CHAPTER - I

General Introduction
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GENERAL INTRODUCTION

Although rapid Urbanization and industrialization lead to economic prosperity and better living standards for some, the process also resulted in increased in lifestyle related diseases like Diabetes, hypertension, obesity, dyslipidemia and cancer(1).

Among mentioned lifestyle related diseases, Diabetes mellitus (DM) is very commonly occurring group of metabolic diseases characterized by hyperglycemia and altered metabolism of carbohydrates, proteins and lipids(1). Chronic hyperglycemia in Diabetes is due to total or subtotal deficiency of insulin or insulin resistance and is associated with long term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessel(2). Diabetic patients usually present with varied symptoms like polyuria, polydipsia and polyphagia. A wide spread pathological change is thickening of capillary basement membrane, increase in vessel wall matrix and cellular proliferation resulting in vascular complications like lumen narrowing, early atherosclerosis, and sclerosis of glomerular capillaries, retinopathy, neuropathy and peripheral vascular insufficiency in long term Diabetes(3).

The word Diabetes comes from the Greek Language meaning to ‘pass through’ or “flow through” and a Latin word mellitus meaning “Sweet”. Hence it is description of what is happening, a fluid
containing sugar passes through the body of a person suffering from Diabetes mellitus\(4\). Due to sweetness of urine charaka and shushruta named it as “\textit{Madhumeha}” (rain of honey).

Diabetes mellitus is associated with increased oxidative stress due to hyperglycemia\(5\). This oxidative stress gives rise to microvascular and macrovascular complications of Diabetes\(6\).

In 1969 German medical student Paul Langerhans point out the role of islet cells in diabetes. Frederick G. Banting and Charles H Best (1921) isolated insulin clinically effective\(7,8\). Reference to disorders with polyuria is found in Egyption papyrus (1500 B.C), Shushruta (400 B.C) and Charaka (6 A.D)\(9\).

\textbf{PREVALENCE:}

According to World Health Organization the prevalence of Diabetes worldwide is 180 million and it will reach to 300 million in 2025. The prevalence of Diabetes in India is 20.9 million and these numbers are expected to increase to 57.2 million by the year 2025 i.e. one sixth of the world’s total population. The revised figures are 80.9 million by year 2030. Every 5\textsuperscript{th} diabetic in the world is an Indian and every 5\textsuperscript{th} and 10\textsuperscript{th} Indian in a metro city like Mumbai is diabetic\(4,9\). The factors for this steep rise include genetic predisposition, urbanization, ethnicity, insulin resistance and central obesity. Hyperglycemia contributes to the increased incidence of \textbf{macrovascular disease} but dyslipidemia and hypertension play a
major role in development of atherosclerosis as complication(7, 8). Distribution of different types of Diabetes in a western setup is well documented by Yeolekar et al(10).

**CLASSIFICATION:**

An International Expert Committee working under the sponsorship of the American Diabetes Association published new classification(10,11).

1. **Type I Diabetes:** (beta cell destruction usually leading to absolute insulin deficiency).

2. **Type II Diabetes:** (many range from predominantly secretary defect with or without insulin resistance).

3. **Other specific Types**
   
   A. Genetic defects of beta cell function(12)
   
   B. Genetic defects of insulin action.
   
   C. Diseases of exocrine pancreas
   
   D. Endocrinopathies
   
   E. Drug / Chemical induced(13)
   
   F. Infections
   
   G. Immune Mediated Diabetes (uncommon forms)
   
   H. Other genetic syndromes
   
   I. Nutrition(14,15).

       - Malnutrition related Diabetes
       - Gestational Diabetes
The term *insulin dependent Diabetes mellitus (IDDM)* and *non-insulin dependent Diabetes mellitus (NIDDM)* are replaced by *Type-I* and *Type-II Diabetes* retained with Arabic numerals respectively.

**DIAGNOSTIC CRITERIA**

In 1997, an International expert committee recommended diagnostic criteria for Diabetes (Impaired Fasting Glucose/Impaired Glucose Tolerance)(16). The use of a fasting plasma Glucose (FPG) test for the diagnosis of Diabetes was recommended and the cut point separating Diabetes from non Diabetes was considered as FPG >126 mg/dl. Oral Glucose Tolerance Test (OGTT) should be done after at least three days of unrestricted diet (>150 gm carbohydrate daily). It should be preceded by 10 to 16 hour of fasting. After collection of fasting blood sample, 75 gm of glucose should be dissolved in 250 to 300 ml. of water and given orally. After 2 hour postmeal blood sample collected(17).

**WHO criteria for diagnosis of Metabolic Syndrome**

<table>
<thead>
<tr>
<th></th>
<th><strong>Abdominal obesity</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>&gt; 102 cm. (waist circumference)</td>
</tr>
<tr>
<td>Women</td>
<td>&gt; 88 cm.(waist circumference)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th><strong>Triglycerides &gt; 150 mg/dl.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>&lt; 40 mg /dl.</td>
</tr>
<tr>
<td>Women</td>
<td>&lt; 50 mg /dl.</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th></th>
<th><strong>HDL – cholesterol.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th><strong>Others</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure</td>
<td>$\geq 130/86$ mm Hg.</td>
</tr>
<tr>
<td>Fasting Glucose(11).</td>
<td>$\geq 110$ mg/dl.</td>
</tr>
</tbody>
</table>
## Diagnosing Diabetes

<table>
<thead>
<tr>
<th>Stage</th>
<th>Fasting Plasma Glucose test (FPG) (Preferred*) mmol/l-mg/dl</th>
<th>Casual Plasma Glucose test mmol/l-mg/dl</th>
<th>Oral Glucose Tolerance Test (OGTT) mmol/l-mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diabetes</strong></td>
<td>FPG greater than or equal to 7.0/126**</td>
<td>Casual plasma glucose greater than or equal to 11.1/200 plus symptoms***</td>
<td>Two-hour plasma glucose (2hPG) greater than or equal to 11.1/200****</td>
</tr>
<tr>
<td><strong>Impaired Glucose Homeostasis</strong></td>
<td>Impaired Fasting Glucose (IFG)= FPG Greater than or equal to 5.6/100 and less than 7.0/126</td>
<td></td>
<td>Impaired Glucose Tolerance (IGT) =2hPG greater than or equal to 7.8/140 (mmol/mg%) and less than 11.1/200 (mmol/mg%)</td>
</tr>
<tr>
<td><strong>Normal</strong></td>
<td>FPG less than 5.6/100</td>
<td>2hPG less than 7.8/140</td>
<td></td>
</tr>
</tbody>
</table>

* The FPG is the preferred test for diagnosis, although any of the three is acceptable. In the absence of unequivocal hyperglycemia with acute metabolic decompensation, one of these three tests should be used on a different day to confirm the diagnosis.

** Fasting is defined as no caloric intake for at least eight hours.

*** Casual is defined as any time of day without regard to time since last meal, are the classic ones of polyuria, polydipsia, and unexplained weight loss.

