results of the biochemical parameters have been shown in Table No.XXII, XXIII and XXIV.

**Table No.XXII indicates the results of the glycemic profile of the CAD patients.** Several recent studies have reported that glucose levels measured as Glycosylated Hemoglobin is a major determinant of future development of CAD among patients with non-insulin dependent Diabetes mellitus(297). The present study, therefore, examines the value of Glycosylated Hemoglobin concentration, a marker of blood glucose concentration in diabetic CAD patients.

Mean values of Glycosylated Hemoglobin (9.23 ± 1.845%) was significantly higher as compared to the control (Table XXII). Elevated Glycosylated Hemoglobin level may be an independent risk factor for CAD which is consistent with earlier reports(115,298). Both the studies also reported that elevated Glycosylated Hemoglobin is an independent risk factor for persons without Diabetes.

Fasting plasma glucose was increased in all diabetic patients (Table XXII) and correlated significantly with cardiovascular complications as also observed earlier(277). David R Jesudason
reported a continuous relationship between FPG and Glycosylated Hemoglobin and cardiovascular risk which correlates with the present study group(278). Postmeal plasma glucose was also significantly increased in all diabetic CAD patients. The recent results suggest that PPG is also important to overall glycemia control and may be a better index of glucose regulation than FPG(279).

Epidemiological studies published in recent years also suggest that postprandial blood glucose might be an independent risk factor of cardiovascular disease(299, 300). Therefore Glycosylated Hemoglobin testing should be added to the other established tests for increased cardiovascular risk, such as blood pressure, cholesterol level, etc.

Table No.XXIII indicates the results of circulating lipids in diabetic CAD patients. The objective was to examine correlation between carbohydrate and lipid metabolism in diabetic CAD patients. Hyperglycemia as reflected by Glycosylated Hemoglobin level was associated with significant increase in serum total cholesterol, triglyceride and LDL levels as compared to the control. No significant change in HDL-c was found as compared to the control (Table XXIII). Diabetologia Journal and Gran T et al also reported the same findings(301,302). He also found that significantly higher cardiovascular risk factors were found in persons with Glycosylated Hemoglobin of more than 6%, 6% may be a threshold value for the metabolic syndrome. Increased Glycosylated Hemoglobin level and atherogenic dyslipidemia predict future coronary heart disease risk.
Table No.XXIV indicates the results of renal function in diabetic CAD patients. Cardiovascular disease is the major cause of death among patients with ESRD. In the ESRD population, CAD mortality is estimated to be thirtyfold greater than among the general population. However, the increase risk of CAD is evident in patients with renal insufficiency (i.e. before the onset of ESRD)(303). The present study was therefore, carried out to evaluate the association between CAD risk markers (glycemia, Diabetes and serum lipids) with renal disease marker (serum creatinine concentration).

Poor glycemic control as reflected by Glycosylated Hemoglobin concentration was associated with increased levels of blood urea (40.975 ± 9.43 mg/dl) and serum creatinine (.89±0.232 mg/dl) (Table XXIV). A serum creatinine level of >1.7 mg/dl (indicative of renal disease) in individuals with hypertension may be an even stronger CAD risk factors than Diabetes, smoking, left ventricular hypertrophy, or systolic blood pressure as reported in American Heart Association Journal(303). Many workers reported earlier an association of CAD risk markers with renal disease markers(304). Renal impairment review reported that improving diabetic control delays the progression of renal disease, and the Glycosylated Hemoglobin should be kept below 7.5% if possible(305). The powerful association of renal disease markers with CAD risk factors confirm a strong link between renal and CAD disease in the early asymptomatic stages of each(306).
VARIOUS PARAMETERS IN DIABETIC CORONARY ARTERY DISEASE

TABLE – XXII

Plasma Glucose and Glycosylated Hemoglobin

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CAD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Plasma Glucose (mg/dl)</td>
<td>175.8±28.41*</td>
<td>89.57±9.31</td>
</tr>
<tr>
<td>Postmeal Plasma Glucose (mg/dl)</td>
<td>261.25±58.08*</td>
<td>129.2±10.6</td>
</tr>
<tr>
<td>Glycosylated Hemoglobin (%)</td>
<td>9.23±1.845*</td>
<td>4.6±0.82</td>
</tr>
</tbody>
</table>

