Material & Methods
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The present study was carried out in the Department of Medicine MLB Medical College Jhansi in 2001 - 02. In this study 30 patients of Type-2 DM whose BMI < 18.5 (low body weight) were enrolled for study group, 25 normal body weight (BMI between 18.5 - 25) and 20 were over body weight (BMI > 25).

Selection of cases
For this study patients were selected from
1. General Medicine OPD
2. Diabetic Clinic OPD
3. Gynaecology & Obstetrics OPD
4. Skin OPD
5. Tuberculosis & Chest OPD
6. Patient admitted in Ward

Criteria for selection
Any individual who volunteered himself or herself fulfilling the criteria either of LB Type-2 DM i.e., BMI < 18.5% kg/m² or non-obese 18.5 - 25 or obese > 25. The Type-1 DM patients were excluded on the basis of age, clinical presentation and insulin dependence from starting
Method of study

Study of clinical profile includes following -

1. Full clinical history about disease and its complication & total duration of illness
2. Family history maternal or paternal and siblings
3. Age and sex of the patient
4. Educational status
5. Dwelling – Rural or Urban
6. Socioeconomic status of the patient dividing into
   i) Lower class
   ii) Middle class
   iii) Upper class
7. Dietary history
8. Habit
   Vegetarian / Non Vegetarian
   Alcoholic / Non Alcoholic
   Smoker / Non Smoker
   Tobacco Chewer / Non Tobacco Chewer
   Other
9. Occupational history
10. General Examination includes:
    - Built and body proportion
    - Nutrition
    - Pallor, Icterus, Cyanosis, Clubbing
    - Edema, Lymphadenopathy
    - Skin, hair, Nails
• Vertebral column and joint
• Temperature
• Pulse
• Blood pressure
• Respiration

11 Systemic Examination

❖ CNS
  • Higher functions
  • C N
  • Motor system
  • Sensory system
  • Other

❖ CVS
❖ Respiratory
❖ Abdomen
❖ Others

12 Investigation

❖ Haemogram
❖ Diabetic profile including
  • Fasting blood sugar
  • HbA1c
  • Anti insulin antibodies
  • Insulin
  • Anti microsomal antibodies
  • C-peptide
Renal parameters
- Urine – Routine and microscopic examination
- 24 hours urinary protein
- Serum creatinine

Lipid profile
- Total serum cholesterol
- High density lipoprotein
- Low density lipoprotein
- Very low density lipoprotein

X-ray Chest PA view

Fundus

ECG

Echo

Specimen collection and handling

A Blood collection
Collect 10 ml of fasting blood sample from venipuncture and dispense it as mentioned below

1. Double OX tube 2ml blood for haemogram
2. Fluoride tube 2ml blood for fasting blood sugar
3. EDTA tube 2 ml blood for HbA1c
4. Plain tube AMD insulin, C-peptide, Serum Creatinine 4ml blood

B Urine collection
Collect 2 ml of urine from 24 hrs urine pool in plain tube and note the total 24 hrs urinary volume for micro albuminurea
C Samples can be stored at 2-8°C upto 24 hours, for longer periods store samples at –20°C Avoid repeated freezing and thawing

Methods used

Haemogram – Biochemistry Dox blood
Fasting blood sugar – Biochemistry Plasma
HbA1c – High performance liquid chromatography
AIA – Radio immuno assay
Insulin – Chemiluminescence Immuno assay
AMA – Haemoglutination assay
C-peptide - Chemiluminescence Immuno assay
Urine – Biochemistry and Chemiluminescence Immuno assay
S Creatinine – Biochemistry
Lipid profile – Biochemistry

These Investigations were conducted at the Thyrocare laboratory

HbA1C ( GLYCATED HEMOGLOBIN )

The other important advancement in diabetes management is the glycosylated hemoglobin assay. In normoglycemic subjects a small proportion of hemoglobin A is attached to a carbohydrate moiety, thus creating what is called glycosylated or glycated hemoglobin. The glycosylated hemoglobin can be separated into three distinct fractions, which are designated A₁a, A₁b, and A₁c. Because of electrophoretic behaviour of these minor hemoglobins, they are referred to as fast hemoglobin
In conditions of sustained hyperglycemia, such as in diabetes mellitus, the proportion of hemoglobin that is glycosylated is increased substantially. This glycosylation is the result of post-translational modification of hemoglobin A molecules, the binding of glucose is a non-enzymatic process that occurs continuously during the life of the red blood cell. Thus, the amount of glycosylated hemoglobin reflects the glycemic control of a patient during the 6 to 8 week period before the blood sample was obtained.

