Pathophysiology of Retina in Type II Diabetes Mellitus: Conventional Diagnostic Parameters Versus Emerging Roles of Growth factors and Cytokines

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IN THE NAME OF ALLAH THE MOST MERCIFUL AND MIGHTY

O my Lord! Open for me my chest

(Grant me Self-confidence,

Contentment and boldness)

And ease my task for me,

And loose the knot from my tongue that they understand my speech (words)

Surah 20, Ta Ha (Al-Quran)

Dedicated to My Family

DECLARATION

The material contained in this thesis is my original work and I have not presented any part of this thesis/work elsewhere for any other degree. I have tried my best to avoid plagiarism. I understand that I may be held responsible in case faulty, non authentic or plagiarized results found in the dissertation.

NARGIS PARVEEN

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ABBREVIATIONS

Ab_5S_1	Absorbance of sample
Ab_5Std	Absorbance of standard
ACE	Acetylcholine esterase
ADA	American Diabetic Association
AGEs	Advanced glycation end products
BMI	Body mass index
BRB	Blood retinal barrier
CDNR	Control diabetic non retinopathy
CTGF	Connective tissue growth factor
20D	20 Diopters
DCCT	Diabetic Complications Control Trial
DME	Diabetic macular edema
DNPDR	Diabetic non proliferative diabetic retinopathy
DNR	Diabetic non retinopathy
DPDR	Diabetic proliferative diabetic retinopathy
DR	Diabetic retinopathy
EDTA	Ethylene diamine tetrachloroacetic acid
ESRD	End stage renal disease
ETDRS	Early Treatment of Diabetic Retinopathy Study
FA	Flourescin angiography
FBS	Fasting blood sugar

HbA1c	Hemoglobin A 1c
HDL	High density lipoprotein
HRP	Horse reddish peroxidase
IL-6	Interleukin-6
LDL	Low density lipoprotein
LepR a-f	Leptin Receptors- a,b,c,d,e,f
LOB	Limit of blank determinant
LOD	Limit of detection
NCCLS	National Council of Clinical Laboratory Services
NIH	National Institute of Health
NKUDIC	National Kidney and Urologic Disease Information Clearing
NO	Nitric oxide
NPDR	Non proliferative diabetic retinopathy
NS	Normal healthy subjects
OB-R	Leptin receptor
PDGF	Platelet derived growth factor
PDR	Proliferative diabetic retinopathy
PKC	Protein kinase C
PMRC	Pakistan Medical Research Council
R/E	Regular examination
RBS	Random blood sugar
ROS	Reactive oxygen species

RPE	Retinal pigment epithelium
TC	Total cholesterol
T-cells	Thymic derived lymphocytes
TG	Triglycerides
UKPDS	United Kingdom Prospective Diabetes Study
VEGF	Vascular endothelial growth factor
VMN	Ventro medial nucleus
WESDR	Wisconsin Epidemiologic Study of Diabetic
WHO	World Health Organization

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ABSTRACT

Until today, type-II diabetes mellitus remains one of the most devastating metabolic disorder affecting millions of people around the world. It is expected that the number of diabetic patients world rise to 70 million 2030. Diabetic retinopathy is one of the three very significant microvascular complications of progressing diabetes that leads ultimately to blindness. Sustained hyperglycemia causes generation of advanced glycation end products thereby forming reactive oxygen species. The resulting stress causes retina to become hypoxic and anemic. As a result, traumatized retinal tissue induces a number of cytokines and growth factors to promote neovascularization in order to counter to supply oxygen to the failing retina. Tortuous growth of blood vessels however impairs the vision, and at times hemorrhagic retina is the complication that appears due to rupturing of fragile vessels. On fundoscopy, retinal artery microaneurysms, dilated veins, hard exudates, edematous retina exhibit in non-proliferative retinopathy. Further worsening and advancement of retinopathic damage leads to proliferative retinopathy characterized by appearance of cotton wool spots, hard exudates and marked neovascularization. Factors like obesity, hypertension, and elevated random and fasting plasma sugar, raised cholesterol level, hyperlipidmia, and serum creatinine contributing to diabetes are very well known risk factors. Situation in Pakistan is no different from the rest of the world. According to relatively recent estimates the prevalence of diabetic retinopathy is 4-5 million.

The current study was designed to determine the specific parameters, viz. serum and vitreous vascular endothelial growth factor (VEGF), interleukin-6 (IL-6), and leptin in diabetic retinopathic patients. Serum levels were also compared with diabetic but non-retinopathic patients and normal healthy subjects. Determination of all major conventional risk factors and complete fundus examination were also carried out to correlate changes in these parameters with the specific parameters. Over 2000 male and female patients of median age of 50 years ranging between 37-65 years were screened in the outpatients departments of four main hospitals, Khyber Teaching Hospital, Hayatabad Medical Complex and Lady Reading Hospital, located in Peshawar city and Al-Shifa Eye Trust Hospital located in Rawalpindi city. Patients with confirmed type-II diabetes mellitus (338) were selected, and patients with complications otherwise were excluded. The duration of the disease and retinopathy was 5-20 years. Normal healthy subjects (39; age range: 35-61; median age 53) were also included in the study to get comparisons with the diseased patients. Standard

methods were followed to determine the body mass index (BMI), fasting (FBSS) and post prandial plasma glucose (RBS), glycated hemoglobin (HbA1c), cholesterol, triglycerides (TG), high-density lipoproteins (HDL), low-density lipoproteins (LDL), serum creatinine, urine creatInine and urinary protein. Commercial kits were used to determine the serum parameters and IL-6, leptin and VEGF concentrations. For obvious reasons, vitreous concentrations of IL-6, leptin and VEGF could not be determined in normal subjects and DNR patients. Data were analyzed statistically to determine correlations between predicator variables with those of specific variables, and differences between males and females were done by one way analysis of variance (ANOVA). Combined analysis was also done to get population estimates.

The results demonstrated significantly higher ($P < 0.001$) concentrations of serum IL-6 (70%), leptin (64%) and VEGF (55%) in DNR, NPDR and PDR patients. Vitreous IL-6, leptin and VEGF concentrations were alarmingly increased (100%, 93% and 100% respectively $P < 0.001$) in NPDR and PDR patients.

For conventional parameters significantly ($P < 0.001$) elevated BMI, RBS, FBS, HbA1c, TG, LDL, serum and urine creatinine and urinary protein concentrations were found in DR, NPDR and PDR patients. Values of these parameters were remarkably low ($P < 0.001$) in normal subjects. All parameters were affected linearly with the severity of the disease. Accordingly highest levels were found in PDR patients. Serum cholesterol concentrations were well in the range. HDL concentrations were significantly reduced ($P < 0.001$) in DNR patients, NPDR and PDR patients; but group comparisons showed slightly greater levels of HDL in NPDR and PDR patients than the DNR patients. TC/HDL ratio and LDL/HDL ratio were also increased in NPDR and PDR patients. Separate male and female comparisons did not show any significant differences with combined male and female analysis demonstrating that the disease prevalence is irrespective of gender; however a small female predisposition is evident from the data.

Most importantly, since all of the above patients were being treated with oral hypoglycemic and several PDR patients had already underwent laser photocoagulation, elevated concentrations of specific and conventional parameters raise questions about the therapy.

Of 338 diabetic patients following were the frequencies of non-retinopathy and retinopathy: DNR (11 %), NPDR (31.95 %) and PDR (56.80 %). Gender-wise, 38 % (129) were

males consisting of 12% DNR patients, 36% NPDR patients, and 52% PDR patients. Of 209 female patients, 11% were DNR patients, 29 % were NPDR patients, and 60% were PDR patients.

The study points out that IL-6, leptin and VEGF can be significant diagnostic factors in clinical settings to predict the probability of retinopathy. They also demonstrated correlations, positive or negative, with some conventional parameters. Alarmingly elevated levels of these factors indicate them to be independent risk factors. Although most conventional parameters can be controlled via intensive treatment but the chain of events that hyperglycemia induces for the first time initiates the vicious cycle of biochemical changes that cannot be controlled with routine therapies and ultimately lead to failure of retina culminating in certain cases into complete blindness. Associated outcomes were obesity, dyslipidemia and microalbiminuria.

The study suggests that ophthalmologists and diabetologists working in the clinical set ups should emphasize on the determination of serum IL-6, VEGF and leptin levels in patients presenting with diabetes and retinal problems to reach an early diagnosis about the severity of the disease and future affliction with retinopathy. This may help for an earlier decision to proceed for invasive therapies like the application of antibodies injections against VEGF and IL-6. Currently, for unknown reasons the role of leptin could not be ascertained. Further detailed studies from around different geographic regions of Pakistan and analyses of even newer retinopathy promoting and inhibiting factors are definitely required to get a more comprehensive data from this region of the world.

INTRODUCTION

INTRODUCTION

1.1 DIABETES MELLITUS

Diabetes mellitus is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia causing significant alterations in carbohydrate, fat and protein metabolism. Diabetes results due to defects in insulin secretion (type I) or insulin synthesis (type II) or both. It is a devastating disease in terms of long term damage, dysfunction and failure of various organs, the eye, kidney, brain and peripheral nerves (Mayfield, 1998; Armulik, 2004). Diabetes arises due to the inability of the body to effectively remove glucose from the blood and extracellular fluid and metabolize it within the cells. The resulting hyperglycemia has a major role in the development of the associated complications (Grant and Lipscomb, 2009). More than seventy eight years after insulin was first introduced as a drug, diabetes remained a major cause of premature disability and death. Insulin is a pancreatic beta cell-derived hormone which helps glucose to be taken up by the cells where it is used as a primary source of energy (Rodriguez-Fontal et al., 2009). Current estimates demonstrate that 285 million people between 20-70 years of age have diabetes and 439 million adults will be affected by 2030 (Shaw et al., 2010; Zhang et al., 2010; Yau et al., 2012; Yeo et al., 2012).

1.1.1 Complications of Diabetes Mellitus

Complications associated with diabetes are on the rise causing considerable health related problems (Bloomgarden, 2002; Ruiz-Ramos et al., 2006). These complications are classified as:

(i). Macrovascular complications

These include large vessel disease, for instance the coronary heart disease and stroke, and are the greatest overall causes of morbidity and mortality in diabetic patients (Fowler, 2008).

(ii). Microvascular complications

These include: (i). Diabetic nephropathy, the most common cause of end-stage renal disease accounting for 40 % of new cases in western countries, (ii). In diabetic neuropathy there occurs substantial damage to peripheral nerves sometimes resulting into amputations mainly because of foot ulcerations. Peripheral

neuropathy leads to neuropathic damage, consequently foot ulcers and amputations are the major causes of morbidity for people with diabetes. iii). Retinopathy, in which there occurs severe damage to the eye (Skyler, 2001; Fowler, 2008; del Cañizo Gómez, 2011).

1.2 RETINOPATHY

Diabetic retinopathy (DR) is a retinal vascular disorder that occurs as a complication of diabetes mellitus and is one of the leading causes of blindness in working–aged adults (Fong et al., 2003; Frank, 2004; Srinivasan et al., 2010). It is characterized by signs of retinal ischemia such as microaneurysms, haemorrhages, cottonwool spots, intraretinal microvascular abnormalities, venous abnormalities and neovascularization and/or signs of increased vascular abnormalities (Bloomgarden, 2002). More recent estimates demonstrate that 93 million people around the world have diabetic retinopathy and 28 million people are at risk of loosing eyesignt due this condition (Yau et al., 2012). Among systemic complications, diabetic retinopathy is one of the most troublesome problems as it is one of the major causes of blindness. It has been estimated that at least 60% of type II diabetic patients develop diabetic retinopathy during the first two decades of diabetes (Fong et al., 2003; Frank, 2004; Sinclair et al., 2005).