TABLE – XXIII

Serum Lipid Profile

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CAD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>189.4±18.30*</td>
<td>169.4±15.8</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>39.67±7.13*</td>
<td>44.6±6.78</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>189.98±22.60*</td>
<td>139.85±10.0</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>147.86±45.97*</td>
<td>97.59±16.45</td>
</tr>
</tbody>
</table>

TABLE – XXIV

Kidney Function Test

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CAD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg /dl)</td>
<td>40.975±9.43*</td>
<td>29.35±5.66</td>
</tr>
<tr>
<td>Creatinine (mg /dl)</td>
<td>0.89±0.232*</td>
<td>0.74±0.225</td>
</tr>
</tbody>
</table>

All values are mean with standard deviation * P < 0.01
Peripheral Arterial Disease (PAD)

Peripheral arterial disease is a common cardiovascular complication in patients with Diabetes. PAD is a manifestation of atherosclerosis. It is characterized by atherosclerotic occlusive disease of the lower extremities. While PAD is a major risk factor for lower-extremity amputation, it is also accompanied by a high likelihood for symptomatic cardiovascular and cerebrovascular disease(307).

Data from the Framingham Heart Study revealed that 20% of symptomatic patients with PAD had Diabetes(308). As well as, it has been reported that of those with PAD, over one-half are asymptomatic or have atypical symptoms, about one-third have claudication and the remainder have more severe forms of the disease(309).

The most common symptom of PAD is intermittent claudication, defined as pain, cramping; or aching in the claves, thighs or buttocks that appears reproducibly with walking exercise and is relieved by rest. More extreme presentations of PAD include rest pain, tissue loss, or gangrene; these limb-threatening manifestations of PAD are collectively termed critical limb ischemia (CLI)(307).

PAD is also a major risk factor for lower extremity amputation, especially in patients with Diabetes. Moreover, even for a symptomatic patient, PAD is a marker for systemic vascular
disease involving coronary, cerebral and renal vessels, leading to an elevated risk of events, such as MI, stroke and death(307).

Close to 30% people over age 50 with Diabetes have peripheral arterial disease. As a result, the American Diabetes Association recommends that all people over age 50 with Diabetes undergo screening for peripheral arterial disease(310).

Among the pathological processes believed to be central in the development of atherosclerosis are biochemical modifications that affect the functional integrity of the LDL particles. Alterations in LDL include more dense smaller particles, glycosylated particles and oxidized and desialiated particles. These biochemical alterations of LDL particles increase the affinity of the endothelium towards the lipoprotein and increase its atherogenic potential in situ after uptake into the vessel wall.

Other lipoproteins i.e. Triglycerides and HDL also present in altered form in Diabetes, potentiating their atherogenic capacities. Triglyceride rich particles tend to have higher cholesterol content, and triglyceride remnant particles are proportionately increased, augmenting the triglyceride, associated contribution toward CAD. The HDL cholesterol subclass distribution trends toward the HDL particles and away from the cardioprotective HDL particles, thus potentially slowing the reversed cholesterol transport(297).
Results and Discussion

Peripheral vascular disease or peripheral arterial disease is the third most common manifestation of macrovascular disease in Diabetes. Chronic hyperglycemia as measured by Glycosylated Hemoglobin contributes to the development of atherosclerosis and subsequent macrovascular events, including peripheral arterial disease in persons with Diabetes(311). The present study was undertaken to assess the level of Glycosylated Hemoglobin in PAD and its correlation with other biochemical parameters. A total of 40 patients were evaluated for study. The mean age of the patient was 50.125 ± 18.79 years and mean duration 17.12±4.12 years.

Fasting blood sample was collected for analysis of Glycosylated Hemoglobin, lipid profile, blood glucose and renal function. Postmeal blood glucose was also recorded. Patients were diagnosed on the basis of anchal branchial index.

Table No.XXV, XXVI and XXVII summarises the results of biochemical parameters in PAD patients.

Table no. XXV show the results of glycemic control in PAD patients.

Chronic hyperglycemia, as measured by Glycosylated Hemoglobin is an established risk factor for Diabetes associated microvascular disease. Recent studies have also suggested that Glycosylated Hemoglobin may be associated with incident large vessel disease
(coronary heart disease, stroke and PAD) in persons with Diabetes(311). The present study thus carried out measurement of Glycosylated Hemoglobin in PAD patients. The results suggest that poor glycemic control, as indicated by elevated Glycosylated Hemoglobin level \((\text{Table XXV})\) in individuals with Diabetes be associated with an increased risk of PAD independently of other known risk factors. It has also shown that Glycosylated Hemoglobin levels predict risk of peripheral arterial disease(311).