Glycosylated hemoglobin can be measured by chromatographic, chemically based, or electrophoretic assays. The advantages and disadvantages of these assays will be reviewed briefly. Falsely high levels of glycohemoglobin can be produced in chromatographic assays by carbamylated hemoglobin (formed in uremia), acetaldehyde addition (in alcoholics), or increased fetal hemoglobin (elevated in thalassemia, aplastic anemia, myeloproliferative disorders, and pregnancy). Hemoglobin variants such as HbS or HbC can cause falsely low results in these assays.

The labile fraction of glycohemoglobin (reflects reversibly bound component) is measured in ion-exchange assays but not in chemical assays employing affinity chromatography or calorimetry. Pretreatment of the sample in ion-exchange assays overcomes this problem.

The electrophoretic method that uses agar gel electrophoresis measures all of HbA1, whereas isoelectric focusing on polyacrylamide gels gives wider separation of the different hemoglobin components and therefore can quantitate HbA1c. In fact, while affinity chromatography measures all glycosylated hemoglobin, it is not
affected by temperature or by the presence of hemoglobin variants or fetal hemoglobin. In general, ion-exchange columns (measure Hb$_{A1}$) correlate quite well with affinity columns (measures all glycosylated hemoglobins) even though they measure two different substances. The glycosylated hemoglobin test gives an estimation of the average glycemic level during the 6 to 8 weeks preceding the test. It correlates well with fasting and postprandial blood glucose levels and 24-hour urinary glucose levels. The glycosylated hemoglobin assay is presently one of the most widely applied tests in the management of diabetes. It is useful for the assessment of glycemic control in both patients with Type-I and Type-II diabetes.

Glycosylated hemoglobin values must be assessed with caution in patients with unstable diabetes. Levels of blood sugar in these patients fluctuate from very low to very high on an almost daily basis, a situation that can lead to unwanted symptoms of hyperglycemia and dangerous episodes of hypoglycemia. The assay of glycosylated hemoglobin should be done every 3 to 4 months, with the goal of adjusting therapy to obtain the lowest value that does not place patients at undue risk for hypoglycemic reactions.

**Hb$_{A1c}$ values**

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<td>&lt; 6%</td>
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<td>Avg Control</td>
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<td>&gt;9</td>
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C-Peptide assays

The pancreatic β-cell's primary function is the production, storage and regulated secretion of insulin. Under normal circumstances, the β-cell maintains a condition where there is always readily available pool of insulin that can be rapidly secreted in response to a stimulus, such as a rise of blood glucose. Any increase in insulin release is compensated for by a corresponding increase in insulin biosynthesis, so that β-cell insulin stores are constantly maintained.

Insulin is produced in the β-cells of the islets of Langerhans by cleavage of its precursors, proinsulin, into one molecule of insulin and one molecule of C-peptide. Insulin is subsequently released into the circulation at concentrations equimolar to those of C-peptide. Small amounts of intact proinsulin and proinsulin conversion intermediates are also released. Their low concentration in serum, however, ensures that under normal physiological conditions, their in vivo effects are negligible. In subjects with non-insulin-dependent diabetes mellitus (NIDDM) and even impaired glucose tolerance, however, there is a disproportionate increase in proinsulin-related peptides to approximately 30% of the total immunoreactive insulin in serum.

In contrast to insulin and proinsulin, C-peptide does not appear to be metabolically active. It is considered to be a good marker of insulin secretion because of its equimolar secretion with insulin, negligible hepatic extraction, and constant peripheral clearance at different plasma concentrations and in the presence of alterations in plasma glucose concentrations. The kidney exclusively excretes it.
and its plasma half-life is approximately 30 minutes contrast sharply with the short plasma half-life (approximately 4 minutes) of insulin.