****OGTT should be performed using a 75-gm glucose load. The OGTT is not recommended for routine clinical use (From the Boston Beaconc)(16).
**Glycemic index of the food and blood glucose level:**

Different dietary carbohydrates and even starches from different sources are not handled identically during ingestion. Some are assimilated at a slower rate(18). Glycemic index is an indicator of the effect of food items on post-prandial blood glucose level. If the value is taken as 100 for glucose, the corresponding values for some food items are as follows: Potato 85%, rice 75%, sucrose 55%, corn 55% and fructose 25%.

The blood glucose fluctuates from minute to minute, hour to hour and day to day(19). However, peaks of glucose appear with each meal and are associated with complications of Diabetes and increased postmeal blood glucose level also depends upon the glycemic index of the food. Higher the glycemic index more will be the blood glucose level and vice versa. Further, it is a tedious job to measure blood glucose levels hour-to-hour and day-to-day. Blood level of Glycosylated Hemoglobin (HbA1c) shows the long-term glycemic control of the patient of last 3 months i.e. lifespan of RBCS and thus serves as the best tool in assessment of blood glucose level(19).

Glycosylated Hemoglobin (total) is total HbA1c, which includes (HbA1a, HbA1b, and HbA1c fractions). World Health Organization and American Diabetes Association (ADA) recommend Glycosylated Hemoglobin level as the best guideline for long-term glycemic control. Higher levels of Glycosylated Hemoglobin are associated with
microvascular and macrovascular and acute metabolic complications of Diabetes.

Glycosylated Hemoglobin is altered by many physiological and pathological states apart from Diabetes namely pregnancy, chronic renal failure, iron deficiency anemia, ageing, hemolytic anemia, hemochromatosis, cancer to mention a few of them(20).

By considering the importance of Glycosylated Hemoglobin in Diabetes, the present study was planned to evaluate the biochemical correlation of Glycosylated Hemoglobin and other blood/serum parameters (Lipid profile, kidney function test, and sensitivity of erythrocytes to peroxide hemolysis) in diabetic patients having microvascular and macrovascular complications.

The experiments were also carried out in other disorders such as chronic renal failure and iron deficiency anemia to study correlation of Glycosylated Hemoglobin level and other parameters in these disorders.

This chapter describes the pathophysiology of Diabetes mellitus and other disorders under study. An account of Glycosylated Hemoglobin and other biochemical parameters and their correlation with Diabetes and the disorders under study is being reviewed with appropriate references.
Assessment of glycemic control

A. Self monitoring of blood glucose (SMBG)

B. Glycosylated Hemoglobin.

By performing Glycosylated Hemoglobin test, health providers can measure a patient’s average glycemia over the preceding 2-3 months and thus, assess treatment efficacy. Glycosylated Hemoglobin testing should be performed routinely in all patients with Diabetes, first to document the degree of glycemic control at initial assessment and then as part of continuing care.

**Glycemic control** is best judged by the combination of the results of the patients SMBG testing and the current Glycosylated Hemoglobin.

<table>
<thead>
<tr>
<th>Glycosylated Hemoglobin(%)</th>
<th>Mean plasma glucose</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>mg/dl.</td>
</tr>
<tr>
<td>6</td>
<td>135</td>
</tr>
<tr>
<td>7</td>
<td>170</td>
</tr>
<tr>
<td>8</td>
<td>205</td>
</tr>
<tr>
<td>9</td>
<td>240</td>
</tr>
<tr>
<td>10</td>
<td>275</td>
</tr>
<tr>
<td>11</td>
<td>310</td>
</tr>
<tr>
<td>12</td>
<td>345</td>
</tr>
</tbody>
</table>
The correlation between Glycosylated Hemoglobin levels and mean plasma glucose levels based on data from the Diabetes control and complications Trial as shown in the above table.

- A lower Glycosylated Hemoglobin is associated with a lower risk of myocardial infarction and cardiovascular death.
- Aggressive glycemic management with insulin may reduce morbidity in patients with severe acute illness, perioperatively, following myocardial infarction and in pregnancy(21,22).

**CLINICAL FEATURES:**

The manifestation of symptomatic Diabetes varies from patient to patient(23,24). Most often symptoms are due to **hyperglycemia like polyuria, polydipsia and polyphagia** but the first event may be an acute metabolic decomposition resulting in **diabetic coma**. Occasionally the initial expression is a degenerative complication such as neuropathy and nephropathy. In the absence of symptomatic hyperglycemias the metabolic derangement of Diabetes due to absolute or relative deficiency of insulin and relative or absolute excess of glucagons.

**Unusual manifestations of Diabetes:**

2. Deafness.
3. Refractory or recurrent infections.
4. Paresthesia, dysthesia, nocturnal cramps.

5. Oculomotor palsy, facial palsy.

6. Anginal chest pain, intermittent claudication.

7. Nonhealing ulcer.

8. Drowsiness, abdominal pain.

**Distinguishing features of Type I and II Diabetes**

<table>
<thead>
<tr>
<th>Character</th>
<th>Type I Diabetes</th>
<th>Type II Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene Locus</td>
<td>Chromosome 6</td>
<td>Unknown</td>
</tr>
<tr>
<td>Age of onset</td>
<td>&lt; 40 years</td>
<td>&gt;40 years</td>
</tr>
<tr>
<td>Body habit</td>
<td>Normal / Wasted</td>
<td>Obese</td>
</tr>
<tr>
<td>Plasma insulin</td>
<td>Low/absent</td>
<td>Normal/high</td>
</tr>
<tr>
<td>Plasma Glucagons</td>
<td>High gap suppressible</td>
<td>High resistant</td>
</tr>
<tr>
<td>Acute complications</td>
<td>Ketoacidosis</td>
<td>Hyperosmolar nonketotic coma</td>
</tr>
<tr>
<td>Insulin therapy</td>
<td>Responsive</td>
<td>Responsive to resistant.</td>
</tr>
<tr>
<td>Response to Sulfonylurea</td>
<td>Unresponsive</td>
<td>Responsive(8).</td>
</tr>
</tbody>
</table>

**Type I Diabetes:**

About 1 to 2% of diabetic patient in India are affected from this Type. It is more severe form of Diabetes. Type-I Diabetes is manifested when functioning beta cells fall below 10% of normal(23). Management of Type I Diabetes include: Diet, Exercise(25,26).
**Type II Diabetes:**

It is major health problem affecting 10 crore million people worldwide. Its pathogenesis is multifactorial involving both genetic (e.g. impaired insulin secretion) and acquired (e.g. insulin resistance) factors, which cause over production and inefficient utilization of glucose(23). There are various modalities of treatment available for management of Type II Diabetes mellitus like diet, exercise and oral hypoglycemic agents(26).