Fasting and postmeal blood glucose was also monitored to assess the degree of glycemic control and as stated earlier they are independent risk factor for development of cardiovascular complications of Diabetes(277-279). Confirming earlier observations, fasting and postmeal plasma glucose shows positive correlation with Glycosylated Hemoglobin(54, 56).

**Table No.XXVI shows the results of lipid profile in diabetic peripheral arterial disease patients.** The aim was to investigate the relationship of traditional risk factor, dyslipidemia with non-traditional risk factor Glycosylated Hemoglobin in PAD patients.

**The serum levels of total cholesterol, triglyceride and LDL were significantly higher as compared to the control indicating dyslipidemic profile in PAD patients (Table XXVI).** This may be due to the probability of LDL to undergo lipid peroxidation in diabetic patients as a result of poor glycemic control. Dyslipidemic profile
characterized by increased triglyceride level, decreased apolipoprotein A level and small dense LDL is associated with uncomplicated PVD in both Type II and non-diabetic subjects have been reported (312).

The results suggest that in Diabetes mellitus patients all risk factor for cardiovascular disease should be controlled.

Table No.XXVII depicts the results of renal function in diabetic PAD patients. Patients with renal impairment have an increase risk of cardiovascular disease which may be the result of advance glycosylation endproducts. The present study thus investigates the association of renal impairment with traditional cardiovascular risk factors. Raised levels of Glycosylated Hemoglobin were associated with increase concentration of serum creatinine in diabetic PAD patients as compared to the control. No significant change in blood urea level was found as compared to the control (Table XXVII). Casper G.Schalkwislk et al. also shown that plasma levels of AGE peptide rise with renal impairment as determined by serum creatinine(79). In another study W.Mlekusch et al. reported that renal impairment as determined by serum creatinine predicts mortality in patients with PAD(313). Wattamakit et al. investigated an association between chronic kidney disease and peripheral arterial disease. These findings are correlated with present study group(314).

This suggests that impaired renal function exerts an unfavourable effect on patient’s outcome, independently of these cardiovascular and renal risk factors.
VARIOUS PARAMETERS IN DIABETIC PERIPHERAL ARTERIAL DISEASE

TABLE – XXV

Plasma Glucose and Glycosylated Hemoglobin

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PAD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Plasma Glucose (mg/dl)</td>
<td>141.5±29.18*</td>
<td>89.57±9.31</td>
</tr>
<tr>
<td>Postmeal Plasma Glucose (mg/dl)</td>
<td>248.47±58.01*</td>
<td>129.2±10.6</td>
</tr>
<tr>
<td>Glycosylated Hemoglobin (%)</td>
<td>9.50±1.86*</td>
<td>4.6±0.82</td>
</tr>
</tbody>
</table>

TABLE – XXVI

Serum Lipid Profile

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PAD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>208.3±44.77*</td>
<td>169.4±15.8</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>37.75±6.78*</td>
<td>44.6±6.78</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>188.77±52.47*</td>
<td>139.85±10.0</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>133.84±43.18*</td>
<td>97.59±16.45</td>
</tr>
</tbody>
</table>

TABLE – XXVII

Kidney Function Test

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PAD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>26.03±7.23</td>
<td>29.35±5.66</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.967±0.225*</td>
<td>0.74±0.225</td>
</tr>
</tbody>
</table>

All values are mean with standard deviation * P < 0.01
Cerebrovascular Disease

Damage to the blood vessels in the brain, resulting in a stroke. The blood vessels become blocked because of fat deposits or they become thick and hard, blocking the flow of blood to the brain. Sometimes, the blood vessels may burst, resulting in a hemorrhagic stroke. **People with Diabetes are at higher risk of cerebrovascular disease**.

Autopsy studies have shown that frequency and severity of cerebral atherosclerosis is twice more common in diabetics than in non-diabetics between the age of 50 to 70. The macroangiopathy of Diabetes is manifested as involvement of large vessels by atherosclerosis. The atheroma of Diabetes involves smaller arteries also. Ischemic strokes occur as a result of occlusion of the small paramedian penetrating arteries supplying the pons, thalamus and basal ganglia.

The frequency of cerebrovascular disease is reported to be 2-3 times higher in diabetics than in non-diabetics.

