CHAPTER 2

LITERATURE SURVEY

Gestational diabetes mellitus is defined as carbohydrate intolerance of varying degrees of severity with onset of first recognition during pregnancy. The policy of screening in the third trimester has resulted in a significant number of pregnant women delivering big babies, despite good glycemia control whereas an early screening for glucose intolerance and care has resulted in the reduction of some of the hyperglycemia related complications (Balaji et al., 2007).

Frequency of congenital malformation among infants of diabetic mothers is estimated to be 6 – 10% factors responsible for these abnormalities are not fully understood but there are reports suggested that increased free radical production and antioxidant depletion in diabetic pregnant female may contribute to risk (Chaudhari et al., 2003).

Women with GDM are at an increased risk of developing Type 2 diabetes mellitus after pregnancy which their offspring are prone to developing childhood obesity, with type 2 diabetes later in life. GDM is a treatable condition and most subjects are treated only with diet modification and moderate exercise but some take medications, including insulin, which effectively decrease these risks (Bian et al., 2000).

In the United States, screening for gestational diabetes typically occurs between 24 and 28 weeks gestation with a one hour glucose challenge test. If this screening test is abnormal, the diagnosis of gestational diabetes is confirmed with a three hour glucosetolerance test sometime in the late second or third trimester.

In clinical practice, however, this can be somewhat challenging because the patients need to be fasting and the additional testing is time-consuming for both patients and the laboratory. Therefore, the diagnosis of gestational diabetes can be delayed or even missed. The pathology may be too sufficiently established for treatment to be effective in reducing significant maternal and fetal/neonatal risks.

Two randomized controlled trials have demonstrated perinatal and maternal benefit from treating gestational diabetes in pregnancy with reductions in preeclampsia,
maternal weight gain, neonatal overgrowth, neonatal fat mass and shoulder dystocia (Crowther et al., 2005 and Landon et al., 2009).

Additionally, a recent systematic review concluded that treatment of GDM is associated with a reduction in the incidence of shoulder dystocia and macrosomia when compared to routine care (Horvath et al., 2010). Even though, earlier detection and treatment of at-risk women has been proposed to improve maternal and fetal outcomes (ACOG, 2001).

2.1 Study of lipids

Diabetes mellitus is known to induce dyslipoproteinaemia (Howard, 1987; Orchard, 1992). Hypertriglyceridaemia in GDM was reported in previous studies (Kuopp et al., 1980; Hollinworth and Grundy, 1982; Metzer et al., 1980). Very few investigations were demonstrated the changes in lipid metabolism during pregnancy complicated by Gestational Diabetes Mellitus (Barakat et al., 1990).

Obesity is the most prominent risk factor for GDM patients and is linked to increasing cardiovascular risk (Ogden et al., 2006). Transport of apolipoproteins and cholesterol is vital adaptive mechanisms in pregnancy that support fetal development. Pregnancy is a hyperlipidemic state in which the placenta and fetal adrenal cortex cooperatively produce cholesterol vital for fetal neuronal and membrane development (Ashwood, 2006).

The biochemical changes in GDM cases reflect fundamental alterations in the balance between carbohydrate and lipid regulation. In GDM, disruption in the balance of VLDL/LDL and HDL-associated apolipoproteins and triglycerides could represent a result or an underlying causative pathology for the associated fetal and maternal morbidities. Placental apolipoprotein D has recently been reported as an adaptation to GDM, possibly providing an important control protecting against oxidative stress (Navarro et al. 2010).

Changes in Apo A-II association with HDL may represent a similar adaptation to GDM affecting cholesterol transport in the blood. Lower apolipoprotein A-II could signal failure in transit of HDL cholesterol, adversely affecting glucose metabolism and lipid-carbohydrate homeostasis (Crowther et al., 2005).

Apo C-III is most closely associated with transporting atherogenic VLDL and LDL cholesterol. The physiological role of Apo C-III sialylation is speculative, but may
include prolonging hepatic clearance or affinity to lipoprotein lipase (Wahrenbrock et al., 2003).

Other recent study have reported that HDL3 is substantially different from controls in GDM subjects while other subtypes show relatively little difference (Merzouk et al., 2000).

Early biomarker testing in pregnancy is also simpler and lends itself to improved patient compliance than the multistep testing currently available. Availability of a reliable clinical test using multiple markers for GDM early in pregnancy could also lead to the development of more effective pharmacological and/or life-style intervention treatment strategies by providing a physiological context for novel pharmaceutical development (Horvath et al., 2010).

Establishing an apolipoprotein pattern unique to gestational diabetes could allow for early detection and intervention well before the onset of clinical symptoms (Nelsestuen et al., 2005). The apolipoproteins are particularly attractive biomarker candidates because of their relative intra-individual stability in the human proteome.

TAG is lower than the normal range for healthy female adults in both normal and diabetic pregnancies. In normal pregnancy, TAG concentrations increase and the other major contributors to TAG are vitamins C and E.

GDM that is characterized by insulin resistance is associated with cardiovascular diseases. Metabolic and biochemical changes that increase the risk of cardiovascular diseases due to GDM include atherogenic dyslipidemia, which is comprised increased blood concentrations of small, dense low-density lipoprotein (LDL) particles, decreased high-density lipoprotein (HDL) particles, and increased triglycerides. (Paradisi et al., 2002).

It was observed that elevated triglycerides and low HDL levels in GDM patients were related to diabetic dyslipidemia. Previous studies have shown that GDM is associated with an increased risk of hypertension. A significant increase in the incidence of hypertension in GDM patients as compared to normo-glycemic subjects was reported and the risk of preeclampsia in pregnant women is increased in subjects with elevated blood sugar and insulin levels (Kvetny et al., 2003, and Solomon et al., 2006).
2.2 Study of Minerals

Diabetes mellitus is characterized by hyperglycemia and is closely related to trace elements. Quite a few pregnant women suffer from impaired glucose tolerance (IGT) or Gestational Diabetes Mellitus (GDM). Investigation of the changes of elemental contents in serum of the pregnant women with IGT and GDM is significant in the etiological research and cure of the diseases.