The current management strategy for diabetic retinopathy requires early detection and optimal glycemic control to slow down the progression of the disease. Adherence to these recommendations is hampered by the fact that the condition is generally asymptomatic at early stages. Current treatment for advanced stage diabetic retinopathy includes laser photocoagulation and application of anti-vascular endothelial growth factor. Several pharmacological therapies are being developed to treat early stages of diabetic retinopathy. The control of diabetes associated metabolic abnormalities, hyperglycemia, hyperlipidemia, and hypertension is also of prime importance in preserving visual function as these conditions have long been identified as risk factors for both the development and progression of diabetic retinopathy (Bhavsar, 2006; Zheng and Wong, 2012).

Diabetic retinopathy is on the rise worldwide with no effective treatment available as yet. The identification of causative factors is therefore of major concern (Hallman et al*.,* 2005). Two common pathological features in diabetic retinopathy

responsible for vision loss are diabetic macular edema and retinal neovascularization. Although exact mechanisms underlying the pathogenesis of diabetic retinopathy are still unclear, experimentation on animals has shown increase of angiogenic stimulators to be one of the major causes (Eming et al*.,* 2007; Joussen and Jores, 2007).

According to the American Diabetes Association (ADA), diabetic retinopathy is the most frequent cause of blindness in the working age group with 12000-24000 diabetics losing their sight each year in USA alone (Ciulla et al., 2003; Lingel, 2007). Studies from other countries show similar and alarming rise in diabetic retinopathy and a maximal increase has been reported in south East Asian countries (Girach and Anderson, 2007; Gupta and Kumar, 2008). Before 1922, diabetes was an undiagnosed disease and patients used to die very young because of various complications (Antonetti et al., 2006; Accurso et al., 2008).

In type I diabetes almost all patients develop signs of retinopathy in the first 20 years while in type II diabetes, up to a third patients have retinopathy at diagnosis increasing to two-third within 20 years (Bates and Jerums, 2003). Hyperglycemia is a prerequisite for the development of microangiopathy. Population studies have shown that patients rarely develop complications below a certain threshold level of glucose as was found In a large cross-sectional study whereby, a highly significant relationship was found between retinopathy and blood glucose concentration (West et al., 2000).

Evidence that hyperglycemia causes microangiopathy has been strengthened by studies which showed that diabetics with poor glucose control were more prone to develop retinopathy than those with good control. Strong evidence that glycemic control is related to the development of microangiopathy has come from much earlier studies. The incidence of clinical complications was significantly associated with glycemia. Each 1 % reduction in updated mean HbA1c was associated with reduction in risk of 21% for any endpoint related to diabetes, 21% for deaths related to diabetes, 14 % for myocardial infarction and 37 % for microvascular complications (Stratton et al., 2000; Massin et al., 2011).

1.2.1 Stages of Retinopathy

Normal Retina

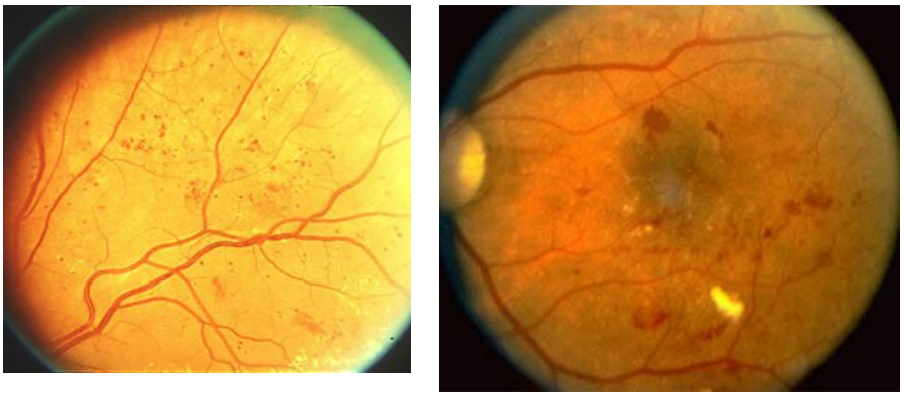
The normal retina is a highly sensitive layer of receptors and neural components which is responsible for normal vision. A disruption of the retinal structure, photoreceptors, blood vessel or neural elements produces visual defects. In diabetic retinopathy, there occurs vascular dysfunction that subsequently leads to ischemic retina in response to growth of abnormal blood vessels (Fong, et al., 2004).

Classification of Diabetic Retinopathy

Different types of classification systems are in use. Diabetic retinopathy is primarily classified into non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) and macular edema (Lingel, 2007; ADA, 2007). The WHO (1999; 2007) classification divides diabetic retinopathy into five stages:

a) No apparent DR
b) Mild non-proliferative DR
c) Moderate NPDR
d) Severe NPDR
e) Proliferative DR

Systems which are more favored worldwide and preferred or used by clinicians are divided into: non-proliferative and proliferative diabetic retinopathy (Fig. 1.1 ab respectively) (Chalam et al., 2005; Lingel, 2007).



a b

Fig.1.1 Non-proliferative and prolifertative diabetic retinopathy.

1.3 PATHOPHYSIOLOGY OF DIABETIC RETINOPATHY

Hyperglycemia causes alterations in the glycolytic pathways causing change in the structure and function of the retina leading ultimately to marked vascular dysfunction and ultimate visual loss. Diabetic retinopathy is characterized by abnormal retinal vascular permeability, microaneurysm formation, retinal haemorrhages, capillary and arteriolar closure, hard exudates, neovascularization with ultimate scarring and retinal detachment if treatment is not instituted (Fong et al., 1999; Antonetti et al., 2006).

The earliest phase of diabetic retinopathy is characterized by capillary leakage with capillary microaneurysm formation, dot and blot intra-retinal haemorrhages and lipid exudates. Extensive capillary leakage can lead to macular edema. The next stage is pre-proliferative retinopathy, which is characterized by the appearance of so-called “cotton wool spots” that marks the beginning of severe capillary closure with resulting ischemia (Frank, 2004). These are infarctions of the retinal nerve fiber layer. Associated morphological abnormalities include venous beading and irregular segmental dilatation of retinal capillaries, collectively called intraretinal microvascular abnormalities (Ciulla et al., 2003). The hallmark of proliferative diabetic retinopathy is neovascularization or growth of new fragile blood vessels from the optic nerve head, neovascularization on the disc or along the retinal venules elsewhere. The new blood vessels extend through internal limiting membrane of the retina along the surface of and into the vitreous body, these vessels can break easily because of any stress with ensuing visual loss due to vitreous hemorrhage. Extensive fibrous tissue formation may subsequently result in severe retinal distortion and detachment (Frank, 2004).

1.3.1 Basement Membrane Thickening and Pericyte Loss

Thickening of the basement membrane appears early in the development of diabetic retinopathy (Hammes, 2002). Pericyte loss from retinal capillaries is often pronounced in diabetic retinopathy and increases with longer duration of the disease. Normally the pericyte/endothelial cell ratio is 1:1 in the retinal microcirculation, but in diabetics the ratio is sometimes reduced to 1:4. As the pericyte loss continues the capillaries show chronic dilatation. In vitro studies have

shown contractility of pericytes, an observation that supports the potential role of pericytes in vasoconstriction (Gardner et al., 2000; Cox et al., 2003; Frank, 2004).

1.3.2 Retinal Non-Perfusion and Ischemia

In diabetic retinopathy areas in the retina with no perfusion are identified. The vascular channels in the capillary system in these areas are devoid of normal epithelial cells, which are replaced with acellular strands or basement membrane tubes, the so called ghost cells. Other retinal changes include loss of ganglion cells (Sinclair et al., 2005; Frank, 2004).

1.3.3 Shunting Dilated Vessels

Although reasons for non-perfusion are not fully known but a possible role of shunting, in which the dilatation of one vessel caused by pericyte loss leads to vessel steal from the nearby vessel thereby leading to occlusion, has been implicated. Several studies have shown clogging of capillaries blood cells, suggesting that primary closure of capillaries might lead to non-perfusion. Increased leukocyte adhesion and deformability have been demonstrated in leukocytes isolated from diabetic patients (Frank, 2004; Joussen et al., 2007).

1.3.4 Effect of Hyperglycemia on Enzyme Pathways

Hyperglycemia leads to excessive glucose entry into the cells with over expression of the transport proteins. The excessive glucose activates enzymatic pathways which cause neuropathy, nephropathy and retinopathy. Prolonged hyperglycemia is a key factor that gives rise to diabetes related complications (Klein et al., 1992; Girach et al., 2006). Several biochemical pathways have been proposed to link hyperglycemia and microvascular complications. These include polyol accumulation, formation of advanced glycation end-products (AGEs), oxidative stress and activation of protein kinase C (PKC). These processes are thought to modulate the disease process through their effects on cellular metabolism, cell signaling and growth factors (Hammes et al., 2002).

1.3.5 Polyol Accumulation

Accumulation of polyol occurs in experimental hyperglycemia, which in rats and dogs is associated with the development of basement membrane thickening, pericyte loss, and microaneurysms formation (Thomas et al., 2003; Hinton et al., 2004). High concentration of glucose increases flux through the polyol pathway with

the enzymatic activity of aldose reductase, leading to an elevation of intracellular sorbitol concentrations. This rise in intracellular sorbitol accumulation has been hypothesized to cause osmotic damage to vascular cells (Schiffelers et al., 2007). Aldose reductase inhibitors have been evaluated for the prevention of retinal and neural damage in diabetes, however clinical trials in human beings have not been effective in preventing the incidence or progression of retinopathy (Klein et al., 2004; Lingel, 2007).

1.3.6 Hexosamine Pathway

There is increasing evidence that pyruvate kinase activation is related to hyperglycemia-induced microvascular dysfunction in diabetes. Activation of PKC results in numerous cellular changes, including increased cellular expression of matrix proteins such as collagen and fibronectin, and increased expression of vasoactive mediators such as endothelin. The changes are seen as thickening of the basement membrane, increased retinal vascular permeability and alteration in retinal blood flow. Although the activity of the isomers of PKC is increased in diabetic retinopathy, experimental evidence demonstrated that the use of PKC – inhibitors does not seem to be much effective (Nathan, 1996; Thomas et al., 2012).

1.3.7 Advanced Glycation Endproducts (AGEs)

Another well recognized pathway is damage resulting from accumulation of the advanced glycation end products. Higher serum glucose concentration can lead to non-enzymatic binding of glucose to protein side chains resulting in the formation of compounds termed AGEs (Thomas et al., 2012). After 26 weeks of induced hyperglycemia, the retinal capillaries of diabetics can have marked accumulation of AGEs and as well as loss of pericytes (Lingel et al., 2007). Inhibitors of AGEs in clinical trials have shown some positive results in reducing pericytes loss, and microaneurysm formation (Ciulla et al., 2003).

