CHAPTER – IV

GLYCOSYLATED HEMOGLOBIN IN

CHRONIC RENAL FAILURE (CRF)

Introduction:

Chronic kidney disease (CKD) is a global threat to health in general and for developing countries in particular, because therapy is expensive and life-long(98). The growing interest in CKD has shed light on the striking incidence of CVD comorbidity that is characteristic of all stages of this illness(337). Insulin resistance is one of the important factors in the causation of CVD(338).

Definition of CKD:

1. Kidney damage for more than 3 months, as defined by structural or functional abnormalities of the kidney with or without decreased GFR, manifest by either:
   a) Pathological abnormalities, or
   b) Markers of kidney damage, including abnormalities in the composition of blood or urine, or abnormalities in imaging tests.

2. GFR less than 60 ml/min/1.73 m² for 3 months or more, with or without kidney damage(339).
Incidence and Prevalence of CKD

The data from the most recent studies suggest that the prevalence of chronic renal failure in India is nearly 0.8% and among the various etiologies, Diabetes has emerged as the most frequent cause (30-40%) followed by hypertension (14-22%)(98).

Clinical Consequences:

An increase in atherogenesis and other cardiac diseases is encountered in CKD patients. Several factors may be responsible for this phenomenon(340). Insulin resistance and the compensatory Hyperinsulinemia might contribute to the development of cardiovascular complications in CKD(341). Postprandial hyperglycemia in the glucose-intolerant uremic patient may, by itself, be risk factors for atherosclerotic cardiovascular disease(100).

Hyperinsulinemia and insulin resistant state may be associated with hypertension, which is a well-recognized risk factor for cardiac disease in CKD(342,343).

Patients with CKD suffer from a secondary form of complex dyslipidemia. The most important abnormalities are an increase in serum triglyceride levels (elevated VLDL-remnants/IDL), small LDL particles and a low HDL cholesterol level. The highly atherogenic LDL subclass, namely LDL-6 or small dense LDL, accumulates preferentially in CKD(344). The contribution of this complex and atherogenic form of dyslipidemia to cardiovascular disease in patients
with CKD is at present unclear(345). *Insulin is an important regulator of lipoprotein lipase activity; this enzyme plays a major role in triglyceride removal. Insulin deficiency or resistance to its action is associated with reduced availability and hence activity of this enzyme and this defect is the primary cause of hypertriglyceridemia in these patients* (346).

Other studies have shown that excess parathyroid hormone (PTH) plays a significant role in the genesis of hypertriglyceridemia of CKD(347). It is possible that the excess PTH exerts its effects by a direct action on the metabolism of both hepatic and lipoprotein lipases and/or through an indirect effect on insulin secretion.

As insulin is an anabolic hormone, it promotes amino acid uptake by skeletal muscle and inhibits protein degradation. Resistance to insulin action in patients with CKD may contribute to protein catabolism in these patients(346).

Insulin is an important regulator of extrarenal disposition of potassium. Since insulin secretion by pancreatic islets in CKD is markedly reduced, this abnormality could contribute to the impairment in the extrarenal disposal of potassium loads in CKD and may contribute to the appearance of hyperkalemia(348). In addition excess PTH in CKD may also interfere with extrarenal disposal of potassium loads. It is possible that this is linked with the impairment in insulin secretion in CKD(346).
Various factors causing abnormal Glycosylated Hemoglobin level in chronic renal failure not secondary to Diabetes mellitus are:

- Chronic renal failure itself.
- Carbonyl stress.
- Impaired glucose metabolism.
- Secondary hyperparathyroidism.
- Presence of glucose in the dialyzed fluid.
- Role of uraemic acidosis.
- Formation of carbamylated hemoglobin.
- Shortened lifespan of erythrocytes.
- Role of lipid peroxidases.

