

**BIOCHEMICAL CHANGES IN GESTATIONAL DIABETES IN
COMPARISON TO HEALTHY PREGNANT WOMEN**



Ph.D THESIS

BY

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**INSTITUTE OF CHEMICAL SCIENCES
UNIVERSITY OF PESHAWAR
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ABSTRACT

Biochemical changes in women with gestational diabetes mellitus (GDM) and healthy pregnant women (HPW) were studied. 103 GDM women and 97 HPW were selected and registered for the study from the admitted pregnant women of Gynea Ward of Khyber Teaching Hospital, Peshawar. Sociodemographic and other pregnancy related information, including monthly-income, age, body mass index (BMI), parity, previous history of gestational diabetes and family history of diabetes, were collected on a well-designed questionnaire. Fasting blood sugar (FBS), random blood sugar (RBS), glycosylated hemoglobin (HbA1c), hemoglobin (Hb), platelet count (PC), total cholesterol (TC), triglycerides (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), liver function tests namely alanine amino transferase (ALT), alkaline phosphatase (ALP), serum bilirubin, renal function tests namely urea, creatinine, and hormones like insulin, prolactin, cortisol, triiodothyronine (T3), thyroxin (T4) and thyroid stimulating hormone (TSH) were determined by standard procedures using recommended kit for each procedure. The data were statistically analyzed using computer software, SPSS version 10. The mean monthly income of GDM and HPW was Rs.30845 $\pm$ 11107 vs Rs.28360 $\pm$ 11511, mean age was 34.01 $\pm$ 4.54 years vs 30.30 $\pm$ 5.86 years, mean BMI was 28.07 $\pm$ 2.90 kg/m² vs 27.30 $\pm$ 1.94 kg/ m², mean parity was 5.44 $\pm$ 2.49 vs 4.95 $\pm$ 2.43, mean systolic BP was 114.80 $\pm$ 17.14 mm Hg vs 107.70 $\pm$ 19.40 mm Hg and mean diastolic BP was 86.50 $\pm$ 7.48 mm Hg vs 80.70 $\pm$ 10.02 mm Hg respectively. Age, BMI, systolic and diastolic BP was significantly higher (P<0.05) according to Pearson Chi- square test in GDM women as

compared to HPW. The mean FBS, RBS and HbA1c of GDM women and HPW were 111.69 ± 8.70 mg/dL vs 86.59 ± 6.91 , 145.45 ± 6.62 mg/dL vs 123.52 ± 9.37 mg/dL and 6.58 ± 1.30 vs 4.95 ± 0.45 respectively. The FBS, RBS and HbA1c of GDM women were significantly higher than the HPW ($P < 0.001$). The mean Hb concentration and PC of GDM women and HPW were $10.98 \pm 1.12\%$ vs $11.01 \pm 1.03\%$ and 226.31 thousand/mL vs 228.14 ± 37.61 thousand/mL. In lipid profile TC and TG of GDM group were significantly higher than the control group ($P < 0.01$). The mean TC of GDM and HPW was 206.01 ± 18.79 mg/dL vs 195.01 ± 24.15 mg/dL, TG was 190.12 ± 19.83 mg/dL vs 172.13 ± 21.66 mg/dL, HDL-C was 55.21 ± 8.20 mg/dL vs 56.20 ± 8.82 mg/dL and LDL-C was 93.13 ± 18.71 mg/dL vs 88.10 ± 16.36 mg/dL respectively. Liver and renal function tests of GDM women were not significantly different ($P < 0.05$) from HPW. In liver function tests the mean ALT, ALP and serum bilirubin values of GDM women were 30.21 ± 12.47 U/L, 190.55 ± 22.20 U/L, 0.67 ± 0.41 mg/dL while of HPW were 29.64 ± 7.96 U/L, 189.95 ± 21.28 U/L, 0.58 ± 0.17 mg/dL respectively. In renal function tests the mean serum urea and serum creatinine values of GDM women were 23.70 ± 8.54 mg/dL and 0.82 ± 0.32 mg/dL while of HPW were 21.97 ± 6.16 mg/dL and 0.74 ± 0.15 mg/dL respectively. The mean insulin level of GDM women were 33.68 ± 3.69 μ IU/mL, which was significantly higher ($P < 0.01$) than the mean insulin level of HPW (29.80 ± 2.80 μ IU/mL). Among placental hormones the mean prolactin values of GDM women and HPW were 135.47 ± 9.83 ng/mL vs 131.80 ± 8.54 ng/mL and mean serum cortisol values were 734.9 ± 51.1 ng/mL vs 719.2 ± 54.7 ng/mL. The mean serum prolactin and serum cortisol values of GDM women were significantly higher ($P < 0.05$) when compared to HPW. No significant differences were

observed in the concentration of thyroid hormones. The mean TSH values of GDM and HPW were 1.72 ± 0.95 mIU/L vs 1.87 ± 0.83 mIU/L, mean T₃ values were 2.51 ± 0.62 nmol/L vs 2.62 ± 0.57 nmol/L, mean T₄ values were 103.86 ± 14.74 nmol/L vs 105.38 ± 13.93 nmol/L. Cesarean sections (P=0.009), still birth rate (P=0.003) and macrosomic babies (P=0.001) were significantly more in GDM group.

Chapter 1

INTRODUCTION

Pregnancy is the fertilization and development of one or more offspring in a woman's uterus. Usual duration of pregnancy is 38 weeks. Pregnancy is a unique normal physiological state where life exists on life. The fetus is entirely dependent on mother for its healthy growth. To keep the fetus growing and the mother healthy, the body of the pregnant woman has to make biochemical and physiological changes in cardiovascular, hematological, renal, metabolic and respiratory systems. All these changes are required in normal pregnancy and during the complications of pregnancy. To cope with the new environment of pregnancy, the body increases its blood sugar, cardiac output and breathing rate. Levels of progesterone, estrogen, cortisol and prolactin also rise continuously throughout normal pregnancy to provide better and nourished environment to the fetus. The biochemical changes during pregnancy result in accumulation of lipid in early gestation, which results in insulin resistance and metabolic syndrome. Metabolic syndrome is a condition which involves central obesity and any two of the following factors; raised triglyceride levels, reduced high density lipoprotein (HDL) cholesterol, raised blood pressure and raised fasting plasma glucose. In the altered physiological state of pregnant woman, many complications can occur. Amongst these complications gestational diabetes, preclampsia, hypertension and obesity are the most serious and fatal ones associated with pregnancy [1].

Gestational diabetes mellitus (GDM) is a condition of pregnant woman where glucose intolerance is found during pregnancy. GDM usually develops during pregnancy and ends after pregnancy. The majority of women with histories of

gestational diabetes are obese; having sedentary life styles and consuming very few vegetables and fruits [2].

GDM women are at higher risk of developing various chronic diseases like metabolic syndrome, hypertension and diabetes mellitus. Gestational diabetic women are at high risk of pre-eclampsia, hypertension, preterm deliveries, caesarian section, still births and insulin treatment. Neonates of the gestational diabetic mothers are usually big in size and large for gestational age [3].

GDM is neither due to abnormal secretion of insulin nor due to disproportionate secretion of pro insulin. It may be the effect of human placental lactogen, cortisol and prolactin. These hormones are anti-insulinogenic and lypolytic in action [4].

The pathophysiology of gestational diabetes is not well understood. This study was an attempt to know the biochemical changes occurring in gestational diabetes and to see to what extent these changes are different from healthy pregnant women (HPW). Moreover, the aim of this project, was to investigate as which factors favor the development of GDM and to find out the effect of GDM on the health of mother and the infant. This study has provided useful information for medical practitioners, educationist and researchers for the better management and treatment of gestational diabetes.

To achieve the above goals, pregnant women were screened for gestational diabetes at 28th week of gestation, and following information were collected and tests performed.

- Information about socioeconomic status, education level, age, body mass index (BMI), blood pressure, previous history of gestational diabetes and family history of diabetes were collected.
- Fasting and random blood sugar, glycosylated hemoglobin (HbA1c), hemoglobin concentration and platelet count were determined in both GDM and healthy pregnant women.
- Lipid profile of both gestational diabetics and healthy pregnant women in terms of total cholesterol, triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) were determined.
- Liver function tests namely alanine amino transferease (ALT), alkaline phosphatase (ALP), bilirubin and renal function tests namely urea and creatinine in both gestational diabetic and healthy pregnant women were determined.
- Insulin, prolactin and cortisol were determined in both gestational diabetic and healthy pregnant women.
- Thyroid function tests including triiodothyronine (T₃), thyroxin (T₄) and thyroid stimulating hormone (TSH) were determined.
- Maternal and neonatal consequences in term of preterm deliveries, still births and macrosomic babies were reported.

Chapter 2

REVIEW OF LITERATURE

2.1 Diabetes Mellitus

Diabetes mellitus is a metabolic disorder of carbohydrate in which a person has high blood sugar level, either because of insufficient production or dysfunction of insulin. The dysfunction of insulin is usually referred to as insulin resistance. The high blood sugar in the body produces the classical symptoms of diabetes. These symptoms are polydipsia (increased thirst), polyphagia (increased hunger) and polyuria (frequent urination). The high blood sugar level of diabetes mellitus is due to abnormal metabolism of carbohydrates, fats, proteins and especially insulin. The metabolic disorder is characterized by hyperglycemia and is associated with high incidence of complications affecting both micro and macro vascular system. Macro vascular disease affects the coronary arteries, the cerebral vessels and the large peripheral arteries of the lower extremities. Microangiopathy affects the kidneys, eyes and nerves. Both forms of complications are major causes of death and disability in diabetes mellitus [3].

Diabetes mellitus results from dysfunction of pancreatic beta cells and insulin resistance. The impaired beta cells carry abnormal partial or total synthesis of insulin. The insulin resistance is basically caused by cell membrane which hinders the transportation of glucose to the cell and prevents oxidation. As glucose is not completely metabolized, high amount of glucose is constantly circulating in the blood. To maintain the normal level of glucose in the blood, the kidney excretes the excess sugar from the blood in the urine. As required amount of glucose is not utilized by the body cells, the

patient is under constant impression of hunger and eats more frequently.

Diabetes mellitus is usually classified into type 1 or insulin dependant diabetes mellitus (IDDM), type 2 or non insulin dependant diabetes mellitus (NIDDM), impaired glucose tolerance (IGT) and gestational diabetes mellitus (GDM).

Type 1 diabetes results from the destruction of beta cells of pancreas, and there is absolute insulin deficiency. Type 1 diabetes can occur at any age; however, it is more common in people of age less than 30 years. Type 1 diabetes is absolutely dependent on exogenous insulin [3].

Type 2 diabetes is the most common type of diabetes, and its prevalence is increasing dramatically throughout the world. The basic cause of type 2 diabetes is insulin resistance with insulin deficiency or insulin secretory defect with insulin resistance. Type 2 diabetes occurs more in adults of age 40 years or above. Both hypoglycemic drugs and exogenous insulin are used for its control [4].

Impaired glucose tolerance (IGT) is a condition where blood glucose level is higher enough but below the diagnostic criteria used for diabetes. IGT is measured by oral glucose tolerance test (OGTT). The 2-hour plasma glucose value in IGT is greater than or equal to 140 mg/dL and up to 199 mg/dL. Majority of the IGT pregnant women are euglycemic in every day life and often have normal glycosylated hemoglobin (HbA1C) levels. IGT is high risk factor for future diabetes mellitus [5].

GDM is any degree of glucose intolerance with onset or first recognition during pregnancy. GDM patients are usually identified during second or third trimester of pregnancy. Glucose tolerance usually returns to normal range within 6 weeks after pregnancy ends. Most GDM patients do not develop diabetes mellitus later in life;

however some will develop IGT and type 2 diabetes. The prevalence rate of GDM is 1–14 percent depending upon the population studied. As GDM is strongly associated with increased fetal mortality and morbidity ratio, so prompt detection and aggressive treatment is important. All pregnant women do not require screening; those exempted must have normal weight before pregnancy, should be of age below 25 years, should be member of an ethnic population with low prevalence of diabetes mellitus, have no history of abnormal glucose tolerance, have no known diabetic history in first-degree relatives and have no history of poor obstetric outcome [6].

2.2 Pregnancy

Pregnancy is defined as the fertilization and development of one or more offspring in a woman's uterus. Length of pregnancy or period of gestation is usually 38 weeks after conception. Pregnancy is a unique physiological state where life nourishes on life, the women must adopt itself to the changed environment. Many physiological changes namely cardiovascular, hematological, renal, metabolic and respiratory occur during pregnancy. All these changes are considered entirely normal in pregnancy. These changes become very important in the event of complications. The body must change its physiological and homeostatic mechanisms in pregnancy to ensure the fetus is provided with all requirements. Physiological and anatomical alterations develop in many organ systems during the course of pregnancy and delivery. Early changes are due, in part, to the metabolic demands brought on by the fetus, placenta and uterus and, in part, to the increasing levels of pregnancy hormones, particularly those of progesterone and estrogen. Later changes, starting in mid-pregnancy, are anatomical in nature and are caused by

mechanical pressure from the expanding uterus. These alterations create unique requirements for the anesthetic management of the pregnant woman [7].

Pregnant women experience adjustments in their endocrine system. Levels of progesterone and estrogens rise continuously throughout pregnancy, suppressing the hypothalamic axis and subsequently the menstrual cycle. Estrogen is mainly produced by the placenta and is associated with fetal well-being. Prolactin level increases due to maternal pituitary gland enlargement by 50%. This mediates a change in the structure of the mammary gland to lobular-alveolar. Parathyroid hormone is increased which leads to increase of calcium uptake in the gut and re-absorption by the kidney. Adrenal hormones such as aldosterone and cortisol also increase. Human placental lactogen is produced by the placenta and stimulates lipolysis and fatty acid metabolism by the woman, conserving blood glucose for use by the fetus. It can also decrease maternal tissue sensitivity to insulin, resulting in gestational diabetes [8].

Woman is the sole provider of nourishment for the embryo and later, the fetus, and so her blood volume and plasma gradually increase by 40-50% over the course of the pregnancy to accommodate the changes. The increase in blood volume and plasma is mainly due to increased aldosterone levels which may result in stroke volume, heart attack and cardiac output. During the first trimester of pregnancy, cardiac output increases by 50 percent. The systemic vascular resistance caused by elevated progesterone drops due to the smooth muscle relaxation and overall vasodilatation, leading to a fall in blood pressure. Diastolic blood pressure consequently decreases between 16-26 weeks, and increases again to pre-pregnancy levels by 36 weeks. If the blood pressure is found to be abnormally high, the woman should be investigated for pre-eclampsia, eclampsia and

other causes of hypertension. This increase in blood pressure is mainly due to an increase in plasma volume through increased aldosterone. Progesterone may also interact with the aldosterone receptor, thus leading to increased levels. Number of red blood cell also increases in pregnancy due to increased erythropoietin level. Cardiac function is enhanced leading to increased heart beat rate and increased stroke volume. Cardiac output rises from 4 to 7 liters in the second trimester. Blood pressure also alters due to decreased sensitivity of angiotensin and vasodilation provoked by increased blood volume [9]. Plasma volume during pregnancy increases by 50% and the red blood cell volume increases only by 20-30%. The number of white blood cell increases and reaches its peak value of 20 mg/mL in stress and strain conditions. A pregnant woman also becomes hyper-coagulable, leading to increased risk of developing embolisms and blood clots due to increased production of coagulation factors by liver. Women are at high risk of developing thrombi or clot, during the weeks following labor. Clots are usually formed in the left leg or the left iliac venous system. The left side is most affected because the left iliac vein is crossed by the right iliac artery. The increased flow in the right iliac artery after birth compresses the left iliac vein leading to an increased risk for thrombosis (clotting). Edema, or swelling, of the feet is common during pregnancy, partly because the enlarging uterus compresses veins and lymphatic drainage from the legs [10].

During pregnancy, both carbohydrate metabolism and protein metabolism are affected. One kilogram of extra protein is deposited, with half going to breast glandular tissue, hemoglobin, plasma protein and uterine contractile proteins, and another half going to the uterus and placenta. Increased amount of nutrients are required for fetus growth and fat deposition. Changes are caused by prolactin, cortisol, lactogen and steroid

hormones. Maternal insulin resistance can result in gestational diabetes mellitus. Nutritionally, caloric requirements of pregnant women increase to 300 kcal/day and protein requirement increases up to 70 or 75 g/day. There is also an increased folate requirement from 0.4 to 0.8 mg/day. On average, a weight gain of 20 to 30 lb (9.1 to 14 kg) is experienced. The use of Omega 3 fatty acids help in visual and mental development of infants [11].

A pregnant woman may experience an increase in kidney and ureter size. The glomerulus filtration rate (GFR) in pregnant women increases by 50%, returning to normal around 20 weeks postpartum. Plasma sodium is not affected by increased GFR so it remains unchanged. There is decreased creatinine, blood urea nitrogen and glucosuria (due to saturated tubular re-absorption). Persistent glucosuria may result in gestational diabetes. The renin-angiotensin system is up regulated, causing increased aldosterone levels [12].

Pregnancy is a unique physiological state which is exposed to many complications. Gestational diabetes is the major complication of pregnancy. Many other complications of pregnancy are because of gestational diabetes. Gestational diabetes is affecting 5–7 percent of all pregnancies and is one of the well-known risk factor for developing type 2 diabetes later in life [13].

2.3 Gestational Diabetes Mellitus

GDM is defined as glucose intolerance recognized for the first time in current pregnancy. It is affecting 5–7 percent of all pregnancies. In US the incidence of GDM is reported 14% of all pregnancies and the rate of incidence are constantly increasing in

multiethnic populations [14]. GDM is one of the well-known risk factor for developing type 2 diabetes later in life. Various factors on the basis of which one can predict that either a pregnant woman will become diabetic in future are: early diagnosis of GDM in pregnancy, need for insulin treatment during pregnancy, high blood glucose level at diagnosis, preterm delivery, macrosomic babies and an abnormal oral glucose tolerance test after two months of delivery [15-16]. Obesity plays an important role in developing diabetes among the women with previous history of gestational diabetes. Recently, it has been reported that GDM has strong association with increased risk of serious perinatal morbidities and mortalities. Poorly controlled GDM consequences are evident, but still no proper consensus exists on either diagnostic criteria or accepted biochemical changes in controlling GDM. Traditionally GDM is taken as a disorder of carbohydrate metabolism; thus, blood glucose level has become the main key player for controlling and directing treatment during pregnancy [17-18].

Normal pregnancy has been considered as a “diabetogenic state” due to change in the pattern of insulin secretion and sensitivity, resulting in elevated postprandial glucose and insulin response in late pregnancy. During the first four months of pregnancy high level of estrogen is released which markedly increases insulin sensitivity. Human placental lactogen, cortisol, prolactin and leptin are mainly involved in insulin secretion and insulin sensitivity. The marked reduction of insulin sensitivity during normal pregnancy is compensated by increased insulin secretion. When this need is not fulfilled, abnormal glucose tolerance develops. Thus pregnancy is characterized as a state of insulin resistance and hyperinsulinemia in response to the diabetogenic effects on sugar metabolism [19]. The major goal of management in pregnancy, complicated by diabetes,

is to control and maintain blood glucose as close to normal as possible [20]. Various methods of glucose monitoring such as plasma, urine strips, capillary, and continuous glucose monitoring have been proposed for the measurement of fasting, pre-prandial, postprandial, and mean 24-hour blood glucose concentrations. Moreover, several authors have reported the association between pregnancy outcome and postprandial glucose determinations [21-24].

2.4 Incidence of Gestational Diabetes Mellitus

Glucose is not properly regulated in 3-10% of all pregnancies. GDM is the glucose intolerance of variable degree with onset or first recognition during pregnancy. It is affecting 90% of cases of diabetes mellitus during pregnancy. Annually 21 million people of the world (7% of the population) are reported with some form of diagnosed diabetes; another 6 million people are reported with undiagnosed particularly type 2 diabetes among women of child bearing age. Currently, type 2 diabetes mellitus are affecting 8% of cases of diabetes mellitus in pregnancy, and preexisting diabetes mellitus accounts for 1% of all pregnancies [25].

American Diabetes Association proposed the Carpenter and Coustan [26] criteria for diagnosis of gestational diabetes mellitus. The Carpenter and Coustan cutoff values are lower than the previously recommended values of National Diabetes Data Group (NDDG) and thus results in higher incidence of GDM. A total of 26,481 women were screened using a 50-g, 1 hour oral glucose tolerance test, and 4,190 women underwent a diagnostic 100 g, 3 hour oral glucose tolerance test after an abnormal screening. Overall, the GDM prevalence among screened women was 4.8% by Carpenter and Coustan

criteria and 3.2% by NDDG. The age-adjusted GDM prevalence by NDDG and Carpenter and Coustan criteria, respectively, was 3.9 and 5.6% in Hispanics, 5.0 and 7.4% in Asians, 2.4 and 3.8% in Whites and 3.0 and 4.0% in African-Americans. On average the incidence of GDM increased by 50% with use of the Carpenter and Coustan thresholds. Relative increments were higher in low-risk age and ethnic groups. The clinical implications of GDM are strongly associated with adverse outcomes in pregnancy, such as hypertension, preeclampsia, macrosomia, cesarean delivery, and birth trauma. Women with GDM are at an increased risk for type 2 diabetes mellitus later in life or postpartum.

An original study by O'Sullivan and Mahan [27] reported the level of glycemia during pregnancy that could identify women at risk for type 2 diabetes in the future. When tested several weeks postpartum most women with GDM exhibit normal glucose tolerance with increased insulin resistance and impaired beta-cell function. Several studies from North America have demonstrated postpartum type 2 diabetes rates ranging from 3% to 40% within one year after delivery, depending on the proportion of participants with severe hyperglycemia during pregnancy and ethnicity [28]. Kjos et al., [29] reported that 9% of Hispanic women with GDM developed type 2 diabetes in early postpartum. Similarly, Catalano et al., [30] found that 3% of white subjects with GDM developed Type 2 diabetes postpartum. Furthermore, Dacus et al., [31] reported a 10% rate of developing diabetes in a population of predominantly black women with GDM.

In Chicago, the cumulative prevalence of type 2 diabetes in women with previous GDM increased rapidly in the first two years after delivery, and approximately 50% of

subjects with GDM developed type 2 diabetes within 5 years postpartum [32]. A systemic review on the development of diabetes in previous GDM cases reported that the incidence of type 2 diabetes increases rapidly in the first 5 years after delivery and appeared to be double after ten years [33]. There are very limited studies on postpartum glucose intolerance in Asian women with previous history of GDM. Kim et al., [34] evaluated 211 South Korean women with GDM at 6 weeks postpartum and found that 9% had impaired glucose tolerance [IGT] while 9.5% had developed type 2 diabetes. After one year of delivery, out of 120 pregnant women, 5% were reported with IGT and 5% with type 2 diabetes mellitus [35]. Another study of 800 Chinese women in Hong Kong, reported that the incidences of diabetes and IGT was 13% and 22%, respectively, at 6 weeks postpartum [36].

The Diabetes Prevention Program demonstrated that subjects who were having previous history of GDM had a 71% increased risk of developing type 2 diabetes, as compared with women without such a history [37]. Lee et al., [38] reported that previous GDM history was a greater risk factor than waist circumference or family history of diabetes for developing type 2 diabetes. The odds ratio for development of Type 2 diabetes in Hong Kong was 3.8 while in south India it was 35.5 [39]. In the united Kingdom, South Asian women had a higher risk of developing diabetes, as compared with Caucasian women (48.6% vs. 25.0%), within a mean period of 4.83 years after delivery [40].

2.5 Diagnosis of Gestational Diabetes Mellitus

Several definitions are currently used for the diagnostic criteria of GDM. The reasons for this variation are complicated, reflecting advances in assay technology,

declines in perinatal mortality, epidemiology, evolving access to care and local cultural practices. Screening for GDM at the time of pregnancy is suggested if conditions like prior history of GDM, glycosuria, severe obesity, polycystic ovarian syndrome, and delivery of an infant that is large for gestational age and family history of type 2 diabetes are present. If these risk factors are absent, women are supposed to undergo diabetes screening at 24–28 week of gestation [41]. The American College of Obstetrics and Gynecology have similar recommendations for screening test if any of the following conditions are present: These are age less than 25 years, obesity, family history of diabetes, non white race/ethnicity, history of abnormal glucose tolerance and poor obstetric outcome. In contrast, the World Health Organization has recommended universal screening of all pregnant women for GDM at 24–28 weeks gestation [42].

Gestational diabetes would be present if one or more values met or exceeded than 5.1 mmol/L fasting glucose, 10.0 mmol/L one hour post glucose and 8.5 mmol/L two hours post glucose. Using the above criteria, 17.9% of the pregnant population will have gestational diabetes. Continuous relation was found between glycemia (fasting, and 1 or 2 hour post glucose load) and primary outcomes such as macrosomia, primary caesarean section, cord C-peptide and neonatal hypoglycemia. However, the Pedersen hypothesis [43] did not show any inflection point indicating clearly increased risk of any of these outcomes with a particular glucose category; rather, risk increased gradually over the glucose range. Crowther et al. [44] reported composite endpoint of shoulder dystocia, bone fracture, fetal death and nerve palsy in women with gestational diabetes. London et al. [45] had also reported a composite but slightly different endpoint, namely fetal death, neonatal hypoglycemia, hyperbilirubinemia and birth trauma. A recent meta-analysis

study demonstrated that treating gestational diabetes significantly decreases frequency of macrosomia and shoulder dystocia, which is the leading cause of birth injury [46]. International Association of Diabetes and Pregnancy Study Groups (IADPSG) [47] reported the incidence of GDM to be 17.8%. Out of which, 8.3% of women were diagnosed on the basis of fasting glucose ≥ 5.1 mmol/L, a further 5.7% were diagnosed on the basis of one hour post challenge glucose ≥ 10.0 mmol/L and 2.1% were diagnosed on the basis of a two hour post-challenge value ≥ 8.5 mmol/L. The IADPSG consensus suggested a single 75 g OGTT at weeks 24–28 of gestation. Testing in early pregnancy for overt diabetes was recommended in populations with a high prevalence of diabetes. Overt diabetes may be diagnosed in pregnancy at fasting glucose level of ≥ 7.0 mmol/L, random glucose level ≥ 11.1 mmol/L and HbA1c $\geq 6.5\%$.

2.6 Sociodemographic Factors involved in prevalence of GDM

The prevalence of GDM has weak but significant relation with socioeconomic status including education level, ethnicity, parity, maternal age, smoking, nutrition and family history of diabetes have been reported to be associated with gestational diabetes [48]. The association between GDM and socioeconomic status is not well established because previous studies have reported conflicting results due to different definitions used for economic status. Two studies have found no association [49-50], and one showed that living in an area of deprivation is positively associated with GDM [51]. Other factors used to determine socioeconomic status were public and private health care sectors. Public sector health care increased association with GDM [52]. Income, health insurance, residential zip codes were not associated with GDM [53]. The importance of maternal social factors on the health of the offspring is well known. Various definitions

of social status is used depending upon monthly income, educational attainment, employment, family influence, type of health care and house hold characteristics. There is a strong association between social class and infant mortality. Social adversity probably represents a wide range of behavioral, environmental, medical and psychological factors that are causally related to pregnancy outcomes. Lower socioeconomic status is associated with an increased risk of various adverse pregnancy outcomes including perinatal mortality, preterm birth and low birth weight. Socioeconomic status, although well established as a risk for obesity and type 2 diabetes, has been less well correlated with GDM [54]. The increasing incidence of GDM, independent of ethnicity, socioeconomic status, or maternal age, has many short-term adverse pregnancy outcomes and long-term future risk of type 2 diabetes. Smoking has been suggested as the key factor underlying socioeconomic differences in low birth weight and infant mortality. Lower socioeconomic status is well recognized as a risk for chronic disease in developed and developing countries. Socially disadvantaged GDM women are less likely to seek perinatal care and thus having more pregnancy complications [55].

Previous descriptive studies have shown an increased association between GDM and many ethnicities, with women from Asian, African, and Hispanic backgrounds being at most risk. Women from across Asia had an increased risk of GDM compared with women born in Australia and New Zealand. Asian Australians are also found to have an increased risk for type 2 diabetes relative to Caucasian Australians [56].

Parity is the number of times a woman has given birth to an infant, dead or alive. Parity is closely related to certain extent with maternal age and socioeconomic status. In

GDM women, the risk of adverse outcome increases with increase in parity. Women of high parity tend to receive inadequate obstetric care owing to delays in seeking care and poor attendance. High parity is associated with abnormal fetal birth and obstetric hemorrhage. Parity, however, does not have a significant effect on the incidence of GDM when maternal age is taken into account [57].

Maternal age is an established risk factor for gestational diabetes mellitus, but there is no consensus on the age above which there is significantly increased risk of GDM. American Diabetes Association recommends the use of age equal to or more than 25 years for screening of gestational diabetes. In clinical practice, maternal age equal to or more than 25 years should be adopted instead of age equal to or more than 35 years. More maternal age is a risk indicator for several pregnancy complications, including spontaneous miscarriage, gestational diabetes, ectopic pregnancy, chromosomal abnormalities, and hypertensive and bleeding disorders [58].

2.7 High Risk Factors involved in incidence of GDM

Women at the greatest risk of developing GDM are those who are obese, older than 25 years, have a previous history of abnormal glucose metabolism or poor obstetric outcome, have first-degree relatives with diabetes, or are members of ethnic groups with high prevalence of diabetes. Women, who are screened on OGCT and OGTT criteria and they exceed the glucose threshold on that criteria, are high risk patients [59]. High risk women must be assessed if any of the following risk factors are present:

2.7.1 Obesity

Obese women are at higher risk of developing GDM. In United States, the prevalence of obesity is increasing day by day which leads to the increased incidence of GDM [60]. GDM and obesity not only increase fetal and maternal complications during pregnancy but it is also associated with increased risk of developing type 2 diabetes later in life, so measures should be adopted for prevention of both obesity and GDM [61-62]. In comparison to normal pregnant women, the risk of becoming GDM among overweight, obese and marked obese women increases by two, four, and eight folds respectively [63].

Pre-pregnancy obesity of the pregnant women results in preterm deliveries and has linear relation with hypertensive disorders of pregnancy [64]. Association between body mass index and the risk of gestational diabetes in a second pregnancy was studied in 22,351 women. For those with GDM in the first pregnancy, the age-adjusted risk of GDM in the second pregnancy was 38.19% and for those whose first pregnancy was not complicated by GDM, the risk was 3.52%. Those women who gained over weight during pregnancy were having increased risk of GDM in the second pregnancy as compared to pregnant women who remained stable during pregnancy [65]. It has also been reported that GDM and preclampsia developed more in obese pregnant women than normal weight pregnant women. Obese and overweight mother also experiences more instrumental delivery, asphyxia, respiratory distress syndrome, congenital anomaly and prenatal death than normal weigh pregnant women. Thus both GDM and obesity results in adverse maternal and perinatal outcomes during pregnancy [66].

2.7.2 Maternal Age

Maternal age is also a known risk factor for developing GDM, but there is certain confusion, that either it is age above 25 or above 30 years which markedly increases risk for GDM. According to American Diabetes Association, the lowest cutoff is equal to or more than 25 years and the relative risk for becoming GDM rose by 4% for each year of age after 25 years [67]. In meta analysis study, pregnancies were categorized according to maternal age, i.e., less than 20 years, 20–24 years, 25–29 years, 30–34 years, 35–39 years and 40 or more than years for statistical analysis. Out of 16,383 women, 96.6% women continued their pregnancies beyond the first trimester, and the prevalence of GDM from the youngest to the oldest category was 1.3, 2.5, 6.2, 10.3, 21.7, and 31.9%, respectively [68].

2.7.3 Ethnicity

Different ethnic groups are at different risks of developing GDM. Asian women and women from South Central Asia are considered to be at the top for developing GDM. However, high risk for GDM is also found among Latin American women. Genetic diversity, dietary traditions, life style modifications, religious and cultural factors among different countries are the major reasons for differences among ethnic groups [69]. South or East Asian, African, Native American and Hispanic populations are reported to be at the high risk of developing GDM. Other risk factors like education, income inequality and discrimination have been reported to influence maternal and perinatal outcome independent of ethnicity [70]. Specific risk factors and their affect on incidence of GDM in a defined population are difficult to quantify, as different studies have shown different

results. It is therefore suggested that high and low risks for GDM in various ethnic populations should be defined on the basis of prevalence of type 2 diabetes [71].

2.7.4 Family History and Genetics

Women having first degree relative with Type 2 diabetes are considered to be at the highest risk of developing GDM, confirming that GDM and type 2 diabetes have the same genetic background [72]. A genetic component is important because GDM gathers in families [73]. However, as mothers of GDM women are often diabetics, it has been considered that the intrauterine environment also contribute to the incidence of GDM [74]. During pregnancy, most of the women experiences temporary glucose intolerance or gestational diabetes which easily reverses to normal after delivery. But it is noteworthy that these women are at increased risk of developing type 2 diabetes later in life [75]. Maturity-onset diabetes of the young is a monogenic form of type 2 diabetes that is inherited and expressed usually before the age of 25 years. Maturity onset diabetes is caused due to mutation or any genetic defect in five different kinds of genes which are strongly associated with pancreatic β -cell dysfunction. In different populations mutations is responsible for 5–6% incidence of GDM [76]. It has been reported that GDM women who develop type 2 diabetes later in life has the same frequency of specific antigens as found in the background population [77]. This is the reason that why most of the women with previous GDM develop type 2 rather than type1 diabetes [78].

2.8 Low Risk Factors involved in development of GDM

Women are classified as having a low risk of becoming GDM if their age is less than 25 years, they are of normal weight before pregnancy, they belong to an ethnic

group with a low prevalence of GDM, they do not have known diabetes in first degree relatives and have no history of poor obstetric outcome. Women of low risk should be tested at 24–28 weeks of gestation. The most common and important low risk factors of GDM are discussed below

2.8.1 Hypertension

Hypertension during first trimester of pregnancy doubles the risk of developing GDM during pregnancy. Mean blood pressure levels significantly increase among the women with previous history of GDM as compared to women who were having normal glucose level during pregnancy [79]. High blood pressure interacts synergistically with GDM in obese women, and results in adverse metabolic effects in pregnancy. Insulin resistance is known to be involved in the pathogenesis of GDM and has been reported as a contributing factor for both gestational and chronic hypertension [80]. Endothelial dysfunction in women with gestational diabetes is also associated with hypertension during and after pregnancy [81].

2.8.2 Low Birth Size

Studies done on children and adults have reported linear and inverse relationship between birth size and prevalence of GDM and other metabolic disorders [82]. In another study, both low and high birth weight were risk factors for GDM development [83]. A significant relationship between gestational diabetes and women of low birth weight has been reported among Americans and Hispanics women [84]. Women of low birth weight were having significantly higher 2-hour glucose concentration when compared to women of normal weight for gestational age [85]. It has also been reported that low birth weight

increases the prevalence of GDM and diabetes in young population. The most acceptable explanation for this is that in past, large birth weight was related to general health condition of the mother and the baby while now-a-days, large birth weight is due to impaired glucose tolerance or diabetes during pregnancy [86-88].

2.8.3 Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is a reproductive condition identified by chronic anovulation, androgen excess and insulin resistance [89]. Legro et al., [90] have reported that PCOS women are more exposed to GDM as compared to non-PCOS women [90] while several other studies have reported increased prevalence of PCOS in women with previous history of GDM [91-93]. Between 2002 and 2004, a large number of pregnant women were randomly screened for gestational diabetes. Out of these 92,933 pregnant women were identified as having GDM. The ethnic distributions of these women were found to be 6.5% black, 18.7% Asian, 24.4% Hispanic and 39.2% white. The percentage of women with diagnosed PCOS was 17%, out of which 97% were identified with other PCOS problems. Women with polycystic ovary syndrome showed 2.4 fold increased risk of developing GDM, independent of weight, age, ethnicity, and multiple gestations. PCOS is also linearly associated with glucose intolerance suggesting higher incidence of GDM among women with PCOS [94]. Obesity also contributes to increased risk of GDM in women having PCOS symptoms [95].

2.8.4 Iron Supplementation

During pregnancy, iron supplementation is beneficial for both mother and fetus. However, in mid pregnancy, iron supplementation may result in hypertension, insulin resistance and glucose impairment. Excessive iron supplements used during pregnancy produces free radicals which later on produces oxidative stress. Oxidative stress reaches to its peak by sixth month of gestation and adversely affects fetal health. Excessive iron intake can lead to impaired glucose metabolism, β -cell dysfunction and β -cell toxicity [96]. In epidemiological studies association between circulating levels of ferritin and GDM has been reported [97-98]. Dietary heme iron increases risk for GDM and Type 2 diabetes [99].

2.8.5 Diet

Diet plays an important role in the development as well in the control of type 2 diabetes and GDM. At the time of diagnosis, patients are usually asked questions about the diet they take. Proper modification in diet can control GDM and type 2 diabetes to some extent. Women considering dietary advice about their diet can prevent the development of GDM [100]. Diet high in fibers and low in glycemic load not only improves glucose tolerance and insulin sensitivity but also reduces the risk of developing GDM. Obesity and decrease intake of polyunsaturated fats increase the prevalence of GDM [101]. High incidence of GDM is strongly associated with high intake of sugar sweetened beverages like Pepsi, Coca-Cola, fruit punch and carbonated beverages. Cane sugar (sucrose) used in the ingredients of sugar sweetened beverages have sharply climbed the rates of obesity, GDM and type 2 diabetes throughout the world [102].

2.9 Biochemical Changes in Gestational Diabetes

Pregnancy is a normal physiological state where different biochemical changes occur for the healthy survival of the mother and fetus. These changes are usually metabolic and hormonal. In human pregnancy, series of metabolic changes take place that promote adipose tissue accumulation in the first four month of pregnancy followed by insulin resistance and facilitated lipolysis in late pregnancy. In early pregnancy, insulin secretion increases, while insulin sensitivity may increase, decrease or remain unchanged. [103-104]. However, in late pregnancy, maternal adipose tissue depots decreases, while postprandial free fatty acid levels increase and insulin- mediated glucose disposal worsens by 60% as compared with pre pregnancy. The decrease ability of insulin to suppress whole body lipolysis during late pregnancy is further reduced in GDM subjects [105-106], contributing to increase in insulin resistance, increase in hepatic glucose production, and increase in postprandial free fatty acid levels [107-108]. Various placental hormones have been reported to reprogram maternal physiology and to meet fetal needs.

2.10 Metabolic Changes

It seems that glucose metabolism is the main variable for controlling and monitoring gestational diabetes. However, the fact that pregnancy outcome is not completely controlled even when strict glycemic control is maintained. Insulin-treated patients are having lower complication rates when compared with diet-treated patients. Changes in carbohydrate, protein and lipid metabolism occur during pregnancy to ensure a continuous supply of nutrients to the growing fetus despite of intermittent maternal

food intake. These metabolic changes are progressive and may accumulate in women who develop gestational diabetes.

