DISSERTATION ON

A COMPARATIVE AND CORRELATIVE STUDY OF SERUM HOMOCYSTEINE LEVEL IN GESTATIONAL DIABETES MELLITUS AND NORMAL PREGNANCY

Dissertation submitted to

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In partial fulfillment of the regulations
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M.D. IN GENERAL MEDICINE

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This dissertation is submitted to Tamilnadu Dr. M.G.R Medical University towards partial fulfilment of requirement for the award of **M.D. Degree (Branch - I) in General Medicine**.

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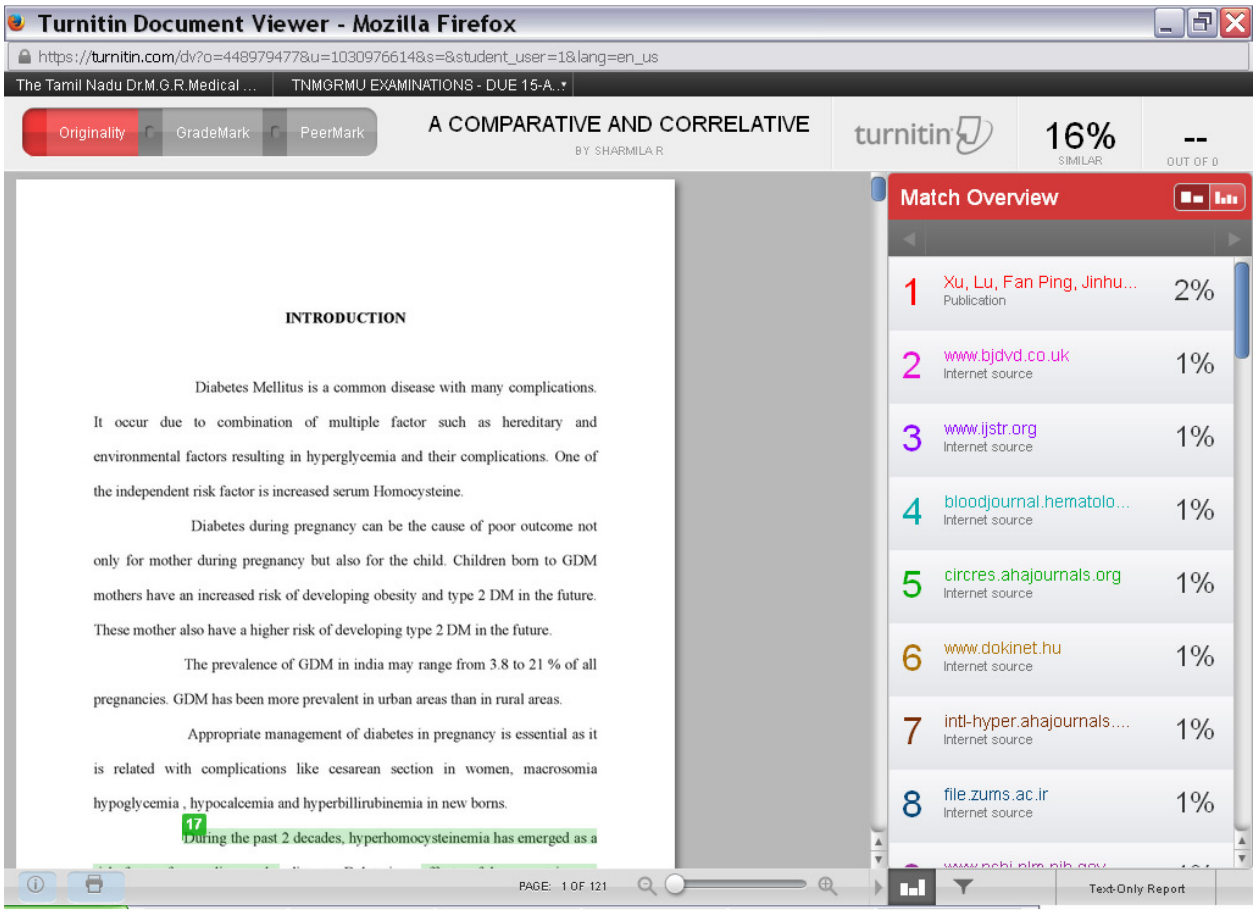
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INTRODUCTION
Diabetes Mellitus is a common disease with many complications. It occur due to combination of multiple factor such as hereditary and environmental factors resulting in hyperglycemia and their complications. One of the independent risk factor is increased serum Homocysteine.
Diabetes during pregnancy can be the cause of poor outcome not only for mother during pregnancy but also for the child. Children born to GDM mothers have an increased risk of developing obesity and type 2 DM in the future. These mother also have a higher risk of developing type 2 DM in the future.
The prevalence of GDM in india may range from 3.8 to 21 % of all pregnancies. GDM has been more prevalent in urban areas than in rural areas.
Appropriate management of diabetes in pregnancy is essential as it is related with complications like cesarean section in women, macrosomia hypoglycemia , hypocalcemia and hyperbillirubinemia in new borns.
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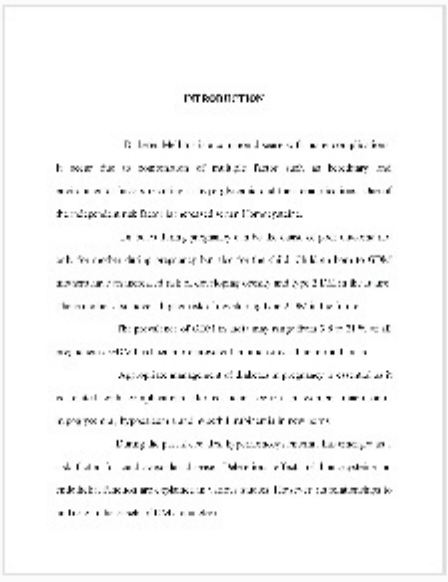

INTRODUCTION

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ABBREVIATIONS AND ACRONYMS:

ADA American Diabetes Association

CRP C- Reactive Protein

CKD Chronic Kidney Disease

DM Diabetes mellitus

DNA Deoxyribonucleic acid

DIPSI Diabetes in pregnancy study group india

FGF21 Fibroblast Growth Factor 21 Level

GMS Grams

GDM Gestational Diabetes mellitus

IGT Impaired glucose tolerance

GLUT Glucose transporter

GCT Glucose Challenge Test

HsCRP Highly sensitivity C - Reactive Protein

HNF Hepatocyte nuclear factors

Hb A1c Glycated haemoglobin

HDL High density lipoprotein

IADPSG International association of diabetes and pregnancy study groups

IRS Insulin Receptor Substrate

Lp (a) Lipoprotein a

MINS Minutes

NO Nitric oxide

OGTT Oral Glucose Tolerance Test

OHA Oral Hypoglycemic Agent

SPARC Secreted Protein Acidic And Rich in Cysteine

SD Standard deviation

TG Triglycerides

TC Total cholesterol

WHO World health organization

WBC Whole blood count

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“A COMPARATIVE AND CORRELATIVE STUDY OF SERUM HOMOCYSTEINE LEVEL IN GESTATIONAL DIABETES MELLITUS AND NORMAL PREGNANCY’’

ABSTRACT

Background :

Gestational diabetes (GDM) is defined as carbohydrate intolerance that begins or is first recognized during pregnancy. Homocysteine is naturally obtained by diet containing methionine which is one of the essential amino acids. The role of homocysteine is an independent risk factor of gestational diabetes mellitus has not been extensively studied in India. Due to multiple factors like dietary, life style, socio economic status and other ethnic differences, the results found in the western studies cannot be applicable to our population.

Aims and Objectives:

The aim of this study is to study the association of serum homocysteine levels in gestational diabetes mellitus and to compare the serum homocysteine levels in gestational diabetes mellitus and in normal pregnancy.

Methods:

This case control study comprised 30 patients with gestational diabetes mellitus and 20 patients of normal pregnancy in the age group of 18 to 35 years attending Obstetrics and Gynaecology department OPD or admitted to Rajamerasutar Hospital, Thanjavur Medical College, Thanjavur,

Serum Homocysteine, Glucose, Urea, Creatinine, Total Cholesterol, Triglycerides, were determined. Data were analyzed with appropriate statistical analyzer.Serum homocysteine levels were estimated in all of them and its correlation to gestational diabetes mellitus and in normal pregnancy was studied.

Results:

The serum homocysteine levels were statistically higher among the cases as compared to the controls. The mean value of serum homocysteine in control group is 3.8 ± 0.95 and in gestational diabetes patients is 16.30 ± 6.09. Its 'p' value is significant (p .000 < 0.05) . Total cholesterol level is significantly elevated in gestational diabetes patients. The mean value of Total cholesterol in control group is 187.70±18.2 and in gestational diabetes patients is 211.50 ± 28.799. Its 'p' value is significant (p=0.002). In our study, 12 patients of gestational diabetes patients had elevated serum Triglyceride level. In control group, all had the normal triglyceride level. The mean value of serum triglyceride level is 113.30 ± 16.10 in control group. In study group (gestational diabetes patients) , the mean value is 140.20 ± 22.15.

Conclusion:

There is significant association between homocysteine with gestational diabetes mellitus. Higher homocysteine levels were observed with gestational diabetes mellitus. Hyperhomocysteinemia is found in 56.66% of patients with gestational diabetes mellitus.

Hyperhomocysteinemia is found to be an independent risk factor for gestational diabetes mellitus patients. The average levels of Total cholesterol, were significantly found to be higher in GDM cases compared to controls.

Keywords Homocysteine; Hyperhomocysteinemia; Gestational diabetes mellitus.

INTRODUCTION

Diabetes Mellitus is a common disease with many complications. It occur due to combination of multiple factor such as hereditary and environmental factors resulting in hyperglycemia and their complications. One of the independent risk factor is increased serum Homocysteine.

Diabetes during pregnancy can be the cause of poor outcome not only for mother during pregnancy but also for the child. Children born to GDM mothers have an increased risk of developing obesity and type 2 DM in the future. These mother also have a higher risk of developing type 2 DM in the future.

The prevalence of GDM in india may range from 3.8 to 21 % of all pregnancies. GDM has been more prevalent in urban areas than in rural areas.

Appropriate management of diabetes in pregnancy is essential as it is related with complications like cesarean section in women, macrosomia hypoglycemia , hypocalcemia and hyperbillirubinemia in new borns.

During the past 2 decades, hyperhomocysteinemia has emerged as a risk factor for cardiovascular disease. Deleterious effects of homocysteine on endothelial function are explained in various studies. However, its relationships to and role in the onsets of DM are unclear.

Meigs et al[1] reported that increased serum Homocysteine is associated with increased insulin level in blood and suggested that it may cause cardiovascular disease risk when it is associated with insulin resistance.

OBJECTIVES

1. To study the association of serum homocysteine levels in gestational diabetes mellitus.

2. To compare the serum homocysteine levels in gestational diabetes mellitus and in normal pregnancy.

REVIEW OF LITERATURE

The earliest description of diabetes was given by the Ancient Greek physician Aretaeus of Cappadocia[2] (2nd century AD). An ancient Egyptian papyrus, explain the diabetes as a disease that caused a person to melt in the loins and the resultant urine to attract ants (due to the high sugar values).

The name itself explains valuable body fluid loss. Diabetes was probably coined by Apollonius of Memphis[2] around 250 BC. Diabetes meaning "to siphon" is from a Greek word, Mellitus, a Latin word, meaning is "sweet taste". *Madhumeha was* identified by the great Indian physician Sushruta[2] and he further identified the risk factor such as obesity and sedentary lifestyle, and he advised exercises to help in curing.

Early diabetes research linked to glycogen metabolism and in 1869, Paul langerhans, a medical student discovered the pancreatic islet cells. In 1916, sharpey–schafer suggested that a single chemical was missing in pancreas and that was insulin. EL scott and Nikolae Paulescu, from the pancreas of experimental dogs, they were extracting insulin successfully.

In 1921, FG Banting discovered the insulin, the extract name is "isletin" and he received nobel prize in 1923. A 14 years boy, leonard was the first patient who received the banting -extract insulin. In 1936 sir Harold Himsworth found that diabetes falls on 2 types based on "insulin insensitivity".

First description of GDM, by the patient fredecia who was admitted in berlin infirmary in 1823 during her 7thmonth of 5th pregnancy[(3)]. The paper titled "ON PUERPERAL SEPSIS" was presented by Mathews Duncan in 1882 .

The real diabetic knowledge came in 1922 with invention of insulin by Best and Banding. He gave the total change in morbidity and mortality associated with GDM.

The management of GDM was an art and science developed by Dr. Elliot proctor Joslin of Boston. Later it was continued by Dr. pricilla white. After that the knowledge about diabetes mellitus is growing dramatically.

"PREDICAMENT" term used by Gilbert and Dunlop[(4)]**.**It denotes the time interval before the disease diagnosis and they retrospectively analyzed in overt diabetic following obstetric history, they found that 50% fetal loss occurs 2 years preceding the diagnosis.

In 1952, jackson was stated that the predicament is clinical diagnosis made by previous obstetrical history. In 1956, Lewis extended this definition based

upon the over weight babies and fetal loss before the diagnosis of increased glucose level and abnormal blood test reports was made.

Miller et al[5] in 1944 and Mengert and Laughlin [6] reported the fetal loss during the pre-diabetic state. Pedowitz and shleva in 1957 and Hagbard in 1958 found that the risk of prenatal death shortly before the diagnosis of diabetes.

In 1967 in Copenhagen, Jorgen Pederson was used first the term gestational diabetes.

DEFINITION:

Gestational diabetes mellitus[7], is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. This definition includes women whose glucose tolerance will return to normal after pregnancy and those who will persist with glucose tolerance and type 2 DM.

INCIDENCE AND PREVALENCE:

In 2012, 371 million people had diabetes, the number may increase to 552 million by 2030[8]. Globally the incidence of type 2 DM is rising. In that eighty percentage of the diabetes population live in, developing countries and common age distribution between 40- 50 years of age. 78,000 children develop type 1 diabetes in each year.

Asian Indians have a higher genetic predisposition for developing DM of type 2 and its complications. A unique combination of clinical and biochemical parameters have identified and labeled as "Asian Indian phenotype"[9]. Because the Asian Indians have a higher degree of central obesity and other risk factors.

The incidence of GDM, depending upon the population studied, may range from o.5 to 12.5 % of all pregnant women[10, 11]. Prevalence is more in African, Hispanic, Native american than white women. The incidence of GDM higher in population with higher type 2 DM prevalence. When compared the Caucasian women and Indian women, Indian women have 11 fold increased glucose tolerance during pregnancy.

Table-1:**PREVAIENCE OF GESTATIONAL DIABETES AROUND THE WORLD:**

COUNTRY	PREVALENCE %	AUTHOR
England	0.15	Lind
Ireland	0.2 – 3.5	Hadden
Australia	0.7	Abell, besicher
Sweden	1.3	Stangenberg
Denmark	1.7	Guttorm
Kenya	1.8	Fraser
Scotland	4.0	sutherland
USA	2.7 – 7.5 in Boston and 12.3 in Los Angeles	O'sullivan Mestman

The increasing prevalence in developing countries is related to increasing urbanization, decreasing physical activity, changes in dietary patterns and increasing prevalence of obesity. Women with GDM and their children are at risk of developing DM in future, so special attention should be paid to these patients in developing countries.

Table-2: Asian prevalence of Diabetes mellitus (urban)

	SRI LANKA	INDIA	PAKISTAN
Diabetes mellitus	12.7%	12.1%	12.0%
IGT	14.4%	14.0%	10.0%

Gomez et al [12] and Das et al [13] found that 25 % and 50% 0f women with GDM had obesity and Family history of diabetic was present in both studies with 77.3% in Gomez and 14.3 % in das studies.

- The classification adapted by World health organization [14] is :

1. Diabetes mellitus.
2. Impaired glucose tolerance
3. Gestational diabetes mellitus.

A Small proportion of type 1 diabetics are difficult to control and are called“ Brittle diabetics or Labile diabetics”[15]. It defined as patients whose life is constantly, disrupted by alternative episodes of decreased or increased glucose level whatever be the cause.

TYPE 2 DIABETES MELLITUS:

Impaired beta cells function, so relative insulin deficiency and insulin resistant. Some of the gene have the positive co-relation with type 2 DM such as Insulin gene hvr, apolipoprotein D, glucocorticoid, glucokinase and complement C4B2.

THEORY OF GLUCOSE TOXICITY:

In Type 2 diabetes mellitus, earliest feature is loss of normal pulsatile pattern of insulin secretion in the basal fasting state, then decrease in the amplitude of insulin secretory pulses in the postprandial phase. Later first phase of insulin release is reduced due to selective glucose unresponsiveness of the beta cell. This is due to direct glucose toxicity of the beta cell. The initial Pancreatic damage, characterized by defective insulin gene expression [16].

ROLE OF GELANIN:

It is a hormone which has inhibitory effects on insulin. This is implicated in etiopathogenesis of type 2 DM and malnutrition related diabetes mellitus.

Nutrition deficiency (zinc and selenium deficiency)

Excess of free radical stress

Lipid peroxidase

Beta cell damage ⟹ protein calorie deficit leads to glucose intolerance and diminished insulin response.

PANCREATIC AMYLOID-AMYLIN IN TYPE 2 DM:

Opie was first to describe pancreatic amylin in 1901. Amylin is produced by beta-cell and is localized with insulin in the secretory granules and is secreted along with insulin and pro-insulin. Amyloid deposits are seen in type 2 DM. Amylin antagonizes insulin action. It inhibits pancreatic insulin secretion, in muscle inhibits glycogen synthesis and causes peripheral insulin resistance [(17)].

❖ **GESTATIONAL DIABETES MELLITUS:** There are two classification method for gestational diabetes mellitus.

A. white classification and

B. ADA classification.

A. WHITE CLASSIFICATION: Named after Priscilla White[18] . This is widely used to assess maternal and fetal risk. It help to distinguish between gestational diabetes (type A) and pre-gestational diabetes. It is further subdivided in to

❖ Type A1: Abnormal oral glucose tolerance test (OGTT), but normal blood glucose levels during fasting and two hours after meals; Diet modification is sufficient to control glucose levels.

❖ Type A2: Abnormal OGTT compounded by abnormal glucose levels during fasting and/or after meals; Additional therapy with insulin or other medications is required.

Pregnant women with pre-existing diabetes is split up into

- Type B: Onset at age twenty or older and duration of less than ten years.
- Type C: Onset at age ten to nineteen or duration of ten to nineteen years.

- Type D: Onset before age ten or duration greater than twenty years.
    - D1- Onset before the age of 10
    - D2- Duration over 20 years
    - D3-Macrovascular disease
    - D4-Microvascular disease/hypertension but not preeclampsia.

- Type E: Overt diabetes mellitus with calcified pelvic vessels.
- Type F: Diabetes nephropathy over 500mg/day proteinuria,
- Type R: Proliferative retinopathy and vitreous haemarrhage,
- Type RF: Both retinopathy and nephropathy.
- Type H: Ischemic cardiac disease,
- Type T: Prior kidney transplant.

B. <u>ADA classification</u>.

- Type 1 DM before pregnancy,
- Type 2 DM before pregnancy OR
- First time becoming diabetic during pregnancy-gestational diabetes mellitus.

However the end results is hyperglycemia, which is responsible for the symptoms such as polyuria, polydipsia, polyphagia, blurred vision, unexplained weight loss, lethargy and inefficient energy metabolism. Insulin resistant is the main cause.

WHAT IS INSULIN SENSITIVITY?

It is a state where a small amount of insulin able to produce severe hypoglycemic effect, in a diabetic patient who has been controlled with OHA or insulin[19].

Mechanism:

1. Decrease in the rate of insulin metabolism and exertion.
2. Decrease secretion of counter regulatory hormone,
3. Increased secretion of insulin or insulin like hormones.

In pregnancy, diabetics on insulin can develop frequent attacks of hypoglycemia in 1st trimester due to poor diet and pregnancy induced vomiting. And also in the first 24- 28 hrs of delivery, insulin requirement suddenly reduces because of the fall in levels of estrogen, progesterone and HPL which have anti-insulin effect.

INSULIN RESISTANCE:

It is a state in which a normal amount of insulin produces a sub normal amount of insulin response[20].. Its fall in to 2 categories:

1. DECREASED SENSITIVITY:

   Where normal response can be obtained with maximum insulin level.

2. DECREASED RESPONSIVENESS:

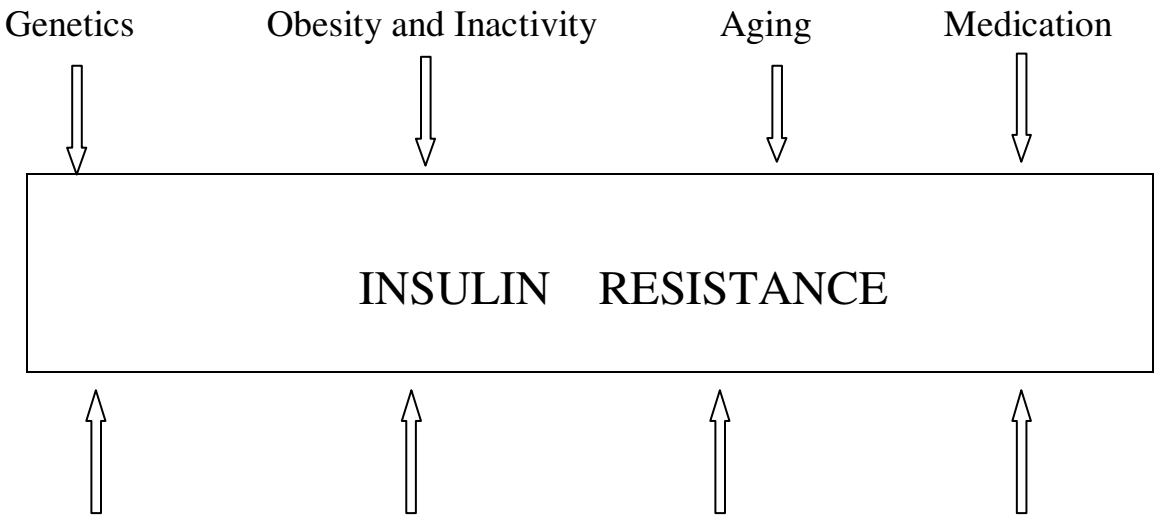
   Even large amount of insulin cannot bring the normal response.

Depending on the molecular mechanisms involved insulin resistance may be at pre-receptor, receptor, and post-receptor. Most common is post-receptor level.

PATHOPHYSIOLOGY OF INSULIN RESISTANCE:

1. Abnormal insulin secretion
2. Resistance to insulin action in target tissues.

<u>Insulin resistance causes and associated conditions</u>:



There are three phase of development of type-2 DM,

- Phase 1- Euglycemia with increased insulin levels,
- Phase 11- Postprandial hyperglycemia with increased insulin levels,
- Phase 111 – Overt diabetes with declining insulin levels.

Insulin resistance in Type 2 DM is at post-receptor level. The substance responsible for this is amylin.

Insulin resistance, hyperinsulinemia and hyperlipidemia often co-exist.

The typical pattern of a lipid profile in Insulin resistance is;

- Decreased serum HDL – cholesterol
- Increased serum very low density lipoprotein(VLDL)
- Less common elevation of LDL ,
- Increase in triglyceride.

❖ The American diabetes association (ADA) in its newer classification retained the state of impaired glucose tolerance and introduce another term impaired fasting glucose [21].

Table-3:

TIME	IMPAIRED GLUCOSE TOLERANCE
Fasting	< 126 mg/dl
Two hour	≥ 140 mg/dl -199 mg/dl

- IMPAIRED GLUCOSE TOLERANCE (IGT):

  Two hours glucose levels of 140- 199mg/dl after 75 gm of oral glucose is called as IGT.

- IMPAIRED FASTING GLUCOSE (IFG):

  Glucose level of 100 –125 mg/dl in fasting patients is called as IFG.

The following risk associated with Impaired glucose tolerance ,

- Coronary artery disease.
- Progression to type 2 DM,
- Cognitive impairment in elderly people
- GDM induces embryopathy as well as increase fetal morbidity and mortality.