**Insulin receptors:**

**Insulin receptors** are heterotetrameric glycoprotein present on cell membrane. It consists of two **135 - Kda alpha subunits**, and two **95 - Kda beta subunits**. Alpha subunits are present extracellularly and beta Subunits is transmembrane units. Alpha subunit has insulin – binding sites. Beta subunit has **tyrosine kinase activity**. When insulin binds to alpha subunit there occur internalization of receptor. It stimulates tyrosine kinase activity. It causes autophosphorylation of beta subunit and formation of insulin substrate 1 and 2. It stimulates phosphorylation and dephosphorylation reactions(27). The **Ras oncoprotein** is one of the most potent mitogen. Ras has been linked to the insulin action pathway, because it is known to activate the cascade of **mitogen activated protein kinases and MAP kinases** are among the many that insulin is known to activate(24).
Glucose Level Sensing Mechanism of Pancreas:

**Glucose** enters the beta cells by facilitated diffusion via glucose transporters. This glucose is metabolized by **beta cells**, which increases **ATP/ADP ratio**. So **ATP dependent k⁺** channel in the cell membrane closes. It causes depolarization of cell membrane. When depolarization reaches threshold value, voltage dependent **ca²⁺** channels open. It causes influx of calcium into the cell. This elevated cytoplasmic calcium cause exocytosis of insulin granules from beta cells(16).
Physiological actions of Insulin:

Insulin stimulates the storage of glucose in the liver as a glycogen and in adipose tissue as triglycerides and in storage of amino acids in muscles as proteins; it also promotes utilization of glucose in muscle for energy. The bold arrows indicate these pathways, which also are enhanced by feeding. Insulin inhibits the breakdown of triglyceride, glycogen and proteins and the conversion of amino acid to glucose (gluconeogenesis) as indicated by hollow arrows. These pathways are increased during the fasting and in diabetic state. The toxic effects of hyperglycemia may be the result of accumulation of non-enzymatically glycosylated products and osmotically active sugar alcohols such as sorbitol in tissues; the effect of glucose on cellular metabolism also may be responsible(28).
GLYCOSYLATED HEMOGLOBIN

Many proteins, particularly those secreted by cell or bound to cell surface are modified by the addition of carbohydrates. **This process of addition of sugar is called glycosylation.** Addition of glucose moieties to protein occurs spontaneously in proportion to the blood glucose concentration. Glycosylation normally takes place in a controlled environment regulated by enzymes. However, sugars can also bind nonenzymatically to proteins under physiologic conditions. This phenomenon is readily observed in the red cell but also takes place in many other tissues. After glycosylation, the cell gets phagocytosed and the hemoglobin gets degenerated.

Of the several pathogenic mechanisms by which hyperglycemia may lead to altered tissue structure and function, is **Non-enzymatic glycosylation of proteins.** Non-enzymatic glycosylation was first identified in 1912 and is known as the Maillard or browning reaction due to the associated yellow brown color when formed endogenously, this reaction is driven forward by hyperglycemia.

Glycosylated Hemoglobin is the first and best-studied example of non-enzymatic glycosylation of protein. It is used as a role model for the glycosylation of other proteins such as lens crystallins, collagen, serum albumin and protein in red cell membrane. Its discovery opened new and still growing avenues of research on Maillard reactions in biological systems including the concept of
advanced glycosylation/ lipooxidation end products (AGES/AIES) and the development of diabetic complications and various diseases associated with aging(33).

Hemoglobin is the molecule in our red blood cells, which carries oxygen around the body(19). This hemoglobin is one of many proteins that undergo nonenzymatic glycosylation. Glycosylated Hemoglobin is a general term for hemoglobin non-enzymatically glycosylated by Glucose(34).

Biochemistry of Glycosylated Hemoglobin:

Hemoglobin in the normal adult consists of HbAO (90%), HbAl (5.8%), HbA2 (2-5%) and HbF (<1%). HbAO, HbA2 and HbF differ from one another in the amino acid sequence of their non-alpha globin chains(31). The synthesis of these minor components is controlled by delta, gamma-chain genes(35). Glycosylation of HbA whereby glucose or other sugars are nonenzymatically bound to the N-terminal valine of the B-chain of HbAO results in the formation of HbA1(31). HbA1 more negatively charged than HbAO may be detected by cation-exchange chromatography and includes HbA1a, HbA1b and HbA1c(34). HbA1a, HbA1b and HbA1c comprise approximately 1.6, .8 and 4% of the total hemoglobin of adult human erythrocytes. HbA1c is the most abundant and most extensively studied of the species of HbA1(36).
Linkage to the N-terminus of the B-chain is of most practical importance since this gives rise to the altered physical properties that are exploited in assays. In particular, chromatographic mobility is increased, hence the name “fast hemoglobin’s” by which these compounds are commonly known(37).

**Structure and nomenclature of hemoglobin Variants**

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure and nomenclature of hemoglobin Variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-glycosylated (“native”) hemoglobins</td>
<td></td>
</tr>
<tr>
<td>HbA(HbAo)</td>
<td>$\alpha_2 \beta_2$</td>
</tr>
<tr>
<td>HbA2</td>
<td>$\alpha_2 \delta_2$</td>
</tr>
<tr>
<td>Hbf</td>
<td>$\alpha_2 \gamma_2$</td>
</tr>
<tr>
<td>Glycosylated derivatives not specifically named</td>
<td>$\alpha$-val-1-deoxyfructose$_2$$\beta_2$</td>
</tr>
<tr>
<td></td>
<td>$(\alpha$ (lys-glucose)$_n\beta_2$</td>
</tr>
<tr>
<td></td>
<td>$(\alpha_2$ (lys-glucose)$_n\beta_2$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Glycosylated Hemoglobin</th>
<th>Often named</th>
<th>Structure and nomenclature of hemoglobin Variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1a1</td>
<td>$(\beta$-val-1-fructose diphosphate)$_2$</td>
<td>$&lt;1%$</td>
</tr>
<tr>
<td>HbA1a2</td>
<td>$(\beta$-val-glucose 6-P)$_2$</td>
<td>$&lt;1%$</td>
</tr>
<tr>
<td>HbA1b</td>
<td>$\alpha_2 (\beta$-val-1-deoxyfructose)$_2$</td>
<td>$&lt;1%$</td>
</tr>
<tr>
<td>Glycosylated as HbA1c</td>
<td>$\alpha_2 (\beta$-val-1-deoxyfructose)$_2$</td>
<td>$4%$</td>
</tr>
</tbody>
</table>
The formation of Glycosylated Hemoglobin is a two-step process. D-Glucose in its aldehyde form reacts rapidly and reversibly with the N-terminal valine residue of the B-chain to form an aldimine compound, a schiff base intermediate (or "pre-HbA1c").