During early pregnancy, glucose tolerance is slightly improved while peripheral (muscle) sensitivity to insulin and hepatic basal glucose production remains normal. Insulin sensitivity is more in the first trimester than in the second and third trimester. The cause of this increased sensitivity is not known, however, these metabolic changes under the influence of cortisol, estrogen, prolactin and progesterone favor lipogenesis and fat storage [109]. GDM is accompanied by alterations in fasting, post prandial 24-hour plasma concentration of glucose, amino acids and lipids. These changes include 3-folds increase in plasma triacylglycerol concentrations during the third trimester of pregnancy, elevation of plasma fatty acids, delayed postprandial clearance of fatty acids, and elevation of branched chain amino acids [110].

Basal endogenous glucose production increases in GDM and in control subjects through out gestation. An increase in first phase insulin response is greater in control subjects. In late pregnancy, insulin suppression of hepatic glucose production is less in GDM women.

In addition to glucose, protein also plays important role in fetal growth. Nitrogen retention is elevated in pregnancy in both maternal and neonatal compartments. It is estimated that at 30 weeks of gestation there is approximately 500-g increase in protein accumulation. Maternal fasting concentrations of most amino acids are significantly decreased in early pregnancy before the accumulation of significant maternal or neonatal tissue. Duggleby and Jackson [111] reported that protein synthesis in the first trimester is similar to that of non pregnant women; however, in second and third trimester the protein

synthesis is increased by 15% and 25% respectively. It appears that the rate of protein breakdown is slightly reduced during fasting while protein turn over is slightly increased in GDM women as compared to healthy pregnant women [112-113]. Kalkhoff et al. [114] reported a direct correlation between maternal amino acid concentrations and birth weight in infants of GDM. In the fetus plasma, the concentrations of amino acids are higher than that of the maternal plasma. This ensures the appropriate availability of all the essential amino acids to the fetus. Amino acids have a greater effect on insulin secretion than glucose, and therefore, change in their delivery to the fetus may have profound consequences on fetal growth. The transport of neutral amino acids in gestational diabetes may be either not affected, decreased, or increased [115-116].

Lipid metabolism is also affected in GDM. Darmady and Postle [117] reported that serum triacylglycerol and serum cholesterol significantly decreased during early gestation and progressively increased in the last trimester. Infants of overweight women were reported to have increased serum free fatty acids, skin fold measurements and birth weights when compared to infants of lean mothers [118]. During the third trimester, GDM experiences an increased triacylglycerol and decreased HDL concentration profile [119]. It has also been reported that GDM women were having lower LDL concentration but higher triacylglycerol levels [120]. The ability of insulin to suppress free fatty acid is significantly lower in women with gestational diabetes. High triacylglycerol and low HDL levels are strongly associated with macrosomic babies in GDM subjects. Moreover, macrosomic babies of GDM patients were having higher lipoprotein and lipid concentrations than those found in healthy pregnant subjects [121-122].

2.11 Hormonal Changes during Gestational Diabetes

Secretion of different hormones changes during pregnancy. Proper balance in various hormones is important for the healthy status of the woman and healthy outcome of the baby. Some of the hormones having a role in mother physiology and fetus development are briefly reviewed.

2.11.1 Insulin

Insulin is a pancreatic hormone and is mainly involved in carbohydrate metabolism. It is a small protein of molecular weight 5808 amu. It is composed of two chains of amino acids connected together by disulfide linkages. When the two amino acid chains are split apart, the functional activity of insulin molecule is lost. The most important function of insulin is to store excess glucose, just after the meal, in the liver in the form of glycogen. Between meals, when food is not available and the blood glucose concentration begins to fall, insulin secretion decreases rapidly and the liver glycogen is broken to glucose which keeps the blood glucose level from falling too low. When the quantity of glucose entering the liver cells is more, then it can be stored as glycogen or can be used for local hepatocytes metabolism. Insulin promotes the conversion of all these excess glucose into fatty acids. These fatty acids are consequently packaged as triglycerides in very low density lipoproteins and transported in this form by the blood to the adipose tissue and deposited as fat [123].

Insulin has several effects that lead to fat storage in adipose tissue. First, insulin increases the utilization of glucose by most of the body's tissue, which automatically decreases the utilization of fat, thus functioning as a fat sparer. However, insulin also

promotes fatty acid synthesis. This is especially true when more carbohydrates are ingested than can be used for immediate energy, thus providing the substrate for fat synthesis. Almost all this synthesis occurs in the liver cells and the fatty acids are then transported to the adipose tissue to be stored [124].

In the absence of insulin, hydrolysis of stored triglycerides occur releasing large quantities of fatty acids and glycerol into the circulating blood. Some of the excess fatty acids in the plasma are converted into phospholipids and cholesterol. These two substances, along with excess triglycerides are discharged into the blood as lipoproteins. The plasma lipoproteins increase three fold in the absence of insulin. This high lipid concentration especially high level of cholesterol promotes the development of atherosclerosis in people with serious diabetes. Insulin mediated glucose disposal is decreased by 50% in normal pregnancy while insulin secretion is increased by 200-250% to maintain euglycemic state in mother. In late pregnancy, fasting glucose level significantly increases and insulin sensitivity decreases in GDM women when compared to healthy pregnant women [125-126].

2.11.2 Human Placental Lactogen

Placental derived hormones are believed to play important role in reprogramming maternal physiology to achieve an insulin-resistant state [127]. During normal pregnancy the level of human placental lactogen is increased up to 30-fold which in turn induces insulin release from the pancreas [128]. It has been reported that human placental lactogen can result in peripheral insulin resistance though not confirmed [129].

2.11.3 Human Placental Growth Hormone.

Another hormone which plays a major role in insulin resistance during pregnancy is human placental growth hormone. Human placental growth hormone level increases six- to eight fold during gestation and easily replaces normal pituitary growth hormone in the maternal circulation by 20th week of gestation [130]. In transgenic mice over expression of human placental growth hormone in third trimester of pregnancy caused severe peripheral insulin resistance [131].

2.11.4 Serum Prolactin

Prolactin is also known as luteotropic hormone and is released from placenta. It is encoded by prolactin gene in humans. Prolactin plays important role in regulating lactation and stimulating proliferation of oligodendrocyte precursor cells. It stimulates the mammary glands to produce milk. Prolactin provides immune tolerance to the fetus and is involved in the surfactant synthesis of the fetal lungs. Prolactin decreases the normal level of estrogen in women and testosterone in men. Milasinovic et al [132] found significantly higher prolactin concentration (205.7 ± 66.4 micrograms/L) in GDM women as compared to mothers with normal pregnancy (172.2 ± 60.7 micrograms/L). In late pregnancy, prolactin concentration remains unchanged in GDM when compared to healthy pregnant women [133]. Dybee et al. [134] demonstrated that amniotic fluid prolactin was having weak but significant correlation with HbA1c, maternal plasma glucose level and insignificant correlation with fasting plasma glucose level. Amniotic fluid prolactin concentrations were significantly higher in patients with pregestational diabetes than in women with gestational diabetes mellitus.

2.11.5 Serum Cortisol

Cortisol is a steroid hormone that occurs naturally in the body. Adrenal system creates cortisol in stress condition which controls the way the body converts sugar into energy. Thus cortisol is indirectly affecting insulin levels. Insulin resistance causes many illnesses like gestational diabetes, type 2 diabetes, cushing's disease, metabolic syndrome and polycystic ovarian syndrome. Exercise is an effective way to reduce cortisol because exercise increases metabolism, improves sleep pattern and relieves anxiety. Several factors, including frequent exercise, a consistent sleep schedule, stress management and a diet filled with foods low in fats and high in fiber supports healthy cortisol levels. A case-control study was undertaken to investigate the state of insulin resistance, which occurs in pregnancy, and the possible role of cortisol in augmentation of this state. A total of 30 pregnant women with gestational diabetes and 30 pregnant women with impaired glucose tolerance were compared with 30 control pregnant women with normal glucose tolerance at Khartoum Teaching Hospital in Sudan. Fasting blood samples were collected for hormonal and glucose analysis. Findings revealed that the GDM and the IGT groups had significantly higher levels of serum cortisol than that of the control group, 937.2 ± 79.4 nmol/L and 794.2 ± 60.5 nmol/L vs 597.3 ± 30.9 nmol/L respectively. Cortisol has a great role to play in the deterioration of glucose tolerance in pregnancy [135]

2.11.6 Thyroid Hormones

Thyroid hormones are triiodothyronine (T_3) and thyroxin (T_4). They are released from thyroid glands and play various functions in the body. Iodide ion from the blood is actively transported to thyroid glands where active thyroid compounds triiodothyronine

(T₃) and thyroxine (T₄) are formed. Production of T₃ and T₄ is controlled by thyroid stimulating hormone (TSH). TSH is released from pituitary gland and regulates the secretion of thyroid hormones. On entering into the blood 99 percent of thyroxine and triiodothyronine combines immediately with plasma proteins, all of which is synthesized by the liver. Thyroid binding globulin has the highest affinity for T₃ and T₄ [136].

Pregnancy is a state of relative iodine deficiency because increased renal loss and transferring of iodine to the developing fetus. To compensate, the thyroid gland increases the uptake of iodine from the blood. Insufficient amount of iodine results in cellular hyperplasia and goiter develops. The increase human chorionogonadotropin level during early pregnancy suppress TSH and increase T₄ levels. The reason for this increase may be to supply sufficient amount of T₄ to the fetus. Little T₄ crosses the placenta after the first trimester and the placenta is relatively impermeable to TSH and T₃. Pregnancy causes an increase in thyroid binding globulin through the effects of estrogen [137].

Hyperthyroidism affects 0.2% of pregnant women. Possible cause of hyperthyroidism includes Grave's disease, iodine treatment and thyroid carcinoma. Hypothyroidism affects 1% of pregnant women and as with hyperthyroidism, many of the symptoms occur in normal pregnancy. Patients with hypothyroidism should be counseled to delay pregnancy until maintenance levels of T₄ have been achieved [138].

2.12 Metabolic Complications of Gestational Diabetes

Pederson postulated that intrauterine exposure could lead to permanent changes in fetal metabolism. During GDM pregnancy, the fetus is exposed to high level of glucose which results in excess fetal growth, impaired insulin secretion and decreased insulin

sensitivity. The short term complications of excess infant birth weight are shoulder dystocia and infant hypoglycemia. The altered fetal metabolism may result in impaired glucose tolerance during early youth and adolescence [139-140]. The association between glucose levels with skin folds and percent body fat are not stronger than associations between glucose levels and birth weight [141]. Catalano et al [142] demonstrated that fat mass was significantly elevated in the GDM pregnancies, while birth weight was not necessarily increased. Link between glucose intolerance during pregnancy and childhood obesity has been reported [143]. It was also reported that youth with diabetes were having a history of exposure to diabetic environment in uterus [144-145]. In contrast, others did not find any association of GDM with childhood obesity [146].

The link between GDM and postpartum diabetes in the mother has been recognized. About 5%–10% of cases of GDM are assumed to be previously undetected cases of diabetes. GDM is due to metabolic stresses of pregnancy along with impaired insulin secretion [147]. Almost one-third of women with diabetes may have been affected by prior GDM. In turn, the increased risk of diabetes is associated with future maternal cardiovascular problems [148-150]. Complications associated with gestational diabetes may be explained by the increased body mass index of GDM women. Majority of the women with GDM are overweight and obese but some women who do not meet body mass index criteria for obesity are also metabolically obese. The most common morbidities of GDM are given below.

2.12.1 Hypertensive Disorders

Women with gestational diabetes have an increased incidence of hypertensive disorders like pre-eclampsia, eclampsia, gestational hypertension and chronic hypertension. Recently it has been reported that in general population 2.5% of women develop chronic hypertension, 4.9% develop gestational hypertension, and 4.8% develop pre-eclampsia [151]. Currently, it is not established whether the GDM and hypertensive disorders shares a common causal pathway. However, both GDM and hypertensive disorders are strongly associated with inflammation, insulin resistance and maternal fat deposition. Increased glucose levels on OGTT were associated with a greater risk of pre-eclampsia, even after adjustment for factors such as weight, height, maternal age, smoking status, gestational age at the time of OGTT, family history of diabetes, parity, infant gender and cord plasma glucose. Elevations in fasting glucose, 1-hour glucose and 2-hour glucose were associated with greater risk of pre-eclampsia [152]. Pregnancies affected by both chronic hypertension and GDM were having higher rates of labor induction when compared with pregnancies affected by GDM alone [153].

2.12.2 Preterm Delivery

Preterm delivery is defined as delivery before 37 weeks of gestation. In a study where 23,316 pregnant women participated, 6.9% experienced preterm delivery (both induced and spontaneous), 9.6% of infants were large for gestational age and 8.0% of infants underwent intensive neonatal care admission. Preterm delivery has statistically significant but relatively weak association with 1-hour glucose level, 2-hour glucose level and maternal blood pressure [154]. The association between GDM and preterm delivery

may be partially explained by the coexistence of other conditions such as pre-eclampsia, placental abruption and intrauterine growth restriction [155].

2.12.3 Shoulder Dystocia

Shoulder dystocia is usually defined as the need for additional maneuvers to deliver the shoulders if gentle traction on the fetal head does not suffice [156]. It has been reported that only 1.3% of women are affected by shoulder dystocia, thus it is considered as one of the least common pregnancy outcome. Shoulder dystocia increases risk of birth trauma to the infant. Brachial plexus palsy occurs in 4-13% of shoulder dystocia deliveries and is often resolved in early infancy [157]. The risk of shoulder dystocia increases with obesity and additionally with GDM. Women with glucose intolerance during pregnancy have slightly increased risk of shoulder dystocia. It has been reported that shoulder dystocia is associated with increased fasting glucose level, 1-hour glucose level and 2-hour glucose after adjustment for maternal weight and height [158].

2.12.4 Risk of Stillbirth

Stillbirth is the delivery of dead baby after 28 weeks of gestation. The rate of still birth in women with gestational diabetes is approximately 1.4 per 1000 births. Association between stillbirth and different glucose levels is difficult because it is one of the uncommon pregnancy outcomes. Untreated GDM increases the risk of stillbirth approximately four-fold [159]. In more industrialized nations stillbirth is an uncommon outcome, even among women with glucose intolerance. Insulin therapy combined with constant monitoring and subsequent induction of labor reduces the rate of stillbirth [160].

2.12.5 Hypoglycemia in the Newborn

Hypoglycemia is diagnosed on the basis of low levels of glucose, defined as less than 30.6 mg/dL in the first 24 hours after delivery or 45 mg/dl glucose after the first 24 hours. Maternal hyperglycemia may results in excess fetal glucose exposure and fetal hyperinsulinemia. In turn, fetal hyperinsulinemia lead to hyperplasia of skeletal muscle, fat tissue, and subsequent neonatal hypoglycemia. Infant hypoglycemia is strongly associated with maternal 1-hour glucose and weakly associated with maternal 2-hour glucose although not significantly associated with maternal fasting glucose level on the oral glucose tolerance test. The prevalence of hypoglycemia is reported to be 5% in GDM not receiving intervention and 7% in GDM receiving intervention [161-162].

2.12.6 Hyperbilirubinemia

Hyperbilirubinemia is said to happen when the infant has bilirubin level ≥ 20 mg/dL. It is usually treated with phototherapy after birth. Hyperbilirubinemia affecting 8.3% of the infants and is more common among women with GDM than in women without GDM. Maternal hyperglycemia, fetal hyperinsulinemia and reduced oxygenation are thought to lead to increased fetal oxygen uptake, hyperbilirubinemia and fetal erythropoiesis [163].

2.12.7 Cesarean delivery

Cesarean delivery is a successful intervention used to decrease complications associated with gestational diabetes such as elevated fetal weight and shoulder dystocia. Cesarean deliveries are common among women with and without GDM. The greater risk

of cesarean sections is particularly due to elevated fetal weight. In Toronto Tri-Hospital Study, it was reported that women with treated gestational diabetes had low rate of macrosomia, while untreated gestational diabetes had two-fold increased risk of cesarean delivery [164]. A Cochrane database review showed that inducing GDM mothers at 38 weeks of gestation would not increase the risk of cesarean delivery [165]. The operation itself is associated with several maternal morbidities, particularly wound infection, deep venous thrombosis, postpartum infection and bleeding [166]. Overweight and obese women had higher infection rates if they underwent a vertical skin incision compared with transverse skin incision. Elective delivery increases rate of transient tachypnea and respiratory distress syndrome in the newborn. Incidence of respiratory distress syndrome can be reduced to less than 10% by accurate pregnancy dating, achieving euglycemia and delaying delivery until term [167].

2.12.8 Macrosomia

The most common neonatal complication significantly associated with gestational diabetes mellitus is macrosomia. Macrosomia is defined as birth weight above the normal mean birth weight or an oversized baby with a birth weight greater than the 90th percentile for gestational age and sex. If maternal glucose level is directly related to fetal macrosomia then strategies to prevent hyperglycemia must be devised to treat GDM women [168]. The incidence of macrosomia is significantly higher in women having higher postprandial glucose concentrations between 29 and 32 weeks of gestation [169]. It has been reported that gestational diabetic women, who only monitored pre-prandial glucose, had a 42% risk of macrosomia, whereas those, who also monitored glucose 1-

hour after eating, decreased their risk of neonatal macrosomia to near normal or 12% [170].

2.12.9 Type 2 Diabetes Mellitus

Type 2 diabetes is the most common type of diabetes and is due to partial lack of insulin or insulin resistance. Its prevalence is increasing dramatically throughout the world. Gestational diabetes is one of the well-known risk factor for developing Type 2 diabetes later in life. Women with a history of GDM will develop Type 2 diabetes within 3–5 years, and 70% will develop Type 2 diabetes if followed for 10 years [171-175].

2.12.10 Cardiovascular Diseases

Women with GDM are at high risk of long-term mortality and morbidity due to cardiovascular disease. The risk of cardiovascular disease in GDM women is the same as that in the diabetic men [176]. Metabolic and cardiovascular changes increase the risk of metabolic syndrome, which is defined by elevated fasting glucose levels, central body adiposity, hypertension and dyslipidemia. Metabolic syndrome has been reported in women with history of GDM [177]. Women with a family history of type 2 diabetes and previous history of gestational diabetes are more prone to cardiovascular diseases. The cardiovascular disease in these women can be diagnosed at very young age. Women with previous history of gestational diabetes showed high fasting insulin levels, high insulin resistance, central adiposity, low HDL cholesterol levels and high LDL cholesterol levels. [178]. Lifestyle modifications and pharmacological interventions are helpful in preventing GDM and type 2 diabetes in high-risk subjects [179].

2.12.11 Elevated Serum Ferritin

Ferritin is one of the major iron storage protein which plays important role in iron metabolism. Serum ferritin level is indirect estimate of body iron stores because it is highly associated with bone marrow iron. In chronic inflammations, elevated serum ferritin levels have been reported [180]. Increase serum ferritin levels increases insulin resistance and risk of cardiovascular diseases in healthy non pregnant women [181]. Gestational diabetes increases the risk of macrosomia for the fetus and risk of type 2 diabetes for the mother. In pregnant Chinese women, serum ferritin level was markedly increases in women with gestational diabetes and impaired glucose tolerance [182-83] Fernandez-Real et al. [184] reported that elevated serum ferritin levels and obesity were independent indicators of insulin sensitivity and suggested that a high serum ferritin concentration could be a component of the insulin resistance syndrome in normal subjects. Lao et al. [185] reported that mean serum ferritin concentrations at 28–30 weeks of gestation was significantly increased in Chinese pregnant women with impaired glucose tolerance. Epidemiological and clinical evidence suggest that both iron overload and iron deficiency affects the production of reactive oxygen species, resulting in systemic inflammation, oxidative stress, and changes in mitochondrial function [186]. GDM risk has been increased several folds in women taking high heme iron in diet. [187].

2.13 Management of Gestational Diabetes

Management during pregnancy consists of blood glucose monitoring, caloric restriction, physical activity, and drug therapy. Management after pregnancy must

include postpartum screening for diabetes, effective contraception that does not affect glucose intolerance, breastfeeding and healthy lifestyle behaviors [188].

Guidelines for weight gain during pregnancy are important to avoid obesity and glucose intolerance. Recommended pregnancy BMI is calculated by pre-pregnancy weight gain as well as rate of weight gain in the second and third trimesters. Weight targets are particularly emphasized if glucose goals are not met. Small reductions in weight and glucose self-monitoring can help in glycemic control. Low carbohydrate diets were associated with fewer macrosomic infants, cesarean deliveries, and drug therapy [189] while low glycemic diet was associated with lower insulin use [190]. The proportion of dietary carbohydrate should be limited to about 40%–45% of total caloric intake [191]. Poly-unsaturated fatty acids may protect GDM from impaired glucose tolerance while saturated fatty acids can increase glucose level and insulin concentration in women with GDM [192-195].

Physical activity may improve glucose tolerance by improving insulin sensitivity involving muscle glucose uptake and glycogen synthesis. Regular exercise can lead to an increase in secretion of insulin, free fatty acids and ketones and decrease glucose levels. [196-197]. General guidelines encourage at least 30 minutes of physical activity on daily basis [198-199].

If women cannot achieve glycemic goals with glucose monitoring, caloric restrictions and physical activity, then drug therapy with insulin is recommended. Usually Neutral Protamine Hagedorn (NPH) insulin injections 2-4 times are used during pregnancy [200] Insulin may be administered according to the woman's pattern of glucose administration. If the fasting glucose is elevated in the morning, evening neutral

protamine hagedorn insulin can be used, at a typical starting dose of 0.2 units/kg body weight. If postprandial glucoses are elevated, short-acting insulin at doses of 1.5 units per 10 g per carbohydrate per breakfast and 1.0 units per 10 g per carbohydrate per lunch and dinner can be used. If both pre- and postprandial glucoses are elevated, four injections per day can be used at 0.9–1.0 units/kg. Insulin can be divided into 50% neutral protamine Hagedorn insulin and 50% as three pre-prandial rapid-acting injections [201]. The use of hypoglycemic agents is beneficial as it improves glucose levels as well as satisfies the patient [202]. However, oral sulfonylureas, particularly glyburide, have not yet been endorsed by ADA or ACOG, due to concerns about impact upon perinatal outcomes [203].

Twice-weekly non stress test is the most used test and consists of continuous external fetal heart rate monitoring and evaluation of amniotic fluid volume. If the results of this testing are not convincing, more specific testing, such as the biophysical profile, contraction stress test, or umbilical artery Doppler evaluations can help determine if fetal hypoxia is present [204-207]. In GDM pregnancies that are managed without drug therapy and are normoglycemic, such testing usually begins at approximately 37 weeks, and in more complicated GDM pregnancies, testing commonly begins at approximately 32 weeks. Intervention in the form of induction can then occur if indicated [208]. Fetal ultrasonography is generally performed for assessment of fetal growth, as well as for detection of anomalies. The first ultrasound usually occurs at diagnosis of GDM. Thereafter, it may occur as often as every three weeks, particularly in the presence of co morbidities that can also affect fetal growth such as hypertension [209-211]. There is no consensus on the timing of induction of labor, risks and benefits in women with GDM.

Risks include cesarean section with its attendant complications, and benefits include decreased fetal growth, dystocia, and stillbirth. During induced and spontaneous labor, insulin requirements generally increase due to the work of the uterus. However, women may still require continuous insulin, particularly if they required pharmacotherapy during the pregnancy [212].

Currently, medications are not advised for the prevention of gestational diabetes. It has been reported that Troglitazone, used in the in prevention of diabetes, not only decrease the risk of diabetes in GDM women but it also has numerous adverse effects, thus banned. Metformin may be the alternate option for GDM women who have impaired glucose tolerance and who are obese [213].

In addition to the above management care, the GDM should make appropriate changes in their life style. The majority of GDM women are overweight or obese. They have sedentary lifestyles and consume less vegetables and fruits. For GDM women, it is recommended that they should have BMI less than 25kg/m^2 . Their physical activity should be more than 2.5 hours/week of moderate activity or 75 minutes/week of vigorous activity. They should consume five or more servings of fruits and vegetables per day [214]. Women with GDM decrease their diabetes risk by weight reduction of 7% of their baseline weight [215]. Also weight loss was achieved by increased physical activity and reduction in caloric intake. Combination of diet and physical activity is associated with weight loss suggesting that both caloric restriction and physical activity are needed for weight reduction [216]. Women with previous histories of GDM have less success rate with lifestyle intervention than women without previous histories of GDM [217].

2.14 Rational of the Present Study

If recent recommendations for diagnosis are adopted, GDM is still on the peak to become one of the most common comorbidities of pregnancy. Even if the current diagnostic criteria remains unchanged, the prevalence of GDM will continue to increase as obesity rate rises. Broad consensus exists on the incidence, risk factors and adverse complications of GDM. However, the actual cause of GDM is still controversial; some studies defend the hypothesis of abnormal production of insulin as the cause of GDM, while others considered it as the affect of increase production of placental hormones. This study was the collection and compilation of many biochemical parameters with the aim of finding out those aspects in which GDM pregnancy differs from healthy ones. This study was not only important in giving clue to the actual cause of GDM but also helpful in indicating those GDM patients which will be at the risk of cardiovascular diseases, hypertension, and type 2 diabetes in future.

Pregnancy complicated by GDM, if not properly diagnosed and controlled in time, leads to still birth which is a great psychological trauma for the mother. This study also provides important information for the obstetrician, physician and GDM mothers to have successful delivery with decreased rate of still birth, macrosomia and birth trauma. Moreover, the population of this study was the admitted hospital patients of Khyber Pukhtonkhwa, majority population of which are poor with high illiteracy rate and low socioeconomic status, thus the results of this study can be implemented on the poor and developing countries for decreasing the incidence of GDM. The information of this study will add to the already available evidences on biochemical changes, maternal and neonatal complications of GDM.

Chapter 3

MATERIALS AND METHODS

3.1 Location of the Study

The study was carried under the supervision of the Institute of Chemical Sciences, University of Peshawar. Gestational diabetic women (GDM) and healthy pregnant women (HPW) for comparison were registered in Khyber Teaching Hospital (KTH), Peshawar. Information was collected from the registered women on an approved questionnaire (Annexure I). Blood sugar, lipid profile, liver function tests and renal function tests were carried out in the main Biochemistry Laboratory of KTH. The hormones namely insulin, prolactin, cortisol, triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) were determined in the Institute of Radioactive and Nuclear Medicine (IRNUM), Peshawar.

3.2 Selection Criteria

The selection criteria for GDM and HPW were:

1. They should be the admitted patient of Gynea Ward of KTH.
2. They should be at the gestational age of 28 weeks or more.
3. They should not have previous history of medical illness like hypertension, cardiac and renal diseases.
4. They should not be on any medical treatment that affects lipid profile and hormones concentration.

3.3 Sample Size and Registration of GDM and HPW

Based on the selection criteria, one hundred and ten gestational diabetic women and one hundred healthy pregnant women were registered for the study. The healthy pregnant women were used as a control group. Both the GDM and HPW were at gestational age of 28 weeks or more. The GDM were the admitted patients of Gynea Ward of KTH. They were admitted for control of gestational diabetes or treatment of its complications. HPW were also registered at the gestational age of 28 weeks or more, and not having any medical problem. Consents from the registered pregnant women, to conduct the study, were obtained (Annexure II). Ethical approval for the study was obtained from Institutional Ethical Medical Board (IEMB) at Post Graduate Medical Institute (PGMI), Hayatabad Medical Complex, Peshawar. Seven GDM patients and 3 HPW were dropped out from the study and the remaining 103 GDM patients and 97 HPW completed the study.

3.4 Information about GDM and HPW

Scociodemographic and other pregnancy related information from GDM and HPW were recorded in a well designed questionnaire (Annexure-1). The recorded information were about economic status, educational level, maternal age, gestational age at screening, parity, previous and family history of gestational diabetes, mode of delivery, previous history of still birth babies, alive birth babies, low birth weight babies and macrosomic babies.

3.5 Calculation of Body Mass Index (BMI)

BMI was calculated from the height and weight of the registered pregnant women using the formula:

$$\text{BMI} = \text{Weight in kg} / \text{Height in (meters)}^2$$

Height of each registered pregnant woman was measured in standing position without shoes using vertical calibrated scale. Heel to head-crown length was measured in centimeters. Weight of each registered pregnant woman was taken in standing position without shoes using an accurate health weighing scale.

3.6 Blood Pressure of GDM and HPW

Both systolic and diastolic blood pressure of the registered pregnant women were measured by standard mercury sphygmomanometer and arm cuff at the right arm. Pregnant subjects were either in sitting or reclining position and the readings were taken after a period of 5 minutes rest in that position.

3.7 Blood Samples Collection

Fasting blood samples were taken from both GDM and HPW. 5 mL fasting blood was taken through routine method applying aseptic technique and tourniquet for a short time as needed. 1mL of blood was transferred to an EDTA vacutainer for measuring hemoglobin concentration and platelet count. The remaining 4mL blood samples were centrifuged at 4000 rpm for 5 minutes for serum separation. Separated serums were divided into two halves and were transferred to proper labeled eppindrof tubes and stored in freezer at -20°C for subsequent biochemical analysis. For determination of random

blood sugar and HbA1c, additional 2mL of blood in fed state were taken from both GDM and HPW.

3.8 Biochemical Analysis of Blood Samples

Biochemical analysis of blood including fasting blood sugar (FBS), random blood sugar (RBS), glycohemoglobin (HbA1c), hemoglobin concentration (Hb) and Platelet count (PC) were determined by fully automated Hematology Analyzed Humacount Plus. Lipid profile, liver function tests and renal function tests were performed on Roche/Hitachi 902 automated analyzer. Concentration of different hormones namely insulin, prolactin, cortisol, thyroxine, triiodothyronine were determined by Immunotech RIA Kit (Beckman Coulter) and thyroid stimulating hormone by Immunotech IRMA Kit (Beckman Coulter).

3.9 Determination of Blood Glucose

Both fasting and random glucose was determined by the enzymatic colorimetric method of Trinder [218]. Auto analyzer (Express plus, Ciba corning USA) and Elitech kit was used.

Principle

In this method, enzymatic reaction was completed in two steps. In the first step, glucose was oxidized to gluconic acid and hydrogen per oxide in the presence of enzyme glucose oxidase. In the second step, red quinine was formed in the presence of peroxidase. The absorbance of this colored substance (red quinine) was taken by autoanalyzer and the concentration of glucose was calculated.

Reagents

Reagent 1:

Phenol	10 mmol/L
Phosphate Buffer, pH: 7.40	100 mmol/L

Reagent 2:

4-Aminoantipyrine	270 μ mol/L
Glucose oxidase	$\geq 10,000$ U/L
Peroxidase	≥ 600 U/L

Sample	Serum
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Procedure

The working reagent was prepared by dissolving reagent 2 in reagent 1. The temperature was maintained at 37°C. For glucose determination cuvette of 1cm path length was used. Before using the auto analyzer, basic modes were selected and both normal and abnormal ranges were given. Wavelength was adjusted to 546 nm and auto analyzer was set to read directly the concentration in units of mg/dL. 300 μ L working reagent and 10 μ L sample was sucked and mixed automatically. After a short incubation period the auto analyzer gave the optical density (OD) of the sample.

Calculations

Factor	n/OD
n	Standard concentration (100 mg/dL or 5.56 mmol/dL)
Glucose concentration	OD of sample x Factor

The calculations were done automatically by the instrument.

3.10 Determination of Glycohemoglobin (HbA1c)

Glycohemoglobin is progressively and irreversibly formed in the erythrocytes throughout the normal cells having life span of 120 days. The concentration of glycohemoglobin in the erythrocytes shows the average blood glucose level of the past 4 to 6 weeks. The measurement of glycohemoglobin provides a very valuable test for evaluating the long term control of diabetic patients [219].

Procedure

Whole blood was mixed with a lysing reagent containing borate ions and a detergent. Elimination of the labile Schiff's base was thus achieved during the hemolysis. The hemolysate was then mixed with a weakly binding cation exchange resin for five minutes. When all the glycohemoglobin was bound to the resin, a special resin separator was used to remove the resin from the supernatant fluid containing HbA1c. The glycohemoglobin percentage of total hemoglobin was determined by measuring the absorbance of the glycohemoglobin and absorbance of total hemoglobin fraction at 415nm.

Step 1: Hemolysis: 100 μ L of standard solution, 100 μ L sample and 1mL of glycohemoglobin control was mixed thoroughly at 15-25°C and incubated in the auto analyzer for 5 minutes.

Step 2: Total HbA1 Determination: 20 μ L hemolysate was pipetted out from the first step and poured into labeled tubes. About 5 mL of distilled water was also added to the

same labeled tubes. All the tubes were vigorously shaken and absorbance determined at 415nm wavelength.

3.11 Determination of Hemoglobin and Platelet Count

Full blood count including hemoglobin concentration and platelet count was measured by using three dimension, fully automated Hematology Analyzed Humacount Plus [220].

Procedure

Fresh whole blood sample collected in K3-EDTA anti-coagulated vacutainer was used for determination of hemoglobin and platelet count. The sample was mixed gently by inverting it eleven times to get homogenous sample. Vigorous and rapid shaking was avoided because it could damage the red blood cells. The cap of the sample tube was removed and the aspiration needle was engrossed into the sample. The sampling bar was pushed to start measuring full blood count. During aspiration process, the sampling tubes were held in a stable position. The instrument sucked 25 μ L of sample, and the aspirating needle was retracted while its outer surface was mechanically rinsed with diluents. When the status LED started flashing and beeps were heard the sampling tubes were removed and covered. An external PC keyboard was attached with the instrument to get results. Blank measurement was used for checking efficiency of the system and reagents.

Calibration is the procedure used to standardize the instrument by applying necessary rectification factors. Special calibrations and three-fold measurements of the control were

used with known parameters. The instrument automatically calculated new factors using the following formula.

New factor = assigned value stored factor / Measured values (S)

Patient results were stored in the memory box in sequential order, and they could be retrieved at any time.

3.12 Determination of Lipid Profile

Lipid profile including total cholesterol, triglyceride, high density lipoprotein cholesterol and low density lipoprotein cholesterol were determined and measured by the following procedures.

3.12.1 Total Cholesterol

Total cholesterol was determined by the enzymatic colorimetric method of Allian et al [221]. Auto analyzer (Express plus, Ciba corning USA) and Elitech kit was used.

Principle

In this method, cholesterol ester is hydrolyzed to fatty acid and free cholesterol by enzyme cholesterol esterase in the first step and in the second step oxidation occurs and hydrogen peroxide is formed by the enzyme cholesterol oxidase. Finally in the presence of peroxidase, red quinone is formed. The absorbance of this colored substance (red quinone) is recorded and then cholesterol concentration is calculated.

Reagents

Reagent1:

Phenol	24 mmol/L
Sodium cholate	0.5 mmol/L
Buffer, pH 6.90	50 mmol/L
Reagent 2:	
Peroxidase	≥ 1000 U/L
4-Aminoantipyrin	0.5 mmol/L
Cholesterol esterase	≥ 200 U/L
Cholesterol oxidase	≥ 250 U/L
Sample	Serum

Procedure

The working reagent was prepared by dissolving reagent 2 in reagent 1. The temperature was maintained at 37°C. For cholesterol determination cuvette of 1cm path length was used. Before using the auto analyzer, basic modes were selected and both normal and abnormal ranges were given. Wavelength was adjusted to 546 nm and auto analyzer was set to read directly the concentration in units of mg/dL. 300 μ L working reagent and 10 μ L sample was sucked and mixed automatically. After a short incubation period the auto analyzer gave the optical density (OD) of the sample.

Calculations

Factor	n/OD
n	Standard concentration (200 mg/dL or 5.17 mmol/dL)
Cholesterol concentration	$\text{OD Sample} \times \text{Factor}$

The calculations were done automatically by the instrument.

3.12.2 Triglyceride

Triglyceride was determined by the enzymatic colorimetric method of Werner et al [222]. Auto analyzer (Express plus, Ciba corning USA) and Elitech kit was used.

Principle

Triglycerides are hydrolyzed to fatty acids and glycerol by lipoprotein lipase. Energy is added to glycerol in the presence of magnesium ion and glycerol kinase. The glycerol is then oxidized to dihydroxyacetone phosphate in the presence of glycerol-3-phosphate oxidase. In the second step, red quinone is formed in the presence of peroxidase. The absorbance of this colored substance is noted and the concentration of triglycerides is calculated.

Reagents

Reagent 1:

Phenol	24 mmol/L
Sodium cholate	0.5 mmol/L
Buffer, pH 7.50	50 mmol/L

Reagent 2:

Peroxidase	≥ 350 U/L
4-Aminoantipyrin	0.7 mmol/L
Lipoprotein Lipase	≥ 1100 U/L
Glycerol Kinase	≥ 800 U/L
Glycerol-3-Phosphate oxidase	≥ 3000 U/L
ATP	0.3 mmol/l
Sample	Serum

Procedure

The working reagent was prepared by dissolving reagent 2 in reagent 1. The temperature was maintained at 37°C. For triglycerides determination cuvette of 1cm path length was used. Before using the auto analyzer, basic modes were selected and both normal and abnormal ranges were given. Wavelength was adjusted to 546 nm and auto analyzer was set to read directly the concentration in units of mg/dL. 300 μ L working reagent and 10 μ L sample was sucked and mixed automatically. After a short incubation period the auto analyzer gave the optical density (OD) of the sample.

Calculations

Factor n/OD

n Standard concentration (200mg/dL or 2.28 mmol/dL)

Triglycerides concentration = OD of sample x factor

The calculations were done automatically by the instrument.

3.12.3 HDL- Cholesterol

Principle

Chylomicron, very low density lipoproteins (VLDL) and low density lipoprotein cholesterol (LDL-C) are precipitated by adding magnesium ion and phosphotungstic acid to the sample. Centrifugation left only the HDL-cholesterol in the supernatant, cholesterol content of which was determined by phosphotungstic acid method [223].

Procedure

200 μ L sample and 500 μ L diluted precipitants was pipetted out into centrifuge tube and allowed to assemble for 10 minutes at room temperature. It was then centrifuged for 10 minutes at 4000 rpm. The clear supernatant was separated within 2 hours and the HDL cholesterol was determined. The working reagent was prepared by dissolving reagent 2 in reagent 1. The working reagent was prepared by dissolving reagent 2 in reagent 1. The temperature was maintained at 37°C. For HDL-Cholesterol determination cuvette of 1cm path length was used. Before using the auto analyzer, basic modes were selected and both normal and abnormal ranges were given. Wavelength was adjusted to 546 nm and auto analyzer was set to read directly the concentration in units of mg/dL. 300 μ L working reagent and 10 μ L sample was sucked and mixed automatically. After a short incubation period the auto analyzer gave the optical density (OD) of the sample.

Calculations

Factor n/OD

n Standard concentration (50mg/dL)

Cholesterol concentration = OD of sample x Factor

The calculations were done automatically by the instrument.