Insulin secretion rates cannot be calculated directly from the peripheral insulin concentrations, because insulin secreted into the portal vein by the pancreas is taken up to a large and variable degree by the liver before it enters the general circulation. Measurements of plasma insulin concentrations are further complicated in insulin-treated patients because of the presence of circulating insulin antibodies, and because insulin immunoassays cannot distinguish endogenous from exogenous insulin. Therefore, peripheral C-peptide concentrations reflect the secretion of the β-cells more accurately than insulin. For these reasons, measurements of C-peptide in plasma have entered general use as a measure of β-cell function.

Clinical applications of C-peptide

1. To assess the residual β-cell function in patients with insulin and to distinguish between IDDM and NIDDM. Of particular interest is its use to indicate the need for progression to insulin therapy in NIDDM.

2. The diagnosis of factitious hypoglycemia. The surreptitious administration of insulin causes high insulin levels in absence of elevated C-peptide concentrations.

3. To diagnose the presence of insulinoma.

4. To assess residual pancreatic tissue after pancreatectomy.
Insulin Assays

Insulin assays play a central role in the investigation of glucose metabolism disorders. Particularly useful in the investigation of the causes of hypoglycemia, insulin assays are also used in the determination of the pathogenesis of Type-I and Type-II diabetes, assessment of β-cell function and for studies on the pharmacology of insulin itself.

Insulin is a polypeptide consisting of two chains linked by disulphide bonds. The A and B chains consist of 21 and 30 amino acids respectively, with disulphide bonds located at positions A7-B7 and A20-B19. The A chain also has an internal disulphide bond bridging the amino acids A6 and A11. Insulin and C-peptide are secreted in equimolar amounts into the portal circulation together with small quantities of proinsulins. 2-6% of the insulin released from the secretory granules is actually in the form of proinsulins and these represent 5-10% of the bioactivity of insulin.

In human circulation, the half-life of insulin is approximately 3-5 minutes. Almost all tissues have the ability to metabolize insulin, but 80% is degraded in the liver and kidneys. In fact 50% of insulins is removed in a single pass through the liver. As the liver does not remove proinsulins and C-peptide, they accumulate in blood and account for 15-20% of the total amount of insulin and proinsulins in the basal state. Glucose is the primary signal that stimulates insulin secretion. Therefore, to correctly interpret an insulin measurement, a simultaneous measurement of the glucose level is also needed.
Clinical uses of insulin measurements

The main clinical application of plasma insulin measurements is in the investigations of the causes of hypoglycemia. This disorder can be caused by hyperinsulinism, insulinoma, insulin autoimmune syndrome or by non-insulin mediated factors. For epidemiological use, insulin assays have been proposed as a marker or risk factor for the development of coronary heart disease and as an early marker for the development of diabetes.

Impaired glucose tolerance (IGT) and diabetes mellitus are diagnosed solely on the basis of chronic hyperglycemia. However, insulin measurements are used in research to study the pathogenesis of these disorders. In Type-1 diabetes, insulin measurements can be useful in pharmacological studies. Type-2 diabetes results from insulin resistance associated with an insulin secretory defect. Specific insulin assays are used to determine the relative insulin deficiency, and the inability of the pancreas to compensate for insulin resistance by adequate insulin secretion. Insulin measurements have a clinical value in the diagnosis of severe insulin resistance. Combined with C-peptide determination, insulin measurements may be used to assess the residual β-cell function, especially in newly diagnosed cases of Type-1 diabetes. They may also aid in the discrimination between Type-1 and Type-2 diabetes.

The routine performance of an insulin assay may be hampered by factors related to the sample material. Insulin auto-antibodies are frequently present in prediabetic states as well as in recent onset IDDM and other antibodies with equally disturbing capabilities have
been reported to be frequently present also in the general population. Heterologous antibodies and rheumatoid factors are also known to be potentially disturbing factors. The influence of such antibodies may be minimized by precipitation with polyethylene-glycol at the time of sampling, followed by the determination of free insulin immunoreactivity in the supernatant. Haemolysis is another sample derived factor which influences most insulin measurements due to the presence of insulin degrading enzymes in erythrocytes. The impact of these types of interference on the individual insulin assay is only weakly predictable and must therefore be studied experimentally. An example is the influence of haemolysis in competitive assays, where an insulin antibody is used in limited concentration, released enzymes may lead to insulin degradation into large fragments (half-molecules) each of which are still capable of reacting in the assay and with similar efficiency as insulin itself. In two-site assays the simultaneous presence of two immunochemical sites on the analyte-molecule are needed to allow determination. Insulin fragments may not be reactive at all. Thus, the analytical design selected has an influence on the impact of a potentially interfering factor.