**Chronic renal failure itself:**

Vandana Menon *et al.* studied the relationship between Glycosylated Hemoglobin and mortality in patients with nondiabetic CKD(45). *They concluded that Glycosylated Hemoglobin is an independent predictor of all cause mortality in non-diabetic CKD.*

De Boer *et al.* studied Glycosylated Hemoglobin in CKD. The level of the Glycosylated Hemoglobin was measured in (i) subjects with normal renal function, (ii) patients with CKD and (iii) patients on intermittent hemodialysis(349). *It was concluded that CKD itself causes an increase in Glycosylated Hemoglobin.*
Kumari K et al. carried out in vivo and in vitro studies to evaluate the clinical application of Glycosylated Hemoglobin plasma proteins in the diagnosis and management of Diabetes mellitus(350). Glycosylated Hemoglobin result in 80% fall in diabetic patients following controlled glycemia for two months, while glycosylated plasma proteins registered an 80% fall in the patients after fifteen days of blood glucose homoeostasis. Human serum proteins glycosylated and glycosylation was linearly proportional to the glucose concentration and albumin and transport proteins that are significantly glycosylated. 

*Glycosylated Hemoglobin and plasma protein levels were elevated in chronic renal failure patients.*

Lantz et al. studied minor hemoglobin fractions in CKD and in diabetic patients(351). Glycosylated Hemoglobin was slightly but significantly elevated in the uremic, non diabetic patients who were not submitted to periodic hemodialysis. It returned, in hemodialyzed patients, to a level not significantly different from the control value.

Sabater J et al. studied nonenzymatic glycosylation of hemoglobin and total plasmatic proteins in end-stage renal disease(352). The levels of Glycosylated Hemoglobin, and total plasmatic glycosylated proteins (PGP) were studied. HbA1 and Glycosylated Hemoglobin fraction were also determined. They concluded that there is an abnormal nonenzymatic glycosylation of proteins in CKD.
Agarwal et al. studied urinary and serum non-enzymatic glycosylated proteins in Diabetes mellitus and renal disorders (353). The mean Glycosylated Hemoglobin in nondiabetic CKD group was found to be significantly increased.

**Carbonyl stress**

*Advanced glycosylation end products (AGES), formed during Maillard or browning reactions by non-enzymatic glycosylation and oxidation (glycooxidation) of proteins are responsible for the pathogenesis of uraemia (354).* AGES such as Pentosidine and carboxymethyl lysine, are markedly elevated in both plasma proteins and skin collagen of uraemic patients. The increased chemical modification of proteins is not limited to AGES because increased levels of advanced lipooxidation end products, such as malondialdehyde lysine, are also detected in plasma proteins in uremia. Uremia may be described as a state of carbonyl overload or “carbonyl stress” resulting from either increased oxidation of carbohydrates and lipids by oxidative and non-oxidative chemistry.

Richard Bucala et al. studied diabetic and renally impaired patients with or without Diabetes and have found high circulating levels of protein bound advanced glycosylation end products (AGES)(257).
Schalwijk CG et al. observed plasma levels of AGE peptides in diabetic patients are associated with serum creatinine and not with albumin excretion rate (possible role of AGE peptide-associated endothelial dysfunction)(79). AGE peptides were increased approximately fivefold in patients with end-stage renal disease.

**Impaired glucose metabolism**

A multitude of abnormalities in carbohydrate metabolism are encountered in CKD. Patients with CKD almost always display resistance to the peripheral action of insulin(355). The normal response of beta cells to the presence of insulin resistance is to enhance their secretion of insulin. If for any reason, the beta cells are unable to augment their secretion of insulin appropriately, an impaired glucose tolerance would ensure. The increase in the blood levels of insulin in response to hyperglycemia in uremic patients may be decreased, normal, or increased(356). These variations may reflect differences in insulin secretion and or in its metabolic clearance. Indeed, glucose intolerance is encountered in CKD patients, who have both resistance to insulin action and impaired insulin secretion by beta cells(357).

Oimomi et al. investigated whether or not the elevation of Glycosylated Hemoglobin values in patients with CKD was related to impaired glucose tolerance(358). Mean Glycosylated Hemoglobin in the CKD group was significantly more than in the controls.
Rufino et al. studied the presence of carbohydrate metabolism anomalies, in a population of non diabetic patients with CKD, by means of the OGTT(359). They concluded that fasting plasma glucose did not predict OGTT results in patients with CKD and the OGTT can be very useful tool to identify states of prediabetes and Diabetes in patients with CKD, specially in those with an elevated pulse pressure, age greater than 65 years, hyperlipidaemia and Glycosylated Hemoglobin above 5.2%.