This labile intermediate can either undergo an irreversible but slow, Amadori rearrangement to form a stable HbA1c with a Ketoamine linkage, or it can dissociate back to D-Glucose and HbAO(36).
The reaction, which takes place in circulating erythrocytes (RBCS), hemolysates and hemoglobin solutions, is almost irreversible and is dependent on the concentration of reactants (39).
(i) **The concentration of glucose that the hemoglobin is exposed to:** Not all cell types in the body require insulin for the uptake of glucose. Red blood cells have “insulin – independent” glucose transporters on their surface. Therefore if glucose levels in the plasma are high, then glucose levels inside the red blood cells will also be high. The higher the blood glucose level, therefore, the more glycosylation of hemoglobin will occur.

(ii) **The length of time that the hemoglobin is exposed to a given concentration of glucose.** The longer the blood glucose level is high, the more glycosylation will occur.

The average lifespan of a red blood cell is about 120 days. **The hemoglobin is continually being glycosylated at a rate, which is proportional to the prevailing blood glucose level.** At any given time, there will be a mixture of old and new red blood cells circulating in the blood stream; old cells will have been exposed to recent and not so recent blood glucose levels; new cells will only have been exposed to recent blood glucose levels. **So the more recent glycemia will have the largest influence on the overall Glycosylated Hemoglobin reading.** Red blood cells in patients with hemolytic anemia have a short lifetime and they are therefore subject to less glycosylation(19).

Not only plasma glucose concentration and erythrocyte lifespan but also **permeability of erythrocytes to glucose, the degree of oxygenation of erythrocytes, and the concentration of 2-3**
Diphosphoglycerate in them can influence the rate of glycosylation of hemoglobin (36, 40).

The biosynthesis of this Glycosylated Hemoglobin was studied in vitro suggesting that the formation of Glycosylated Hemoglobin is a posttranslational modification. The formation of Glycosylated Hemoglobin in vivo was determined and the results indicate that Glycosylated Hemoglobin is slowly formed during the 120-day life span of the erythrocyte, probably by a non-enzymatic process (35).

The formation of Glycosylated Hemoglobin is conveniently expressed by a simple equation:

\[
\text{Glucose + Hemoglobin } \xrightleftharpoons{K_1}{K_2} \text{Labile intermediate } \xrightarrow{K_3} \text{HbA1c}
\]

in which \( K_1, K_2 \) and \( K_3 \) are the reaction rate constants (37).

The presence of Glycosylated Hemoglobin was first shown in 1950; it was later separated into different fractions by the technique of cation-exchange chromatography and shown to be present in increased amounts in blood from patients with Diabetes mellitus (20).

Structural studies by Holmquist and Schroeder, Bookchin and Gallop in 1968 and Bunn et al. subsequently reported that the largest of these minor fractions, designated HbA1c, had a glucose moiety linked to the N-terminal valine of the B-globin chain (41).

Rahbar first noted an unusual hemoglobin fraction in hemolysates prepared from the blood of certain diabetic subjects on
agar gel electrophoresis at pH 6.2 in citrate buffers. Later it was found to be the same as Glycosylated Hemoglobin(42).

**Clinical usefulness of Glycosylated Hemoglobin:**

Glycosylated Hemoglobin (HbA1c) assay, is the most widely accepted laboratory test for the measurement of glycemic control and is recommended for routine use in the management of patients with Diabetes mellitus. Although the lifespan of erythrocytes is approximately 120 days, Glycosylated Hemoglobin levels, represented a ‘weighted’ average of blood glucose levels, with youngest red blood cells, reflecting mean glucose levels over the past month, contributing to a greater extent than older ones(43).

The final and crucial observation, which led to the use of assays of abnormal hemoglobin as a method of assessing diabetic control, was the demonstration by Koening et al. that Glycosylated Hemoglobin concentration was proportional to fasting blood glucose concentration and glucose tolerance. Furthermore, they showed that Glycosylated Hemoglobin concentration fell when diabetic control was improved by treatment(37).

The importance of Glycosylated Hemoglobin as an index of diabetic control was reinforced by the Diabetes control and complications trial (DCCT). This study demonstrated a direct correlation between glycemic control as indicated by Glycosylated
Hemoglobin and the likelihood of developing long-term Diabetes related complications.

Glycosylated Hemoglobin is independent of the sugar level. Although reading for good control is same for both(19).

Because the formation of stable Glycosylated Hemoglobin is an essentially irreversible reaction, the steady state concentration of Glycosylated Hemoglobin reflects a balance between the rate of its formation and its removal (i.e. the lifespan of the erythrocyte). Thus, subnormal concentration of Glycosylated Hemoglobin can often be seen in patients with shortened erythrocyte survival – e.g. hemolytic anemia(36).

**Usefulness of Glycosylated Hemoglobin test:**

1) Glycosylated Hemoglobin is an important marker of glycemic control in patient with Diabetes(44).

2) Measurement of Glycosylated Hemoglobin does not require fasting glucose load, easy to perform and does not require any special preparation(45).

3) Glycosylated Hemoglobin provides a more comprehensive picture of glycemic status and is more indicative of chronic hyperglycemia than single plasma glucose measurement(45).
4) Glycosylated Hemoglobin reflects both fasting and postprandial hyperglycemia. Hence it is a better prognostic marker than fasting hyperglycemia(45).

5) Glycosylated Hemoglobin is not subjected to the wide variations that are inherent in single measure of blood glucose(45).

6) In subjects with IFG, Glycosylated Hemoglobin is better than glucose to evaluate Diabetes risk, and it could be used to select subjects for intensive early intervention(46).

7) It is correlated with FPG and 2-h plasma glucose(46).

8) Glycosylated Hemoglobin is more reproducible than FPG and within subject coefficients of variation are 1.7 and 5.7% respectively(46).

9) It is done on the spot in some hospital clinics(19).

10) Glycosylated Hemoglobin test can help confirm self-testing results or blood tests results by the doctor(47).

11) Judge whether a treatment plan is working(47).

12) Show you how healthy choices can make a difference in Diabetes control(47).

13) It is a good resource to use along with your daily blood sugar checks, to work for the best possible control(47).
**Glycosylated Hemoglobin and Diabetes:**

Clinical studies supporting the concept that Glycosylated Hemoglobin levels reflect long-term glucose control are of two types(40). The first type involves an assessment of the correlation of various parameters of glucose control with Glycosylated Hemoglobin measurement. The second type is the longitudinal study in which diabetic patients who are clearly in poor control are brought under good control with careful therapy. The time lapse that occurs between attainment of control and normalization of Glycosylated Hemoglobin values in such studies support the concept that a single Glycosylated Hemoglobin measurement reflects time averaged blood glucose concentration over preceding several weeks.

Huisman and Dozy in 1962 showed association between Glycosylated Hemoglobin fraction and Diabetes for the first time(48). They reported a two to three fold increase in HbA1 in diabetic patients.

Trivelli et al. in 1971 confirmed these earlier observations. They observed mean value of Glycosylated Hemoglobin in normal adults 6.5% and in diabetic adults mean Glycosylated Hemoglobin value was 11.1%(42).