Pregnancy with the mitochondria-rich placenta, is a condition that favours oxidative stress. Transitional metals, especially iron, which is particularly abundant in the placenta, are important in the production of free radicals. Protective mechanisms against free radical generation and damage increase throughout pregnancy and protect the fetus, which, however, is subjected to a degree of oxidative stress.

2.2.1 Role of iron in metabolism

Iron is both an essential nutrient and a potential toxicant to cells; as such, it requires a highly sophisticated and complex set of regulatory approaches to meet the demands of cells as well as prevent excess accumulation.

A sufficient supply is essential for the functioning of many biochemical processes, including electron transfer reactions, gene regulation, binding and transport of oxygen, and regulation of cell growth and differentiation. Iron is a strong pro-oxidant and high body iron levels are associated with increased level of oxidative stress that may elevate the risk of type 2 diabetes (Rajpathak *et al.*, 2009).

The homeostasis involves the regulation of iron entry into the body, regulation of iron entry into cells, storage of iron in ferritin, incorporation into proteins an regulation of release from cells for transport to other cells and organs. Many reviews explain the basic biology of iron (Webb, 1992) and the biological aspects of the role of iron in immune system function (Hershko, 1996), the biology of iron in neural functioningand the role of iron in muscle function and energy metabolism (Beard and Dawson 1996).

In biological systems the oxidation states are ferrous (+2), ferric (+3) and ferryl (+4). The inter-conversion of iron oxidation states is not only a mechanism whereby iron participates in electron transfer but also a mechanism whereby iron can reversibly bind ligands. Iron can bind to many ligands by virtue of its unoccupied \( d \) orbitals.
The preferred biological ligands for iron are oxygen, nitrogen and sulphur atoms. The electronic spin state and biological redox potential (from +1000 mV for some heme proteins to -550 mV for some bacterial ferredoxins) of iron can change according to the ligand to which it is bound. By exploiting the oxidation state, redox potential and electron spin state of iron, nature can precisely adjust iron’s chemical reactivity. Thus iron is particularly suited to participate in a large number of useful bio-chemical reactions (Webb, 1992). The general classification of these reactions is oxygen transport and storage, electron transfer and substrate oxidation-reduction.

In iron-sulphur enzymes, iron participates in single-electron transfer reactions primarily in energy metabolism. In the third category, iron is bound to various forms of heme and participates again in electron transfer reactions when associated with various cofactors (e.g., cytochrome P450 complexes).

Iron is essential ion for life, playing a central role in many metabolic processes. Many enzymes in metabolic pathways are iron dependent, thus making iron necessary for essential processes such as DNA synthesis and myelin production, and synthesis of ATP (adenosine triphosphate), as well as several neurotransmitters (i.e., serotonin, and dopamine) (Wigglesworth et al., 1988; Forge et al., 1998; He et al., 2007).

The most important property of free iron is its capacity to reversibly oxidized and reduced, but at the same time this makes it a highly prooxidant molecule. In this regard, iron is able to generate powerful reactive oxygen species (ROS) Gutteridge, 1990; Fridovich, 1978). Therefore, the maintenance of iron homoeostasis in the organism is crucial, and high levels of free iron could be harmful.

Excess iron has been implicated in the pathogenesis of diabetes and its complications (Fernández – Real et al., 2002a; Loebstein et al., 1998; Sempos et al., 2000, Fernández – Real et al., 2002b; Ford and Cogswell, 1999). Free iron serves as a catalyst for lipid and protein oxidation and the formation of reactive oxygen species. In addition iron indices are correlated with obesity and insulin sensitivity (Fernández – Real et al., 2002a). These factors have led some to promote iron chelation as a possible adjunctive therapy in diabetes (Fernández – Real et al., 2002a).
Increased tissue iron levels are associated with diabetes, both in human hereditary hemochromatosis (HH) and in dietary iron overload (Fernandez – Real et al., 2002b; Ford and Cogswell, 1999; McClain et al., 2006; Fleming et al., 2001) Although this is at least partially the result of decreased insulin secretion (McClain et al., 2006; Cooksey et al., 2004; Jouihanet al., 2008), tissue iron overload also results in significant changes in glucose metabolism in skeletal muscle (Huang et al., 2007). However, the effect of iron is to increase glucose uptake, a change that would be predicted to be protective of diabetes. Iron is a strong pro-oxidant and high body iron levels are associated with increased level of oxidative stress that may elevate the risk of type 2 diabetes.

2.2.1 Changes in iron level with reference to Diabetes Mellitus

Conditions of iron overload in various tissues, including the pancreas, might precipitate the destruction of the β cell leading to the onset of diabetes via a ROS mechanism. Indeed there are strong links between increased Fe status and diabetes. Overt diabetes occurs in about 60% of patients with idiopathic, haemochromatosis (Powell, 1985). Normal human ferritin protein is required for iron stimulation of the hepatic synthesis of hepcidin, a peptide that regulates iron absorption through the downregulation of iron channel ferroprotein (Nemeth et al, 2004).

Failure to down regulate ferroprotein in hemochromatosis leads to unlimited iron entry into the circulation from duodenal cells and macrophages (Nemeth et al., 2004b). While the prevalence of idiopathic haemochromatosis in the general population is 2-5/1000, the prevalence previously unrecognized idiopathic, haemochromatosis among diabetic patients was 9.6/1000 (Pheleps et al., 1989). Insulin resistance is markedly depletion of body Fe stores by phlebotomy, resulting in lower insulin requirements in those patients with IDDM and improvement in glucose tolerance in about 50% of patients with NIDDM (Stremmel et al, 1987).