1.3.8 Oxidative Damage

Diabetes and hyperglycemia can also lead to oxidative stress and generation of reactive oxygen species (Ros) or free radicals of oxygen, leading to vascular damage (Ciulla et al., 2003; Matough et al., 2012). Production of Ros may result from glucose auto oxidation, protein glycation and increased flux through the polyol pathway and protanoid production. Normalization of glucose–stimulated superoxide

production has been found to block at least three independent pathways of hyperglycemia- induced vascular damage (Fong et al., 2004). Experiments using animal models have suggested that blocking the oxidative damage through anti-oxidants such as vitamin-E may prevent some of the vascular dysfunction associated with diabetes. The polyol pathway activation, diacylglycerol-protein kinase C pathway activation, stimulation of cell oxidative stress and changes in macromolecule structure and function via the formation of AGEs are all responsible for the vascular dysfunction seen in diabetic retinopathy (Nathan, 1996; Ciulla et al*.,* 2003; Joussen and Jores, 2007; Schiffler et al., 2007). Despitre these facts the exact mechanism for the development of diabetic retinopathy is still not known. A recent study by Hinten et al. (2007) shows that the amino-terminal CTGF content is increased in the vitreous of patients having proliferative diabetic retinopathy (PDR). The biochemical changes observed due to chronic hyperglycemia leads to change in the endothelial intercellular junction resulting in dysfunction of vascular endothelial cells (Joussen and Jores, 2007).

The impaired anti-thrombotic function of endothelial cells, the interaction between leukocytes and endothelial cells, the vasoconstriction caused by over produced endothelia and the reduced function of vasodilating factors such as prostacyclin and nitric oxide causing thrombosis and closure of retinal capillaries resulting into failure the of retinal vascular function and regional hypoxia in the retina (Creager et al., 2003; Porta, 2005). Hyperglycemia decreases endothelium-derived nitric oxide (NO). Nitric oxide is normally produced by the action of endothelial nitric oxide synthetase. It is responsible for vasodilatation and also protects the blood vessels from injury. Lack of nitric oxide causes atherogenic predisposition and blood vessel occlusion (Creager et al., 2003).

Several common pathological changes occur in diabetic retinopathy; these include appearance of microaneurysms, increased vascular permeability, capillary occlusion and retinal ischemia (Gardner, 2000; Ciulla et al*.,* 2003; Frank, 2004). The earliest histological change encountered in diabetic retinopathy is the loss of pericytes and subsequent dilatation of capillaries that can cause microaneurysms, which is the earliest visible lesion of diabetic retinopathy (Hammes et al., 2002). Under ophthalmoscopy, micro aneurysms appear as red dots of various diameters

ranging in size from 16-60 µm (Frank, 2004). Although the pathogenesis of microaneurysms is unclear, increase of microaneurysms has been shown to associate with the progression of retinopathy (Srinivasan, 2010).

1.3.9 Blood-Retinal Barrier

The blood retinal barrier plays an important role in maintaining normal physiological functions of retina. It is composed of two spatially distinct barriers limiting the flow of macromolecules and fluid into the retina. The inner barrier is the vascular endothelium mainly residing at the tight junction between adjacent endothelial cells. The outer barrier is the tight junction between the retinal pigment endothelial cells. The tight junction between the endothelial cells contains an assembly of unique proteins such as occludin, claudins and zonnula occludins (Kondo et al., 2004; Girach et al., 2006).

The structural interconnections among these proteins constitute the tight junction and limit the fluid flow. Impaired inner blood retinal barrier has been found to play major role in the evolution of diabetic macular edema and diabetic retinopathy (Cox et al., 2003; Olmos et al., 2009). The increased vascular permeability resulting from the breakdown of blood-retinal barrier (BRB) allows the leakage of plasma macromolecules and the fluid into retina resulting into the formation of microexudates (Frank, 2004; Girach et al., 2006). The appearance of diabetic macular edema represents a more advanced stage of diabetic retinopathy and can cause significant impairment of central vision (Fig. 1.2) (Gardner et al., 2000; Frank, 2004).

Fig. 1.2 Visual changes due to retinal edema (WHO, 2002)

The exact mechanism is although not known but occlusion of the capillaries gives rise to focal retinal ischemia and hypoxia (Hernandez et al*.,* 2001; Caldwell, et al., 2005; Ding and Wong, 2012). The local hypoxia then induces the overproduction of angiogenic stimulators to stimulate new blood vessel formation in order to improve oxygenation in the retinal tissue. These new vessels cross over both the normal veins of the retina and in advanced stage, these can grow into the vitreous body resulting in pre retinal neo-vascularization (Fong et al*.,* 1999; Hammes, 2002; Frank, 2004), The abnormal structure of new blood vessels can lead to leakage of plasma proteins and hemorrhage into the retina or vitreous, consequently compromising vision (Fong et al*.,* 1999; Frank, 2004; Olmos et al., 2009). Some animal studies have shown that unbalanced expression of antigenic factors play an important role in the development of diabetic macular edema (Joussen and Jores, 2007).

Diabetic macular edema (DME) can occur at any stage of diabetic retinopathy, however the incidence of DME is closely correlated with the severity of diabetic retinopathy and it is the single greatest cause of vision loss in diabetic patients with a change in the intercellular junctions causing endothelial cell death, a resultant change in permeability and retinal edema leading to blindness (Joussen and Jores*,* 2007; Schiffler et al*.,* 2007).

The incidence of diabetic macular edema is 40 % and 71 % for patients with non proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) respectively. As diabetic macular edema directly affects the function of the macula it often results in significant vision impairment (Frank, 2004; Lingel, 2007). The current treatment for diabetic macular edema is transient and is far from satisfactory (Frank, 2004; Girach and Anderson, 2007). The ETDRS study shows that vitreous hemorrhage is the commonest cause of visual loss and it can be reduced to a certain extent by treatment options like photocoagulation and vitrectomy. However, these treatments are not much effective or long lasting and can damage the retinal pigment epithelium leading to visual loss and decrease in color vision (Fong et al*.,* 1999; Ciulla et al*.,* 2003). In more advanced stages of the disease, fluid can accumulate in the retina around the fovea causing diabetic maculopathy (Zhang et al*.,* 2001).

Retinal blood flow increases in early diabetic retinopathy and this may be due to autoregulation in response to decreased oxygen utilization caused by hyperglycemia. Increased blood flow can damage the endothelial cells lining the vessel by shear stress and contribute to the progression of small retinal hemorrhages (Dodson, 2007). Another process that begins early in the development of diabetic retinopathy is progressive capillary closure. The endothelial surface, however changes in diabetes ultimately reducing its ability to carry out its function. Platelet levels are also altered and show increased coagulability, blood viscosity increases while fibrinolysis decreases. These changes result in the formation of micro thrombi in the capillaries, leading to capillary closure and retinal hypoxia. As the number of hypoxic areas increases, the disease progresses into a more severe form (Gardner et al., 2000).

Intensive glycemic control, as demonstrated by the Diabetic Complication Control Trial (DCT, 1993) can decrease the incidence of the diabetic macular edema by at least 23 %. The ETDRS (1991) demonstrated that treatment of diabetic macular edema by photocoagulation is less beneficial and reduces visual loss by 50%. In addition, the laser burns cause atrophy of retinal pigment epithelium leading to visual loss and the loss of color vision (Thomas et al*.,* 2012).

1.4 RETINAL HYPOXIA

The occlusion of retinal capillaries leading to the development of large ischemic areas in the inner retina is a critical step in the development of proliferative diabetic retinopathy (PDR). The requirement for the tissue oxygenation in the retina is greater than anywhere else in the body because of the very high metabolic rate of the retinal tissue, therefore, even a small decrease in the oxygen supply would result in retinal ischemia (Hammes et al., 2002). The significance of retinal hypoxia in the neovascular response is demonstrated by the fact that other conditions which predispose to proliferative retinopathy all produce ischemic retinal tissue, that include the central retinal vein occlusion, sickle cell anemia, retrolental fibroplasias and both Earle's disease and sarcoidosis (Ferrara, 2004).

The release of a vasculogenic factor from ischemic retina was first proposed by Michaelson 1948. Such a factor can be released physiologically in response to a decrease in pH in the tissue, due to increased lactic acid production or due to an

impairment of cytochrome oxidase activity and changes in cell respiration. The cells likely to be affected by hypoxia in diabetic retinopathy are those of the inner retina because cells in the outer nuclear layer, rods, cones and pigment epithelium receive oxygen from choroidal circulation (Wangsa-Wirawan and Linsenmeier, 2003).

1.5 RETINAL NEOVASCULARIZATION

Retinal neo-vascularization is another central feature of diabetic retinopathy and is a major cause of blindness in diabetics. The appearance of neo-vascularization represents the progression of the disease from NPDR to PDR (Frank, 2004). The retinal pathology described thus far in response to hyperglycemia includes pericyte loss, basement membrane thickening, capillary leakage, decreased retinal blood flow, changes in endothelial cell metabolism and particularly the development of microthrombi leading to increased areas of hypoxic retina all contributing to the progression of the disease (Hammes et al., 2002).

Neo-vascularization is characterized by the formation of new blood vessels which originate mostly from the venous side of the circulation on or around the optic disc (Cunha, 1978; Ciulla et al., 2003; Joussen et al., 2007). They break through the inner limiting membrane of the retina and grow at the boundary of the posterior cortical vitreous gel. The latter may provide a scaffold on which the endothelial cells can proliferate (Frank, 2004). The new vessels are often leaky and develop tortuous paths. Their development is accompanied by the production of an epiretinal membrane of fibrous tissue which contains other retinal cells; retinal glia, fibroblasts and retinal pigment epithelium. Bleeding from the new vessels and fibrotic traction retinal detachment are the main causes of visual loss in proliferative diabetic retinopathy (Gardner et al., 2000; Zimmerman, 2010; Thomas et al., 2012). In severe NPDR, the extensive area of capillary closure caused by drop out of pericytes and the loss of endothelial cells results in local retinal hypoxia which in turn stimulate the release of angiogenic factors leading to neovascularization. However the newly formed blood vessels are malformed with fragile basement membrane, deficient in tight junctions between endothelial cells and lack of pericytes, all resulting in hemorrhage into vitreous and loss of vision (Stefánsson et al., 2000; Hammes et al., 2002; Wangsa-Wirawan and Linsenneier, 2003).

A major obstacle in studying progressive diabetic retinopathy is the lack of ideal animal models since diabetic rodent models examined thus far do not develop typical neovascularization identical to that seen in progressive diabetic retinopathy patients. On the other hand the number of people with diabetic retinopathy is rising alarmingly specifically in developing countries like Pakistan and India (Agrawal et al., 2003; Frank, 2004; Kazi et al., 2005).

1.6 RISK FACTORS AND THE DEVELOPMENT OF DIABETIC RETINOPATHY

A number of factors viz. persistently high plasma glucose, smoking, hyperlipidemia, hypertension, and proteinuria, duration of diabetes, age, sex and race are considered to be risk factors for diabetic retinopathy (Xiao-ling et al., 2006; Yau et al., 2012).

1.6.1 Duration of Diabetes

The duration of diabetes is strongly associated with the development and severity of retinopathy. The WESDR study, 2003 (Brown et al., 2003) shows that the prevalence of retinopathy increases from 0% at 3 years duration to 25% at 15 years duration (Donald et al., 2003). Modern effective control of diabetes has lessened the duration specific risk factor (Harding et al., 2003; El-Maskari and El-Sadig, 2007).