PATHOPHYSIOLOGY OF IGT:

Abnormality may be intracellular glucose metabolism namely defective non-oxidative glucose storage. The loss of first phase of insulin secretion is a well known early changes in IGT. But in second phase of insulin secretion remained normal. So person with IGT have higher than normal insulin level but insignificant to overcome the resistance. IGT is characterized by hyperglycemia with hyperinsulinemia.

Elevated level of fasting insulin level associated with impaired fibrionlysis and hypercoagulopathy. Hyperglycemia dependent oxidative stress leads to endothelial dysfunctions. Increased triglyceride level could cause decreased insulin sensitivity via raised nonesterified free fatty acids.

❖ RISK FACTOR ASSOCIATED WITH GDM:

❖ Maternal factors such as :

- Maternal age
- Higher parity
- Pre-pregnancy weight
- Pregnancy weight gain
- BMI > 27

- Short stature
- Smoking
- Non white ethnic origin
- α-Thalassaemia trait
- Polycystic ovary syndrome
- High intake of saturated fat

❖ Family history:

- Family history of type 2 diabetes
- GDM in woman

❖ Previous obstetric outcome:

- Congenital malformation
- Stillbirth
- Macrosomia
- Caesarean section
- Previous GDM
- Previous Low birth weight baby

❖ Pregnancy factors:

- High blood pressure in pregnancy
- Multiple pregnancy
- Polyhydraminosis

❖ Protective factors:

- Young age
- Alcohol use

CARBOHYDRATE METABOLISM IN NON PREGNANT STATE:

ACTION OF INSULIN:

1. It promotes glucose uptake by skeletal muscle

2. Glycogen synthesis

3. Suppression of gluconeogenesis

4. Lipolysis by liver.

The body tissue obtained the energy from glucose in the systemic circulation. Carbohydrate are the most important source. Brain cells derive energy from glucose.

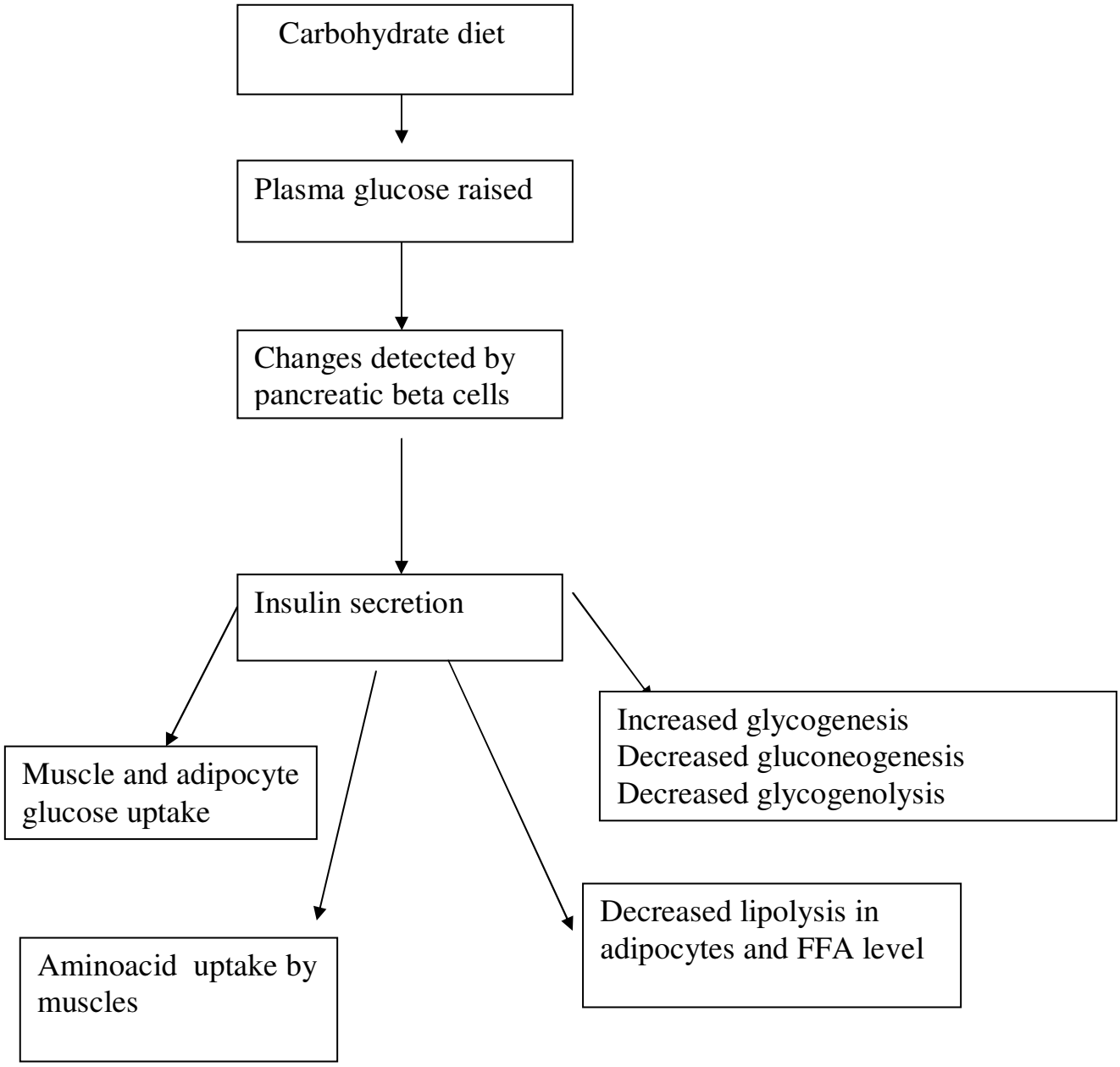
Glucose after entering the cells has 2 metabolic pathway.

1. Oxidative metabolism = glucose ⟶ glucose -6 - phosphate

   glycolysis ⟶ ATP production

2. Non oxidative metabolism: glycogen formation.

   Prime regulator is insulin.



POST PRANDIAL PHASE:

Insulin production decreased after 2 to 3 hours of meal, and also after prolonged fasting. Gluconeogenesis pathway activated during fasting to produce glucose by using protein and fat breakdown. This endogeneous glucose production maintains the energy.

FASTING PHASE:

- After 12 hour of diet, Gluconeogenesis is the source of energy. it needs aminoacid so protein catabolism will occur.
- And also fat breakdown leads to production of glycerol and fatty acids resulting in glucose and ketone body formation
- But ketone bodies rapidly cleared from circulation.
- Insulin acts as both anabolic and catabolic in well fed state.
- FFAs important for maintaining insulin secretion during prolonged fast.

In 1950, Burt, Frenkel and Goodner found that pregnancy was associated with certain physiological changes in carbohydrate metabolism. Continuous supply of glucose and other nutrients to fetus through placenta.

CARBOHYDRATE METABOLISM IN GDM AND IN PREGNANCY:

Pregnancy characterized by gradual increasing insulin resistance that starts near mid pregnancy and progresses through the third trimester. It is important to note that insulin resistance return to near normal in post delivery, suggesting the placental hormones plays important role in the pathogenesis.

The cumulative effects of maternal adiposity and placental influences results in insulin signaling pathway dysfunction, which lead to decreased glucose uptake and an increase in insulin resistance. The placenta produces human

chorionic somatomammotropin or the human placental lactogen which stimulates pancreatic secretion of insulin in the fetus and inhibits peripheral uptake of glucose in the mother.

All pregnant women become insulin resistance out of that only 10% will have GDM. Buckman and co workers found that in pregnant women first phase of intravenous glucose response significantly reduced compare to normal pregnancy. Catalano and coworker[22,23] found the hepatic insulin resistance in GDM.

- **Effect of pregnancy on DM on various stages**:

Table-4:

	Changes noted	**Possible causes**
First trimester	Improve-carbohydrate metabolism,Hypoglycemic reaction, increased sensitivity to insulin.	Poor intake due to hyperemesis gravidorum, maternal glucose enter into foetus
Second trimester	Insulin need increases, ketosis prone	Anti-insulin hormone are acting and degenerarion of insulin by placenta
Third trimester	Greater intensification of diabetic status	Anti-insulin hormone are acting.
Labour	Hypoglycemia,Increased sensitivity to insulin	Due to increased physical activity,Insulin sensitivity increases
postpartum	Remission in DM , insulin need falls	Once the placenta is out anti-insulin effect has gone.

- **HORMONAL RESPONSES TO GDM:**

❖ ESTROGEN: Increasing the insulin level and also its binding capacity[24].

❖ PROGESTRONE:

1. Insulin response to glucose is increased.
2. Glucose transport decreased[24].
3. Insulin receptor number decreased
4. Insulin response is decreased to suppress the endogenous production of glucose.

- **CORTISOL:**

1. Insulin resistance by post receptor mechanism[25] is due to increased Maternal cortisol level in last trimester.
2. Hepatic glucose production is increased.
3. Promotes lipolysis ⟶ increase FFA
4. Protein breakdown ⟶ increase aminoacids.

- **HUMAN PLACENTAL LACTOGEN:**

1. Its level increases as pregnancy advances
2. Maximum role for insulin resistance.
3. It directly acts on pancreatic beta cell to stimulate insulin secretion.

4. Glucose transports are decreased.
5. It stimulates IGF-1production through cell surface receptor.

- **PLACENTAL GROWTH FACTOR:**

Secreted by placenta. It has anti insulin action.

- **PROLACTIN**:

It increases 10 fold of normal level. In women with hyperprolactinemia have increase basal insulin and decreased glucose transport.

After the mid trimester, the placental size increases with increasing the above hormones, which results in a more insulin resistant state. Insulin sensitivity also affected by elevated level of estrogen, progesterone and prolactin.

In non diabetic pregnant mother, compensatory response occur such as beta cell hypertrophy and hyperplasia.

- **LIPOTOXICITY:**

Besides the hyperglycemia, raised levels of free fatty acid is implicated in acquired defect in pancreatic beta cells and progression to diabetes from impaired glucose tolerance and its complication.

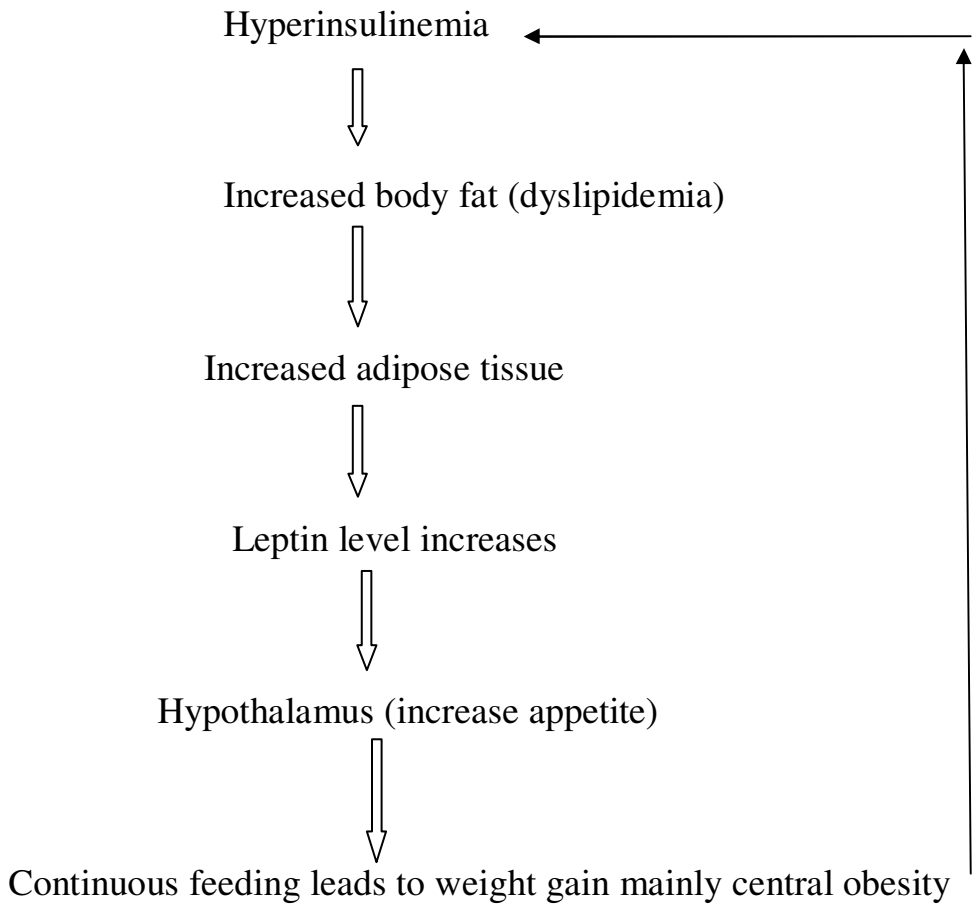
- **TUMOUR NECROSIS FACTOR *α*:**

TNF α is a cytokines produced by adipocytes, neutrophils, monocytes. fibroblast and macrophages. Increased level of this cytokine is associated with hyperinsulinemia.

It impairs the insulin signaling by increasing serine phosphorylation of IRS-1 which in turn inhibits tyrosine kinase activity of insulin receptor.Catalano et al reported that 25% of increase in TNF α in association with body fat and insulin sensitivity changes.

- **LEPTIN:**

1. It is a polypeptide, secreted from adipocytes and it is a OB gene.
2. It inhibits food intake and decrease the body fat.
3. It stimulates energy expenditure.
4. It acts on hypothalamus to produce the above action.
5. Possibly modulation of insulin sensitivity.



So hypothalamus mediated leptin resistance causes a rise in leptin and initiates hyperinsulinemia and insulin resistance in obesity.

Highman et al[(26)] reported that in pregnancy the leptin level increased significantly before the physiological changes occur and also plasma level become normal after 24 hours of placental delivery. Leptin has a role in fetal growth and maternal carbohydrate metabolism.

- **THE INSULIN SIGNALING SYSTEM:**

Insulin receptor tyrosine kinase activity[27]is needed for signaling. It will be decreased in GDM patient more than the obese pregnant patient.

- **PROTEIN TYROSINE PHOSPHATASE:**

It regulates the phosphorylation and dephosphorylation reaction at cellular level. Some of the studies showed this enzyme modulates the insulin sensitivity and fuel metabolism.

- **INSULIN RECEPTOR SUBSTRATE PROTEIN:**
  - The level of insulin receptor substrate protein and insulin mediated tyrosine phosphorylation are necessary for insulin sensitivity.
  - Decreased expression of IRS-1 in skeletal muscle of pregnant women
  - Increased IRS-2 level[27] which has the primary progesterone elements. So insulin resistance may be exerted by decreasing the signaling cascade at the level of IRS.

- **PHOSPHATIDYL INOSITOL 3 KINASE:**

This protein activation is essential for glucose transport. This level will increase in both pregnant and GDM patients skeletal muscle.

➢ GLUCOSE TRANSPORTERS:

Insulin mediated glucose uptake is mediated by GLUT-4. In pregnant women, adipocytes has decreased expression of GLUT-4 leads to hyperglycemia.

Plasma glucose level between pregnant mother and their fetus is only 0.5 mmol/l. Fetal glucose utilization rates (5-7 mg/kg/min) are higher than in adults (2-3 mg/kg/min).

The three key points in this complex regulation of fetal glucose metabolism[28] are:

1. Maintenance of maternal glucose concentration by increasing maternal glucose production and development of relative maternal glucose intolerance and insulin resistance.
2. Placental transfer of maternal glucose to the fetus, buffered by placental glucose utilization.
3. Fetal insulin production and enhancement of glucose utilization in sensitive tissues

Oakley et al[29] reported that hyperglycemia induces facilitated diffusion across the placenta.

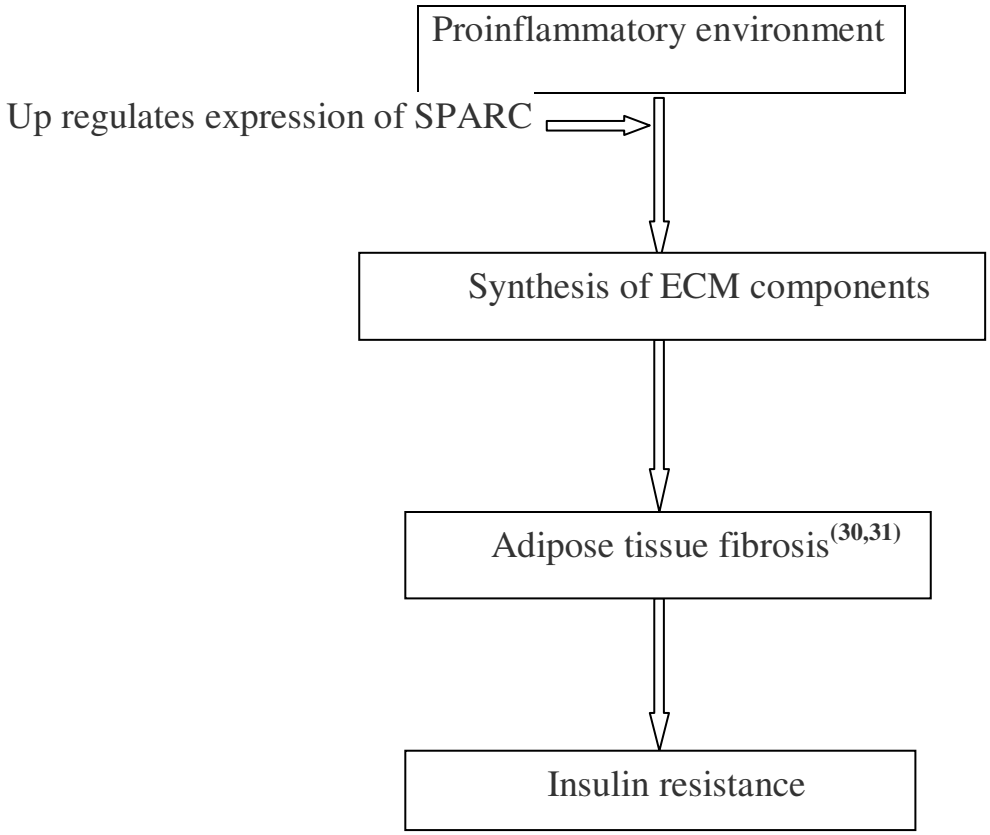
Fetal pancreas secretes the insulin as early as 10 to 12 weeks of gestation. In second trimester, fetal insulin level is elevated. Fetal pancreas is highly sensitive to maternal hyperglycemia.

Beta cell dysfunction in women diagnosed with GDM may fall into autoimmune, monogenic or insulin resistance (common cause).

- **SECRETED PROTEIN ACIDIC AND RICH IN CYSTEINE (SPARC):**

It is a newly identified adipokine, is a main regulator in the pathogenesis of obesity and T_2DM. Recent studies determined circulating levels of SPARC in pregnant women and found that SPARC levels were increased significantly in GDM group of population compared with normal glucose tolerance test group of pregnant women and correlated significantly with insulin resistance. It is an independent indicator of insulin resistance.

Levels of SPARC are significantly elevated in T2DM patients compared with normal controls in Chinese and Japanese populations. A recent study showed that human placenta villi[32] could express and secrete SPARC, suggesting that levels of SPARC may be affected by gestational age. SPARC may be connected to the inflammation and glucose intolerance by excessive synthesis of extra cellular matrix components.



The co-existence of high SPARC level and increased inflammatory markers in GDM and their close relevance with each other and with insulin resistance suggest that their interaction may play an important role in the development and progression of GDM.

- **FIBROBLAST GROWTH FACTOR 21 LEVEL**:

The levels of FGF21 in German GDM women assessed by Stein et al at mid-pregnancy (24–28th week of gestation) and showed that serum FGF21 levels[33] was not significantly different between patients with GDM and healthy pregnant controls.

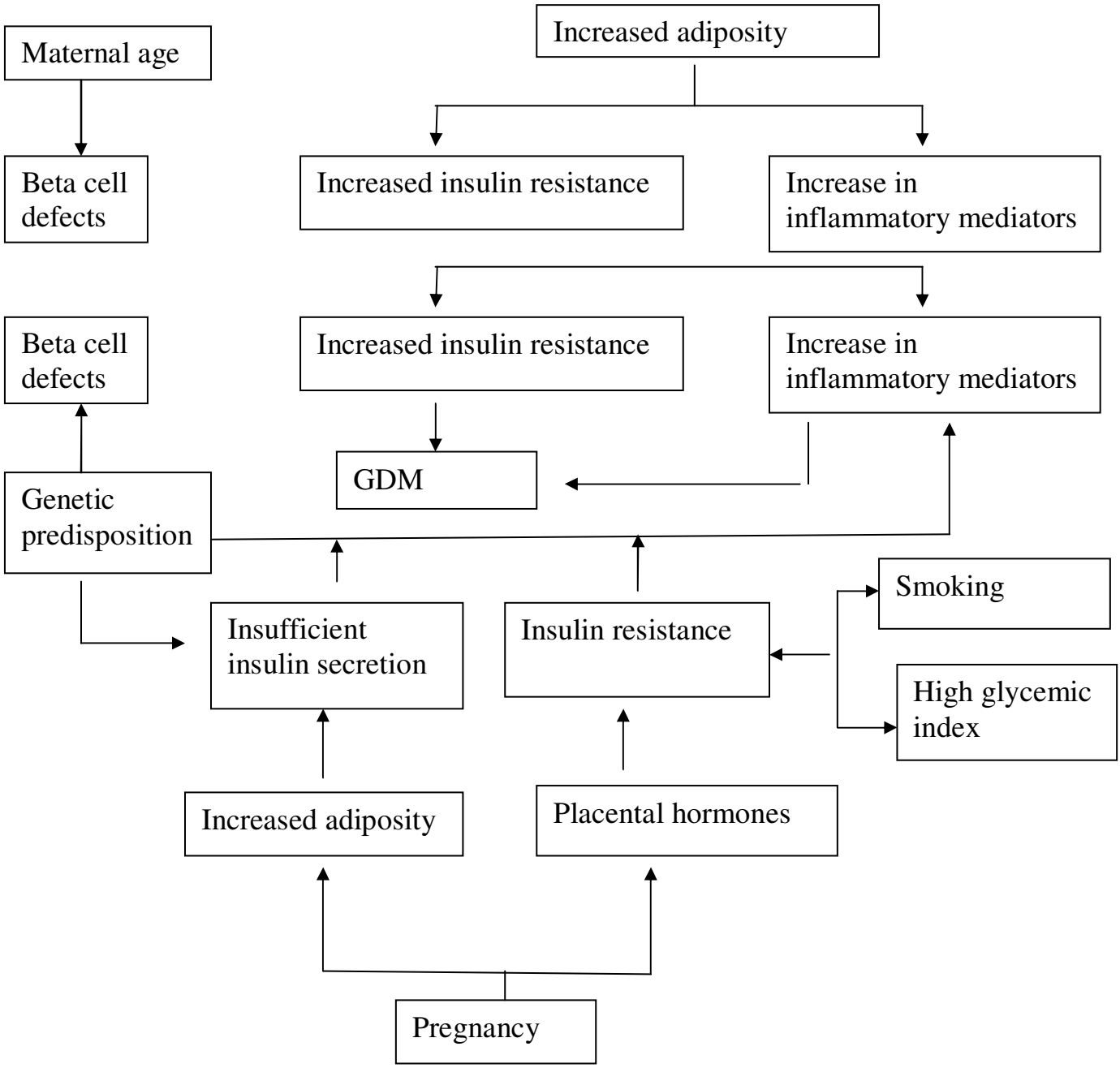
But another recent study done in UK in GDM women at end of third trimester of gestation reported that GDM women had significantly higher plasma levels of FGF21 than controls. The role of FGF21 in lipid metabolism and insulin resistance during pregnancy, need further study.

- **C- REACTIVE PROTEIN**:

Increased CRP levels and leukocyte count in the first trimester have been demonstrated to independently predict the subsequent development of GDM later in pregnancy. In one study, showed that circulating SPARC levels in the second trimester positively correlated with hsCRP levels, and with WBC count that were detected in the earlier trimester of pregnancy. Another previous study[34] also expressed a strong correlation between serum hsCRP and SPARC expression in adipose tissue .

PATHOGENESIS OF GESTATIONAL DIABETES MELLITUS:

It is a heterogenous disorder. Multiple factor responsible for the pathogenesis.