Glucose intolerance overt diabetes are also observed in secondary forms of Fe-loading disease. (Merkel et al, 1988) suggested that insulin resistance and increased insulin secretion develop in older children with thalassaemia treated with long-term hyper-transfusion therapy before the development of diabetes apart from impaired insulin secretion caused by selective deposition of Fe in β cell of the pancreas of the other
possible mechanisms which may explain these findings include the development of insulin resistance due to Fe accumulation in the liver and muscle.

It is tempting to speculate that the increased Fe status obese women (Fricker et al., 1990) and those with greater body mass index (Micozzi et al., 1989) might give increased risk of NIDDM. Although associated with increased serum triacylglycerols, Fe deficiency does not seem to have a significant effect on glucose homeostasis (Mertz, 1982).

### 2.2.2 Role of calcium in metabolism

The lowered Ca2+ of the diabetes was associated with increased serum triacylglycerols, and some of the calcium complexes may possibly have physiological importance. Lactate, β-hydroxybutyrate, acetoacetate, and non-esterified fatty acids were probably involved, as they are increased in diabetes mellitus (Wildenhoff, 1975; Burrin et al., 1981; Hansen et al., 1970). Non-esterified fatty acids increase the binding of calcium by albumin (Ladenson and Shyong, 1977; Aguanno and Ladenson, 1980); nevertheless, protein-bound calcium was normal. Hypomagnesemia probably increased calcium-binding by decreasing the binding competition between calcium and magnesium.

#### 2.2.2.1 Changes in calcium with reference to Diabetes Mellitus

There are multiple potential cellular sites of insulin resistance; one such site is dysfunctional regulation although the relationship between Ca2+ and insulin signal transduction is complex which was poorly understood.

Impaired Ca2+ - ATPase mediated Ca2+ efflux was a result rather than a cause of insulin resistance (Aviv and Leder, 1972; Levy et al., 1989; Zemelet al., 1993; Sowers et al., 1991). Draznin et al. (1987) demonstrated an optimal range of Ca2+ for maximizing insulin stimulated glucose transport, with elevations beyond this range causing marked decreases in adipocyte insulin sensitivity. Similarly, data from a number of studies indicate the increasing Ca2+ in isolated adipocytes results in significant inhibition of insulin-stimulated glucose transport (Reusch et al., 1993; Begum et al., 1992; Begum et al., 1991) and oxidation (Kelly et al., 1989). In addition, Ca2+ entry blockade in obese
elderly humans resulted in significant increase in peripheral insulin sensitivity (Byyny et al., 1992; Zemelet al., 1991).

Calcium is essential for insulin-mediated intracellular processes in insulin-responsive tissues such as skeletal muscle and adipose tissue (Ojuka, 2004; Wright et al., 2004; Williams et al., 1990) with a very narrow range of \( \text{Ca}^{2+} \) needed for optimal insulin-mediated functions (Draznin et al., 1987). Changes in \( [\text{Ca}^{2+}] \) in primary insulin target tissues may contribute to peripheral insulin resistance (Draznin et al., 1987; Segal et al., 1990; Byyny et al., 1992; Ohno et al., 1999; Draznin et al., 1988b; Draznin et al., 1989) via impaired insulin signal transduction (Williams et al., 1990) leading to decreased GLUT-4 activity (Reusch et al., 1991).

Serum Ca may be released from the surface of the bones and be responsible for the increase in glucose due to low serum phosphorus levels, which stimulate bone resorption. It has been suggested that diabetic patients should be checked for hypercalcemia at appropriate intervals (Taylor and Khaleeli, 2001). A signaling pathway for the link between energy metabolism and bone remodeling has been presented (Gravenstein et al., 2011).

The correlation revealed between osteocalcin and insulin resistance might include changes in both serum phosphate and serum Calcium, and it would have been interesting to know if a high concentration of serum Ca together with high Serum Glucose is associated with lack of osteocalcin. The signalling pathway, with increase in leptin due to obesity and decrease in osteocalcin, may help to regulate, primary or secondary ionic changes.

High serum Ca but low serum Mg without involvement of serum Phosphorus can predict all-cause mortality in type 2 diabetes, although this is stronger for men than for women (Haglin et al., 2007). Advanced research studies also describe high mortality with high Sulfur-Calcium (Leifsson and Ahren, 1996; Lundgren et al., 2001). Women with type 2 diabetes had in addition to their lower serum Mg, significantly higher serum Ca.
compared to men with type 2 diabetes and non-diabetic women at baseline.

2.2.3 Role of zinc in metabolism

It was reported that zinc is involved in the synthesis, storage, secretion and conformational integrity of insulin monomers and that zinc assembles to a dimeric form for storage and secretion as crystalline insulin (Chausmer, 1998). Lower levels of zinc may affect the ability of pancreatic islet cells responsible for the production and secretion of insulin, such as in type 2 diabetes (DiSilvestro, 2000).

Epidemiological studies have reported decreased plasma and intracellular zinc concentrations in conjunction with increased urinary zinc excretion in diabetic patients. In subjects with type – 2 diabetes mellitus with low zinc intake, the risk of coronary heart disease increases by a factor of two to four times and is a major cause of mortality among diabetic subjects (Singh et al., 1998).

2.2.3.1 Changes of zinc with reference to diabetes

A physical chemical relationship exists between insulin and zinc. Long before there was biochemical evidence for the relationship between zinc and insulin in the beta cell and it was clear that the addition of zinc to insulin would change the time course of the effect of a given dose of insulin. As early as the 1930s, when insulin was just becoming available for commercial use, zinc was being added in vitro to make protamine zinc crystalline insulin which prolonged the duration of action of the insulin by delaying its absorption from the subcutaneous injection site thus requiring fewer insulin injections.

