Discussion

5.1 Evaluation of biochemical, haematological and immunological alterations in type 2 diabetics.

5.1.1 Evaluation of biochemical alterations in type 2 diabetics.

This chapter deals with the biochemical alterations associated with type 2 diabetic patients. Fasting and postprandial blood glucose were found to be significantly elevated in type 2 diabetics (group II) when compared to normal subjects (group I). This is mainly due to hyperglycemia occurring in group II subjects. Increased hepatic glucose output predominantly accounts for increased FBG levels, whereas decreased peripheral glucose usage results in postprandial hyperglycemia (Satiel et al., 1996).

In type 2 diabetes, hepatic insulin resistance is believed to underlie the elevated basal rates of EGP and subnormal postprandial suppression of EGP. In a recent study elevated EGP was found to be a primary mechanism for postprandial hyperglycemia in type 2 diabetes (Waerle et al., 2006)

Hyperglycemia may induce the onset and development of macro vascular disease thereby affecting the three determinants of atherosclerosis: arterial dysfunction, hematological disturbances and lipid abnormalities. Hyperglycemia induces such effects through two mechanisms oxidative stress and non-enzymatic glycation.

The sources of oxidative stress in diabetes include the following: glucose auto oxidation with generation of hydrogen peroxide and superoxide; protein glycosylation and amadori auto oxidation with consequent production of superoxide oxidation and reduced antioxidant status due to reduced levels of vitamin C and E. Activation of sorbitol pathway leads to low levels of reduced glutathione (Ceriello and Gingliano, 1997)

Non enzymatic glycation generates advanced glycation end products (AGE’s) that can alter the structural and functional properties of proteins thereby contributing to the processes that lead to atherogenesis. In the arterial wall, hyperglycemia produces
structural and functional alterations in all three constitutive elements: endothelium, smooth muscle cells and matrix. Hyperglycemia induces apoptosis of endothelial cells with consequent loss of intact endothelium and exposition of sub endothelium which facilitates thrombosis.

Various investigators have reported that this effect is dependent on glucose concentration and time. (Baumgartner Parzer et al., 1995; Curcio and Ceriello, 1992). This confirms that metabolic control plays a crucial role in the prevention of macro vascular complications. Recent evidence has also demonstrated that apoptosis is generated by activation of nitrogen-activation protein kinase ($\text{p}^{38} \text{MAPK}$) secondary to the generation reactive oxygen species (Igarashi et al., 1998).

Endothelial permeability at the tight junction level is increased when the glucose concentration is high. This effect of glucose seems to be moderated by activation of protein kinase Cα (Holler, 1997).

Adhesion of monocytes to the endothelium is the central event in the development of atherosclerosis. Long term exposure of endothelial cells to high glucose levels increases the binding of monocytes to the endothelium. This event seems to be mediated by the activation of PKC α. Advanced glycation end products are increased in type 2 diabetic patients and they enhance the binding of monocytes to the endothelium (Kim et al., 1994).

Hyperglycemia enhances endothelial generation of thrombin and tissue factor and leading to the expression of plasminogen activator inhibitor-1 (PAI-I) and reduced tissue plasminogen activator (t-PA). Glycated lipoprotein (a) seems to be responsible for the alterations in PAI-I and t-PA levels (Schor, 1997).

Vascular smooth muscle cells participate in atherogenesis in two ways: Cellular proliferation with successive migration from the media to the intima and cholesterol engagement with consequent transformation into foam cells.

Reduced vascular dispensability associated with increased wall thickness is characteristic of arterial wall disturbance in diabetic macro vascular disease. These
effects seem to be a result of glucose induced AGE formation producing cross linking of matrix proteins such as elastin and collagen.

Collagen to collagen cross linking may explain the increased tissue rigidity and the cross linking processes may decrease the proteolytic enzyme digestion of matrix proteins, leading to increased thickness. (Vlassara, 1997)

High levels of blood glucose are associated with shifts in coagulation towards thrombophilia due to enhanced coagulation and impaired fibrinolysis. (Winocous et al., 1990).

Population studies have shown glucose to be an independent cardiovascular risk factor besides other well-known risk factors such as elevated blood pressure and cholesterol levels.

Urea and creatinine levels were elevated in type 2 diabetic patients due to renal insufficiency. As the disease progresses, more albumin leaks into the urine. This condition is referred to as overt diabetic nephropathy or macroalbuminuria. As a result, the filtering function begins to drop. The body retains various wastes as filtration falls. Creatinine is one such waste and it is a measure of decline in kidney function. Hypertension is a major factor in the development of kidney problems in people with type 2 diabetics.

Hyperuricemia has been reported to be associated with increased risk of renal insufficiency in type 2 diabetic patients. Epidemiological studies have found that hyperuricemia is an independent risk factor for renal dysfunction in a general population in patients with hypertension and in patients with diabetes. (Tseng, 2005).

Group II subjects have elevated level of serum uric acid. The present study correlates well with the study of Bo et al (2002) who reported that hyperuricemia is associated with the insulin resistant syndrome and with early onset or increased progression to overt nephropathy in patients with type 2 Diabetes Mellitus.
The main pathophysiological mechanism by which uric acid causes renal dysfunction involves an initiation of endothelial nitric oxide bioavailability (Khosla et al., 2005) activation of the rennin angiotensin system and direct actions an endothelial cells and VSMC (Kang et al., 2005). Uric acid has been shown to stimulate the production of IL-6, TNF-α and CRP by human vascular cells (Kanellis et al., 2005). Protein glycation is widespread and glycation of hemoglobin (HbA1c) probably reflects the level of glycation of other proteins. Protein glycation is probably important in leading to diabetic microvascular complications and inhibition of glycation can prevent the development of these complications.

Glycation of hemoglobin occurs as a two step reaction resulting in the formation of a covalent bond between the glucose molecule and the terminal valine of the beta chain of the Hb molecule. The rate at which this reaction occurs is related to the prevailing glucose concentration. Glycated hemoglobin is expressed as a percentage of the normal hemoglobin. This test provides an index of the average blood glucose concentration over the life of the hemoglobin molecule.

In the present study, the level of glycosylated Hb was found to be significantly elevated in type 2 subjects when compared to normal subjects. Hence HbA1c tests may be considered as one of the most reliable prognostic markers for vascular and neurological complications in type 2 diabetic population. Higher the HbA1c level the worse is the prognosis.

Insulin is a key hormone for the regulation of blood glucose and generally normoglycemia is maintained by the balanced interplay between insulin action and insulin secretion.

In type 2 diabetic patients, hyperglycemia is produced because of beta - cell dysfunction and is an actual component in the pathogenesis of type 2 diabetes. This correlates well with the results of the present study in group II subjects (type 2 diabetics).
This concept has been verified not only in cross-sectional studies but also longitudinally in pima Indians progressing from normal to impaired glucose tolerance in type 2 diabetes. (Weyer et al., 1999)

Cross-sectional studies have reported that patients with type 2 diabetes and cardiovascular disease have fasting hyperinsulinemia compared with those without cardiovascular disease (Ronnemaa et al., 1999). Because hyperinsulinemia is often clustered with other cardiovascular risk factors, the presence of endogenous hyperinsulinemia combined with hypertriglyceridemia, increased body mass index and a decreased HDL-C increases the risk of CHD death in patients with type 2 diabetes (Lento et al., 2000). Depres et al (1996) also reported that people with hyperinsulinemia and high TG have an increased risk for CHD.