EFFECTS OF PREGNANCY ON DIABETIC CONTROL:

Insulin resistance and resultant hyperglycemia and consequent enhanced lipolysis are of use in a non diabetic pregnant women as it enhances nutrient transfer to the growing fetus.

However in a diabetic women, this can be seen as a form of accelerated starvation and predisposes to ketosis. With growing fetus, the diaphragm is pushed upwards, resulting in a relative increase in alveolar ventilation with consequent respiratory alkalosis and compensatory renal tubular loss of bicarbonate.

A decrease in serum bicarbonate and loss of acid buffering capacity partly explains the occurrence of diabetic ketoacidosis in pregnancy with normal or mild to modest elevation of glucose.

EFFECTS OF MATERNAL DIABETES ON THE PREGNANCY:

Developmental malformation and altered islet cell development and accelerated growth.

- Increased congenital anomalies
- Increased risk of miscarriage and late uterine death
- Pre-eclampsia
- Macrosomia
- Premature delivery and cesarean section rates
- Perinatal mortality rates

- anxiety to the patient
- increased cost of care
- The teratogenic effects of diabetes occur in the first 8 weeks of gestation, when the major organogenesis occur. These abnormalities seen in the heart, musculoskeletal system and nervous systems.

Congenital malformation can be related to the degree of hyperglycemia in early pregnancy and Hb A1c levels in the pre-pregnancy state. Enhanced delivery of glucose and other nutrients to the fetus results in accelerated fetal growth and macrosomia. This stimulates the islets and induces fetal hyperinsulinemia resulting in enhanced abdominal fat deposition, skeletal growth and organomegaly.

COMPLICATION ON THE NEONATE:

Fetal life risk:

- Intrauterine death
- Macrosomia
- Shoulder dystocia
- Hypoxia ,acidosis
- Nerve palsy

- Prematurity and
- Malformation

Neonatal life:

- Hypoglycemia
- Hypocalcemia
- Hypomagnesemia
- Respiratory distress syndrome
- Jaundice
- Polycythemia
- Cardiomyopathy.

Adult life:

- Obesity
- Type 2 DM
- Hypertension and cardio vascular disease.

For every 18mg/dl increase in fasting glucose level incident of macrosomia doubles.

- **IN 1993-1995 DENMARK STUDY SHOWED THE OUTCOME AND MATERNAL EFFECT OF 1215 WOMEN WITH TYPE 1 DM IN PREGNANCY:** Table-5:

MATERNAL OUTCOME	FOETAL AND NEWBORN OUTCOME
Caesarian – 56%	Respiratory distress syndrome -17%
Pre-termed delivery -42%	Neonatal jaundice -18%
Pre-eclampsia -18%	Congenital malformation -5%
	Perinatal mortality -3%
	Fetal macrosomia -63%

SCREENING FOR GDM:

Practically all pregnant women should undergo screening for glucose tolerance test. Screening test should be well defined, easily administered and reproducible and that should be significant sensitivity and some specificity. History is more important for early detection of case.

INDICATION FOR SCREENING:

HIGH RISK FOR GDM:

I. Family history of diabetic

II. Glucose in second fasting urine sample

III. History of unexplained fetal loss

IV. History of large for gestational age infant

V. History of congenitally malformed infant

VI. Maternal obesity.

LOW RISK FOR GDM:

I. Not known DM in first degree relatives

II. No history of abnormal OGTT

III. Age < 25 years

IV. Normal weight women before conception

V. Member of ethnic group with low prevalence population of GDM

VI. No prior history of poor outcome obstetric history.

- **METHOD USED FOR SCREENING:**
  - **1.HISTORIC RISK FACTOR:**

O"sullvian and coworker[35] found that 53% of 19 GDM had the history of risk factors compare to 43% without GDM. History like age, family history of DM, previous large baby birth and other obstetric complication. Sacks and coworkers studied history with age >25, weight >150 pounds and other risk factor was showed 97% of sensitivity for gestational diabetic. But it is difficult in case of first pregnancy. history and clinical risk factor have low sensitivity compare to other screening test.

- **2.SIMPLEST SCREENING PROCEDURE :**

It is detection of glucose in the urine. Low sensitivity and high specificity. It is not a good screening because renal glycosuria common in pregnancy. Lactosuria in 3rd trimester also give the positive results. Screening test can be accepted by demonstrating glycouria in the second fasted urine sample.

- **3.RANDOM BLOOD GLUCOSE LEVEL:**

Simple and easy to perform. Strangenberg et al(36) studies 1500 patients capillary blood sample without GDM. Identification rate was o.7%. Another study in kuwait, done with 276 patients without GDM. After the random blood glucose level, 250 patients under went GTT for conformation.3 had GDM and 46 patients had impaired glucose tolerance. So this test had insufficient screening test.

- **4.FASTING GLUCOSE AND POSTPRANDIAL GLUCOSE LEVEL;**

FBS is > 95mg/dl needed further diagnostic test. Sacks et al and daniele et al reported that 70% of the women did not need the diagnostic test and 19% of cases were missed.2 step approach used for diagnosis and preferable to challenge test.

- **5.ADA RECOMMENDS 2 STEP APPROACHS;**

A . One step approach[37]; Diagnostic method and doing oral glucose tolerance test without previous plasma glucose screening.

C. Two step approach: Initially by doing glucose challenge test (GCT) and followed by perform a diagnostic oral glucose tolerance test[38] (OGTT)

- **GCT**: Done by measuring the plasma glucose one hour after 50 grams of glucose. It has positive predictive value.

If the GCT more than 140 mg/dl, then go to OGTT with 100 gm of glucose.

- **O'sullivan and Mohan criteria**: Table-6:

TIME(hour)	100g OGTT(mg/dl)
Fasting	90
One hr	165
Two hrs	145
Three hrs	125

- **National and Diabetic data group**: Table-7:

TIME(hour)	100g OGTT(mg/dl)
Fasting	105
One hr	195
Two hrs	165
Three hrs	145

- 100 g glucose load (O'sullivan and Mohan criteria modified by Carpenter and Causen)- Table-8:

TIME(hour)	100g OGTT(mg/dl)	100g OGTT(mmol/l)
Fasting	95	5.3
One hr	180	10
Two hrs	155	8.6
Three hrs	140	7.8

- **BY USING 75 GMS OF GLUCOSE:** Table-9:

TIME (hour)	75g OGTT(mg/dl)	75g OGTT(mmol/l)
Fasting	95	5.3
One hr	180	10
Two hrs	155	8.6

Drawback of ADA guideline is same cutoff value with different glucose load, but in USA used this method. Other countries follow the WHO guide line. Schmidt et al found that prevalence of GDM was 2.4% with ADA criteria but 7.2% with WHO criteria in a pregnant population.

- 4^{th} international workshop on GDM and European association for study of DM concluded the following values with one with 75 gm and another study with 100 gm of glucose. Table-10:

4 TH INTERNATIONAL WORKSHOP ON GDM		**EUROPEAN ASSOCIATION FOR STUDY OF DM**	
75 gm OGTT dose(mg/dl)		100 gm OGTT dose(mg/dl)	
Fasting	95	Fasting	95
Two hrs	155	Two hrs	162

IADPSG: International association of diabetes and pregnancy study groups and DIPSI [39]: Diabetes in pregnancy study group india , values are -Table-11:

IADPSG (mg/dl)	**DIPSI (mg/dl)**
Fasting plasma glucose >92 mg/dl	_
1 hour post glucose >180 mg/dl	_
2 hour post glucose >153 mg/dl	Two hour post glucose >140 mg/dl

Disadvantages of OGTT:

- Non – reproducibility ,
- Time taken for test.

- **6.WHO CRITERIA**: It is simple and cost effective.
- WHO criteria---FPG ≥ 6.1 mmol/l and 2 Hour PPG is ≥ 7,8 mmol/l. WHO CRITERIA FOR 75 G OGTT : Table-12:

TIME	**IMPAIRED GLUCOSE TOLERANCE**(mg/dl)	**DIABETIC(mg/dl)**
Fasting	< 126	140—200
Two hrs	≥ 140 -199	>200

Pregnant women classified as GDM, who either meet IGT or Diabetes criteria. Impaired glucose tolerance and impaired fasting glucose are a stage before development of frank diabetes, when higher than normal values of blood glucose are observed.

- **7.GLYCOSYLATED HB A1C:**

Glucose tolerance during pregnancy only brief period of time before testing. In early pregnancy, erythropoiesis increased so new Hb which has not reached sufficient glycosylation.

- **8.FRUCTOSAMINE ASSAY:**

Fructosamine is associated with glycemic control over 1 to 3 weeks. Hoffman demonstrated fetal hyperinsulinemia associated with fructosamine >2.6mm in women with GDM. it is used for fetal screening not for routine GDM screening test.

- **9. INTRAVENOUS GLUCOSE TOLARENCE TEST:**

❖ Indication:

- It can be done in those with abnormal intestinal absoption.
- In pregnant women who do not tolerate the glucose tolerance test.

25 gms of glucose in 50% solution give IV over 3 min. samples collected every 10 mins for 1 hour and a graph of blood glucose against time plotted. The rate constant is calculated by a formula K = o.693 × 100 /1.5. Normally K is 0.9 to 2.3, if K is below 0.9, it indicates diabetes.

Advantage: is shorter procedure and eliminates irregular oral absorption. **Disadvantage**: is frequent blood collection.

MANAGEMENT :

Medical therapy:

Nutritional supplementation is main for GDM management. All patient with GDM should receive nutritional counseling. Treatment should be planned to achieve glycemic goals without weight loss or undue weight gain.

- Divide their caloric consumption, especially the breakfast atleast 2 hour interval to avoid undue peak of plasma glucose level.
- Dietary components in pregnant women:
  - Calories in 1st trimester—30 to 32 Kcal/kg IBW and
  - II , III trimester -38 Kcal/kg IBW
  - Carbohydrates---50-55% of calories (not < 200 gm)
  - Fats--< 30 % of calories.
  - Protein—1.5 – 2.0 gm/kg IBW
  - Fiber –20-40 g/day.

Fasting level should be maintained with 3.3 to 5.0 mmol/L and postprandial glucose level at 1 hour should be maintained with < 7.8 mmol/l.

- **INSULIN:**

If the nutrient therapy fail to achieve glycemic control by 2 weeks, insulin therapy should be started.

Indication for insulin therapy:

According to American diabetes association (ADA) is

1. Fasting plasma glucose >105 mg/dl
2. One hour post glucose >155 mg/dl,
3. Two hour post glucose >130 mg/dl,

NPH insulin can be started with low dose of 4 units and adjusted during follow up. Or combination of 2/3 rd of intermediate acting with 1/3 rd of short acting before breakfast.

INSULIN ANALOGS IN PREGNANCY[(40)]:

One complication of insulin therapy during pregnancy is insulin antibodies. Compare to regular insulin, the rapid acting insulin analogs are useful in GDM, as they are able to reduce postprandial hyperglycemia more efficiently. Lispro and Aspart and Glulisine are assigned in the pregnancy category "B".

- Demonstrated clinically more effectiveness
- No evidence of teratogenesis.
- Low antigenicity.

Long acting insulin like glargine are contraindicated in pregnancy as there is insulin like growth factor receptor affinity and mitogenic potency.

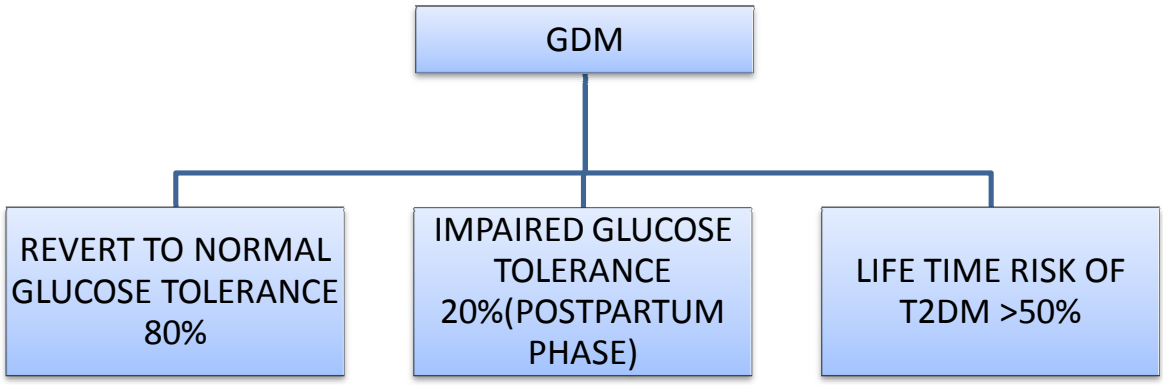
Insulin glargine and insulin detemer are considered category "C" by food and drug administration.

MANAGEMENT OF DIABETIC DURING LABOUR:

- Elective delivery and induction of labour at 38 to 39 weeks in poorly controlled blood sugar.
- **CESAREAN SECTION:** needed in case of
    1. Fetal weight is **>** 4.5 kg
    2. Malpresentation
    3. Disproportion
    4. Pre-eclampia
    5. History of previous still birth
    6. Poor maternal compliance
- During labour, blood sugar should be maintained between 72 mg/dl - 126mg/dl with regular insulin. Monitor blood glucose level every 1-2 hours.
- Following delivery, insulin requirement is sharply falls, so insulin should be reduced 25-40% of the pre-delivery dose to prevent hypoglycemia.

MANAGEMENT OF DIABETES DURING POSTPARTUM PERIOD:

- GDM patients should be reassessed at 6-12 weeks postpartum.
- The ADA recommends screening at 6-12 weeks of postpartum with a seventy five grams of oral glucose tolerance test.
- After delivery who have normal glucose level should be evaluated at least every 3 years or subsequent pregnancy.
- NATURAL COURSE OF GDM AFTER PREGNANCY:



HOMOCYSTEINE:

Butz and du vigneud[41] was coined the word Homocysteine and homocystine 70 years ago at Illinosis university.

- It is a non-protein forming sulfur amino acid .

- Metabolism of homocystine is interaction between the metabolic pathways of two reactions namely remethylation and trans-sulfuration respectively.
- The sulfur has an atomic weight of 32,064 and atomic number of 16. In 1977, Antoine Lavoisier indentified this element.
- "THIOL" refers to compounds containing sulfur of both the reduced (sulfhydryl) and oxidized (disulfide) forms.

An abnormality of this homocysteine metabolism explained by Carson and Naill in 1962. They were first identified from Northern Ireland siblings as a cause of mental retardation. In these patient vascular pathology such as smooth muscle proliferation, progressive stenosis of artery, and haemostatic changes.

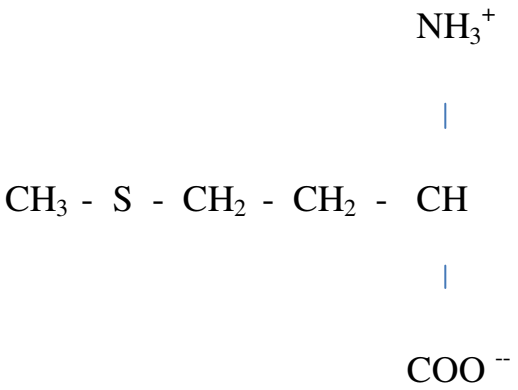
In last 20 years, lot of studies documented that moderate hyperhomocysteinemia is a harmful factor for arterial occlusive disease and thrombosis of veins. More than 50 percent of the cerebrovascular accident, chronic kidney disease and diabetes mellitus have the moderate rise in this level.

In pregnancy this homocysteine have the lot of complication in both mother and the fetus such as placental vasculopathy, congenital abnormalities – cleft lip, cleft palate and cardiac abnormalities, and spontaneous abortion when compare to control groups.

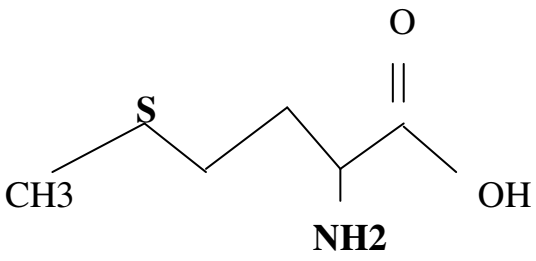
In some studies found that an association between increasing homocysteine level and cognitive impairment. And also an association with depression and other neuropsychiatric disorders was found. The recent identification of polymorphism of gene involving in homocysteine metabolism and there decreased enzyme activity has lent the research.

Formula of homocysteine is HSCH2CH2CH(NH2)CO2H. It is same like cysteine but it has extra methylene (-CH2-) groups.

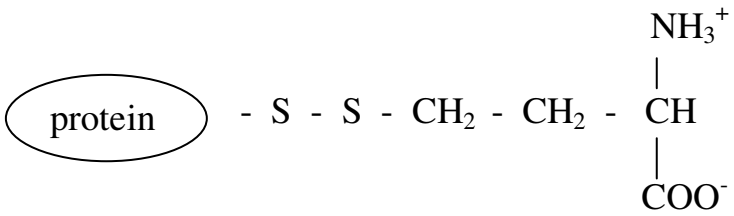
- **STRUCTURE**:-



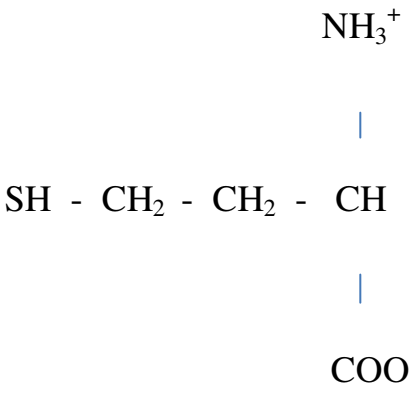
- **METHIONINE:**



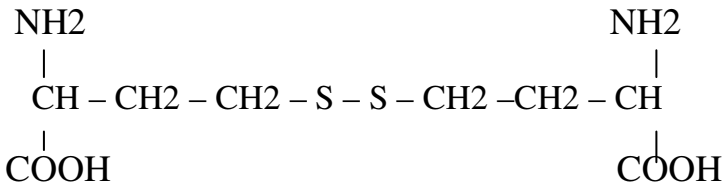
❖ **Protein bound homocysteine (70-80%)**



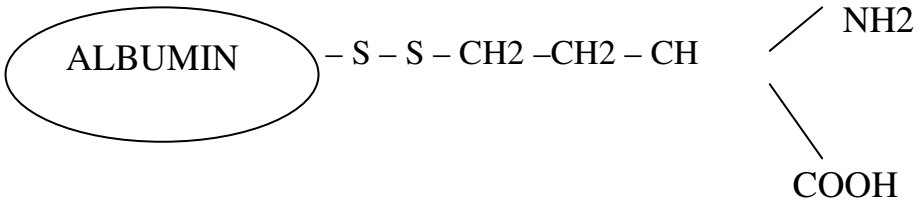
❖ **Reduced form:-**



❖ Mixed disulphide form:



❖ Albumin (protein)-homocysteine mixed disulphide:



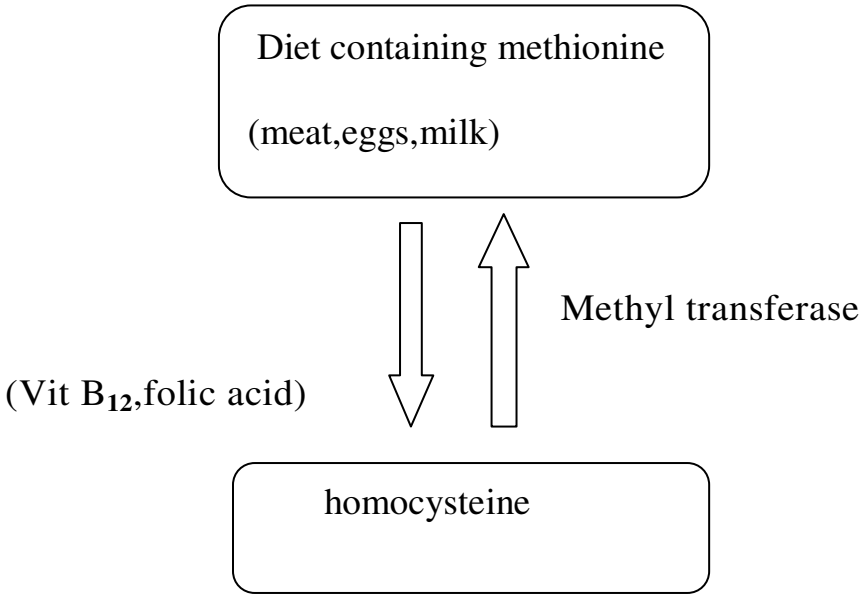
METABOLISM OF HOMOCYSTEINE[42,43]:

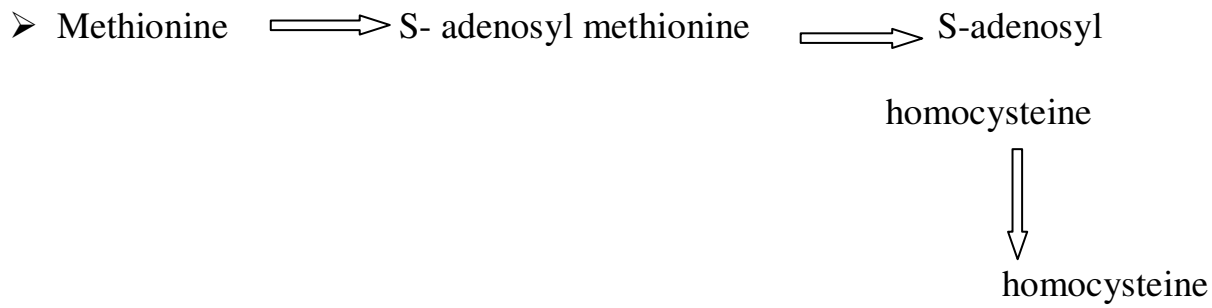
3 steps are there.

- Demethylation
- Transmethylation
- Transulphuration.

1. DEMETHYLATION;

Methionine is an one of the 9 essential amino acid. Human cannot synthesis it must be supplied by the diet, Methionine.



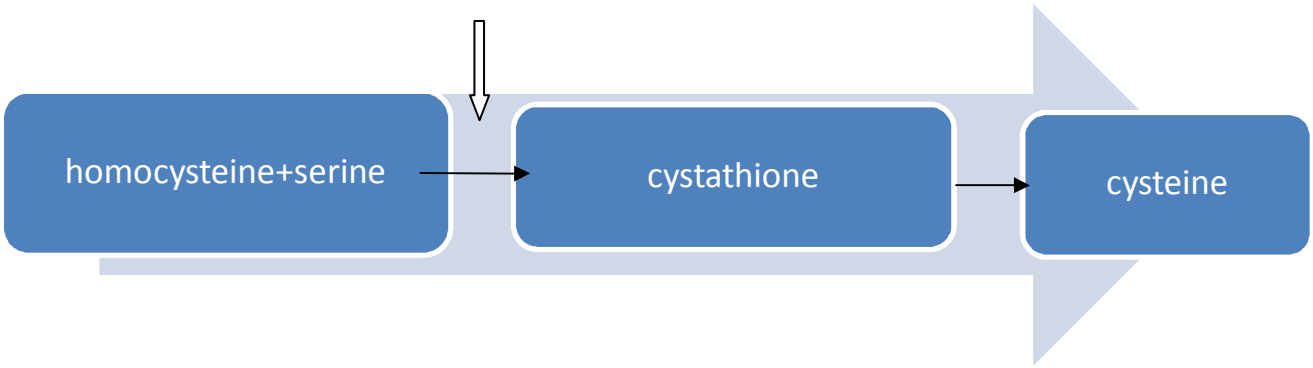


Methionine first converts into S-adenosyl methionine and become S-adenosyl homocysteine (SAH). SAH undergo the hydrolysis reaction to produce homocysteine and adenosine. Depending on the level of the methionine, homocysteine enter either transmethylation or transulphuration.

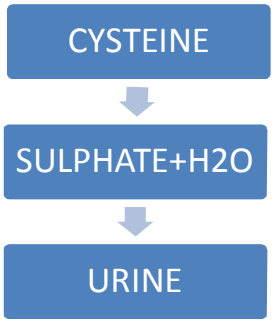
2.TRANSULPHURATION:

This pathway convert the homocysteine which condense with serine molecules to form cystathione which is not a reversible reaction. In the presence of cystathione beta synthetase and cystathione is hydrolysed by gamma cystathionase into cysteine and alpha keto butyrate. Both are vitamin B_6 dependent enzyme (pyridoxal -5-phosphate).