Since the 1970s investigations the biochemical pathways and the structures for insulin been known. Insulin is produced by the beta cell of the pancreatic islets as a single chain peptide that is bent around itself and two inter – chain disulfide bonds. This proinsulin is cleaved by the removal of an intra cellular chain fragment known as the “C-peptide” to form two peptide chain molecules of 51 amino acids cross – linked to each other by inter – chain disulfide bonds.
In the presence of zinc within the cell, insulin monomers assemble to a dimeric form for storage and secretion as the zinc crystal. It was shown that high concentration of glucose decrease the islet cell labile zinc and video fluorescence analysis showed zinc concentrated in the islet cells was related to the synthesis, storage and secretion of insulin.

In the presence of zinc and at neutral pH, dimeric insulin assembles further into a hexamer consisting of three dimeric units. Which relatively stable and it is this hexameric crystal which is the commonly used pharmacologic form. The size of the crystal is, at least in large part, the determinant of dissolution rate. Antigenic determinants are altered by the removal of zinc from insulin by changing the conformation of the molecule. In one series of studies, zinc free insulin was much less immunologically active than zinc insulin in immune hemolysis inhibition assays while there was little difference in radioimmune assay determinations (Arquilla et al., 1978).

With the development of genetic engineering, it has become possible to develop analogues of insulin which do not form zinc insulin hexamers which result in a more rapid absorption from the injection site (Bristow, 1993).

Crystalline insulin contains Zn but the effect Zn deficiency on impaired glucose tolerance or insulin secretion is controversial and remains unproven (Mooradian and Morley, 1987). It has been suggested that the insulin-like effects of Zn is adipocytes involve the ability of Zn to modulate peroxide generation (May and Contoreggi, 1982), but any effects of Zn on insulin secretion are biphasic with higher concentrations impairing insulin secretion (Mooradian and Morley, 1987).

Diabetes per se and appears to alter zinc metabolism. Urinary zinc excretion elevated in diabetic patients compared with control subjects (Canfield et al., 1984; Heiseet al., 1988). The increase is associated with urinary protein loses. Urinary zinc was not associated with the excretion of any aminoacid and peptides chelated with zinc contributed to the increased urinary zinc losses a decline in serum zinc may also be related to diabetic control (McNair et al., 1981).

Zinc originating from bone loss and exogenous insulin accounted for a small part of the zinkemia. Other homeostatic adjustments must have occurred. Rats made diabetic by the administration of Streptozotocin did not have an increase in zinc absorption but the amount of zinc excreted into the intestine was reduced (Johnson and Canfield, 1984).
The decreased endogenesis loss of zinc into the intestine may be a homeostatic response to the increased urinary excretion of endogenous zinc in diabetic patients.

The role of zinc in Gestational Diabetes is not well established. Studies in diabetic pregnant rats suggest the zinc transport to the foetus is reduced either because of a decrease in placental transport or altered maternal or foetal zinc-binding ligands (Uriu–Hare et al., 1992). In humans no difference in serum zinc were observed between insulin requiring diabetic women and control pregnant women if the diabetic women are maintaining careful control (Wibell et al., 1985). Further research is needed to establish the marginal zinc status on glucose homeostasis in women during pregnancy.

2.2.4 Role of magnesium in metabolism

Magnesium is the second most plentiful in human living cells. The body of an adult human weighing 70 kg contains 25 gm (1200 mol) of Mg. About 55% is present in bone combined with phosphate and carbonate and 27% is present in muscle. The remainder is in soft tissues and body fluids. However, less than 1% of total body Mg is present in the blood (Elin, 1985). Magnesium in the plasma exists in free (55%), complexed (13%) and protein-bound forms (32%).

Magnesium is the fourth most abundant cation in the human body and the second most abundant intracellular cation. It may exist as a protein-bound, complexed, or free cation. It serves as a co-factor for all enzymatic reactions that require ATP and as a key component in various reactions that require kinases.

Magnesium has been found to regulate and improve the blood sugar control, play a vital role in the secretion and function of insulin and is necessary for insulin to open cell membranes for glucose and helps the body digest, absorb, and utilize proteins, fats, and carbohydrates.

Magnesium (Mg) is one of the most abundant ions present in living cells and its plasma concentration is remarkably constant in healthy subjects. Plasma and intracellular Mg concentrations are tightly regulated by several factors. Among them, insulin seems to be one of the most important. In vivo studies have demonstrated that insulin may modulate the shift of Mg from extracellular to intracellular space.
Intracellular Mg concentration has also been shown to be effective in modulating insulin action (mainly oxidative glucose metabolism), offset calcium-related excitation-contraction coupling, and decrease smooth cell responsiveness to depolarizing stimuli. A poor intracellular Mg concentration, as found in non insulin-dependent diabetes mellitus (NIDDM) and in hypertensive patients, may result in a defective tyrosine-kinase activity at the insulin receptor level and exaggerated intracellular calcium concentration. Both events are responsible for the impairment in insulin action and a worsening of insulin resistance in noninsulin-dependent diabetic and hypertensive subjects.

Magnesium plays a key role as a prosthetic ion in many essential enzymatic reactions which are pivotal in the metabolism of carbohydrate, lipid and protein. It activates more than 300 enzymes in the body and it is crucial in the transfer, storage and utilization of energy (Lehninger, 1950). By contributing to RNA synthesis and DNA structure, Mg plays a vital role in cell growth and cell membrane structure (Walker and Duffus, 1983). The role of Mg in regulating mitochondrial membrane permeability and mitochondrial functions is well known (Heaton and Elie, 1984; Ebel and Gunhert, 1980).

Magnesium has also an important role in neurochemical transmission and muscle contraction and is essential for the metabolism of calcium, sodium and potassium in humans. Recently, it has been shown that Mg influences natural and adaptive immunity and that both cellular and humoral immune reactions are adversely affected by hypomagnesemia (McCoy and Kenney, 1985).