Homocysteine is a sulfur containing aminoacid formed during the metabolism of methionine. The present study shows elevated levels of plasma homocysteine in type 2 diabetic patients as indicated in table 2. This is found to be associated with high levels of fibrinogen, microalbuminuria and hypertension.

Dietary deficiencies of the B vitamins (B6,B12) and folic acid increase plasma homocysteine concentrations produced by mutations affecting genes encoding enzymes involved in methionine metabolism.

Subclinical renal dysfunction can be another cause of this elevation. Plasma homocysteine levels rise in parallel to serum creatinine as the glomerular filtration rate falls. (Arnadottir et al., 1996).

HbA1c values correlated positively with homocysteine concentration in poorly controlled type 2 diabetic subjects. It was thus concluded that chronic poor metabolic control in type 2 diabetes is characterized by elevation of plasma homocysteine concentration which also inversely correlates with endogenous insulin levels. These results may add to the understanding of the increased frequency and mechanisms of vascular damage in diabetes mellitus (Drzewoski et al., 2000)
The present study reveals elevated levels of TG, total cholesterol and LDL in group II diabetic subjects as given in Table 3. Hypertriglyceridemia is common in type 2 diabetes and is usually accompanied by low HDL-C. Fasting hypertriglyceridemia is associated with abnormal prolonged postprandial lipidemia which is associated with increased cardiovascular risk. Hypertriglyceridemia leads to significant decrease in LDL levels. Increased PAI-1 correlate positively with plasma TG levels. Hypertriglyceridemia can be produced by two mechanisms.

(i) Increased synthesis of VLDL TG.

(ii) Decreased clearance of plasma TG.

Hepatic triglyceride has been shown to be strongly associated with hepatic insulin resistance in type 2 diabetes (Krassak et al., 2004). The exact mechanism by which hepatic triglyceride induces hepatic insulin resistance is unknown but is thought to relate to accumulation of intracellular fatty acid metabolites and consequent activation of serine kinase cascade and induction of cellular insulin resistance.

The total cholesterol levels get significantly elevated in type 2 diabetic patients (group II) because of hyperglycemia. Free fatty acids are believed to play a major role in increased hepatic gluconeogenesis and overproduction of triglyceride rich VLDL which in turn lead to higher levels of small dense atherogenic LDL and decreased cardioprotective HDL (Lewis et al., 2002).

The abnormal lipoprotein metabolism that results from the metabolic syndrome negatively influences endothelial function and the atherogenic process.

Low HDL-cholesterol is a typical symptom of diabetic dyslipidemia together with hypertriglyceridemia and small dense LDL (Tadkinen, 2003). As HDL-Cholesterol is frequently low in patients with hyperglycemia, low HDL-C can be considered to be an indirect surrogate marker of a metabolic situation, which favors β-cell death.

HDL has mutual relationships with both diabetes mellitus and inflammation. These mutual relationships result in vicious cycles in which both insulin resistance in the prediabetic state and inflammation cause qualitative and quantitative changes in HDL.
which contribute to the escalation of the clinical presentation into manifestations of diabetes mellitus and organ failure.

As diabetes mellitus, inflammation and low HDL-cholesterol also play important pathogenetic roles in atherosclerosis and as obesity importantly contribute to all of these three risk factors, these vicious cycles may also explain the high cardiovascular risk of patients with the metabolic syndrome. Being in the intersection of these mutual relationships, HDL is an important target to prevent atherosclerosis and potentially diabetes mellitus and organ failure.

Fatty acids are key mediators of storage and release of adipocyte derived signaling factors affecting insulin sensitivity. FFA levels were found to be elevated in type 2 diabetic patients (group II) given in table 3 of the present study. High levels of free fatty acids have been linked to the induction of insulin resistance because increased FFA metabolism in the liver leads to increased gluconeogenesis.

FFA induces insulin resistance at the level of insulin stimulated glucose transport or phosphorylation by impairing the insulin signaling pathway. Another mechanism by which free fatty acids can cause insulin resistance is by increasing oxidative stress. Reactive oxygen species can activate PKC and the NF-κB pathway and thereby contribute to insulin resistance (Itani et al., 2002). FFA also affect the functioning of insulin in the liver and thus contribute to hepatic overproduction of glucose and to elevated circulating blood glucose levels.

High levels of FFA have emerged as a major link between obesity, insulin resistance and type 2 diabetes. Elevated levels of FFA activate the proinflammatory and proatherogenic nuclear factor kappa beta pathway. Thus elevated plasma levels of FFA in obese subjects can produce a low grade inflammatory state which may contribute to accelerated atherosclerosis.

The level of hepatic enzymes such as ALT, AST and ALP were found to be above the normal range in group II diabetic patients. Mild chronic elevations of transaminases often reflect the underlying insulin resistance.
Aminotransferases such as alanine aminotransferase (ALT) and aspartate amino transferase (AST) measure the concentration of intracellular hepatic enzymes that have leaked into the circulation and serve as a marker of hepatocyte injury. Alkaline phosphatase (ALP) act as marker of biliary function and cholestasis.

The liver helps to maintain normal blood glucose concentration in the fasting and postprandial states. Loss of insulin effect on the liver leads to glycogenolysis and an increase in hepatic glucose production. Abnormalities of triglyceride storage and lipolysis on insulin sensitive tissues such as the liver are early manifestations of conditions characterized by insulin resistance and are detectable earlier than fasting hyperglycemia (Lewis et al., 2002).

In animal models, chronic hyperinsulinemia is found to predispose the liver to relative resistance to insulin. This is characterized by a failure of insulin to signal an increase in insulin receptor substrate -2. Upregulation of sterol regulatory element binding protein 1C (SREBP - IC) also occurs, leading to increased lipogenesis (Shimomura et al., 2000). Despite down regulation of the insulin receptor substrate -2 mediated insulin signaling pathway in insulin resistant states, the upregulation of SREBP-1C and subsequent stimulation of the de novo lipogenesis in the liver leads to increased intracellular availability of triglycerides, promoting fatty liver. This also increases VLDL assembly and secretion. (Lewis et al., 2002). Thus hyperinsulinemia might directly lead to hepatic insulin resistance with associated fatty changes.

The excess amount of free fatty acids found in the insulin resistant state is known to be directly toxic to hepatocytes. The putative mechanisms include cell membrane disruption at high concentration, mitochondrial dysfunction of metabolism (Neuschwander et al., 2003).