Cystathione β synthetase & B_6



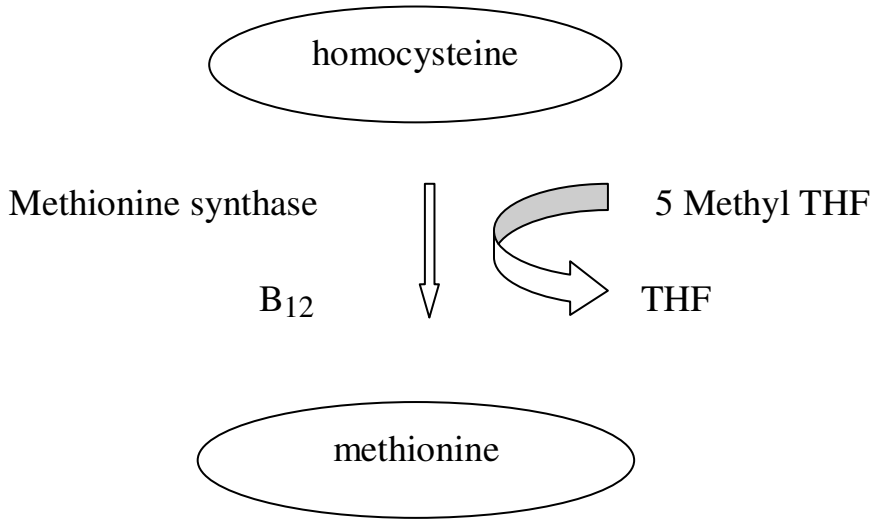
Cysteine either excreted into urine or in-cooperated with glutathione.



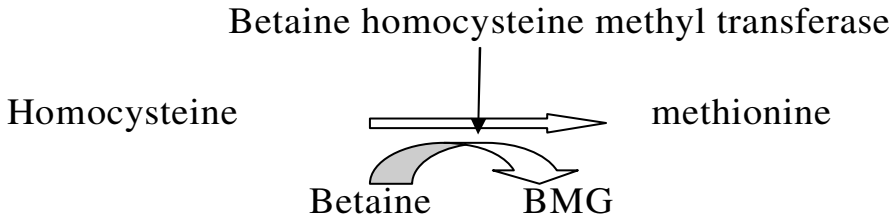
3.TRANSMETHYLATION:

In this reaction, homocysteine acquires a methyl group from the N-5-methyl tetrahydro folate (MTHF) or from betaine (trimetyl glycine) to form methionine. In our body, MTHF reaction occur in all tissues and it is vitamin B_{12} dependent process where as the reaction with betaine occurs mainly in liver and it is not vitamin B_{12} dependent.

B_{12}dependent pathway:



B_{12} independent pathway:



Metabolic abnormality of any of these reaction leads to accumulation of homocysteine and it produces lot of complications. So sulphuration reaction catabolizes excess homocysteine and delivers sulfate to synthesis of heparin, heparin sulfate, chondroitin sulphate and dermatine sulphate, because of the existence of a homocysteine catabolism plasma contain only a very small amount of homocysteine.

Total homocysteine is the sum of protein bound and free homocysteine. 80% of total homocysteine in circulation is bound to protein by disulphide bonds. In plasma rapidly it become oxidized and mostly present as a mixed disulfide with albumin, and small amount of free circulating disulfide forms.

S-adenosyl methionine provide methyl group to multiple reaction including methylation of DNA, RNA, proteins, phospholipids and myelin. So defect in methylation leads to defect in cellular growth, differentiation and function.

Neurochemical processes are delayed as in ageing, depression, and neuropsychiatric disorders. Congenital abnormality carcinoma due to DNA repair problems. Demyelination in severe inborn error metabolism due to lack of synthesis of methyl groups.

Glutathione which is an important endogenous antioxidants properties, and synthesis depends on the homocysteine transulphuration reaction. It helps to protect cellular component against vascular damage. It also have the possible vascular productive effect by interaction with nitric oxide.

HYPERHOMOCYSTEINEMIA:

Kang et al[44] described the abnormal homocysteine in normal individual. In healthy individual, fasting homocysteine level < 15 micromol/l. kang and coworkers, classify it into mild, moderate and severe according to the level. Table-13:

MILD	15 -3O µmol/l
MODERATE	30-100 µmol/l
SEVERE	>1OO µmol/l

FACTORS RESPONSIBLE ELEVATED SERUM HOMOCYSTEINE LEVEL:

- Inherited cause.
- Acquired cause

PRIMARY HYPERHOMOCYSTEINEMIA :

It is due to inherited causes. Genetic defect in genes encoding enzymes involved in homocysteine metabolism or depletion of important co-factors or co-substances for those enzymes, including vitamin B_{12}, folate,

and vitamin B_6 may results in elevated homocysteine level in plasma.

3 types of homocysturia : Depending upon the enzymes involvement, type I, II, and III.

- **Cystathionine beta synthase deficiency:**

  Commonest type of all three, and inherited as AR (autosomal recessive).It is mainly characterized by mental retardation, downward dislocation of eyes (ectopic lentis), marfanoid features and premature atherosclerosis.

  In these individuals, fasting plasma homocysteine concentrations can be as high as 400 micromol/l.

  Several CBS mutations are known at present, the most frequent are 833TC, and 919GA located in exon 8 and 1224-2AC which causes the entire exon 12 being deleted. 833TC is spread in several ethnic groups.

  The 919GA mutation has been almost exclusively reported in patients of celtic region. There are several novel mutation s indentified such as 146 CT, 172CT, 262CT, 346GA, 374GA, 376AG,869CT, and 904GA.

- **5,10 methylene tetra hydro folate reductase deficiency**:

  Abnormal in gene in chromosome 1 implicated as a cause of MTHFR reduction. More prevalence in Mediterranean region and 12% prevalence in Europeans. The thermolabile form of the enzyme MTHFR is a genetic

abnormality that occurs in 4% to 10% of the general population as a homozygous form.

C677T gene point Mutation leads to elevated homocysteine and low plasma folate level. It is commonly due to alanine-valine substitution. Person with homozygous mutation to have hyperhomocystenemic response with depletion of folic acid which leads to increased cardiovascular risk. And also A1298C mutation in MTHFR gene also indentified.

It have a worse prognosis than cystathionine-3- synthase deficiency, due to complete lack of effective therapy.

- **Methylene tetra hydro folate homocysteine methyl transferase deficiency**.

ACQUIRED CAUSES:

Secondary hyperhomocystenemia due to either physiological or secondary to organ dysfunctions.

- Physiologically elevated level seen in
  - ✓ Elder person (due to malabsorption of vitamins and amino acids),
  - ✓ Male gender,
  - ✓ Post menopausal women and coffee intake.

- Dietary deficiency of vitamins such as vitamin B12, B6 and folate.
- Smoking interfere with synthesis of pyridol phosphate .
- Tobacco also influence the homocysteine level.

Disorder associated with elevated level, systemic disorder such as

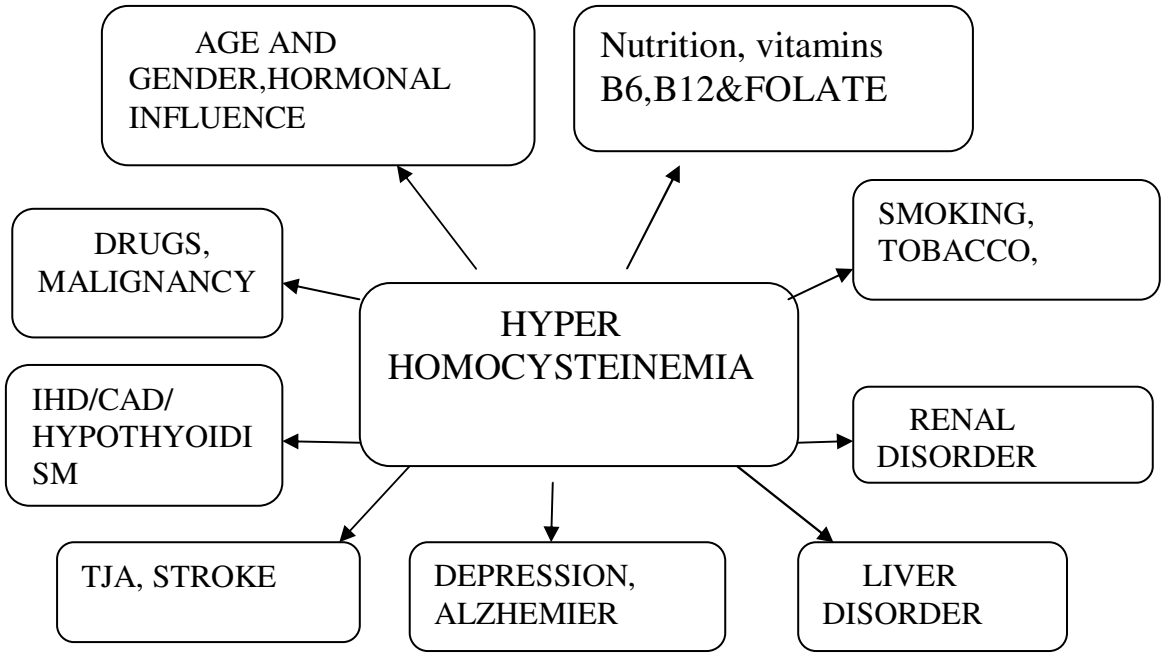
- Chronic renal disorders
- Chronic liver diseases
- Coronary artery disease
- Pernicious anemia –intrinsic factor deficiency.
- Alzhemier disease
- Diabetes mellitus
- Systemic lupus erythematosus
- Hypothyroidism – A number of studies suggesting a potential mechanism for vascular disease in hypothyroidism.
- Organ transplantation (cardiac and kidney)
- Drugs,
- Malignancy such as ovarian, breast and pancreatic cancer. ALL also associated with elevation, after chemotherapy for this disorder, it will be reduced.

Hyperhomocysteinemia has been reported in :Table-14:

Western people	11-22%
Chinese in Hong Kong	23-36%(premature coronary artery disease) 29% (nonselective series of coronary subjects)

.

Severe hyperhomocysteinemia is very rarely seen, but mild increase can occur in 5 to 7 % of general population. Further studies reveal that the prevalence estimates of hyperhomocysteinemia vary between 5% -30% in the general population.



AGE AND GENDER:

In both male and female plasma homocysteine level increases with increasing age. This may be due to differences intake of diet like vitamins and aminoacids in various age of people. Pre pubertal stage both gender have the same value. During puberty it is increasing but girls have lower value than boys. The gender disparity may be due to muscle mass, hormonal status and life style differences.

Postmenopausal women have elevated homocysteine level compare to menstruating women. Premenopausal women have decreased homocysteine level when compare to male. This may be cause of sex hormones.

In elderly patients, higher level of homocysteine due to

- ❖ Malabsorption food due to atrophic gastritis
- ❖ Inadequate nutritional intake
- ❖ Inadequate supplies of vitamins
- ❖ Reduced metabolism
- ❖ Lower kidney functions

NUTRITIONAL FACTORS:

Vegetarian diet containing very low amount of vitamins such as B_6, B_{12}. So vegetarian diet[45] patients, found with elevated level of homocysteine.

LIFE STYLE:

It determines the level of homocysteine.

- Lower level seen in patient with moderate amount intake of alcohol and normal physical activity[46].
- Higher level seen in excess alcohol intake, caffeine and smoking.
- Lack of physical activity, obesity and even stress leads to hyper homocysteinemia.
- In alcoholic person, may be due to vitamin deficiency or excess alcohol affects the one carbon metabolism.

PREGNANCY:

During pregnancy its level will be reduced owing to

- ✓ Increased plasma volume, or
- ✓ Increased metabolic rate and
- ✓ Glomerular filtration rate.

LIVER DISEASE:

Synthesis and metabolism of homocysteine take place in the liver. So it has important role. In chronic liver disorder[47]its level elevated. It is mainly by

1. Decreased and utilization of B-complex vitamins
2. Gene expression defect which is involved in its metabolisms.

Turkish researches evaluated, they found that homocysteine were significantly elevated in non alcoholic fatty liver disease. They published in a September 2005 edition of gastroenterology and hepatology.

RENAI DISEASE[48]: Elevated homocysteine in CKD are independent risk factor for coronary heart disease. Elevated level in chronic kidney disease due to

- ❖ Reduced systemic clearance
- ❖ Enzyme inhibition
- ❖ Presence of MTHFR thermolabile polymorphism
- ❖ Absolute or relative deficiency of folate
- ❖ Reduced folate absorption
- ❖ Enhanced folate excretion in haemodialysis patients

ORGAN TRANSPLANTATION:

Approximately 54 – 87 % of orthotopic heart transplant recipients develop hyperhomocysteinemia, which emerge after three months of transplant. In kidney transplantation as many as 29 % of patients having elevated level depending upon the folate level and degree of renal impairments. The changes in homocysteine level after renal transplantation, reported by In 1981 Wilcken et al[49]. Immunosuppression used in after transplant also important implication. Another study done by Massy et al and also Bostom et al[50]explained the increased prevalence of hyperhomocysteinemia in post renal transplant patients compared to control group.

HOMOCYSTEINE IN MALIGNANCY:

Methionine –dependent malignant cells in organs of lung, kidney, breast, bladder and colon cannot convert the homocysteine to Methionine. It results in homocysteine accumulations. So in lung cancer it can be used in early cancer biomarker. DNA methylation plays a role in gene regulation for control of normal growth of cell, differentiation and apoptosis. Microarray- based studies explained that hypomethylation contribute to cancer initiation and progression. its level also increased in ovarian, pancreatic, colorectal , head and neck squamous cell carcinomas.

DRUGS: Many drugs influence the Plasma homocysteine level.

The following drugs elevates the homocysteine level:

- ✓ Immunosuppressive methotrexate which is folate antagonist,
- ✓ Anti epileptic such as phenytoin and carbomazepine.
- ✓ Theophylline, PDE inhibitor which antagonizing the synthesis of pyridol phosphate .
- ✓ Cholesterol lowering drugs[(51)]like cholestyramine, colestipol interferes with absorption of vitamin B_{12} and folic acid.
- ✓ Metformin which is used in the treatment of diabetes decreases the absorption of cobalamin and increases the serum homocysteine level.
- ✓ Bezafibrate and fenofibrate alters renal function so increases the serum homocysteine level.
- ✓ Nitrous oxide increase serum homocysteine by causing impairment of the enzyme methionine synthase.
- ✓ Levodopa used in the treatment of Parkinson disease increases the serum homocysteine level by increasing the formation of S-adenosyl homocysteine.
- ✓ Niacin and theophylline causes vitamin B6 deficiency and hence increases homocysteine level.

SOME OF THE DRUGS LOWER THE HOMOCYSTEINE LEVEL:

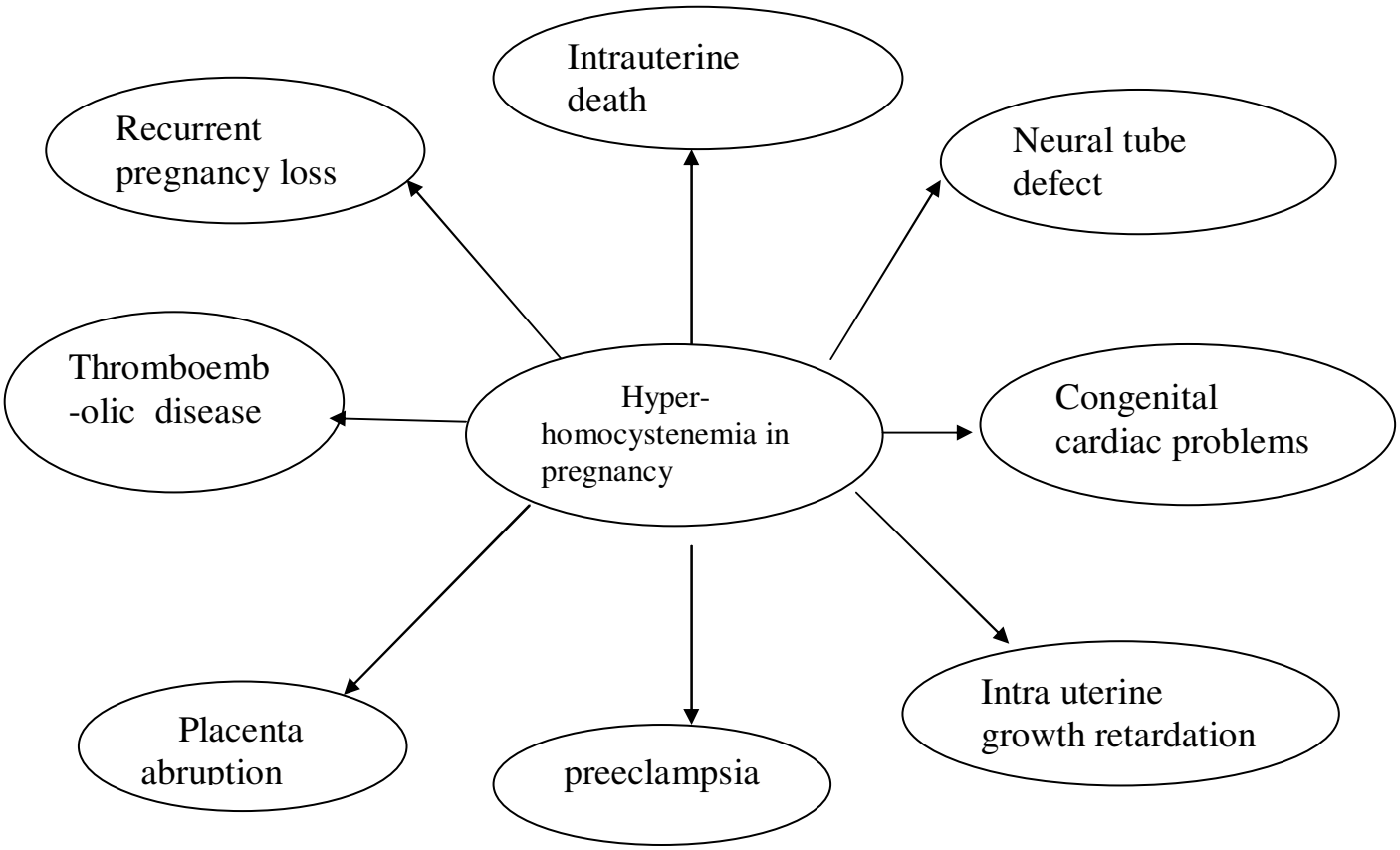
- ✓ Penicillamine, Acetylcysteine are interferes with disulphide exchange reactions.
- ✓ Oestrogen[52] containing oral contraceptives may have a beneficial effect on plasma homocysteine level.
- ✓ Tamoxifen is the drug used in the treatment of breast cancer also showed to reduce plasma homocysteine level.
- ✓ Betaine increases vitamin B_{12} independent remethylation of homocysteine thereby reducing its level in plasma.

COMPLICATIONS OF HYPERHOMOCYSTEINEMIA:-

- ➢ Coronary artery disease
- ➢ Ischemic heart disease
- ➢ Venous thromboembolism
- ➢ Peripheral vascular disorder
- ➢ Hypertension
- ➢ Diabetes vascular complication
- ➢ Osteoporosis
- ➢ Dementia and alzhemier disease
- ➢ In eye, central retinal artery occlusions and

- **In pregnancy complications** ,such as
    - Recurrent fetal loss,
    - Intra uterine death
    - Thrombo-embolic disease in pregnancy
    - Neural tube defect,
    - Congenital cardiac complication
    - Pre-eclampsia,
    - Placental abruption
    - Intra uterine growth retardation.

In pregnancy complications:



HOMOCYSTEINE AND DIABETES MELLITUS:

Moderate elevation of plasma homocysteine level was observed in lot of studies in diabetic patients[53]. Plasma homocysteine level in adolescent DM patient without vascular complication had similar level when compare to non diabetic control group. The homocysteine levels were not dependent of vitamin status and it reflect the various factor of the patients, including patients with poor glucose control, longer duration of diabetes and its complications.

DETERMINANTS :

- **Genetic factors :**

  The most common genetic cause is the C677T mutation of the methylenetetrahydrofolate reductase (MTHFR) gene. The prevalence of this mutation among different ethnic groups with a prevalence of 0–2% in Africans,12% in Whites and 20% in Asians. A recent meta-analysis has shown a significantly higher risk of ischemic heart disease in people with the MTHFR mutation

  In a study of 354 patients with type 1 diabetes, the C677T mutation has no significant changes in plasma homocysteine levels. Although the MTHFR polymorphism has been observed more frequently in patients with diabetes with nephropathy compared with those without nephropathy.

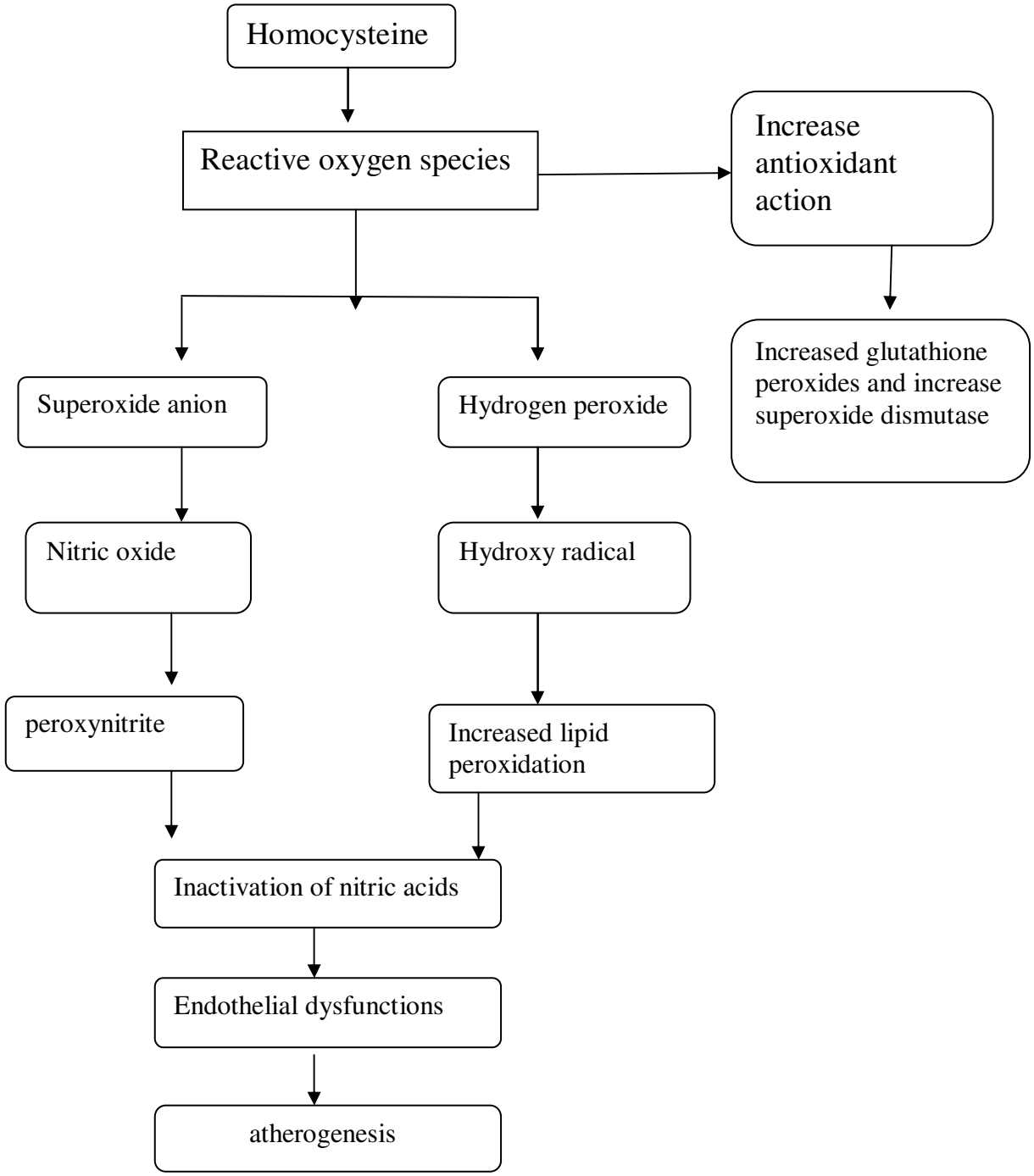
The incidence of heterozygotes for cystathione beta-synthase deficiency in the general population is less than 1% and has not been studied in diabetes.