Magnesium is involved in glucose homeostasis at multiple levels. It is a co-factor in the glucose transport system of hepatocyte plasma membranes and it regulates hepatocyte mitochondrial functions. It catalyzes the various enzymes involved in the phosphorylation of glucose in its anaerobic metabolism, as well as, in its oxidative decarboxylation in the citric acid cycle and can modulate the mechanisms of energy transfer from high energy phosphate bonds (Goldman and Fisher, 1983). Magnesium also plays a role in the release of insulin and the maintenance of the pancreatic β-cell cycle (Durlach and Altura, 1983).

In experimental animals, severe and prolonged Mg deficiency caused exhaustion and loss of the β-cell in the pancreas. On the other hand, insulin acts as a Mg-sparing
hormone, both directly and indirectly by stimulating vitamin D hydroxylation. It also enhances the shift of Mg muscle cells (Mellerup, 1974). It has been shown that main action of insulin on target tissues involves an ionophore effect on Mg and calcium. The translocation of Mg seems particularly correlated to the peptide mediator and to transphosphorylation reactions. Insulin deficiency induces a drop in 1,25-dihydroxycholecalciferol and modifies secretion of parathyroid, calcitonin and gastrointestinal tract peptide hormones; which in turn favour the occurrences of Mg depletion (Durlach et al., 1983).

Magnesium deficiency in IDDM is related to the duration of the disease and to the degree of the metabolic control (Ewaldet al., 1983; Gebre – Medhinet al., 1985; Sjogrenet al., 1986b; Speech et al., 1986; Fort and Lifshitz, 1986; Kobbahet al., 1988). Marked blood and tissue hypomagnesemia is common in poorly controlled diabetic patients and with diabetic ketoacidosis (Kreisberg, 1978; Durlach and Rayssiguier, 1983). Residual insulin secretory status has no effect on plasma Mg levels in IDDM (Menzelet al., 1985).

Furthermore, in experimentally induced diabetes mellitus hypomagnesemia occurred only when the Mg intake was restricted to the physiological requirements of the control animals (Schneider and Schedl, 1974). However, in a study of diabetic children with hypomagnesemia and matched healthy controls the mean daily intake of Mg for the diabetic children was 50% higher than that of the control group(Kylberget al., 1985).

Increased urinary Mg excretion in connection with glucosuria is probably the most important factor (McNair et al., 1982; Johansson et al., 1982). Mather et al. (1982) demonstrated an inverse correlation between diurnal levels of plasma Mg and blood glucose in diabetic subjects. Similarly, a linear correlation between urinary loss of Mg and blood glucose concentration in human IDDM has been shown (McNair et al., 1982).

Other mechanisms such as hyposecretion of insulin and adrenaline, hypourinemia, modification of vitamin D metabolism, lack of pyridoxine, increase of nicotinic acid, ascorbic acid and glutathione turnovers and decreased intestinal absorption of Mg have been implicated (Mather et al., 1981b; Miller and Schedl, 1976; Awadallahet al., 1978; Emerson et al., 1964; Sulimovici and Roginsky, 1980; Bachemet al., 1980; Durlach and Collery, 1984; Mooradian and Morely, 1987; Kuoppala, 1988; Mather and Levin, 1979).
Hypomagnesemia has been associated with diabetic microvascular disease in several studies (Mather and Levin, 1979; Mather et al., 1981a). Hypomagnesemia was found to be more marked in diabetic subjects with Hypertension and Ischemic heart disease compared with diabetic without the above said complications (Seelig and Heggtviet, 1974).

Chronic low magnesium homeostasis is associated with chronic diseases such as diabetes, hypertension, cardiovascular disorders, neurological disorders and osteoporosis whereas acute magnesium deficiency has been associated with hypocalcaemia and hypokalaemia as well as asthma, stroke, cardiac arrhythmias and neurological dysfunction (Swaminathan, 2003; Fox et al., 2001; Sinert et al., 2007; Mutlu et al., 2007; Cillerler et al., 2007).

Ionic magnesium is the biologically active form of magnesium, a number of studies have accordingly suggested that only the ionic magnesium pool accurately reflects magnesium status and have recommended the routine use of ionic magnesium analyzers for magnesium assessments in serum samples (Resnick et al., 1993; Saha et al., 1998; Barbagallo et al., 2007).

A number of reports have suggested that the total serum magnesium pool does not accurately reflect changes in the ionic Mg pool (Sinert et al., 2007; Resnick et al., 1993; Maj-Zurawska, 1997; Barrera et al., 2000; Johansson and Whiss, 2007), and vice versa. Several studies have now reported correlations between the two parameters in various disease states.

There is a considerable evidence to suggest that hypomagnesemia may adversely affect various aspects of cellular physiology. Available data suggest that low Mg levels may promote endothelial cell dysfunction and thrombogenesis via increased platelet aggregation and vascular calcifications (Rayssignier, 1984). Low Mg levels also may lead to the induction of proinflammatory and profibrogenic response (Shivakumar, 2002; Kurantsin – Mills et al., 1997; Maier et al., 2004), reduction of protective enzymes against oxidative stress (Zhou et al., 1999), induction or augmentation of vasoconstriction and hypertension (Chakrabortiet al., 2002; Altura et al., 1984; Rude et al., 1989), and stimulation of aldosterone (Fakunding et al., 1979; Ichihara et al., 1993) Moreover, because Mg is crucial in DNA synthesis and repair (Hartwig, 2001), it is
possible that Mg deficiency may interfere with normal cell growth and regulation of apoptosis.

Saha et al. (1998) (1996) have published several reports showing strong correlations between total and ionic Mg concentration in serum taken from hemodialysis patients, patients with intestinal disease, alcoholic liver disease, and chronic renal disease. In contrast, a poor correlation was noted in critically ill patients (Barrera et al., 2000; Johansson and Whiss, 2007).

Mikhail and Ehsanipoor (1999) have reported a correlation between the total and ionic Mg, although their study reported that serum ionic but not serum total magnesium declined in diabetes. This is in contrast with the widely reported phenomenon of decreased serum total Mg in diabetes, which has been reported by a number of different authors (Kao et al., 1999; Rodriguez – Moron and Guerrero – Romero, 2001; Chambers et al., 2006).