1.6.2 Age and Sex as Related to Diabetic Retinopathy

Some studies show an increased incidence of retinopathy and blindness in females as compared to males. Smoking has been associated with visual loss in diabetics and higher systolic blood pressure recordings show more severe changes in type II diabetics (Fukuda, 1994). In the elderly population throughout high prevalence of diabetes mellitus is seen, the prevalence of vision threatening diabetic retinopathy is low (Hiervela and Laatikainein, 1997). Other studies show diabetic retinopathy as an increasing public health problem around the age of 40 and over with one out of every twelve persons with diabetes mellitus having vision threatening retinopathy (Kerpen et al., 2004). Recovery rate in diabetic retinopathy patients with intensive treatment is very satisfactory and more than 50% of cases show recovery from vision threatening retinopathy level (DCCT, 1998). Early diagnosis and early treatment is prevention of visual loss in diabetic retinopathy (Fong et al., 2003).

1.6.3 Race Distribution of Diabetes

Racial differences in risks of developing diabetic retinopathy are seen in type II diabetic patients that can be related to the genetic factor making the individuals more sensitive to blood glucose level or high systolic blood pressure. Some races show a higher diabetes incidence along with its various associated complications as compared to others (Chew et al., 1999).

1.6.4 Smoking

In a multinational study conducted by the WHO, increase in mortality and morbidity in diabetes was shown to be correlated with tobacco smoking. Smoking leads to the progression in severity of diabetic retinopathy and cataract (Belkin, 2006). Studies on diabetic non-smokers and smokers show a more severe visual loss in smokers with diabetic retinopathy as well as more vulnerability to a high incidence of cardiovascular disease, nephropathy and other complications (Gulliford et al., 2003). It has been studied that diabetic visual loss decreased from 70 %to 30 % after strict control of smoking (Chaturvedi et al., 1995). A study on diabetic smokers having diabetic retinopathy showed that the severity of retinopathy was more obvious in older patients (Klein et al., 1994). Some studies show only a borderline significance and smoking not a significant risk factor in the progression of diabetic retinopathy (Klein and Klein, 1990).

1.6.5 Hypertension

Hypertension and dyslipidemia are other risk factors that become worse during diabetic retinopathy in the case of an associated hypertensive state. A strict glycemic control can improve the blood pressure profile thereby reducing the risk of visual loss in diabetic retinopathy (DCCT, 1993; 1998; UKPDSG, 1998; Estacio, 2000). Studies on rats show an exacerbated inflammatory response in the retina in hypertensive rats with proliferative diabetic retinopathy (Moreno and Fuster, 2004; Pinto et al., 2007). Many other studies from around the world show similar worsening of diabetic retinopathy in hypertension (Klein et al., 1992; UKPDS, 1998; Klein et al., 2004; Colucciello, 2004; Feldstein et al., 2008; Yau et al., 2012). Retinal microvascular abnormalities in the form of narrowing of retinal arterioles are related with the duration of hypertension in elderly patients (Adler et al., 2000; Wong and Aiello, 2000).

1.6.6 Hyperglycemia and Glycemic Control

Much work has been done on the association of hyperglycemia with visual loss and, it is an established fact that earlier diagnosis and a strict control of blood glucose are essential to prevent diabetic complications: like retinopathy, nephropathy and neuropathy (Basit et al., 2005, Benoit et al., 2005). In diabetic rats vascular and biochemical changes in the retina are observed as early as 1-2 months of hyperglycemia (Accurso et al., 2008).

HbA1c provides important information about blood glucose control and the development of various complications (Brown, 2003; Basit et al., 2005; Benoit et al., 2005). A decrease in 1 % mean HbA1c reduces diabetic complications by 21-35 % (Irene et al., 2000; Sinclair et al., 2005). The critical level at which there is a risk of developing diabetic complications is not fixed but several studies show that levels of Hb-A1c below 7 % reduces the risk of microvascular complications (Irene et al., 2000; Stratton et al., 2000) The normal range being 4-6 % according to a study conducted in United States (ADA, 2002).

A poorly controlled HbA1c leads to worsening of retinopathy (Agrawal, 2003), whereas lowering of glycemic level to 8 % or less is associated with regression of the condition. All these studies show that HbA1c is a very important indicator for progression of the retinopathy and this parameter is therefore required to be routinely analyzed in long standing diabetics' status to keep a check on the diabetic state of the patients (Krishanamurti and Steffes, 2001; Massin et al., 2011). It has been observed that people who know their glycated hemoglobin concentration control their glucose level better (Sinclair et al., 2005).

1.6.7 Proteinuria

Chronic hyperglycemia leads to damage of glomerular mirovasculature causing albuminuria, commonly seen associated with retinopathy (Hong et al., 2000). Proteinuria describes a condition in which urine contains an abnormal amount of proteins. Thus increased urinary albumin is associated with nephropathy in patients with diabetes. Studies show that more than 65 % of diabetic patients tend to develop albuminuria and increased creatinine level in the serum due to nephropathy (Bloomgarden, 2002; Wong and Aiello, 2000).

1.6.8 Hyperlipidemia

A strong association exists between diabetes and dyslipidemia (Klein et al., 2004). Patients with elevated cholesterol, triglycerides and LDL levels have twice the chance of developing diabetic retinopathy and studies show that 30-40 % of diabetics have additional impaired lipid metabolism because of associated nephropathy. In contrast, some studies have reported no change in total cholesterol, triglycerides and HDL-cholesterol concentration in PDR, NPDR and no diabetic retinopathy groups (Klein et al., 2004). Groups of diabetics with associated nephropathy showed higher levels of serum triglycerides and serum cholesterol associated with the duration and severity of diabetic retinopathy (Hadjadj et al., 2004).

1.7 GROWTH FACTORS AND THEIR ROLE IN DIABETIC RETINOPATHY

Endothelial cells and mural cells are two main factors determining the stability and regulation of the blood vessel wall and its proper functioning. Abnormal interaction between these two cell types leads to marked vascular changes in the form of microangiopathy and angiogenesis with involvement of certain growth factors like vascular endothelial growth factor (VEGF), advanced glycation end products (AGEs), platelet derived growth factor (PDGF), adipose tissue-derived protein leptin and a cytokine Interleukin-6 (IL-6), all leading to abnormal changes that are characteristic of diabetic retinopathy. Under hyperglycemic state, vitreous VEGF levels are strongly correlated to the presence of retinopathy. VEGF plays a major role in the noevascularisation which is a very strong feature of diabetic retinopathy (Caldwell et al., 2003).

1.7.1 Vascular Endothelial Growth Factor

Vascular endothelial growth factor (VEGF) belongs to a multipotent family of cytokines together with VEGF-B, C, D and E. The role of VEGF in embryonic vasculogenesis and angiogenesis is highlighted by the VEGF knockout mice phenotype where inactivation of a single allele resulted in embryonic lethality at day 11 and 12 and exhibited developmental abnormalities associated with defective vascularization and hematopoisis and reduced angiogenic sprouting. VEGFs are

secreted as disulphide bonded homoclimers. Fully processed VEGFs bind in an overlapping manner to three plasma membrane bound receptor tyrosine kianses: VEGF R-1, 2 and 3 (Jeltsch et al., 2006).

The development of methods for endothelial cell culture and models of angiogenesis has led to the isolation of many substances capable of influencing endothelial cell growth, hypoxia being the main inducing factor. VEGF is such an angiogenic inducer as has been shown in in vivo and in vitro studies. In some animal models loss of a single VEGF allele results in reduced and defective angiogenesis and vascularization on one end and certain tumor conditions due to VEGF overproduction on the other (Ferrara, 2004). Other studies have demonstrated that transplantation of tumor tissue shows a strong neo-vascular response and VEGF is one of the factors involved amongst the angiogenic factors (Ferrara et al., 2003). Previous studies indicate that patients with DR have a high concentration of VEGF in their ocular fluids (Aiello et al., 1994).

VEGF is an important neuroprotective agent as well and exposure of this factor to retina results in reduction of retinal neuronal apoptosis. VEGF and their receptors pay a key role in angiogenesis and lymphangiogenesis. Two receptors in this regard have been identified including the VEGF-1 and VEGF-2 (Jeltsch et al*.,* 2006). The main mechanism of how VEGF exerts its action is although not well understood but it has been postulated that it increases vascular permeability and causes macular edema which is the cause of blindness in diabetic patients. In diabetic retinopathy VEGF is increased in eye fluids. Moreover, injection of VEGF in the eyes of healthy animals produces retinopathic changes as seen in diabetic retinopathy (Starita et al., 2007). Normally VEGF is absent from retina, but in DR VEGF staining is apparent in most tissues particularly the endothelial cells and perivascular tissue and reduces to basal levels after laser surgery (Boulton et al., 1998). New vessel growth occurs due to stimulation by the VEGF in tissues facing hypoxia in order to meet metabolic demands of the tissue. In vitro models have also allowed the testing of known hormones and cytokines some of which have been found to affect endothelial cell proliferation (Lacono et al., 2002; Leske et al., 2003).

1.7.2 Interleukin-6

Cytokines are groups of proteins that regulate various immune responses by evoking a response in the form of upregulation or downregulation of several genes and their transcription factors. The cytokines act like hormones by binding to cell surface receptors and then producing a complex which alters the cell permeability and changes in cell function. Mostly these are soluble glycoproteins of 8-30 KDa molecular mass. In contrast to hormones, cytokines are released by the cells and are important in innate and adaptive immune response. They are involved in a number of immunological, inflammatory and infectious diseases and also are involved during embryogenesis (Zhang et al., 2011).

IL-6 is an interleukin that acts both as pro-inflammatory and anti-inflammatory cytokine. Human interleukin-6 has a 21-28 KDa peptide depending on the post-translational modification and contains 212 amino acids. The human IL-6 gene located on chromosome 7P21 (95-97) is approximately of 5 kb length and consists of four introns and five exons. Regulation of IL-6 expression is increased during times of stress and steroids are said to have a major role in its control. It is secreted by T-cells and macrophages to stimulate immune response to trauma, arthritis, reproduction, neoplasia and aging (Keller et al., 1996). IL-6 levels rise in serum and vitreous in patients with diabetic retinopathy (Dogan et al., 2006; Citirik et al., 2012). Experimental work has shown that IL-6 is involved in production of VEGF (Omori et al., 2004) and plays important role in the pathogenesis of retinal neo-vascularization in DR (Ohara et al., 2001, Funatsu et al., 2002, Grant et al., 2004). IL-6 is also myokine, a cytokine produced from muscle and is significantly elevated with exercise and precedes the appearance of other cytokines in the circulation. During exercise it is thought to act in a hormone like manner to mobilize extravascular substrates. Aadditionally osteoblasts secreted IL-6 to stimulate osteoclast formation (Mitamura et al., 2005).

IL-6 is one of the most important mediators of fever as it stimulates energy mobilization in the muscle and fatty tunic which leads to increased body temperature. In addition, IL-6 is secreted by macrophages in response to specific microbial molecules referred to as pathogen associated molecular patterns (Basu et

al., 2005). It is involved in a myriad of biologic processes like autoimmune diseases such as liver disease and multiple myeloma that explains its characteristics of differentiation factors. In addition, IL-6 appears to play an important role in bone metabolism through induction of osteoclastic activity and IL-6 inhibitors are used to treat osteoporosis in post-menopausal women. At times of stress, IL-6 levels are increased and steroids are said to have a major role in its control. Expression of IL-6 is reduced in stromal cells of fertile endometrium and in menopause or ovariectomy results in increased IL-6 secretion by mononuclear cells (Keller et al., 1996).