Other potential explanations for elevated transminases in insulin resistant states include oxidative stress from reactive lipid peroxidation, peroxisomal beta oxidation and recruited inflammatory cells. The insulin resistant state is also characterized by an increase in proinflammatory cytokines such as tumor necrosis factor - α (TNF - α) which may also contribute to hepatocellular injury. In preliminary studies an increased
frequency of specific TNF-α promoter polymorphisms was found in nonalcoholic steatohepatitis (NASH patients) suggesting a possible genetic link or predisposition to fatty liver found in insulin resistant states (Grove et al., 1997).

The above mechanisms attribute elevated transaminis to direct hepatocyte injury. It is also hypothesized that elevation in ALT, a gluconeogenic enzyme whose gene transcription is suppressed by insulin could indicate an impairment in insulin signaling rather than purely hepatocyte injury (O Brein and Granner, 1991).

Vozaroza et al (2002) followed 451 non-diabetic pima Indians for an average of 6.9 years to determine whether hepatic enzyme elevations could be linked to the development of type 2 diabetes. At baseline, ALT and AST were related to percent body fat. Prospectively, increasing ALT concentrations were associated with a decline in hepatic insulin sensitivity and risk of type 2 diabetes. The authors concluded that higher ALT is a risk factor for type 2 diabetes and indicates a potential role of increased hepatic gluconeogenesis and/or inflammation in the pathogenesis of type 2 diabetes.

Qualitative analysis of urine in type 2 diabetic patients (group II) showed positive results for both glucose and protein and negative result for urinary deposits. Microalbuminuria (MAU) refers to urinary albumin excretion rate of more than 20 mg/min and less than or equal to 200 mg/min. As MAU is predictive of glomerular damage, its early detection helps in reversion and/or preventing progression to kidney damage by medical intervention. Microalbuminuria develops with 10-15 years from the onset of hyperglycemia and usually progresses with 3 to 7 years to overt proteinuria.

MAU was found to be above the normal range in group II subjects. There is now overwhelming evidence that hyperglycemia is the major initiating factor in the pathogenesis of diabetic complications including microalbuminuria. However most adverse effects of glucose are mediated indirectly through diverse metabolic pathways involving AGE formation, polyol pathway activation of protein kinase C and increased flux through the hexosamine pathway.
The development of microalbuminuria in individuals with type 2 diabetes mellitus is associated with a 10 fold increase in the risk of progression to overt nephropathy and essential end stage renal failure. Studies in pima Indians (Nelson et al., 1995) with type 2 diabetes have identified age, duration of diabetes, HbA1c, serum cholesterol and initial level of urinary albumin excretion as risk factors for progression from normoalbuminuria to either micro or macro albuminuria.

Emerging evidence suggests that animal models with insulin resistance have increased production of the highly atherogenic apoB-48 by the intestine, which may explain the profound postprandial lipemia observed in insulin resistant states. (Ginsberg et al., 2005 and Federico et al., 2006).

The study subjects with microalbuminuria had higher by almost two fold insulin resistance compared with subjects without MAU and the increase of postprandial lipemia was mainly attributable to the increase of intestinally derived TG. Therefore the findings support the notion that TG-rich lipoprotein assembly and secretion in the intestine may be different in patients with MAU.

5.1.2 Evaluation of haematological alterations in type 2 diabetics.

This chapter deals with the hematological alterations associated with type 2 diabetes. There was no alteration in the number of erythrocytes among type 2 diabetic patients. This concept was reflected in the results obtained for RBC count in group II subjects.

In the present study the leukocyte count was found to be significantly elevated above the normal range in group II subjects. This is due to the close association between WBC count and both micro and macro vascular complications through inflammation which serves as a common linking factor.
The raised leukocyte count reflects the effects of low grade inflammation initiated by obesity or other mechanisms. Because cytokines can raise leukocyte count, leukocyte count may act as a surrogate marker for such cytokines.

A recent study has also demonstrated a relationship between albuminuria and WBC count (Cavalot et al., 2002) as seen in the present study. Leukocyte count is positively correlated to CVD incidence and mortality as well as to traditional cardiovascular risk factors such as smoking, hyperlipidemia and insulin resistance (Danesh et al., 2000; Huang et al., 2001).

All the type of leukocytes were found to be elevated in group II subjects except basophils which was found to be within the normal range. Eosinophils act as first line defense cells and the reduction of their functional activity contributes to the high susceptibility and severity of infections in diabetes mellitus.

Neutrophil count increases postprandially in type 2 diabetics when TG and glucose rise (Van Oostrom et al., 2003). This neutrophil increase is associated with the production of pro inflammatory cytokines and oxidative stress and it has been suggested that these changes may contribute to endothelial dysfunction (Van wijk et al., 2005).

Monocytes which are present as macrophages in adipose tissue are likely to contribute to the production of inflammatory mediators either alone or in concert with adipocytes, which suggests the potential influence of macrophages in promoting insulin resistance. Monocytes and macrophages were found to contribute significantly to LDL oxidation.

ESR was significantly elevated above the normal range in group II subjects. ESR measures the tendency of red blood cells to aggregate. It is a time-honored, routine analysis mainly used to screen for the presence of hidden inflammation. ESR was directly associated with age, serum cholesterol, hypertension, heart rate, BMI and diabetes but inversely related to alcohol consumption.

The major determinants of ESR are the concentration of positively charged inflammatory proteins such as fibrinogen, IgM and alpha-2-macroglobulins (Festa et al.,
2000). Elevated ESR and high white blood cell count had been related to a worsening of insulin sensitivity or increased incidence of type 2 diabetes.

5.1.3 Evaluation of immunological alterations in type 2 diabetics.

This chapter deals with the immunological alterations associated with type 2 diabetes. The levels of immunoglobulin’s such as IgA, IgG, IgM and IgE were found to be significantly elevated in group II subjects. The immunoglobulin concentrations of IgA, IgG, IgM and IgE classes have previously been reported to be higher in those with type 2 diabetes. (Arsawi et al., 1994).

In the present study higher levels of gamma globulin, a nonspecific measure of the adaptive immune system was found to be associated with higher incidence of type 2 diabetes. These observations support a number of recent observations suggesting a role of inflammation or infection in the pathogenesis of type 2 diabetes.

The increased levels of IgG and IgM that accompanies albuminuria in type 2 diabetes mellitus might be due to alteration of the size selective properties of the glomerular capillary wall (Bakoush et al., 2002).

C reactive protein is a plasma protein an acute phase protein produced by the liver and by adipocytes. It is a member of the pentraxin family of proteins (Lan et al., 2005). The level of CRP was found to be significantly elevated above the normal range in group II subjects. This is regulated by circulating levels of IL-6 although IL-1 and TNF-α can also induce hepatic CRP mRNA expression. Mendell et al (2002) showed that elevated CRP levels were positively associated with elevated serum levels of IL-6.

More recently, two large cross-sectional prospective studies have reported that CRP and IL-6 levels predict the development of diabetes in both obese men and women implicating CRP as a possible link in the causal relationship between obesity and diabetes (Freeman et al., 2002).

CRP is not merely an inflammatory marker but directly participates in the process of atherogenesis by modulating endothelial function (Pasceri et al., 2000). CRP may also
play a coordinating role by amplifying the proinflammatory activity of other adipokines. For example it increases the expression and activity of PAI-1 in endothelial cells (Devaraj et al., 2003).