It is also an essential enzyme activator for neuromuscular excitability and cell permeability, a regulator of ion channels and mitochondrial function, a critical element in cellular proliferation and apoptosis, and an important factor in both cellular and humoral immune reactions (Sanders et al., 1999; Saris et al., 2000; Elamin and Tuvemo, 1990; White and Campbell, 1993; Sales and de Fatima Campos Pedrosa, 2006).

Magnesium antagonizes calcium on the atrio-ventricular node (Fawcett et al., 1993) and myocardial Mg$^{2+}$ deficiency decreases intracellular potassium, resulting in a less negative resting membrane potential and enhanced vulnerability to ventricular arrhythmia (Abbot and Rude, 1993; Parikka et al., 1999; Chakraborti et al., 2002).

Hypomagnesemia is an established risk factor for polymorphic ventricular tachycardia. (Rammeeet et al., 1985). Low serum Mg has been associated with T2DM, but not metabolic states preceding T2DM (Simmons et al., 2010). As T2DM is the most common condition associated with low Mg (Rude and Shils, 2006) and diabetes significantly increases risk of ventricular arrhythmias (Escobedo and Caspersen, 1997).
2.2.4.1 Changes of magnesium with reference to Diabetes Mellitus

Magnesium is involved in glucose intolerance and Sulfur-Magnesium and incidence of type 2 diabetes are inversely associated, it is important to consider whether the serum levels of magnesium are connected to metabolic control (Mather et al., 1979). The disposal rate of glucose in diabetes was related to fasting plasma magnesium concentrations after a standard glucose tolerance test (Yajnik et al., 1984). Low serum magnesium may be a predictor of both metabolic syndrome and type 2 diabetes (Hjelmesaeth et al., 2009).

Glucose leads induce urinary excretion of both calcium and magnesium (Linderman et al., 1967) due to decreased resorption in the proximal tubule (Iseki et al., 2004). This mechanism can be illustrated by the comparative effects from diuretic drugs, which may result in magnesuria and acidosis, reflected by an increased urinary excretion with or without changes in S-Mg (McBain et al., 1988; Milionis et al., 2002). It has been suggested that the metabolic disturbances in type 2 diabetes causes hypomagnesemia (Tosiello, 1996).

Hypomagnesemia, defined by low stream Mg concentrations, has been reported to occur in 13.5 to 47.7% of non-hospitalized patients with type 2 diabetes compared with 2.5 to 15% among their counterparts without diabetes (Pham et al., 2005; McNair et al., 1982; Mather et al., 1979; De Lordeset et al., 1998; Waltiet al., 2003).

The wide range in the reported incidence of hypomagnesemia most likely reflects the difference in the definition of hypomagnesemia, techniques in Mg measurements, and the heterogeneity of the selected patient cohort. In terms of gender difference, it is interesting to note that independent studies have reported a higher incidence of hypomagnesemia in women compared with men, at a 2-to-1 ratio (Pham et al., 2005; Sheehan, 1991). In addition, men with diabetes may have higher ionized levels of Mg (Mikhail and Ehsanipoor, 1999).

Not only has hypomagnesemia been associated with type 2 diabetes, but also numerous studies have reported an inverse relationship between glycemic control and serum Mg levels (Pham et al., 2005; Mather et al., 1979; Sjogrenet al., 1986b; Pon and Ho, 1989; Paolissoet al., 1992; Resnicket al., 1993). Although many authors have
suggested that diabetes *per se* may include hypomagnesemia, others have reported that higher Mg intake may confer a lower risk of type 2 diabetes (Kao et al., 1999; Lopes – Ridaura et al., 2004; Song et al., 2008; van Dam et al., 2006). In patients with type 2 diabetes, oral Mg supplementation during a 16 week period was suggested to improve insulin sensitivity and metabolic control (Rodriguez – Moron and Guerrero - Romero, 2003).

The mechanisms whereby hypomagnesemia may induce or worsen existing diabetes are not well understood. Nonetheless, it has been suggested that hypomagnesemia may induce secretion, defective postreceptor insulin signaling, and/or altered insulin-insulin receptor interactions (Dzurik et al., 1991; Grafton and Baxter, 1992; Durlach et al., 1983; Tonyai et al., 1985). A correlation between glycemic control and serum Mg levels or improvement of diabetic control with Mg replacement was observed in many studies (De Lordes Lima et al., 1998; Garber et al., 1996; Schnacket al., 1992; Eibblet al., 1995). The conflicting data may reflect study designs and populations studied.

GDM subjects with higher fasting glucose levels had lower blood magnesium levels and higher urine levels of magnesium. Decreases in serum magnesium and increased urinary losses of magnesium were reported in type 1 diabetes and Gestational Diabetes.

**2.2.5 Role of copper in metabolism**

Copper ion is involved in the pathogenesis of various diseases (Uriu – Adams and Keen, 2005; Goodman et al., 2005; Walshe, 2007). Copper ion plays an important role in the neovascularization and that the treatment with a copper chelating agent inhibits the neovascularization and the development of tumors. It is also known that copper ion is involved in the pathogenesis of Wilson disease which is a copper excess accumulation disorder and that a copper chelating agent exerts beneficial effects on the disease.

Furthermore, it has been reported that a copper chelating agent tetrathiomolybdate exerts beneficial effects on a variety of diseases including Wilson disease, autoimmune disease, angiogenesis and fibrosis (Brewer and Merajver, 2002; Brewer et al., 2006; Song et al., 2008; Houet al., 2008; Gong et al., 2008).
2.2.5.1 Changes of copper with reference to Diabetes Mellitus

Historically, one of the earliest recognized effects of Cu deficiency, was the impaired glucose tolerance in animals observed by Keil and Nelson (1934). More recently it was demonstrated that the diabetogenic effects of Cu deficiency dependent on the type of carbohydrate fed to rats with fructose being more diabetogenic than glucose (Fields et al., 1984). Copper deficiency can also increase the severity of STZ – induced diabetes (Cohen et al., 1982) and the susceptibility of the exocrine pancreas to oxidative damage (Dubicket al., 1989).