Later on Paulsen et al. in 1976 confirmed these findings in pediatric IDDM patients. HbA1 levels in control children were found to be 6.9±1.2% and in diabetic children, it was 13.7±2.4%(49).
Fitzgibbon et al. in 1976 observed that amount of HbA1a+b and HbA1c are higher in old RBCs as compared to young RBCs in both normal and diabetic subjects(41). They concluded that formation of Glycosylated Hemoglobin is a continuous process throughout the cell life. Davis et al.(40), Cole et al(50), Simon et al(51), Bouriotis et al(52) and many other workers also reported similar findings.

Compagnucci et al. showed that measurement of stable Glycosylated Hemoglobin is particularly useful for longitudinal control of unstable diabetics(53).

Peterson et al. confirmed that Glycosylated Hemoglobin assay provide a useful means of showing the degree of control of glucose metabolism in diabetic patients(54).

Skelton CW et al. observed that Glycosylated Hemoglobin levels are a reflection of a patient’s average blood sugar for the two preceding months(55). Knowledge of Glycosylated Hemoglobin is helpful in the management of Diabetes and hence in diabetic complications. The use of Glycosylated Hemoglobin should motivate patients with Diabetes to maintain higher degrees of control and help doctors in their treatment plans.

**Glycosylated Hemoglobin and fasting blood glucose:**

Glycosylated Hemoglobin was 6.3% in normal glucose tolerance (NGT), 7.3% in impaired glucose tolerance (IGT) and 8.11% in Type II
and was significantly correlated to fasting and post-prandial plasma glucose in Type II group(56).

Verrillo A et al. found that a significant correlation was observed between Glycosylated Hemoglobin values and fasting blood glucose(57). **These results provide evidence that Glycosylated Hemoglobin levels are influenced by slightly reduced carbohydrate tolerance. Glycosylated Hemoglobin may be a useful test to improve the specificity of the oral glucose load to select and to follow up subjects with impaired glucose tolerance.**

Glycosylated Hemoglobin could offer several practical advantages over the OGTT for assessing glucose metabolism(58). Of these subjects with IGT, a significantly greater percentage of subjects with elevated Glycosylated Hemoglobin at baseline showed worsening for Diabetes than those with normal Glycosylated Hemoglobin. The Glycosylated Hemoglobin level has correlation with fasting blood glucose in Type II. Thus, in subjects with IGT Glycosylated Hemoglobin may be a useful predictor of progression to Diabetes.

Mannucci et al. stated that use of Glycosylated Hemoglobin alone or combined with FPG has been suggested for the screening of Diabetes and impaired glucose tolerance (IGT)(59).

There was also a slight inverse correlation between Glycosylated Hemoglobin and uricemia(60). **No correlation resulted with total lipemia, serum triglyceride levels, serum total cholesterol levels**
and HDL cholesterol concentrations. HbA1 measurement is a simple and objective test of metabolic control in diabetic subjects and can serve as a valuable adjunct to blood glucose determinations in epidemiological studies. Further the correlation between urinary glucose and HbA1 or fasting blood glucose were highly significant(61).

Koenig et al. in 1976 studied correlation between Glycosylated Hemoglobin, fasting blood sugar and response to oral glucose tolerance test(62). A significant correlation was also found between fasting blood sugar and Glycosylated Hemoglobin levels in diabetics. This suggests that Glycosylated Hemoglobin is a better measure of overall degree of glucose intolerance as compared to fasting blood glucose.

Koenig et al. (NEJM) in 1976 in his another study estimated fasting glucose and Glycosylated Hemoglobin levels before and after diabetic control(54). Before control of Diabetes mean fasting blood sugar was 343 mg% (range 280 – 450 mg %) and Glycosylated Hemoglobin level 9.8% (range 6.8 to 12.1%). During optimal diabetic control blood sugar concentration was 84 mg% (range 70-100 mg %) and Glycosylated Hemoglobin concentration 5.8% (range 4.2 to 7.6%). This concludes that Glycosylated Hemoglobin concentration appears to reflect mean blood sugar concentration best over previous weeks to months.
Gonen et al. in 1977 also observed significant correlation between Glycosylated Hemoglobin and mean fasting glucose(63). They concluded that Glycosylated Hemoglobin measurement is simple, rapid and objective procedure to assess diabetic control and serves as screening test for uncontrolled Diabetes and as an indicator of the efficacy of various therapeutic regimes.

In another study, HbA1 levels showed a high correlation with fasting and 2 hours postmeal glucose levels(64). This study showed that HbA1 levels are bimodally distributed in population with high prevalence of Diabetes and may be used to identify diabetic and non diabetic subgroups.

Lev Ran et al. in 1979 also studied correlation between Glycosylated Hemoglobin concentration and various points of glucose tolerance curve in 167 patients who were divided into four groups - normal GTT, abnormal GTT with normoglycemia, abnormal GTT with hyperglycemia and known mild diabetics(39). They concluded that the Glycosylated Hemoglobin levels that distinguish between latent and overt Diabetes correlated strongly with values of fasting plasma glucose and glucose tolerance and reflected changes in control of Diabetes.

Chandaliya et al. in 1980 estimated Glycosylated Hemoglobin, fasting and 2-hour postprandial blood glucose(65). They found significant correlation between Glycosylated Hemoglobin with the
mean of fasting and 2 hours postprandial sugar values obtained over a period of 2 months preceding Glycosylated Hemoglobin measurement.

Goldstein *et al.* in 1982 observed that closer the Glycosylated Hemoglobin level is to the normal range, lower the mean plasma glucose value(66).

In 1979, criteria for the diagnosis of Diabetes were selected based on levels of glycemia on the oral glucose tolerance test (OGTT) that were associated with the subsequent development of retinopathy(67). Since, then, five long-term studies have demonstrated that when Glycosylated Hemoglobin levels are maintained below 7% (normal 6%), development of retinopathy and microalbuminuria is practically nil. This article offers an alternative approach to diagnosis using both FPG and Glycosylated Hemoglobin values.

A study was performed to determine the relationships between FPG and 2-hour PPG levels over the normal Glycosylated Hemoglobin range and to assess the need to control FPG and 2-hour PPG levels to achieve Glycosylated Hemoglobin targets recommended by the American Diabetes Association, International Diabetes Federation, and American college of Endocrinology (ACE)(68). **Most individuals with Glycosylated Hemoglobin values between 6.0% and 7% have normal FPG levels but abnormal 2-hour PPG levels, suggesting that an upper limit of normal for FPG at 110 mg/dl is too high**
and that attempts to lower Glycosylated Hemoglobin in these individuals will require treatment preferentially directed at lowering postprandial glucose levels.

Study were performed in diabetic patients, indicating that FPG, PPG and especially mean plasma Glucose (MPG) concentrations defined by the average of multiple measurements of glucose taken throughout the day, are highly correlated with Glycosylated Hemoglobin(69).