CRP binds with the phosphocholine of oxidized LDL (Ehang et al., 2002) and upregulates the expression of adhesion molecules in endothelial cells. It increases LDL uptake into macrophages inhibits endothelial cells (Venugopal et al., 2002) and increases PAI-1 expression and activity.

The level of cortisol was significantly elevated in group II subjects. The presence of retinopathy, neuropathy and microangiopathy is directly correlated with cortisol secretion (Roy et al., 1998).

In type 2 diabetic subjects hypothalamic pituitary adrenal activity is enhanced in patients with diabetic complications and the degree of cortisol secretion is related to the presence and the number of diabetic complications. Hypercortisolinemia results in hyperplasia, central obesity, high concentration of VLDL, insulin resistance and predisposition to diabetes.

Non-insulin dependent diabetic patients have elevated levels of PAI-1 (Juhan-Vagne et al., 1989). This correlates well with the result obtained for type 2 diabetic subjects in group II.

Synthesis of PAI-1 is increased by several stimuli including endotoxins, cytokines (IL-1, TNF-α), growth factors (TGF-β) and hormones (Glucocorticoids). TNF-α is a potent inducer of PAI-1 synthesis in insulin resistance syndrome. (Hotamistigil et al., 1993).

Plasminogen activator inhibitor 1 is a prothrombotic factor secreted from endothelial cells, mononuclear cells, hepatocytes, fibroblasts and adipocytes that negatively regulates fibrinolysis by inhibiting tissue plasminogen activator. The connection between PAI-1 and cardiovascular disease is more firmly established and PAI-1 has also been associated with insulin resistance (Alessi et al., 1997) obesity.
glucose intolerance (Meigs et al., 2000) and type 2 diabetes (Leurs et al., 2002) in cross-sectional studies.

In addition adipocytes from the visual fat depot produces significantly more PAI1 messenger RNA than adipocytes derived from subcutaneous tissue from the same individual (Morange et al., 1999). Thus PAI-1 has been suspected to provide a link between the increased predisposition of atherosclerosis in patients with the metabolic syndrome (Bastard et al., 2000).

Elevated PAI-1 levels was found to be an independent risk factor for the development of type 2 diabetes in healthy subjects in the IRAS study (Festa et al., 2002) suggesting that they may be a very early risk marker for the development of the metabolic syndrome and type 2 diabetes.

Plasma fibrinogen is an important component of the coagulation cascade as well as a major determinant of blood viscosity and blood flow. It is influenced by many factors such as age, BMI, smoking, diabetes, LDL cholesterol and leukocyte count. Epidemiological studies consistently have found a significant association to exist between fibrinogen levels, insulin levels in glucose tolerant women only. (Meigs et al., 2000), body mass index (Folson et al., 1991) and reduced HDL.

Plasma fibrinogen was significantly elevated in group II subjects of the present study because of inflammation. In the atherosclerosis risk in communities study fibrinogen was significantly higher in subjects with diabetes than those without diabetes in men and women (Misser et al., 1996). A positive correlation between fibrinogen and Hb A1C has been observed in various studies (Ceriello et al., 1998)

Fibrinogen is a soluble glycoprotein found in plasma with a molecular weight of 340 KDA. (Daolittle et al., 1998). It comprises of three parts of non-identical polypeptide chains linked to each other by disulphide bonds (Herrick et al., 1998). Fibrinogen has a biological half-life of about 100 hrs and is synthesized predominantly in the liver (Haidaris et al., 1989).
A broad range of abnormalities in thrombosis and fibrinolysis is associated with cardiovascular dysmetabolic syndrome and insulin resistance. Recent evidence suggest that inflammation may play an important role in the pathogenesis of CVD in type 2 diabetes. Fibrinogen may be an acute phase reactant and similar to CRP is increased in type 2 diabetes and insulin resistance. It is converted to fibrin and thus promotes thrombosis.

Increased levels of fibrinogen and PAI-1 indicate a prothrombotic, proinflammatory state that may result in arterial lesion progression and subsequent coronary events (Fuster et al., 1992).

High C3 levels have been reported in group II subjects with type 2 diabetes in the present study. This is in agreement with the study of (Weyer et al., 2000).

C3 is mainly produced in the liver in response to proinflammatory cytokines such as IL-6 (Ritchie et al., 2004). The relation between C3 and incidence of diabetes could reflect a systemic low-grade inflammation and the actions of IL-6 and TNF-α. ASP is a proteolytic fragment of C3 and diabetes. ASP stimulates glucose uptake and lipid storage in adipose tissue (Maslowska et al., 1997)

Increased C3 concentrations may indicate the progression of atherosclerosis. It has been postulated in pima Indians that C3 is the mediator linking adiposity, insulin resistance, hyperinsulinemia and possibly atherosclerosis (Weyer et al., 2000).

Cross-sectional studies have reported strong correlations between plasma levels of C3, insulin and glucose (Engstrom et al., 2005). C3 is inversely and independently associated with insulin sensitivity (Weyer et al., 2000).

Ceruloplasmin is a circulating blue multicopper oxidase that contains greater than 95% of copper in plasma. Ceruloplasmin is synthesized mainly in the liver as a single chain polypeptide and then secreted into the plasma as an α2 glycoprotein.

An increase in serum ceruloplasmin levels has been reported in type 2 diabetes (Walter et al., 1991). The increase is due to hyperglycemia in type 2 diabetic patients. A
increase in serum ceruloplasmin in type 2 diabetes could generate excess oxidized LDL which causes atherosclerosis (Ethrenwald et al., 1994).

An increased level of oxidized LDL is known to impair the endothelium-dependent relaxation of arteries resulting in atherosclerosis. Elevated ceruloplasmin levels may also cause vascular injury by generating free radicals such as hydrogen peroxide (Starkebaum et al., 1986).

Alpha-1-antitrypsin is produced in liver monocytes and alveolar macrophages. α₁AT is an acute phase reactant. It is upregulated during acute phase response to tissue necrosis and inflammation.

The level of α₁AT was found to be significantly elevated in type 2 diabetic subjects (group II) when compared with group I control individuals. This is mainly mediated by activation of inflammatory cascade in type 2 diabetic patients.

The level of Haptoglobin was also found to be elevated in group II cases when compared with group I subjects. Haptoglobin (HP) is a glycoprotein involved in the acute phase response to inflammation. A strong association has been documented between insulin sensitivity and inflammatory response so that a direct relationship between age and serum HP levels can be explained by changes in insulin sensitivity (Yudkin et al., 1999).

A strong positive correlation was found between circulating haptoglobin and BMI. The findings are in line with those of (Hannerz et al., 1995) who reported that serum haptoglobin was moderately increased in a group of 20 obese female subjects compared with controls and point to haptoglobin as a possible novel marker of adiposity.