There is additional evidence from human studies of link between Cu deficiency and diabetes. Decreased glucose tolerance was observed in two men during experimental Cu depletion (0.78 mg/d) but improved Cu repletion (6 mg/d) beyond the glucose tolerance observed before the initiation of depletion (Klevay et al., 1986).

In general, STZ – induced diabetes increases copper content associated with metallothionein of rat liver and kidney tissues (Uriu – Hare et al., 1988). The near normal copper concentration found in the diabetic obese animal, however, is probably due to the opposing influences of the obese and diabetic conditions on the hepatic concentration of copper (Donaldson et al., 1987).

In general serum copper and ceruloplasmin levels are increased in IDDM and NIDDM patients (Mooradian and Morley, 1987). It is probable that these increases reflect the greater inflammatory conditions in diabetes as ceruloplasmin is an acute phase reactant (DiSilvestro, 1990).

Increased ceruloplasmin – ferroxidase activity and Fe – bonding proteins in diabetic serum may be a response to oxidative stress (Jones et al., 1988). It was observed by (DiSilvestro1990) that increased blood copper levels do not necessarily reflect increased body copper status. Similar observations were made by (Sjogrenet al., 1986a) who observed that level of copper in striated muscle was lower in IDDM patients compared with healthy controls even though much higher levels of plasma copper were observed in these patients compared with controls.

It is also known that production of reactive oxygen species (ROS) is facilitated in the presence of copper ion through the Fenton reaction (Hunt et al., 1990; Kobayashi et al., 1995; Masadet al., 2007). On the other hand, ROS are induced under diabetic conditions (Dandonael et al., 1996; Ihara et al., 1999; Takahashi et al., 2008) and are likely
associated with the development of type 2 diabetes (Matsuoka et al., 1997; Rudich et al.,
1998; Tanaka et al., 1999; Tirosh et al., 1999; Kaneto et al., 2001; Kaneto et al., 2002;
Evans et al., 2002; Kawamori et al., 2003; Robertson, 2004; Wellen and Hotamisligil
2005; Kaneto et al., 2008).

It has been reported that the treatment with antioxidants exerts beneficial effects on
the pathogenesis of type 2 diabetes (Kaneto et al., 1999; Tanaka et al., 1999; Haber et al.,
2003; Yamamoto et al., 2008). Therefore, it is likely that copper ion is also involved in
the development of type 2 diabetes.

2.3 Status of Antioxidants

Oxidative stress peaks by the second trimester of pregnancy, ending what appears
to be a vulnerable period for fetal health and gestational progress. Conditions restricted
to pregnancy, such as gestational hypertension, insulin resistance and diabetes, exhibit
exaggerated indications of free radical damage.

Free radical mediated oxidative stress has been implicated in the pathogenesis of
diabetes mellitus and its complications (Kalansooriya et al., 2004; Orhan et al., 2003;
Dorge 2002). Elevated glucose levels can induce oxidative stress in gestational diabetic
mothers (Kamath et al., 1998; Bis – gluchowska et al., 2001; Nasrat et al., 1996; Xinhua
et al., 2005).

Low insulin sensitivity has been suggested to be the cause of oxidative stress in
diabetes which eventually leads to free radical generation (Hunt and Wolff, 1991). Several
studies found associations among diabetes in pregnancy and different markers of
oxidative stress (Carone et al., 2001; Kinalska et al., 2001). Scavenging enzyme activities
reflect antioxidant defense status among Glutathione is the most abundant intracellular
antioxidant (Jentzsch et al., 1996).

Oxidative stress is associated with a pro – oxidative shift of the GSH redox state in
the blood (De Mattia et al., 1988). GST would act as a biomarker of oxidative stress upon
sudden increase in oxygenation (Orhan et al., 2003). During pregnancy, the synthesis rate
of lipoperoxides appears to exceed their decomposition rate, causing oxidative stress.
Lipoperoxides are also increased in the fetus as it develops, but to lesser extent than that
of GDM mother (Yoshioka et al., 1987). Diabetes induced oxidative stress might result
from the underlying metabolic abnormalities rather than the direct causes of the disease
itself (Zadehet et al., 1997).
It has been demonstrated that non-pregnant patients with Type I diabetes have a lower total antioxidant activity and low levels of vitamin C (Maxwell et al., 1997).

The reduction of antioxidant levels may be linked to an increase in free-radical-mediated lipid peroxidation. Our present findings in normal pregnancy of elevated LHP concentrations allow us to confirm observations made by others (Little et al., 1999). The oxidative damage is higher in healthy pregnant women than in non-pregnant women. In addition, differences in LHP concentrations between women with diabetes and those without were observed from Type1Diabetes. The difference was significantly greater in women with Type II diabetes ($P<0.05$). This suggests that these changes are perhaps not pregnancy related and oxidative stress is present in Type II diabetic subjects before pregnancy (Sagol et al., 1999).

### 2.4 Hematological parameters

Gestational Diabetes mellitus (GDM) is influenced by higher hemoglobin (Hb) level and Mean Corpuscular Volume (MCV) before 14 weeks gestation in GDM women and healthy women. The incidence of GDM in women with iron deficiency anemia has been reported to be 50% of that seen in non-anemic women (Lao and Ho, 2004)

In developed countries, not only maternal anemia but also high hemoglobin concentration during pregnancy has been reported to increase the risks of unfavorable outcomes such as small – for – gestational – age (SGA) birth, preterm birth, and prenatal death the association between hemoglobin concentration in early pregnancy, changes in hemoglobin concentration during pregnancy and risk of still birth are not known (Stephansson et al., 2000).