- **NUTRITION:**
- Folate and vitamin B_{12} deficiency lead to reduced remethylation of homocysteine to methionine and increased plasma homocysteine levels . In most studies of plasma homocysteine levels in diabetes, serum B_{12} and folate levels was within the normal range.
- Riboflavin (vitamin B_2) is a co-factor for MTHFR and deficiency of riboflavin associated with increased plasma homocysteine levels, particularly in patient with low folate status and the MTHFR C677T mutation.
- vitamin B_6 deficiency, the trans-sulphuration pathway is only mildly impaired and most studies have found that in vitamin B_6 deficiency , elevation of homocysteine after oral methionine loading. There is no evidence of riboflavin or vitamin B_6 deficiency in patients with diabetes. Nutritional deficiencies may lead to mild hyperhomocysteinaemia are more common in elderly patients and strict vegetarians.

➢ CORONARY ARTERY DISEASE AND HOMOCYSTEINE:

✓ ENDOTHELIALDYSFUNCTIONBYHOMOCYSTEINE:

Endothelium necessary in maintaining vascular tone and the endothelial permeability. Nitric oxide (NO) is the prime regulator for the vascular tone.



Its role in coagulation cascade through thrombomodulin- protein C and heparin sulphate and anti thrombin III interactions. Fibrinolytic functions of the Endothelium which are mediated through plasminogen activator inhibitor -1, tissue plasminogen activator (t-PA). another action influenced by endothelial cells is the sub-endothelial matrix composition and smooth muscle cell proliferation. Homocysteine causes disruption of endothelial layer and changes it character to pro-coagulant form from anti-coagulant properties.

- It causes direct injury to endothelial cells by generating free oxygen radicals. So promotes the vascular endothelial injuries.
- It Impairs the endothelium-dependent relaxation. Homocysteine impairs the vasodilation mediated by endothelial cells. This endothelium dependent vasodilation is due to production of nitric oxide (NO).
- In the presence of increased homocysteine levels, nitric oxide is decreased and thereby affecting endothelium dependent vasodilation . Homocysteine promotes lipid peroxidation, that in turn decreases the expression of NO synthase enzyme in endothelial cells. It also increases the degradation of NO.

- Alters the coagulant properties of blood.
- HCY undergoes auto oxidation to form homocysteine mixed disulphides and homocysteine thiolactate. During this auto oxidation, superoxide anion, hydrogen peroxide, hydroxyl radicals are produced. These free radicals causes vascular injury.

- Superoxide radicals causes oxidation of low density lipoprotein. High serum levels of homocysteine also increase reduced homocysteine level inside the cells. It leads to reduces intracellular glutathione and NAD^+ and alters the ratio between the reduced glutathione and oxidized glutathione.
  - Homocysteine help to enhance the expression of monocyte chemoattractant protein -1(MCP-1) in endothelial cells. This MCP-1 increases the transmigration of monocytes from sub-endothelial matrix and promotes atherosclerosis.
  - Homocysteine increases the proliferation of smooth muscles present in sub-endothelial matrix. In an animal experimental study conducted in rat showed that aortic smooth muscle cells, increased DNA synthesis were noted after exposure to homocysteine. During this studies, increased cyclin D1 and cyclin A mRNA levels also were noted. It increases collagen deposition in the atherosclerotic plaque.

Homocysteine and hypertension[54]:

Vascular risk associated with elevated Homocysteine observed in hypertensive individual, because of the positive correlation of homocysteine with hypertension. Now a days most of the studies has been focused on the relations between plasma homocysteine and blood pressure.

The observational study of the third National Health and Nutrition Examination Survey (NHANES III), persons in the highest quintile of plasma homocysteine had a 2- to 3-fold increased prevalence of hypertension relative to those in the lowest quintile. Role for homocysteine in the pathogenesis of elevated blood pressure is explained by the demonstration that homocysteine-lowering treatment is associated with a reduction in systolic and diastolic blood pressures.

A role for plasma homocysteine in the pathogenesis of hypertension:

- Increased arterial stiffness
- Impaired endothelial integrity
- Reduced vasodilatory capacity
- Insulin resistance

Study conducted in Srilanka, by Mendis S and et al identified the association between hypertension and homocysteine level. They concluded that serum homocysteine level was significantly higher in patients with hypertension. Also Bortolotto LA et al demonstrated that increased homocysteine levels in hypertensive patients was associated with increased arterial stiffness.

HOMOCYSTEINE AND CEREBRO VASCULAR DISEASE:

A elevated homocysteine level is associated with a higher risk of strokes. Carotid stenosis appears to role in response to increased levels of homocysteine. Increased carotid plaque thickness often associated with high homocysteine and also low B_{12} levels.

Yoo et al studied in both intracranial and extracranial vessels by MR angiography and reported that homocysteine levels was higher in patients with 2- or 3-vessel stenosis than in those with 1-vessel stenosis.

Boushey et al [(55)] done a meta-analysis including many studies regarding the association between the homocysteine and cerebrovascular disease. Out of that 9 studies showed that increased homocysteine were an independent risk factor for stroke.

In a prospective study conducted from Netherlands, positive association between hyperhomocysteinemia and stroke was demonstrated. Also graded linear association between homocysteine and risk of stroke has been observed in the prospective study, British Heart study.

LIST OF STUDIES : Regarding the association between homocysteine and cardiovascular disease, peripheral vascular disease, stroke;

This showed study conducted in the year of 1992 and 1994.: Table-15:

Year ,Author	Population	Endpoint of study	Follow-up (years)	Co-relation
In 1992 Stampfer et al	U.S physicians	MI / death	5 years	Positive
In 1994 Alfhan et al	Community	MI	9years	Negative
In 1994 Verhoef et al	U.S physicians	Stroke	5years	Negative

Studies from 1995 to 1996: Table-16:

Year ,Author	Population	Endpoint of study	Follow-up (years)	Co-relation
In 1995 Perry et al	Community	Stroke	13	Positive
In 1995 Arnesan et al	Community	MI	3-4	Positive
In 1996 Petri et al	SLE patients	Stroke/ CVD	4.8	Positive
In 1996 Chasan taber et al,	U.S physicians	MI /death	7.5	Negative

Studies from 1997 to 1999: Table-17:

Year ,Author	Population	Endpoint of study	Follow-up (years)	Co-relation
In 1997 Ridker et al	U.S physicians	DVT	10	Positive
In 1997 Evans et al	Community	MI /death	20	Negative
In 1997 Verhoer et al	U.S physicians	Angina/CABG	9	Negative

In 1998 Folsom et al	Community	CAD /death	3.3	Negative
In 1998 Moustapha et al,	ESRD	CVD /death	1.5	Positive
In 1999 Taylor et al	PVD	CVD /death	3	Positive
In 1999 Bots et al	Community	Stroke /MI	4	Positive
In 1999 Bostom et al	Framingham cohort	CVD / death	10	Positive
In 1999 Nahlawi et al	Heart transplant patients	CVD / death	2.4	Negative

HOMOCYSTEINE AND PERIPHERAL VASCULAR DISEASE[56]:

A study by Clarke et al, Hyperhomocysteinemia was observed

a) In 7 of 25 with peripheral vascular disease (in 28% of patients with PVD),
b) In 16 of 38 patients with cerebrovascular disease (42% of patients with cerebrovascular disease) and
c) In 18 of 60 with coronary vascular disease (30% of patients with CAD).

In the present issue of *Circulation Research*, Chang et al showed that a specific homocysteine-induced down regulation of fibroblast growth factor (FGF)2

involved in the disruption of endothelial integrity by both reducing endothelial cell proliferation and inducing endothelial cell apoptosis. Recently, new studies providing insight into the regulatory effects of elevated homocysteine levels are necessary for the development of new diagnostic and therapeutic research.

At the molecular level studies proposed several potential mechanisms: including

- Induction of endoplasmic reticulum stress
- Unfolded protein response (UPR),
- Protein *N*-homocysteinylation, and
- Epigenetic effects concerning methylation status

And also homocysteine reduces the expression levels of endothelin-1, a well-known potent vasoconstrictor.

The recent observational studies showed that hyperhomocysteinemia inhibits reverse cholesterol transport by reducing circulating HDL via inhibiting the antioxidant apolipoprotein A-I protein synthesis and increasing HDL cholesterol clearance seems special relevant.

HOMOCYSTEINE AND VENOUS THROMBOSIS:

In many of the studies found that increased homocysteine level is an independent risk factor for venous thromboembolism. Approximately 25 retrospective studies, two recent prospective studies have demonstrated that hyperhomocysteinemia is an independent risk factor for premature arteriosclerotic disease in the coronary, cerebral, and peripheral arteries.

Brattström et al found that no significant difference in plasma homocysteine concentrations between 42 patients with VTE and healthy control subjects. Bienvenu et al reported that 7 of 23 patients with VTE was significantly increased (>2 SD) homocysteine levels and concluded that hyperhomocysteinemia is a risk factor for venous and arterial thromboses.

Den Heijer and et al[57] demonstrated high levels of serum homocysteine with recurrent venous thrombosis patients. They estimated homocysteine level in patients with age less than 70 years with first episode of deep vein thrombosis and concluded that mild hyperhomocysteinemia is a risk factor for deep vein thrombosis.

A plasma homocysteine level more than 22µmol/liter increases the odds ratio of 4 for recurrent venous thromboembolism.

HOMOCYSTEINE HAS BEEN LINKED TO VASO-OCCLUSIVE DISEASES IN THE EYE[58] :

Ocular complications associated with Homocysteine include

- Ectopia lentis,
- Secondary glaucoma,
- Optic atrophy,
- Age-related macular degeneration (ARMD),
- Central retinal vein occlusion (CRVO), and
- Diabetic retinopathy

HOMOCYSTEINE AND OSTEOPOROSIS[59]:

Homocysteine elevation may weaken the bone by interfering with collagen cross linking, thereby increasing the risk of osteoporotic fracture.

Methods used for Measuring Homocysteine Concentrations :

Measurement of Homocysteine levels by using

- High-Performance Liquid Chromatography (HPLC): it can be detected during HPLC as a fluorescent derivative or by direct electrochemical detection. Competitive immunoassays for homocysteine are available in the clinical laboratory enabled more laboratories to perform this test. These

assays are based on quantitative enzymatic conversion of Homocysteine to S-adenosylhomocysteine.

- CE-LIF- capillary electrophoresis with laser induced fluoresce detection, electrochemical detection, EIA-enzyme immune assay[60], LC-liquid chromatography, and UV-ultra violet.
- Simple colorimetric enzyme assays for Homocysteine, to be performed on routine clinical chemistry analyzers. These are based on either an enzymatic cycling assay or on enzymatic release of hydrogen sulfide which reacts to form a chromogen.
- Another option is Tandem mass spectrometry.

All methods appear to offer adequate analytical performance for routine clinical use. Choice of method therefore usually will depend on practical considerations of cost, labor efficiency, and which analyzers are already available in the laboratory. There is a trend for an increasing number of clinical laboratories to use routine chemistry analyzers rather than immunoassay or HPLC methods.

TREATMENT OF HYPERHOMOCYSTEINEMIA

Plasma homocysteine level is influenced by many factors like genetic determinants, lifestyle habits and physiological conditions. Among these factors dietary intake of vitamin B_6, B_{12} and folate is a major determinant.

Plasma homocysteine level is inversely related to dietary intake of these vitamins. Persons following strict vegetarian diet may develop vitamin B_{12} deficiency. Studies have shown increased serum homocysteine level in vegetarians compared to non-vegetarians [(61)].

Several studies have been conducted to assess the reduction in plasma homocysteine level with supplementation of vitamin B_6, B_{12} and folate.

A study conducted by Martin den Heijer, Ingeborg A. Brouwer and et al[(62)]showed that supplementation of 0.5µg of folic acid reduces homocysteine level by25% (range -54% to 40%) and supplementation of 0.4mg of hydroxyl cobalamine reduces homocysteine level by 10% (range -21% to 41%).

TREATMENT REGIMENS:-

- **Nutritional supplementation**:
- B_{12} vitamin is found in foods of animal origins. Food sources: Table-18:

FOOD(B_{12})	**AMOUNT (mcg)/100 Gms of food**
Liver	70.58
Seafood	6.88
Eggs	1.11
Beef Hamburger	2.50
Salmon	2.80

- **Folic acid** – found in
  - Green leafy vegetables such as spinach, broccoli,
  - Legumes such as lentils, chick peas, lima beans
  - orange.
- **Vitamin B_6** - found in
  - Meat, poultry, fish, green leafy vegetables,
  - legumes, seeds, potatoes, cantaloupe, milk,
  - egg yolks, cereals, grains, wheat, wheat germ.
- **Betaine**, a choline derivative, found in
  - Wheat Bran, Wheat Germ, Spinach, Beef Liver, Dried soybeans and Pork**.**

Treatment of hyperhomocysteinemia is with supplementation of vitamin B_6, B_{12} and folic acid. Effect of vitamin B_{12} and folic acid in the treatment of hyperhomocysteinemia is documented well in many studies. But the effect of vitamin B_6 is not well established.

- Regimen I:
  - 650µg folic acid ,
  - 100mg vitamin B_6 along with
  - 400µg vitamin B_{12} for 6 weeks.

- Regimen II:
  - 5mg folic acid
  - 250mg vitamin B_6 for 12 weeks.

CHOLESTEROL LEVEL IN PREGNACY:

Total plasma lipids is 400-600 mg/dl. The lipids are insoluble in water, so it complex with proteins to form lipoproteins. The protein part of lipoprotein called apolipoprotein.

Classification of lipoproteins:

Depending upon the density, or electrophorotic mobility, the lipoprotein classified into 5 major types.

1. Chylomicrons
2. Very low density lipoprotein or pre-beta lipoprotein
3. Intermediate density lipoprotein or broad- beta lipoprotein

4. Low density lipoprotein or beta lipoprotein

5. High density lipoprotein or alpha-lipoprotein**.**

Total cholesterol = High density lipoprotein **+** Low density lipoprotein **+** (0.2×triglycerides).

VALUES : Table-19:

	Total cholesterol(MG/DL)	**LDL(MG/DL)**	**TG(MG/DL)**
Normal	Less than 200	100 to 129	Less than 150
Border line	200 to 239	130 to 159	150 to 199
high	More than 240	More than 160	More than 200

Marked elevations of total plasma cholesterol and triglyceride levels[(63)] is present during pregnancy due to increased liver synthesis of triglycerides(TG). TG in particular rise disproportionately in comparison to other lipid fractions reaching two to four times pre-pregnancy levels by the third trimester[(64)].

And in response to elevated oestrogen levels Very Low Density Lipoprotein-Cholesterol (VLDL-C) also increased. Reduction in Lipoprotein lipase (LPL) activity due to the down regulation of LPL gene expression by oestrogen

during pregnancy decreases the clearance of VLDL-C. Maternal factors[65] are highly influence on lipid profiles such as

- Body mass index,
- Weight gain of pregnant women,
- Nutrition of the pregnant women,
- Pre-pregnancy lipid levels and
- Medical complications of pregnancy also have significantly affects the lipid metabolism and plasma lipid levels.

During the first trimester, there is marked deposition and hypertrophy of maternal adipocytes with increased expression of insulin receptors such that glucose is available to meet the metabolic demand of the growing fetus.

- ❖ Increase in maternal insulin and progesterone leads to
  - o Lipogenesis with diminished lipolysis, and
  - o Increased production of lipids, which are transported across the placenta and metabolized. This signifies the essential role of lipids to normal fetal development.

Cholesterol levels start to rise in the second trimester of gestation and peak during the third trimester. However, these changes are generally non-atherogenic, and fall precipitously to pre-pregnancy levels around 4 weeks of post

partum period. Cholesterol levels dropped most quickly in women who is breastfeeding their babies.

LIPIDS AND HYPERTENSIVE COMPLICATIONS IN PREGNANCY:

1. Chronic hypertension, where elevations in blood pressure precede the conception.
2. Gestational hypertension, is elevated blood pressure without proteinuria occurring during pregnancy.
3. Preeclampsia is defined by new-onset gestational hypertension and proteinuria occurring in approximately 5-8% of pregnancies and is a major source of maternal and fetal morbidity and mortality[66,67]

PATHOGENESIS:

- Inadequate remodeling of the placental vasculature, leading to reduced placental perfusion.
- Uterine vascular hypoplasia along with maternal factors of genetics, hypertension, diabetes, obesity, androgen secretion, and black race, then lead to the maternal manifestation of preeclampsia, with associated endothelial dysfunction and marked systemic inflammation.

- Preeclampsia that is associated with more pronounced hypertension, oliguria, non-cardiogenic pulmonary edema, elevated liver enzymes, thrombocytopenia of less than 100,000/m^3, and neurologic deficits is classified as severe disease.

LIPIDS AND DIABETES MELLITUS IN PREGNANCY:

Lipid profiles in women with preexisting uncomplicated DM of type 1 is similar to healthy women without diabetes[(68)]. But risk factors such as obesity, hypertension, poor glycemic control, and preeclampsia, type 1 DM is associated with higher elevations in first trimester TG levels and lower levels of HDL-C in comparison to normal pregnancy.

Women with preexisting type 2 DM have higher TG and lower HDL-C levels during the first trimester without significant change in LDL-C and Lp(a) levels in comparison to normal[(69)].

Women with gestational diabetes may have increased to unchanged TG and TC levels and stable LDL fractions throughout gestation although these results have been equivocal.

Maternal obesity, on the other hand, with or without overt gestational diabetes, is associated with atherogenic lipid profiles and poor pregnancy outcomes, due to inflammation and endothelial dysfunction[(70)]. Obese pregnant

women is more frequently associated with elevated TG and small, dense LDL fractions with low HDL-C levels. Obese mothers will give large for gestational age, and may risk for cardiovascular events later in life.

AIM AND OBJECT

1. To estimate serum homocysteine in gestational diabetes mellitus.
2. To compare the serum homocysteine levels in gestational diabetes mellitus and in normal pregnancy.

MATERIAL AND METHODS

SETTING : Inpatients,

Thanjavur medical college hospital,

Obstetrics and Gynecology department,

Thanjavur.

ETHICAL COMMITTEE APPROVAL : Obtained.

DESIGN OF STUDY : Single centre prospective Observational study.

PERIOD OF THE STUDY : December 2013 to June 2014

SAMPLE SIZE : 50 patients.

METHOD OF COLLECTION OF DATA:

The present study was done on 50 patients admitted to RMH department of Obstetrics and Gynaecology department Thanjavur medical college, Thanjavur. Out of 50 patients, 20 patients were normal pregnancy and 30 patients were diagnosis as GDM with glucose challenge test and confirmed by OGTT as per WHO criteria and ADA criteria. A detailed history and thorough clinical examination was done as per proforma and investigated further.

SELECTION OF THE PATIENT:

INCLUSION CRITERIA:

- Gestational diabetic mellitus in primi gravida
- Gestational diabetic mellitus in multi gravida and
- Normal pregnancy.

EXCLUSION CRITERIA:

- Type 1 diabetes mellitus
- Known case of type 2 diabetes mellitus
- Family history of diabetes mellitus
- Coronary artery disease
- Chronic kidney disease.

STUDY METHODOLOGY:

Totally 50 patients were estimated for serum homocysteine level. In that 20 patients of normal pregnancy were under the control groups. Out of that 10 primi and 10 multi gravid. Another 30 patients were newly diagnosed gestational diabetes mellitus, out of that 15 patients were primi and another 15 patients were multigravida.

Only newly diagnosed GDM patients were included in this study and excluding other risk factor such as Type 1 diabetes mellitus, Known case of diabetes mellitus, Family history of diabetes mellitus, Coronary artery disease and Chronic kidney disease. Diagnosis of GDM was made by characteristic history, urine sample for sugar, random blood sugar. and confirmed with 1^{st}Glucose challenge test , if 1 hour post glucose test value >14o mg /dl then, we did oral glucose tolerance test.

INVESTIGATIONS;

The following investigations were done .

- Fasting Serum homocysteine level,
- Complete hemogram,
- Fasting blood sugar, post prandial blood sugar,

- Renal function test (urea, creatinine),
- Lipid profile –Total cholesterol and Triglyceride,
- Glucose challenge test,
- Oral Glucose tolerance test,
- Urine sugar ,
- VCTC,
- Ultra sonogram for fetal well being and ECG.

MEASUREMENT OF HOMOCYSTEINE LEVEL:

Serum homocysteine level were estimated with using Flurorescense Polarisation Immuno Assay(FPIA)

PRINCIPLE OF THE PROCEDURE:

First step is oxidized form of homocysteine is reduced to free form of homocysteine by use of dithiothreitol.

Second step is, in the presence of SAH hydrolase and excess adenosine, the reduced homocysteine converted into S-adenosyl- L –homocysteine(SAH).

$$\text{Free form of homocysteine + adenosine} \xrightarrow{\text{SAH hydrolase}} \text{SAH} + \text{H2O}.$$

This mixture containing SAH, antibody, FPIA diluent buffer and a tracer tagged with a fluorescent chromophore were added to the cuvette. There will be competition between SAH from the serum sample and the fluorescent tagged tracer to bind with the antibody. Then the intensity of the polarized fluorescent light is measured using FPIA optical assembly.

SPECIMEN COLLECTION AND STORAGE:

From each patients Four ml of blood collected in EDTA coated tube. Samples were stored in 2-8 degree if testing was delayed. Centrifugation used for serum separation.

STATISTICAL ANALYSIS: Data were analyzed statistically described with

1. Mean ± standard deviation (± SD),
2. Range,
3. Frequencies (number of cases) and relative frequencies (percentages) when appropriate.

For comparing categorical data,

1. Chi square (X 2) test was performed.
2. A probability value (*p* value) less than 0.05 was considered statistically significant.

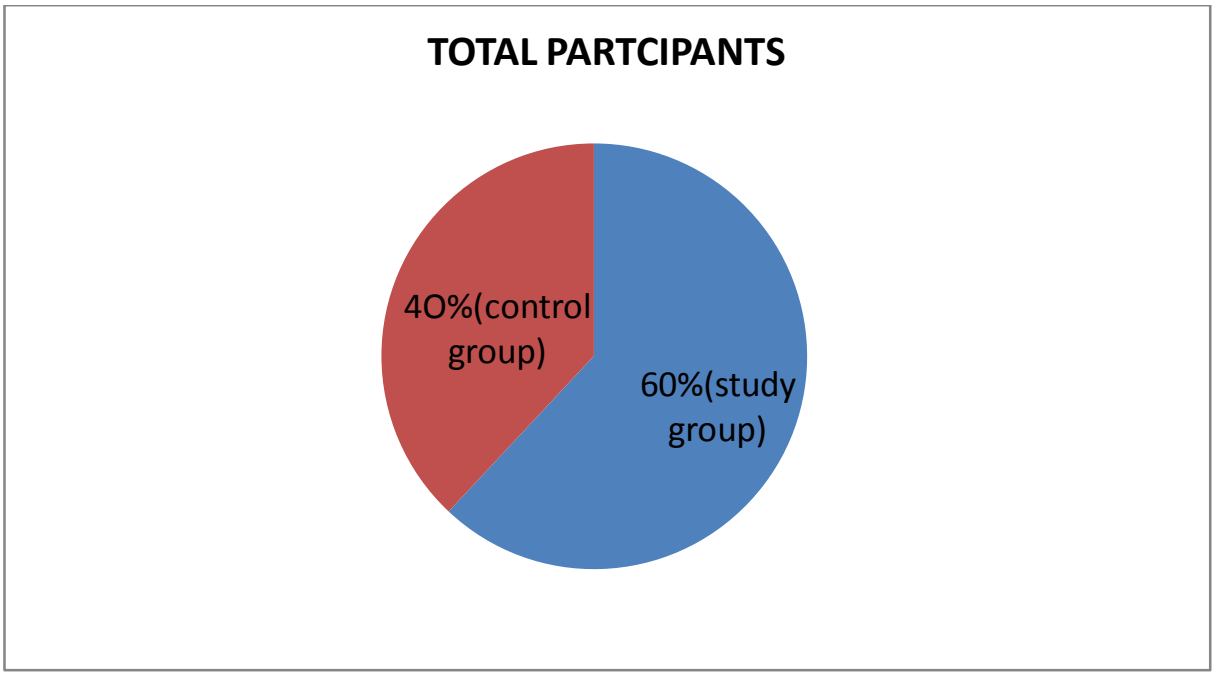
OBSERVATION AND RESULTS

50 patients were included in this study, serum homocysteine level estimated in all patients. Among 50, 20 normal pregnant women were control group. 30 patients were newly diagnosis as gestational diabetes mellitus.