The simple increase in the adipose mass of obese subjects due to an hypertrophy and hyperplasia of the tissue may account for the higher level of serum haptoglobin (Hausman et al., 2001). Hence Haptoglobin could constitute an important link between obesity and its comorbidities by mediating some of the inflammatory effects associated with the obesity status in type 2 diabetes.
Haptoglobin is synthesized in liver and IL-6 is thought to be the main cytokine that induces the synthesis of haptoglobin in the liver (Castell et al., 1989).

IL-6 has been considered as an important proinflammatory cytokine. IL-6 plasma concentrations correlate with the development of type 2 diabetes mellitus (Tsigsu et al., 1997). The results of the present study also reveal elevated levels of IL-6 in type 2 diabetics as given in table 8.

Plasma concentration of IL-6 increase with obesity, unlike those of TNF-α, which acts in an autocrine and paracrine fashion. In obese individuals, adipose tissue is a major determinant of plasma IL-6 concentrations contributing to as much as 30% of total body production. IL-6 increases lipolysis and fat oxidation in humans (Ran Hall et al., 2003) and plasma IL-6 concentrations correlate with insulin resistance (Kern et al., 2001). Elevated IL-6 concentration is a predictor for the development of type 2 diabetes and for myocardial infarction.

Among the putative cytokines involved in atherosclerosis IL-6 appears to link local systemic inflammation and the hepatic acute phase response, acting as a messenger molecule, causing rise in CRP and fibrinogen.

TNF-α or cachectin exists as a trimer and is one of the products of activated macrophages, fibroblasts, T cells and NK cells (Smith and Baglioni, 1987). TNF-α has proinflammatory property TNF-α also shares an important inflammatory property with IL-6 (i.e.) induction of acute phase reactant protein production by the liver TNF-α exert secondary inflammatory effects by stimulating IL-6 synthesis in several cell types.

There is accumulating data to suggest that TNF-α plays a direct role in the metabolic syndrome. Patients with type 2 diabetes demonstrate high expression of TNFα in skeletal muscle and in plasma (Mishima et al., 2001). The results of the present study also show similar results in type 2 diabetic patients (group II). TNF-α was found to impair insulin stimulated rates of glucose storage in cultured human muscle cells and impairs insulin mediated glucose uptake in rats (Yound et al., 2000).
TNF-α has direct inhibitory effects on insulin signaling (Paraldi et al., 1996) and in addition, it has been proposed that TNF-α cause insulin resistance in vivo indirectly by increasing the release of free fatty acids from adipose tissue (Souzar et al., 1998). TNF-α increases lipolysis in humans (Ryden et al., 2002). Recently it was found that TNF-α has no effect on muscle fatty acid oxidation but increased fatty acid incorporation into diacylglycerol, which may be involved in the development of TNF-α induced insulin resistance in skeletal muscle. (Bruce et al., 2004).

TNF-α exert its effects in insulin signaling in three ways firstly it reduces tyrosine phosphorylation by increasing serine or threonine phosphorylation of the insulin receptor (Hotamisligil et al., 1996). Secondly it down regulates GLUT 4 and the enzyme responsible for insulin signal transduction (Stephens et al., 1997). It has also been shown that TNF-α increases ceramide, a lipid which down regulates GLUT-4 gene transcription in adipocytes (Long and Pekala, 1996). Thirdly, TNF-α increases release of free fatty acids by stimulation of lipolysis. This process is dependent on the down regulation of the lipid droplet associated protein peril pin. Perilipin is thought to prevent the accession of hormone sensitive lipase to the surface of the fat droplet where lipid degradation takes place.

TNF-α can disturb normal endothelial functioning. This disturbance in turn can impair insulin mediated vasodilatation resulting in delayed trans endothelial insulin transport reduced glucose disposal and eventually insulin resistance (Pinkney et al., 1997 and Bhagat et al., 1997).)

TNF-α may cause insulin resistance by impairing insulin signaling and indirectly by stimulating FFA production which may also increase insulin resistance. Furthermore TNF-α, IL-6 and FFA may affect β cell functioning. (Searim et al., 1997).

Adiponectin also known as AdipoQ and Acrp 30 is a complement factor (C1q) abundantly expressed in adipocytes that increases fat oxidation and insulin sensitivity. It is a 244 amino acid protein which has been shown to be associated with insulin sensitivity better lipid profile, decreased inflammation and improved glycaemic control. (Kadowaki et al., 2006).
In this study low levels of adiponectin were observed in type 2 diabetic subjects similar to that reported in a south Indian study (Mohan et al., 2005). Adiponectin may stimulate fatty acid oxidation in skeletal muscle, decreasing intramyocellular accumulation of TG and potentially accelerating the catabolism of TG rich lipoproteins (Yamuchi et al., 2002). Adiponectin may also decrease FFA flux to the liver and hepatic glucose output (Berg et al., 2001).

Hypertriglyceridemia, low HDL cholesterol and decreased LDL particle size were shown recently in humans to be correlated with low plasma adiponectin levels independent of the degree of intra abdominal fat and insulin resistance (Baratta et al., 2004).

The strength of the correlations in the present study between adiponectin and elevated TG on the one hand and low HDL cholesterol on the other hand suggests that low adiponectin may be associated with increased generation of small dense LDL particles as well as higher catabolism of HDL apo A1 (Packard, 2003). Adiponectin has recently been demonstrated to have several antiatherogenic functions, involving antiadhesive, antiproliferative and antioxidant properties.

5.2 Effect of rosiglitazone in type 2 diabetic patients.

5.2.1 Effect of rosiglitazone on biochemical parameters in type 2 diabetic patients.

This chapter deals with the effect of rosiglitazone on biochemical parameters in type 2 diabetic patients. Thiazolidinediones, a new class of oral antidiabetic agents reduce hyperglycemia by decreasing insulin resistance in peripheral tissues.

Rosiglitazone is a potent member of the thiazolidinedione class with a binding affinity for PPAR Y that is ~ 100 fold greater than that of pioglitazone and 190-fold greater than that of Troglitazone (Sirtori et al., 1977). Rosiglitazone therapy is not associated with either hypoglycemia or GIT intolerance. The benefits of rosiglitazone in reducing glucose levels apply to wide spectrum of patients with type 2 diabetes.
In addition to the effects on glucose metabolism, *rosiglitazone* has effects on lipid metabolism, inflammatory responses and cellular proliferation (Desvergne *et al*., 1999). The efficacy and safety of *Rosiglitazone* as monotherapy have been established in many studies. (Patel *et al*., 1999; Heboritz *et al*., 2001).

Studies suggest that the thiazolidinedione *rosiglitazone* in addition to lowering blood glucose concentrations, has potentially beneficial effects on overall cardiovascular risk (Haffner *et al*., 2002). The cardio protective effect of *rosiglitazone* may be indicated by inhibiting the activation of JNK-AP-1 pathway.

*Rosiglitazone* improves glycemic control primarily by increasing insulin sensitivity in skeletal muscle, liver and adipose tissue (Wagstaff *et al*., 2002). *Rosiglitazone* has a beneficial impact on a number of factors associated with insulin resistance and cardiovascular disease, including microalbuminuria, hypertension, dyslipidemia, visceral fat and elevated PAI-1 levels (Viberti *et al*., 2003).