Recent studies on the course of various diseases and experimental work on laboratory animals have shown that IL-6 is active in a great number of physiologic and pathophysiologic processes. A wide variety of factors have been demonstrated to modulate IL-6 expression. While many of these processes stimulate IL-6 expression, only a few factors have been demonstrated to inhibit IL-6 expression. Corticosteroids, estrogens and androgens are among the inhibitors of IL-6 expression. Similarly in the process of ageing process IL-6 levels are increased and even greater levels are seen in Alzheimer's disease (Dor et al., 2001).

1.7.3 Leptin

Leptin is a 16 KDa protein hormone that plays a key role in regulating energy intake and energy expenditure, including the regulation of appetite and metabolism (Rahmouni and Haynes, 2005). The effects of leptin were observed in mutant obese mice that arose at random, these mice were massively obese and hyperphage (Baranova et al., 2006). Leptin gene is located on chromosome 7 in humans. Leptin protein is produced by the adipose tissue and interacts with six types of receptors (Lep Ra-f). Lep Rb is the only receptor present in a number of hypothalamic nuclei where it exerts its action. This is the only receptor that contains active intracellular signaling domains. Leptin binds to receptors located on neurons of ventral medial nucleus (VMN) of the hypothalamus known as satiety centre. Binding of leptin to this nucleus signals to the brain that the body has had enough to eat, thereby producing a sensation of satiety. Circulating leptin levels provide information to the brain about energy storage for the purpose of regulating appetite and metabolism. Leptin works by inhibiting the activity of neurons that regulate appetite. Alterations in immune

and inflammatory responses are present in leptin or leptin receptor deficient animals as well as during starvation and malnutrition, two conditions characterized by low levels of circulating leptin. Both leptin and its receptors share structural and functional similarities with the interleukin-6. Leptin exerts proliferative and anti-apoptotic activities in a variety of cell types including T-lymphocytes, leukemia cells and hematopioetic progenitors (Fantuzzi and Faggioni, 2000).

Leptin also affects cytokine production during the activation of monocytes /macrophages, wound healing, angiogenesis and hematopoiesis (Gariano et al., 2000). Moreover leptin production is acutely increased during infection and inflammation (Fantuzzi and Faggioni, 2000). Experiments on mice in 1950 identified a genetic defect that caused a severely obese phenotype due to over eating and decreased energy expenditure in mice. It was postulated that these obese mice were unable to produce a satiety factor. Leptin reversed the obesity syndrome in the obese mice and resulted in decreased food intake and increased activity when administered to normal mice. Leptin receptor (OB-R) was identified shortly after the discovery of leptin itself. The most important role for leptin is considered to be its inhibitory effect on appetite. Both the leptin deficient (ob/ob) and leptin receptor deficient (db/db) mice are not only obese they also develop a complex syndrome characterized by abnormal reproductive function, hormonal imbalances, and alterations in the hematopioetic and immune system. Similar alterations have been described in leptin-deficient humans. In diabetes there is resistance to leptin and not deficiency of leptin (Taniguchi et al., 2006; Snijder et al., 2006).

Furthermore, leptin directly regulates the production of several cytokines in vitro. Leptin displays proliferative and anti-apoptotic activity in a variety of cell types. It stimulates proliferation of tracheal epithelial cells, squamous cells of lungs and has a role in glomerulosclerosis. Leptin causes proliferation of endothelial cells and causes angiogenesis. Both in vitro and in vivo assays show that leptin has angiogenic activities including neo-vascularization and formation of capillary like structures (Ohara et al., 2001).

1.8 Situation in Pakistan

Pakistan is a developing country and has a population of over 170 million. Around 70% of its population lives in rural areas, illiterate and are mainly agriculturists. Larger cities have industries and, currently unhygienic conditions and extreme level of pollution, ill planned urbanization, and low economic conditions have all contributed over the past several decades to increasing incidence of devastating diseases like diabetes, cardiovascular problems, stroke, kidney diseases, skin problems, pulmonary diseases, hepatitis, chronic infections, neurological problems, bone disorders, genetic mutations and several others. All over the country, hospitals and dispensaries remain crowded each day with patients presenting variety of diseases and ailments.

As far as diabetes is concerned, like the rest of the world, diabetes mellitus is a major problem in South Asia (Gupta and Kumar, 2008) and is on the rise in Pakistan. According to National Diabetes of Pakistan, 10-11% of its population has diabetes (Shera et al., 1995; Shera et al., 1999ab; Shera et al., 2004), the country ranks 6th among countries with the highest burden of diabetes (Wild et al., 2004). Authenticated population-based data on the prevalence of diabetic retinopathy in Pakistan and on the visual impairment due to diabetic retinopathy are lacking. All available data are mostly hospital-based (Haider and Obaidullah, 1981; Jahangir, 1989; Khan 1990; 1991; Aziz 1996; Kayani et al., 2003; Basit et al., 2004; Shera et al., 2004; Afghani et al., 2007; Khanzada et al., 2011). In a recent study by Jamal-ud-Din et al. (2006), of 912 patients, 17.5% were diabetes, of which 1.8% had type-I diabetes and 15.7% had type-II diabetes. The commonest form of diabetic retinopathy was non-proliferative (76.5% [mild: 35.3 %, moderate: 29.4 %, and severe: 11.8 %), followed by maculopathy (17.6 %) and proliferative diabetic retinopathy (5.9 %). In addition to the above there are a number of studies conducted on Pakistani diabetic retinopathic patients, however, none of the studies addressed the significance of cytokines pertaining specifically to retinopathy, and their relationship with conventional diagnostic parameters.

Since data on clinical parameters in diabetic retinopathic patients and the involvement of growth factors and cytokines especially with reference to Pakistan do not exist, the present study was therefore designed to investigate samples of

diabetic retinopathic and non-retinopathic patients to further delineate the association of predictor variables with retinopathy.

1.9 Aim

The aim of the present study was to gain a further insight into the role of three significant molecules VEGF, IL-6 and leptin as predictors in a sample from local population of diabetic retinopathic patients.

Objectives

The objectives were to:

1. Measure the levels of VEGF, IL-6 and leptin in vitreous and serum of NPDR and PDR patients, in normal subjects and diabetic but non-retinopathic subjects.
2. To measure conventional serum parameters, body mass index, fasting and two post-prandial blood glucose levels, glycated hemoglobin, lipid profile (total cholesterol, HDL, LDL, triglycerides), renal profile (serum and urine creatinine, and urinary protein), systolic and diastolic blood pressure and visual aquity.
3. To carry out fundus examination for determining the severity of the disease
4. To compare and contrast conventional parameters among the four groups.
5. To correlate levels of serum and vitreous VEGF, IL-6 and leptin with all above conventional parameters, levels of which may predict the status or future onset of the disease.
6. To compare differences between male and female subjects.

MATERIALS AND METHODS

MATERIALS AND METHODS

2.1 STUDY DESIGN

2.1.1 Patients

The study was cross sectional. A total of 2000 male and female patients with known Type-II diabetes mellitus were screened for the presence of diabetic retinopathy over a period of two years. Sampling was random and no preference was given to specific geographical area, race or ethnic background in order to remove bias. Finally, 338 patients with confirmed diabetes and with or without retinopathy were selected. Patients' selection was purely random and ethnic or regional backgrounds were not given any preference. Retinopathic groups were designated as diabetic non-proliferative retinopathy (NPDR) and diabetic proliferative retinopathic (PDR). Positive and negative controls were diabetic but non-retinopathic (DNR, n = 38) and normal healthy subjects (NS, n = 39) respectively. Details of subjects and patients are being provided in Fig. 2.1 and Fig. 2.2. The study was carried out over a period of two years in the Outpatients Departments of four main hospitals, Khyber Teaching Hospital, Hayatabad Medical Complex and Lady Reading Hospital, located in Peshawar city and Al-Shifa Eye Trust Hospital located in Rawalpindi city.

Written informed consents were obtained from all patients presented for complaints of diabetes or diabetes related visual impairment. Patients' examinations were carried out in the presence of qualified diabetologist and ophthalmologist. The study design was approved by the Pakistan Medical Research Council (PMRC) and National Institute of Health (NIH), Islamabad. Ethical guidelines as given by the ethics committees of PMRC, NIH and Ministry of Health, Islamabad on scientific research on human subjects or human samples were strictly followed.

Performa (Appendix-I) were designed to record anthropomorphic and clinical case histories such as age, weight, height etc. Exclusion criteria were systemic diseases, kidney or heart disease, liver malfunctioning, respiratory or gastrointestinal disorders. Inclusion criteria were marked diabetes, adult onset of the disease and visual symptoms. Only those patients who had hypertension related with diabetes were included, and were excluded otherwise. Detailed history was followed by