TOTAL PARTICIPANTS: Table-20:

PARTICIPANTS	N=50	PERCENTAGE (100%)
Control	20	40.0
Study	30	60.0

FIGURE : 1



CONTROL GROUP 20 PATIENTS: Table-21:

Total normal pregnant women	20
Primi gravid	10
Multi gravid	10

GESTATIONAL DIABETES 30 PATIENTS: Table-22:

Total normal pregnant women	30
Primi gravid	15
Multi gravid	15

AGE GROUP: By using T-test, we calculated the mean, standard deviation and 'p 'value. Table-23:

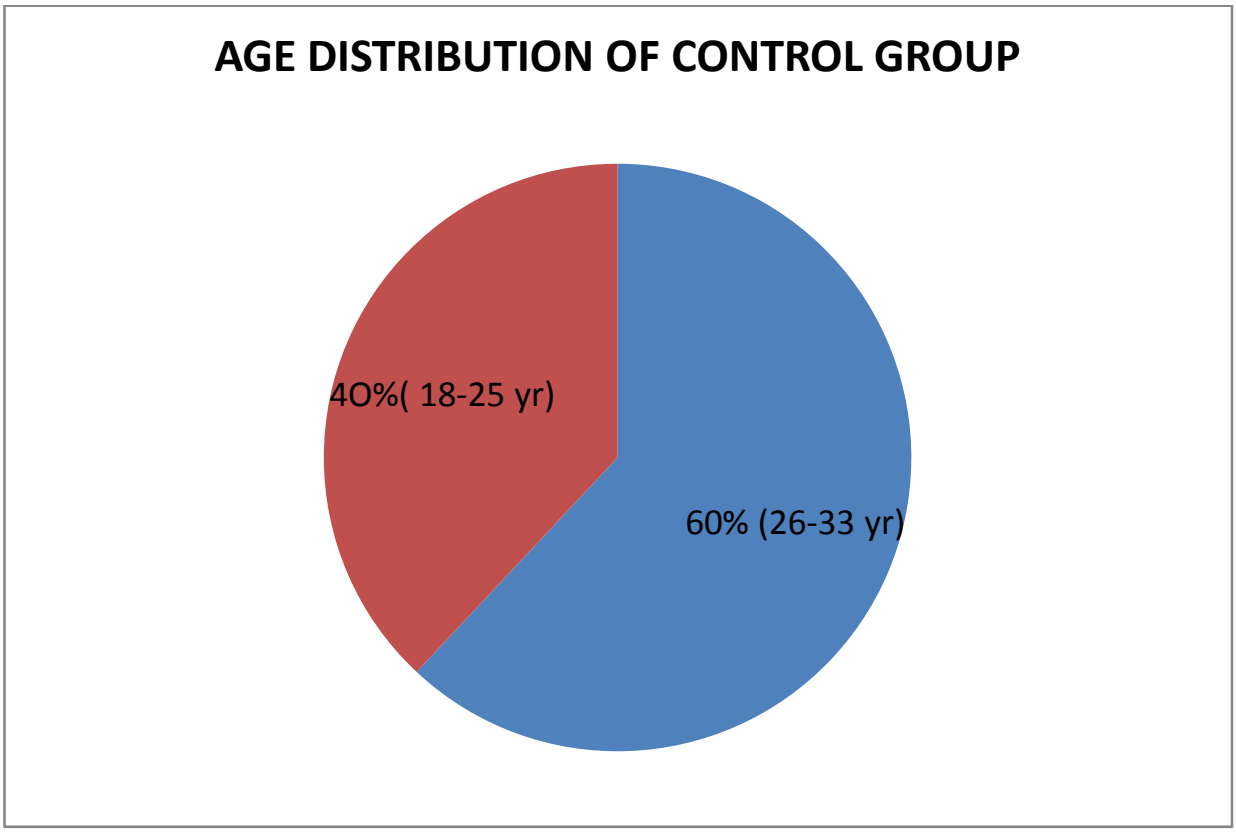
AGE	**MEAN**	**S.D**	**STATISTICAL INFERENCE**
Control(n=20)	24.25	4.102	T=-1.451 Df=48 .153>0.05 Not Significant
Study(n=30)	25.83	3.553	

Mean age group of control group is 24.25 years. Among them, 12 patients are the age between 18 to 25years.And 8 patients are between 26 to 33 years ofage.

Table-24:

AGE OF 18 TO 25 YEARS	12 (60 %)
AGE OF 26 TO 33 YEARS	8 (40%)

FIGURE : 2

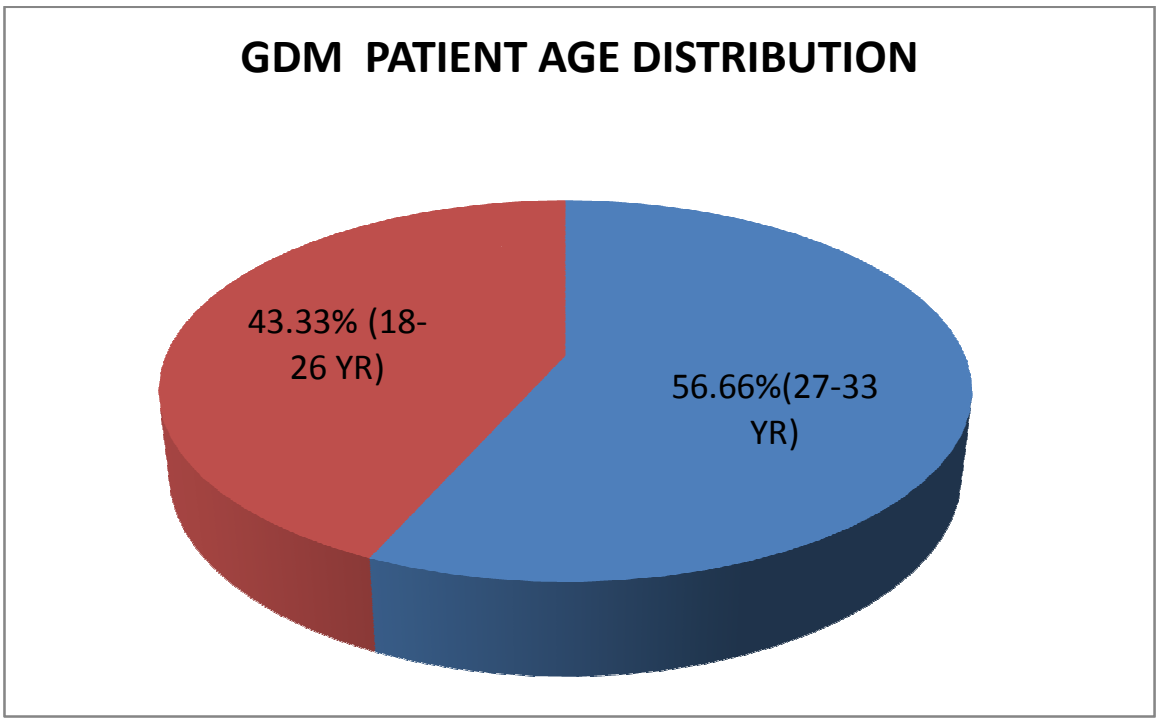


IN GDM PATIENTS ;

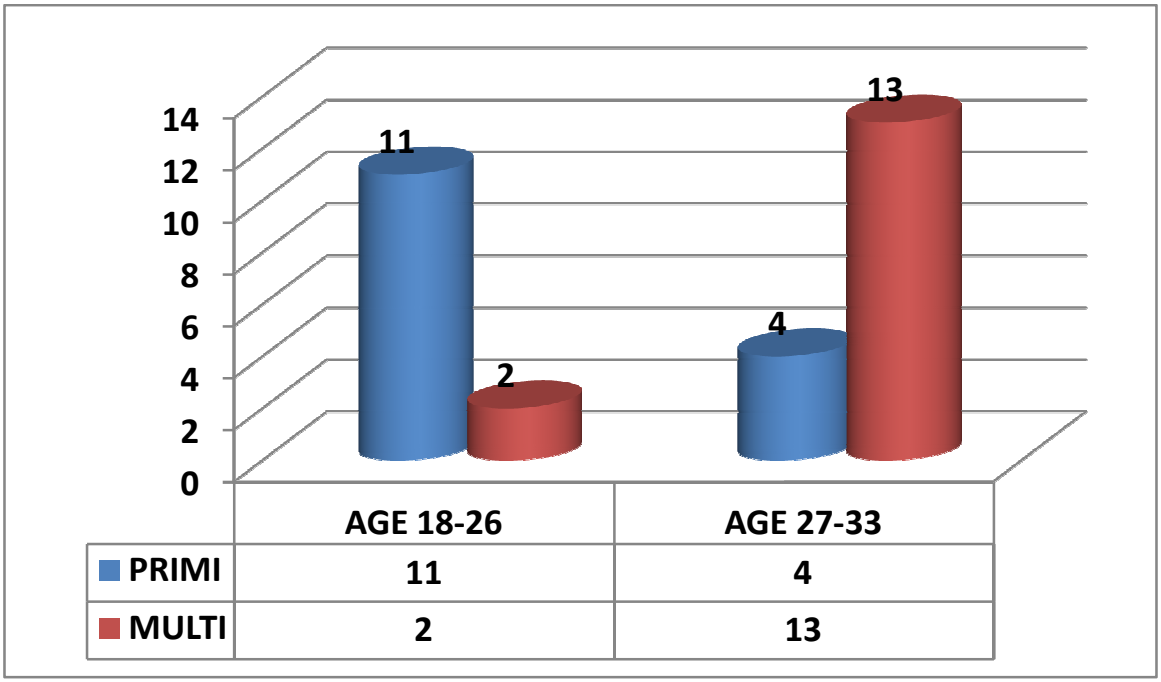
Mean age group of gestational diabetes patients is 25.8. Among them,13 patients are the age between 18 to 26 years, and 17 patients are between 27 to 32 years of age. Table-25:

AGE OF 18 TO 26 YEARS	13(11 primi,2 multi)(43.33%)
AGE OF 27 TO 33 YEARS	17(4 primi,13 multi)(56.66%)

FIGURE : 3



In the age group between 18 to 26 years, there are 11 primi and 2 multi gravida and between 27 to 33 years, there are 4 primi and 13 multi gravida. FIGURE : 4



GLUCOSE CHALLENGE TEST:

Screening test of glucose challenge test done in all patients with 50 g of glucose and its values are statistically interpreted. 'P' values are significant (<0.005). Table-26:

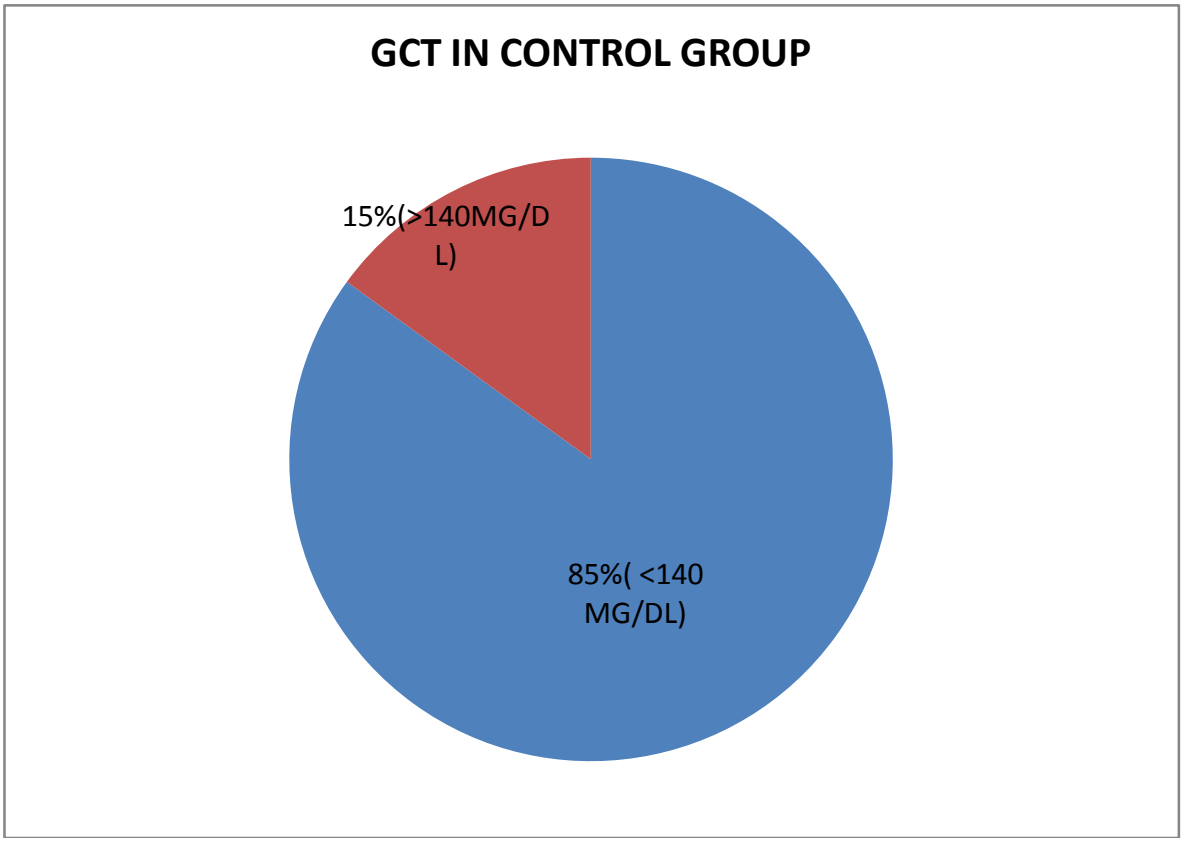
GCT MG.DL	MEAN	S.D	STATISTICAL INFERENCE
Control(n=20)	114.5000	20.87683	T=-8.797 Df=48 .000<0.05 Significant
Study(n=30)	162.4667	17.46274	

In normal pregnancy , three women's had > 140 mg of blood sugar value, others had normal values of <140 mg/dl. The mean GCT values is 114.50. The standard deviation is 20.87.

Table-27:

< 140 mg /dl of blood sugar at 1 hour	17 (85%)
> 140 mg /dl of blood sugar at 1 hour	3 (15%)

FIGURE : 5



In GDM: patients the mean values of GCT is 162.42. the standard deviation is 17.46.

GCT values : 17 patients had >140 to 160 mg/dl. 13 patients had > 160mg/dl.

Table-28:

>140 to 160 mg/dl	17 (56,6 %)
➢ 160 mg/dl	13 (43.3%)

FIGURE : 6

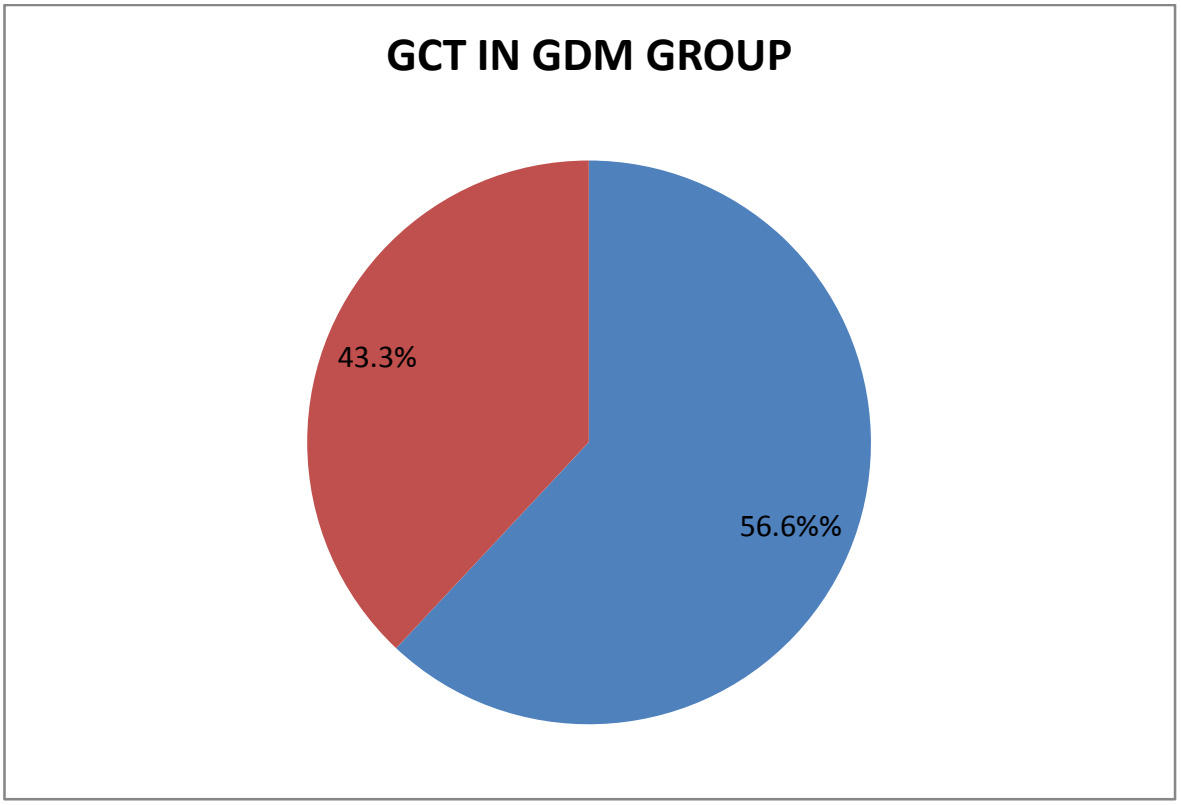
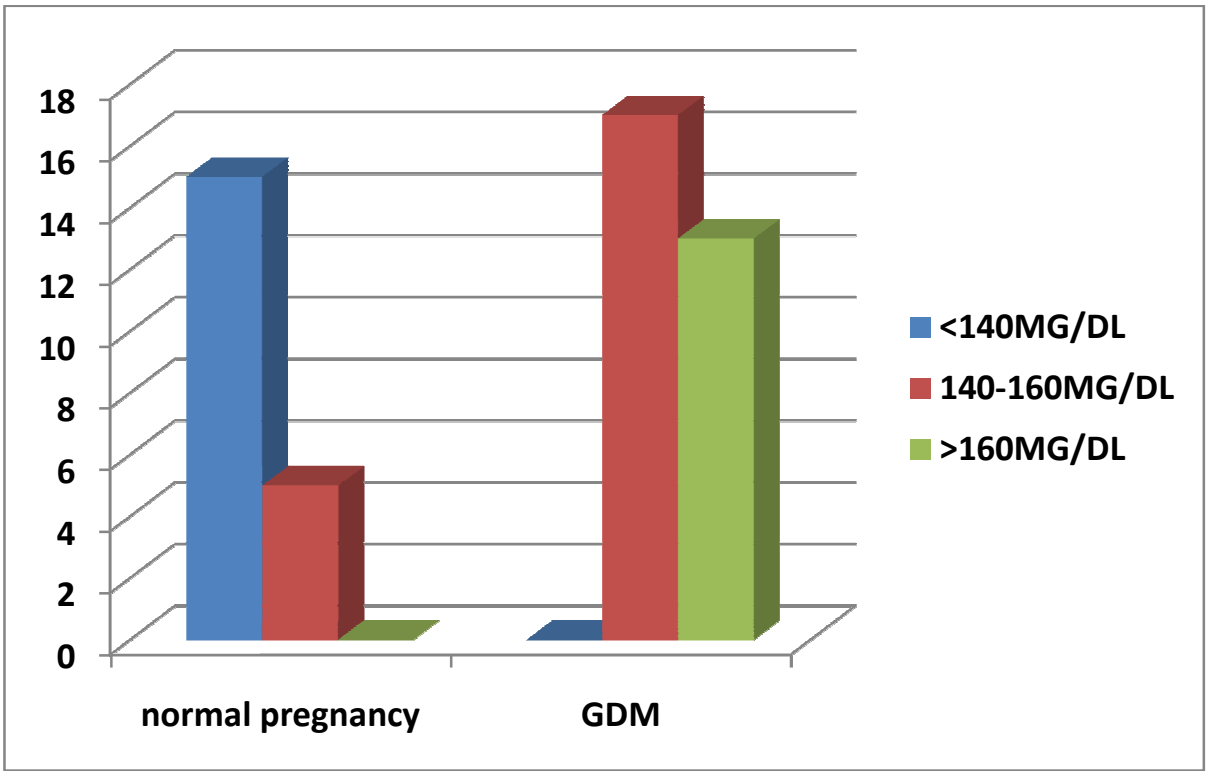


FIGURE : 7



After glucose challenge test ,we did oral glucose tolerance test after overnight fast with 100 gm of glucose to conform the glucose intolerance, Almost all the gestational diabetes group patients had abnormal OGTT test reports .Among 30 patients , 2 patients is under diet control and 28 patients are started on insulin therapy .

And we did fasting and postprandial blood sugar in all patients of both groups. And results were interpreted along with OGTT.

FASTING BLOOD SUGAR:

We collect the blood sample at 8 am for fasting blood sugar. Normal value is < 95 mg/dl. In control group mean values of fasting blood glucose level 85.60 and standard deviation is 8.531. In case of GDM, mean of 116.4 and SD is 10.85 with significant 'p' values.

Table-29:

FBS	MEAN	SD	STATISTICAL INFERENCE
Control(n=20)	85.60	8.531	T=-10.667 Df=48 .000<0.05 Significant
Study(n=30)	116.40	10.858	

POST PRANDIAL BLOOD SUGAR :

We collected the blood sample from all patients after 2 hours of glucose. The report showed that normal pregnant women had less than 140 mg/dl .

In control group mean values of post prandial blood glucose level 127.95 and standard deviation is 9.944. In case of GDM, mean of 193.23 and SD is 12.32 with significant 'p' values.

Table-30:

PPBS	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control(n=20)	127.95	9.944	T=-19.768 Df=48 .000<0.05 Significant
Study(n=30)	193.23	12.322	

HOMOCYSTEINE LEVEL :

In normal pregnant women had the values of **<** 5 micro mol/l. Among the GDM patients 17 patients had elevated homocysteine level and 13 patients had normal .But when compare to normal pregnant women 29 patients elevated level that is **>** 8.micromol/l.

Table-31:

Homocysteine	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control(*n=20)*	3.8600	.95038	T=-9.024 Df=48 .000 < 0.05 Significant
Study*(n=30)*	16.3040	6.09711	

The mean value of homocysteine in control group is 3.8 ± 0.95 and in gestational diabetes patients is 16.30 ± 6.09. Its 'p' value is significant.