*Rosiglitazone* decreases fasting plasma FFA levels (Miyazaki *et al*., 2001) probably due to improved peripheral fat storage, but it has only minor effects in fasting plasma triglycerides (Van *et al*., 2005).

The lowering of free fatty acids, lipids and TG by *rosiglitazone* have a beneficial effect on the survival of the β cell agents of great value in preserve β-cell function (walter *et al*., 2005). This has been shown to be the case in animal models such as the zucker diabetic obese rats and is also probably operative in humans.

*Rosiglitazone* act both directly and indirectly on the β cell. The indirect effects are through decreased lipotoxicity glucose toxicity and TNF - α (Leboritz *et al*., 2001). The direct effects on the β cell are produced via the PPAR- receptor. Studies show that *Rosiglitazone* may modulate insulin secretion (Juhl *et al*., 2003).

In humans, *rosiglitazone* reduces whole body insulin resistance by its insulin sensitizing effect on muscle adipose tissue and liver (Lozzo *et al*., 2003).
In the current study, *rosiglitazone* therapy decreased FFA levels and the improvement in glucose uptake was significantly associated with the decrease in the circulating FFA levels.

Chronic hyperglycemia has been demonstrated to cause the formation of free radicals, thus provoking oxidative stress in cardiomyocytes, leading to apoptosis, cell loss, myocardial thinning and compensatory hypertrophy (Flordaliso *et al.*, 2004). *Rosiglitazone* removed such toxic chronic overload as shown by reduced HbA1c and fasting glucose levels.

Microalbuminuria is an early feature of diabetic nephropathy and indicates intracranial endothelial damage. It is a marker of inflammation and an independent risk factor for cardiovascular mortality and it is strongly related to insulin resistance.

In response to defective responsiveness of peripheral tissues, vascular plasma insulin may rise to supranormal concentrations that may sustain glomerular hyperfiltration, endothelial dysfunction and increased vascular permeability that eventually result in increased albumin ultrafiltration and leakage into the urine. Furthermore, impaired insulin sensitivity is associated with altered renal cellular metabolism and electrolyte composition, mesangial hyperplasia, renal hypertrophy and increased endothelial cell proliferation.

*Rosiglitazone* ameliorated renal glomerular endothelial dysfunction as evidenced by the changes in filtration fraction and by improved bioavailability of nitric oxide. Both together may yield decreased microalbuminuria and perhaps nephroprotection.

A decrease in liver function tests was demonstrated with *rosiglitazone* in the present study. This may be due to normalization of liver function by *rosiglitazone*. A study by Neushwander *et al* (2004) used *rosiglitazone* 4 mg three daily for 48 weeks in the treatment of diabetes of the 25 patients who completed the study all had significant improvement in mean serum ALT levels, changing from a baseline of 104 units/l to 42 units/l at 48 weeks. This also confirms the absence of hepatotoxicity due to the administration of *rosiglitazone* in type 2 diabetic patients.
5.2.2 Effect of *rosiglitazone* on hematological parameters in type 2 diabetic patients.

This chapter deals with the effect of *rosiglitazone* on hematological parameters in type 2 diabetic subjects. The levels of RBC and WBC were found to be within the normal range in group III subjects. Differential count was also found to be normal in all the group III subjects.

Normal level of RBC indicates normal erythrocyte status thereby ruling out anemia during *rosiglitazone* administration. White blood cells and ESR were found to be within the normal range as *rosiglitazone* was involved in the control of inflammation in type 2 diabetic subjects. *Rosiglitazone* also attenuated the increase of neutrophils in patients with type 2 diabetes. This effect may contribute to control of inflammation and cardiovascular risk reduction.

5.2.3 Effect of *rosiglitazone* on immunological parameters in type 2 diabetic patients

This chapter deals with the effect of *rosiglitazone* on the immunological alterations in type 2 diabetes mellitus. The levels of immunoglobulins such as IgA, IgE, IgM and IgG were found to be within the normal range in group III subjects. This is mainly mediated by the antiinflammatory effect of *rosiglitazone* in group II subjects.

With *rosiglitazone* treatment, fasting C3 level was decreased, producing a little change in fasting ASP with the most striking change being a loss of postprandial ASP production.

In the present study, the levels of ceruloplasmin, haptoglobin and α 1 - antitrypsin were also brought to normal range by *rosiglitazone* treatment in group III subjects. This may be attributed to improvement of inflammatory status in type 2 diabetic subjects by *rosiglitazone* therapy.

The levels of CRP, IL - 6 and TNF - α were found to be within the normal range in group III subjects. This is due to the anti-inflammatory action of *rosiglitazone*.

The results of the present study confirms the data of Haffner *et al* (2002) who measured serum biomarkers - CRP, IL-6 and WBC in 357 patients with type 2 diabetes.
mellitus who completed a 26-week randomized, double blind, placebo-controlled study to assess the safety and efficacy of *rosiglitazone* at two different doses, 2 mg twice daily and 4 mg twice daily.

The present study clearly demonstrated that treatment with *rosiglitazone* in type 2 diabetic patients increased plasma adiponectin levels (Table 8). This effect may potentially protect diabetic patients from macro vascular complications and may improve their insulin sensitivity and glycemic control.

The improvement of the parameters of the metabolic syndrome in the study was accompanied with a significant (two-fold) increase of the serum adiponectin concentrations during *rosiglitazone* treatment.

A correlation between adiponectin and the components of the metabolic syndrome (serum insulin, triglycerides, glucose and systolic blood pressure) were also established.

Adiponectin plays an important role in the control of the insulin sensitivity of the peripheral organs as well as in the maintenance of the glucose homeostasis (Kahn *et al*, 2000).

The obtained results are in agreement with other studies, performed with experimental animals or cell cultures that indicate an increase of the adiponectin levels as a result of PPAR gamma agonists treatment (Maeda *et al*, 2001).

Sharabe *et al* (2007) detected an increase in adiponectin gene expression in the adipose tissue of fucntose fed rats. Iwaki *et al* (2003) and combs *et al* (2002) suggest that the mechanism of action of PPAR-gamma agonists is accomplished through activation of the peroxisome proliferator response element of the adiponectin gene, thus inducing an increase of its expression and the adipose tissue is stimulated to produce more adiponectin. A recent invivo study shared that the expression of one or two recently identified adiponectin receptor, AdipoR, is upregulated in adipose tissue but, down regulated in skeletal muscle by *rosiglitazone* (Tan *et al.*, 2005).

### 5.3 Effect of *pioglitazone* in type 2 diabetic patients
5.3.1 **Effect of pioglitazone on biochemical parameters in type 2 diabetic patients.**

This chapter deals with the effect of *pioglitazone* on biochemical parameters in type 2 diabetic patients. *Pioglitazone* is a very attractive candidate for first line glycaemic management and prevention of primary and secondary adverse outcomes in patients with metabolic syndrome, cardio diabetes and type 2 diabetes mellitus.