Previous studies have shown that MPV was increased in subjects with arterial thrombosis and in patients at a high risk for cardiovascular disease associated with diabetes and hypertension (Muscari et al., 2009). It was reported that there is association between elevated MPV and acute myocardial infarction, mortality after infarction, and restenosis after coronary angioplasty (Nadar et al., 2004). It was demonstrated that patients with hypertension have increased MPV, which is associated with target organ damage (Nadar et al., 2004).
MPV was increased in both gestational diabetic patients and in pregnant women with abnormal glucose tolerance tests, but they showed an inverse relationship between MPV values and platelet numbers.

2.5 Study of Insulin hormone and receptors

Pregnancy is characterized by peripheral insulin resistance, which is compensated by an increase in insulin secretion to maintain glucose homeostasis. Gestational diabetes mellitus (GDM), which occurs in about 4% of pregnancies, develops if insulin secretion fails to overcome insulin resistance.

Therefore, GDM shares many features with Type 2 Diabetes Mellitus, including not only glucose intolerance, and impaired insulin secretion, but also association with similar risk factors, as well as obesity and family history of diabetes.

In the United States nearly 4% of patients were reported to have diabetes. Eighty eight percent of these women have gestational diabetes mellitus (GDM, 450,000 women per year), and the remaining 12% have either Type 1 (12,000) or Type 2 diabetes (50,000) pregnancy is a diabetogenic condition characterized by insulin resistance with a compensatory increase in β-cell response and hyperinsulinemia the placental secretion of hormones is a major contributor to the insulin resistance, which likely plays a role in ensuring that the fetus has an adequate supply of glucose. Pregnant women with diabetes are associated with an increased incidence of congenital abortions in women with poor glycemic control (Gorge et al., 2003).

Genetic analysis of IRS-1 gene has shown that a glycine-to-arginine substitution at codon 972 (Gly972Arg mutation), which occurs in about 6% of the general population, significantly impairs IRS-1 function and is associated with insulin resistance, lipid abnormalities, and Type 2 Diabetes Mellitus.

Pregnancy is potentially a diabetogenic condition. Normal human pregnancy is associated with hyperinsulinemia and a progressive decline in insulin sensitivity (Friedman et al., 1999). There is an average 60% decrease in insulin sensitivity with advancing gestation in women with normal as well as abnormal glucose tolerance (Catalano, 1999).
The underlying abnormality in GDM and type 2 diabetes includes either insulin resistance, impaired insulin secretion or a combination of both. When the insulin resistance of pregnancy is superimposed on a predisposed individual, glucose intolerance develops. Several genes have been investigated for association with GDM development.

A sixfold increase of the insulin infusion rate still showed a more marked insulin resistance in pregnancy. Obese patients with previous GDM had significantly greater insulin resistance compared with lean patients. All women had NGT at postpartum investigations (Catalona et al., 1986).

A measure of the insulin sensitivity and the insulin response to glucose was investigated. (Bergman et al., 1985). Insulin sensitivity was found to be similarly reduced in pregnant women with NGT and women with GDM both groups displayed an insulin sensitivity that was only about one-third that of a group of non-pregnant women of similar age and relative weight.

This marked insulin resistance was compensated by reciprocal enhancement of the first- and second-phase insulin responses to the intravenous glucose injection in the pregnant women with NGT, whereas the mean first-phase insulin response was significantly reduced in the women with GDM compared with that of pregnant women with NGT.

Decreased insulin-receptor binding has been observed in human obesity and non-insulin-dependent diabetes mellitus, which are, like pregnancy, characterized by decreased glucose tolerance and increased insulin resistance (Pedersen et al., 1984).

Insulin-receptor binding increased significantly in the women with GDM after dietary treatment had been instituted (Andersen et al., 1986). The changes in insulin-receptor binding to monocytes and adipocytes may be secondary to various pregnancies associated endocrine and metabolic factors, which again may have different effects on different tissues. In adipocytes, insulin-receptor binding is increased by estradiol (Ballejo et al., 1983 and Ryan et al., 1988) and relaxin (Jarrett et al., 1984). Progesterone may increase (Mendes et al., 1985) or decrease the binding, and cortisol decreases or does not affect the binding (Watanabe et al., 1984). Prolactin decreases the insulin binding to adipocytes (Jarrett et al., 1984), whereas gastric inhibitory polypeptide exerts an increase (Starich et al., 1989). But the present hormone is decreased during
pregnancy (Hornes et al., 1981). Human chorionic gonadotropin and human placental lactogen do not influence insulin-receptor binding to adipocytes (Ryan et al., 1988).

2.6 Study of LDL particle

Some investigators attributed the increased risk of developing Type 2 Diabetes Mellitus and hypertension in women with history of GDM to their dyslipidemic profiles (Enquobahrie et al. 2005, Lauenborg et al. 2005, AbouGhalia et al. 2003). Low density lipoprotein (LDL) particles are the major carriers of plasma cholesterol in humans LDL exhibits substantial heterogeneity based on measures of size, density, and chemical composition. Krauss and Burke (1982), with the use of analytical procedures such as gradient ultracentrifugation, identified many distinct subclasses of LDL in plasma samples from healthy humans. Austin et al. (1988) categorized LDL particles into two major classes phenotypes denoted as A and B. LDL particles and subclass phenotype B is characterized LDL particles. Subjects with subclass phenotype B tend to have atherogenic lipoprotein profiles, including higher plasma triglycerides, very low density lipoprotein (VLDL), apoprotein B concentrations and lower concentrations of apoprotein AI and high-density lipoprotein (HDL), and an increased fraction of small, dense LDL particles are frequent lipoprotein abnormalities noted in subjects with insulin resistance and Type 2 Diabetes Mellitus (Selby et al., 1993, Austin et al., 1995, Syvanne and Taskinen 1997, Goff et al., 2005).