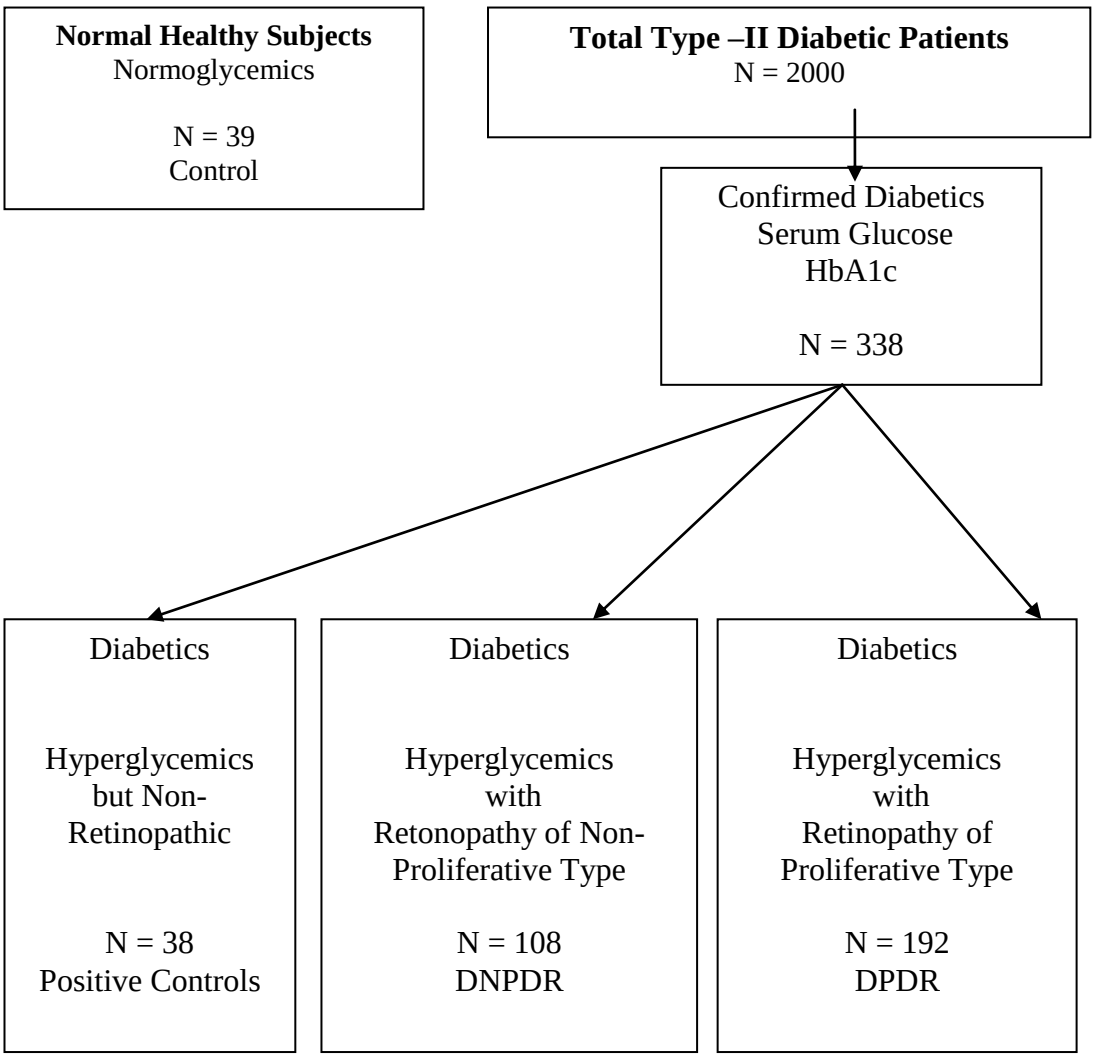


Fig. 2.1 Flow Chart diagram demonstrating total number of subjects screened and those found having type-II diabetes on the basis of pre-examination and plasma glucose levels. Further diagnostics tests revealed patients having retinopathy who were subjected to further standard clinical and fundoscopic examination for confirmation of having either non-proliferative or proliferative retinopathy. Diabetic patients not having retinopathy were taken as controls against retinopathic patients. Normal completely healthy subjects were taken as pure controls for comparison purpose and gathering standard data of Pakistani population.

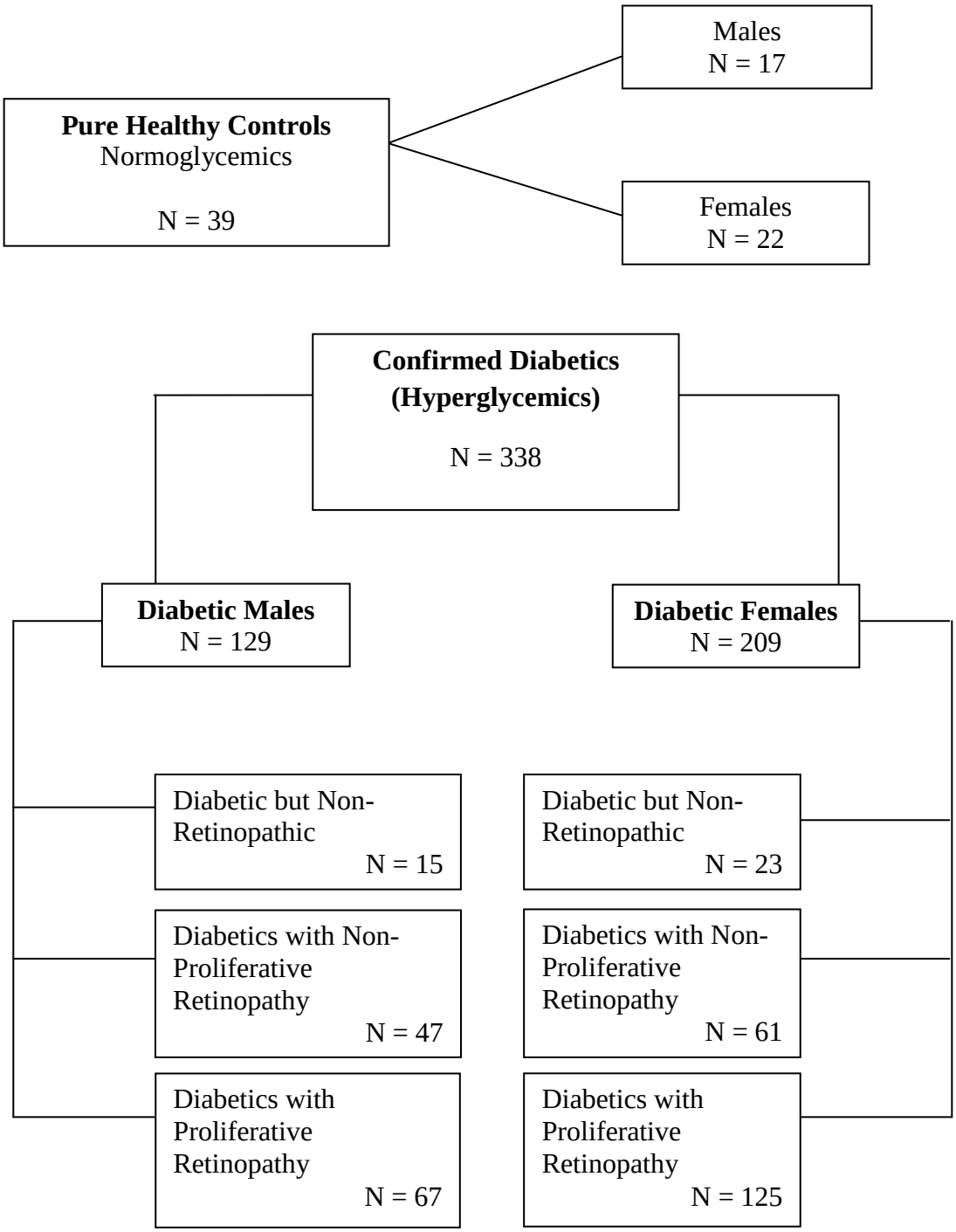


Fig. 2.2 Flow-chart diagram showing sex-wise distribution of healthy control subjects, diabetics not having retinopathy and diabetics with either non- proliferative or proliferative retinopathy.

Standard physical examination included the measurement of blood pressure, testing of visual acuity and fundus examination. Then blood was aspirated for serum separation to determine glucose levels, HbA1c, lipid profile, VEGF, IL-6 and leptin using kit obtained from commericial suppliers.

2.1.2 Clinical History

Detailed information of presenting complaints, symptoms, history of presenting illness, past history, social history, family history and personal history was recorded for each patient in the prescribed performa.

2.1.3 General Physical Examination

General physical examination of diabetic patients was carried out according to the standard criteria as laid down in Talley and O'Conner (2006).

2.1.3.1 Body Mass Index (BMI)

Patients' height and weight were recorded through standard procedures. BMI was calculated using the standard formula:

$$\frac{Weight\ (kg)}{Height\ (m^2)}$$

Reference values of BMI values range between 20-25 kg/m^2. Following were the recommendations of the BMI for Asia-Pacific region defined by the WHO in the year 2000:

Reference values:	Under weight	<18.5 kg/m^2
	Normal range	18.5-24.9 kg/m^2
	Over weight	25 - 29.9 kg/m^2
	Obese	> 40 kg/m^2

In Pakistani population it has been described that BMI values of ≥25 and ≥30 define overweight and obesity, respectively (Nanan, 2001).

2.1.3.2 Skin Examination

Skin color, hydration and pigmentation were examined and relevant information was recorded.

2.1.3.3 Leg examination

Skin of legs was inspected for necrobiosis, hair loss, infection, pigmented scars, atrophy, ulceration and for evidence of injection sites. Muscle wasting was

examined in both legs with measuring tape. Temperature of feet and legs were recorded by palpation. Femoral, popliteal, posterior tibial, and dorsalis pedis pulses were felt and recorded for each patient.

2.1.3.4 Arm Examination

Skin of arms was inspected for injection site and any skin lesions.

2.1.3.5 Determination of Radial Pulse

Radial pulse was examined for rate, amplitude, character and volume. These were recorded appropriately.

2.1.3.6 Measurement of Blood Pressure

Seated systolic and diastolic blood pressures were measured using the conventional mercury sphygmomanometer. The first reading was discarded and three readings were taken for each patient and values are presented as arithmetic mean. Blood pressures values beyond which the patients were considered hypertensive were: > 130 mmHg and pressure > 90 mmHg for systolic and diastolic pressures respectively,

2.2 SYSTEMIC EXAMINATION

2.2.1 Cardiovascular Examination

Patients were examined for jugular venous pressure, radial and carotid pulses, inspection and palpation of apex beat. Auscultation of heart was done for any abnormal heart sounds or murmurs.

2.2.2 Respiratory System

Patients were examined for any signs of cyanosis. Shape of the chest and breathing pattern were examined. Chest was auscultated for breath sounds.

2.2.3 Gastrointestinal Examination

Abdomen was inspected for scars, distention or abnormal vein bruising or pigmentation. Deep palpation was done for liver and kidneys screening. Percussion was done for ascites.

2.2.4 Genitourinary Examination

Detailed history and genitourinary examination performed.

2.3 Urine Examination

2.3.1 Urine Regular Examination (Urine R/E)

Urine appearance, smell, specific gravity and pH were recorded. Urine samples were assessed for epithelial casts and presence of RBCs. Bile pigment, bile salts and ketone bodies were also measured.

2.3.2 Estimation of Urine Creatinine

Principle

In alkaline medium, creatinine reacts with picrate and forms a red-orange colored complex. The transformation speed of the colored complex, measured with a spectrophotometer during the few minutes of the reaction, is proportional to the creatinine concentration in the sample.

Procedure

Urine samples were diluted 1:50 with distilled water. Working reagent was made by mixing equal volumes of reagent 1 (sodium hydroxide 0.8 mol/L) with reagent 2 (picric acid 15 mmol/L). Analysis was done when working reagent acquired the chosen temperature (37^0C). Samples were mixed and incubated for 30 sec at the test temperature. Absorbance of the sample (AbsS1) and the standard (Abs Std 1) were read. After exactly 1 min from the first reading, the sample (AbsS2) and the standard (AbsStd2) absorbance was read again.

The concentrations in the sample were calculated by using the following formula:

[gr/24h] creatinine = (AbsS2-AbsS1) / (AbsStd2-AbsStd1) ×1×L/24h

[mmol/24h] creatinine = (AbcsS2-AbsS1) / (AbsStd2-AbsStd1) ×8.85×L/24h

The sensitivity of the method was 0.07 mg/dl while the linearity was up to10 mg/dl (884 µmol/l).

Reference Values: 14-26 mg /24 hrs Men

11-20 mg /24 hrs Women

Urinary protein (albumin in this case) was also determined. Normal values were considered to be between 0-225 mg/24 hrs.

2.4 OPHTHALMIC EXAMINATION

Detailed ophthalmoscopic examination was carried for both eyes on each patient.