EnRico Cagliero et al. described that the Glycosylated Hemoglobin assay provides the most objective and reliable information about long-term glucose control in diabetic patients(70). It is widely used to guide hypoglycemic therapy and has been demonstrated to be effective in identifying patients with unacceptably poor glycemia control and to facilitate their improvement. The Glycosylated Hemoglobin assay can be useful to verify the accuracy of self-glucose monitoring and provides essential information for patients who do not self monitor their glucose. For these reasons, routine determinations of Glycosylated Hemoglobin values have become an essential component of the standard of care for diabetic patients. Clinical trials such as the Diabetes control and complications Trial and the U.K. Prospective Diabetes study have established specific Glycosylated Hemoglobin goals that result in substantial reductions in long term complications.
PM Hall et al. determined total and stable glycosylated hemoglobins and glycosylated plasma proteins in 53 patients referred for a glucose tolerance test(71). Significant correlations were found with fasting blood glucose, 2 - hour glucose and area under the glucose tolerance curve. The measurement of Glycosylated Hemoglobins and glycosylated plasma proteins by the simple, precise, affinity- chromatography method is potentially a quick, accurate and simple screening test for patients with DM and IGT and deserves consideration as criteria for their diagnosis.

**Correlation of Glycosylated Hemoglobin with urinary glucose**

Gabby et al. in 1977 studied the correlation of total Glycosylated Hemoglobin levels to the antecedent urinary glucose excretion(72). The Glycosylated Hemoglobin levels significantly correlated with the amount of glucose excreted in 24 hours urinary collection obtained immediately before one month, 2 months, and 3 months prior to blood sampling. Highest correlation coefficient was found between Glycosylated Hemoglobin levels and amount of glucose excreted in urine collections obtained 2 months previously. **These data suggest that Glycosylated Hemoglobin levels reflect blood glucose levels over previous few months and hence represent a potentially vulnerable index of long term blood glucose control.**

MC Donald et al., have also shown a significant correlation between Glycosylated Hemoglobin and urinary glucose(40).
Glycosylated Hemoglobin and Lipid profile:

Gabby et al. in 1977 studied relationship of blood glucose control to plasma cholesterol levels(72). They found a significant correlation between plasma cholesterol levels and levels of Glycosylated Hemoglobin on it blood samples. The correlation coefficient was higher for the females than the male diabetic patients. These findings suggested that long term hyperglycemia is associated with hypercholesterolemia and it is suggested that Glycosylated Hemoglobin measurement is good index of long term blood glucose levels in diabetic patients.

SoSenko et al. in 1978 studied relationship of Glycosylated Hemoglobin with cholesterol in Type I Diabetes mellitus(73). They showed that the plasma cholesterol a known risk factor of atherosclerosis was positively correlated with degree of hyperglycemia and correlation coefficient between Glycosylated Hemoglobin and plasma cholesterol was significant.

Mc Donald et al. have reported that Glycosylated Hemoglobin levels significantly correlates with serum cholesterol and triglyceride levels, the known risk factors for atherosclerotic disease(40).

Dorchy H et al. have shown that the measurement of Glycosylated Hemoglobin gives an “objective” estimate of the degree of metabolic control of Diabetes during the erythrocyte lifespan(74). Glycosylated Hemoglobin levels parallel the clinical evaluation of the
degree of control. HbAl concentrations are correlated with the duration of Diabetes, triglyceridemia and glycemia.

H Aleyassine et al. monitored the levels of Glycosylated Hemoglobin, fasting plasma glucose (FPG), urine and HDL – cholesterol in several hundred patients attending an adult diabetic clinic(75). A weak but positive correlation was found between Glycosylated Hemoglobin and serum triglycerides and cholesterol.

Odetti P et al., obtained positive correlation between Glycosylated Hemoglobin and triglycerides and between HbA1 and mean diurnal plasma glucose (MDPG)(76). There was a negative correlation between HbA1 and high-density lipoproteins (HDL) cholesterol. No significant correlation was found between HbA1 and either uric acid or total cholesterol. Correlations were also found between MDPG and triglycerides, MDPG and HDL cholesterol, triglycerides and HDL - cholesterol, uric acid and HDL – cholesterol.

**Glycosylated Hemoglobin and Renal function:**

Masao Kanauchi et al. evaluated serum levels of AGEs in diabetic nephropathy(77). In renal biopsy specimens from diabetic subjects with a normal renal function, the severity of glomerular lesions was assessed morphometrically. The serum AGE concentrations increased with the severity of glomerular lesions in DM. The serum AGE levels measured by the new enzyme-linked immunosorbent assay reflected the severity of glomerulopathy and
therefore, it may be a clinically useful tool for assessing diabetic renal complications.

Manouchehr Nakhjavani et al. studied Albuminuria and its correlates in an Iranian diabetic population and found that albuminuria was associated with dyslipidemia (Low-HDL-C), long duration of Diabetes, and uncontrolled glycemia revealed by higher Glycosylated Hemoglobin(78).

Casper G. Schalkwijk et al. in their study have shown that plasma levels of AGE peptides in diabetic patients are associated with serum creatinine and not with albumin excretion rate(79). Patients with renal impairment have an increased risk for cardiovascular disease, which may be the result of advanced glycosylation end products (AGES). AGE peptides were increased approximately fivefold in patients with end stage renal disease, without difference between patients with or without Diabetes.

Frank Stam et al. evaluated that patients with end-stage renal disease as well as mild renal impairment have an increased risk for the development of cardiovascular disease(80). It has been suggested that advanced glycosylation end products are involved in atherogenesis, possibly through induction of endothelial dysfunction and low grade inflammation. Plasma concentrations of AGE-peptides are associated with creatinine clearance but not with biochemical markers of endothelial dysfunction and inflammatory activity in non-
diabetic patients over a wide range of renal function. This suggests that the atherogenic effects of AGE-peptides in individuals with renal functional impairment are not mediated by endothelial dysfunction or inflammatory activity as estimated by the markers used.

**Glycosylated Hemoglobin and complications of Diabetes:**

Not only are Glycosylated Hemoglobin levels so tightly linked to diabetic retinopathy, nephropathy and neuropathy, but recent reports have also demonstrated that blocking the production of advanced glycosylation end products beyond the formation of Glycosylated Hemoglobin (and therefore independent of hyperglycemia) markedly retards the development of these complications.

**Glycosylated Hemoglobin levels are a better measure of glycemia than values on the OGTT for two reasons:**

First, they reflect months of prevailing glucose concentration rather than one instance of time. Second, there have been five studies in several thousand diabetic patients carried out over 6-9 yrs relating the average Glycosylated Hemoglobin level to the development and progression of the microvascular complications of Diabetes(67). **Average values >2% above the Upper limit of normal was associated with much higher risk for the microvascular complications.**

The American Diabetes Association recommends determining Glycosylated Hemoglobin levels every 3-6 months to monitor glycemic
Reducing the Glycosylated Hemoglobin to as close to normal as possible is directly related to the reduction of the chronic complications of Diabetes in several studies.