The atherothrombotic brain infections (ABI) were attributable to Diabetes. In other studies, cerebrovascular disease was reported as the underlying cause of death in 12-16% of all diabetic deaths. In Japan, where cerebrovascular disease is very common and CAD mortality relatively low, cerebrovascular disease is the leading cause of death among diabetics. The Whitehall study reported cerebrovascular
mortality to be two times higher in diabetic men than in non-diabetic men (212).

People with Diabetes have an increased risk of dying from a stroke, according to first time findings from a large community, based study reported in today’s rapid access issue of stroke (316). The death from cerebrovascular disease is higher in the patients with Diabetes than in the general population.

A number of mechanisms are responsible for ischemic stroke in diabetics –


2. Cardiac embolism. Diabetic cardiomyopathy, myocardial infarction.


Diabetes has higher chances of myocardial infarction and complications after it as compared to normal subjects. The presence of coronary artery disease in diabetics increases the prevalence of stroke. The presence of distinct diabetic cardiomyopathy leads to the development of intracardiac thrombi and cardiac arrhythmia that increase stroke risk. As many as 39% of the myocardial infarctions in
the diabetic subjects may be silent. Thus cerebral embolism and arrhythmias are more common in diabetics(212).

Epidemiological studies show that Diabetes is a risk factor for ischemic stroke. The pathogenesis of Diabetes-associated stroke appears to be linked to excessive glycosylation and oxidation, endothelial dysfunction, increased platelet aggregation, impaired fibrinolysis and insulin resistance(317).

Here, hyperglycemia induced formation of AGES is responsible for the pathogenesis of cerebrovascular disease.

**Hyperglycemia causes following effects:**

The first is that under the hypoxic conditions caused by a stroke, glucose is anaerobically metabolized to form lactic acid and the resultant cerebral intracellular and extracellular acidosis causes damage to neurons, glial tissue and vascular tissue.

Secondly, during ischemia, extracellular concentration of neurotransmitters, glutamate and aspartate increases. Glutamate causes stimulation of a nerve at a post receptor site and depolarization. In presence of hyperglycemia and hypoxia, glutamate levels rise which literally stimulate neurons to death.

Lastly, ischemia along with hyperglycemia and neuronal hyperstimulation increases intracellular calcium which also causes neuronal damage(212).
Results and Discussion

*Cerebrovascular disease* after coronary heart disease is the second leading cardiovascular cause of death among diabetics(315).

Individuals with Diabetes have a raised risk of stroke, but it is unclear whether sustained hyperglycemia contributes to the development of cerebrovascular disease. Glycosylated Hemoglobin, a measure of long term glycemia, is strongly related to retinopathy, nephropathy and neuropathy in Diabetes. The aim is to assess the level of Glycosylated Hemoglobin in diabetic cerebrovascular disease and its correlation with other biochemical parameters. Forty patients with cerebrovascular disease were included in the study. The mean age of the patient was $45.02 \pm 14.56$ years and mean duration of Diabetes was $9.57 \pm 4.0$ years.

Venous blood samples collected from all the subjects after at least 8 hours fasting for the analysis of Glycosylated Hemoglobin, blood glucose and lipid profile and renal function, whereas the blood samples from the non-fasted subjects were collected for the analysis of postmeal blood glucose. Patients were diagnosed on the basis of cerebral angiography.

Biochemical analysis of the diabetic cerebrovascular disease patients and control group is summarized in Table No.XXVIII, XXIX and XXX.
Table No.XXVIII shows the results of glycemic control in diabetic cerebrovascular disease patients.

Glycosylated Hemoglobin, fasting and postmeal blood glucose were determined in diabetic cerebrovascular disease patients to assess the severity of the disease and diabetic control of the patient. Patients had poor glycemic control as reflected by Glycosylated Hemoglobin concentration. The mean value of Glycosylated Hemoglobin 9.96±2.44% was significantly higher (p<0.01) in all diabetic patients as compared to the control (Table XXVIII). It has been reported that raised Glycosylated Hemoglobin could be an independent risk factor for stroke with similar relative risk as for coronary heart disease(318). In another study, J. Kuusisto et al. evaluated that non-insulin dependent Diabetes mellitus; its metabolic control and the duration of Diabetes are important predictors of stroke in elderly subjects(319).

Carlo Bruno Giorda et al. observed that age and previous stroke are the main predictors of stroke in Diabetes(320). The combined role of Glycosylated Hemoglobin, microvascular complications, low HDL cholesterol and treatment with insulin plus oral agents highlights the importance of diabetic history and clinical background in the development of stroke. These findings are well correlated with result of the present study.
Table No.XXIX shows the results of lipid profile in diabetic cerebrovascular disease patients. The aim of the study was to assess the correlation of hyperglycemia with cardiovascular risk factor, dyslipidemia in diabetic cerebrovascular patients.