The results of the present study suggest that *pioglitazone* therapy in type 2 diabetic patients decreases fasting and postprandial plasma glucose levels by improving hepatic and peripheral muscle tissue sensitivity to insulin.

The mechanism of the antidiabetic action of *pioglitazone* involves activation of insulin receptors and/or high affinity PPAR-γ. Hydroxylation of the phenyl and pyridine rings in the chemical structure of *pioglitazone* may facilitate the scavenging of hydroxyl radicals. The direct antioxidant effect of *pioglitazone* may contribute to its effect on insulin resistance. The hypoglycemic and hypolipidemic effects of *pioglitazone* are likely to reduce the expression of TNF-α.

The reduction in oxidative stress may lead to the suppression of TGF-β and collagen accumulation. A decrease in collagen content is likely to improve left ventricular diastolic function and distensibility of the aortic wall. Reduction in the oxidative stress may prevent the proliferation of vascular smooth muscle cells and contribute to the decrease in the aortic wall stiffness. (Mezushige *et al.*, 2002).

*Pioglitazone* treatment improved fasting and postprandial glycemia principally via inhibition of gluconeogenesis (Gastaldelli *et al.*, 2007). *Pioglitazone* has been shown to decrease gluconeogenesis (Nishimura *et al.*, 1997) and to inhibit expression of key genes involved in gluconeogenesis (Way *et al.*, 2001). In addition free fatty acids are potent stimulators of gluconeogenesis and *pioglitazone* decrease FFA levels. (Bajaj *et al.*, 2004).

*Pioglitazone* also markedly increases adiponectin levels and adiponectin has been shown to decrease glyconeogenesis and EGP (Zhong *et al.*, 2005). *Pioglitazone* is postulated to increase tissue fatty acid oxidation through its effect on increasing
adiponectin and consequent activation of adenosine monophosphate activated protein kinase.

In patients with type 2 diabetes, the level of HbA1C was significantly decreased by pioglitazone therapy. In a 26 meek clinical trial by (Aronoff et al., 2000) comparing pioglitazone monotherapy in a dose range of 15-45 mg day with placebo in patients with type 2 diabetes, pioglitazone treatment resulted in significant improvements in glycosylated hemoglobin and fasting plasma glucose and appeared to confer additional benefit with respect to lipid parameters and fasting insulin.

Pioglitazone treatment produces significant decrease in urea and creatinine levels in type 2 diabetic patients as shown in group IV (Table 1). Pioglitazone serves as a potential therapeutic agent for diabetic nephropathy that may prevent glomerular dysfunction independent of their insulin sensitizing action through the inhibition of the DAG-PKC-ERK pathway. In cultured mesangial cells, pioglitazone prevented the high glucose induced activation of the DAG-PKC pathway activating DAG kinase (Isshiki et al., 2000).

Pioglitazone significantly decreases TG and FFA levels. Pioglitazone directly affect adipose tissue by enhancing differentiation of preadipocytes into mature adipocytes and the regulation of gene expression in adipose tissue leading to the coordinated regulation of lipid metabolism.

The anti-diabetic efficacy of pioglitazone correlates well with their rank order of binding affinity to PPAR-γ. Hence it is inferred that most of the anti-diabetic effects of pioglitazone result from PPAR-γ mediated regulation of adipocyte gene expression and the subsequent improvement in adipose physiology.

Investigations into the mechanisms of plasma TG lowering showed that abolition of hypertriglyceridemia by pioglitazone involves removal of TG from VLDL particles and decreased hepatic TG production.

Pioglitazone treatment results in decrease of homocysteine and MAU to normal range. This is mainly mediated by hypoglycemic effect of pioglitazone.
The decrease in LFT demonstration with pioglitazone therapy in type 2 diabetic patients has been shown in pilot studies using thiazolidinediones to treat NASH, a surrogate for insulin resistance. One study by Promrat et al (2003) placed 18 non-diabetic patients with NASH on pioglitazone, 30 mg daily for 48 weeks. By the end of the study serum ALT levels decreased in all the patients and normalized in 72% of them. Serum ALT levels fell from an average of 99 units/l at baseline to 40 units/l at 48 weeks. The results of the present study also shows similar improvement in liver function. Pioglitazone, a PPAR gamma ligand, greatly retards the progression of hepatic fibrosis through inhibition of HSC activation and amelioration of hepatocyte necroinflammation.

5.3.2 Effect of pioglitazone on hematological parameters in type 2 diabetic mellitus.

This chapter deals with the effect of pioglitazone on hematological parameters in type 2 diabetic mellitus. The results of the present study reveal a normal RBC, WBC and differential count in group IV subjects (Table 5). ESR was also found to be within the normal range in group IV subjects. This may be mainly due to anti-inflammatory property of pioglitazone.

5.3.3 Effect of pioglitazone on immunological parameters in type 2 diabetes mellitus.

This chapter deals with the effect of pioglitazone on immunological parameters in type 2 diabetes mellitus. The levels of immunoglobulins were found to be within the normal range in group IV subjects. This indicates the normalization of immune status by pioglitazone in type 2 diabetic subjects.

The major findings of the present study are that pioglitazone caused significant reduction in the markers of inflammation - CRP, fibrinogen, C3, ceruloplasmin, haptoglobin and α1 anti-trypsin.

Pioglitazone also produces considerable reduction in the values of IL-6 and TNF-α, the adiposytokine that play a prominent role in the pathophysiology of type 2 diabetes mellitus (Table 8).
The attributable reduction in the levels of the above inflammatory markers were not attributable to glycemic control alone but also mediated by the increase in the levels of adiponectin.

*Pioglitazone* treatment in type 2 diabetes causes a three fold increase in plasma adiponectin concentration. The increase in plasma adiponectin is strongly associated with a decrease in hepatic fat content and improvements in hepatic and peripheral insulin sensitivity. The increase in circulating adiponectin by *pioglitazone* is related to enhanced production by smaller adipocytes and decreased lipotoxicity.

Adiponectin turn exerts insulin sensitizing effect through AMP-activated protein kinase (AMPK) in liver, muscles and adipocytes improving tissue lipid oxidation resulting in reduced lipotoxicity (Kadowakin and yamanchi, 2005 ; Wu *et al.*, 2004).

The effects of adiponectin on energy metabolism, insulin sensitivity and atherogenesis are mainly mediated through ability to increase the phosphorylation and activation of AMPK/malonylCOA signaling and to modulate the nuclear factor kβ pathway in metabolically active tissues.(Goldstein and Scalia, 2004). These effects result in increased fatty acid oxidation, increased glucose utilization, reduced endogenous glucose production and subsequently improved insulin sensitivity and circulating carbohydrate and lipid profiles (Chandran *et al.*, 2003 ; Diez and Iglesias , 2003)

Recently, adiponectin has been shown to be transported through the blood brain barrier to act centrally and to increase energy expenditure and weight loss, combined with a reduction in serum glucose and lipid levels. This effect is partially synergistic with that of leptin and hence is likely to be mediated through a neuroendoceine feedback loop (Qi *et al.*, 2004).