Dimitrakov et al. studied the disturbances in carbohydrate metabolism in pre-dialysis patients with CKD(360). In patients with first degree CKD the level of Glycosylated Hemoglobin was 5.9±0.5%. In patients with second and third degree CKD there was a trend towards higher Glycosylated Hemoglobin levels 6.3±0.6%, as compared with the controls (5.5±0.4%).

**Secondary hyperparathyroidism**

Several studies indicate that the impairment of insulin secretion in uremia is mediated, in major part, by the state of secondary hyperparathyroidism that is commonly encountered in CKD(361). Excess PTH in CKD interferes with the ability of beta cells to augment insulin secretion appropriately in response to the insulin-resistant state.

The severity of secondary hyperparathyroidism varies greatly among patients with CKD. It is possible that those with mild secondary
hyperparathyroidism have appropriate response in insulin secretion during hyperglycemia as dictated by the insulin-resistant state and hence normal glucose metabolism. In contrast, impaired insulin secretion and glucose intolerance will be present in those with moderate to marked hyperparathyroidism. This postulate would provide an explanation for the variability in the presence of glucose intolerance among CKD patients.

Available data indicate that the chronic excess of PTH in the presence or absence of CKD causes a sustained rise in basal levels of cytosolic calcium (Ca\(^{2+}\)) of pancreatic islets and this abnormality plays an important role in the impaired insulin secretion\(^{(362)}\).

**Presence of glucose in the dialyzed fluid:**

Hirszel P et al. reported that Glycosylated Hemoglobin test was used to evaluate the role of dialysate glucose in the development of carbohydrate intolerance and hyperlipidemia in chronic hemodialysis patients and chronic peritoneal dialysis patients\(^{(193)}\). Glycosylated Hemoglobin levels were significantly elevated in all groups of patients. HbAl levels were not ameliorated with 8 weeks of glucose free hemodialysis. There was no correlation between HbAl and serum glucose, triglyceride, or cholesterol. Thus, HbAl elevation cannot be explained solely by glucose reabsorption from the dialysate.
Role of uremic Acidosis:

De Marchi S et al. have shown that major role is played by uremic acidosis in the increased HbA1 fraction in chronic renal failure(363).

Mak R.H stated that metabolic acidosis is frequent in uremia but not in hemodialysis patients(364). Treatment of metabolic acidosis increases insulin sensitivity and insulin secretion. The presence of a highly significant correlation between HbA1 and arterial blood PH and between HbA1 and plasma bicarbonate indicated a major role for acidosis in increasing the HbA1 levels in uremic patients on long term hemodialysis.

Formation of carbamylated hemoglobin:

Some studies indicate that the apparent Glycosylated Hemoglobin in uremia is due to the increased formation of carbamylated hemoglobin. The nonenzymatic formation of carbamylated hemoglobin in uremia has several similarities to Glycosylated Hemoglobin in patients with Diabetes mellitus. However specific affinity chromatography methods rather than ion-exchange chromatography free from interference by carbamylated hemoglobin detect HbA1 specifically and have found elevated levels in CRF patients.

Assessment of glycemias in uremic patients by measurement of hemoglobin glycosylation is not permitted by either chromatographic
or chemical means. However, measurement of hemoglobin carbamylation (i.e., valine hydantoin content) permits assessment of the uremic state in a way analogous to the use of hemoglobin glycosylation to monitor glycemia.

Kwan JT et al. observed that Carbamylated hemoglobin arises from the non-enzymatic modification of hemoglobin monomers by isocyanate derived from the spontaneous dissociation of urea(191). Carbamylated hemoglobin levels were found to be raised in uraemic subjects, but were independent of age, sex, and glycemic state and hemodialysis procedure. The formation of carbamylated and Glycosylated Hemoglobin was studied by the use of cyanate and glucose respectively(365). The formation of glycosylated hemoglobin is more favourable than that of carbamylated hemoglobin. The effect of glucose in reducing the formation of carbamylated hemoglobin is greater than the effect of cyanate on the formation of Glycosylated Hemoglobin.