Uncontrolled Diabetes as reflected by Glycosylated Hemoglobin was associated with significant increase in total cholesterol, triglycerides and LDL-c levels as compared to the control. The levels of HDL-c were reduced as compared to the control (Table XXIX).

Atherosclerosis is a primary cause of deaths in patients with Diabetes. The pathophysiology of development of atherosclerosis is complex and multifactorial(321). Diabetic dyslipidemia accounts for around 80% diabetic deaths due to cardiovascular complications. There is a growing body of evidence to show that hyperglycemia and dyslipidemia are connected with excess of cardiovascular risk(322-325).

A. Arsovska et al have also shown that Diabetes is a significant risk factor for stroke development(326). These patients also have lipid status and coagulation impairment. Hyperglycemia is associated with higher morbidity and mortality and is a bad prognostic sign.

This suggests that lipid abnormality as shown by hypertriglycerideremia, elevated levels of LDL and total cholesterol and reduced levels of HDL may be associated with twofold increase in risk of stroke.
Table No.XXX shows the results of lipid profile in diabetic cerebrovascular disease patients.

The major risk factor for stroke is hypertension. There is acceleration of the vascular complications of Diabetes in the presence of hypertension. In spite of 40% increased incidence of hypertension in the diabetic population, Diabetes and glucose intolerance are still independent risk factors for stroke(212).

The combined presence of hypertension and Diabetes concomitantly accelerates the decrease in renal function, the development of diabetic retinopathy and the development of cerebral diseases. This is evident by the increase in levels of blood urea and serum creatinine. The mean values of blood urea and serum creatinine were significantly on the higher side (p<0.01) as compared to the control (Table XXX and Figure 30). The improving diabetic control delays the progression of renal disease and the Glycosylated Hemoglobin should be kept below 7.5% if possible.
VARIOUS PARAMETERS IN DIABETIC CEREBROVASCULAR DISEASE

TABLE – XXVIII
Plasma Glucose and Glycosylated Hemoglobin

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cerebrovascular Disease</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Plasma Glucose (mg/dl)</td>
<td>177.025±26.08*</td>
<td>89.57±9.31</td>
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<tr>
<td>Postmeal Plasma Glucose (mg/dl)</td>
<td>294.15±77.09*</td>
<td>129.2±10.6</td>
</tr>
<tr>
<td>Glycosylated Hemoglobin (%)</td>
<td>9.96±2.44*</td>
<td>4.6±0.82</td>
</tr>
</tbody>
</table>

TABLE – XXIX
Serum Lipid Profile

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cerebrovascular Disease</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>200.675±44.89*</td>
<td>169.4±15.8</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>41.5±10.35*</td>
<td>44.6±6.78</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>202.92±50.66*</td>
<td>139.85±10.0</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>118.44±40.13*</td>
<td>97.59±16.45</td>
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</tbody>
</table>

TABLE – XXX
Kidney Function Test

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cerebrovascular Disease</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg /dl)</td>
<td>149.825±54.86*</td>
<td>29.35±5.66</td>
</tr>
<tr>
<td>Creatinine (mg /dl)</td>
<td>1.30±0.523*</td>
<td>0.74±0.225</td>
</tr>
</tbody>
</table>

All values are mean with ±standard deviation  * P < 0.01
Sensitivity of erythrocytes to peroxide hemolysis in diabetic patients having micro and macrovascular complications