3.12.4 LDL-Cholesterol

LDL Cholesterol was calculated by using the following formula; [223]

$$\text{LDL Cholesterol (mg/dL)} = \frac{\text{Total Cholesterol} - \text{TGL}}{5\text{-HDL Cholesterol}}$$

$$\text{LDL Cholesterol (mmol/L)} = \frac{\text{Total Cholesterol} - \text{TGL}}{2.2\text{-HDL Cholesterol}}$$

3.13 Liver Function Tests (LFTs)

Liver function tests including alanine amino transferase (ALT), alkaline phosphatase (ALP) and bilirubin were determined by the following procedures:

3.13.1 Alanine Amino Transferase (ALT)

Alanine amino transferase catalyzes the conversion of amino acids to corresponding alpha-keto acids through the transfer of amino groups. ALT also catalyzes the reverse process. Higher activities of ALT exist in the liver however, minor activity can also be observed in the heart, kidney, skeletal muscle, spleen, pancreas and lungs. Increased levels of transaminases are investigative of myocardial infarction,

hepatopathies, muscular dystrophy and damage of the internal organs. ALT activity in the serum is a precise indicator of damage to the liver parenchyma. ALT was determined by Roche kit without pyridoxal phosphate activation. This method is on the procedure of Bregmeryer [224].

Principle

ALT was determined with optimal substrate concentrations, use of TRIS (hydroxyl methyl-ammino methane), simultaneous pre incubation of serum with buffer to prevent opposing reactions with NADH. ALT is the enzyme which catalyzes the equilibrium reaction. The increase pyruvate was measured in a subsequent indicator reaction which was catalyzed by lactate dehydrogenase. In the second reaction, NADH is oxidized to NAD⁺. The rate of decrease in NADH is directly proportional to the rate of formation of pyruvate, and thus the ALT activity.

Reagents

Reagent 1:

TRIS buffer pH: 7.3	125 mmol/L
L-alanine	625 mmol/L
NADH (yeast)	0.23 mmol/L
LDH (microorganism)	1.5 U/L

Reagent 2:

Alpha ketogluterte	94 mmol/L
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Sample	serum
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Procedure

The wavelength was adjusted at 340 nm and the temperature was set at 37°C. Cuvette of 1cm path length was used. Before using the auto analyzer, it was calibrated and both normal and abnormal control ranges were given. If the sample reading was between the two normal ranges and the auto analyzer shows the current calibration, then it is in the position to work properly and to give precise results. 10 µL sample was mixed with 250 µL of reagent 1 and 50 µL of reagent 2 automatically and the analyzer (Roche/Hitachi 902 automated analyzer) gave the concentration of ALT in U/L after a short period of incubation.

3.13.2 Alkaline Phosphatase (ALP)

Alkaline phosphatase in serum consists of four structural genotypes; the kidney type, the liver bone type, intestinal type and the placental type. ALP occurs in hepatocytes, osteoblast, leukocytes, spleen, prostate, kidney, spleen, placenta, prostate and small intestine. A rise in ALP occurs with all form of cholestasis, hyperparathyroidism, rickets, osteomalacia, as well as with fractures and malignant tumors.

In 1983 the International Federation of Clinical Chemistry (IFCC) suggested a standardized method for ALP determination [225] using an optimized substrate concentration and 2- amino-2-methyl-1 propanol as buffer plus magnesium and zinc cations.

Principle

In the presence of magnesium and zinc ions, p-nitro phenyl phosphatase was cleaved by phosphatases into phosphate p- nitro phenol. The released phosphate p-nitro phenol was proportional to the ALP activity and measured photochemically.

Reagents

Reagent 1:

2-amino-2-methyl-1-propanol. pH: 10.44	1.12 mol/L
N-(2-hydroxyethyl)-ethyldiamine triacetic acid	2.49 mmol/L
Zinc sulphate	0.50 mmol/L
Magnesium acetate	2.49 mmol/L

Reagent 2:

p- Nitrophenylphosphate, pH 8.50	99.5 mmol/L
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Sample	Serum
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Procedure

The wavelength was adjusted at 450 nm and the temperature was kept at 37°C. Cuvette of 1cm path length was used. Before using the auto analyzer, it was adjusted for both normal and abnormal control ranges. If the sample reading falls between these two control ranges and the auto analyzer shows the current calibration, then it is in the position to work properly and to give accurate results. 250 µL of reagent 1 and 50 µL of reagent 2 were mixed with 11 µL sample automatically and the analyzer (Roche/Hitachi 902 automated analyzer) gave the concentration of ALP in U/L.

3.13.3 Serum Bilirubin

Bilirubin is produced during normal and abnormal degradation of erythrocytes in the reticoendothelial system. Bilirubin determinations are used in the recognition of hemolytic anemia, diagnosis of liver diseases, and in the assessment of the degree of severity in jaundice. Bilirubin was determined by the procedure of Wahlefeld et al [226].

Principle

In this method, the reaction is catalyzed by a detergent to prevent the precipitation of protein. The diazo reagent 2, 5-dichlorophenyl diazonium tetrafluoroborate (DPD), was used which under the acidic conditions couples very rapidly with bilirubin.

Reagents

Reagent1:

Detergent/Hydrochloric acid	120 mmol/L
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Reagent 2:

2,5-dichlorophenyl diazonium salt	3 mmol/L
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Sample	Serum
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Procedure

The working reagent was prepared by dissolving reagent 2 in reagent 1. The temperature was maintained at 37°C. For serum bilirubin determination cuvette of 1cm path length was used. Before using the auto analyzer, basic modes were selected and both normal and abnormal ranges were given. Wavelength was adjusted to 450 nm and auto

analyzer was set to read directly the concentration in units of mg/dL. 250 μ L of reagent 1 and 50 μ L of reagent 2 were mixed automatically with 7 μ L sample and the analyzer (Roche/Hitachi 902 automated analyzer) gave the concentration of bilirubin in mg/dL after short incubation time.

3.14 Renal Function Tests (RFTs)

Renal functional tests including urea and creatinine were determined by the following procedures:

3.14.1 Serum Urea

The determination urea is the most widely used for the evaluation of kidney functions. This test is recurrently used in conjunction with the creatinine determination for the differential diagnosis of perennials hyper uremia (cardiac decompensation, increased protein catabolism and water depletion,), renal hyper uremia (glomerulonephritis, polycystic kidney, chronic nephritis and nephrosclerosis tubular necrosis) and post renal hyper uremia(obstruction of urinary tract). Urea is the final degradation product of amino acid and protein metabolism. In metabolism, proteins are deaminated and broken down to amino acids. The ammonia formed in this process is synthesized to urea in the liver. This is the most important metabolic pathway for excreting excess nitrogen from the human body. Urea was determined by enzymatic procedure of Schubert who used the coupled urease/ glutamate dehydrogenase (GLDH) enzyme system [227].

Principle

Urea is hydrolyzed by urease to form carbondioxide and ammonia. The ammonia formed is then reacted with alpha ketogluterate and NADH in the presence of glutamate dehydrogenase and NAD⁺. The decreased absorbance due to utilization of NADH is measured kinetically.

Reagents

Reagent 1:

CAPSO buffer, pH 9.65	5 mmol/L
NADH (yeast)	0.23 mmol/L

Reagent 2:

BICIN buffer, pH 7.6	1000 mmol/L
Urease	≥120 μkat/L
GLDH	≥15.0 μkat/L
Alpha ketogluterate	≥8.3 μkat/L
CAPSO = 3 (cyclohexylamino)-2-hydroxyl-1-propanesulphonic acid	
BICIN = N, N bis (2-hydroxyethyl)-glycine	

Sample	Serum
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Procedure

The working reagent was prepared by dissolving reagent 2 in reagent 1. The temperature was maintained at 37°C. For serum urea determination cuvette of 1cm path length was used. Before using the auto analyzer, basic modes were selected and both normal and abnormal ranges were given. Wavelength was adjusted to 450 nm and auto

analyzer was set to read directly the concentration in units of mg/dL. 300 μ L working reagent and 10 μ L sample was sucked and mixed automatically. After a short incubation period the auto analyzer gave the optical density (OD) of the sample.

250 μ L of reagent 1 and 50 μ L of reagent 2 were mixed automatically with 4 μ L of sample and the analyzer (Roche/Hitachi 902 automated analyzer) gave the concentration of urea in mg/dL after short incubation time.

3.14.2 Serum Creatinine

In the muscle metabolism, creatinine is synthesized endogenously from creatine and creatinine phosphate. Creatinine determinations are performed for the analysis and the monitoring of chronic and acute renal disease as well as for the monitoring of renal dialysis. Creatinine concentration in urine can be used as reference values for the excretion of certain analytes like alpha amylase and albumin. Creatinine was determined by the method of Bartles [228].

Principle

In alkaline solution, creatinine with picrate formed a yellow-orange complex. The intensity of this coloured complex was directly proportional to the creatinine concentration and measured photometrically.

Reagents

Reagent 1:

Sodium hydroxide	0.2 mol/L
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Reagent 2:

Picric acid 25 mmol/L

Sample Serum

Procedure

The working reagent was prepared by dissolving reagent 2 in reagent 1. The temperature was maintained at 37°C. For serum creatinine determination cuvette of 1cm path length was used. Before using the auto analyzer, basic modes were selected and both normal and abnormal ranges were given. Wavelength was adjusted to 450 nm and auto analyzer was set to read directly the concentration in units of mg/dL. 250 µL of reagent 1 and 50 µL of reagent 2 were mixed automatically with 15µL of the sample and concentration of serum creatinine was determined in mg/dL after short incubation time by Roche/Hitachi 902 automated analyzer.

3.15 Determination of Hormones

There are many hormones but in this study insulin, prolactin, cortisol, triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) were determined by the following procedures.

3.15.1 Insulin

Both free insulin and immunoreactive insulin were determined by immunoradiometric assay [229] using insulin IRMA Kit of Beckman Coulter.

Principle

Serum samples, controls and calibrators, are incubated in tubes encrusted with the first monoclonal antibody in the presence of the second monoclonal antibody which is labeled with iodine 125. After incubation, the content of tubes is rinsed so as to remove unbound ^{125}I -labeled antibody. The bound radioactivity is then determined in gamma counter. The insulin concentration in the sample is obtained by interpolation from the standard curve. The insulin concentration in the samples is directly proportional to the radioactivity.

Reagents

Anti-insulin monoclonal antibody coated tubes: 50 tubes

^{125}I - labeled monoclonal antibody: One 11mL vial. (The iodine vial contains 640 kBq of ^{125}I -labeled immunoglobulin in buffer containing bovine serum albumin, sodium azide and dye).

Calibrators: Five 1 mL vials and one 2mL “zero calibrator” vial (The calibrator vials contain from 0 to 300 $\mu\text{IU/mL}$ of human insulin in sodium azide).

Control sera: Two vials (lyophilized)

Wash solution: One 50 mL vial

Procedure

Antibody coated tubes were taken and 50 μL of calibrator or 100 μL of supernatant for controls and samples and 100 μL of tracer was added to it. The tubes were shaken thoroughly at $>280\text{rpm}$ and incubated for 2 hours at $18\text{-}25^\circ\text{C}$. The content of

the tubes were aspirated carefully and washed two times with 2 mL wash solution. The activity of the solution was determined for one minute. Results were obtained from the standard curve by interpolation.

3.15.2 Serum Prolactin

Serum prolactin was determined by immunoradiometric assay [230] using prolactin IRMA Kit of Beckman Coulter.

Principle

Samples or calibrators are incubated in tubes coated with first monoclonal antibody in the presence of the second monoclonal antibody which is labeled with iodine 125. After incubation for a short time, the content of tubes is rinsed so as to remove unbound ¹²⁵I-labeled antibody. The bound radioactivity is then determined in gamma counter. The radioactivity bound is directly proportional to the concentration of prolactin in the sample. Prolactin concentration in the samples is obtained by interpolation from the standard curve.

Reagents

Anti-polactin monoclonal antibody coated tubes: 50 tubes

¹²⁵I- labeled monoclonal antibody: One 55 mL vial. (The iodine vial contains 370kBq of ¹²⁵I-labeled immunoglobulin in buffer containing bovine sodium azide, serum albumin, and dye).

Calibrators: Five 1 mL vials and one 5 mL “zero calibrator” vial (The calibrator vials contain from 0 to 180 ng/mL of human prolactin in sodium azide).

Control sera: Two vials (lyophilized)

Wash solution: One 50 mL vial

Procedure

For prolactin determination, 50 μ L of calibrator, control or sample and 500 μ L of tracer were added successively to antibody coated tubes. The tubes were shaken for an hour at 350 rpm for thorough mixing. The contents of the tubes were aspirated carefully and washed with 2 mL of wash solution. The count bound (B) and total count bound (T) for 1 minute were determined. Results were obtained from the standard curve by interpolation. The curve serves for the determination of prolactin concentrations (ng/mL) in sample.

3.15.3 Serum Cortisol

Cortisol was determined by radioimmunoassay [231] using cortisol RIA Kit of Beckman Coulter.

Principle

Serum is incubated in tubes coated with the first monoclonal antibody in the presence of the second monoclonal antibody which is labeled with iodine 125. After incubation for a short time, the liquid contents of the tubes are aspirated to remove unbound ^{125}I -labeled antibody. The bound radioactivity is then determined in gamma

counter. The radioactivity bound is directly proportional to the concentration of cortisol in the sample.

Reagents

Anti-cortisol monoclonal antibody coated tubes: 50 tubes

^{125}I - labeled cortisol tracer: One 55 mL vial. (The iodine vial contains 185kBq of ^{125}I -labeled cortisol in buffer containing sodium azide, bovine serum albumin and dye).

Calibrators: Five vials with 0.5 mL and one vial with 5 mL “zero calibrator” vial (The calibrator vials contain from 0 to 2000 nM of cortisol in buffer of bovine serum albumin and sodium azide).

Control sera: Two vials (lyophilized)

Wash solution: one 50 mL vial

Procedure

For cortisol determination, 50 μL of calibrator, control or sample and 500 μL of tracer were added successively to antibody coated tubes. The tubes were shaken thoroughly at 400 rpm and incubated for an hour at 18-25°C. The contents of the tubes were aspirate carefully and washed with 2 mL of wash solution. The count bound (B) and total count bound (T) were determined for 1 minute. Results were obtained from the standard curve by interpolation. The curve serves for the determination of cortisol concentrations (ng/dL) in sample.

3.15.4 Triiodothyronine (T₃)

T₃ was determined by radioimmunoassay [232] using T₃ RIA Kit of Beckman Coulter.

Principle

Serum is incubated in tubes coated with the first monoclonal antibody in the presence of the second monoclonal antibody which is labeled with iodine 125. After incubation for a short time, the liquid contents of the tubes are aspirated to remove unbound ¹²⁵I-labeled antibody. The bound radioactivity is then determined in gamma counter. The radioactivity bound is directly proportional to the concentration of T₃ in the sample.

Reagents

Anti- T₃ monoclonal antibody coated tubes: 50 tubes

¹²⁵I- labeled T₃ tracer: One 22 mL vial.

The iodine vial contains 165kBq of ¹²⁵I-labeled T₃ in buffer with proteins sodium azide and dye.

Calibrators: Six 0.5 vials.

The calibrator vials contain from 0 to 12 nmol/L of T₃ in human serum with sodium azide.

Control sera: Two vials (lyophilized)

Wash solution: One 50 mL vial

Procedure

For T₃ determination, 25 µL of calibrator, control or sample and 200 µL of tracer were added successively to antibody coated tubes. The tubes were shaken thoroughly at less than 280 rpm and were incubated for an hour at 18-25°C. The contents of the tubes were aspirated carefully and washed with 2 mL of wash solution. The count bound (B) and total count bound (T) were determined for 1 minute. Results were obtained from the standard curve by interpolation. The curve serves for the determination of triiodothyronine concentrations (nmol/L) in sample as well as in the calibrator.

3.15.5 Thyroxine (T₄)

T₄ was determined by radioimmunoassay [233] using T₄ RIA Kit of Beckman Coulter.

Principle

Serum is incubated in tubes coated with the first monoclonal antibody in the presence of the second monoclonal antibody which is labeled with iodine 125. After incubation for a short time, the liquid contents of the tubes are aspirated to remove unbound ¹²⁵I-labeled antibody. The bound radioactivity is then determined in gamma counter. The radioactivity bound is directly proportional to the concentration of T₄ in the sample.

Reagents

Anti- T₄ monoclonal antibody coated tubes: 50 tubes

^{125}I -labeled T_3 tracer: One 55 mL vial.

The iodine vial contains 110 kBq of ^{125}I -labeled T_4 in buffer with sodium azide, proteins and dye.

Calibrators: Six 0.5 vials.

The calibrator vials contain from 0 to 400 nmol/L of T_4 in human serum with sodium azide.

Control sera: Two vials (lyophilized)

Wash solution: One 50 mL vial

Procedure

For T_4 determination, 20 μL of calibrator, control or sample and 500 μL of tracer were added successively to antibody coated tubes. The tubes were shaken thoroughly at less than 280 rpm and incubated for an hour at 18-25°C. The contents of the tubes were aspirated carefully and washed with 2 mL of wash solution. The count bound (B) and total count bound (T) were determined for 1 minute. Results were obtained from the standard curve by interpolation. The curve serves for the determination of thyroxine (nmol/L) in sample as well as in the calibrator.

3.15.6 Thyroid-Stimulating Hormone (TSH)

TSH was determined by immunoradiometric assay [234] using TSH IRMA Kit of Beckman Coulter.

Principle

Samples or calibrators are incubated in tubes coated with first monoclonal antibody in the presence of the second monoclonal antibody which is labeled with iodine 125. After incubation for a short time, the content of tubes is rinsed so as to remove unbound ^{125}I -labeled antibody. The bound radioactivity is then determined in gamma counter. The radioactivity bound is directly proportional to the concentration of TSH in the sample. TSH concentration in the samples is obtained by interpolation from the standard curve.

Reagents

Anti-TSH antibody coated tubes: 50 tubes

^{125}I - labeled monoclonal anti-TSH antibody: One 22mL vial.

The iodine vial contains 515 kBq of ^{125}I -labeled T_3 in buffer with sodium azide, proteins and dye.

Calibrators: Seven 1mL vials.

The calibrator vials contain from 0 to 50m IU/L of TSH in bovine serum with sodium azide.

Control sera: Two vials (lyophilized)

Wash solution: One 50 mL vial

Procedure

For TSH determination, 100 μL of calibrator, control or sample and 100 μL of tracer were added successively to antibody coated tubes. The tubes were shaken

thoroughly at less than 280 rpm and incubated for an hour at 18-25°C. The contents of the tubes were aspirated carefully and washed with 2 mL of wash solution. The count bound (B) and total count bound (T) were determined for 1 minute. Results were obtained from the standard curve by interpolation. The curve serves for the determination of thyroid stimulating hormone (nmol/L) in sample as well as in the calibrator.

Chapter 4

Results and Discussion

Human pregnancy is a normal physiological condition with many biochemical changes which ranges from alteration in electrolyte concentration to more complicated hormonal changes. Thus pregnancy is a unique, normal physiological episode in women's life. However, unexpected illness or preexisting disease of the mother or the fetus can make complications during pregnancy. High risk pregnancy is the one which has increase probability of adverse outcomes for both the mother and the child among the general pregnant population. Diabetes mellitus has long been associated as risk factor with maternal and perinatal mortality. Diabetes in pregnant women could either be pregestational or gestational. Gestational diabetes mellitus (GDM) accounts for about 80% of cases and could results in extreme manifestation of metabolic changes during pregnancy and development of type 2 diabetes later in life.

4.1 General Information about GDM and HPW

This study was basically carried out for the determination of various biochemical changes that occurs during pregnancy in GDM women in comparison to healthy pregnant women. The HPW group was considered as the control group of the study. During the study, information about monthly income, education level, age, body mass index, parity, blood pressure, previous history of gestational diabetes and family history of diabetes were collected from both GDM and healthy pregnant women. These basic information were important in providing some positive clues for increase incidence of GDM, therefore, in addition to the major work i.e. data on biochemical changes in GDM

women, these additional information were also included in this dissertation and are presented in Table-4.1 through Table-4.11 and Figure-4.1 through Figure-4.9. Both the general information and the data on biochemical changes in GDM women were compiled and compared with healthy pregnant women.

Table-4.1: Some Important Information about GDM and HPW

Parameters	Groups	Mean $\pm$ SD ¹	P-Value ²
Monthly-Income (Rs.)	GDM	30845 $\pm$ 11107	0.12
	HPW	28360 $\pm$ 11511	
Age (years)	GDM	34.01 $\pm$ 4.54	< 0.001
	HPW	30.30 $\pm$ 5.86	
BMI (kg/m ²)	GDM	28.07 $\pm$ 2.90	0.001
	HPW	27.30 $\pm$ 1.94	
Parity	GDM	5.44 $\pm$ 2.49	0.172
	HPW	4.95 $\pm$ 2.43	
Systolic Blood Pressure(mm of Hg)	GDM	114.80 $\pm$ 17.14	0.005
	HPW	107.70 $\pm$ 19.40	
Diastolic Blood Pressure(mm of Hg)	GDM	86.5 $\pm$ 7.48	0.002
	HPW	80.7 $\pm$ 10.02	

1. Data are the means and standard deviations of 103 GDM and 97 HPW.

2. P values determined by Chi square test are given in column 4.

The monthly income of the GDM women was Rs.30845 $\pm$ 11107/- which was little more than the monthly income of the HPW (Rs.28360 $\pm$ 11511/-). However, the increase in monthly income was not statistically significant at P<0.05. The mean age of the GDM women was 34.01 $\pm$ 4.54 years and of HPW was 30.30 $\pm$ 5.86 years. The mean age of GDM women was significantly higher than the control group at P<0.001, indicating that elder pregnant women were more prone to gestational diabetes. The mean

BMI value of GDM group was also significantly higher than the healthy pregnant women group at $P < 0.05$. The mean BMI of GDM and HPW were $28.07 \pm 2.90 \text{ kg/m}^2$ and $27.30 \pm 1.94 \text{ kg/m}^2$ respectively. Parity of the GDM group was slightly higher than the HPW group but was not significant at $P < 0.05$. The above data showed that over-weight and obese pregnant women were at risk for development of gestational diabetes. The increase incidence of obesity in U.S. mirrors a worrisome rise in the prevalence of GDM [235]. Obesity and GDM not only increase the risk of adverse pregnancy outcomes [236] but also are associated with a higher risk of developing type 2 diabetes later in life [237].

The mean systolic blood pressure of GDM group was $114.80 \pm 17.14 \text{ mmHg}$ as compared to the mean systolic blood pressure $107.70 \pm 19.40 \text{ mmHg}$ of the HPW (control) group. Similarly the mean diastolic blood pressure of the GDM group was $86.50 \pm 7.48 \text{ mmHg}$ as compared to the mean diastolic blood pressure $80.70 \pm 10.02 \text{ mmHg}$ of HPW group. The data showed that GDM individuals as a whole were having significantly higher ($P < 0.05$) blood pressure than the control individuals.

4.1.1 Monthly Income of GDM Women

The monthly income levels of GDM and of healthy pregnant women are given in Table-4.2 and shown in Figure-4.1. The data in the Table-4.2 indicates that 14.5% individuals of the GDM group and 21.6% individuals of the HPW group were having income level of Rs.15000/- or less per month. In the income range of Rs.15001-30000/-, there were 42.7% individuals of GDM group and 49.4% of HPW group. The number of healthy pregnant women in low monthly income group was greater than the number of pregnant women in GDM group, indicating that high income favors onset of gestational

diabetes. There were 27.2% individuals of the GDM group and 16.5% individuals of the HPW group who were in the income range of Rs.30001-45000/- per month. There were 15.5% individuals of the GDM group and 12.4% individuals of the HPW group who were having income level of more than Rs.45000/- per month. The data showed that those women who were having high monthly income were more exposed to the onset of gestational diabetes. In other words, the increased mean monthly income of GDM group showed that gestational diabetes was common in those families who were having high economic status.

Table-4.2: GDM and HPW having Different Monthly Income Levels

Monthly-Income (Rs)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
≤ 15000	15	14.5	21	21.6	0.184
15001-30000	44	42.7	48	49.4	
30001- 45000	28	27.2	16	16.5	
>45000	16	15.5	12	12.4	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

The current study identified a strong inverse association between socioeconomic status and GDM. Lower socioeconomic status is well recognized as a risk for chronic disease in developed and developing countries [238]. The association between GDM and socioeconomic status is less well established, with conflicting results seen in previous studies. These studies cannot easily be compared because of different definitions of socioeconomic status, but two studies have used indexes of relative deprivation. Tanaka et al. [239] found no association, while Clausen et al. [240] showed that living in an area

of deprivation was positively associated with GDM. The importance of maternal social factors on the health of the mother and the offspring is well-known. Lower socioeconomic status is associated with an increased risk of various adverse pregnancy outcomes such as perinatal mortality, miscarriages, preterm birth, and lower birth weight.

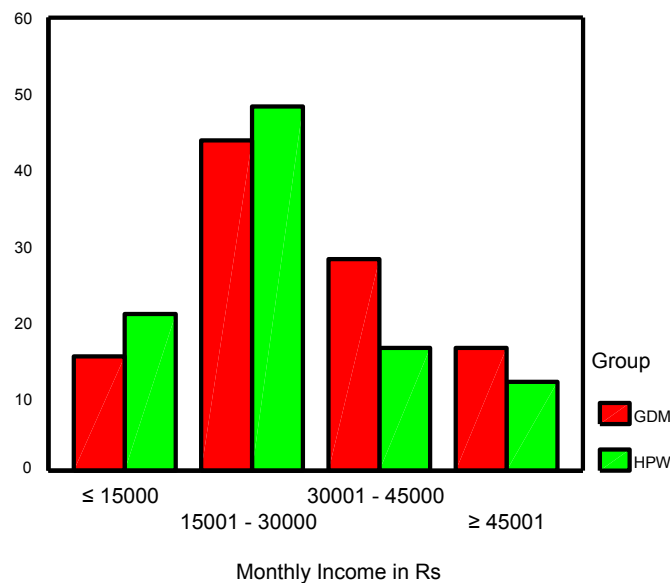


Figure-4.1: GDM and HPW having different monthly income levels.

4.1.2 Education Level of GDM and HPW

The education levels of GDM and HPW groups are presented in Table-4.3 and Figure-4.2. The education level of GDM and HPW were almost similar. There were 60.2% and 69.1% illiterate women in the GDM and HPW groups respectively. However, in GDM group, 24.3% women had completed school level education while in HPW group 18.5% women had completed school level education. 15.5% GDM women and 12.4% healthy pregnant women were reported to have completed education above school

level. The chi square test indicated no significant difference in the education levels of both GDM and HPW groups.

Table-4.3: Education Levels of GDM and HPW

Education Levels	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Illiterate	62	60.2	67	69.1	0.422
School level education	25	24.3	18	18.5	
Above school level education	16	15.5	12	12.4	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

In different countries different measures of social status and education levels are used. These include income type, educational attainment, and family education levels. There is a strong association between social class and infant mortality rate [241].

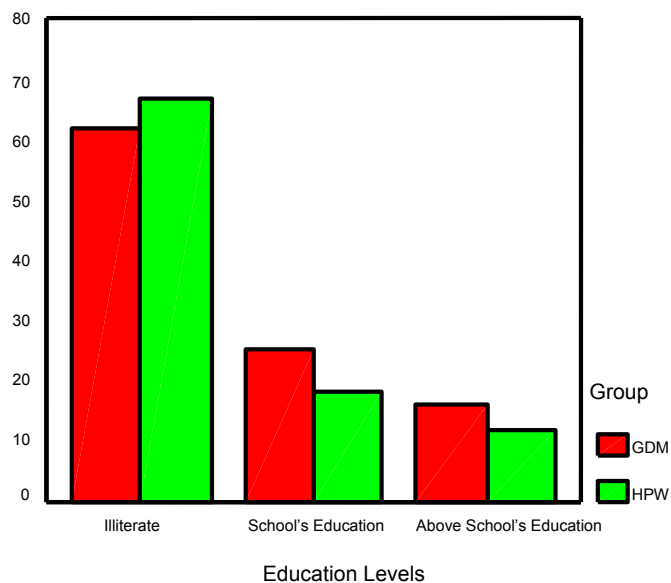


Figure-4.2: GDM and HPW having different education levels.

4.1.3 Age of GDM Women

GDM and HPW who were having different ages are given in Table-4.4 and Figure-4.3. The data in Table-4.4 shows that no GDM women was present in the age group of 25 years or less, 30.1% GDM women were in the age group of 25-30 years, 42.7% GDM women were in the age group of 31-35 years and 27.2% GDM women were in the age group of more than 35 years. In the HPW group, 17.5% HPW were in the age group of 25 years or less, 32.9% HPW were in the age group of 25-30 years, 28.9% HPW were in the age group of 31-35 years and 20.6% HPW were in the age group of more than 35 years. The data in Table-4.4 and Figure-4.3 indicated that incidence of GDM increases with the age of pregnant women.

Table-4.4: Age Wise Distribution of GDM and HPW

Age (Years)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
≤ 25	0	0	17	17.5	0.001
26-30	31	30.1	32	32.9	
31-35	44	42.7	28	28.9	
> 35	28	27.2	20	20.6	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

In the last decades, there has been a trend towards deferred child bearing, especially among healthy, wealthy and well educated women with career opportunities. Our findings are not supporting later age pregnancy as it increases the chances of gestational diabetes. However, it supports the American Diabetes Association

recommendation that instead of age of ≥ 35 years, age ≥ 25 years, should be considered as a risk factor for developing gestational diabetes [242].

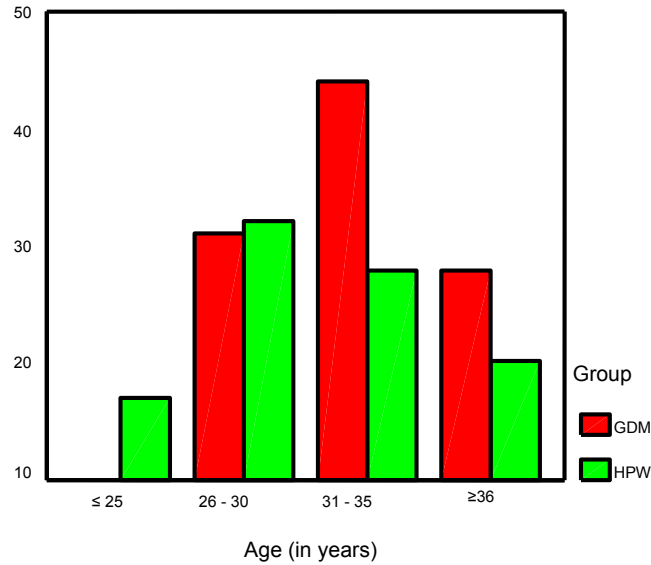


Figure-4.3: Age wise distribution of GDM and HPW.

4.1.4 Body Mass Index of GDM Women

Body mass index (BMI) is the most common measure used for health evaluation. Many health complications are associated with over weight/obesity. In this study, the mean BMI of the GDM women was compared with the mean BMI of HPW (control). The mean BMI of the GDM was significantly higher than the mean BMI of the HPW at $P < 0.005$ as shown in Table-4.1. In Table-4.4 and Figure-4.3, the percentages of GDM and HPW in the various BMI groups are presented. No GDM women were under weight while 9.3% HPW were under weight. In the normal BMI range, 30.1% GDM and 41.2% HPW were present. In GDM group 42.7% women were overweight while 27.2% women were obese in comparison to HPW group in which 28.9% women were overweight and

20.6% obese. The data indicates a positive association between obesity and incidence of GDM.

Table-4.5: GDM and HPW having Different BMI Levels

BMI (Kg/m ²)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Underweight (≤18.50)	0	0	9	9.3	0.002
Normal (18.50-22.90)	31	30.1	40	41.2	
Overweight (23.00-27.00)	44	42.7	28	28.9	
Obese (>27.00)	28	27.2	20	20.6	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

The prevalence of both obesity and gestational diabetes mellitus is rising worldwide. The complications of diabetes affecting the mother and fetus are well known. Maternal complications include preterm labor, pre-eclampsia, nephropathy, birth trauma, cesarean section, and postoperative wound complications. Fetal complications include fetal wastage from early pregnancy loss or congenital anomalies, macrosomia, shoulder dystocia, stillbirth, growth restriction, and hypoglycemia. The presence of obesity among diabetic patients compounds these complications. The above-mentioned short-term complications can be controlled by achieving and maintaining the desired level of glycemic control during pregnancy [243].

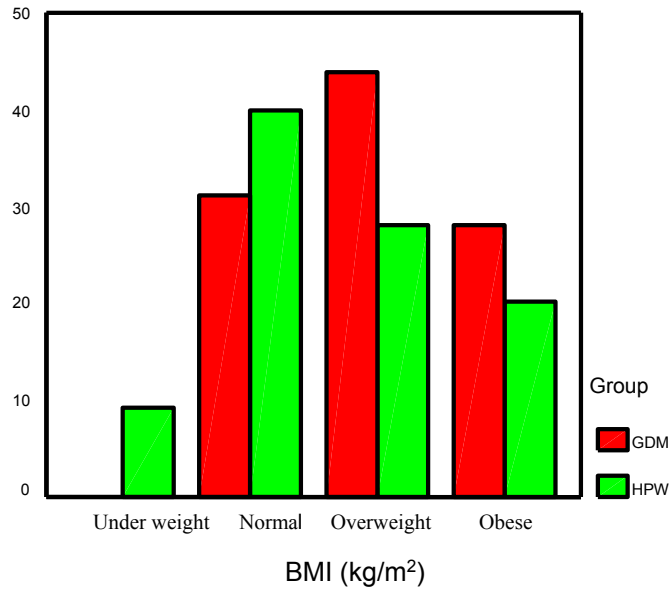


Figure-4.4: GDM and HPW having different BMI levels.

4.1.5 Parity of GDM Women

Parity is defined as the number of times a woman has given birth to the infant, alive or dead. Parity wise distribution of GDM and HPW groups is given in Table-4.6 and Figure-4.5. In the GDM group, 9.7% women were primiparous, 35.9% were multiparous and 54.4% were grand multiparous. On the other hand, in HPW group, 13.4% women were primiparous, 49.4% were multiparous and 37.2% women were grand multiparous. The data on parity indicates that the chances of gestational diabetes increases with increasing parity. Our findings were in accordance with the findings of Ben-Haroush et al [244] who had reported that parity, smoking, obesity and family history of diabetes are risk factors for gestational diabetes.

Table-4.6: GDM and HPW having Different Parity

Parity	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Primiparous	10	9.7	13	13.4	0.05
Multiparous (2-5)	37	35.9	48	49.4	
Grand Multiparous (>5)	56	54.4	36	37.2	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

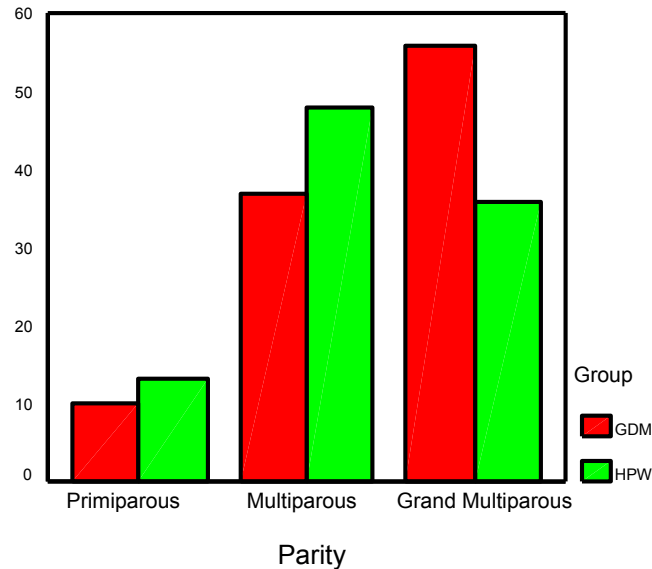


Figure-4.5: GDM and HPW having different parity.

4.1.6 Blood Pressure of GDM Women

The mean systolic and diastolic blood pressure of GDM and HPW are given Table-4.1. The mean values of both the systolic and diastolic blood pressure of the GDM and of the HPW were in the normal range of blood pressure. However, the mean values of systolic and diastolic blood pressure of the GDM women were significantly higher

than the mean values of systolic and diastolic blood pressure of the HPW at $P < 0.05$. Though the mean values of systolic and diastolic blood pressure were in the normal range as shown in Table-4.1, but almost 33.1% GDM women and 20.6% healthy pregnant women were having higher systolic blood pressure than normal as shown in Table-4.7. Similarly, 34.9% GDM women and 22.7% healthy pregnant women were having higher diastolic blood pressure than normal (Table-4.8). The data indicates that both systolic and diastolic blood pressures are associated with GDM.

Women exposed to gestational diabetes are at higher risk of hypertension in the years after pregnancy, even after adjusting for other major risk factors of hypertension. Previous research has demonstrated that women who developed gestational diabetes had an underlying high susceptibility to insulin resistance and β cell impairment and hence GDM women are more likely to develop GDM when facing the different metabolic changes during pregnancy. Defects in both insulin sensitivity and insulin secretion in GDM women increase the risk of hypertension [245-246].

Table-4.7: GDM and HPW having Normal and High Systolic Blood Pressure

Systolic BP (mm of Hg)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
≤ 120 (Normal)	69	66.9	77	79.4	0.05
> 120 (High)	34	33.1	20	20.6	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

GDM and HPW classified on the basis of normal and high systolic blood pressure are shown in Table-4.7 and Figure-4.6. The number of GDM women in normal range of

systolic blood pressure was 69 (66.9%) and number of HPW in the normal range of systolic blood pressure was 77 (79.4%). There were 34 (33.1%) GDM women and 20 (20.6%) HPW in the high range of systolic blood pressure. The greater number of HPW in normal range of systolic blood pressure and greater number of GDM women in high range of systolic blood pressure provides us a clue that women with high systolic blood pressure might be more prone to the development of gestational diabetes.

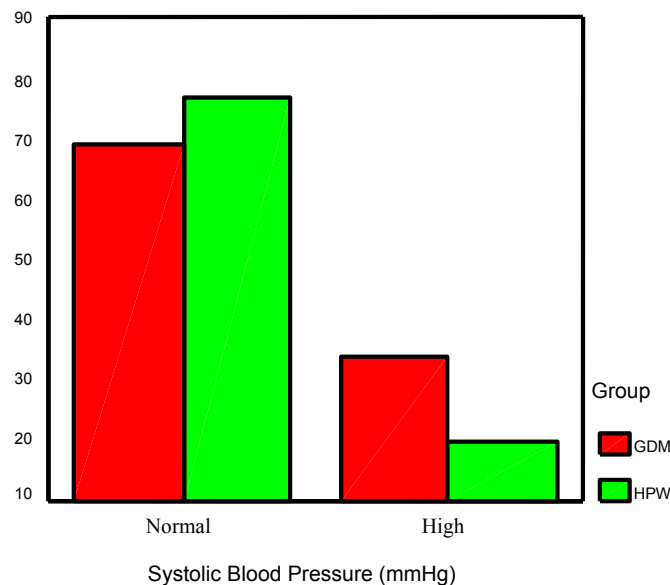


Figure-4.6: GDM and HPW having normal and high systolic blood pressure.

Our results are generally in accordance with the findings of Lao and Ho [247] who reported that systolic blood pressure above the median (109 mmHg) had a fourfold increased risk of GDM in high risk women.