Chachou A et al. studied influence of in vivo hemoglobin carbamylation of Glycosylated Hemoglobin measurements by various methods(366). Their results show that Hb adducts whether due to carbamylation or to other chemical reactions, interfer a variable extent with different HbA1 assay methods and confirm that Glycosylated Hemoglobin values should be interpreted with caution in uremic patients.
Data from Smith WG et al. study strongly suggests that the apparent elevation of chromatographically determined glycosylated hemoglobin in uraemia is due to the increased formation of carbamylated hemoglobin (367). However, in patients with Diabetes mellitus, independent of renal function, both the chromatographic and colorimetric methods of determining glycosylated hemoglobin are equally valuable and reliable. Carbamylated hemoglobin may have a clinical role as a marker of uraemia and may also have a pathophysiological relevance.

Glycosylated Hemoglobin is present in increased amounts in patients with Diabetes mellitus as a consequence of the elevated blood glucose (127). HbA1 is elevated in uremia because of the presence of nonglycosylated hemoglobin which is detected by column chromatography but not by a chemical method specific for the detection of glycosylation. The increased HbA1 level in uremia correlates with the level of BUN and results at least in part from the carbamylation of hemoglobin by urea - derived cyanate.

**Shortened lifespan of erythrocytes:**

Some studies suggest that there is a direct linear relationship between hemoglobin concentration and HbA1 level (195). Due to the shortened lifespan of erythrocytes, Glycosylated Hemoglobin level decreases in CRF patients. The primary effect of CRF on Glycosylated Hemoglobin is that of a reduction due to shortened red blood cell
survival. The superimposition of hyperglycemia on CRF could lead to a normal or raised Glycosylated Hemoglobin.

**Role of lipid peroxidases:**

N. Selvaraj *et al.* evaluated the relationship between Glycosylated hemoglobin and lipid peroxidation in non-diabetic CKD patients(368). They found that the percentage of HbA1 concentrations and plasma malondialdehyde (MDA) were significantly increased in CKD patients compared to the control subjects and MDA was found to be a significant determinant of HbA1 in patients with CKD.

**Results and Discussion**

Abnormalities of carbohydrate metabolism in CKD not related to preexisting Diabetes mellitus have been recognized since the mid 20th century(132). Until recently, this subject has been the purview of purely experimental studies. However, the recent recognition of the importance of asymptomatic abnormalities in glucose metabolism in the pathogenesis of cardiovascular disease has made this subject a focus of intense clinical investigation, especially in view of the high CVD mortality in CKD(100).

Glycosylated Hemoglobin is an important marker of glucose metabolism. Until recently, the elevated Glycosylated Hemoglobin level in nondiabetic CKD was considered unreliable, owing in major part to the confounding effect of carbmylated hemoglobin. However, recent
advances in clinical chemistry have improved the reliability of this marker in this group of patients.

The aim of the present study was to evaluate Glycosylated Hemoglobin level in chronic renal failure patients and its correlation with other biochemical parameters. A total number of 40 patients who were on conservative treatment with chronic kidney disease (as defined by NKFK/DOQI Guidelines, 2005) were selected for the study. The mean age of the patients was 45.95±16.73 years. Patients having Diabetes mellitus, family history of Diabetes mellitus, patients on maintenance hemodialysis, patients having anemia or hemoglobinopathies were excluded from the study.

Table No.I, II and III summarises the results of biochemical parameters in chronic renal failure patients.

Table No.I shows the results of glycemic control in chronic renal failure patients. The levels of Glycosylated hemoglobin ranged from 3.9% to 8.5%. The mean value of Glycosylated hemoglobin in CRF patients was 6.00 ±1.16% which is significantly higher as compared to the control (Table I).

The mean Glycosylated hemoglobin level in the present study is comparable with that of previous other studies(45,349,351,353,358-360).

The level of fasting plasma glucose in the patient shows statistically significant difference as compared to the control (Table I).
The mean fasting plasma glucose among the subjects was 105.62 ± 44.77 mg/dl which is comparable with the studies of Menon et al. and Rufino et al. (45, 359). There was a positive correlation of Glycosylated Hemoglobin with fasting plasma glucose which is similar to earlier reports of David E Bruns et al and Nuttall et al. who have shown that Glycosylated hemoglobin better correlates with other indices of glycemic control when measured by affinity chromatography method (369, 370, 371). In contrast de Boer et al. have shown that there was no correlation between Glycosylated hemoglobin and fasting blood glucose (349).