Homocysteine level in gestational diabetes mellitus group: Table-32:

< 15 µmol/l	13 (43.33%)
➢ 15 µmol/l	17 (56.66%)

FIGURE : 8

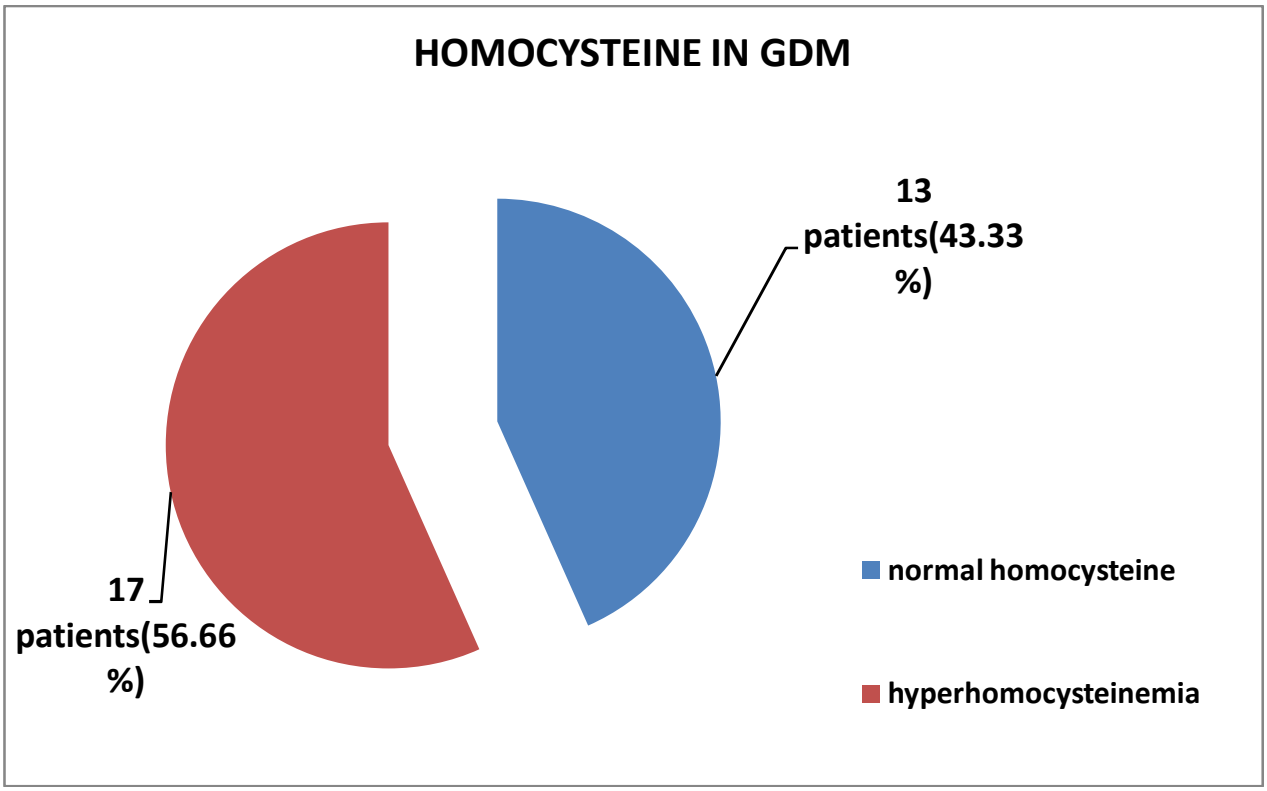
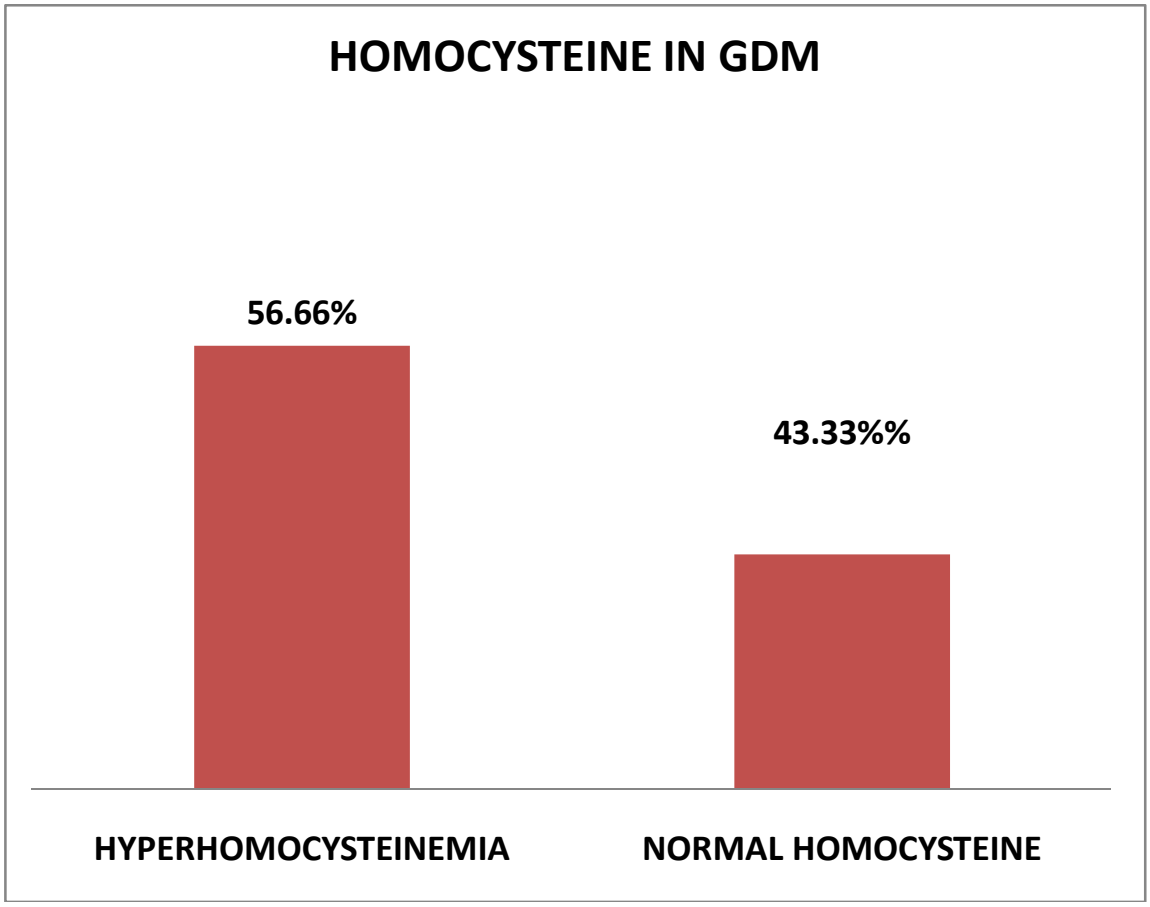


FIGURE : 9



HYPERHOMOCYSTEINEMIA COMPARING PRIMI AND MULTI PARA:

Out of 13 patients with normal homocysteine, 7 patients are primi and 6 patients are multi gravid. And among the 17 patients with elevated homocysteine, 8 patients are primi and 9 patients are multigravida.

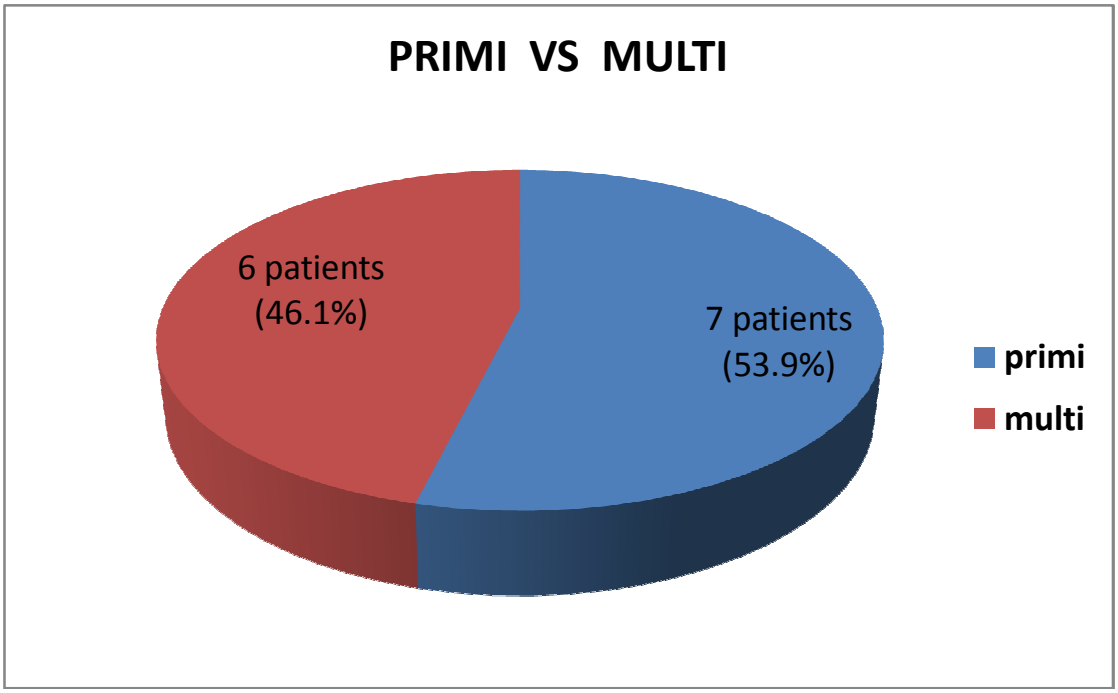
Normal homocysteine in GDM : primi vs multi:

13 PATIENTS → 7 PRIMI(53.8%)

13 PATIENTS → 6 MULTI(46.1%)

Normal homocysteine level in gestational diabetes mellitus: This pie diagram showed primi vs multi with normal HCY level.

FIGURE : 10

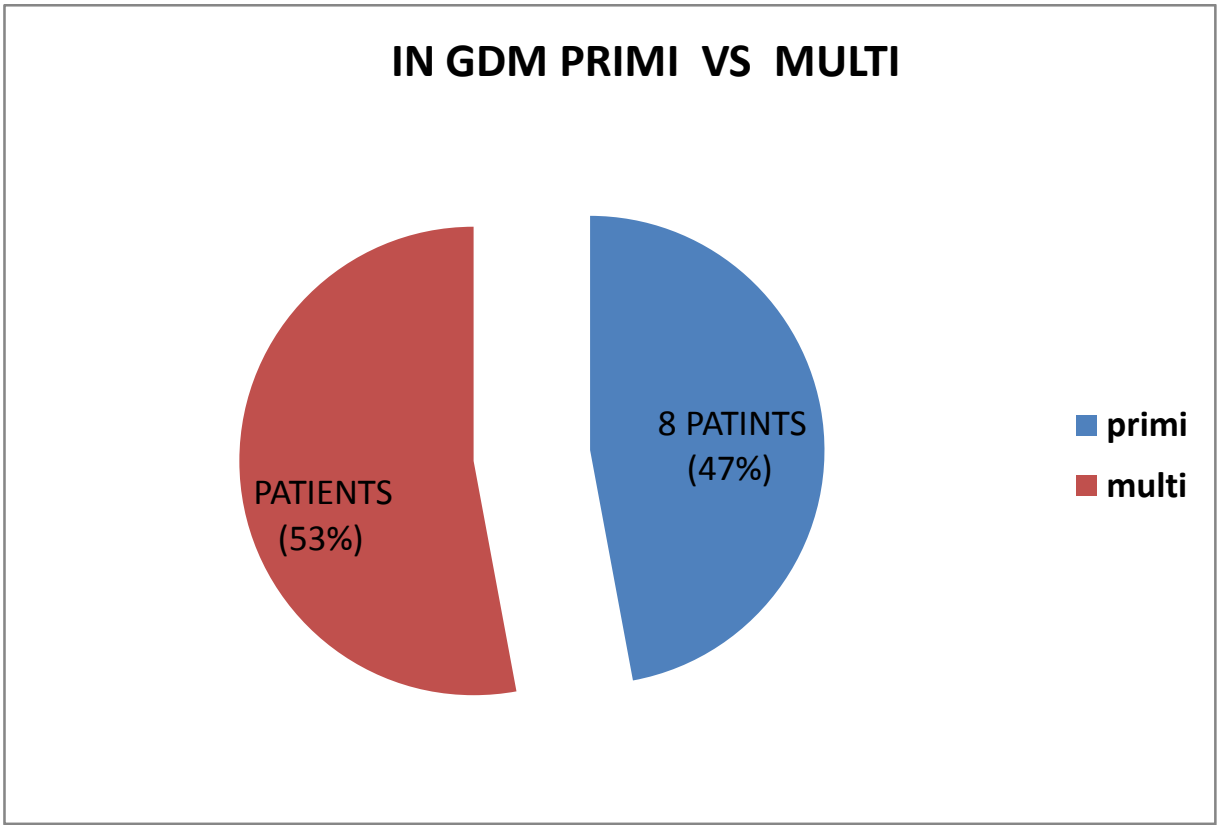


HYPERHOMOCYSTEINEMIA IN GDM : PRIMI VS MULTI:

17 PATIENTS ⇒ 8 PRIMI (47%)

17 PATIENTS ⇒ 9 MULTI (53%).

FIGURE : 11



TOTAL CHOLESTEROL LEVEL IN PREGNANCY:

Total cholesterol level is significantly elevated in gestational diabetes patients.The mean value of Total cholesterol in control group is 187.70±18.2and in gestational diabetes patients is 211.50 ± 28.799. Its 'p' value is significant (p=0.002).

Table-33:

TC	MEAN	SD	STATISTICAL INFERENCE
Control(n=20)	187.70	18.270	T=-3.276 Df=48 .002<0.05 Significant
Study(n=30)	211.50	28.799	

Total cholesterol level is elevated in 18 patients of gestational diabetes patients,

Table-34:

TOTAL CHOLESTEROL	< 200 MGS/DL	> 200 MGS/DL
Control group(20 patients)	15 (75 %)	5 (25 %)
Study group(30 patients)	12 (40%)	18 (60%)

In control group: among the 20 patients, 5 patients had mild elevation of Total cholesterol level(25%) and 15 patients had the normal level of Total cholesterol(75%).

FIGURE : 12

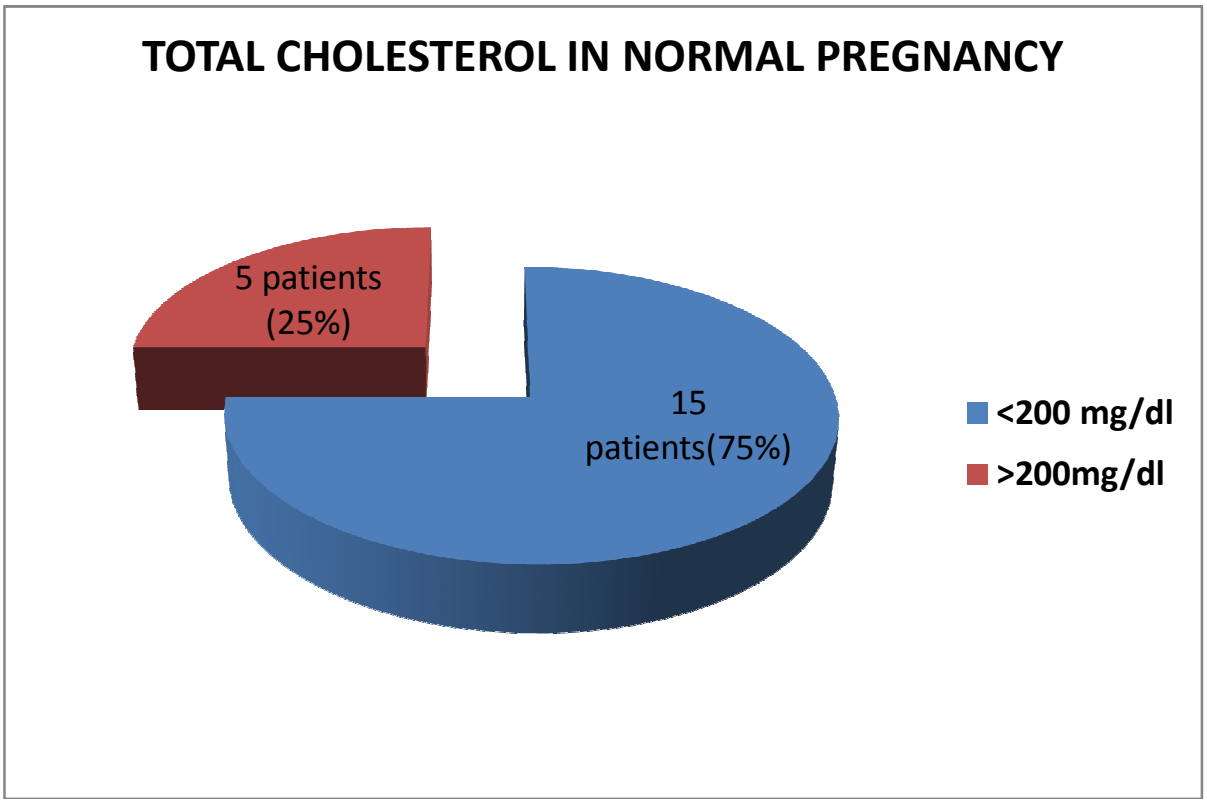


FIGURE : 13

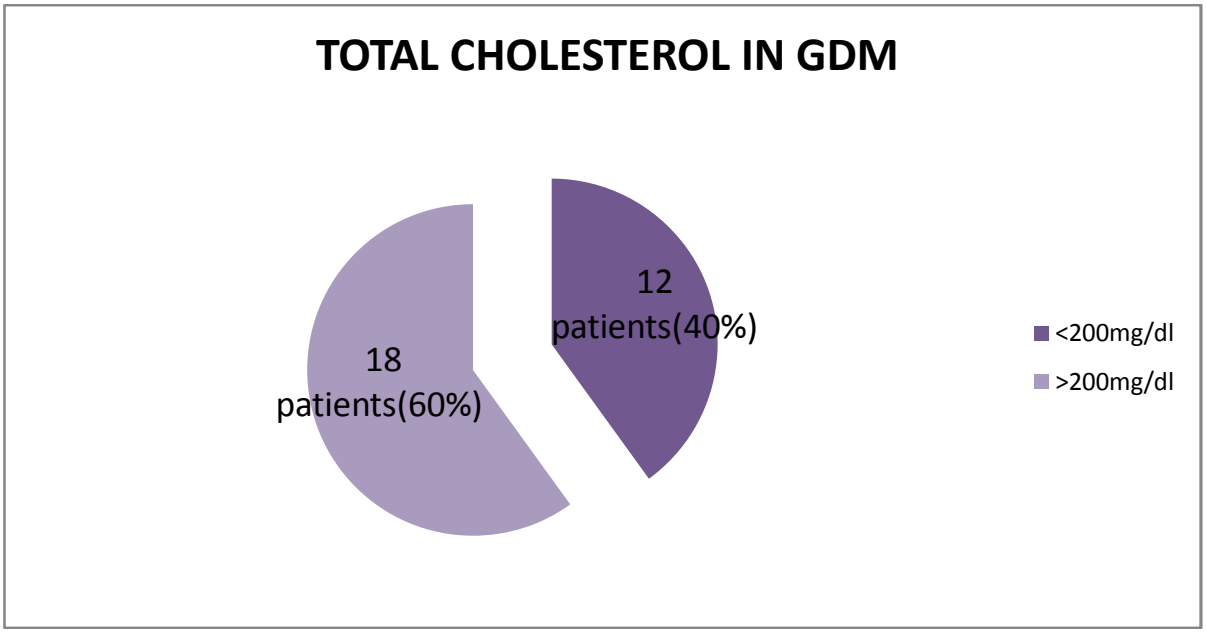
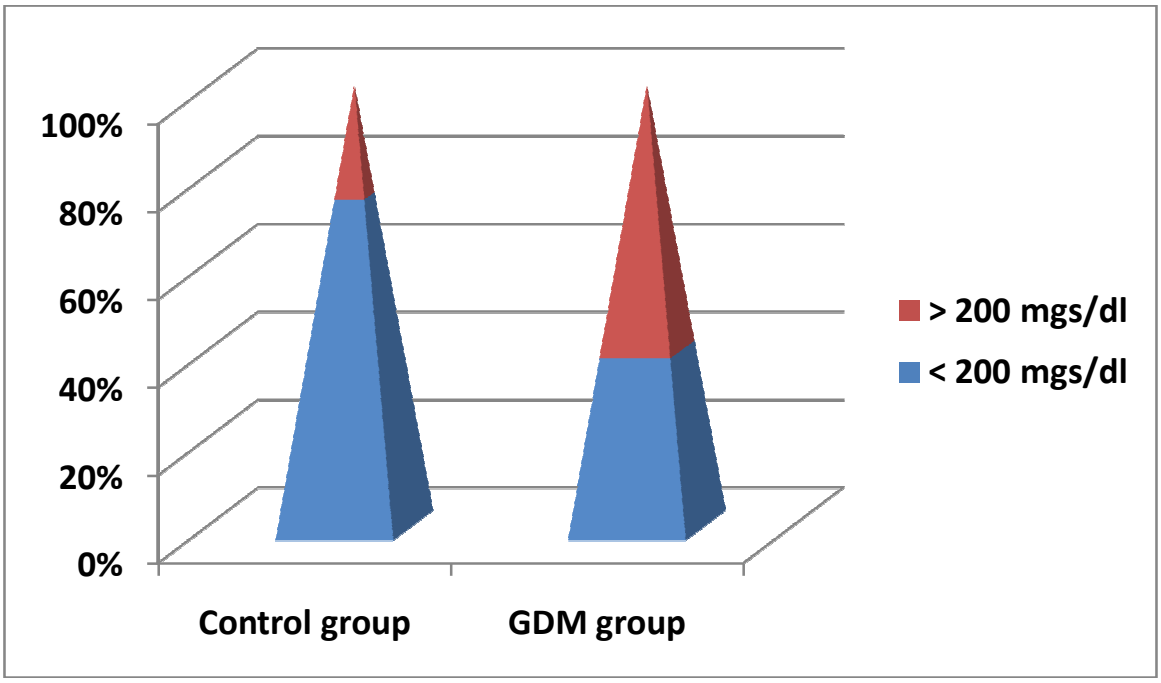


FIGURE : 14



TRIGLYCERIDE LEVEL IN PREGNANCY:

In our study, 12 patients of gestational diabetes patients had elevated TG level. In control group, all had the normal triglyceride level.

The mean value of triglyceride level is 113.30 ± 16.10 in control group. In study group (gestational diabetes patients) the mean value is 140.20 **±** 22.15. 'p' value is **<** o.oo5.