Table-4.8: GDM and HPW having Normal and High Diastolic Blood Pressure

Diastolic BP (mm of Hg)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
≤ 89 (Normal)	67	65.1	75	77.3	0.05
> 89 (High)	36	34.9	22	22.7	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

The data on distribution of GDM and HPW based on diastolic blood pressure is given in Table-4.7 and Figure-4.6. In the normal range of diastolic blood pressure the number of HPW was 77.3% while the number of GDM women was 65.1%. In the high range of diastolic pressure, the number of GDM women was 34.9% while the number of HPW was 22.7%. Thus the number of GDM women was more in high range of diastolic blood pressure while the number of HPW was more in normal range of diastolic blood pressure.

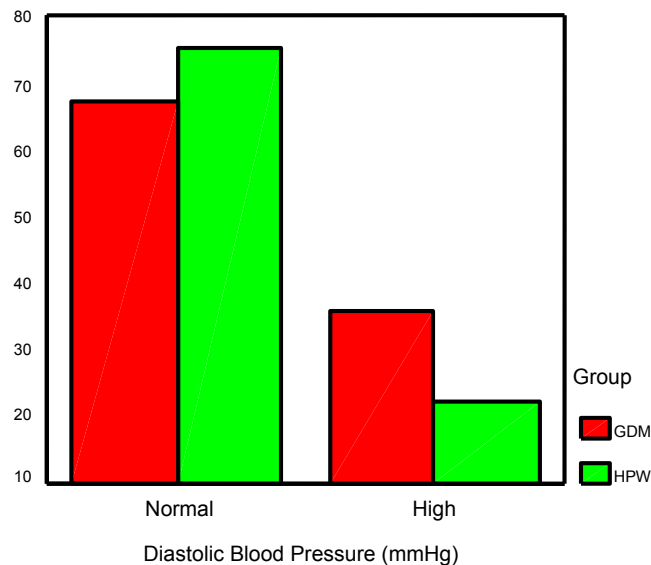


Figure-4.7: GDM and HPW having normal and high diastolic blood pressure.

The above vice versa position showed that hypertension is a risk factor for future gestational diabetes. This is in correspondence to the study which showed that women with hypertension either during the 5 years before pregnancy or during the first trimester of pregnancy had a twofold increased risk of developing GDM [248]. These associations persisted after adjusting for BMI, suggesting that the association is independent of BMI. However, the association between blood pressure and GDM was stronger among women who were overweight and obese. Crowther et al. [249] reported that treatment of glucose intolerance in mid pregnancy effectively reduced both perinatal and maternal complications. Therefore, blood pressure should be measured at each medical visit as it would be an inexpensive and easy clinical way for the identification of women who may be at high risk of GDM.

Table-4.9: Blood Pressure of GDM and HPW in Relation to BMI

Blood Pressure (mm Hg)	Groups	Mean BMI $\pm$ SD ¹				P-value ²
		Underweight	Normal	Overweight	Obese	
Systolic BP	GDM	00	108 $\pm$ 19	112 $\pm$ 16	128 $\pm$ 18	0.01
	HPW	100 $\pm$ 18	100 $\pm$ 16	110 $\pm$ 16	125 $\pm$ 17	
Diastolic BP	GDM	00	86 $\pm$ 5	86 $\pm$ 8	91 $\pm$ 9	0.001
	HPW	79 $\pm$ 13	74 $\pm$ 6	85 $\pm$ 6	87 $\pm$ 9	

1. Data are the means and standard deviations of 103 GDM and 97 HPW.

2. P values determined by Chi square test are given in column 7.

The mean blood pressure data of the various BMI groups of GDM and HPW are shown in Table-4.9. The systolic BP of the GDM women in overweight and obese groups was 112 $\pm$ 16 mmHg and 128 $\pm$ 18 mmHg respectively, and was significantly higher at $P < 0.05$ than the systolic blood pressure of the HPW in the same overweight and obese

group. The systolic blood pressure of HPW in the overweight and obese groups were 110 ± 16 mmHg and 125 ± 17 mmHg respectively. The diastolic blood pressure of GDM women was also significantly higher at $P < 0.05$ than the diastolic blood pressure of the HPW. The data in Table-4.9 indicates that association between high blood pressure and GDM is stronger among overweight and obese women, suggesting that high blood pressure and GDM would interact synergically resulting in metabolic syndrome during pregnancy. There are various common pathogenic pathways to gestational diabetes and hypertension. Insulin resistance is one of the known contributing factors to both gestational and chronic hypertension [250], and it is also known to be involved in the pathogenesis of GDM [251]. Endothelial impairment has been reported in GDM women both during [252] and after [253] pregnancy and is also strongly related to hypertension [254]. C-reactive protein, markers of inflammation has been related with increased blood pressure levels [255]. The elevated C-reactive protein levels during early pregnancy have been related to increased risk of GDM [256].

4.1.7 Previous History of Gestational Diabetes of GDM Women

The previous history of gestational diabetes of GDM and HPW are given in Table-4.10 and Figure-4.8. Out of 103 GDM women, 75.7% were having previous history of gestational diabetes, which means that they were having gestational diabetes in their previous pregnancies. Only 24.3% GDM women were not having previous history of gestational diabetes but they developed it in the present pregnancy. Similarly in the HPW group, which was the control group of the study, previous history of gestational diabetes was not reported by any subject. Precautionary measures are required for those GDM women who have experienced gestational diabetes in their previous pregnancies.

Table-4.10: Previous Gestational Diabetic History of GDM and HPW

Previous History of GDM	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Yes	78	75.7	0	0	<0.001
No	25	24.3	97	100	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

It is important to recognize and treat gestational diabetes as soon as possible to minimize the risk of complications in the baby. In addition, it is important for women with a history of gestational diabetes to be tested for diabetes after pregnancy because of an increased risk of developing type 2 diabetes in the years following delivery [257].

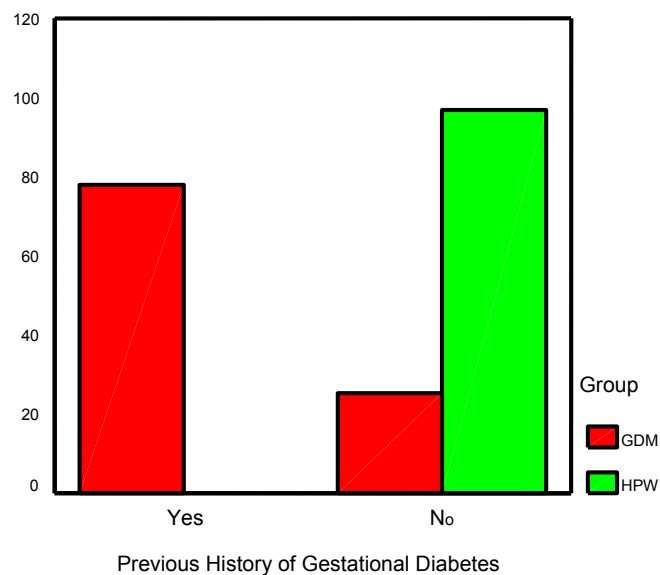


Figure-4.8: Previous gestational diabetic history of GDM and HPW.

4.1.8 Family History of Diabetes of GDM Women

The family history of diabetes of GDM and HPW are given in Table-4.11 and Figure-4.9. There were 84.5% GDM women who were having family history of diabetes mellitus and 15.5% GDM women were not having family history of diabetes mellitus. In the HPW group, 26.8% pregnant women were having family history of diabetes mellitus but they did not develop gestational diabetes in their present pregnancy. This shows that though family history has a strong association with gestational diabetes, yet it is not necessary that every pregnant woman who was having family history of diabetes would become gestational diabetic in her present pregnancy, however, she should be alert about her health care.

Table-4.11: Family History of Diabetes of GDM and HPW

Family History of Diabetes	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Yes	87	84.5	26	26.8	<0.001
No	16	15.5	71	73.2	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

Established risk factors for GDM are relevant in women with family history of diabetes but may not be the principal determinants of gestational hyperglycaemia in women without family history of diabetes. Moreover, family history of diabetes may be more relevant to risk of GDM in nulliparous women than in multiparous subjects. These findings highlight the complex relationship between family history of diabetes and

gestational hyperglycaemia, and may hold implications for selective screening for GDM [258].

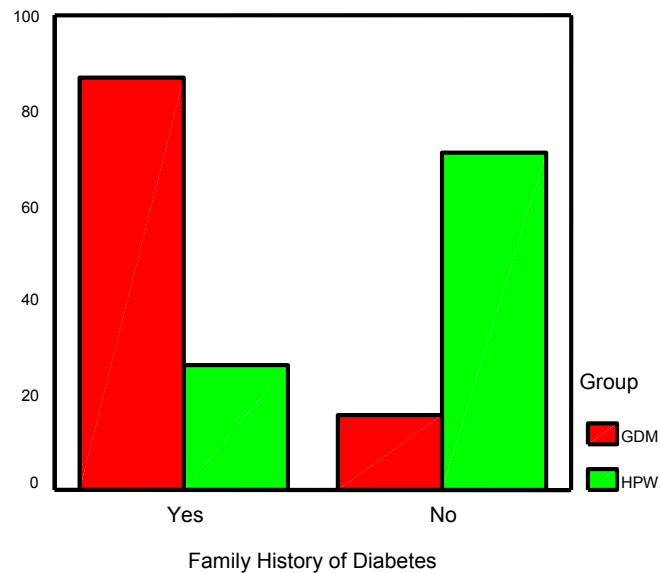


Figure-4.9: Family history of diabetes of GDM and HPW.

4.2 Blood Sugar, Hemoglobin and Platelet Count

The major objective of the study was to note and report the biochemical changes during gestational diabetes. These changes may appear in blood sugar, lipid profile, liver functions, renal functions and in concentration of various hormones. Blood samples from GDM and HPW were taken and the above parameters were determined in the laboratory and the results were statistically analyzed and compared. The data were tabulated and presented in Table- 4.12 through 4.43 and Figure 4.10 through 4.31.

4.2.1 Blood Sugar Levels in GDM Women

The mean fasting blood sugar, random blood sugar, glycosylated hemoglobin (HbA1c), hemoglobin concentration and platelet count of GDM and HPW are given in Table-4.12. The mean fasting blood glucose level of GDM women was 111.69 ± 8.70 mg/dL while of HPW was 86.59 ± 6.91 mg/dL. The mean fasting blood sugar of GDM women was significantly higher at $P < 0.001$ as compared to HPW (control group). Similarly, the mean random blood sugar (145.45 ± 6.62 mg/dL) and mean HbA1c (6.58 ± 1.30) values of GDM women were significantly higher at $P < 0.001$ than the mean random blood sugar (123.52 ± 9.37 mg/dL) and HbA1c (4.95 ± 0.45) values of HPW. This was expected as all the GDM were having sugar problem, while the HPW were the healthy pregnant women who were considered as control group.

Table-4.12: Blood Sugar, Hemoglobin and Platelet Count of GDM and HPW

Blood Parameter	Groups	Mean $\pm$ SD ¹	P- value ²
Fasting Blood Sugar (mg/dL)	GDM	111.69 ± 8.70	< 0.001
	HPW	86.59 ± 6.91	
Random Blood Sugar (mg/dL)	GDM	145.45 ± 6.62	< 0.001
	HPW	123.52 ± 9.37	
HbA1c	GDM	6.58 ± 1.30	< 0.001
	HPW	4.95 ± 0.45	
Hemoglobin (%)	GDM	10.89 ± 1.12	0.55
	HPW	11.01 ± 1.03	
Platelet Count (thousand/mL)	GDM	226.31 ± 38.20	0.49
	HPW	228.14 ± 37.61	

1. Data are the means and standard deviations of 103 GDM and 97 HPW.

2. P values determined by Chi square test are given in column 4.

Both low and high fasting glucose values at 22–30 weeks of gestation are associated with increased risk of macrosomia or babies large for gestational age (LGA). The presence of a large fetus is associated with multiple risks for both the mother and the newborn. The short-term risks include a higher probability of operative delivery, two- to three fold increase in intrauterine death rate and several neonatal morbidities (namely shoulder dystocia and brachial plexus injuries) and increased risks of maternal injuries like perineal laceration. There are also long-term risks for the newborn, such as diabetes later in life, obesity, and the development of metabolic syndrome [259]. The hemoglobin and platelet concentration in both groups were non significant.

In Table-4.13 and Figure-4.10, the GDM and HPW were classified as those who were having mean fasting sugar less than 105 mg/dL, they were classed as having normal sugar level and those GDM and HPW who were having sugar level equal or more than 105 mg/dL, they were classed as having high sugar level, meaning that they were hyperglycemic.

Table-4.13: GDM and HPW having Normal and High Fasting Blood Sugar

Fasting Blood Sugar (mg/dL)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Normal (< 105)	10	9.7	93	95.9	< 0.001
High (≥ 105)	93	90.3	04	4.1	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

Gestational diabetes is defined by the fasting plasma glucose of 105mg/dL or above, or a random plasma glucose of 140mg/dL or above, or abnormal glucose tolerance

test. In this study the fasting blood sugar level was significantly different at $P < 0.001$ between the two study groups. In HPW group, which was the control group, out of 97 healthy pregnant women 95.9% pregnant individuals were normoglycemic while 4.1% were hyperglycemic. However, they were not declared as gestational diabetes by the standard criteria of diabetes. In GDM group, out of 103 subjects, 90.3% pregnant women were hyperglycemic while 9.7% individuals were having normal fasting blood sugar level indicating that these 9.7% GDM women were strictly controlling their sugar level. In the normal range of sugar level, HPW were significantly ($P < 0.001$) more than the GDM women, while in hyperglycemic class, the GDM women were significantly more than HPW at $P < 0.001$.

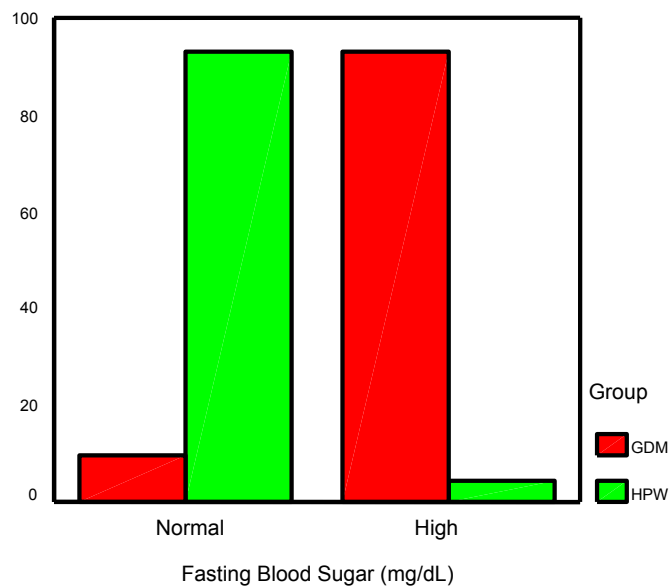


Figure-4.10: GDM and HPW having normal and high fasting blood sugar.

The consequences of high blood sugar in pregnant women are that it results in increased morbidity of the fetus and also the mother. By knowing this parameter, one can predict the severity of the disease and can plan to provide better antenatal care by

institutional treatment to save the mother and the child from all the fatal complications of gestational diabetes. Similar reports of elevated blood glucose levels in gestational diabetes have been reported by Taricco et al. [260].

The random blood sugar of GDM and HPW are given in Table-4.14 and Figure-4.11. In GDM group, 92.2% pregnant women were having high random glucose level greater than 140 mg/dL while 7.8% pregnant women were having random sugar less than 140 mg/dL. Perhaps they were controlling their random sugar level either by diet, medicine or exercise. In the HPW group, 87.6% women showed normal random blood glucose level while 12.4% were having high random sugar level. However, they were not diabetic by the standard criteria for diabetes. The data indicated that majority of GDM women were hyperglycemic while majority of HPW were normoglycemic on the criteria of random sugar test. The percent of HPW in normal random sugar level were significantly ($P < 0.001$) more than the GDM women. In the hyperglycemic range of random sugar, the percent of GDM women were significantly ($P < 0.001$) more than the HPW.

Table-4.14: GDM and HPW having Normal and High Random Blood Sugar

Random Blood Sugar (mg/dL)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Normal (< 140)	08	7.8	85	87.6	< 0.001
High (≥ 140)	95	92.2	12	12.4	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

Random blood sugar level is important in predicting the risk of future type 2 diabetes and fetal macrosomia. Women with mildly elevated oral glucose tolerance test

(OGTT) are more likely to have pre-eclampsia, eclampsia, shoulder dystocia, cesarean delivery, miscarriages, still births and preterm birth. Similarly, women with one abnormal OGTT value have a greater risk of macrosomia, preeclampsia, and cesarean delivery [261].

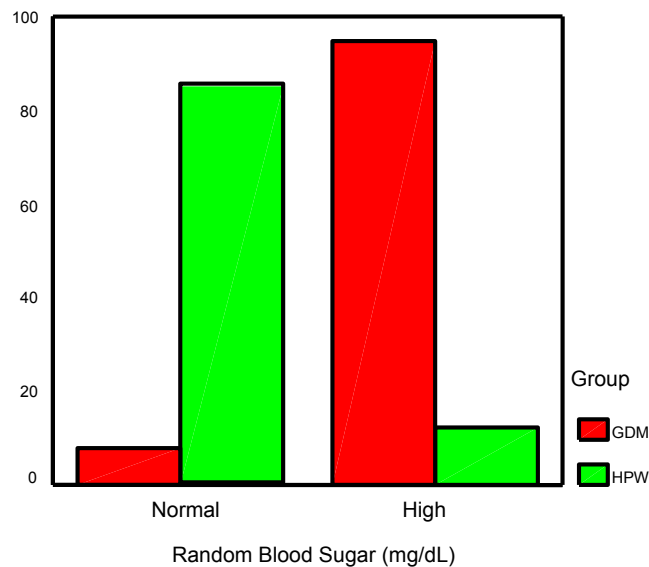


Figure-4.11: GDM and HPW having normal and high random blood sugar.

4.2.2 Glycosylated Hemoglobin in GDM Women

Glycosylated hemoglobin, HbA1c in GDM provides an objective and accurate measure of glycemic control over a period of weeks to months. This test can be utilized as an assessment of glycemic control in patients with known diabetes but is not sufficiently good and sensitive to make diagnosis of gestational diabetes and is usually normal in patients with impaired glucose tolerance.

Table 4.15: GDM and HPW having Normal and High HbA1c Levels

HbA1c	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Normal (< 6)	59	57.3	97	100	< 0.001
High (≥ 6)	44	42.7	00	00	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

The GDM and HPW who were having normal and high HbA1c level are given in Table-4.15 and Figure-4.12. In GDM group, 57.3% pregnant women were having normal HbA1c values while 42.7% were having high HbA1c values. In HPW group, all pregnant women were having normal HbA1c values. The data indicated that sugar levels of 57.3% GDM women were in the control range and 42.7% were above the normal range.

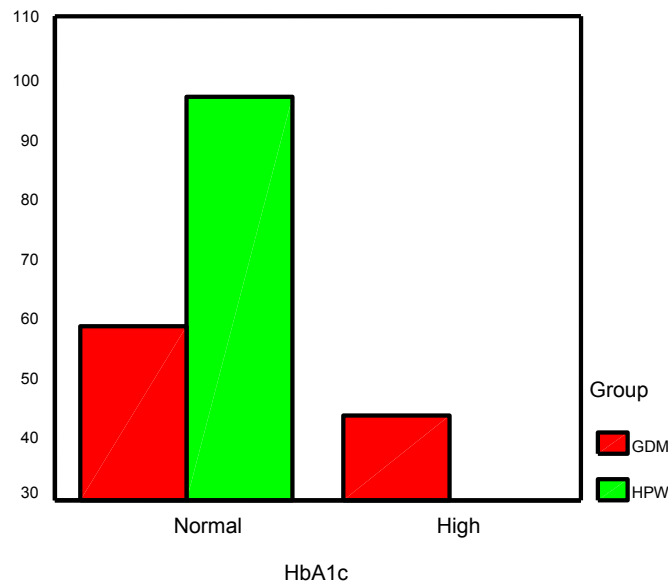


Figure-4.12: GDM and HPW having normal and high HbA1c levels.

It is very much important that pregnant women with gestational diabetes must control their glucose level in the normal range to avoid complications during pregnancy and delivery. HbA1c provides the extent to which the blood glucose has been controlled in the previous 8-12 weeks. So, by determining this parameter, one can prevent further worsening of the disease by providing better treatment at appropriate time. Significantly elevated levels of glycosylated hemoglobin in gestational diabetes were also reported by researchers in several studies supporting our findings [262-263].

4.2.3 Hemoglobin Concentration in GDM Women

Anemia is defined as hemoglobin concentration that is lower than the threshold of two standards deviation below the median value for a healthy matched population. The World Health Organization (WHO) defines anemia in pregnancy as hemoglobin concentration of less than 11g/dL [264].

Table-4.16: GDM and HPW having Low and Normal Hemoglobin Levels

Hemoglobin g/dL	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Low (< 11) (Anemia)	30	29.1	23	23.7	0.240
Normal ($\geq$ 11)	73	70.9	74	76.3	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

Both the GDM and HPW who were having low and normal hemoglobin levels are given in Table-4.16 and Figure-4.13. The difference in hemoglobin status was not significant among GDM and healthy pregnant women. In HPW group, out of 97 healthy

pregnant women 23.7% individuals developed anemia while 76.3 % remains normal. Similarly in GDM group 29.1% pregnant women develop anemia while 70.9% pregnant women have normal hemoglobin concentration.

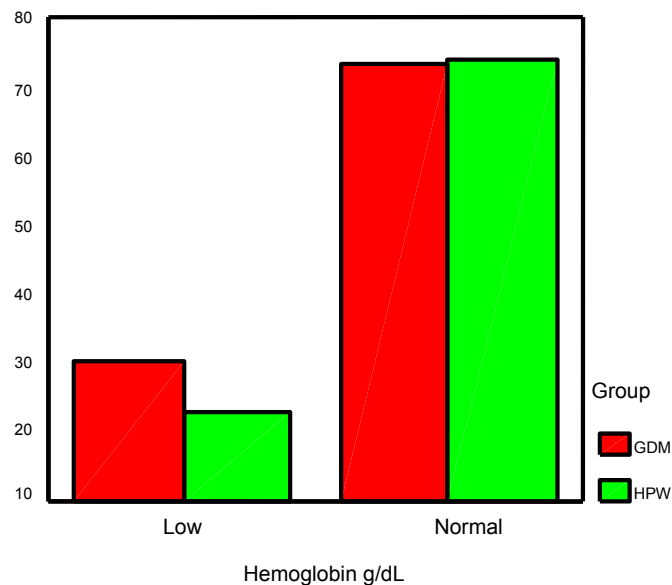


Figure-4.13: GDM and HPW having low and normal hemoglobin levels.

Increase incidence of gestational diabetes is associated with elevated iron stores in the general population. In non pregnant subjects, strong association between red cell count and high hemoglobin (Hb) concentration results in elevated HbA1c level in diabetic patients. It has been reported that the glycation of hemoglobin A (Hb A) does not affect oxygen transport. However, glycosylated Hb has increased affinity for oxygen, and higher levels of glycosylated Hb could results in sufficient tissue hypoxia [265-266].

4.2.4 Platelet Count in GDM Women

The GDM and HPW who were having low and normal platelet count are given in Table-4.17 and Figure-4.14. The normal range for peripheral blood platelet count in pregnant individuals is generally reported as 150 to 400 thousand/ μL [267].

Table-4.17: GDM and HPW having Low and Normal Platelet Count

Platelet Count (thousand/ μL)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Low (<150) (Thrombocytopenia)	12	11.7	12	12.4	0.57
Normal (≥ 150)	91	88.3	85	87.6	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

The platelet count of GDM and healthy pregnant women were not significantly different and thus platelet count has no direct correlation with gestational diabetes. In GDM group, 88.3% pregnant women and in control group, 87.6% pregnant women were having normal platelet count. Thrombocytopenia was shown by 11.7% GDM women and 12.4% healthy pregnant women. This study showed that GDM was doing nothing with thrombocytopenia but pregnancy itself may results in low platelet count. Studies about platelet counts during normal pregnancy differed in their conclusion, with some reporting no effect of pregnancy on platelet count and other illustrating a mild reduction during late pregnancy [268]. In this study, no significant changes were observed in the concentration of hemoglobin and platelet count of the GDM women and HPW.

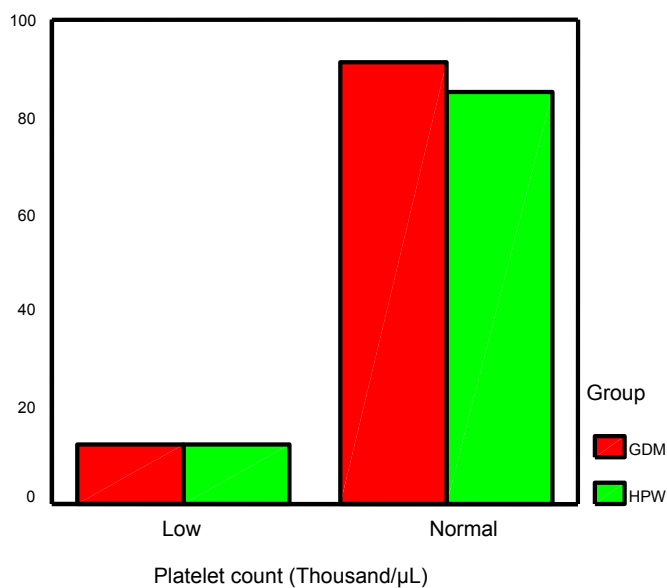


Figure-4.14: GDM and HPW having low and normal platelet count.

4.3 Lipid Profile

Lipid profile is important in investigating the biochemical changes in gestational diabetes in comparison to healthy pregnant women. The lipid profile of GDM women and HPW are given in Table-4.18. Mean total cholesterol (TC) and mean triglyceride (TG) were significantly higher at $P < 0.05$ in GDM group as compared to HPW group. The mean value of total cholesterol in GDM group was 206 ± 18.79 mg/dL while in HPW (control group) it was 195 ± 24.15 mg/dL. The mean TG level of GDM women was 190 ± 19.83 mg/dL while of HPW was 172 ± 21.66 mg/dL. The mean HDL cholesterol (HDL-C) was 55 ± 8.20 mg/dl in gestational diabetic individuals and 56 ± 8.82 mg/dL in control group. The HDL-C was not significantly different in GDM and HPW groups.

Table-4.18: Lipid Profile of GDM and HPW

Parameter	Groups	Mean $\pm$ SD ¹	P- value ²
TC (mg/dL)	GDM	206.01 $\pm$ 18.79	0.001
	HPW	195.06 $\pm$ 24.15	
TG (mg/dL)	GDM	190.12 $\pm$ 19.83	0.001
	HPW	172.13 $\pm$ 21.66	
HDL-C (mg/dL)	GDM	55.21 $\pm$ 8.20	0.25
	HPW	56.21 $\pm$ 8.82	
LDL-C (mg/dL)	GDM	93.13 $\pm$ 18.71	0.06
	HPW	88.10 $\pm$ 16.36	
TC: HDL-C	GDM	3.9.01 $\pm$ 1.05	0.006
	HPW	3.5.12 $\pm$ 1.00	

1. Data are the means and standard deviations of 103 GDM and 97 HPW.

2. P values determined by Chi square test are given in column 4.

The mean LDL cholesterol (LDL-C) of GDM women and HPW were 93 $\pm$ 18.71 mg/dL and 88 $\pm$ 16.36 mg/dL respectively. The TC: HDL-C ratio was significantly higher in GDM individual as compared to healthy pregnant women (P < 0.05).

4.3.1 Total Cholesterol Levels in GDM Women

In normal person, total cholesterol level varies from 150-220 mg/dL. It should be preferably below 200 mg/dL. A concentration below 200-240 mg/dL is considered borderline and a concentration above 240 mg/dL increases risk for atherosclerosis and heart attack [269]. GDM and HPW having different cholesterol levels are presented in Table-4.19 and Figure-4.15.

Table-4.19: GDM and HPW having Different Cholesterol Levels

Total Cholesterol (mg/dL)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
< 200 (Desirable)	50	48.5	65	67	0.03
200-240 (Acceptable)	41	39.8	24	24.7	
> 240 (Undesirable)	12	11.7	08	8.3	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

In GDM group, 50 (48.5%) pregnant women were having desirable cholesterol level, 41 (39.8%) were having acceptable level of cholesterol and 12 (11.7%) were having undesirable level of cholesterol. Those 11.7% GDM women in the undesirable range of cholesterol were more prone to obesity, hypertension and heart attack. In the control group (HPW), 65 (67%) women were having desirable level of cholesterol, 24 (24.7%) were having acceptable level of cholesterol and only 8 (8.3%) were having the undesirable level of cholesterol. These 8.3% healthy pregnant women were at high risk of cardiovascular diseases. In Pakistan, Cholesterol level of 200 mg/dL is already considered high, because of higher incidence of risk factors such as lack of exercise, smoking and high fat diet. GDM women, who were having acceptable and undesirable cholesterol level, were significantly more in number than the healthy pregnant women. Women with previous history of GDM had greater central adiposity, higher LDL cholesterol levels, higher fasting insulin levels and a significantly increased prevalence of hypertension as compared with women without a history of GDM [270].

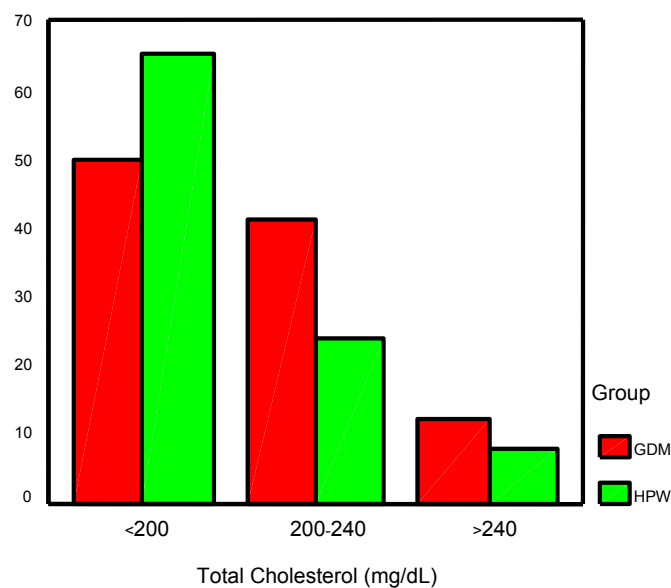


Figure-4.15: GDM and HPW having different cholesterol levels.

Studies performed in other populations have shown that women with a history of gestational diabetes are more obese, have greater waist circumferences, and are dyslipidemic and insulin resistant [271].

4.3.2 Triglyceride Levels in GDM Women

Triglyceride (TG) is a non polar, water insoluble compound with normal range of 40-150 mg/dL in healthy individuals [272]. The mean desirable, acceptable and undesirable level of TG in gestational diabetes and healthy pregnant women are given in Table-4.20 and Figure-4.16. In GDM group, 9.7% women were in the desirable range of TG, 59.2% were in the acceptable range of TG and 31.1% were in the undesirable range of TG. In the HPW group, 27.8% women were in the desirable range of TG, 51.6% were in the acceptable range of TG and 20.6% were in the undesirable range of TG. On Comparison, significantly ($P < 0.05$) high numbers of HPW were in the desirable range of TG than GDM (27.8% vs 9.7%). But in the acceptable and undesirable range of TG,

GDM were significantly ($P < 0.05$) more than HPW (59.2%, 31.1% vs 51.6%, 20.6%) respectively. The data indicates that most of the GDM are having higher level of TG.

Table-4.20: GDM and HPW having Different TG Levels

Triglycerides (mg/dL)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
<150 (Desirable)	10	9.7	27	27.8	0.003
150-200 (Acceptable)	61	59.2	50	51.6	
>200 (Undesirable)	32	31.1	20	20.6	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

Higher TG level is one of the risk factor for heart diseases. On average basis, more GDM women were having acceptable and undesirable range of TG. The elevated TG is deposited in subcutaneous tissues and is leading cause of xanthomas.

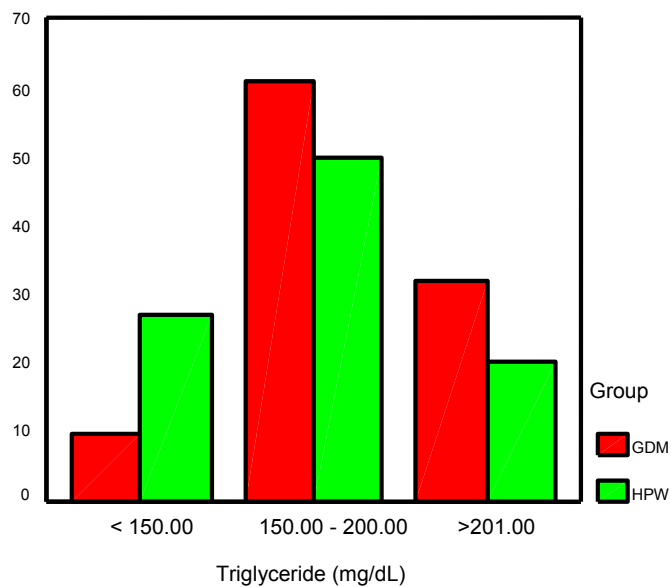


Figure-4.16: GDM and HPW having different triglycerides levels.

Women with gestational diabetes have a significant high risk of long-term morbidity and mortality due to cardiovascular disease, with heart disease being the leading cause of death. Furthermore, type 2 diabetes ignores the protective effect of being female; thus, the risk of cardiovascular disease is similar in women with type 2 diabetes to that in men [273]. Elevated cholesterol and TG level also result in metabolic syndrome, dyslipidemia, central body adiposity, hypertension, and elevated fasting glucose levels. Several studies have demonstrated evidence of the metabolic syndrome in women with a history of GDM [274]. Similar reports of elevated TG levels in gestational diabetes have been reported by Kjos et al [275]. In contrast to our findings, Sobki et al [276] reported lower levels of triglycerides in patients with gestational diabetes when compared to controls.

4.3.3 High Density Lipoprotein-Cholesterol Levels in GDM women

High density lipoprotein cholesterol (HDL-C) level above 60 mg/dL protect against heart diseases. A level below 40 mg/dL increases the risk of cardiovascular diseases. The mean desirable, acceptable and undesirable levels of HDL-C of GDM and HPW are presented in Table-4.21 and Figure-4.17. The result in Table-4.21 shows that the number of GDM and HPW in different categories of HDL-C is not significantly different with each other. In GDM group, 35.0% pregnant women were in the desirable range, 49.5% were in the acceptable range and 15.5% were in the undesirable range of HDL-C. In the control group (HPW), 49.5% pregnant women were having desirable HDL-C level, 39.2% were having acceptable range of HDL-C and 11.3% pregnant women were having the undesirable range of HDL-C. The data indicated that HDL-C of

GDM and HPW were not significantly different from each other, meaning that gestational diabetes was not affecting HDL-C in pregnant women.

Table-4.21: GDM and HPW having Different HDL-Cholesterol Levels

HDL-Cholesterol (mg/dL)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
>60 (Desirable)	36	35.0	48	49.5	0.13
40-60 (Acceptable)	51	49.5	38	39.2	
< 40 (Undesirable)	16	15.5	11	11.3	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

It has been reported that every 1 mg/dL drop in HDL cholesterol increases the risk of heart diseases by 3%. Women with previous history of gestational diabetes usually have greater central adiposity, higher LDL cholesterol levels, higher fasting insulin levels, and a significantly increased risk of hypertension.

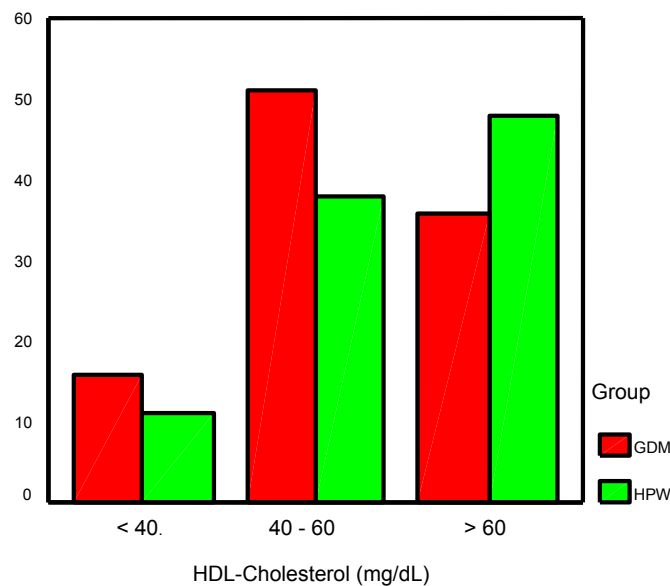


Figure-4.17: GDM and HPW having different HDL-cholesterol levels.

4.3.4 Low Density Lipoprotein-Cholesterol Levels in GDM Women

Low density lipoprotein cholesterol (LDL-C) concentration below 100 mg/dL is considered to be desirable. Increased LDL-C concentration has direct association with incidence of cardiovascular diseases [276]. The mean desirable, acceptable and undesirable LDL-C of GDM and HPW are given in Table-4.22 and Figure-4.18.

Table-4.22: GDM and HPW having Different LDL-Cholesterol Levels

LDL-Cholesterol (mg/dL)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
< 100 (Desirable)	71	68.9	73	75.2	0.58
100-130 (Acceptable)	20	19.4	16	16.5	
>130 (Undesirable)	12	11.7	08	8.3	
Total	103	100	97	100	

2. *n* stands for number of GDM and number of HPW.

3. *P* value determined by Chi square test is given in column 6.

The data in Table-4.22 indicates that the number of GDM and healthy pregnant women, who were having different levels of LDL-C, were not significantly different from each other at $P < 0.05$. There were 68.9% GDM women and 75.2% healthy pregnant women, who were having desirable LDL-C. Among the GDM and HPW groups, 19.4% and 16.5% women were having acceptable range of LDL-C respectively. In the undesirable range of LDL-C, there were 11.7% GDM women and 8.3% HPW. The data demonstrates that there were more GDM women in the acceptable and undesirable range of LDL-C showing that GDM women were at higher risk of heart problems, though this increase was statistically not significant. Kadare has reported that serum HDL-C and serum LDL-C level of GDM women were not significantly different from healthy pregnant women [277].

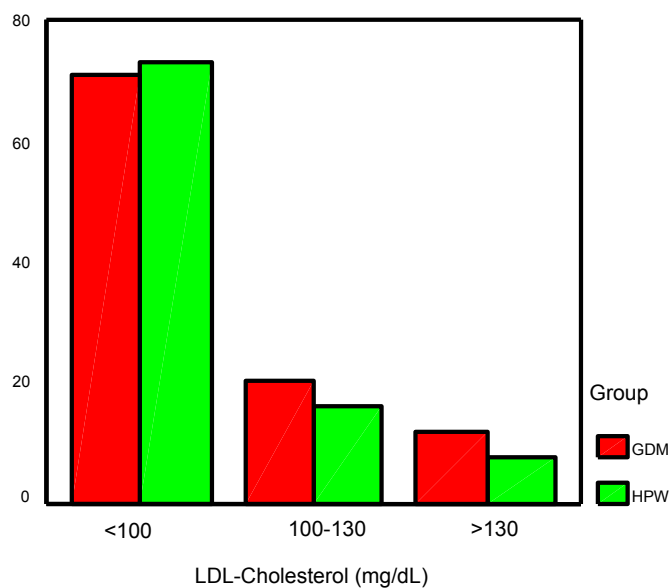


Figure-4.18: GDM and HPW having different LDL-cholesterol levels.