Glycosylated Hemoglobin has recently been reported to correlate with certain established risk factors for atherosclerotic disease. Levels of Glycosylated hemoglobin, fast hemoglobin levels have been correlated with both serum cholesterol and triglyceride levels in at least four separate studies. This correlation could be interpreted to imply that **Glycosylated Hemoglobin levels may be of some value in predicting macrovascular disease.**

When Glycosylated Hemoglobin and fasting blood sugar levels were studied in patients with various complications of Diabetes like neuropathy, nephropathy, retinopathy, keto-acidosis, cardiac and respiratory complications, it was found that there was a significant correlation between fasting blood sugar and Glycosylated Hemoglobin in normal subjects as well as in diabetic patients(81). Further, there was a significant correlation between levels of Glycosylated Hemoglobin and blood sugar over preceding 4-6 weeks. Most frequent complication being retinopathy and keto-acidosis was associated with maximum Glycosylated Hemoglobin with poor metabolic control.

**Hyperglycemia is associated with an accumulation of sorbitol in vascular tissue and with the appearance of small amounts of Glycosylated Hemoglobin(82).** It is sometimes implied
that these biochemical changes are responsible for the alterations in vascular basement membrane that lead to diabetic microangiopathy. Bondy and Felig have critically reviewed the effect of lowering blood glucose on the vascular complications of Diabetes.

Dr. Meena Verma et al. reported that Diabetes mellitus is a lifelong disease, which makes many people worry about the quality and longevity of their life after being diagnosed with it(83). The complications of Diabetes are influenced not only by the duration of Diabetes but also by the average level of chronic glycemia, which is measured most reliably with Glycosylated Hemoglobin assay.

Glycosylated Hemoglobin has been postulated as a biochemical model for the pathogenesis of diabetic sequelae through the glycosylation reactions(84). Glycosylated Hemoglobin has special affinity for oxygen thereby causes tissue anoxia and plays a role in causation of micro and macroangiopathy. Boucher et al. documented that levels of Glycosylated Hemoglobin above 12.6% indicate risk for development of microangiopathy. They also observed a positive correlation of Glycosylated Hemoglobin with fasting blood glucose levels as reported earlier in diabetics with microangiopathy.

Thus 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 secondary sequelae of Diabetes.

The long term microvascular and neurologic complications cause major morbidity and mortality in patients with Type I Diabetes Mellitus(85).

Manley S reported that Glycosylated Hemoglobin is the pre-eminent factor for quantifying the risk of complications in patients with Diabetes and for monitoring glycemia(86).

Diabetes is associated with increased mortality following acute myocardial infarction compared to the general population(87). Elevated Glycosylated Hemoglobin is also a risk marker for short-term mortality following acute myocardial infarction in non-diabetic subjects.

Glycosylated Hemoglobin value has been shown to predict the risk for the development of many of the chronic complications in Diabetes(88).

Svein Skeie et al. studied the relationships between Glycosylated Hemoglobin and blood glucose concentrations and late complications(89). Glycosylated Hemoglobin measurements have become routine practice and it has been shown that long-term regular measurement of Glycosylated Hemoglobin leads to improved metabolic control.
Maike C Eppens et al. compared the prevalence of Diabetic complications and their risk factors in youth with Type I versus Type II Diabetes(90).

Kilpatrick et al. reported their analysis of the large Diabetes control and complications Trial (DCCT) database on the relationship between glucose variability and relative risk of developing microangiopathic complications in Diabetes(91). They found that glucose variability (intraday blood glucose excursions) does not play a role and conclude that only elevation of mean blood glucose over time associates with proportionally greater risk of developing microangiopathy long term.

Rahman et al. studied the extent of non-enzymatic glycosylation in diabetic patients with or without chronic complications(92). All patients were selected on clinical grounds. Fasting plasma glucose was increased in all diabetic patients and correlated significantly with Glycosylated Hemoglobin, glycosylated plasma protein and serum fructosamine concentrations. There was no significant difference between diabetic patients with or without chronic complications in the levels of fasting plasma glucose, glycosylated plasma proteins, Glycosylated Hemoglobin, serum fructosamine, mucoprotein, hexosamine, sialic acid and fucose.
It has been demonstrated that the mean values of body mass index, hemoglobin A1, serum protein, total lipids, triglycerides, Lactate dehydrogenase, alkaline phosphatase, amylase and beta-glucuronidase and heart rate and blood pressure and blood urea levels of Libyan diabetic patients with secondary complications are significantly higher than those of the patients without secondary complications(93). However, the mean values of fasting blood glucose, serum cholesterol and beta-N-acetylglucosaminidase of patients without complications are higher than those of the patients with secondary complications.

The possible association between blood glucose control and the appearance and progression of vascular complications was studied(94). **The most frequent vascular complications both at the beginning and at the end of the study were retinopathy, hypertension, and angina Pectoris.**

Karabun PM studied the level of Glycosylated Hemoglobin in erythrocytes of patients with Diabetes mellitus with regard to compensation of disease, the presence and a degree of expression of diabetic angiopathies(95). An elevated level of Glycosylated Hemoglobin in decompensation of Diabetes decreased after achieving normoglycemia and a glycemia without reaching the normal level. **The development of severe diabetic angiopathies, especially nephro and retinopathies was accompanied by a decrease in the level of**
Glycosylated Hemoglobin in decompensation up to values which could be observed during compensation of Diabetes mellitus. A conclusion is that the level of Glycosylated Hemoglobin in erythrocytes of patients with Diabetes mellitus as an indicator of carbohydrate metabolic compensation can be used only in persons with unaffected development of vascular affection.

Kolarov P et al., reported a positive correlation between the serum Glycosylated Hemoglobin level and blood sugar before meals(96). Glycosylated Hemoglobin could be used as a marker of synthesis of HDL cholesterol and to a lesser degree of triglycerides and total cholesterol.

Glycosylated Hemoglobin and other diseases:

Uptil now studies have performed addressing the role of Glycosylated Hemoglobin in Diabetes as a measure of glycemic control. However, studies addressing the role of Glycosylated Hemoglobin in non-diabetic disorders such as chronic renal failure and iron deficiency anemia are scanty.

Chronic renal failure:

Chronic Kidney Disease (CKD) is a devastating disease with clinical, economic and ethical dimensions and is emerging as a major public health problem globally(97).
Over 1 million people worldwide are alive on dialysis or with a functioning graft. Incidence of Chronic Kidney Disease has doubled in the last 15 years. Although Diabetes mellitus is the leading cause of CKD worldwide; non-diabetic patients still constitute 60-65% of the disease burden (98).

It has been recognized that glucose intolerance is a common finding in patients with CKD. Insulin resistance and impaired insulin secretion contribute to the pathogenesis of glucose intolerance (99).

Although the majority of uremic patients are insulin resistant and about half of them are glucose intolerant, they are rarely diabetic (100). But, there are clinical implications of abnormal insulin metabolism in uremia.