TG	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control(n=20)	113.30	16.102	T=-4.664 Df=48 .000 **<** 0.05 Significant
Study(n=30)	140.20	22.154	

Table-35:

TRIGLYCERIDE LEVEL	< 150 mg/dl	>150 mg/dl
Control group(20 patients)	20	Nil
Study group(30 patients)	18 (60%)	12 (40%)

FIGURE : 15

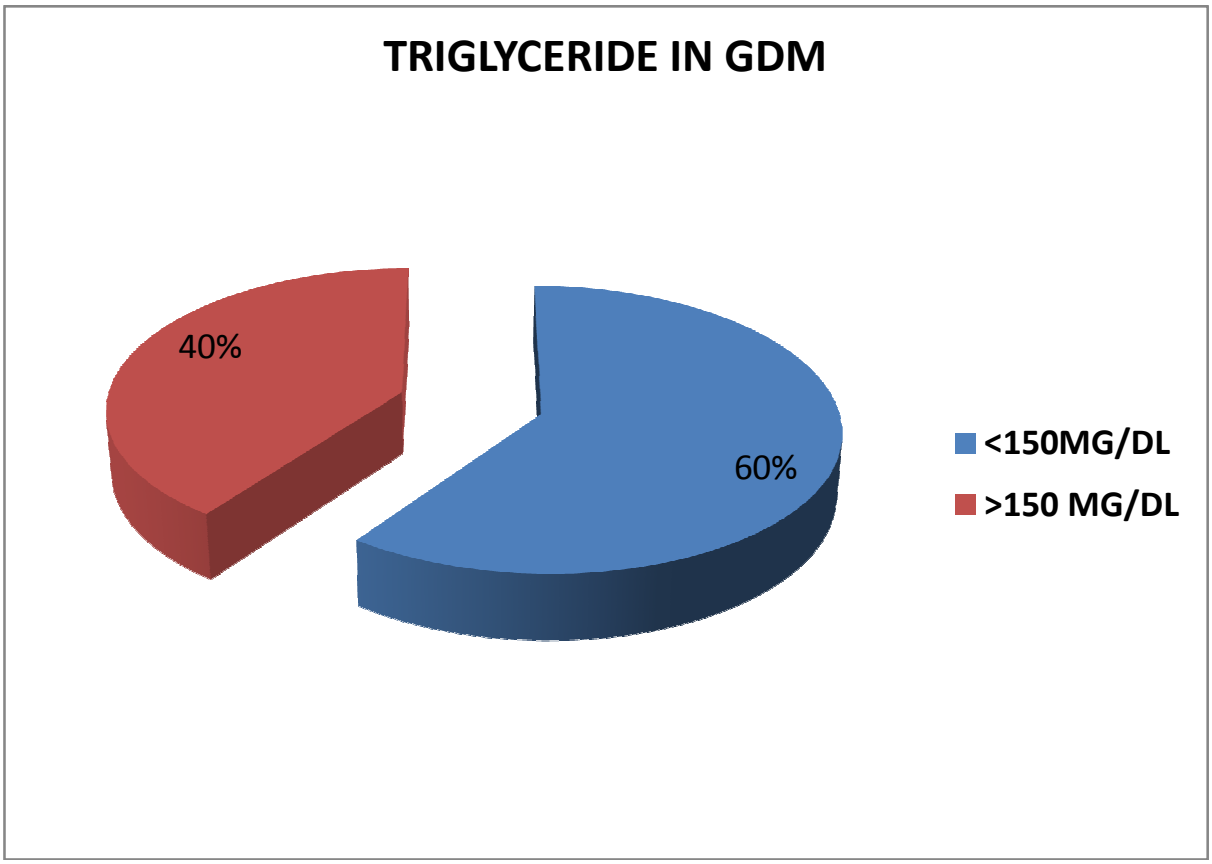
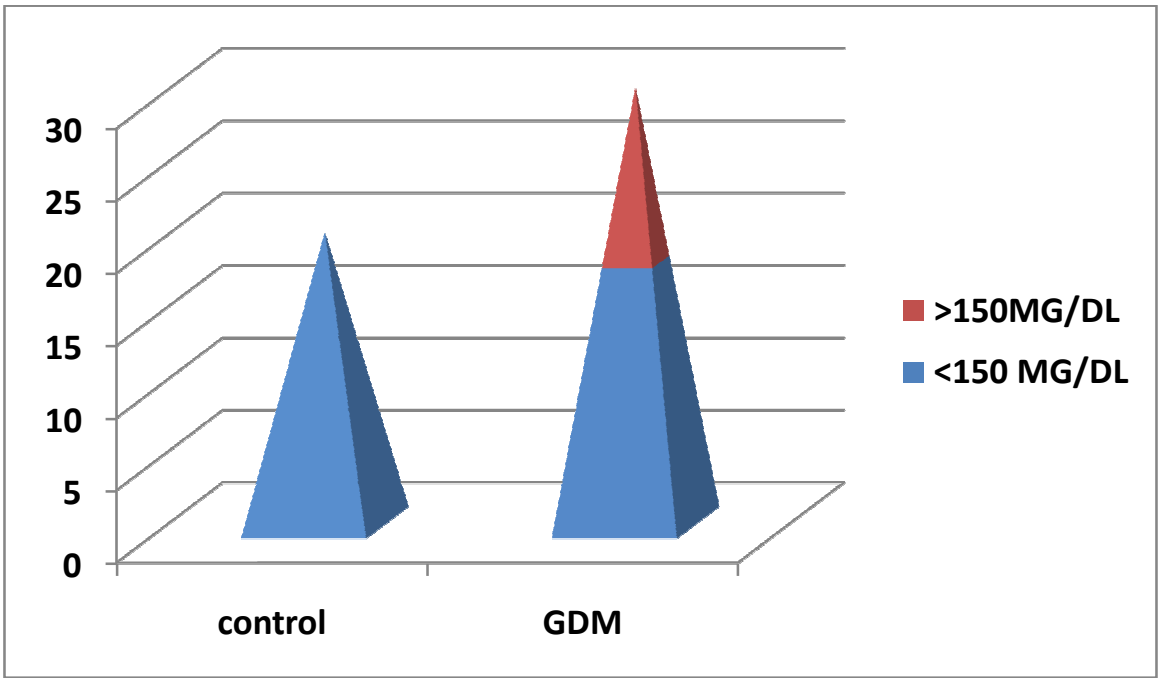


FIGURE : 16



DISCUSSION

Homocysteine is naturally obtained by diet containing methionine which is one of the essential amino acid. The role of homocysteine as an independent risk factor of gestational diabetes mellitus has not been extensively studied in India. Due to multiple factor like dietary, life style, socio economic status and other ethnic differences, the results found in the western studies cannot be applicable to our population.

The mean homocysteine level in control group is (3.8 ±0.95 μmol/L) is similar to that reported by Walker and associates in a Canadian population between 20 and 28 weeks' gestation (2.7±1.3μmol/L) in normal glucose tolerance test.

The mean serum homocysteine concentration in women with gestational diabetes mellitus in our study was significantly higher than that in normal pregnant controls between 24-28 weeks gestation (16.30 ± 6.09 μmol/L and 2.7 ± 1.3 μmol/L respectively. These results are comparable to the levels detected in GDM patients in previous analysis.

Also, Guven group[71] found higher homocysteine levels GDM patients compared to normal pregnant women, but they reported lower estimates (9.0 ± 3.1μmol/L and 7.4 ± 1.6μmol/L respectively).

Another the Cotton's study[72] explained the higher levels, which demonstrated that homocysteine level may show a various range in a various population and may elevate up to sixteen μmol/L for 97.5 percentile in normal healthy individual.

However, Vitoratos et al[73]was not find a statically significant different homocysteine levels between GDM patients (n=15), and glucose tolerant pregnant controls (n=21), it may due to their studied population which was small and was late in the third trimester.

In our present study, significant positive correlation was found between, total cholesterol, triglyceride and homocysteine levels in GDM patients. It has been well documented by older studies that in diabetes, serum homocysteine levels are directly related to insulin resistance, especially when associated with the presence of vascular complications.

In Some studies there was reduction in serum homocysteine level in gestational diabetes after supplementation with vitamin B_{12} , folate and B_6. But it requires large scale study and long term follow up to evaluate the outcome.

Gestational diabetes mellitus is associated with abnormal endothelial function. Schaich et al[74]reported that even slightly elevated homocysteine levels also to be of crucial importance for the endothelial dysfunction, especially when associated with other risk factors such as hypercholesterolemia co-exist.

Additionally, it has been reported that even in normal ranges cholesterol levels may be associated with impaired homocysteine metabolism and endothelial dysfunction.

There was significant elevation of total cholesterol concentrations in gestational diabetes mellitus compared with control group of normal pregnant. In our study, GDM significantly alters serum cholesterol metabolism leading to dyslipidemia. These findings co-relate with reports by Amraei and Azemati done in pakistan who reported significant difference in total cholesterol levels between pregnancy complicated by GDM and normal pregnancy.

. These changes of increased levels of serum triglycerides, total cholesterol and LDL cholesterols observed in GDM is due to the result of increased fat storage and progesterone level in the second trimester of pregnancy. This action may be due to the reset of the lipostat in the hypothalamus leading to increase in the lipids concentration .

STRENGTHS OF THE STUDY

- In our study patients without conventional risk factors presented with gestational diabetes mellitus were selected and the role of serum homocysteine was studied in these patients. So, the role of serum homocysteine has been assessed as an independent risk factor for gestational diabetes mellitus.
- We included patients of both primi and multi gravida with age group of 18 to 33 years and compared the role of serum homocysteine level in patients with primi and multi gravida. Homocysteine is found to be a significantly elevated in multi gravida (53 %) compare to primi (47%) in gestational diabetes mellitus.
- We also compared the homocysteine levels between normal pregnancy and in gestational diabetes mellitus. Hyperhomocysteinemia is significantly elevated among gestational diabetes mellitus (56.66%).
- We compared the total cholesterol level between normal pregnancy and in gestational diabetes mellitus. Total cholesterol level is significantly elevated among gestational diabetes mellitus (60 %).
- And also triglyceride level between normal pregnancy and in gestational diabetes mellitus, triglyceride level elevated in 40 % of the gestational diabetes mellitus.

LIMITATIONS OF THE STUDY

- Sample size is very small , and
- Our study lack the knowledge of homocysteine status of the patient before the diagnosis of the gestational diabetes mellitus.
- In this study we did not evaluate the role of other risk factors of gestational diabetes mellitus like insulin level, sensitivity of insulin, vitamin B_{12} , B_6 and folate level. whether these factors also plays a role in development of gestational diabetes mellitus in the absence of conventional risk factors is not studied. This is the limitation of the study.

- We did not evaluate the association between severity and outcome of maternal status and also fetal outcome with serum homocysteine levels. Also we did not evaluate whether the reduction of serum homocysteine by treatment will prevent the future type 2 DM, or recurrence of gestational diabetes mellitus in subsequent pregnancy. Further studies involving large sample will be needed to confirm this.

CONCLUSION

- The mean age groups of controls and cases were 24.25 ± 4.102 and 25.83 ± 3.553 years.
- The mean serum homocysteine level was significantly higher in diabetic patients compared to controls (P=0.000).
- Hyperhomocysteinemia is found in 56.66% of patients with gestational diabetes mellitus.
- Hyperhomocysteinemia is found to be an independent risk factor for gestational diabetes mellitus patients.
- Among the control group of normal pregnant women and the study group of gestational diabetes mellitus, serum homocysteine level is found to be significantly elevated in gestational diabetes mellitus.
- Among the study groups, the incidence of hyperhomocysteinemia is 53% in multi gravida and 47% in primi gravida.
- Whether serum homocysteine is directly associated with GDM or it may cause deterioration of glucose tolerance, remains Unclear.
- Only well-designed large trials with vitamin B_{12}, vitamin B_6 and folic acid supplementation will be able to give more clear explanations.

- Vitamin B_{12}, vitamin B_6 and Folic acid supplementation with higher dose throughout pregnancy will change the plasma homocysteine concentration. This simple, safe and inexpensive intervention may therefore play a preventive role.
- The average levels of Total cholesterol, were significantly found to be higher in GDM cases compared to controls.
- The average levels of Triglyceride were elevated in 40% of GDM cases.

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CONSENT FORM

I _____ hereby give consent to participate in the study conducted by **DR.R.SHARMILA,** post graduate in department of internal medicine ,Thanjavur medical college & Hospital, Thanjavur – 613001 and to use my personal clinical data and result of investigation for the purpose of analysis and to study the nature of disease. I also give consent for further investigations.

Place :

Date : Signature of participant

A COMPARATIVE AND CORRELATIVE STUDY OF SERUM HOMOCYSTEINE LEVEL IN GESTATIONAL DIABETES MELLITUS AND NORMAL PREGNANCY

PROFORMA

PATIENT NAME; DOA:

AGE: DOD:

SEX: OP/IP NO:

OCCUPATION:

ADDRESS:

- **CHIEF COMPLAINT:**

- **HISTORY OF PRESENTING ILLNESS:**

- Frequent urination:
- Excessive thirst:
- Unexplained weight loss:
- Extreme hunger:
- Sudden vision changes:
- Tingling or numbness in the hands or feet:
- Feeling very tired much of the time:
- Very dry skin:

- **Gravida : Primi / G1 / G2 / G3**
- **Para: P1 / P2 / P3**
- **living child:**
- **History of Abortion:**
- **Past History :Hypertension / DM / CAHD**
- **Family History:**
- **Personal History:**
- **Diet:**

EXAMINATION OF THE PATIENT:

- **GENERAL EXAMINATION:**
  - Build And Nourishment:
  - Pallor;
  - Icterus:
  - Clubbing:
  - Cyanosis:
  - Pedal edema:
- **VITAL SIGNS:**

Pulse: Blood pressure:

Respiratory rate: Temp:

- **Cardiovascular System Examination:**

- **Respiratory System Examination:**

- **Per Abdominal Examination:**

- **Central Nervous System Examination:**

INVESTIGATION

Urine; Albumin:

Sugar:

Deposits:

Complete Hemogram:

HB: RBC: PCV:

TC: PLT:

DC: ESR:

Random Blood Sugar:

Renal Function Test:

Urea:

Creatinine:

Serum Electrolytes:

Pottasium:

Sodium**:**

Serum Homocysteine Level:

VCTC: Reactive / Non Reactive

Oral Glucose Tolerance Test:

Fasting:

1 Hour:

2 Hour:

3 Hour:

Electrocardiogram:

Ultra sonogram for fetus:

NAME	AGE	IP NO	GRAVIDA	FAMILY H/O	URINE ALBUMIN	URINE SUGAR	HB GMS %	GCT MG.DL	OGTT	FBS	PPBS	UREA	CREATININE
Poovizhi	24 YR	291885	PRIMI	NIL	NIL	NIL	11	106	NORMAL	78	114	30	0.8
Rajalakshmi	20 YR	291571	PRIMI	NIL	NIL	NIL	9.2	150	NORMAL	100	138	30	0.8
Janisha	26 YR	291781	PRIMI	NIL	NIL	NIL	9	91	NORMAL	94	127	30	0.9
Suriyakala	20 YR	291547	PRIMI	NIL	NIL	NIL	9.6	101	NORMAL	90	130	30	0.8
Elavarasi	20 YR	291240	PRIMI	NIL	NIL	NIL	9.8	100	NORMAL	64	110	30	0.8
Akilandeswari	18 YR	286447	PRIMI	NIL	NIL	NIL	9	112	NORMAL	82	120	27	0.8
Umarani	20yr	301234	PRIMI	NIL	NIL	NIL	8.9	118	NORMAL	89	128	27	0.7
Veni	23yr	311728	PRIMI	NIL	NIL	NIL	12	150	NORMAL	90	138	28	0.8
Lakhsmi	22yr	321546	PRIMI	NIL	NIL	NIL	10.4	98	NORMAL	84	136	28	0.8
Thanya	21yr	327132	PRIMI	NIL	NIL	NIL	8	152	NORMAL	80	130	29	0.7
Valarmathi	30 YR	286470	MULTI	NIL	NIL	NIL	12.1	98	NORMAL	88	120	29	0.7
Uma	22 YR	281344	MULTI	NIL	NIL	NIL	13	97	NORMAL	82	123	28	0.8
Shanthana	34 yr	284231	MULTI	NIL	NIL	NIL	9.6	87	NORMAL	90	110	27	0.7
Revathy	28yr	301382	MULTI	NIL	NIL	NIL	8	149	NORMAL	94	139	28	0.8
Kalaivani	24yr	301378	MULTI	NIL	NIL	NIL	8.2	112	NORMAL	76	140	25	0.9
Vandarkulali	27yr	301989	MULTI	NIL	NIL	NIL	9.4	126	NORMAL	84	136	29	0.9
Sabeetha	26yr	318903	MULTI	NIL	NIL	NIL	11.3	104	NORMAL	94	134	29	0.8
Kaliyammal	24yr	324768	MULTI	NIL	NIL	NIL	8.4	100	NORMAL	96	140	28	0.7
Seetha	27yr	325089	MULTI	NIL	NIL	NIL	10	117	NORMAL	79	128	30	0.9
Megala	29yr	320126	MULTI	NIL	NIL	NIL	9.7	122	NORMAL	78	118	28	0.7

NAME	HOMOCYSTEINE	LIPID PROFILE/TC/TG	USG ABDOMEN	VCTC	ECG	ON INSULIN
Poovizhi	3.6	170/110	SLIUF.26 WEEKS	NON REACTIVE	WNL	NO
Rajalakshmi	4.69	167/102	SLIUF.28 WEEKS	NON REACTIVE	WNL	NO
Janisha	4.7	180/130	SLIUF.27 WEEKS	NON REACTIVE	WNL	NO
Suriyakala	5.1	193/120	SLIUF.24WEEKS	NON REACTIVE	WNL	NO
Elavarasi	2.4	187/105	SLIUF.28WEEKS	NON REACTIVE	WNL	NO
Akilandeswari	5.11	192/122	SLIUF.26 WEEKS	NON REACTIVE	WNL	NO
Umarani	2.77	200/129	SLIUF.27 WEEKS	NON REACTIVE	WNL	NO
Veni	3.11	210/140	SLIUF.24WEEKS	NON REACTIVE	WNL	NO
Lakhsmi	4.23	229/122	SLIUF.27WEEKS	NON REACTIVE	WNL	NO
Thanya	4.21	192/103	SLIUF.26WEEKS	NON REACTIVE	WNL	NO
Valarmathi	4.71	182/102	SLIUF.28WEEKS	NON REACTIVE	WNL	NO
Uma	3.3	170/100	SLIUF.24WEEKS	NON REACTIVE	WNL	no
Shanthana	3.6	190/124	SLIUF.25WEEKS	NON REACTIVE	WNL	NO
Revathy	4.9	160/98	SLIUF.24WEEKS	NON REACTIVE	WNL	NO
Kalaivani	3.3	183/104	SLIUF.28WEEKS	NON REACTIVE	WNL	NO
Vandarkulali	2.3	179/98	SLIUF.25WEEKS	NON REACTIVE	WNL	NO
Sabeetha	3.67	213/140	SLIUF.24WEEKS	NON REACTIVE	WNL	NO
Kaliyammal	4.1	210/134	SLIUF.25WEEKS	NON REACTIVE	WNL	NO
Seetha	5	187/99	SLIUF.26WEEKS	NON REACTIVE	WNL	NO
Megala	2.4	160/84	SLIUF.27WEEKS	NON REACTIVE	WNL	NO

NAME	AGE	IP NO	GRAVIDA	FAMILY H/O	URINE ALBUMIN	URINE SUGAR	HB GMS %	GCT MG.DL	OGTT	FBS	PPBS	UREA	CREATININE
GESTATIONAL DIABETIC MELLITUS													
Ananthajothy	32 YR	250712	MULTI	NIL	NIL	.++	9	180	130/210/180/150	138	190	29	0.7
Lakhmi	31 YR	283546	MULTI	NIL	NIL	.++	10.4	167	138/200/190/164	110	200	28	0.8
Sugamathy	24 YR	385523	MULTI	NIL	NIL	.+++	9	240	128/208/190/170	148	210	28	0.7
Suganya	27 YR	286484	MULTI	NIL	NIL	.+++	9	160	140/220/198/174	100	190	28	0.7
Giltamary	29 YR	281344	MULTI	NIL	NIL	.++	11.6	150	130/198/170/158	100	198	25	0.6
Valarmathi	30 YR	286470	MULTI	NIL	NIL	++	12.1	142	128/190/168/140	120	179	29	0.7
Suganthi	29 YR	281344	MULTI	NIL	NIL	.+	11.6	150	110/200/175/148	100	198	25	0.6
Alagumeena	26YR	291781	MULTI	NIL	NIL	.+++	8.8	167	130/217/198/167	110	210	28	0.8
Kavitha	28YR	292765	MULTI	NIL	NIL	.++	12	156	126/198/170/156	114	187	28	0.7
Sangeetha	30YR	309811	MULTI	NIL	NIL	.+++	8.4	170	136/218/210/170	124	202	29	0.6
Devi	29YR	311056	MULTI	NIL	NIL	.++	8	156	112/198/174/150	110	197	28	0.8
Kaliyatha	25YR	333527	MULTI	NIL	NIL	.+++	8.6	168	126/200/188/156	128	200	29	0.9
Podum ponnu	28YR	323098	MULTI	NIL	NIL	.++	9.6	156	114/198/168/150	118	190	27	0.7
Yasothi	27YR	341234	MULTI	NIL	NIL	.++	8	154	127/198/168/140	110	180	30	0.7
Sivakami	26YR	321000	MULTI	NIL	NIL	.+++	9	168	124/210/198/164	110	184	30	0.9

NAME	HOMOCYSTEINE	LIPID PROFILE/TC/TG	USG ABDOMEN	VCTC	ECG	ON INSULIN
GESTATIONAL						
Ananthajothy	22.1	220/150	SLIUF, 23 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Lakhmi	20.9	260/140	SLIUF, 30 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Sugamathy	18.34	210/110	SLIUF, 28 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Suganya	19.11	234/182	SLIUF, 29 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Giltamary	13	160/98	SLIUF ,25 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Valarmathi	19.8	220/164	SLIUF, 28 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Suganthi	10.9	210/160	SLIUF, 22 WEEKS	NON REACTIVE	WNL	DIET
Alagumeena	12.7	194/140	SLIUF,21 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Kavitha	13.5	190/128	SLIUF,19 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Sangeetha	11.34	199/130	SLIUF,30 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Devi	20.8	260/140	SLIUF,27 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Kaliyatha	20.2	250/153	SLIUF,30 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Podum ponnu	17	228/160	SLIUF,27 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Yasothi	9.44	167/144	SLIUF,25 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Sivakami	14.9	240/154	SLIUF,26 WEEKS	NON REACTIVE	WNL	INSULIN STARTED

NAME	AGE	IP NO	GRAVIDA	FAMILY H/O	URINE ALBUMIN	URINE SUGAR	HB GMS %	GCT MG.DL	OGTT	FBS	PPBS	UREA	CREATININE
Anitha	27 yr	290496	PRIMI	NIL	NIL	NIL	11.9	150	110/180/158/150	120	160	27	0.5
Mutharasi	22 YR	289576	PRIMI	NIL	NIL	TRACE	9.8	168	134/200/170/160	120	198	28	0.9
Sathya	20 YR	286484	PRIMI	NIL	NIL	.+	9.8	156	120/190/160/150	120	210	29	0.8
Ponmani	24YR	290999	PRIMI	NIL	NIL	.+++	9.3	170	128/218/188/170	120	200	29	0.9
Suganthi	29YR	297687	PRIMI	NIL	NIL	.++	11.3	164	128/206/189/160	112	190	30	0.9
Vinoliya	23YR	310098	PRIMI	NIL	NIL	.+++	9.6	178	120/220/200/184	130	206	29	0.7
Jayamalini	28YR	321167	PRIMI	NIL	NIL	.+	10.3	140	110/190/160/148	120	180	29	0.7
Srimathy	21YR	322169	PRIMI	NIL	NIL	.++	9.6	156	124/190/174/158	114	187	27	0.8
Kurhir begam	21YR	312576	PRIMI	NIL	NIL	.+++	9.5	160	130/208/178/150	126	190	28	0.7
Senduravalli	20YR	317342	PRIMI	NIL	NIL	.++	8.3	154	126/196/168/144	110	184	29	0.9
Meenatchi	24YR	314829	PRIMI	NIL	NIL	TRACE	7.9	150	110/189/160/140	100	190	28	0.8
Mangalam	23YR	323671	PRIMI	NIL	NIL	.++	10	158	122/196/170/150	112	185	28	0.8
Devaki	20YR	351213	PRIMI	NIL	NIL	.+++	12.3	170	130/240/200/179	120	220	29	1
Chinnaponnu	29YR	325678	PRIMI	NIL	NIL	.+++	8.9	160	128/208/180/158	116	204	30	0.8
Mariyammal	23YR	333935	PRIMI	NIL	NIL	.++	9.4	156	117/189/178/154	112	178	28	0.8

NAME	HOMOCYSTEINE	LIPID PROFILE/TC/TG	USG ABDOMEN	VCTC	ECG	ON INSULIN
Anitha	4.8	190/110	SLIUF, 28 WEEKS	NON REACTIVE	WNL	DIET
Mutharasi	10	183/117	SLIUF, 29 WEEKS	NON REACTIVE	WNL	DIET
Sathya	22.4	231/179	SLIUF, 28 WEEKS	NON REACTIVE	WNL	DIET
Ponmani	24	188/133	SLIUF,24 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Suganthi	12	187/130	SLIUF,30 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Vinoliya	22.24	211/120	SLIUF,31 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Jayamalini	17.9	200/152	SLIUF,26 WEEKS	NON REACTIVE	WNL	DIET
Srimathy	13.4	190/146	SLIUF,25 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Kurhir begam	8.77	160/90	SLIUF,27 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Senduravalli	9	222/130	SLIUF,24 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Meenatchi	18.4	240/136	SLIUF,29 WEEKS	NON REACTIVE	WNL	DIET
Mangalam	32	244/155	SLIUF,28 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Devaki	9	190/130	SLIUF,30 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Chinnaponnu	15.88	210/167	SLIUF,27 WEEKS	NON REACTIVE	WNL	INSULIN STARTED
Mariyammal	25.3	257/160	SLIUF,28 WEEKS	NON REACTIVE	WNL	INSULIN STARTED