The mean TC, TG, HDL-C and LDL-C in different age groups of GDM and HPW are given in Table-4.23. The data indicates that the mean TC of GDM women is not significantly different between the age group of 25-30 years and 31-35 years, but the mean TC (235 ± 27.3 mg/dL) of GDM in the age group of more than 35 years is significantly higher than the two age groups of GDM i.e. 25-30 years and 31-35 years at $P < 0.001$. In the HPW (control group) the TC level is 183 ± 15.7 mg/dL in the age group of less than 25 years, 197 ± 23.2 mg/dL in the age group of 25-30 years, 196 ± 16.3 mg/dL in age group of 31-35 years and 207 ± 16.2 mg/dL in the age group of more than 35 years. In the HPW, TC in the age group of more than 35 years was significantly ($P < 0.001$) higher than the mean TC level in the age group of less than 25 years and age group of 25-30 years. Though the mean TC level in the HPW in the age group of 35 years was higher than the mean TC level of the age group of 31-35 years, but the increase was not significant. The data indicates that old age did affect TC level in both the groups.

The mean TG levels of both GDM and HPW in different age groups increased with increase in age. However, the increase in TG of GDM women in the age groups of more than 35 years was significantly high at $P < 0.001$ than HPW, the control group. The mean TG levels of both GDM and HPW, in the age groups of less than 25 years, were 0.00 and 140 ± 7.29 mg/dL, 164 ± 16.6 mg/dL and 165 ± 12 mg/dL in the age groups of 25-30 years, 186 ± 12.0 mg/dL and 190 ± 12 mg/dL in the age group of 30-35 years and 218 ± 9.8 mg/dL and 206 ± 8.2 mg/dL in the age group of more than 35 years respectively.

Table-4.23: Lipid Profile of GDM and HPW in Relation to Age Groups

Lipid Profile	Groups	Mean ± SD ¹				P-value ²
		Age (Years)				
		<25	25-30	31-35	>35	
TC (mg/dL)	GDM	00	206±19.9	200±16.9	235±27.3	<0.001
	HPW	183±15.7	197±23.2	196±16.3	207±16.2	
TG (mg/dL)	GDM	00	164±16.6	186±12.0	218±9.8	0.001
	HPW	140±7.2	165±12	190±12.1	206±8.2	
HDL-C (mg/dL)	GDM	00	51±8.9	52±4.2	52±8.8	0.82
	HPW	54± 13.9	56±8.4	53±4.5	53±4.5	
LDL-C (mg/dL)	GDM	00	91±13.9	95±22.5	93±19.1	0.80
	HPW	85±8.54	96±20.9	81±7.7	90±17.1	

1. Data are the means and standard deviations of 103 GDM and 97 HPW.

2. P values determined by Chi square test are given in column 7.

HDL-C and LDL-C of GDM and HPW were not significantly different from each other among the various age groups. Maternal age is an established risk factor for gestational diabetes and cardiovascular diseases, but there is no consensus on the age above which the risk of gestational diabetes is significantly increased. It has been reported that in youngest to oldest age group women the prevalence of gestational

diabetes increases from 1.3% to 31.9%, respectively. Another study indicated that one-third to one half of women with a history of GDM will develop type 2 diabetes within 3–5 years, and 70% will develop type 2 diabetes if followed for 10 years. Women with type 2 diabetes have a significant risk of long-term morbidity and mortality due to altered TC, TG, HDL-C and LDL-C values [279].

4.4 Liver Function Tests

Liver function tests (LFTs) are really non specific tests of liver cell damage. They only show the extent to which hepatocytes are damaged. The present study demonstrated that alanine amino transferase (ALT), alkaline phosphatase (ALP) and serum bilirubin values were not significantly different from GDM and HPW (control) groups. The mean ALT, ALP and serum bilirubin levels are given in Table-4.24.

Table-4.24: Liver Function Tests of GDM and HPW

Liver Function Tests	Groups	Mean $\pm$ SD¹	P- value²
ALT (U/L)	GDM	30.21 $\pm$ 12.47	0.71
	HPW	29.64 $\pm$ 7.96	
ALP (U/L)	GDM	190.55 $\pm$ 22.20	0.84
	HPW	189.95 $\pm$ 21.28	
Serum Bilirubin (mg/dL)	GDM	0.67 $\pm$ 0.41	0.64
	HPW	0.58 $\pm$ 0.17	

2. Data are the means and standard deviations of 103 GDM and 97 HPW.

3. P values determined by Chi square test are given in column 4.

The mean ALT, ALP and serum bilirubin level in GDM group were 30.21 $\pm$ 12.47 U/L, 190.55 $\pm$ 22.20 U/L and 0.67 $\pm$ 0.41 mg/dL respectively. In the healthy

pregnant women the mean ALT, ALP and serum bilirubin values were 29.64 ± 7.96 U/L, 189.95 ± 21.28 U/L and 0.58 ± 0.17 mg/dL respectively. The data in Table-4.24 indicates that diabetes in pregnancy does not affect liver functions. Pregnancy itself may decrease all markers of liver functions due to expansion of extra cellular fluid. Hence transaminases, serum bilirubin and serum albumin are low as compared with the non pregnant state. The only exception is ALP, which is increased due to ALP of placental origin.

4.4.1 Alanine Amino Transferase Levels in GDM Women

The number of GDM and HPW who were having normal and high mean values of ALT are given in Table-4.25 and Figure-4.19. ALT is one of the most sensitive markers for liver cell and has an average value of 5-40 U/L. In the state of pregnancy its level is reduced to 30 U/L.

Table-4.25: GDM and HPW having Normal and High ALT Levels

ALT (U/L)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Normal (≤ 40)	95	92.2	88	90.7	0.80
High (> 40)	08	7.8	09	9.3	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

The present study showed that in GDM group, the number of pregnant women in normal range of ALT were 92.2% while in HPW group it was 90.7%. The raised ALT values were noted in 7.8% gestational diabetic women and in 9.3% healthy pregnant women. No significant difference was present in ALT levels of both GDM and control

group. The mildly abnormal level of transaminases may be highly significant in the evaluation of preeclampsia and almost all liver diseases [280].

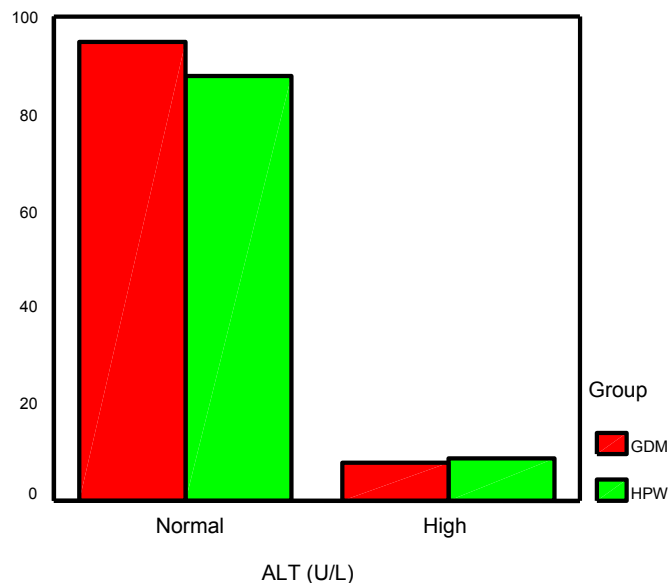


Figure-4.19: GDM and HPW having normal and high ALT levels.

ALT is the enzyme produced within the cells of the liver. ALT level is abnormally increased in conditions where cells of the liver are inflamed or gone cell death as in hepatitis. The highest elevation in ALT occurs during severe hepatitis; toxin induced hepatic necrosis and circulatory shocks. Though the enzyme level may reflect the extent of hepatocellular necrosis, but it may not correlate with eventual outcome. In fact declining of ALT has no direct relation with gestational diabetes but it may indicate either good prognosis or recovery of hepatic failure.

4.4.2 Alkaline Phosphatase Levels in GDM Women

GDM and HPW who were having normal and high mean ALP values are given in Table-4.26 and Figure-4.20. The data shows that 89.3% GDM and 95.9% HPW were in

the normal range of ALP. In GDM group, 10.7% pregnant women and in the control group (HPW) 4.1% pregnant subjects were having high values of ALP.

Table-4.26: GDM and HPW having Normal and High ALP Levels

ALP (U/L)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Normal (≤ 125 -250)	92	89.3	93	95.9	0.08
High (> 250)	11	10.7	04	4.1	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

The increase in ALP values is mainly due to extra hepatic and intra hepatic biliary obstruction. The overall difference in ALP levels was not significant between GDM and control groups. The normal values of ALP are 45-115U/L which rises steadily throughout pregnancy reaching to the peak value of 125-250U/L in the last trimester [281].

ALP values also vary with age and are relatively higher in child hood and puberty, lower in middle age and higher in old age. The ALP level is directly associated with weight of the person and inversely with the height of the person. Highest levels of alkaline phosphatase occur in cholestatic disorders. Alkaline phosphatase levels are likely to be very high in alcoholic hepatitis and biliary cirrhosis [282].

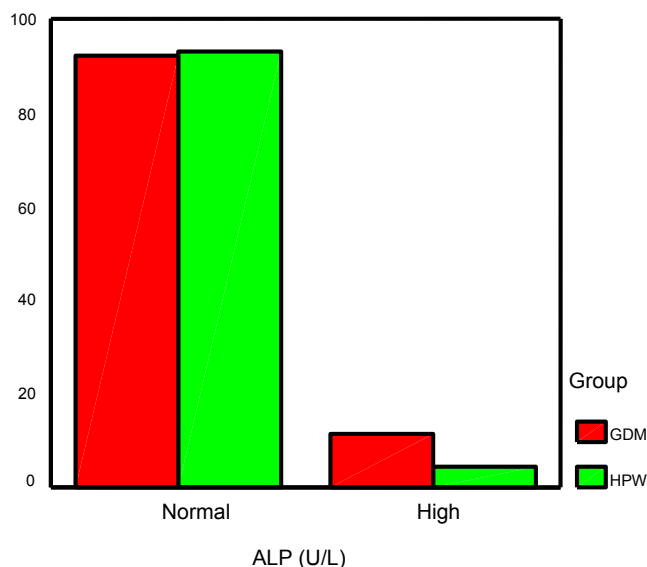


Figure-4.20: GDM and HPW having normal and high ALP levels.

4.4.3 Serum Bilirubin Levels in GDM Women

The GDM and HPW who were having normal and high levels of serum bilirubin are given in Table-4.27 and Figure-4.21. The data indicated that the number of GDM and HPW who were having normal serum bilirubin values were not significantly different.

Table-4.27: GDM and HPW having Normal and High Serum Bilirubin Levels

Serum Bilirubin (mg/dL)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Normal (≤ 1)	87	84.5	89	91.8	0.11
High (> 1)	16	15.5	08	8.2	
Total	103	100	97	100	

2. n stands for number of GDM and number of HPW.

3. P value determined by Chi square test is given in column 6.

In GDM group 84.5% women were having normal values of serum bilirubin while 15.5% pregnant women were having abnormally high values of bilirubin. In HPW

group 91.8% individuals were having normal range of serum bilirubin while 8.2% pregnant women were having high serum bilirubin concentration which may be due to hyperlipidemia, one of the major complications of gestational diabetes. The data showed that GDM is not affecting the serum bilirubin level.

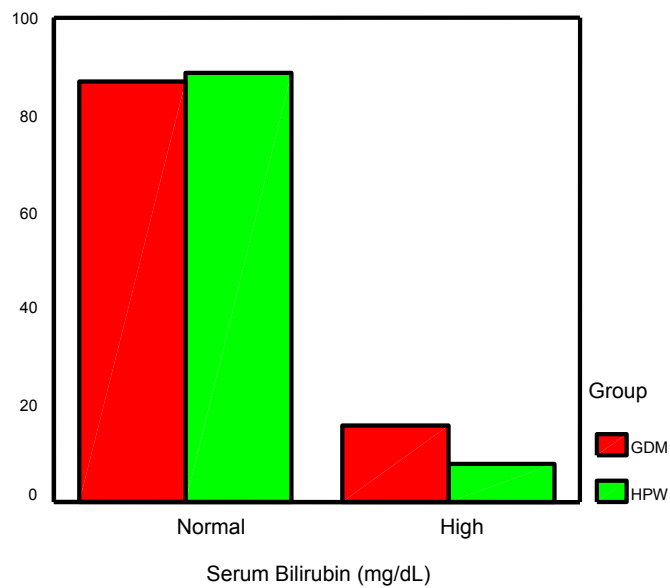


Figure-4.21: GDM and HPW having normal and high serum bilirubin levels.

Hyperbilirubinemia is the raised amount of bilirubin in blood and results from over production and impaired uptake of conjugated and unconjugated bilirubin from hepatocytes to bile duct. The normal value of serum bilirubin in pregnancy is 0.1-1mg/dL. In few pregnant women serum bilirubin rises up to 6mg/dL due to intra hepatic cholestasis, however, gestational diabetes is having no direct correlation with bilirubin [283].

4.5 Renal Function Tests

Many conditions can affect the ability of the kidneys to carry out their important functions. Some conditions can lead to rapid and some to gradual decline in the kidney functions. A number of clinical laboratory tests that measure the levels of substances normally regulated by the kidneys can help to determine the cause and extent of kidney functions. These tests are called renal function tests (RFTs).

Table-4.28: Renal Function Tests of GDM and HPW

Renal Function Tests	Groups	Mean $\pm$ SD ¹	P- value ²
Serum Urea (mg/dL)	GDM	23.70 $\pm$ 8.54	0.104
	HPW	21.97 $\pm$ 6.16	
Serum Creatinine (mg/dL)	GDM	0.82 $\pm$ 0.32	0.02
	HPW	0.74 $\pm$ 0.15	

1. Data are the means and standard deviations of 103 GDM and 97 HPW.

2. P values determined by Chi square test are given in column 4.

Renal function tests (RFTs) during pregnancy can be adequately evaluated by the serum urea and serum creatinine. The mean serum urea and serum creatinine values of GDM and HPW are given in Table-4.28. The mean serum urea of GDM women was 23.70 $\pm$ 8.54 mg/dL and of the HPW was 21.97 $\pm$ 6.16 mg/dL. Statistically these values were not different from each other. The mean serum creatinine in GDM women was 0.82 $\pm$ 0.32 mg/dL, which was significantly higher than 0.74 $\pm$ 0.15 mg/dL in healthy pregnant women.

4.5.1 Serum Urea Levels in GDM Women

GDM and HPW who were having normal and high levels of serum urea are given in Table-4.29 and Figure-4.22. In GDM group, 89.3% women were having normal serum urea while 10.7% women were having high serum urea. Similarly, in HPW group, 91.8% pregnant women were having normal serum urea while 8.2% women were having high level of serum urea.

Table-4.29: GDM and HPW Having Normal and High Serum Urea Levels

Serum Urea (mg/dL)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Normal (20-40)	92	89.3	89	91.8	0.55
High (> 40)	11	10.7	08	8.2	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

The normal value of serum urea is 20-40 mg/dL, however, in pregnancy its level is reduced to 10-25 mg/dL. In the present study, serum urea was not significantly different between GDM and healthy pregnant women. The higher urea level in some GDM individuals and normal pregnant women may be due to dehydration. Serum urea is poor guide to renal functions as it varies with liver metabolic capacity, protein intake and renal perfusion.

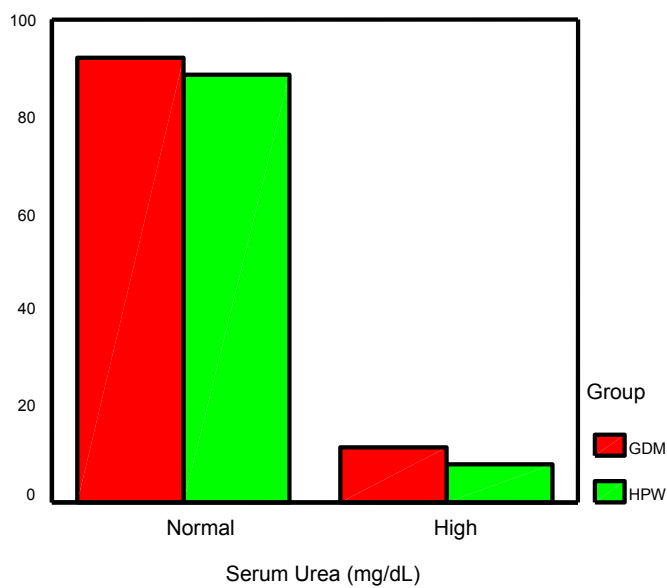


Figure-4.22: GDM and HPW having normal and high serum urea levels.

4.5.2 Serum Creatinine Levels in GDM Women

The number of GDM and HPW with normal and high levels of serum creatinine is given in Table-4.30 and Figure-4.23. In GDM group, 88.3% women were in the normal range of serum creatinine and 11.7% women were in the high range of serum creatinine. In HPW group there were 92.8% pregnant women were having normal serum creatinine and 7.2% women were having high level of serum creatinine. Thus serum creatinine of GDM women is not significantly different from control group. Serum creatinine is more reliable guide to predict renal functions as it is produced at constant rate and completely filtered at the glomerulus.

The serum creatinine test measures blood levels of creatinine, a by-product of muscle energy metabolism that is similar to urea. Creatinine is filtered from the blood by the kidneys and excreted into urine. Production of creatinine depends upon muscle's mass of an individual.

Table-4.30: GDM and HPW having Normal and High Serum Creatinine Levels

Serum Creatinine (mg/dL)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Normal (≤ 0.8)	91	88.3	90	92.8	0.28
High (> 0.8)	12	11.7	07	7.2	
Total	103	100	97	100	

2. *n* stands for number of GDM and number of HPW.

3. *P* value determined by Chi square test is given in column 6.

With normal kidney functions, creatinine level in the blood remains relatively constant and normal. For this reason, and because creatinine is affected, to very little extent by the liver functions, an elevated blood creatinine level is more sensitive test of impaired kidney function than the urea.

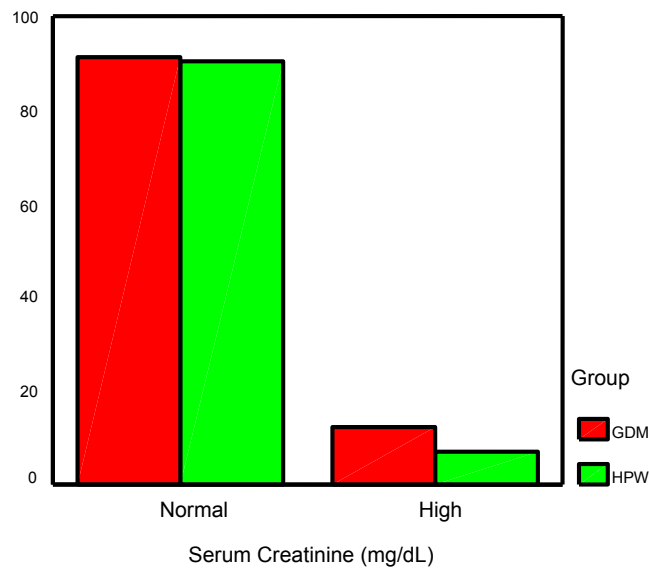


Figure-4.23: GDM and HPW having normal and high serum creatinine levels.

4.6 Insulin and Placental Hormones

Human pregnancy is characterized by a series of metabolic changes that promote adipose tissue accumulation in early pregnancy, followed by facilitated lypolysis and insulin resistance in late pregnancy. In first four months of pregnancy, insulin secretion increases, while insulin sensitivity may increase, decrease or remain unchanged [284].

The mean insulin concentration of GDM and HPW is given in Table-4.31. The mean concentration of insulin in GDM women was 33.68 ± 3.69 μ IU/mL and the mean concentration of insulin in HPW was 29.80 ± 2.80 μ IU/ml. The data indicated that serum insulin level in GDM group was significantly lower than the serum insulin level in HPW at $P < 0.05$. In gestational diabetes, insulin is either not functioning properly or its secretion is not sufficient to cope with the increased requirement of insulin in gestational diabetes as compared to healthy pregnant women.

Table-4.31: Insulin Values of GDM and HPW

Hormone	Groups	Mean $\pm$ SD ¹	P- value ²
Insulin (μ IU/mL)	GDM	33.68 ± 3.69	0.001
	HPW	29.80 ± 2.80	

1. Data are the means and standard deviations of 103 GDM and 97 HPW.

2. P value determined by Chi square test is given in column 4.

Many previous studies have shown that pregnancy results in state of insulin resistance and ladies with gestational-onset diabetes appear to have greater degree of insulin resistance [285].

4.6.1 Serum Insulin Levels in GDM Women

Gestational diabetic women and HPW, with normal and high levels of serum insulin are given in Table-4.32 and Figure-4.24.

Table-4.32: GDM and HPW having Normal and High Insulin Levels

Serum Insulin	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Normal (≤ 30)	62	60.2	77	79.4	0.003
High (>30) (Hyperinsulinemia)	41	39.8	20	20.6	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

In the GDM group, 39.8% women were having high insulin values while 60.2% women were having normal values of insulin. Similarly in HPW group, 20.6% women were having high insulin levels while 79.4% women were having normal insulin concentration. The number of GDM women with normal insulin levels was significantly higher than the number of HPW having normal insulin levels. On the other hand, the number of GDM women having high serum insulin levels were significantly lower at $P < 0.05$ than the number of HPW, who were having high levels of serum insulin. The insulin resistance in both GDM and control groups appears due to the combination of increased maternal adiposity and placental secretion of hormones. To overcome this insulin resistance, most pregnant women increase their insulin secretion. However, when the capacity of insulin secretion is not sufficiently large to meet the resistance, glucose intolerance occurs and the pregnant women develop gestational diabetes.

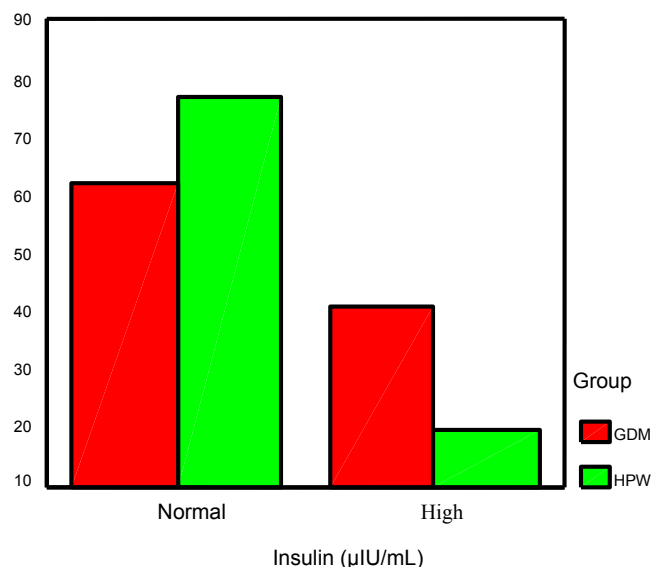


Figure-4.24: GDM and HPW having normal and high insulin levels.

During pregnancy, placenta secretes various hormones, amongst which prolactin and cortisol play a major role in normal pregnancy and fetus development. These two hormones were studied in GDM and HPW to investigate if there was any change in their concentration.

The mean prolactin and mean cortisol values of GDM and HPW are given in Table-4.33. The mean serum prolactin of GDM women was 135.47 ± 9.83 ng/mL and of the HPW was 131.80 ± 8.54 ng/mL. The normal serum prolactin concentration in non pregnant women is 8 ng/mL, but during pregnancy, it rises many folds [286]. The mean concentration of serum prolactin in GDM women was significantly increased which results in insulin resistance. The mean serum cortisol of GDM women was 734.9 ± 51.8 ng/mL and of the HPW was 719.5 ± 54.7 ng/mL. Though the cortisol level of GDM women was significantly higher ($P < 0.05$) than the cortisol level of healthy pregnant women, however it was in the normal range.

Table-4.33: Concentration of Placental Hormones in GDM and HPW

Placental Hormones	Groups	Mean $\pm$ SD ¹	P- value ²
Prolactin (ng/mL)	GDM	135.47 $\pm$ 9.83	0.005
	HPW	131.80 $\pm$ 8.54	
Cortisol (ng/mL)	GDM	734.9 $\pm$ 51.1	0.04
	HPW	719.2 $\pm$ 54.7	

1. Data are the means and standard deviations of 103 GDM and 97 HPW.

2. P values determined by Chi square test are given in column 4.

The importance of GDM as a clinical entity has been demonstrated by Australian randomized trial in which the treatment and identification of GDM significantly reduced the rate of macrosomia and adverse perinatal outcomes. Our data demonstrated that gestational diabetes was associated with hyperinsulinemia and hence results in abnormal intrauterine environment which has many short term consequences for the neonates. The elevated secretion of insulin may not be able to resist the insulin resistance produced by placental hormones. Crowther et al. [287] reported that 9% of GDM women and 58% of women having type 2 diabetes required 200 units of insulin and hence they used as insulin pump. This is a warning for those physicians who are not used to such populations and who may be inclined to dismiss the importance of GDM.

4.6.2 Serum Prolactin Levels in GDM Women

GDM and HPW with normal and high levels of serum prolactin are given in Table-4.34 and Figure-4.25.

Table-4.34: GDM and HPW having Normal and High Prolactin Levels

Serum Prolactin (ng/mL)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Normal (120-150)	78	75.7	89	91.7	0.002
High (>150)	25	24.4	08	8.3	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

There were 75.7% GDM and 91.7% HPW in the normal range of serum prolactin. In the normal range of prolactin no significant difference was observed between GDM and HPW. There were 24.4% GDM and 8.3% HPW in the high range of serum prolactin. The high values of serum prolactin in GDM women may add to insulin resistance [288].

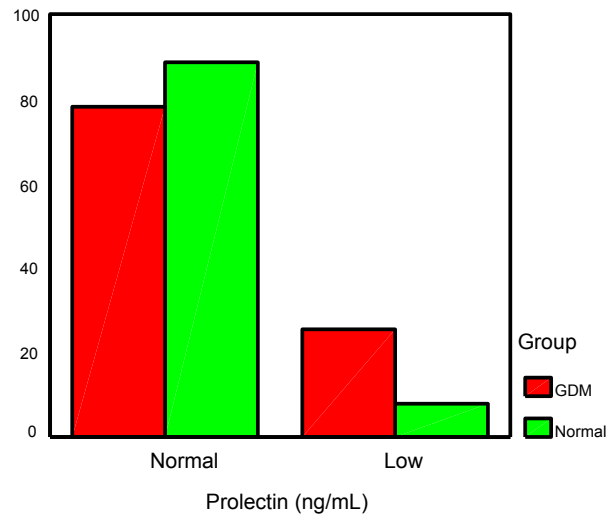


Figure-4.25: GDM and HPW having normal and high prolactin levels.

4.6.3 Serum Cortisol Levels in GDM Women

Serum cortisol plays vital in providing appropriate intrauterine environment to the fetus. However, its abnormally increased secretion causes insulin resistance which may result in gestational diabetes.

Table-4.35: GDM and HPW having Normal and High Serum Cortisol Levels

Serum Cortisol (ng/mL)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
≤ 750 (Normal)	35	34.0	47	48.5	0.03
> 750 (High)	68	66.0	50	51.5	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

Individuals having normal and high levels of serum cortisol are given in Table-4.35 and Figure-4.26. Serum cortisol levels were significantly different at $P < 0.05$ between the GDM and healthy pregnant women. In GDM group, 34.0% women were having normal cortisol values while 66% were having elevated cortisol values. In control group, 48.5% healthy pregnant women were having normal serum cortisol values while 51.5% were having high cortisol values. Statistically, cortisol concentration of GDM and HPW were significantly different. Overall gestational diabetes was slightly affecting serum cortisol levels as earlier reported by Grogorakis et al [289].

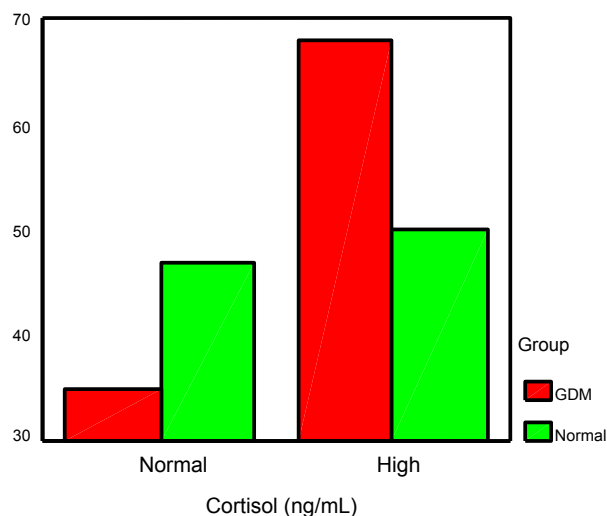


Figure-4.26: GDM and HPW having normal and high serum cortisol levels.

4.7 Thyroid Hormones

Thyroid hormones are triiodothyronine (T3) and thyroxine (T4). These hormones are secreted from thyroid gland and its secretion is under the influence of thyroid stimulating hormone (TSH) which is secreted from the anterior pituitary gland in 1-2 hourly cycles. TSH increases both release and synthesis of T3 and T4. Thyroid disease occurs in more than 1% of general population and is the most common persisting endocrine disorder in pregnant women. T3 and T4 are mostly bound to thyroid-binding-globulin (TBG). In pregnancy the increased TBG level increases T3 and T4 secretion which in turn suppress TSH level [290].

The mean concentration of T3 and T4 are given in Table-4.36. The mean concentration of T3 in GDM and HPW were 2.51 ± 0.62 nmol/L and 2.62 ± 0.57 nmol/L respectively. The mean concentration of T4 in GDM and HPW were 103.86 ± 14.74 nmol/L and 105.38 ± 13.93 nmol/L Both T3 and T4 in GDM and HPW were not

significantly different at $P < 0.05$, indicating that concentration of T3 and T4 was not changed in GDM.

Table-4.36: Concentration of Thyroid Hormones in GDM and HPW

Thyroid Hormones (nmol/L)	Groups	Mean $\pm$ SD ¹	P- value ²
T3	GDM	2.51 $\pm$ 0.62	0.15
	HPW	2.62 $\pm$ 0.57	
T4	GDM	103.86 $\pm$ 14.74	0.45
	HPW	105.38 $\pm$ 13.93	

1. Data are the means and standard deviations of 103 GDM and 97 HPW.

2. P values determined by Chi square test are given in column 4.

4.7.1 Triiodothyronine Levels in GDM Women

GDM and HPW who were having different levels of T3 are given in Table-4.37 and Figure-4.27.

Table-4.37: GDM and HPW having Different Triiodothyronine Levels

T3 (nmol/L)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Low (< 1.2)	04	3.9	00	00	0.14
Normal(1.2-2.8)	96	93.2	93	95.9	
High (> 2.8)	03	2.91	04	4.1	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

In GDM group, 3.9% women were in the lower range of T3, 93.2% were in the normal and 2.9% were in the higher range of T3. Similarly, in HPW group, no women were having low level of T3 while 95.9% were having normal and 4.1% were having

high level of T3. All the low normal and high concentration of T3 in GDM group was not statistically different from the concentration of T3 in HPW. T3 concentration was not affected by gestational diabetes.

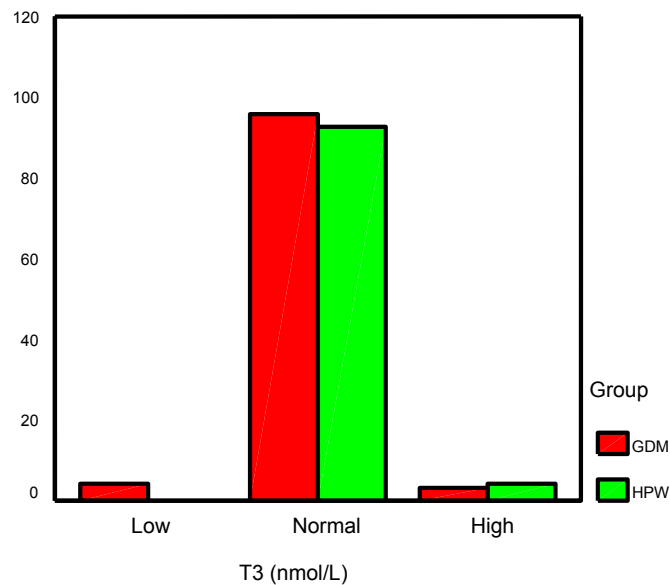


Figure-4.27: GDM and HPW having different triiodothyronine levels.

During pregnancy treatment of thyroid disease in-time is of utmost importance to prevent adverse maternal and fetal outcomes. Thyroid disorders are very often subclinical in nature and can not be easily recognized without specific screening tests. Mild deficiency of maternal thyroid hormone may lead to neurodevelopment complications in the fetus. The main diagnostic indicator of thyroid disease is the measurement of serum thyroid-stimulating hormone and free thyroxine levels. Availability of gestation-age-specific thyroid-stimulating hormone thresholds is an important aid in the accurate diagnosis and treatment of thyroid dysfunction. Pregnancy- specific free thyroxine thresholds not presently available are also required. Large-scale intervention trials are urgently needed to assess the efficacy of preconception or early pregnancy screening for

thyroid disorders. Accurate interpretation of both antepartum and postpartum levels of thyroid hormones is important in preventing pregnancy-related complication secondary to thyroid dysfunction. [291-292]

4.7.2 Thyroxine Levels in GDM Women

GDM and HPW having different levels of thyroxin (T4) are given in Table-4.38 and Figure-4.28. In the low level of T4, 3.9 % GDM women were reported. No healthy pregnant women were having low level of T4

Table-4.38: GDM and HPW having Different Thyroxin Levels

T4 (nmol/L)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Low (< 60)	04	3.9	00	00	0.146
Normal (60-120)	95	92.2	93	95.9	
High (> 120)	04	3.9	04	4.1	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

In the normal range of T4, there were 92.2% GDM women and 95.9% HPW. Only 3.9% GDM women and 4.1% HPW were in the high level of T4. There was no significant difference between GDM and HPW in all levels of T4. Also T4 and T3 concentration was similar in both GDM and HPW.

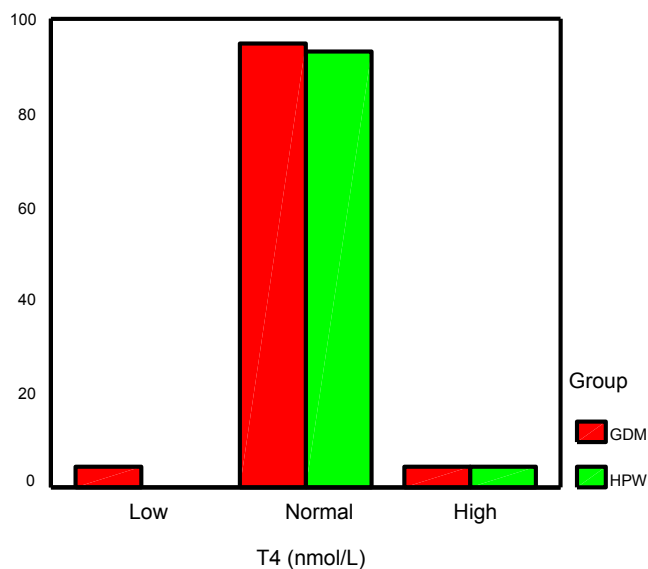


Figure-4.28: GDM and HPW having different thyroxin levels.

Thyroid stimulating Hormone (TSH) is secreted from anterior pituitary gland and regulates the secretion of T3 and T4 hormones. The mean concentration of TSH of GDM and HPW is given in Table-4.39. The mean concentration of TSH in GDM group was 1.72 ± 0.95 mIU/L and the mean concentration of TSH in HPW group was 1.87 ± 0.83 mIU/L. There was no significant difference in the TSH concentration of GDM and HPW.

Table-4.39: Concentration of TSH in GDM and HPW

Hormone	Groups	Mean $\pm$ SD ¹	P- value ²
TSH (mIU/L)	GDM	1.72 ± 0.95	0.084
	HPW	1.87 ± 0.83	

1. Data are the means and standard deviations of 103 GDM and 97 HPW.

2. P values determined by Chi square test are given in column 4.

4.7.3 Thyroid Stimulating Hormone Levels in GDM Women

GDM and HPW with different levels of TSH are given in Table-4.40 and Figure-4.29.

Table-4.40: GDM and HPW having Different TSH Levels

TSH (mIU/L)	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Low (< 0.17)	04	3.9	04	4.1	0.559
Normal (0.17-4.05)	98	95.14	90	92.8	
High (>4.05)	01	0.97	03	3.1	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

There were 04 (3.9 %) GDM women and 04 (4.1%) HPW in the low levels of TSH. In the normal range of TSH, there were 95.1% GDM and 92.8% HPW. Only 0.97% GDM and 3.1% HPW were in the high range of TSH. There was no statistical difference in the TSH level of the two groups. Thus TSH concentration was not changed due to gestational diabetes. Our result does not confirm the findings of Prilozi [293], who had reported the increased prevalence of hypothyroxinemia in GDM pregnancies. However, both GDM and healthy pregnant women should undergo routine screening for thyroid abnormalities to prevent its maternal and neonatal complications.

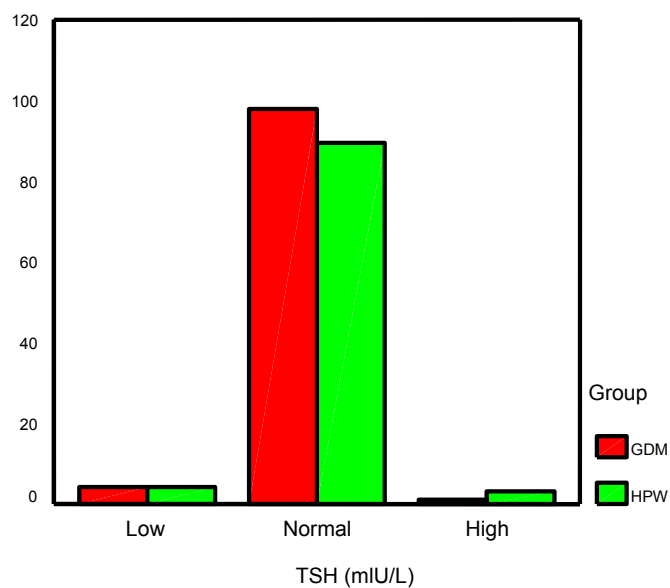


Figure-4.29: GDM and HPW having different TSH levels.

4.8 Mode of Deliveries

GDM and HPW having different mode of deliveries are given in Table-4.41 and Figure-4.30. There were 41.7% GDM women and 62.9% HPW who gave birth by normal vaginal deliveries (NVD) and 23.3% GDM women and 12.4% HPW by caesarian section deliveries (C/Section).