Thus *pioglitazone* prevented coronary arteriosclerosis, possibly by its antiinflammatory effects. The anti inflammatory and anti arteriosclerotic effects of *pioglitazone* may be mediated by downregulation of CCR 2 in circulating and lesional monocytes. Inhibition of the CCR-2 mediated inflammation may represent novel antiinflammatory actions of pioglitazone beyond metabolic effects.
5.4 Comparative study of *rosiglitazone* and *pioglitazone* in type 2 diabetes mellitus.

5.4.1 Comparative study of *rosiglitazone* and *pioglitazone* on biochemical parameters in type 2 diabetic patients.

This chapter deals with the comparative study of *rosiglitazone* and *pioglitazone* an biochemical parameters in type 2 diabetic patients.

Peroxisome proliferators activated receptors (PPARS) play an important role in regulating both glucose and lipid metabolism agonists for both PPAR-α and PPAR-γ have been used to treat dyslipidemia and hyperglycemia respectively. Thiazolidinediones belong to the category of PPAR-γ agonists.

The glucose lowering effects of thiazolidinediones appear to be related to their ability to reduce insulin resistance in liver, skeletal muscle and adipose tissue thereby increasing the effectiveness of insulin (Parulkar *et al.*, 2001).

These pharmacological actions are mediated through peroxisome proliferators activated receptors that control genes involved in adipocyte differentiation, fatty acid metabolism and insulin regulation (Olesky, 2000).

Treatment with *pioglitazone* was associated with greater beneficial effects on blood lipid levels that treatment with *rosiglitazone*, whereas glycemic control was equivalent between the two treatments. A significant reduction in insulin levels was observed with both *rosiglitazone* and pioglitazone.

In combination studies with sulphonylureas or metformin, *pioglitazone* has resulted in an improvement in glycemic control. A group of 560 patients who were receiving a stable dose of sulphonylurea were randomized to receive *pioglitazone* 15 mg once daily *pioglitazone* 30 mg once daily or placebo. (Kipnes *et al.*, 2001). After 16 weeks HbA1c was 0.9% and 1.3 % lower than placebo with 15mg and 30 mg doses respectively.

*Rosiglitazone* has been studied in combination with sulphonylureas. In a trial of 574 patients with type 2 diabetes who mere receiving therapy with sulphonyl urea, low
dose *rosiglitazone* or placebo was added to *glibenclamide*, *gliclazide* or *glipizide*. The maximum dose of rosiglitazone in the study was 2 mg twice daily which resulted in the greatest decrease in Hb A\textsubscript{1C} of 1\% compared to placebo plus sulphonylurea at 26 weeks (Wolffen buttel *et al*., 2000).

Goldberg *et al* (2005) compared the effects of *pioglitazone* and *rosiglitazone* in patients with type 2 diabetes mellitus and dyslipidemia on non lipid altering medications.

*Pioglitazone* therapy was associated with a reduction in fasting triglycerides throughout the study. The decrease in triglycerides with pioglitazone was associated with a decrease in large VLDL and intermediate density lipoproteins (IDL).

As expected, both medications increased HDL cholesterol was significantly greater with *rosiglitazone* therapy (14.9\% and 7.8\% respectively). Again there was a difference in HDL particle subclasses between the medications *pioglitazone* increased total large and medium HDL while decreasing small HDL concentration. *Rosiglitazone* in contrast decreased total, large and small HDL while increasing medium HDL particle concentration. These suggest that there are differences in HDL metabolism with these two agents.

One potential difference, which may account for the difference is the effect on apolipoprotein C111. Two studies have demonstrated that *pioglitazone* decreases and *rosiglitazone* increases apolipoprotein C111 (Nagashima *et al*., 2005).

A decrease in apolipoprotein C111 would lead to an increase in lipoprotein lipase activity and hence an increase in the hydrolysis of TG and catabolic rate of triglyceride rich lipoproteins including CM and VLDL. This hypothesis is supported by the observation that *pioglitazone* increases the lipolysis of VLDL triglycerides without affecting the removal of VLDL particles. Conversely, *rosiglitazone* increases the production and reduces the catabolism of triglyceride-rich lipoprotein including both VLDL and CM (Duez *et al*., 2008).

Another possibility is that genetic difference may contribute to the different lipid effects. Polymorphism of the PPAR-2 gene influences of the glycemic response to
rosiglitazone but not to pioglitazone (Bluher et al., 2003). A lipoprotein lipase variant influences the glycemic effect of pioglitazone (Wang et al., 2007), while a polymorphism of the adiponectin and perilipin gene influences the glycemic and weight gain responses respectively to rosiglitazone.

It is possible that pharmacokinetic differences between pioglitazone and rosiglitazone may account for the differences in lipid effects; however, this is an unlikely contributor since the gene expression and pharmacodynamic effects of both agents exceed the presence of active drug in the serum.

The difference between the effects of rosiglitazone and pioglitazone in lipids cannot be attributed to differences in their effects in serum FFA concentrations which decreased by similar amounts approximately 20-30% pioglitazone seems to act like a partial PPAR-α agonist invitro, whereas rosiglitazone seems to be a pure PPAR-γ agonist (Miyazaki et al., 2001).

It could be speculated that cardiac risk reduction would be more likely with pioglitazone than rosiglitazone treatment because only pioglitazone combined a significant decrease in LDL-C levels with a significant increase in HDL-C levels.

*Pioglitazone* and *rosiglitazone* have been shown to decrease the levels of hepatic enzymes such as ALT, AST and ALP as tighter blood glucose levels are achieved in the present study.

### 5.4.2 Comparative study of rosiglitazone and pioglitazone on hematological parameters in type 2 diabetic patients.

This chapter deals with the comparative study of *rosiglitazone* and *pioglitazone* in hematological parameters in type 2 diabetic patients. In the present study *pioglitazone* showed significant decrease in the hematological parameters when compared with *rosiglitazone*. This is mainly mediated by the structural, genetic and pharmacokinetic differences between *rosiglitazone* and *pioglitazone*. 
5.4.3. **Comparative study of rosiglitazone and pioglitazone on immunological parameters in type 2 diabetic patients.**

This chapter deals with the comparative study of *rosiglitazone* and *pioglitazone* on immunological parameters in type 2 diabetic patients. *Pioglitazone* treatment produced a significant decrease in the levels of immunoglobulins and inflammatory markers when compared with *rosiglitazone*.

The increase in the levels of adiponectin, an anti-inflammatory marker, was also found to be greater in the case of *pioglitazone* when compared with *rosiglitazone* treatment.

It was found that *pioglitazone* can promote cell survival at doses that induce optimal PPAR-γ transcriptional activity. The results suggest that type 2 diabetic patients taking *pioglitazone* may not be at risk for further impairment of their immune function. The ability of PPAR-γ to promote cell survival under conditions of growth factor withdrawal might even improve immune cell functions at vascular or necrotic sites such as diabetic ulcers.

Thus structural, genetic and pharmacokinetic differences between *rosiglitazone* and *pioglitazone* contribute to their action on immune status in type 2 diabetes mellitus.