Cardiovascular complications are the most important consequences and significant cause of mortality in these patients (101). Hyperinsulinemia, insulin resistance and glucose intolerance are being recognized as important contributors to cardiovascular as well as many other complications of CKD (102).

The aim of the present study was to evaluate Glycosylated Hemoglobin level in chronic renal failure patients.
**Iron deficiency anemia:**

Iron deficiency is still a big problem today. In fact, the world Health Organization lists iron deficiency as one of the ‘Top Ten Risk factors contributing to Death’. As many as 4-5 billion people, of the world’s population, may be iron deficient, 2 billion people over 30% of the world’s population- are anemic, mainly due to iron deficiency.

Iron deficiency anemia is not just a problem in developing countries of the world. Although iron deficiency is more common in developing countries, a significant prevalence was observed in the United States during the early 1990s among certain populations, such as toddlers and females of child bearing age. 7% of toddlers aged 1-2 years and 9-16 % of adolescent and adult females aged 12- 49 years found to have iron deficiency.

Also at risk are premature babies who are born with low stores of iron in their bodies.

Treatment of Iron deficiency anemia is important because iron deficiency anemia significantly impairs mental psychomotor development in infants, children, leading to developmental delays and behavioral disturbances can also increase the risk for a preterm delivery and delivering a low birth weight baby in pregnant women who are iron deficient(103).

Gould BJ *et al.* investigated the mechanism underlying the variability of Glycosylated Hemoglobin in non diabetic subjects not
related to glycemia(104). They identified two groups of non-diabetic individuals, low and high glycators. Glycosylated albumin and fructosamine measurements gave comparable classifications. The glycosylated albumin was positively correlated with mean blood-glucose concentration. Fasting plasma glucose concentration was greater than the intra-erythrocyte concentration, but their ratio was reduced in low compared to high glycators. No differences between groups were found for plasma insulin, urea or non-esterified fatty acids; plasma or intra-erythrocyte inorganic phosphate or vitamin C; nor plasma, erythrocyte or urinary total aminoacids. *Erythrocyte 2, 3 diphosphoglycerate, a catalyst of glycosylation, was elevated in high compared to low glycators.*

With this background, the present study was carried out to evaluate Glycosylated Hemoglobin level in iron deficiency anemia patients.

**Recent trend:**

Although traditionally considered as an indicator of glycemic control in established Diabetes, Glycosylated Hemoglobin has recently been shown to be in itself a good diagnostic test for Type II Diabetes mellitus(105). It correlates well with the fasting plasma glucose(106) and it may make the oral glucose tolerance test obsolete in the near future(107).
In addition, Glycosylated Hemoglobin has been shown to be a predictor for the future development of Diabetes as well as the metabolic syndrome in patients with normal fasting glucose levels(108). Glycosylated Hemoglobin is a marker for intracellular glycooxidation and peroxidation reactions that result in the formation of Advanced Glycosylation End products (AGES).

**AGES have been implicated in the initiation and progression of atherosclerosis**(45). Studies in non-diabetic individuals have shown a relationship between Glycosylated Hemoglobin and prevalent coronary artery disease(109-111).

It has been associated with abnormalities of cellular antioxidant mechanisms(112) and increased circulating levels of oxidized LDL, a highly atherogenic form of LDL cholesterol. In patients with coronary atherosclerosis Glycosylated Hemoglobin levels in the high normal range were associated with higher levels of several inflammatory markers(113).

**Raised Glycosylated Hemoglobin is an independent risk factor for stroke in people with and without Diabetes** (114). Population-based prospective studies have also demonstrated an association between Glycosylated Hemoglobin values in the non-diabetic range and cardiovascular (CVD) mortality(115).
In addition to its relationship with CVD mortality, the association between Glycosylated Hemoglobin and all-cause mortality may reflect the relationship between abnormalities of glucose metabolism and cancer(116).

Several population based studies have shown an association of impaired glucose tolerance and Diabetes with mortality from cancers of the breast, prostate, colon, pancreas, liver and gall bladder(117-119).

The biologic link between hyperglycemia and the development of cancer may involve stimulation of IHF-1, which has been shown to promote tumor cell growth.
AIMS AND OBJECTIVES

The present study was carried out in subjects suffering from Diabetes (with various complications), chronic renal failure and iron deficiency anemia. The results were compared with healthy control subjects.

The following serum/blood parameters were determined to evaluate the risk factors in diabetic and chronic renal failure patients (Chapter III and IV).

1. Glycemic Control
   - Glycosylated Hemoglobin.
   - Fasting plasma glucose.
   - Postmeal plasma glucose.

2. Lipid profile
   - Serum cholesterol.
   - Serum triglycerides.
   - Serum HDL-C
   - Serum LDL-C

   - Blood urea.
   - Serum Creatinine
The following blood parameters were determined in iron deficiency anemia (Chapter V).

1. Glycosylated Hemoglobin.
3. Random sugar.

The patients were divided into 3 major study groups.

Group : 1) Diabetic patients with micro and macrovascular complications. These patients were further subdivided into 10 groups (Chapter III):

1. Retinopathy  
2. Cataract
3. Nephropathy  
4. Neuropathy
5. Hypertension  
6. Dyslipidemia
7. Ishemic Heart Disease  
8. Coronary Artery Disease
9. Peripheral Arterial Disease  
10. CerebroVascular Disease

Group: 2) Chronic renal failure patients (Chapter IV).

Group: 3) Iron deficiency anemia patients (Chapter V).

For comparison with test group study purpose, healthy control subjects without Diabetes, chronic renal failure and iron deficiency anemia were treated as control.
The present study was undertaken with the following aims and objectives:

**Aims and Objectives:**

1. To study the level of Glycosylated Hemoglobin in healthy control population.

2. To study the level of Glycosylated Hemoglobin in diabetic patients having micro and macrovascular complications.

3. To study the ‘diabetic control’ status of the patient having micro and macrovascular complications (with determination of Glycosylated Hemoglobin and plasma glucose estimation).

4. To study the relationship between raised Glycosylated Hemoglobin levels and late complications in diabetic patients.

5. To study the correlation of Glycosylated Hemoglobin with other biochemical parameters (in diabetic patients with micro and macrovascular complications) viz fasting and postmeal plasma glucose, lipid profile and kidney function test.

6. To study sensitivity of erythrocytes to peroxide hemolysis in diabetic patients with micro and macrovascular complications.

7. To study the levels of Glycosylated Hemoglobin in chronic renal failure patients.
8. To study the correlation of Glycosylated Hemoglobin with other biochemical parameters (in chronic renal failure patients) viz fasting and postmeal plasma glucose and lipid profile.

9. To study the level of Glycosylated Hemoglobin in iron deficiency anemia patients and its correlation with other biochemical parameters such as random sugar.

Subsequent chapter deals with methodology used, followed by the chapters presenting the results of the experiments conducted and the discussion related to the results. Summary and Overall conclusions are included in the last chapter.