Table-4.41: GDM and HPW having Different Modes of Deliveries

Mode of Delivery	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
NVD	43	41.7	61	62.9	0.009
C/Section	24	23.3	12	12.4	
Instrumental	36	35.0	35	24.7	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

35% GDM and 24.7% HPW were having instrumental deliveries. NVD rate was significantly more in HPW as compared to GDM indicating that gestational diabetes was creating problems in normal deliveries. Deliveries by C/Section and instruments method were significantly higher in GDM than HPW. The data indicates that gestational diabetic women experienced greater delivery complications.

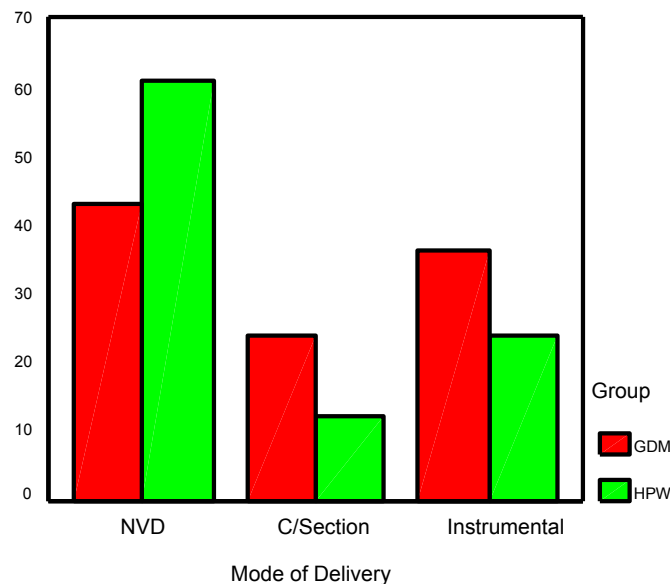


Figure-4.30: GDM and HPW having different mode of deliveries.

Cesarean delivery has been successfully employed as an intervention used to reduce complications associated with gestational diabetes, particularly shoulder dystocia. However, as a major surgery in pregnancy, it poses many risks to both the fetus and the mother. Thus, the elevated rate of cesarean sections among GDM women can be interpreted as both an unfortunate side effect of diagnosis, as well as an appropriate response to the other morbid conditions associated with GDM, particularly elevated fetal growth weight and shoulder dystocia. Our findings were in accordance with the findings of Senanayake and Gunawardene [294] who reported that elective cesarean section rates

were much higher than emergencies in less developed countries of South [295]. In our environment women usually register late for antenatal check-up and hence the acceptability of operative deliveries is very low.

4.9 Still Births and Alive Births

GDM and HPW who gave births to still and alive babies are given in Table-4.42 and Figure-4.31. In GDM group, 9 (8.7%) women gave birth to still babies and 94 (92.3%) women gave birth to alive babies. However, in HPW group, none of the pregnant women gave birth to still babies. All the babies of HPW were alive at the time of birth. The still birth rate was significantly high at $P < 0.05$ in GDM women as compared to healthy pregnant women while alive birth rate was significantly high at $P < 0.05$ in HPW as compared to GDM women. The data demonstrates that GDM increases the incidence of still births.

Table-4.42: GDM and HPW giving Birth to Still and Alive Babies

Type of Birth	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Still	09	8.7	0	0	0.003
Alive	94	92.3	97	100	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

Despite of good glycemic control in pregnancy, there is a persisting increased risk of still birth in pregnancy complicated by diabetes. This increased risk of still birth has led to a generally accepted policy of induction of labor at around 38 weeks of gestation to

avoid risk of still births, which has invariably contributed to the higher C/section rate experienced by women with diabetes. Our findings are in accordance with O’Sullivan et al. [296] who reported that untreated GDM women can experience an increased risk of stillbirth by approximately four-fold.

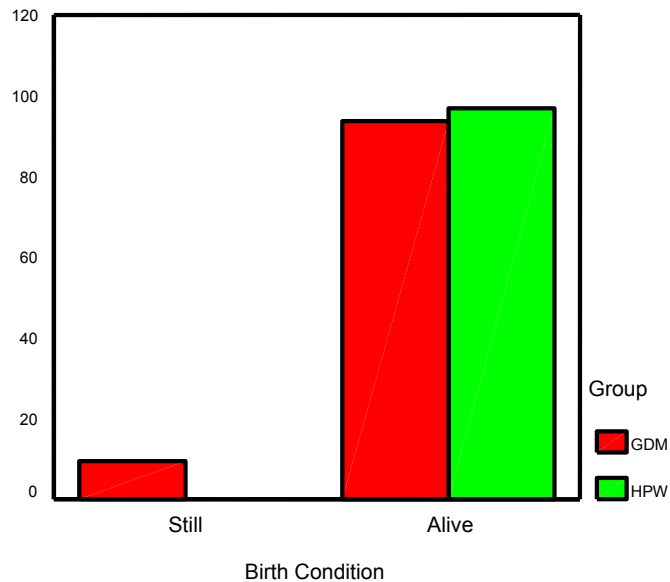


Figure-4.31: GDM and HPW giving birth to still and alive babies.

In more industrialized nations, stillbirth is an uncommon outcome, even among women with glucose intolerance and gestational diabetes. But in our set up the increase rate of still birth in GDM patients is mainly due to irregular antenatal check up and poor glycemic control. The causes of still birth may be multi factorial. Fetal hypoxia, acidosis and hypokalemia may result in fetal cardiac dysrhythmias as well as placental dysfunction. The increased risk of still birth was shown to be correlated with poor glycemic control during late pregnancy [297].

4.10 Macrosomic Babies and Gestational Diabetes

GDM and HPW who gave birth to macrosomic babies are given in Table-4.43 and Figure-4.32. There were 71.8% GDM women and 89.7% HPW who gave birth to babies of normal weight while 28.2% GDM women and 10.3% HPW gave birth to babies of increased weight. In the birth of normal weight babies, there was no significant difference between GDM and HPW. However, birth of increased weight babies were significantly ($P < 0.05$) more in GDM as compared to HPW. The data demonstrates that gestational diabetes increases the chances of big babies, medically termed as macrosomia. This is because high sugar level of GDM supplies the child with more sugar and thus the baby gains more weight.

Table-4.43: GDM and HPW giving Birth to Babies of Different Weights

Birth Weight	GDM		HPW		P-Value ²
	n ¹	%	n ¹	%	
Normal (< 4Kg)	74	71.8	87	89.7	0.001
High (≥ 4Kg)	29	28.2	10	10.3	
Total	103	100	97	100	

1. n stands for number of GDM and number of HPW.

2. P value determined by Chi square test is given in column 6.

Large for gestational age (LGA) is defined as birth weight of more than 90th percentile and macrosomia is absolute birth weight of more than 4 kg. A macrosomic fetus is however, variously defined as birth weight greater than either 4kg or 4.5kg regardless of gestational age. This is recognized clinically by a disproportionate increase in abdominal circumference on ultrasound. Excessive fetal growth is associated with increased infant and maternal morbidity. The increased proportion of macrosomic infants

and morbidity is in accordance with findings already established in a group of women with borderline glucose tolerance [298]. Fetal macrosomia is associated with delivery problems, such as shoulder dystocia and increased risk of cesarean section. An increased proportion of LGA and macrosomia is a major outcome in GDM, even when treated, and is often used as a reason to detect and manage GDM women [299].

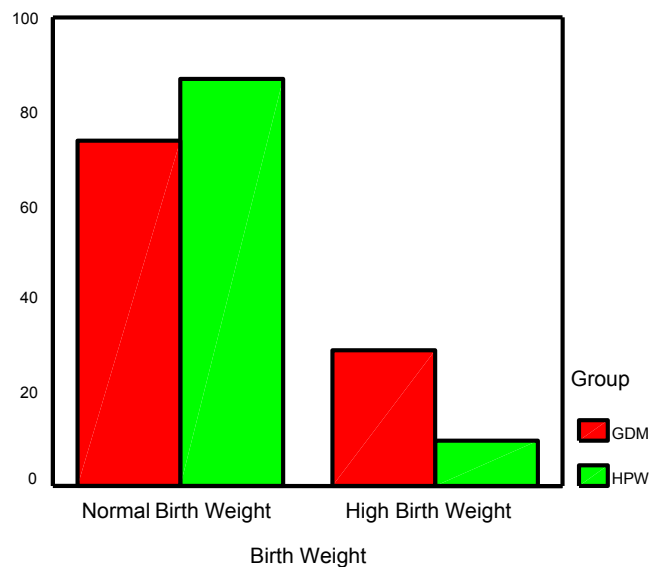


Figure-4.32: GDM and HPW giving birth to babies of different weights.

Fetal macrosomia was considered to be driven by maternal glucose control in the last trimester with the 1-hour postprandial glucose level strongly associated with fetal birth weight. There is now confirmation to suggest that diabetic control in the first and early second trimester is also important in the development of macrosomic infants. Increased maternal blood glucose levels in early pregnancy may result in hyperplasia of fetal adipose cells that contribute to fetal macrosomia [300].

4.11 Coefficient of Correlation

Coefficient of correlation 'r' was determined between different biochemical indices in GDM women by using Pearson Product Moment correlation on SPSS-10 software computer package. Table-4.44 defines correlation between different biochemical tests in GDM women. The magnitude of alteration of one value by changes in the other is determined by the value of 'r'. P value of $r < 0.05$ indicates that correlation shown is significant. Age, BMI, fasting blood sugar, random blood sugar, total cholesterol, triglycerides, and HDL-cholesterol were having significant correlation with insulin concentration in GDM women. Prolactin has mild but significant correlation ($P < 0.05$) with insulin while cortisol, triiodothyronine, thyroxine and thyroid stimulating hormones were non significantly correlated with insulin in GDM women. Hemoglobin and platelet count were inversely but non significantly correlated with insulin in GDM women.

Table-4.44: Correlation between Insulin Concentration and Different Biochemical Indexes in GDM Women

Variables on X-axis	Variables on Y-axis	Correlation 'r'	P -value
Insulin	Age	0.389	0.001
Insulin	BMI	0.512	0.001
Insulin	Systolic BP	0.137	0.169
Insulin	Diastolic BP	0.760	0.448
Insulin	Fasting blood sugar	0.429	0.001
Insulin	Random blood sugar	0.417	0.001
Insulin	Platelet Count	-0.159	0.108
Insulin	Hemoglobin	-0.079	0.427
Insulin	Cholesterol	0.391	0.001
Insulin	Triglycerides	0.544	0.001
Insulin	HDL-Cholesterol	-0.386	0.001
Insulin	LDL-Cholesterol	-0.011	0.911
Insulin	ALT	-0.081	0.417
Insulin	ALP	0.05	0.615
Insulin	Serum Bilirubin	0.006	0.954
Insulin	Serum Urea	0.004	0.972
Insulin	Serum Creatinine	0.002	0.983
Insulin	Serum Prolactin	0.201	0.042
Insulin	Serum Cortisol	0.084	0.398
Insulin	Triiodothyronine	0.109	0.273
Insulin	Thyroxine	0.032	0.747
Insulin	TSH	0.106	0.286

4.12 Conclusion

This study revealed that among sociodemographic characters age, BMI, systolic and diastolic BP was significantly higher in GDM women as compared to HPW. The mean fasting blood sugar (FBS), random blood sugar (RBS) and HbA1c of GDM women and HPW were significantly higher than the HPW ($P>0.001$). Total cholesterol (TC) and triglycerides (TG) of GDM group were significantly higher than the control group ($P=0.001$). Liver and renal function tests of GDM women were not significantly different from HPW while the mean insulin level of GDM women was 33.68 ± 3.69 $\mu\text{IU/mL}$, which was significantly higher ($P=0.001$) than the mean insulin level of HPW. Among placental hormones the mean serum prolactin and serum cortisol of GDM women were significantly higher at $P< 0.05$, when compared to HPW. No significant differences were observed in the concentration of thyroid hormones. GDM women were more exposed to cesarean sections ($P=0.009$) as compared to HPW. The still birth rate and number of macrosomic babies were significantly more at $P=0.003$ and $P=0.001$ in GDM group as compared to HPW.

4.13 Suggestions and Recommendations

Gestational diabetes (GDM) is a condition of pregnant women where glucose intolerance is found. GDM usually develops during pregnancy and ends after pregnancy. Thus GDM is one of the most attention seeking public health problem during pregnancy. Along with its numerous maternal complications it is adversely affecting the health of the infants, thus GDM should be properly controlled to save the lives of newborns and suffering GDM mothers.

Pregnant women with central obesity, advanced maternal age, positive family history of diabetes and previous history of GDM are at the high risk of GDM. American Diabetes Association recommends the control of obesity for the decrease incidence of GDM. However, early detection and diagnosis of GDM will markedly reduce its maternal and neonatal complications. Maternal complications of GDM includes pregnancy induced hypertension, pre-eclampsia, hyperglycemia, hyperinsulinemia, hyperprolactinemia, nephropathy, increased still birth rates, cesarean deliveries, instrumental deliveries and preterm labour. Neonatal complication includes macrosomia, birth trauma, jaundice, hyperbilirubinemia and congenital anomalies.

To prevent all the above mentioned maternal and neonatal complications, pregnant women have to be diagnosed for GDM early in pregnancy. In this way, due to proper monitoring, the pregnant women will have more chances to have an uncomplicated delivery as compared to the undiagnosed GDM women. For the early detection of GDM, an oral glucose tolerance test is performed at 24 to 28 weeks of gestation. In this test, 75g glucose is orally given to the pregnant women and the blood glucose levels are recorded at 1 hour and 2 hour. GDM is diagnosed in pregnancy by fasting glucose level of ≥ 7.0 mmol/L, 2 hour glucose level ≥ 11.1 mmol/L. Once the woman has been diagnosed it is important to maintain the blood glucose in normal range (fasting blood glucose ≤ 105 mg/dL and random blood glucose ≤ 140 mg/dL) to prevent its maternal and neonatal complications. The blood glucose level can be controlled by doing vigorous exercise for 45 minutes thrice a week, and by taking food of low glycemic index, excluding beverages and pepsis colas as they contain high calories of sugar. Herbal medicines like cinnamon, curcumin, green tea and cloves are gaining importance in the

control of diabetes. If pregnant women fails to maintain blood glucose in normal range by exercise, weight reduction and using herbal medicines, then they are advised to take medical treatment, metformin 850 mg twice a day or insulin injection (dose depends upon the blood glucose concentration). Early detection, proper control and better management options for GDM will be helpful in the prevention of cardiovascular diseases and reduces the chances of GDM women to develop type 2 diabetes in future.

LITERATURE CITED

1. Damm, P.; Kuhl, C.; Bertelsen, A.; Molsted-Pedersen, L. Predictive factors for the development of diabetes in women with previous gestational diabetes mellitus. *Am. J. Obstet. Gynecol.* **1992**, *167*. 607–616.
2. Kjos, S.L.; Buchanan, T.A.; Greenspoon, J.S; Montoro, M; Bernstein, G.S; Mestman, J.H. Gestational diabetes mellitus: the prevalence of glucose intolerance and diabetes mellitus in the first two months post partum. *Am. J. Obstet. Gynecol.* **1990**, *163*. 93–98.
3. Carey, V.J.; Walters, E.E.; Colditz, G.A.; Solomon, C.G.; Willett, W.C.; Rosner, B.A.; Speizer, F.E.; Manson, J.E. Body fat distribution and risk of non-insulin-dependent diabetes mellitus in women. *Am. J. Epidemiol.* **1997**, *145*. 614- 619.
4. Engelgau, M.M.; Herman, W.H.; Smith, P.J.; German, R.R.; Aubert, R.E. The epidemiology of diabetes and pregnancy in the US. *Diabetes Care* **1995**, *18*.1029-1033.
5. Harris, M.I.; Flegal. K.M.; Cowie, C.C. Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults. *Diabetes Care.* **1998**, *21*, 518-524.
6. Chu, S.Y.; Callaghan, W.M.; Kim, S.Y.; Schmid, C.H.; Lau, J.; England, L.J.; Dietz, P.M. Maternal obesity and risk of gestational diabetes mellitus. *Diabetes Care.* **2007**, *30*. 2070-2076.

7. Oruc, S.; Saruc, M.; Koyuncu, F.M. Changes in the plasma activities of protein C and protein S during pregnancy. *Aust. N Z. J. Obstet. Gynaecol.* **2000**, *40*, 448-450.
8. Chesnutt, A.N. Physiology of normal pregnancy. *Crit. Care. Clin.* **2004**, *20*, 609-615.
9. Butte, N.F. Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. *Am. J. Clin. Nutr.* **2000**, *71*, 1256-1261.
10. Nomura, R.M.; Paiva, L.V.; Costa, V.N.; Liao, A.W.; Zugaib, M. Influence of maternal nutritional status, weight gain and energy intake on fetal growth in high-risk pregnancies. *Gynecol. Obstet.* **2012**, *34*, 107-112.
11. Thornburg, K.L.; Jacobson, S.L.; Giraud, G.D. Hemodynamic changes in pregnancy. *Semin. Perinatol.* **2000**, *24*, 11-14.
12. Butte, N.F.; Wong, W.W.; Treuth, M.S. Energy requirements during pregnancy based on total energy expenditure and energy deposition. *Am. J. Clin. Nutr.* **2004**, *79*, 1078-1087.
13. Kim, C.; Newton, K.M.; Knopp, R.H. Gestational diabetes and the incidence of type 2 diabetes. *Diabetes Care* **2002**, *25*, 1862-1868.
14. Gaudier, F.L.; Hauth, J.C.; Poist, M.; Corbett, D.L.; Cliver, S.P. Recurrence of gestational diabetes mellitus. *Obstet. Gynecol.* **1992**, *80*, 755– 758.

15. Damm, P.; Kuhl, C.; Bertelsen, A.; Molsted-Pedersen, L. Predictive factors for the development of diabetes in women with previous gestational diabetes mellitus. *Am. J. Obstet. Gynecol.* **1992**, *167*. 607–616.
16. Kjos, S.L.; Buchanan, T.A.; Greenspoon, J.S; Montoro, M; Bernstein, G.S; Mestman, J.H. Gestational diabetes mellitus: the prevalence of glucose intolerance and diabetes mellitus in the first two months post partum. *Am. J. Obstet. Gynecol.* **1990**, *163*. 93–98.
17. Carey, V.J.; Walters, E.E.; Colditz, G.A.; Solomon, C.G.; Willett, W.C.; Rosner, B.A.; Speizer, F.E.; Manson, J.E. Body fat distribution and risk of non-insulin-dependent diabetes mellitus in women. *Am. J. Epidemiol.* **1997**, *145*. 614- 619.
18. Engelgau, M.M.; Herman, W.H.; Smith, P.J.; German, R.R.; Aubert, R.E. The epidemiology of diabetes and pregnancy in the US. *Diabetes Care* **1995**, *18*.1029-1033.
19. Rao, A.K.; Cheng, Y.W.; Caughey, A.B. Perinatal complications among different Asian- American subgroups. *Am. J. Obstet. Gynecol.* **2006**, *194*. 39-41.
20. Langer, O.; Yogev, Y.; Most, O.; Xenakis, E.M. The consequences of not treating gestational diabetes. *Am. J. Obstet. Gynecol.* **2005**, *192*. 989-997.
21. Langer, O.; Rodriguez, D.A.; Xenakis, E.; McFarland, M.B.; Berkus, M.D.; Arrendondo, F. Intensified versus conventional management of gestational diabetes. *Am. J. Obstet. Gynecol.* **1994**, *170*. 1036-1047.

22. Langer, O. A spectrum of glucose thresholds may effectively prevent complications in the pregnant diabetic patient. *Semin. Perinatol.* **2002**, 26. 196 -205.
23. Jovanovic-Peterson, L.; Peterson, C.M.; Reed, G.F.; Metzger, B.E.; Mill, J.L.; Knopp, R.H.; Aarons, J.H. Maternal postprandial glucose levels and infant birth weight in Diabetes. *Am. J. Obstet. Gynecol.* **1991**, 164. 103–111.
24. Veciana, M.; Major, C.A.; Morgan, M.A.; Asrat, T.; Toohey, J.S.; Lien, J.M.; Evans, A.T. Postprandial versus preprandial blood glucose monitoring in women with gestational diabetes mellitus requiring insulin therapy. *N. Engl. J. Med.* **1995**, 333. 1237–1241.
25. Ali Z.; Alexis, S. Occurrence of diabetes mellitus after gestational diabetes mellitus in Trinidad. *Diabetes Care.* **1990**, 13. 527–529.
26. Carpenter, M.W.; Coustan, D.R. Criteria for screening tests for gestational diabetes. *Am. J. Obstet. Gynecol.* **1982**, 144. 768 -773.
27. O’Sullivan, J.B.; Mahan, C.M. Criteria for the oral glucose tolerance test in pregnancy. *Diabetes.* **1964**, 13. 278-285.
28. Metzger, B.E.; Bybee, D.E; Freinkel, N.; Phelps, R.L.; Radvany, R.M.; Vaisrub, N. Correlations between the phenotypic and genotypic characteristics of the mother and abnormal glucose tolerance during the first year postpartum. *Diabetes* **1985**, 34. 111-115.
29. Kjos, S.L.; Buchanan, T.A.; Greenspoon, J.S.; Montoro, M.; Bernstein, G.S.; Mestman, J.H. The prevalence of glucose intolerance and diabetes mellitus in the first two months post partum. *Am. J. Obstet. Gynecol.* **1990**, 163. 93-98.

30. Catalano, P.M.; Vargo, K.M.; Bernstein, I.M.; Amini, S.B. Incidence and risk factors associated with abnormal postpartum glucose tolerance in women with gestational diabetes. *Am. J. Obstet. Gynecol.* **1991**, *165*. 914-919.
31. Dacus, J.V.; Meyer, N.L.; Muram, D.; Stilson, R.; Phipps, P.; Sibai, B.M. Postpartum glucose tolerance testing in gestational diabetes. *Am. J. Obstet. Gynecol.* **1994**, *171*. 927-931.
32. Nohira, T.; Kim, S.; Nakai, H.; Okabe, K.; Nohira, T.; Yoneyama, K. Recurrence of gestational diabetes mellitus: rates and risk factors from initial GDM and one abnormal GTT value. *Diabetes. Res. Clin. Pract.* **2006**, *71*. 75–81.
33. Mohamed N.; Dooley, J. Gestational diabetes mellitus and subsequent development of NIDDM in aboriginal women of northwestern Ontario. *Int. J. Circumpolar Health.* **1998**, *57*. 355–358.
34. Kim, Y.L.; Cho, Y.W.; Park, S.W.; Lee, S.K.; Ahn, I.S.; Na, B.W.; Lee, J.; Cho, Y.K.; Lee, H.Y.; Lee, S.J. Antepartum characteristics predicting persistent postpartum glucose intolerance in the patients with gestational diabetes mellitus (GDM). *J. Korean Diabetes. Assoc.* **2000**, *24*. 46-59.
35. Krishnaveni, G.V.; Hill, J.C.; Veena, S.R.; Geetha, S.; Jayakumar, M.N.; Karat, C.L.; Fall, C.H. Gestational diabetes and the incidence of diabetes in the 5 years following the index pregnancy in South Indian women. *Diabetes. Res. Clin. Pract.* **2007**, *78*. 398-404.
36. Ko, G.T.; Chan, J.C.; Tsang, L.W.; Li, C.Y.; Cockram C.S. Glucose intolerance and other cardiovascular risk factors in Chinese women with a

- history of gestational diabetes mellitus. *Aust. NZ. J. Obstet. Gynaecol.* **1999**, 39. 478-483.
37. Ratner, R.E.; Christophi, C.A.; Metzger, B.E.; Dabelea, D.; Bennett, P.H.; Pi-Sunyer, X.; Fowler, S.; Kahn, S.E. Diabetes Prevention Program Research Group. Prevention of diabetes in women with a history of gestational diabetes: effects of metformin and lifestyle interventions. *J. Clin. Endocrinol. Metab.* **2008**, 93. 4774-4779.
38. Lee, H.; Jang, H.C.; Park, H.K.; Metzger, B.E.; Cho, N.H. Prevalence of type 2 diabetes among women with a previous history of gestational diabetes mellitus. *Diabetes. Res. Clin. Pract.* **2008**, 81. 124-129.
39. Lee, A.J.; Hiscock, R.J.; Wein, P.; Walker, S.P.; Permezel, M. Gestational diabetes mellitus: clinical predictors and long-term risk of developing type 2 diabetes--a retrospective cohort study using survival analysis. *Diabetes Care.* **2007**, 30. 878-783.
40. Oldfield, M.D.; Donley, P.; Walwyn, L.; Scudamore, I.; Gregory, R. Long term prognosis of women with gestational diabetes in a multiethnic population. *Postgrad. Med. J.* **2007**, 83. 426-340.
41. Metzger, B.E.; Lowe, L.P.; Dyer, A.R. Hyperglycemia and adverse pregnancy outcomes. *N. Engl. J. Med.* **2008**, 358.1991–2002.
42. Metzger, B.E.; Gabbe, S.G.; Persson, B. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care.* **2010**, 33. 676-682.

43. Pedersen, J.; Bojsen-Moller, B.; Poulsen, H. Blood sugar in newborn infants of diabetic mothers. *Acta. Endocrinol.* **1954**, *15*. 33–52.
44. Crowther, C.A.; Hiller, J.E.; Moss, J.R.; McPhee, A.J.; Jeffries, W.S.; Robinson, J.S. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. *N. Engl. J. Med.* **2005**, *352*. 2477–2486.
45. Landon, M.B.; Spong, C.Y.; Thom, E. A multicenter, randomized trial of treatment for mild gestational diabetes. *N. Engl. J. Med.* **2009**, *361*. 1339–1348.
46. Horvath, K.; Koch, K.; Jeitler, K. Effects of treatment in women with gestational diabetes mellitus: systematic review and meta-analysis. *Br. Med. J.* **2010**, *340*. 1395.
47. American Diabetes Association. Gestational diabetes mellitus. *Diabetes Care.* **2004**, *27*, 88–90.
48. Spong, C.Y.; Guillermo, L.; Kuboshige, J.; Cabalum, T. Recurrence of gestational diabetes mellitus: identification of risk factors. *Am. J. Perinatol.* **1998**, *15*. 29–33.
49. Janghorbani, M.; Stenhouse, E.A.; Jones, R.B.; Millward, B.A. Is neighbourhood deprivation a risk factor for gestational diabetes mellitus? *Diabet. Med.* **2006**, *23*. 313–317.
50. Tanaka, M.; Jaamaa, G.; Kaiser, M.; Hills, E.; Soim, A.; Zhu, M.; Shcherbatykh, I.Y.; Samelson, R.; Bell, E.; Zdeb, M.; McNutt, L.A. Racial disparity in hypertensive disorders of pregnancy in New York state: A 10-year longitudinal population-based study. *Am. J. Public. Health.* **2007**, *97*. 163–170.

51. Clausen, T.; Oyen, N.; Henriksen, T. Pregnancy complications by overweight and residential area: A prospective study of an urban Norwegian cohort. *Acta. Obstet. Gynecol.* **2006**, *85*. 526-533.
52. Berkowitz, G.S.; Lapinski, R.H.; Wein, R.; Lee, D. Race/ethnicity and other risk factors for gestational diabetes. *Am. J. Epidemiol.* **1992**, *135*. 965-973.
53. Shen, J.J.; Tymkow, C.; MacMullen, N. Disparities in maternal outcomes among four ethnic populations. *Ethn. Dis.* **2005**, *15*. 492-497.
54. Bo, S.; Menato, G.; Bardelli, C.; Lezo, A.; Signorile, A.; Repetti, E.; Massobrio, M.; and Pagano, G. Low socioeconomic status as a risk factor for gestational diabetes. *Diabetes Metab.* **2002**, *28*. 139-140.
55. Popkin, B.M.; Gordon-Larsen, P. The nutrition transition: Worldwide obesity dynamics and their determinants. *Int. J. Obstet.* **2004**, *28*. 2-9.
56. Thow, A.M.; Waters, A.M. Diabetes in Culturally and Linguistically Diverse Australians: Identification of Communities at High Risk. *Aust. Inst. Health Welf.* **2005**, *17*. 222-227.
57. Hanley, A.G.; McKeown-Eyssen, G.; Harris, S.B.; Hegele, R.A.; Wolever, T.; Kwan, J.; Zinman, B. Association of parity with risk of type 2 diabetes and related metabolic disorders. *Diabetes Care.* **2002**, *25*. 690-695.
58. Danilenko-Dixon, D.R.; Van Winter, J.T.; Nelson, R.L.; Ogburn, P.L. Universal versus selective gestational diabetes screening: Application of 1997 *Am. J. Obstet. Gynecol.* **1999**, *181*. 798-802.
59. ACOG Practice Bulletin. Clinical management guidelines for obstetrician-gynecologists. *Obstet. Gynecol.* **2001**, *98*. 525-538.

60. Ferrara, A.; Kahn, H.S.; Quesenberry, C.P.; Riley, C.; Hedderson, M.M. An increase in the incidence of gestational diabetes mellitus. *Obstet. Gynecol.* **2000**, *103*. 526–533.
61. Thorpe, L.E.; Berger, D.; Ellis, J.A.; Bettgowda, V.R.; Brown, G.; Matte, T.; Bassett, M.; Frieden, T.R. Trends and racial/ethnic disparities in gestational diabetes among pregnant women in New York City. *Am. J. Public Health.* **2001**, *95*. 1536–1539.
62. Casey, B.M.; Lucas, M.J.; McIntire, D.D.; Leveno, K.J. Pregnancy outcomes in women with gestational diabetes compared with the general obstetric population. *Obstet. Gynecol.* **1997**, *90*. 869–873.
63. Saydah, S.H.; Chandra, A.; Eberhard, M.S. Pregnancy experience among women with and without gestational diabetes in the US. *Diabetes Care.* **2005**, *28*. 1035–1040.
64. Gillman, M.W.; Rifas-Shiman, S.; Berkey, C.S.; Field, A.E.; Colditz, G.A. Maternal gestational diabetes, birth weight, and adolescent obesity. *Pediatrics.* **2003**, *111*. 221–226.
65. Ehrlich, S.F.; Hedderson, M.M.; Ferrara, A. Change in body mass index between pregnancies and the risk of gestational diabetes in second pregnancy. *Obstet. Gynecol.* **2011**, *117*. 1323–1330.
66. Mazumder, U.; Sarker, S.; Riaz, B.K. Maternal over weight and obesity: It's effect on pregnancy outcome. *Mymensingh. Med. J.* **2011**, *2*. 213–218.

67. Williams, M.A.; Qiu, C.; Mui-Rivera, M. Plasma adiponectin concentrations in early pregnancy and subsequent risk of gestational diabetes mellitus. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 2306–2311.
68. Terence, T.; Ben, C.P. Maternal age and prevalence of gestational diabetes mellitus. *Diabetes Care.* **2006**, *29*, 948-949.
69. Savitz, D.A.; Janevic, T.M.; Engel, S.M.; Kaufman, J.S.; Herring, A. H. Ethnicity and gestational diabetes in New York City. *Br. J. Obstet. Gynecol.* **2008**, *115*, 969- 978.
70. Naylor, C.D.; Serme, M.; Chen, E.; Farine, D. Selective screening for gestational diabetes mellitus. *N. Engl. J. Med.* **1997**, *337*, 1591–1596.
71. Coustan, D.R. Gestational diabetes. *Obstet. Gynecol.* **2001**, *98*, 525–538.
72. Dornhorst, A.; Bailey, P.C.; Elkeles, R.S.; Johnston, D.G.; Beard, R.W. Abnormalities of glucose tolerance following gestational diabetes. *J. Med.* **1990**, *77*, 1219–1228.
73. Martin, A.O.; Simpson, J.L.; Ober, C.; Freinkel, N. Frequency of diabetes mellitus in mothers with gestational diabetes. *Am. J. Obstet. Gynecol.* **1985**, *151*, 471-475.
74. Karter, A.J.; Rowell, S.E.; Ackerson, L.M.; Mitchell, B.D.; Ferrara, A.; Selby, J.V.; Newman, B. Excess maternal transmission of type 2 diabetes. *Diabetes Care.* **1999**, *22*, 938–943.
75. Damm, P.; Kuhl, C.; Bertelsen, A.; Molsted-Pedersen, L. Predictive factors for the development of diabetes in women with previous gestational diabetes mellitus. *Am. J. Obstet. Gynecol.* **1992**, *167*, 607–616.

76. Horikawa, Y.; Iwasaki, N.; Hara, M.; Furuta, H.; Hinokio, Y.; Cockburn, B.N. Mutation in hepatocyte nuclear factor-1 gene (TCF2) associated with MODY. *Nat. Genet.* **1997**, *17*. 384–385.
77. Saker, P.J.; Hattersley, A.T.; Barrow, B.; Hammersley, M.S.; McLellan, J.A.; Lolds, R.J.; Gillmer, M.D.; Holman, R.R.; Turner, R.C. High prevalence of a missense mutation of the glucokinase gene gestational diabetic patients due to a founder-effect in a local population. *Diabetologia.* **1996**, *39*. 1325–1328.
78. Damm, P.; Kuhl, C.; Buschard, K.; Jakobsen, B.K.; Svejgaard, A.; Sodoyez-Goffaux, F.; Shattock, M.; Bottazzo, G.F.; Molsted-Pedersen, L. Prevalence and predictive value of islet cell antibodies and insulin autoantibodies in women with gestational diabetes. *Diabet. Med.* **1994**, *11*. 558–563.
79. Verma, A.; Boney, C.M.; Tucker, R.; Vohr, B.R. Insulin resistance syndrome in women with prior history of gestational diabetes mellitus. *J. Clin. Endocrinol. Metab.* **2002**, *87*. 3227–3235.
80. Ferrannini, E.; Buzzigoli, G.; Bonadonna, R.; Giorico, M.A.; Oleggini, M.; Graziadei, L.; Pedrinelli, R.; Brandi, L.; Bevilacqua, S. Insulin resistance in essential hypertension. *N. Engl. J. Med.* **1987**, *317*. 350–357.
81. Masding, M.G.; Stears, A.J.; Burdge, G.C.; Wootton, S.A.; Sandeman, D.D. Premenopausal advantage in postprandial lipid metabolism are lost in women with type 2 diabetes. *Diabetes Care.* **2003**, *26*. 3243–3249.
82. Hales, C.N.; Barker, D.P. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia.* **1992**, *35*. 595–601.

83. McCance, D.R.; Pettitt, D.J.; Hanson, R.L.; Jacobsson, L.H.; Knowler, W.C.; Bennett, P.H. Birth weight and non-insulin dependent diabetes: thrifty genotype, thrifty phenotype, or surviving small baby genotype. *Br. Med. J.* **1994**, *308*. 942–945.
84. Savona-Ventura, C.; Chircop, M. Birth weight influence on the subsequent development of gestational diabetes mellitus. *Acta. Diabetol.* **2003**, *40*. 101-104.
85. Moses, R.G.; Moses, J.; Knights, S. Birth weight of women with gestational diabetes. *Diabetes Care.* **1999**, *22*. 1059–1062.
86. Bo, S.; Marchisio, B.; Volpiano, M.; Menato, G.; Pagano, G. Maternal low birth weight and gestational hyperglycemia. *Gynecol. Endocrinol.* **2003**, *17*. 133–136.
87. Moses RG, Moses J, Knights S. Birth weight of women with gestational diabetes. *Diabetes Care.* **1999**, *22*. 1059–1062.
88. Knowler, W.C.; Pettitt, D.J.; Saad, M.F. Diabetes mellitus in the Pima Indians: incidence, risk factors and pathogenesis. *Diabetes. Metab. Rev.* **1990**, *6*. 1–27.
89. Ehrmann, D.A. Polycystic ovary syndrome. *N. Engl. J. Med.* **2005**, *352*. 1223–1236.
90. Legro, R.S.; Gnatuk, C.L.; Kusanman, A.R.; Dunaif, A. Changes in glucose tolerance over time in women with polycystic ovary syndrome: a controlled study. *J. Clin. Endocrinol. Metab.* **2005**, *90*. 3236– 3242.

91. Haakova, L.; Cibula, D.; Rezabek, K.; Hill, M.; Fanta, M.; Zivny, J. Pregnancy outcome in women with PCOS and in controls matched by age and weight. *Hum. Reprod.* **2003**, *18*. 1438–1441.
92. Mikola, M.; Hiilesmaa, V.; Halttunen, M.; Suhonen, L.; Tiitinen, A. Obstetric outcome in women with polycystic ovarian syndrome. *Hum. Reprod.* **2001**, *16*. 226– 229
93. Turhan, N.O.; Seckin, N.C.; Aybar, F.; Inegol, I. Assessment of glucose tolerance and pregnancy outcome of polycystic ovary patients. *Int. J. Gynaecol. Obstet.* **2003**, *81*. 163– 168.
94. Selby, J.V. Linking automated databases for research in managed care settings. *Am. Intern. Med.* **1997**, *127*. 719– 724.
95. Lo, J.C.; Feigenbaum, S.L.; Yang, J.; Pressman, A.R.; Selby, J.V.; Go, A.S. Epidemiology and adverse cardiovascular risk profile of diagnosed polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.* **2006**, *91*. 1357– 1363.
96. Knottnerus, J.A.; Delgado, L.; Knipschild, P.G.; Essed, G.M.; Smits, F. Maternal hemoglobin and pregnancy outcome. *Lancet.* **1986**, *2*. 282.
97. Steer, P.; Alam, M.A.; Wadsworth, J.; Welch, A. Relation between maternal haemoglobin concentration and birth weight in different ethnic groups. *Br. Med. J.* **1995**, *310*. 489– 491.
98. Lao, T.T.; Ho, L.F. Impaired glucose tolerance and pregnancy outcome in Chinese women with high body mass index. *Hum. Reprod.* **2000**, *15*. 1826– 1829.

99. Lao, T.T.; Chan, L.Y.; Tam, K.F.; Ho, L.F. Maternal hemoglobin and risks of gestational diabetes mellitus in Chinese women. *Obstet. Gynecol.* **2002**, *99*, 807–812.
100. Schulze, M.B.; Liu, S.; Rimm, E.B.; Manson, J.E.; Willett, W.C.; Hu, F.B. Glycemic index, glycemic load, and dietary fiber intake and incidence of type 2 diabetes in younger and middle-aged women. *Am. J. Clin. Nut.* **2004**, *80*, 348–356.
101. Stevens, J.; Ahn, K.; Juhaeri, K.; Houston, D.; Steffan, L.; Couper, D. Dietary fiber intake and glycemic index and incidence of diabetes in African-American and white adults: the ARIC study. *Diabetes Care.* **2002**, *25*, 1715–1721.
102. Montonen, J.; Knekt, P.; Jarvinen, R.; Aromaa, A.; Reunanen, A. Whole-grain and fiber intake and the incidence of type 2 diabetes. *Am. J. Clin. Nut.* **2003**, *77*, 622–629.
103. Catalano, P.M.; Tyzbir, E.D.; Wolfe, R.R.; Calles, J.; Roman, N.M.; Amini, S.B.; Sims, E.A. Carbohydrate metabolism during pregnancy in control subjects and women with gestational diabetes. *Am. J. Physiol.* **1993**, *264*, 60–67.
104. Catalano, P.M.; Huston, L.; Amini, S.B.; Kalhan, S.C. Longitudinal changes in glucose metabolism during pregnancy in obese women with normal glucose tolerance and gestational diabetes mellitus. *Am. J. Obstet. Gynecol.* **1999**, *180*, 903–916.