2.4.1 Visual Acuity and Ophthalmoscopy

Visual acuity was carried out through Snellen's Eye chart using standard procedures. Indirect ophthalmoscopy provided a stereoscopic view of the fundus. 20D (magnification ×3; field about 45°) was used for general examination of the fundus. Both pupils were dilated with 1 % tropicamide and 10 % phenylephrine to prevent constriction when exposed to bright light during the eye examination. Locking screw unlocked to allow side tilting of illumination column, then anaesthetic drops were instilled and coupling fluid (high viscosity methylcellulose or equivalent) was inserted into the cup of the contact lens: it should be no more then half full. The patient is asked to look up; the inferior rim of the lens is inserted into the lower fornix and pressed quickly against the cornea so that the coupling fluid has no time to escape and the illunination column should always be tilted except when viewing the 12 0'clock position in the fundus (i.e. with the mirror at 6 o' clock). When viewing horizontal meridians (i.e. 3 and 9 o' clock positions in the fundus) the column should be kept central. When viewing the vertical meridians (i.e 6 and 12 o' clock positions) the column was positioned left or right of centre. When viewing oblique meridians (i.e. 1.30 and 7.30 o' clock) the column was kept to the right of centre, and vice versa when viewing the 10.30 and 4.30 o' clock positions. When viewing different positions of the peripheral retina the axis of the beam is rotated so that it is always at right angles to the mirror. To visualize the entire fundus the lens was rotated at 360 degrees, using first the equatorial mirror and then the peripheral mirrors. To obtain a more peripheral view the lens was tilted to the opposite side asking the patient to move the eyes to the same side. For example, to obtain a more peripheral view of 12 o' clock (with mirrors at 6 o' clock) tilt the lens down and the patient was

asked to look up. The vitreous cavity was examined with the central lens using both a horizontal and vertical slit beam. The posterior pole was examined.

2.4.2 Fluorescein Angiography

Principle

Fluorescence is the property of certain molecules to emit light of a longer wavelength when stimulated by light of a shorter wavelength. The excitation peak for one such dye named fluorescein is about 490 nm (blue part of the spectrum) and represents the maximal absorption of light energy by fluorescein. Molecules stimulated by this wavelength will be excited to a higher energy level and will emit light of a longer wavelength at about 530 nm (green part of the spectrum). Filters of two types are used to ensure that blue light enters the eye and only yellow-green light enters the camera. The emerging blue light enters the eye and excites the fluorescein molecules in the retinal and choroidal circulations, which then emit light of a longer wavelength (yellow-green). A yellow-green filter then blocks any reflected blue light from the eye, allowing only yellow-green light to pass through.

Fluorescein is an orange colored water-soluble dye that, when injected intravenously remains largely intravascular and circulates in the blood stream. Fluorescein angiography (FA) involves photographic surveillance of the passage of fluorescein through the retinal and choroidal circulations following the intravenous injection. On intravenous injection, 70-85% of fluorescein molecules bind to serum proteins (bound fluorescein); the remainder remains unbound (free fluorescein).

The major choroidal vessels that form the outer blood-retinal barrier are impermeable to both bound and free fluorescein. However, the walls of the choriocapillaries are extremely thin and contain multiple fenestrations through which free fluorescein molecules escape into the extravascular space. They then pass across Bruch membrane but on reaching the retinal pigment epithelium (RPE) encounter tight junctional intercellular complexes termed zonula occludens, which prevent the passage of free fluorescein molecules across the RPE. The inner blood-retinal barrier that is composed of the tight junctions between retinal capillary endothelial cells and retinal layer is the next across which neither bound nor free fluorescein can pass. Fluorescein is therefore confined within the lumen of the retinal capillaries. The basement membrane and pericytes play only a minor role in

the leakage of fluorescein. Disruption of the inner blood-retinal barrier permits leakage of both bound and free fluorescein into the extravascular space.

Phases of the Angiogram

When intravenous fluorescein is injected it enters the eye through the ophthalmic artery, passing into the choroidal circulation through the short posterior ciliary arteries and into the retinal circulation through the central retinal artery. Because the route to the retinal circulation is slightly longer than the choroidal, the latter is filled about one second before the former. The choroidal (pre-arterial) phase occurs 8-12 sec after the dye injection and is characterized by the patchy filling of the choroid due to the leakage of free fluorescein through the fenestrated choriocapillaries. A cilioretinal artery will fill at this time since it is derived from the posterior ciliary circulation. The arterial phase shows arterial filling and the continuation of choroidal filling. The arteriovenous (capillary) phase shows complete filling of the arteries and capillaries with early laminar flow in the veins in which the dye appears to line the venous wall, leaving an axial hypofluorescent strip. Choroidal filling continues and background choroidal fluorescence increases as free fluorescein continues to leak from the choriocapillaries into the extravascular space.

The venous phase

(i) The early phase exhibits complete arterial and capillary filling and more marked laminar venous flow.

(ii) The mid- phase displays almost complete venous filling.

(iii) The late phase shows complete venous filling with reducing concentration of dye in the arteries.

The late (elimination) phase demonstrates the effects of continuous recirculation, dilution and elimination of the dye. With each succeeding wave, the intensity of fluorescence gets weaker. Late staining of the disc is a normal finding. Fluorescein is absent from the angiogram after 5-10 min and is usually totally eliminated from the body within several hrs. The dark appearance of the fovea is caused by three phenomena:

i. Absence of blood vessels in the foveal avascular zone
ii. Blockage of background choroidal fluorescence due to increased density of xanthophyll at the fovea.

Technique

During the angiographic examination, the patients were allowed to be seated in front of the fundus camera. Fluorescein, 3 ml of a 25 % solution, was injected intravenously. Images of the fundus of both eyes were taken at approximately 1 sec intervals, 5-25 sec after the injection. Where appropriate, photographs were taken after 10 min and occasionally at 20 min especially if leakage was anticipated.

Adverse Effects

Patients were kept under strict observation for mild side effects like nausea, vomiting, flushing of the skin, itching, hives and excessive sneezing. Necessary arrangements were kept ready for serious but rare problems such as syncope, laryngeal edema, bronchospasm and anaphylactic shock.

2.4.3 Fundus Photography

Fundus photography of the affected eyes was done in all the patients. Prior to fundus photography pupil dilation was made with one or more drops of 1% tropicamide, a fundus camera (CF-60UV; Canon Europa NV, Amstelveen, The Netherlands) was set at 60° angular field of view using 35-mm color transparency film (Ektachrome Elite 100; Eastman Kodak, Rochester, NY). A stereoscopic set of photographs was recorded from the macular region of each eye.

2.4.4 Vitrectomy for Advanced Proliferative Retinopathy

The patients were admitted 24 hrs prior to the surgical procedure in the opthalmology ward. The FBS, RBS, blood clotting time, bleeding time and urine examination were repeated. The patients were given light meal followed by overnight fast. Lignocaine injection was given prior to the surgical procedure. During the limbal peritomy an infusion cannula was secured to the sclera 3.5 mm behind the limbus in the affected eye at the level of the inferior border of the lateral rectus muscle. Two further sclerotomies were made at the 10 and 2 o'clock positions. These can be standard stab incisions made with an MVR blade or self-sealing sclerotomies. The cutter and fiber optic light pipe enter through the upper two sclerotomies. Vitreous fluid was with drawn through standard size cannula. The fluid was collected in the 5 ml syringes and immediately stored at -30°C. The central vitreous gel and posterior hyaloid face were excised.

The above basic steps apply to all vitrectomies. After the withdrawal of vitreous fluid, the flat retinal breaks were treated with photocoagulation. To bring the eye back to normal stable position, silicone oil (EM-2180 amino silicon) was introduced into the eye.

2.5 Serum Analyses

Patients were advised to have an overnight fast for serum preparation.

Serum preparation

Blood samples collected from normal healthy subjects, diabetic non-retinopathic and retinopathic patients were processed for serum preparation through standard procedures. Briefly, the blood was allowed to stand at room temperature for about an hour and then centrifuged (Eppendorf 5417C, Germany) at 1250 $\times g$ for 10 min. Serum thus collected was aliquoted and stored at -20°C until analyzed for related serum parameters and growth factors.

Clinical laboratory examination included determination of the random blood sugar (RBS); fasting blood glucose / sugar (FBS); glycated hemoglobin (HbA1c); serum total cholesterol (TC); triglycerides (TG); high density lipoprotein (HDL); low density lipoprotein (LDL) and serum creatinine.

2.5.1 Estimation of Random and Fasting Blood Sugar/ Glucose

Random blood sugar (RBS) was collected 2 hrs post prandial, (2 hrs after the first meal in the morning). For measurement of fasting blood sugar (FBS), patients were put to overnight fast and serum was collected and stored at -20 °C until analyzed. Glucose levels were measured with a multi-channel analyzer (Hitachi Model 737) using the kits obtained from commercial suppliers (Boerhinruger Mannheim, Indianapolis, IN) following the standard enzymatic colorimetric method for the determination of glucose.

Procedure

Reagent 1 (phosphate buffer 100 mmol/L of pH 7.4, phenol 10 mmol/L) was mixed with reagent 2 (glucose oxidase 10000 µl, peroxidase 600 µl, 4-Aaminoantipyrine 270 µmol/L). Absorbance was measured at 500 nm at the room temperature 37°C after 10 min of incubation. The final color was stable for at least 1hr.

Fasting blood glucose upto 126mg/dl and above was taken as diabetes and levels between 101 and 125 mg/dl was impaired fasting glucose (American Diabetes Association, 2007). The same criteria are used by WHO for diagnosis of diabetes mellitus in epidemiological studies (WHO, 1999).

2.5.2 Estimation of Creatinine

Principle

In alkaline medium, creatinine reacts with picrate and forms a red-orange colored complex. The transformation speed of the colored complex, measured with a spectrophotometer during the few minutes of the reaction, is proportional to the creatinine concentration in the sample.

Procedure

Working reagent was made by mixing equal volumes of reagent 1(sodium hydroxide 0.8 mol/L) with reagent 2 (picric acid 15 mmol/L). Analysis was done when working reagent acquired the chosen temperature (300 or 37°C). Samples were mixed and incubated for 30 sec. Absorbance of the sample (AbsS1) and the standard (AbsStd 1) was read. After exactly 1 min from the first reading, the sample (AbsS2) and the standard (AbsStd2) absorbence was read again.

The concentrations in the sample were calculated by using the following formula.

[mg/dl] creatinine= (AbsS2-AbsS1)/ (AbsStd2-AbsStd1) ×2

[µmol/L] creatinine= (AbsS2-AbsS1)/ (AbsStd2-AbsStd1) ×176.8

The sensitivity of the method was 0.07 mg/dl while the linearity was up to 10 mg/dl (884 µmol/L).

Reference Value: 0.8-1.2 mg/dl

2.5.3 Estimation of Glycated Hemoglobin – HbA1c

Principle

Calibrators, controls and hemolyzed whole blood samples are mixed with reagent 1 containing anti-HbA1c antibody to form a soluble antigen-antibody complex. Unbound anti-HbA1c antibody reacts with polyhapten (hexapeptide-glycan, A1c reagent 2) to form an insoluble antibody-polyhapten immune complex, which is measured turbidimetrically at 340 nm. After a calibratation has been performed for each reagent lot, the HbA1c concentration in each unknown sample can be

determined using the stored calibration curve and the measured absorbance obtained in the assay of the hemolyzed sample. The % HbA1c is calculated from the quantitative measurements of hemoglobin and HbA1c in the hemolyzed sample.

Procedure

Whole blood free of clots collected in EDTA, vaccoutainers was used. Whole blood samples were mixed properly, 350 µl was pipetted into a sample cup.

Absorbance was measured at 340 nm after incubation time.