The levels of postmeal plasma glucose in subjects show a significant rise as compared to the control (Table I). Glycosylated hemoglobin correlated significantly with postprandial blood glucose levels as reported by Paisey R et al. confirming the results of present study (372).

**Table No.II shows the results of lipid profile in chronic renal failure patients.** Although increased levels of glycosylated hemoglobin and lipid concentrations have been shown in diabetic nephropathy patients, their impairment is not clear in both groups with impaired glucose tolerance without apparent Diabetes mellitus (373). Therefore, serum lipid profile in chronic renal failure patients is determined.

The levels of serum total cholesterol in the patients were slightly elevated as compared to the control (Table II). Serum triglyceride and
serum LDL-C levels were also slightly elevated as compared to the control. HDL-C level was significantly decreased as compared to the control (Table II). A positive correlation was found between Glycosylated Hemoglobin and serum cholesterol and triglyceride, indicating that Glycosylated Hemoglobin resembles blood cholesterol in continuous relation as a risk factor for cardiovascular disease. Argani et al. also studied the relationship of impaired glucose tolerance with the other atherosclerotic risk factors, fasting blood sugar and the standard two hour glucose tolerance test, serum triglyceride and serum cholesterol. They concluded that although fasting blood sugar is normal in non-diabetic renal transplant and hemodialysis patients, impaired glucose tolerance was accompanied by other atherosclerotic risk factors such as hyperlipidemia in hemodialysis patients (with hyper-triglyceridemia) and renal transplant recipients (with hypercholesterolemia)(373). Menon et al. concluded that Glycosylated Hemoglobin is an independent predictor of all-cause mortality in nondiabetic CKD(45).

**Table No.III shows the results of renal function test in chronic renal failure patients.** The levels of blood urea and serum creatinine in the subjects were significantly higher as compared to the control (TableIII). The mean value of serum creatinine was 4.69±2.4mg/dl. Patients with renal impairment have an increased risk of cardiovascular disease was reported(79).
Schalwijk CG et al. also observed that plasma levels of AGE peptides in diabetic patients are associated with serum creatinine and not with albumin excretion rate(79). S. kumar et al studied HbA1 in cases of CKD of various etiologies. In patients with more severe renal insufficiency HbA1 levels were significantly increased(374).

Impaired Fasting Glucose and elevated Glycosylated Hemoglobin observed in the present study could stratify patients for aggressive monitoring and reduction of other cardiovascular risk factors. Hyperglycemia may be a potential therapeutic target to reduce CAD risk in this patient population.
### VARIOUS PARAMETERS IN CHRONIC RENAL FAILURE

#### TABLE – I

Plasma Glucose and Glycosylated Hemoglobin

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<th>Parameters</th>
<th>CRF</th>
<th>Control</th>
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<tr>
<td>Fasting Plasma Glucose (mg/dl)</td>
<td>105.62±44.77**</td>
<td>89.57±9.31</td>
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<tr>
<td>Postmeal Plasma Glucose (mg/dl)</td>
<td>149.65±68.41**</td>
<td>129.2±10.6</td>
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<td>Glycosylated Hemoglobin (%)</td>
<td>6.00±1.16*</td>
<td>4.6±0.82</td>
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#### TABLE – II

Serum Lipid Profile

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<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>173.73±41.38</td>
<td>169.4±15.8</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>39.43±16.54**</td>
<td>44.6±6.78</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>143.89±61.4</td>
<td>139.85±10.0</td>
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<tr>
<td>LDL (mg/dl)</td>
<td>108.6±38.37</td>
<td>97.59±16.45</td>
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#### TABLE – III

Kidney Function Test

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<th>Parameters</th>
<th>CRF</th>
<th>Control</th>
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<tbody>
<tr>
<td>Urea (mg /dl)</td>
<td>120.44±52.36*</td>
<td>29.35±5.66</td>
</tr>
<tr>
<td>Creatinine (mg /dl)</td>
<td>4.69±2.4*</td>
<td>0.74±0.225</td>
</tr>
</tbody>
</table>

All values are mean with ±standard deviation  

*P < 0.01  **P < 0.05