105. Homko, C.J.; Sivan, E.; Reece, E.A.; Boden, G. Fuel metabolism during pregnancy. *Semin. Reprod. Endocrinol.* **1999**, *17*, 119–125.
106. Catalano, P.M.; Nizielski, S.E.; Shao, J.; Preston, L.; Qiao, L.; Friedman, J.E. Down regulated IRS-1 and PPAR gamma in obese women with gestational diabetes: relationship to FFA during pregnancy. *Am. J. Physiol. Endocrinol. Metab.* **2002**, *282*, 522–533.
107. Friedman, J.E.; Ishizuka, T.; Shao, J.; Huston, L.; Highman, T.; Catalano, P. Impaired glucose transport and insulin receptor tyrosine phosphorylation in skeletal muscle from obese women with gestational diabetes. *Diabetes Care.* **1999**, *48*, 1807–1814.
108. Metzger, B.E.; Cho, N.H.; Roston, S.M.; Radvany, R. Pre pregnancy weight and antepartum insulin secretion predict glucose tolerance five years after gestational diabetes mellitus. *Diabetes Care.* **1993**, *16*, 1598–1605.
109. Langer O, Yogev Y, Most O, Xenakis EM: Gestational diabetes: the consequences of not treating. *Am. J. Obstet. Gynecol.* **2005**, *192*, 989–997.
110. American Diabetes Association: Position statement on gestational diabetes mellitus. *Diabetes Care.* **2004**, *27*, 88–90.
111. Duggleby, S.C.; Jackson, A.A. Relationship of maternal protein turnover and lean body mass during pregnancy and birth weight. *Clin. Sci.* **2001**, *101*, 65–72.
112. Debenoist, B.; Jackson, A.A.; Hall, J.S.; Persaud, C. Whole body protein turnover in Jamaican women during normal pregnancy. *Hum. Nutr. Clin. Nutr.* **1985**, *39*, 167–179.

113. Fitch, W.L.; King, J.C. Protein turnover and 3-methylhistidine excretion in non-pregnant, pregnant and gestational diabetic women. *Hum. Nutr. Clin. Nutr.* **1987**, *41*, 327–339.
114. Kalkhoff, R.K.; Kandaraki, E.; Morrow, P.G.; Mitchell, T.H.; Kelber, S.; Borkowf, H.I. Relationship between neonatal birth weight and maternal plasma amino acid profiles in lean and obese non diabetic women and in type I diabetic pregnant women. *Metabolism*. **1998**, *37*, 234–239.
115. Kuruvilla, A.G.; D'Souza, S.W.; Glazier, J.D.; Mahendran, D.; Maresh, M.J.; Sibley, C.P. Altered activity of the system A amino acid transporter in micro villous membrane vesicles from placentas of macrosomic babies born to diabetic women. *J. Clin. Invest.* **1994**, *94*, 689–695.
116. Dicke, J.M.; Henderson, G.I. Placental amino acid uptake in normal and complicated pregnancies. *Am. J. Med. Sci.* **1988**, *295*, 223–227.
117. Darmady, J.; Postle, A. Lipid metabolism in pregnancy. *Br. J. Obstet. Gynecol.* **1982**, *89*, 211–215.
118. Kliegman, R.; Gross, T.; Morton, S.; Dunnington, R. Intrauterine growth and post natal fasting metabolism in infants of obese mothers. *J. Pediatr.* **1984**, *104*, 601–607.
119. Knopp, R.H.; Chapman, M.; Bergelin, R.; Wahl, P.W.; Warth, M.R.; Irvine, S. Relationship of lipoprotein lipids to mild fasting hyperglycemia and diabetes in pregnancy. *Diabetes Care*. **1980**, *3*, 416–420.

120. Koukkou, E.; Watts, G.; Lowy, C. Serum lipid, lipoprotein and apolipoprotein changes in gestational diabetes mellitus: a cross-sectional and prospective study. *J. Clin. Pathol.* **1996**, *49*. 634–637.
121. Catalano, P.M.; Nizielski, S.; Shao, J.; Preston, L.; Qiao, L.; Friedman, J.E. Downregulated IRS-1 and PPARgamma in obese women with gestational diabetes: relationship to FFA during pregnancy. *Am. J. Physiol. Endocrinol. Metab.* **2002**, *282*. 522–533.
122. Merzouk, H.; Bouchenak, M.; Loukidi, B.; Madani, S.; Prost, J.; Belleville, J. Fetal macrosomia related to maternal poorly controlled type 1 diabetes strongly impairs serum lipoprotein concentrations and composition. *J. Clin. Pathol.* **2000**, *53*. 917–923.
123. Merzouk, H.; Madani, S.; Korso, N.; Bouchenak, M.; Prost, J.; Belleville, J. Maternal and fetal serum lipid and lipoprotein concentrations and compositions in type 1 diabetic pregnancy: relationship with maternal glycemic control. *J. Lab. Clin. Med.* **2000**, *136*. 441–448.
124. Catlano, P.M.; Tyzbir, E.D.; Roman, N.M. Longitudinal changes in insulin release and insulin resistance in non-obese pregnant women. *Am. J. Obstet. Gynecol.* **1991**, *165*. 1667-1672.
125. Catalano, P.M.; Tyzbir, E.D.; Wolfee, R.R.; Roman, N.M.; Amini, S.B.; Sims, E.H. Longitudinal changes in basal hepatic glucose production and suppression during insulin infusion in normal pregnant women. *Am. J. Obstet. Gynecol.* **1992**, *167*. 913-919.

126. Kalhan, S.; Rossi, K.; Gruca, L.; Burkett, E.; Oberin, A. Glucose turnover and gluconeogenesis in human pregnancy. *J. Clin. Invest.* **1997**, *100*. 1775-1781.
127. Brelje, T.C.; Scharp, D.W.; Lacy, P.E.; Ogren, L.; Talamantes, F.; Robertson, M.; Friesen, H.G.; Sorenson, R.L. Effect of homologous placental lactogens, prolactins, and growth hormones on islet B-cell division and insulin secretion in rat, mouse, and human islets. *Endocrinology*. **1993**, *132*. 879–887.
128. Beck, P.; Daughaday, W.H. Human placental lactogen: studies of its acute metabolic effects and disposition in normal man. *J. Clin. Invest.* **1967**, *46*. 103–110.
129. Ryan, E.A.; Enns, L. Role of gestational hormones in the induction of insulin resistance. *J. Clin. Endocrinol. Metab.* **1988**, *67*. 341– 347.
130. Handwerger, S.; Freemark, M. The roles of placental growth hormone and placental lactogen in the regulation of human fetal growth and development. *J. Pediatr. Endocrinol. Metab.* **2000**, *13*. 343–356.
131. Barbour, L.A.; Shao, J.; Qiao, L.; Pulawa, L.K.; Jensen, D.R.; Bartke, A.; Garrity, M.; Draznin, B.; Friedman, J.E. Human placental growth hormone causes severe insulin resistance in transgenic mice. *Am. J. Obstet. Gynecol.* **2002**, *186*. 512–517.
132. Milasinovic, L.; Djurdjevic, J.; Djordjević, A.; Petrovic, D.; Milasinovic, L.; Kopitovic, V. Prolactin levels in pregnant women with glucose intolerance at full-term delivery. *Br. J. Med.* **2008**, *50*. 269-273.

133. Skouby, S.O.; Kühl, C.; Hornnes, P.J.; Andersen, A.N. Prolactin and glucose tolerance in normal and gestational diabetic pregnancy. *Obstet. Gynecol.* **1986**, *67*. 17-20.
134. Dybee, D.E.; Metzger, B.E.; Freinkel, N.; Dooley, S.L. Amniotic fluid prolactin in the third trimester of pregnancies complicated by gestational or pregestational diabetes mellitus. *Metabolism*. **1990**, *39*. 714-718.
135. Ahmed, S.A.; Shalayer, M.H. Role of cortisol in the deterioration of glucose tolerance in Sudanese pregnant women. *East. Afr. Med. J.* **1999**, *76*. 465-467.
136. Baba, K.A.; Azar, S.T. Thyroid dysfunction in pregnancy. *Int. J. Gen. Med.* **2012**, *5*. 227-230.
137. Rashid, M.; Rashid, M.H. Obstetric management of thyroid disease. *Obstet. Gynecol. Surv.* **2007**, *62*. 680-688;
138. Pedersen, J.; Lazarus, J.H. Thyroid disorders associated with pregnancy: etiology, weight and length at birth in infants of diabetic mothers. *Acta. Endocrinol.* **1954**, *16*. 330–342.
139. Henry, O.; Beischer, N.; Sheedy, M. Gestational diabetes and follow-up among immigrant Vietnam-born women. *Aust. NZ. J. Obstet. Gynaecol.* **1993** *33*. 109 –114.
140. Catalano, P.; Thomas, A.; Huston-Presley, L.; Amini, S.; Increased fetal adiposity: A very sensitive marker of abnormal in-utero development. *Am. J. Obstet. Gynecol.* **2003**, *189*. 1698–1704.

141. HAPO Study Cooperative Research Group. Hyperglycemia and Pregnancy Outcome Study: Associations with neonatal anthropometrics. *Diabetes*. **2009**, 58. 453–459.
142. Catalano, P.; Kirwan, J.; Haugel-de Mouzon, S.; King, J. Gestational diabetes and insulin resistance: Role in short- and long-term implications for mother and fetus. *J. Nutr.* **2003**, 133. 1674–S1683.
143. Dabelea, D. The predisposition to obesity and diabetes in offspring of diabetic mothers. *Diabetes Care*. **2007**, 30. 169–S174.
144. Pettitt, D.; Nelson, R.; Saad, M.; Bennett, P.; Knowler, W. Diabetes and obesity in the offspring of Pima Indian women with diabetes during pregnancy. *Diabetes Care*. **1993**, 16. 310–314.
145. Silverman, B.; Metzger, B.; Cho, N.; Loeb, C. Impaired glucose tolerance in adolescent offspring of diabetic mothers: Relationship to fetal hyperinsulinism. *Diabetes Care*. **1995**, 18, 611–617.
146. Gillman, M.; Rifas-Shiman, S.; Berkey, C.; Field, A.; Colditz, G. Maternal gestational diabetes, birth weight and adolescent obesity. *Pediatrics*. **2003**, 111. 221–226.
147. Buchanan, T.; Xiang, A.; Peters, R. Response of pancreatic beta-cells to improved insulin sensitivity in women at high risk for type 2 diabetes. *Diabetes*. **2000**, 49. 782–788.
148. Bellamy, L.; Casas, J.; Hingorani, A.; Williams, D. Type 2 diabetes mellitus after gestational diabetes: A systematic review and meta-analysis. *Lancet*. **2009**, 373. 1773–1779.

149. Carr, D.; Utzschneider, K.; Hull, R. Gestational diabetes mellitus increases the risk of cardiovascular disease in women with a family history of Type 2 diabetes. *Diabetes Care*. **2006**, *29*. 2078–2083.
150. Shah B.; Retnakaran R.; Booth, G. Increased risk of cardiovascular disease in young women following gestational diabetes. *Diabetes Care*. **2008**, *31*. 1668–1669.
151. Kim, S.; England, L.; Wilson, H.; Bish, C. Percentage of gestational diabetes mellitus attributable to overweight and obesity. *Am. J. Public. Health*. **2010**, *100*. 1047–1052.
152. Rowan, J.; Hague, W.; Gao, W.; Battin, M.; Moore, M. MiG Trial Investigators. Metformin versus insulin for the treatment of gestational diabetes. *N. Engl. J. Med*. **2008**, *358*. 2003–2015.
153. Seely, E.; Solomon, C. Insulin resistance and its potential role in pregnancy-induced hypertension. *J. Clin. Endocrinol. Metab*. **2003**, *88*. 2393–2398.
154. Anyaegbunam, A.; Scarpelli, S.; Mikhail, M. Chronic hypertension in gestational diabetes: Influence on pregnancy outcome. *Gynecol. Obstet. Invest*. **1995**, *39*. 167–170.
155. ACOG Committee on Practice Bulletins – Obstetrics. ACOG practice bulletin. Management of preterm labor. *Int. J. Gynaecol. Obstet*. **2003**, *82*. 127–135.
156. Sokol, R.; Blackwell, S. American College of Obstetricians and Gynecologists Committee on Practice Bulletins. *Int. J. Gynaecol. Obstet*. **2003**, *42*. 80-92.

157. Dumont, C.; Forin, V.; Asfazadourian, H.; Romana, C. Function of the upper limb after surgery for obstetric brachial plexus palsy. *J. Bone. Joint. Surg. Br.* **2001**, *83*. 894–900.
158. Metha, S.; Blackwell, S.; Bujold, E.; Solol, R. What factors are associated with neonatal injury following shoulder dystocia? *J. Perinatol.* **2006**, *26*. 85–88.
159. O’Sullivan, J.; Charles, D.; Mahan, C.; Dandrow, R. Gestational diabetes and perinatal mortality rate. *Am. J. Obstet. Gynecol.* **1973**, *116*. 901–904.
160. Girz, B.; Divon, M.; Merkatz, I. Sudden fetal death in women with well-controlled intensively monitored gestational diabetes. *J. Perinatol.* **1992**, *12*. 229–233.
161. ACOG Practice Bulletin. Clinical management guidelines for obstetrician-gynecologists. *Obstet. Gynecol.* **2001**, *98*. 525–538.
162. Landon, M.; Spong, C.; Thom, E. A multicenter, randomized trial of treatment of mild gestational diabetes. *N. Engl. J. Med.* **2009**, *361*. 1339–1348.
163. Ferrara, A.; Weiss, N.; Hedderston, M. Pregnancy plasma glucose levels exceeding the American Diabetes Association thresholds, but below the National Diabetes Data Group thresholds for gestational diabetes mellitus, are related to the risk of neonatal macrosomia, hypoglycaemia, and hyperbilirubinaemia. *Diabetologia.* **2007**, *50*. 298–306.
164. Sermer, M.; Naylor, C.; Kenshole, A. The Toronto Tri-Hospital Gestational Diabetes Project: A preliminary review. *Diabetes Care.* **1998**, *21*. 833–842.

165. Witkop, C.; Neale, D.; Wilson, L.; Bass, E.; Nicholson, W. Active compared with expectant delivery management in women with gestational diabetes: A systematic review. *Obstet. Gynecol.* **2009**, *113*. 206–217.
166. Declercq, E.; Barger, M.; Cabral, H. Maternal outcomes associated with planned primary cesarean births compared with planned vaginal births. *Obstet. Gynecol.* **2007**, *109*. 669–677.
167. Kennare, R.; Tucker, G.; Heard, A. Chan A. Risk of adverse outcomes in the next birth after a first cesarean delivery. *Obstet. Gynecol.* **2007**, *109*. 270–276.
168. Jovanovic, L.; Peterson, C.M.; Reed, G.F.; Metzger, B.E. The National Institute of Child Health and Human Development–Diabetes in Early Pregnancy Study. *Am. J. Obstet. Gynecol.* **1991**, *164*. 103–111.
169. Combs, C.A.; Gunderson, E.; Kitzmiller, L.; Gavin, L.A.; Main, E.K. Relationship of fetal macrosomia to maternal postprandial glucose control during pregnancy. *Diabetes Care.* **1992**, *15*. 1251–1257.
170. Mills, J.L.; Knopp, R.H.; Simpson, J.L.; Jovanovic, L.; Metzger, B.E. Lack of relation between malformation rates in infants mof diabetic mothers to glycemic control during organogenesis. *N. Engl. J. Med.* **1988**, *318*. 671–676.
171. Mokdad, A.H.; Ford, E.S.; Bowman, B.A.; Dietz, W.H.; Vinicor, F.; Bales, V.S.; Marks, J.S. Prevalence of obesity, diabetes, and obesity related health risk factors, 2001. *J. Am. Med. Assoc.* **2003**, *289*. 76 –79.
172. Dabelea, D.; Snell-Bergeon, J.K.; Hartsfield, C.L.; Bischoff, K.J.; Hamman, R.F.; McDuffie, R.S. Increasing prevalence of gestational diabetes mellitus

- (GDM) over time and by birth cohort: Kaiser Permanente of Colorado GDM Screening Program. *Diabetes Care*. **2005**, 28. 579–584.
173. Mestman, J.H.; Andersen, G.V.; Guadalupe, V. Follow-up study of 369 subjects with abnormal carbohydrate metabolism during pregnancy. *Obstet. Gynecol.* **1972**, 39. 421–425.
 174. Kim, C.; Newton, K.M.; Knopp, R.H. Gestational diabetes and the incidence of type 2 diabetes. *Diabetes Care*. **2002**, 25.1862–1868.
 175. Cheung, N.W.; Byth, K. Population health significance of gestational diabetes. *Diabetes Care*. **2003**, 26. 2005–2009.
 176. Gu, K.; Cowie, C.C.; Harris, M.I. Diabetes and decline in heart disease mortality in US adults. *J. Am. Med. Assoc.* **1999**, 281. 1291–1297.
 177. Clark, C.J.; Chunfu, Q.; Amerman, B.; Porter, B.; Fineberg, N.; Aldasouqi, S.; Golichowski, A, Gestational diabetes: should it be added to the syndrome of insulin resistance. *Diabetes Care*. **1997**, 20. 867–871.
 178. Ward, K.W.; Johnston, C.W.; Beard, J.C.; Benedetti, T.J.; Halter, J.B.; Porte, D.J. Insulin resistance and impaired insulin secretion in subjects with histories of gestational diabetes mellitus. *Diabetes*. **1985**, 34. 861–869.
 179. Knowler, W.C.; Barrett-Connor, E.; Fowler, S.E.; Hamman, R.F.; Lachin, J.M.; Walker, E.A.; Nathan, D.M. The Diabetes Prevention Program Research Group: Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N. Engl. J. Med.* **2002**, 346. 393–403.

180. Lim, M.K.; Lee, C.K.; Ju, Y.S.; Cho, Y.S.; Lee, M.S.; Moon, H.B. Serum ferritin as a serologic marker of activity in systemic lupus erythematosus. *Rheumatol. Int.* **2001**, *20*:89–93.
181. Tuomainen, T.P.; Nyysönen, K.; Salonen, R.; Ervahauta, A.; Korpela, H.; Lakka, T.; Kaplan, G.A.; Salonen, J.T. Body iron stores are associated with serum insulin and blood glucose concentrations: population study in 1,013 eastern Finnish men. *Diabetes Care.* **1997**, *20*. 426–428.
182. Lao, T.T.; Tam, K.F. Maternal serum ferritin and gestational impaired glucose tolerance. *Diabetes Care.* **1997**, *20*. 1368–1369.
183. Lao, T.T.; Chan, P.L.; Tam, K.F. Gestational diabetes mellitus in the last trimester—a feature of maternal iron excess. *Diabet. Med.* **2001**, *18*. 218–223.
184. Fernandez-Real, J.M.; Ricart-Engel, W.; Casamitjana-Abella, R.; Cabrero, R. Serum ferritin as a component of the insulin resistance syndrome. *Diabetes Care.* **1998**, *21*. 62–68.
185. Lao TT, Chan PL, Tam KF. Gestational diabetes mellitus in the last trimester—a feature of maternal iron excess. *Diabet. Med.* **2001**, *18*. 218–223.
186. Galan, P.; Vergnaud, A.C.; Tzoulaki, I. Low total and nonheme iron intakes are associated with a greater risk of hypertension. *J. Nutr.* **2010**, *140*. 75–80.
187. Cade, J.E.; Moreton, J.A.; O'Hara, B. Diet and genetic factors associated with iron status in middle-aged women. *Am. J. Clin. Nutr.* **2005**, *82*. 813–820.

188. Ellis, S.; Speroff, T.; Dittus, R.; Brown, A.; Pichert, J.; Elasy, T. Diabetes patient education: A meta-analysis and meta-regression. *Patient. Educ. Couns.* **2004**, *52*, 97–105.
189. Magee, M.; Knopp, R.; Benedetti, T. Metabolic effects of 1200-kcal diet in obese pregnant women with gestational diabetes. *Diabetes.* **1990**, *39*, 234–240.
190. Algert, S.; Shragg, P.; Hollingsworth, D. Moderate caloric restriction in obese women with gestational diabetes. *Obstet. Gynecol.* **1985**, *65*, 487–491.
191. Major, C.; Henry, M.; Veciana, M.; Morgan, M. The effects of carbohydrate Restriction in patients with diet-controlled gestational diabetes. *Obstet. Gynecol.* **1998**, *91*, 600–604.
192. Romon, M.; Nuttens, M.; Vambergue, A. Higher carbohydrate intake is associated with decreased incidence of newborn macrosomia in women with gestational diabetes. *J. Am. Diet. Assoc.* **2001**, *101*, 897–902.
193. Moses, R.; Barker, M.; Winter, M.; Petocz, P.; Brand-Miller, J. Can a low-glycemic diet reduce the need for insulin in gestational diabetes mellitus? A randomized trial. *Diabetes Care.* **2009**, *32*, 996–1000.
194. Franz, M.; Bantle, J.; Beebe, C. Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications. *Diabetes Care.* **2003**, *24*, 51–61.
195. Ilic, S.; Jovanovic, L.; Pettitt, D. Comparison of the effect of saturated and monounsaturated fat on postprandial plasma glucose and insulin concentration

- in women with gestational diabetes mellitus. *Am. J. Perinatol.* **1999**, *16*. 489–495.
196. Perseghin, G.; Price, T.; Petersen, K. Increased glucose transport-phosphorylation and muscle glycogen synthesis after exercise training in insulin-resistant subjects. *N. Engl. J. Med.* **1996**, *335*. 1357–1362.
 197. Artal, R.; Catanzaro, R.; Gavard, J.; Mostello, D.; Friganza, J. A lifestyle intervention of weight-gain restriction: Diet and exercise in obese women with gestational diabetes mellitus. *Appl. Physiol. Nutr. Metab.* **2007**, *32*. 596–601.
 198. Bung, P.; Artal, R.; Khodiguian, N.; Kjos, S. Exercise in gestational diabetes. An optional therapeutic approach. *Diabetes*. 1991;**40** 182–185.
 199. Artal, R.; Lockwood, C.; Brown, H. Weight gain recommendations in pregnancy and the obesity epidemic. *Obstet. Gynecol.* **2010**, *115*. 152–155.
 200. Hirsch, I. Insulin analogues. *N. Engl. J. Med.* **2005**, *352*. 174–183.
 201. Nachum, Z.; Ben-Shlomo, I.; Weiner, E.; Shalev, E. Twice daily vs four times daily insulin dose regimens for diabetes in pregnancy: Randomised controlled trial. *Br. Med. J.* **1999**, *319*. 1223–1227.
 202. Gabbe, S.; Graves, C. Management of diabetes mellitus complicating pregnancy. *Obstet. Gynecol.* **2003**, *102*. 857–868.
 203. Langer, O.; Conway, D.; Berkus, M.; Xenakis, E.; Gonzales, O. A comparison of glyburide and insulin in women with gestational diabetes mellitus. *N. Engl. J. Med.* **2000**, *343*. 1134–1138.

204. Evertson, L.; Gauthier, R.; Schiffrin, B.; Paul, R. Antepartum fetal heart rate testing. Evolution of the nonstress test. *Am. J. Obstet. Gynecol.* **1979**, *133*. 29–33.
205. Manning, F.; Platt, L.; Sipos, L. Antepartum fetal evaluation: Development of a fetal biophysical profile. *Am. J. Obstet. Gynecol.* **1980**, *136*. 787–795.
206. Freeman, R.; Anderson, G.; Dorchester, W. A prospective multi-institutional study of antepartum fetal heart rate monitoring. II. Contraction stress test versus nonstress test for primary surveillance. *Am. J. Obstet. Gynecol.* **1982**, *143*. 778–781.
207. Weiner, C. The relationship between the umbilical artery systolic/ diastolic ratio and umbilical blood gas measurements in specimens obtained by cordocentesis. *Am. J. Obstet. Gynecol.* **1990**, *162*. 1198–1202.
208. Ben-Haroush, A.; Chen, R.; Hadar, E.; Hod, M.; Yogev, Y. Accuracy of a single fetal weight estimation at 29–34 weeks in diabetic pregnancies: Can it predict large-for-gestational-age infants at term. *Am. J. Obstet. Gynecol.* **2007**, *197*. 491–496.
209. Wolfe, H.; Sokol, R.; Martier, S.; Zador, I. Maternal obesity: A potential source of error in sonographic prenatal diagnosis. *Obstet. Gynecol.* **1990**, *76*. 339–342.
210. Lantz, M.; Chisholm, C. The preferred timing of second-trimester obstetric sonography based on maternal body mass index. *J. Ultrasound. Med.* **2004**, *23*. 1019–1022.

211. Conway, D.; Langer, O. Elective delivery of infants with macrosomia in diabetic women: Reduced shoulder dystocia versus increased cesarean delivery. *Am. J. Obstet. Gynecol.* **1998**, *178*. 922–925.
212. Flenady, V.; MacPhail, J.; Gardener, G. Detection and management of decreased fetal movements in Australia and New Zealand: A survey of obstetric practice. *Aust. NZ. J. Obstet. Gynaecol.* **2009**, *49*. 358–363.
213. Buchanan, T.; Xiang, A.; Peters, R. Preservation of pancreatic beta-cell function and prevention of Type 2 diabetes by pharmacological treatment of insulin resistance in high-risk Hispanic women. *Diabetes*. **2002**, *51*. 2796–2803.
214. Ratner, R.; Christophi, C.; Metzger, B. . Prevention of diabetes in women with a history of gestational diabetes; effects of metformin and lifestyle interventions. *J. Clin. Endocrinol. Metab.* **2008**, *93*. 4774–4779.
215. Lovelady, C.; Nommsen-Rivers, L.; McCrory, M.; Dewey, K. Effects of exercise on plasma lipids and metabolism of lactating women. *Med. Sci. Sports. Exerc.* **1995**, *27*. 22–28.
216. Lovelady, C.; Garner, K.; Moreno, K.; Williams, J. The effect of weight loss in overweight, lactating women on the growth of their infants. *N. Engl. J. Med.* **2000**, *342*. 449–453.
217. O’Toole, M.; Sawicki, M.; Artal, R. Structured diet and physical activity prevent postpartum weight retention. *J. Women’s Health*. **2003**; *12*. 991–998.
218. Trinder, P. Determination of Blood glucose by enzymatic calorimetric method. *Clin. Biochem.* **1969**, *12*. 6-24.

219. Korolev, V.A. Experience and methodology of glycosylated hemoglobin determination. *Lik. Sprava*. **2010**, 2. 57-69.
220. Ständker, L.; Zachgo, V.; Hillemanns, P.; Rösinger, M.; Forssmann, W.G.; Hass, R. Quantitative enzyme-linked immunosorbent assay determination of an abundant hemoglobin-derived anti-infective peptide in human placenta. *Anal. Biochem*. **2010**, 401. 53-60.
221. Allian, C.; Poon, C. L.; Chon, C. S.; Richmond, U. Enzymatic determination of total serum cholesterol. *Clin. Chem*. **1974**, 20. 470-475.
222. Werner, M.; Garielson, D. G.; Estman, G. Enzymatic determination of triglycerides. *Clin. Chem*. **1981**. 21. 268.
223. Bonny, A.E.; Ziegler, J.; Harvey, R.; Debanne, S.M.; Secic, M.; Cromer, B.A. Weight gain in obese and nonobese adolescent girls initiating depot medroxyprogesterone, oral contraceptive pills, or no hormonal contraceptive method. *Arch. Pediatr. Adolesc. Med*. **2006**, 160. 40–45.
224. Sinha, B.; Brydon, P.; Taylor, R.; Hollins, A.; Munro, A.; Jenkins, D.; Dunne, F. Maternal ante-natal parameters as predictors of persistent postnatal glucose intolerance. *Diabet. Med*. **2003**, 20. 382–386.
225. Bergmeyer, H. U.; Horder. M.; Rej. R. Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzyme. *Clin. Chem*. **1986**. 24, 481-495.
226. Wahlefeld, A. W.; Hertz, G.; Bernt. E. Modification of the Malloy method for simple and reliable determination of total bilirubin in serum. *J. Clin. Lab. Invest*. **1972**, 29. 11-12.

227. Lapolla, A.; Mazzon, S.; Marini S, Burattin, G.; Grella, P.; Fedele, D. A screening program for gestational diabetes in a North Mediterranean Area. *Diabetes Nutr. Metab.* **1995**, *8*, 33–41.
228. Di Cianni, G.; Miccoli, R.; Volpe, L.; Lencioni, C.; Ghio, A.; Giovannitti, M.G.; Cuccuru, I.; Pellegrini, G.; Chatzianagnostou, K.; Boldrini, A.; Del Prato, S. Maternal Creatinine levels and newborn weight in pregnant women with normal glucose tolerance. *Diabet. Med.* **2005**, *22*, 21–25.
229. Burgi, W.; Briner, M.; Franken, N.; Kessler, A. One step sandwich enzyme immunoassay using monoclonal antibodies. *Clin. Biochem.* **1988**, *21*, 311-314.
230. Read, C.H.; baryan, G.T. The immunological assay of human growth hormone. Recent progress in hormone research. *Clin. Biochem.* **1960**, *16*, 187-218.
231. Ganjam, V.; Desjardins, C.; Ewing, L.L. A quantitative procedure for the determination of cortisol in blood plasma. *Steroids*. **1970**, *16*, 227-250.
232. Tai S.S.; Bunk, D.M.; White, E.; Welch, M.J. Development and evaluation of a reference measurement procedure for the determination of total triiodothyronine in human serum. *Anal. Chem.* **2004**, *76*, 5092-5096.
233. Holm, S. S.; Hansen, S. H.; Faber, J.; Staun-Olsen, P. Reference methods for the measurement of free thyroid hormones in blood: evaluation of potential reference methods for free thyroxine. *Clin. Biochem.* **2004**, *37*, 85-93.
234. Seth, J.; Kellett, H.A.; Caldwell, G.; Sweeting, V.M.; Beckett, G.J.; Gow, S.M.; Toft, A.D. A sensitive immunoradiometric assay for serum thyroid

- stimulating hormone: a replacement for the thyrotrophin releasing hormone test. *Br. Med. J.* **1984**, 289. 1334-1336.
235. Dabelea, D.; Snell-Bergeon, J.K.; Hartsfield, C.L.; Bischoff, K.J.; Hamman, R.F.; McDuffie, R.S. Increasing prevalence of gestational diabetes mellitus (GDM) over time and by birth cohort: Kaiser Permanente of Colorado GDM Screening Program. *Diabetes Care.* **2005**, 28. 579 –584.
 236. Ferrara, A.; Kahn, H.S.; Quesenberry, C.P.; Riley, C.; Hedderson, M.M. An increase in the incidence of gestational diabetes mellitus: Northern California, 1991–2000. *Obstet. Gynecol.* **2004**, 103. 526–533.
 237. Ben-Haroush, A.; Yogev, Y.; Hod, M. Epidemiology of gestational diabetes mellitus and its association with type 2 diabetes. *Diabet. Med.* **2004**, 21. 103–113.
 238. Popkin, B.M.; Gordon-Larsen, P. The nutrition transition: worldwide obesity dynamics and their determinants. *Int. J. Obes.* **2004**, 28. 2–9.
 239. Tanaka, M.; Jaamaa, G.; Kaiser, M.; Hills, E.; Soim, A.; Zhu, M.; Shcherbatykh, I.Y.; Samelson, R.; Bell, E.; Zdeb, M.; McNutt, L.A. Racial disparity in hypertensive disorders of pregnancy in New York state: a 10-year longitudinal population-based study. *Am. J. Public Health.* **2007**, 97. 163–170.
 240. Clausen, T.; Oyen, N.; Henriksen, T. Pregnancy complications by overweight and residential area: a prospective study of an urban Norwegian cohort. *Acta. Obstet. Gynecol.* **2006**, 85. 526 –533.

241. Dunstan, D.W.; Zimmet, P.Z.; Welborn, T.A.; Courten, M.P.; Cameron, A.J. The rising prevalence of diabetes and impaired glucose tolerance. *Diabetes Care*. **2002**, *25*, 829–834.
242. Yogeve, Y.; Visser, G.H. Obesity, gestational diabetes and pregnancy outcome. *Semin. Fetal. Neonatal. Med.* **2009**, *64*, 1477-1484.
243. Knopp, R.H.; Warth, M.R.; Charles, D. Lipoprotein metabolism in pregnancy, fat transport to the fetus, and the effects of diabetes. *Biol. Neonate*. **1986**, *50*, 297–317.
244. Ben-Haroush, A.; Yogeve, Y.; Hod, M. Epidemiology of gestational diabetes mellitus and its association with type 2 diabetes. *Diabet. Med.* **2004**, *21*, 103–113.
245. Metzger, B.E.; Buchanan, T.A.; Coustan, D.R. Summary and recommendations of the Fifth International Workshop-Conference on Gestational Diabetes Mellitus. *Diabetes Care*. **2007**, *30*, 251–260.
246. Buchanan, T.A. Pancreatic B-cell defects in gestational diabetes: implications for the pathogenesis and prevention of type 2 diabetes. *J. Clin. Endocrinol. Metab.* **2001**, *86*, 989–993.
247. Lao, T.T.; Ho, L.F. First-trimester blood pressure and gestational diabetes in high-risk Chinese women. *J. Soc. Gynecol. Investig.* **2003**, *10*, 94–98.
248. Monique, M.; Hedderson, P.D.; Assiamira Ferrara, M.D. High blood pressure before and during early pregnancy is associated with an increased risk of gestational diabetes mellitus. *Diabetes Care*. **2008**, *31*, 2362-2367.

249. Crowther, C.A.; Hiller, J.E.; Moss, J.R.; McPhee, A.J.; Jeffries, W.S.; Robinson, J.S. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. *N.Engl. J. Med.* **2005**, *352*. 2477–2486.
250. Carpenter, M.W. Gestational diabetes, pregnancy hypertension, and late vascular disease. *Diabetes Care.* **2007**, *30*. 246–250.
251. Buchanan, T.A.; Xiang, A.H. Gestational diabetes mellitus. *J. Clin. Invest.* **2005**, *115*. 485–491.
252. Knowler, W.C.; Barrett-Connor, E.; Fowler, S.E.; Hamman, R.F.; Lachin, J.M.; Walker, E.A.; Nathan, D.M. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N. Engl. J. Med.* **2002**, *346*. 393–403.
253. Anastasiou, E.; Lekakis, J.P.; Alevizaki, M.; Papamichael, C.M.; Megas, J.; Souvatzoglou, A. Stamatelopoulos SF: Impaired endothelium-dependent vasodilatation in women with previous gestational diabetes. *Diabetes Care.* **1998**, *21*. 2111–2115.
254. Gokce, N.; Holbrook, M.; Duffy, S.J.; Demissie, S.; Cupples, L.A.; Biegelsen, E.; Keaney, J.F.; Loscalzo, J.; Vita, J.A. Effects of race and hypertension on flow-mediated and nitroglycerin-mediated dilation of the brachial artery. *Hypertension.* **2001**, *38*. 1349–1354.
255. Hu, F.B.; Meigs, J.B.; Li, T.Y.; Rifai, N.; Manson, J.E. Inflammatory markers and risk of developing type 2 diabetes in women. *Diabetes Care.* **2004**, *53*, 693–700.

256. Qiu, C.; Sorensen, T.K.; Luthy, D.A.; Williams, M.A A prospective study of maternal serum C-reactive protein (CRP) concentrations and risk of gestational diabetes mellitus. *Pediatr. Perinat. Epidemiol.* **2004**, *18*. 377–384.
257. Steinhart, J.; Sugarman, J.; Connell, F. Previous history of gestational diabetes is a herald of NIDDM in Navajo women. *Diabetes Care.* **1997**, *20*. 943– 947.
258. Lam, K.; Li, D.; Lauder, I.; Lee, C.; Kung, A.; Ma, J. Prediction of persistent carbohydrate intolerance in patients with gestational diabetes. *Diabetes. Res. Clin. Pract.* **1991**, *12*. 181– 186.
259. Henriksen, T. The macrosomic fetus: a challenge in current obstetrics. *Acta. Obstet. Gynecol. Scand.* **2008**, *87*. 134– 145.
260. Taricco, E.; Radaelli, T.; Rossi, G.; Bulfamante, G.P.; Avagliano, L.; Cetin, I. Effects of gestational diabetes on fetal oxygen and glucose levels in vivo. *Br. J. Obstet. Gynecol.* **2009**, *116*. 1729-1735.
261. McLaughlin, G.; Cheng, Y.; Caughey, A. Women with one elevated 3-hour glucose tolerance test value: are they at risk for adverse perinatal outcomes. *Am. J. Obstet. Gynecol.* **2006**, *194*. 16-19.
262. Baxi, L.; Barad, D.; Reece, E.A.; Farber, R. Use of glycosylated hemoglobin as a screen for macrosomia in gestational diabetes. *Obstet. Gynecol.* **1984**, *64*. 347-350.
263. Bacigalupo, G.; Langner, K.; Saling, E. Glycosylated hemoglobin (HbA1), glucose tolerance and neonatal outcome in gestational diabetic and nondiabetic mothers. *J. Perinat. Med.* **1984**, *12*. 137-139.

264. Mody, R.J.; Brown, P.I.; Wechsler, D.S. Refractory iron deficiency anemia as the primary clinical manifestation of celiac disease. *J. Pediatr. Hematol. Oncol.* **2003**, *25*, 169–72.
265. Tuomainen, T.P.; Korpela, H.; Nyysönon, K.; Lakka, T.; Salonen, R. Body iron stores are associated with serum insulin and blood glucose concentrations. *Diabetes Care.* **1997**, *20*, 426–428.
266. Salonen, J.T.; Tuomainen, T.P.; Nyysönon, K.; Lakka, H.M.; Punnonen, K. Relationship between iron stores and non-insulin dependent diabetes in men: case-control study. *Br. Med. J.* **1998**, *317*, 727.
267. Abbassi-Ghanavati, M.; Greer, L.G.; Cunningham, F.G. Pregnancy and laboratory studies: a reference table for clinicians. *Obstet. Gynecol.* **2009**, *114*, 1326-1231.
268. Bozkurt, N.; Yilmaz, E.; Biri, A.; Taner, Z.; Himmetoğlu, O. The mean platelet volume in gestational diabetes. *J. Thromb. Thrombolysis.* **2006**, *22*, 51-57.
269. Arcaro, G.; Zamboni, M.; Rossi, L.; Turcato, E.; Covi, G.; Armellini, F.; Bosello, O.; Lechi, A. Body fat distribution predicts the degree of endothelial dysfunction in uncomplicated obesity. *Int. J. Obes. Relat. Metab. Disord.* **1999**, *23*, 936–942.
270. Verma, A.; Boney, C.M.; Tucker, R.; Vohr, B.R. Insulin resistance syndrome in women with prior history of gestational diabetes mellitus. *J. Clin. Endocrinol. Metab.* **2002**, *87*, 3227– 3235.