Chronic elevation of plasma glucose causes many complications in Diabetes mellitus. People with Diabetes mellitus develop characteristic microvascular complications such as retinopathy, nephropathy and neuropathy. There is also an increased risk of Macrovascular complications such as cardiovasculopathy, cerebrovasculopathy and peripheral vasculopathy(10). A variety of hematological abnormalities are seen in Diabetes. These include increased erythrocyte aggregation, decreased deformability of erythrocytes, increased platelet aggregation and adhesion predisposed to sluggish circulation, endothelial damage and focal capillary occlusion(327). De novo oxidative damage, a result of increased protein glycosylation could participate in the mechanism, whereby diabetic erythrocytes may acquire membrane abnormalities(328). Enhanced glycosylation by elevated glucose concentration may induce the formation of oxygen derived free radicals through protein glycosylation, which releases early and late glycosylation end products, contributing to enhancement of oxidative stress(329). Both protein glycosylation and protein oxidation are biochemical alterations occurring in diabetics(330). Under physiological conditions, autooxidation of glucose leads to hydrogen peroxide, reactive oxygen species and reactive ketoaldehydes, which modify the cellular proteins
leading to their fragmentation by free radical mechanism. This protein fragmentation is inhibited by antioxidants confirming that tissue damage associated with Diabetes has an oxidative origin(331). Evidence has accumulated indicating that the generation of reactive oxygen species (oxidative stress) plays an important role in the etiology of diabetic complications. This hypothesis is supported by evidence that many biochemical pathways strictly associated with hyperglycemia (glucose auto-oxidation, polyol pathway, prostanoid synthesis, protein glycosylation) can increase production of free radicals. Diabetes causes dyslipidemia and increases susceptibility to lipid peroxidation(332).

**Results and Discussion**

The present study was undertaken to evaluate sensitivity of erythrocytes to peroxide hemolysis and its correlation with glycemic control. Forty patients of Diabetes (mean age 44.56 ± 17.03 years) with various complications were selected for the study and sensitivity of erythrocyte to peroxide hemolysis was determined by the method as given in Chapter II. The blood sample was collected in EDTA tube for analysis.

As shown in Table XXXI the sensitivity of erythrocyte to peroxide hemolysis in diabetic patients was significantly higher (p<0.01) as compared to the control and shows statistically significant correlation with glycemic control. Previous studies have also shown
association of lipid peroxide and glucose concentration, which may be also thought to play a role in increased lipid peroxidation in Diabetes mellitus. Jyoti M. Sawant et al. reported association of poor glycemic control with increased lipid peroxidation and reduced antioxidant vitamin status in diabetic neuropathy(226). S.K. Jain et al. studied membrane lipid peroxidation in erythrocytes of diabetic subjects and its possible relationship with hyperglycemia(333). There was a significantly increased membrane lipid peroxidation in diabetic erythrocytes compared with non-diabetic erythrocytes.

The degree of membrane lipid peroxidative damage in erythrocytes was significantly correlated with the level of glycosylated hemoglobin, an index of mean glucose level for the preceding 3-4 month. This suggests that peroxidation of membrane lipids occurs in erythrocytes, of diabetic patients. Sushil K. Jain documented an increased membrane lipid peroxidation in erythrocytes treated with high levels of glucose(334, 335). These studies showed that glucose-induced lipid peroxidation in erythrocytes was blocked with fluoride, an inhibitor of glucose metabolism; with vitamin-E, an antioxidant, with parachlorom ercuribenzoate and metyrapone, inhibitors of the cytochrome P-450 system; and with dimethylfurane, diphenylamine and thiourea, scavengers of oxygen radicals. These studies suggest that increased glucose oxidation leads to increased levels of glucose metabolites such as NADPH, which stimulates the cytochrome P-450-like activity of hemoglobin on erythrocytes or of microsomes in various
tissues, resulting in an increased production of oxygen radicals leading to cellular lipid peroxidation(335).

Another cause of the increased lipid peroxidation in the hyperglycemic condition is that the generations of free radicals inhibit the activity of superoxide dismutase enzyme leading to accumulation of superoxide radicals which accelerate the lipid peroxidation, which may lead to damage of the erythrocyte membrane resulting in their hemolysis(336).

**TABLE – XXXI**

**Percent sensitivity of erythrocytes to lipid peroxide hemolysis in Complications of Diabetes**

<table>
<thead>
<tr>
<th>Group</th>
<th>Sensitivity%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.6 ± 0.82</td>
</tr>
<tr>
<td>Diabetes</td>
<td>19.65 ± 12.25*</td>
</tr>
</tbody>
</table>

All values are mean with ± standard deviation * P < 0.01
The findings of the present study strongly support the observations that the measurement of Glycosylated Hemoglobin not only shows promise of being a successful approach to the monitoring of diabetic patient but also provides a conceptual framework for the pathogenesis of microvascular as well as macrovascular complications of Diabetes. The findings of the present study also observed that Glycosylated Hemoglobin is not only a marker of glycemic control but also a marker of dyslipidemia and renal dysfunction in microvascular as well as macrovascular complications of Diabetes.

As the percent sensitivity of erythrocytes to lipid peroxide hemolysis in Diabetes is increased, assessment of this parameter will be helpful in diabetic patients with hematologic abnormalities.