**Anti Insulin Antibodies**

Anti-insulin antibodies interfere in insulin RIAs and IMAs. They are present in the serum of patients with insulin autoimmune syndrome and are frequently found in the serum of insulin-treated diabetic patients, even when the patients receive biosynthetic human insulin. Studies have indicated that 30% of Type-1 diabetic patients have anti-insulin antibodies prior to insulin administration, and 50% of
diabetic patients treated with human insulin have these antibodies. In the serum of these patients, insulin circulates in both the bound form and also as the free, unbound and biologically active moiety. In RIAs the size of the effect of anti-insulin antibody interference depends mainly on the technique used to separate free and bound radioligand and can thus yield either falsely – elevated or falsely – low insulin values. In two-site immunoassays, if the affinity of the antibodies used in the assay exceeds that of the anti-insulin autoantibodies, falsely high free insulin values may be produced. On the other hand, if the assay antibodies are less avid than the autoantibodies, the final result will tend to reflect the free insulin concentration, since little or no displacement of the autoantibody-insulin complex occurs during the incubation time.

**Anti Microsomal Antibody (AMA or anti-TPO antibodies)**

Anti Microsomal Antibody are those that are directed against the microsomes in the thyroid gland. Abnormal values are seen in Hashimoto’s thyroiditis (100%), Graves disease (80%), hypothyroidism and atrophic thyroiditis. Estimation of AMA would help the physician in planning a long term treatment strategy for a patient of thyroid dysfunction.

**Serum creatinine**

Creatinine, an end product of creatine, which is excreted by kidneys and it is used to monitor renal diseases. In patients with renal dysfunction, the serum creatinine is disturbed resulting in accumulation of creatinine in serum. In diabetics, high serum creatinine is of bad prognostic value and signals deterioration of renal function.
**Urinary Albumin / Urinary Creatinine Ratio**

Whenever urinary concentration of an analyte is to be correlated, it is essential to know what volume of urine output in 24 hours is collected. However, in certain emergencies or in a situation, where collection of 24 hours urine is not practical, estimation of urinary analytes is done on random samples. It is expected that urinary creatinine levels (which co-relates to urine volume) would be indicating the role of urinary volume changes in albumin concentration and therefore in such circumstances albumin to creatinine ratio is demanded for any meaningful clinical co-relation.

- **Normal Albumen**  
  < 30 µg/mg creatinine  
- **Micro Albumen**  
  30 – 300 µg/mg creatinine  
- **Macro Albumen**  
  > 300 µg/mg creatinine

**Micro Urinary Albumin**

Diabetic patients are prone to develop kidney damage as the disease progresses. The damage caused to the kidney if known in time can be reduced or eliminated by giving the patient certain drugs and full blown diabetic kidney damage can be avoided. Detection of increased levels of albumin in the urine at an early stage serve this purpose as it is the earliest marker of diabetic marker of diabetic kidney disease. It may be worth noting that various other disorders also result in nephropathy (kidney disorders).

- **Normal Albumin Urea**  
  < 20 µg/min  
- **Micro Albumin Urea**  
  20-200 µg/min  
- **Macro Albumin Urea**  
  >200 µg/min
### Working Proforma

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CVA
Cataract
Retinopathy
Nephropathy
Peripheral Neuropathy
  Sensory Neuropathy
  Motor Neuropathy
  Mixed Neuropathy
Autonomic Neuropathy
Impotency
GI Symptoms
Diabetic Foot
Tuberculosis
Skin Infection
Fungal Infection
Other

17 General Examination
Pulse rate /min
Blood Pressure
  Supine position mm/Hg
  Standing (After 3 min) mm/Hg

18 Anthropometry
  Weight Kg  Height mt  BMI
  Waist cm  Hip cm  W/H

19 Systemic Examination
CNS
  Higher Function
  Motor System
  Others
Crani al Nerves
Sensory System
CVS
Respiratory System
Abdomen
Other

20 E C G
21 ECHO
22 X-ray
23 Fundus

24 Biochemical

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<td>Anti Insulin Antibodies (%)</td>
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25 Treatment

26 Treatment Complains

27 Diabetic Control Poor / Average / Good