271. Kautzky-Willer, A.; Krssak, M.; Winzer, C.; Pacini, G.; Tura, A. Increased intramyocellular lipid concentration identifies impaired glucose metabolism in women with previous gestational diabetes. *Diabetes*. **2003**, 52. 244 –251.
272. Peterson, C.M.; Jovanovic-Peterson, L.; Mills, J.L.; Conley, M.R. The Diabetes in Early Pregnancy Study: changes in cholesterol, triglycerides, body weight, and blood pressure. The National Institute of Child Health and Human Development--the Diabetes in Early Pregnancy Study. *Am. J. Obstet. Gynecol.* **1992**, 166. 513-518.
273. Natarajan, S.; Liao, Y.; Cao, G.; Lipsitz, S.R.; McGee, D.L. Sex differences in risk of coronary heart disease mortality associated with diabetes and established coronary heart disease. *Arch. Intern. Med.* **2003**, 163.1735-1740.
274. Kjos, S.L.; Buchanan, T.A.; Montoro, M.; Coulson, A.; Mestman, J.H. Serum lipids within 36 week of delivery in women with recent gestational diabetes. *Diabetes*. **1991**, 40. 142-146.
275. Sobki, S.H.; Al-Senaigy, A.M.; Al-Shammari, T.A.; Inam, S.S.; Al-Gwiser, A.A., Bukhari, S.A. Impact of gestational diabetes on lipid profiling and indices of oxidative stress in maternal and cord plasma. *Saudi. Med. J.* **2004**, 25. 876-880.
276. Lippi, G.; Albiero, A.; Montagnana, M.; Salvagno, G.L. Lipid and lipoprotein profile in physiological pregnancy. *Clin. Lab.* **2007**, 53. 173-177.
277. Kadari, G.S. Status of Glycosylated Hemoglobin and lipid profile in gestational diabetes patient. *Rec. Res. Sci.* **2010**, 2. 04-07.

278. Terence, T.; Lao, T. Maternal age and prevalence of gestational diabetes mellitus. *Diabetes Care*. **2006**, *29*. 948-949.
279. Kim, C.; Newton, K.M.; Knopp, R.H: Gestational diabetes and the incidence of type 2 diabetes. *Diabetes Care*. **2002**, *25*.1862–1868.
280. Sherman, K.E. Alanine amino transferase in clinical practice. *Arch Int.Med*. **1991**, *151*. 260-265.
281. Tomassini, A.; Razzini. M.; Deledda, R. Placental alkaline phosphatase in pregnancy at term. *Minerva. Gynecol*. **1975**, *27*. 711-716.
282. Gordon, T.F. Actris associated with serum alkaline phosphatase level. *Arch. Pathol. Lab. Med*. **1993**, *11*. 187-193.
283. Thapa, B.R.; Walia, A. Division of the pediatric gastroenterology, hepatology and nutrition. *J. Pediatr*. **2007**, *74*. 663-671.
284. Bandyopadhyay, G.K.; Yu, J.G.; Ofrecio, J.; Olefsky, J.M. Increased expression and decreased phosphatidylinositol 3-kinase activity in insulin-resistant human skeletal muscle. *Diabetes*. **2005**, *54*. 2351– 2359.
285. Catalano, P.M. Obesity, insulin resistance, and pregnancy outcome. *Reproduction*. **2010**, *140*. 365-371.
286. Shalayel, M.H.; Saadelin, A.D.; Mohammad, S.M. Prolactin and insulin estimates in pregnancy with glucose intolerance. *Kharton. Med. J*. **2009**, *02*. 200-205.
287. Crowther, C.A.; Hiller, J.E.; Moss, J.R.; McPhee, A.J.; Jeffries, W.S, Robinson, J.S. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. *N. Engl. J. Med*. **2005**, *352*. 2477–2486.

288. Innes, K.E.; Wimsatt, J.H.; McDuffie, R. Relative glucose tolerance and subsequent development of prolacynemia in pregnancy. *Obstet. Gynecol.* **2001**, *97*, 905–910.
289. Grigorakis, S.I.; Alevizaki, C.C.; Beis, C.; Alevizaki, C.C. Hormonal parameters in gestational diabetes mellitus during the third trimester. *Gynecol. Obstet. Invest.* **2000**, *49*, 106-109.
290. Baba, K.A.; Azar, S.T. Thyroid dysfunction in pregnancy. *Int. J. Gen. Med.* **2012**, *5*, 227-230.
291. Rashid, M.; Rashid, M.H. Obstetric management of thyroid disease. *Obstet. Gynecol. Surv.* **2007**, *62*, 680-688;
292. Lazarus, J.H. Thyroid disorders associated with pregnancy: etiology, diagnosis, and management. *Treat. Endocrinol.* **2005**, *4*, 31-41.
293. Prilozi, M.; Velkoska Nakova, V.; Krstevska, B.; Dimitrovski ,C.H.; Simeonova ,S.; Hadzi-Lega, M. Prevalence of thyroid dysfunction and autoimmunity in pregnant women with gestational diabetes and diabetes type *Br. J. Med.* **2010**, *31*, 51-59.
294. Senanayake, M.P.; Gunawardene, M.S. Neonatal morbidity following control of maternal diabetes mellitus with human insulin. *Sri Lanka J. Child Health.* **2000**, *29*, 11–14.
295. Awoyinka, B.S.; Ayinde, O.A.; Omigbodun, A.O. Acceptability of Caesarian delivery to antenatal patients in a tertiary health facility in South-West Nigeria. *J. Obstet. Gynaecol.* **2006**, *26*, 208–210.

296. .O'Sullivan, J.; Charles, D.; Mahan, C.; Dandrow, R. Gestational diabetes and perinatal mortality rate. *Am. J. Obstet. Gynecol.* **1973**, *116*. 901–904.
297. IADSPG Consensus Panel. International Association of Diabetes and Pregnancy Study Groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care.* **2010**. *33*. 676–682.
298. Svare, J.A.; Hansen, B.B.; Molsted-Pedersen. L. Perinatal complications in women with gestational diabetes mellitus. *Acta. Obstet. Gynecol. Scand.* **2001**, *80*. 899–904,
299. Aberg, A.; Rydhstroem, H.; Frid, A. Impaired glucose tolerance associated with adverse pregnancy outcome: a population-based study in southern Sweden. *Am. J. Obstet. Gynecol.* **2001**, *184*. 77–83,
300. Crowther, C.A.; Hiller, J.E.; Moss, J.R. Effect of treatment of gestational diabetes on pregnancy outcome. *N. Engl. J. Med.* **2005**, *352*. 2477-2486.

APPENDICES

Annexure I

Case NO:

Date:_____/_____/_____

Registered Un-registered OPD

1. Name: _____
2. Age: a. 20-30 yrs b. 30-40yrs c. 40-50yrs
3. Education: a. Illiterate b. Educated c. Highly Educated
4. Occupation: a. House Wife b. Govt.Job c. Earning at home
5. Husband's Name: _____
6. Husband Education: a. Illiterate b. Educated c. Highly Educated
7. Husband Occupation: a. Jobless b. Govt.Job c. Private Job
8. Monthly Income Rs: a.10, 000- 15,000 b. 15,000-2500 c. 30,000-50,000
9. Address: _____

Maternal History:

10. Age at marriage: _____yrs
11. No of pregnancies: _____
12. No of alive births: _____
13. No of still birth: _____
14. No of Abortions: _____
15. No of C- sections: _____
16. No of macrosomic babies: _____
17. No of alive children: _____
18. Prior Gestational Diabetes Yes No DK
19. Any complications in the last pregnancy: Yes No DK

Present History

20. EDD: _____

- | | | | |
|--|-------------------------|-------------------------------|----|
| 21. Gestation week: | a. < 28 wks | b. $\geq$ 28wks | |
| 22. Body Mass Index | a. <25kg/m ² | b. $\geq$ 25kg/m ² | |
| 23. Blood Pressure | a. < 140/90 | b. $\geq$ 140/90 | |
| 24. Previous history of Gestational Diabetes | Yes | No | DK |
| 25. Family history of Diabetes | Yes | No | DK |
| 26. History of Hypertension | Yes | No | DK |
| 27. History of renal disease | Yes | No | DK |
| 28. History of liver disease | Yes | No | DK |

Annexure II

Consent Form

Affidavite

Biochemical Changes in Gestational Diabetes in Comparison to Healthy Pregnant Women

The protocol and purpose of the study were explained to me. I fully understand the aims and objectives of the study.

I, Ms. _____ **w/o** _____

Address _____

Contact No. _____

Here by volunteer myself for the above stated study. I will abide by the instructions given by the researcher.

Researcher Signature

Advisor Signature

Dated; _____

Annexure III

Correlations

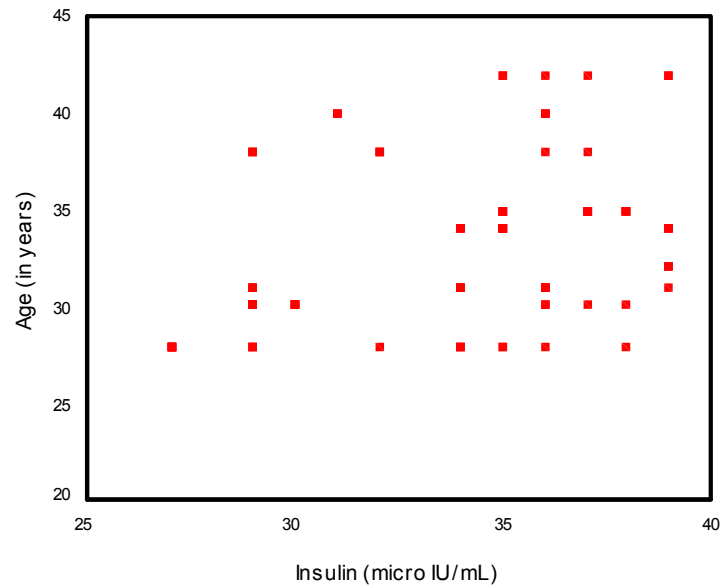


Figure-4.33: Correlation between insulin and age of GDM women.

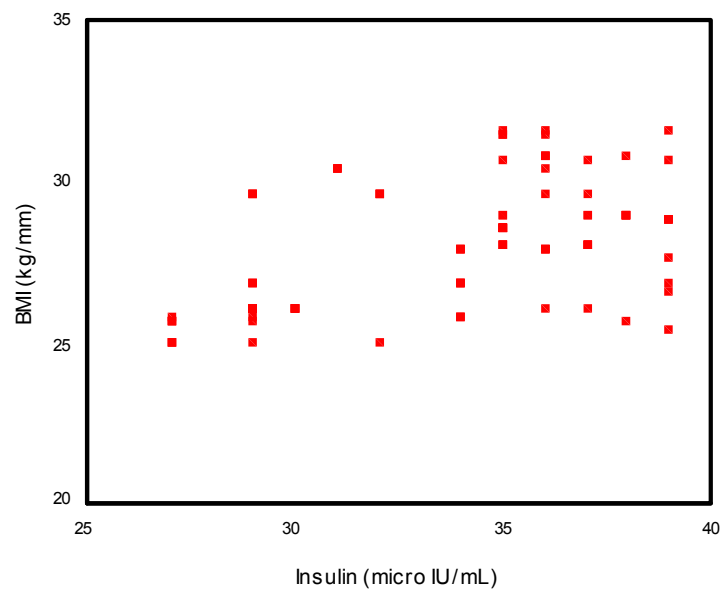


Figure-4.34: Correlation between insulin and BMI of GDM women.

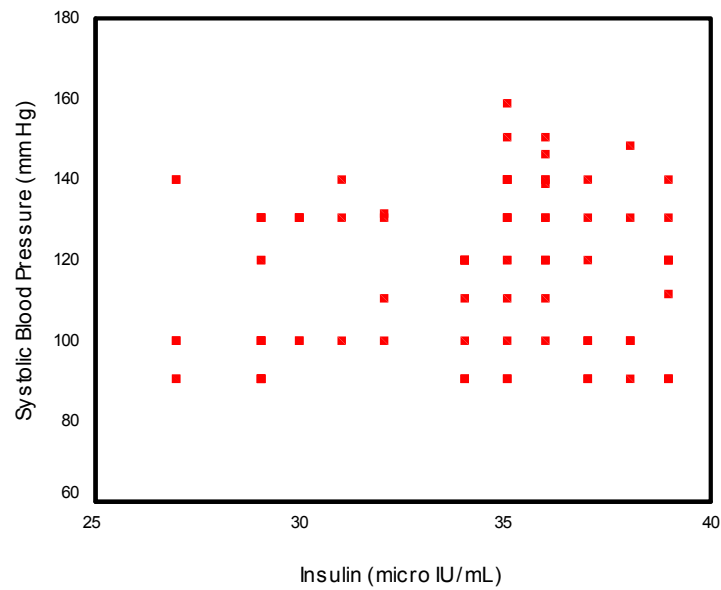


Figure-4.35: Correlation between insulin and systolic BP of GDM women.

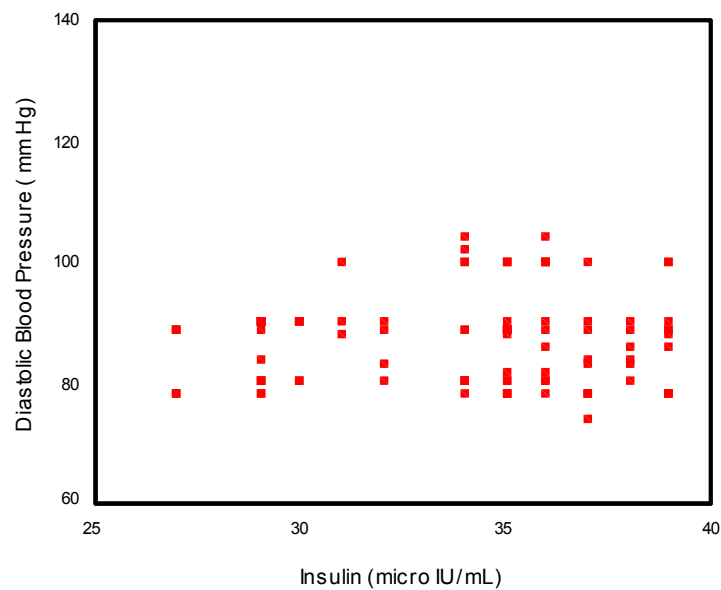


Figure-4.36: Correlation between insulin and diastolic BP of GDM women.

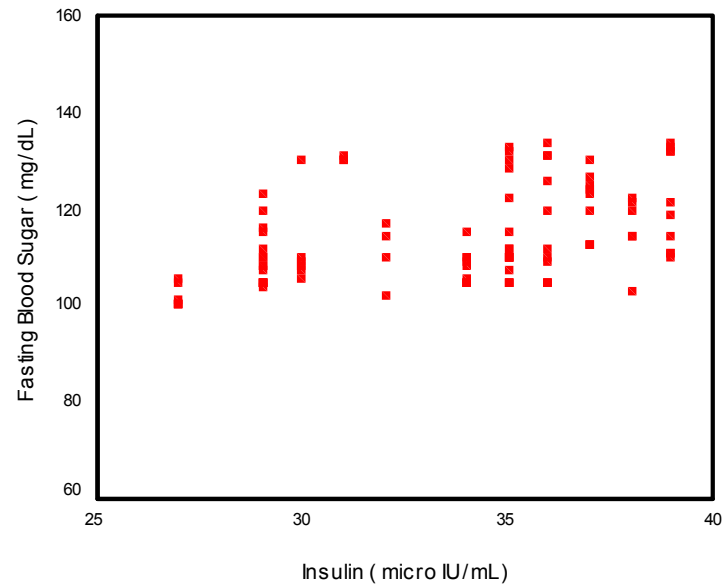


Figure-4.37: Correlation between insulin and FBS of GDM women.

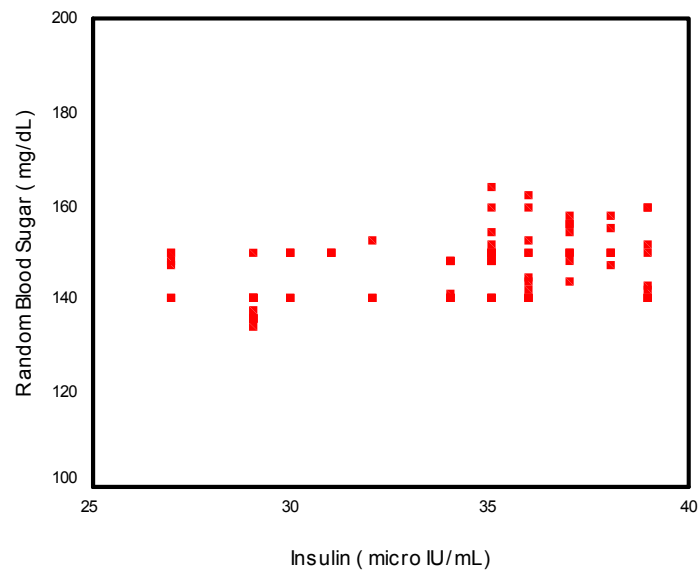


Figure-4.38: Correlation between insulin and RBS of GDM women.

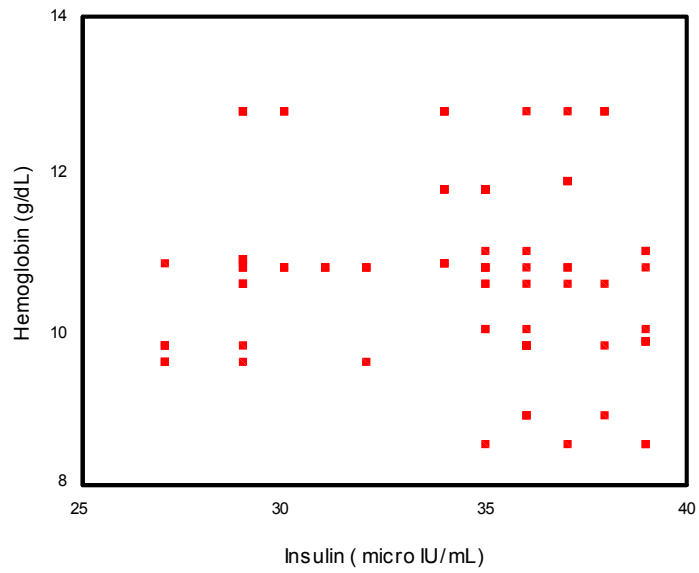


Figure-4.39: Correlation between insulin and hemoglobin of GDM women.

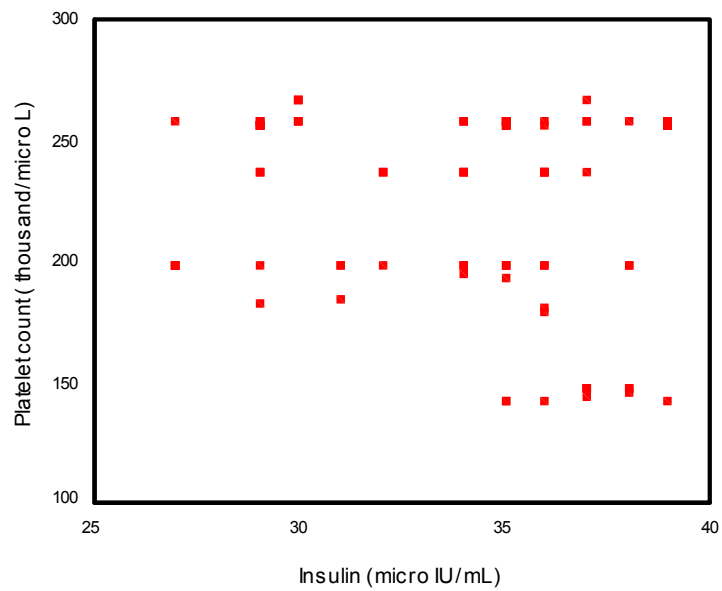


Figure-4.40: Correlation between insulin and platelet count of GDM women.

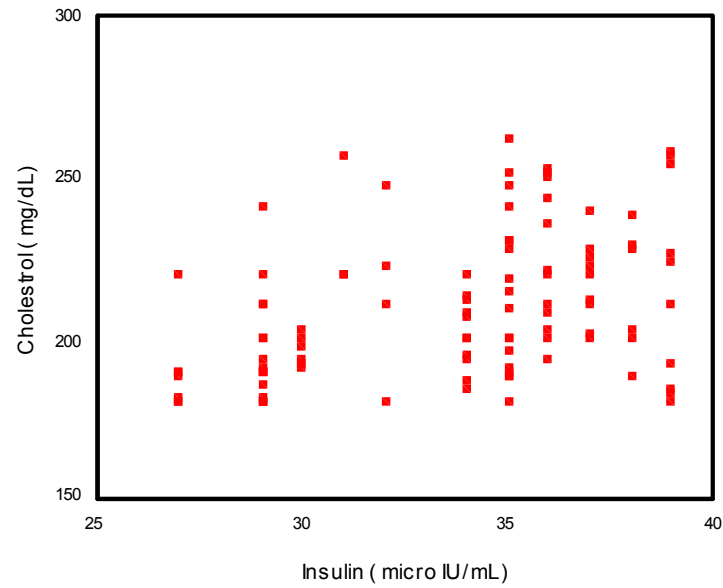


Figure-4.41: Correlation between insulin and cholesterol of GDM women.

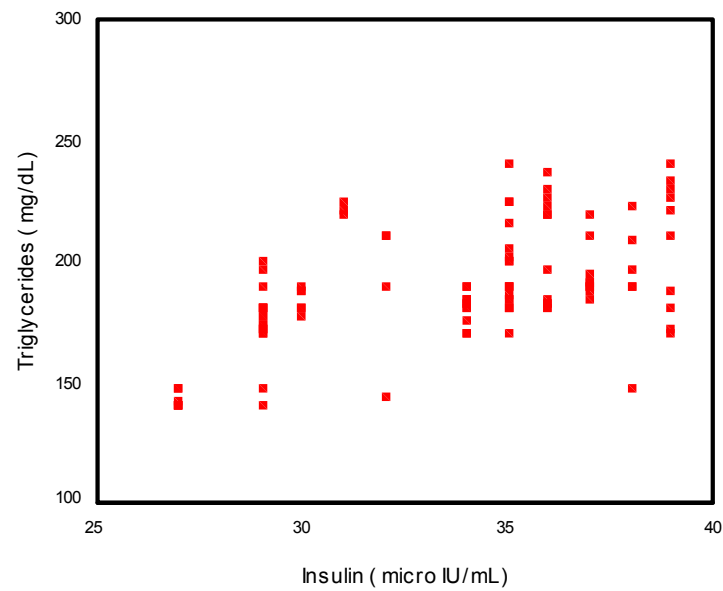


Figure-4.42: Correlation between insulin and triglycerides of GDM women.

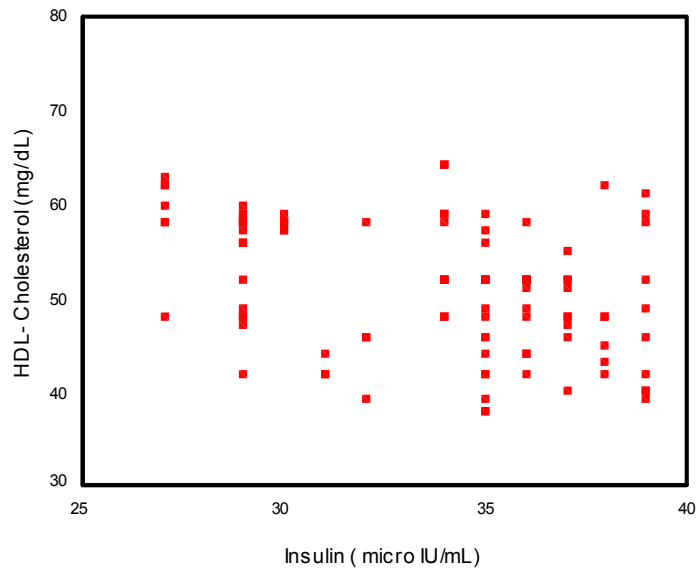


Figure-4.43: Correlation between insulin and HDL-cholesterol of GDM women.

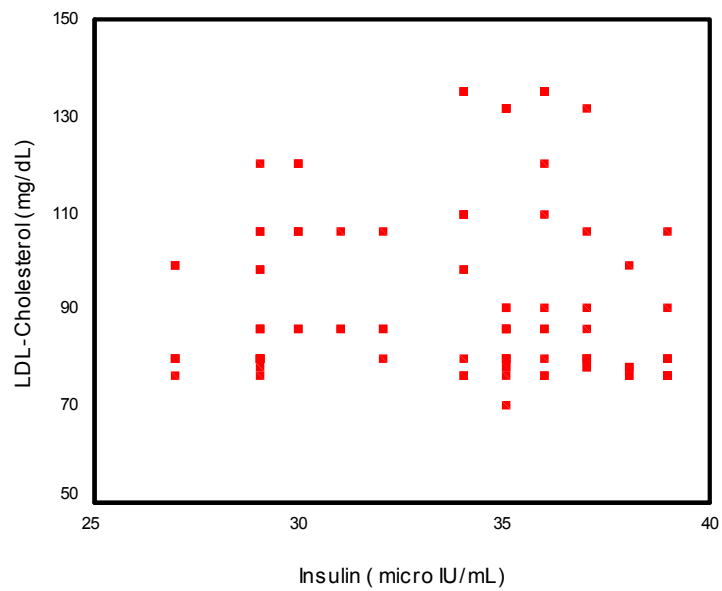


Figure-4.44: Correlation between insulin and LDL-cholesterol of GDM women.

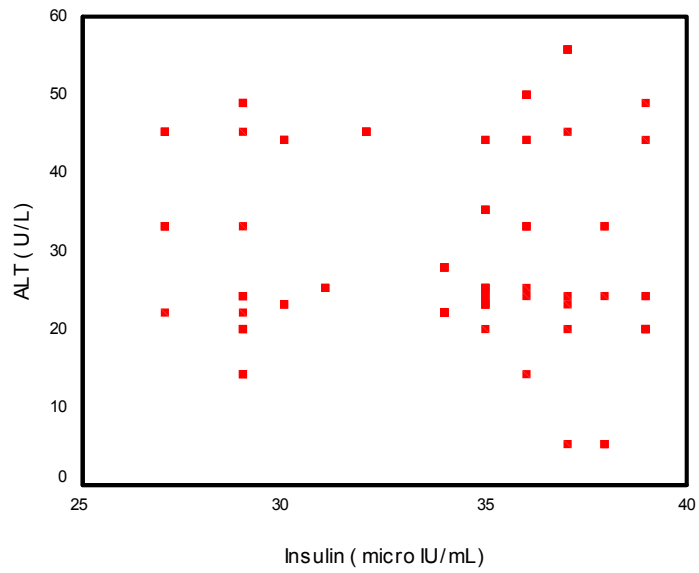


Figure-4.45: Correlation between insulin and ALT of GDM women.

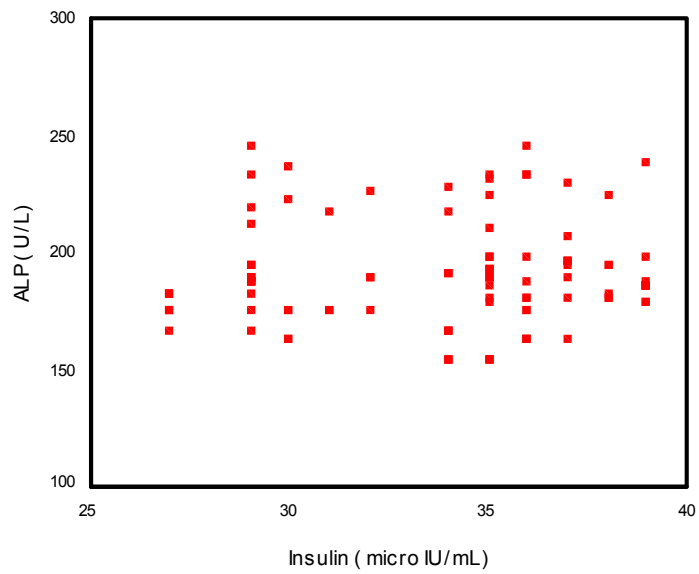


Figure-4.46: Correlation between insulin and ALP of GDM women.

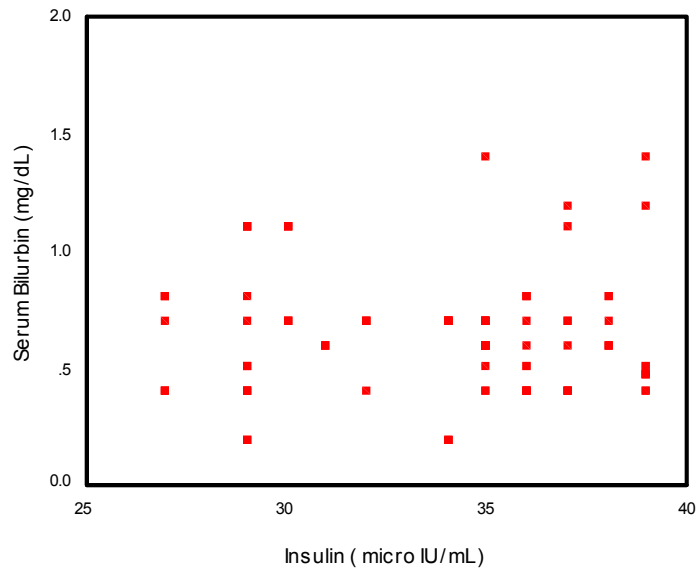


Figure-4.47: Correlation between insulin and bilirubin of GDM women.

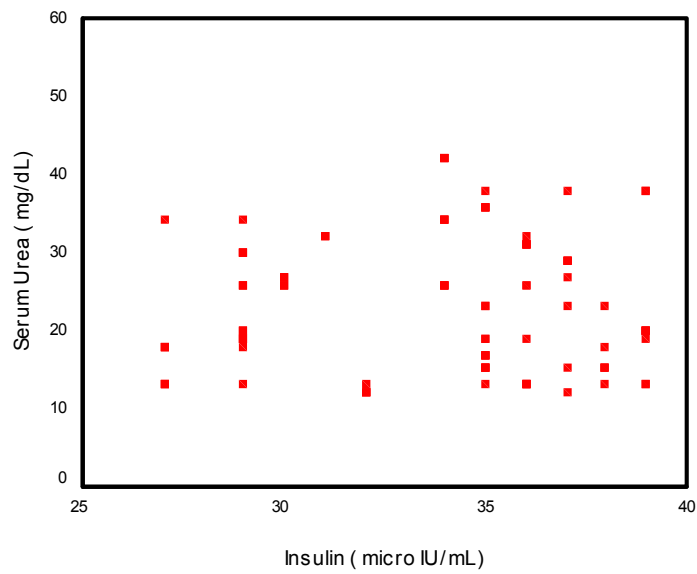


Figure-4.48: Correlation between insulin and serum urea of GDM women.

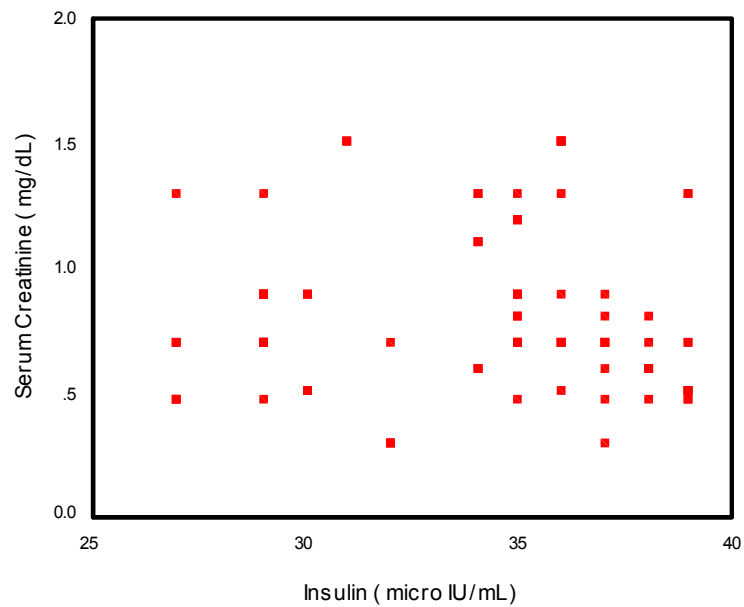


Figure-4.49: Correlation between insulin and serum creatinine of GDM women.

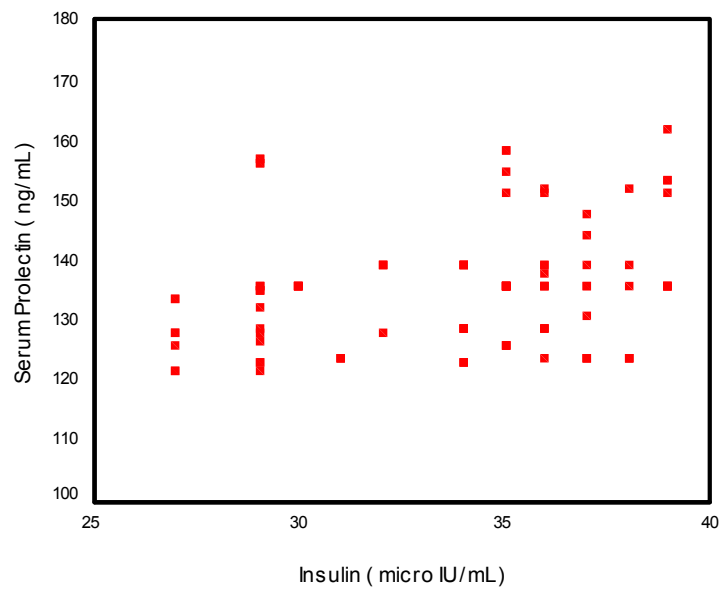


Figure-4.50: Correlation between insulin and serum prolactin of GDM women.

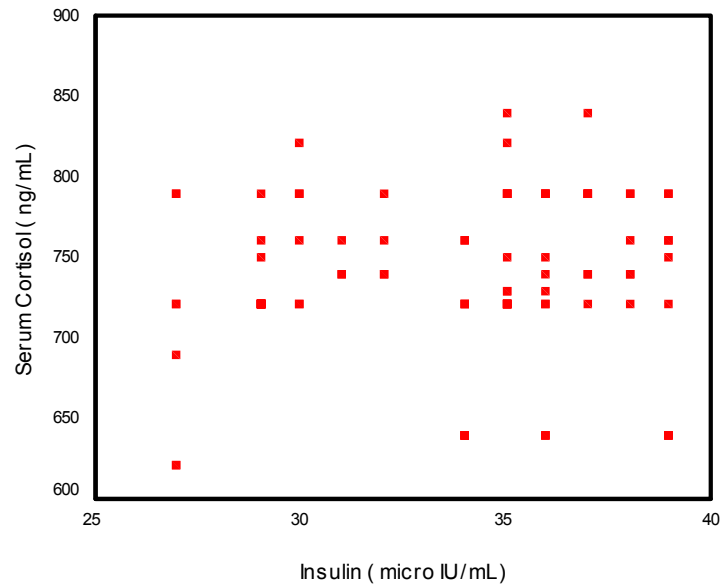


Figure-4.51: Correlation between insulin and serum cortisol of GDM women.

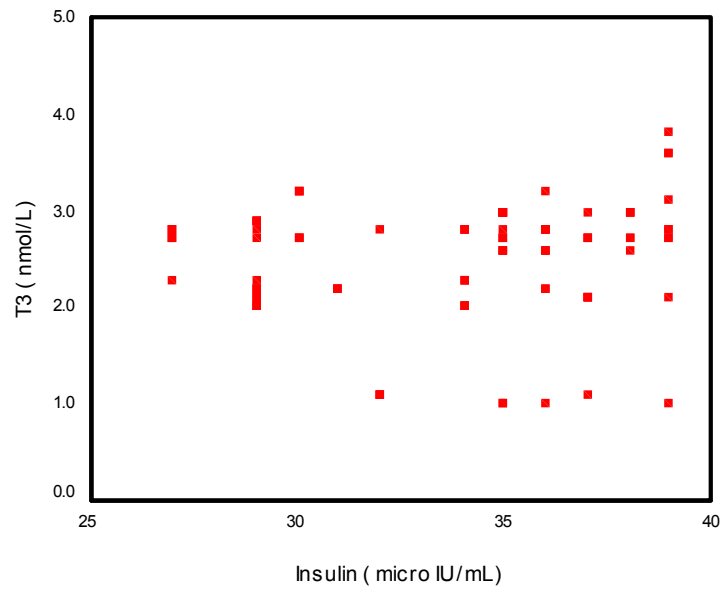


Figure-4.52: Correlation between insulin and T3 of GDM women.

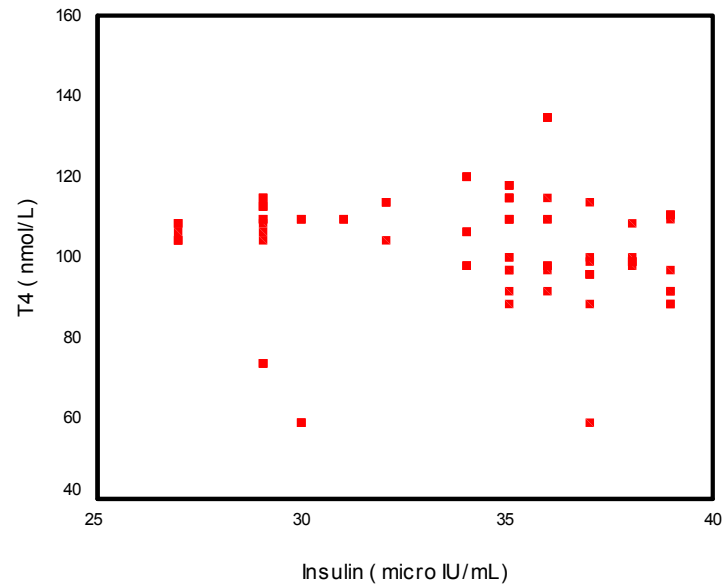


Figure-4.53: Correlation between insulin and T4 of GDM women.

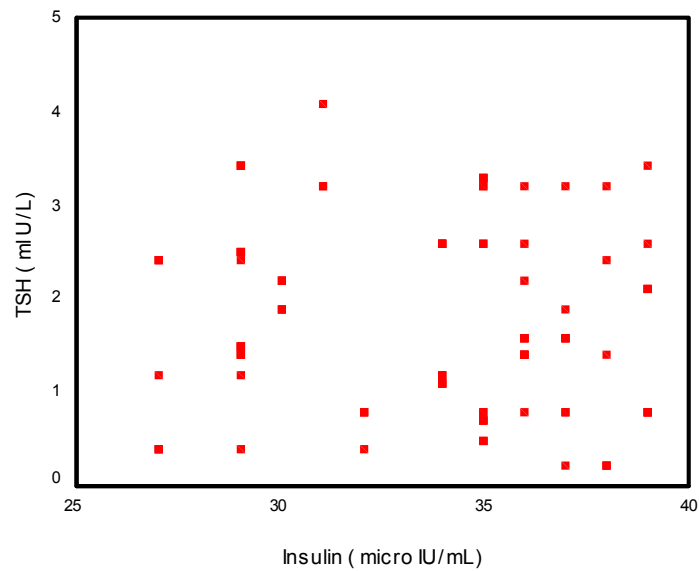


Figure- 4.54: Correlation between Insulin and TSH of GDM women.