%A1c = HbA1c [g/dl] / Hb[g/dl] ×100

Reagents

HbA1c reagent 1(R1): HbA1c antibody (ovine serum) ≥ 0.5 mg/ml

HbA1c reagent 1(R2): HbA1c polyhapten ≥ 8 µg/ml

Precision

Precision was evaluated with quality control materials (hemolysate and blood-based) on the vitros 5, 1FS chemistry system following NCCLS protocol EP-5A.

Specificity

Rheumatoid factor (RF) up to 750 IU/ml did not interfere. The anti-HbA1c antibodies used in this kit did not cross react with HbA0, HbA1a, HbA1b, acetylated hemoglobin, carbamylated hemoglobin and glycated albumin. This method was unaffected by the presence of labile glycated hemoglobin. The substances acetominophen, acetlisted, acetylcysteine, ascorbic acid, bilirubin, ascorbic acid, Ca-Dobesilate, cefoxitin, intralipid, levodopa, rifampicin were tested with the vitros chemistry products d% A1c assay values of approximately 5.0 % to 7.0 % and were found not to interfere.

Reference Value: < 6.5%

Cut off value: > 7.0 %

2.5.3 Estimation of Lipids and Lipoproteins

The patients were advised a light meal before overnight fast. Total cholesterol was measured using the commercial kit (Ecoline, Germany) on Autoanalyzer II (Technicon) by the Lisermann Burchard Reaction using un-extracted sample. High density lipoprotein (HDL) cholesterol was measured in the supernatant obtained by precipitate of low density lipoprotein (LDL) with heparin and manganese chloride according to the previously described method of Burstein et al. (1970).

Principle

Chylomicrons, VLDL and LDL are precipitated by adding phosphtungstic acid and manganese ions to the samples, centrifugation leaves only the HDL in the supernatant, Their cholesterol content is determined enzymatically using Ecoline S + Cholesterol.

Procedure

For serum total cholesterol, serum (200 µl) was mixed with 500 µl precipitation reagent (phospotungstic acid 1.4 mmol/L, magnesium chloride 8.6 mmol/L). The mixture was incubated for 15min at room temperature and then centrifuged for 20 min at 2500×g. After 2 hrs of centrifugation transfer 0.1 ml of the clear supernatants were transferred to the reaction solution for the determination of cholesterol.

Serum with triglyceride contents >1000 mg/L tends to produce turbid supernatants or HDL cholesterol was measured in the supernatant obtained after precipitation of VLDL by sodium dodecyl sulphate while the LDL cholesterol was obtained by difference.

Triglycerides were measured by enzymatic hydrolysis using a commercial kit (Beohringer Mennheim Corporation, Germany) on an automated spectrophotometer. While the supernatants after the centrifugation should be clear. In this case sample was diluted as 1:1 with 0.9 % saline solution and precipitation was performed.

Reference Values: Total cholesterol: 120 – 200 mg/dl

HDL cholesterol: > 60 mg/ dl

LDL cholesterol: < 100 mg/dl

2.5.4 Estimation of Vascular Endothelial Growth Factor (VEGF)

Vascular endothelial growth factor concentration in serum and vitreous samples was estimated through a standard solid phase sandwich enzyme linked-immunosorbant assay (ELISA) using a commercial kit obtained from AssayPro (Belgium).

Principle

A polyclonal antibody specific for human VEGF coated onto the wells of the microtiter strips is used. Samples, including standards of known human VEGF

content, control specimens, and unknowns, are pipetted into these wells. During the first incubation, the Hu VEGF antigen binds to the immobilized (capture) antibody on one site. After washing, a biotinylated monoclonal antibody specific for Hu VEGF is then added. During the second incubation, this antibody binds to the immobilized Hu VEGF captured during the first incubation. After removal of excess secondary antibody, streptavidin-peroxidase is added which binds to the biotinylated antibody to complete the four-member sandwich. After a third incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of Hu VEGF present in the original specimen. To prepare the standard curve, for human VEGF, dilutions ranging from 0-1500 pg/ml, while for streptavidin-HRP dilutions ranging from 2-12 ml were made.

Assay method: Procedure and Calculation

All reagents were brought to room temperature approximately 30 min before use. Standard curve was prepared simultaneously with the measurement of test samples. Wells for reagent blank were determined and 100 µl each of 'tube-4' EIA buffer was put into it. Wells for test sample blank, test sample and diluted standard were determined. 100 µl was each of the test sample blank (tube 8), test sample and dilutions of standard (tube-1-7) were added to the appropriate wells. Precoated plate was incubated for 60 min at 37 ^{0}C or overnight at 4 ^{0}C. Each well of the precoated plate was washed vigorously with wash buffer. Wash buffer was removed completely from the precoated plate by snapping them onto paper towel. Labeled antibody solution of 100 µl was pipetted into the wells of test samples, diluted standard and test samples blank. The precoated plate was incubated for 30 min at 4 ^{0}C after covering it with plate lid, washed the plate nine times as above. Required quantity of 100 µl "6, chromogen" was taken and pipetted into the wells. The precoated plate was incubated for 30 min at room temperature in the dark. The liquid was turned blue by the addition of "6, chromogen". Stop solution (1N H_2SO_4) of 100 µl was pipetted into the wells and mixed by tapping the side of precoated plate. The liquid was turned yellow by the addition of stop solution. Samples were run on the plate reader and absorbance was recorded at 450 nm. The measurements were done within 30 min after addition of the stop solution. Absorbance of

standards was plotted against the standard concentration. Hu VEGF concentration was read for unknown samples and controls from the standard curve. Same assay procedure was used for both the vitreous and serum. No background problem or cross reactivity was seen.

Limitations of the Procedure

Standard curve did not extrapolate beyond the 1500 pg/ml and the minimum detectable dose of VEGF is < 5 pg/ml. Intra and inter-assay coefficients were 4.7 and 8.1 respectively.

Specificity

The following substances were tested and found to have no cross reactivity: human IL-1b, IL-2, IL-6. IL-8, IL-10, IL-13, IL-15, EGF,FGF basic, FGF acidic ,G-CSF,GM-CSF, IFN-g, RANTES, SCF, TGF-a, TNF-a; mouse IL-1b, IL-6,IL-10, G-CSF, GM-CSF, IFN-g, TNF-a; rat IL-1b,IL-6, IL-10,GM-CSF,IFN-g, TNF-a. Mouse and rat VEGF-165 showed 0.25 % and 0.11% cross-reactivity, respectively. Human VEGF-121 showed 100 % cross-reactivity and complete parallelism with huVEGF-165.

Reference Values:

Serum:	33-86 pg/ml
Vitreous:	$\leq$ 138 pg/ml

2.5.5 Estimation of Interleukin-6

Interleukin-6 concentration in serum and vitreous samples was estimated through a standard solid phase sandwich enzyme linked-immunosorbant assay (ELISA) using a commercial kit obtained from Immunotech Immunotech SAS (France).

Principle

The immunotech IL-6 enzyme immunoassays (IM1120, IM11120) are intended for quantification of human interleukin 6 in plasma, serum or culture supernatants. Samples and calibrators are incubated in microtiter plate coated with the first monoclonal antibody anti IL-6, in the presence of the second anti-IL-6 monoclonal anti body linked to acetylcholinesterase (ACE). After incubation, the wells are washed and the bound enzymatic activity is detected by addition of a chromogenic substrate. The intensity of the coloration is proportional to the IL-6 concentration in the sample or calibrator.

Calibration Curve

A quadratic mode curve fit with absorbance taken on vertical axis and the IL-6 concentration of the calibrators taken on the horizontal axis (0-1000 pg/ml) was drawn.

Procedure

After solubilization of lyophilized reagent and 10 min wait, components were mixed gently to avoid foaming. The samples were diluted with 50 ml of the wash solution (20 x) with 950 ml of distilled water. The lyophilized conjugate was reconstituted with the volume of distilled water stated on the vial label. Calibrator or sample of 100 µl and 100 µl conjugate were added per well. Incubated for 2 hrs at 18-25 ^{0}C and were shaken. Substrate (200 µl) was added and incubated for 30 min at 18-25 ^{0}C. 50 µl stop solution was added and absorbance was read at 450 nm. Same assay procedure was used for both the vitreous and serum. No background problem or cross reactivity was seen.

Specificity

The IL-6 ELISA kit was obtained from Immunotech SAS, (France). The assay measures natural or recombinant, human IL-6 no cross-reactivity or interface with other cytokines or cytokine receptors is known.

Precision According to manufacturer, intra-assay coefficient of variation ranged between 1.6 and 6.8 %, while the Inter-assay coefficient of variation ranged between 7.9 and 14.6 %.

Accuracy

a. Dilution Test

Sera containing IL-6 were diluted to 1:8 using the IL-6 diluent 2. The observed recovery was between 95 and 109 %.

b. Recovery Test

IL-6 was added at different concentrations using different samples. The observed recovery average was ranged between 97 and 105 %.

Reference Values:

Serum:	< 4.0 pg/ml
Vitreous:	< 100 pg/ml

2.5.6 Estimation of Leptin

Leptin concentration in serum and vitreous samples was estimated through a standard solid phase sandwich enzyme linked-immunosorbant assay (ELISA) using a commercial kit obtained from Immunotech SAS (France).

Principle

The enzyme immunoassay test followed a typical two-step capture or "sandwich" type assay by using AssayMax Human Leptin ElISA Kit (Assaypro, Belgium). The assay makes use of two highly specific monoclonal antibodies: a monoclonal antibody specific for leptin is immobilized onto the micro well plate and another monoclonal antibody specific for a different epitope of leptin is conjugated to biotin.

During the first step, leptin present in the samples and standards is bound to the biotinylated antibody, thus forming a sandwich complex. Excess and unbound biotinylated antibody is removed by a washing step. In the second step, streptavidin –HRP is added, which binds specifically to any bound biotinylated antibody. Again, unbound streptavidin –HRP is removed by a washing step.

Next the chromogen/substrate is added, forming a blue colored product that is directly proportional to the amount of leptin present. The enzymatic reaction is terminated by the addition of the stop solution, converting the blue color to a yellow color. The absorbance is measured on a microtiter plate reader at 450 mm. A set of standards is used to plot a standard curve from which the amount of leptin samples and control can be directly read.

Procedure

Working solutions of the streptavidin-HRP conjugate and washing solution were prepared. Each calibrator of 20 µl control and serum samples was pipetted in duplicate into correspondingly labeled wells. Monoclonal anti-leptin-biotin conjugate of 80 µl were pipetted out into each well. Samples were incubated on a plate shaker

(approximately 200 rpm) for 1 hour at room temperature. The wells were washed three times with prepared washing solution (300 µl/well for each wash). Streptavidin-HRP conjugate of 100 µl was pipetted out into each well and incubated on a plate shaker (approximately 200 rpm) for 30 min at room temperature. Chromogen/substrate of 100 µl was pipetted out into each well at timed intervals

and incubated on a plate shaker for 10-15 min at room temperature. Stop solution of 50 µl was pipetted out into each well and the plate was read on a micro well plate reader at 450 nm within 20 minutes after addition of the stop solution. Same assay procedure was used for both the vitreous and serum. No background problem or cross reactivity was seen.

Sensitivity

The limit of detection (loD) for leptin is 0.05 ng/ml, as determined by use of a NCCLS protocol and with proportions false positives (α) less than 5% and false negative (β) less than 5% based on 82 blank determinations LoB= 0.42 ng/ml.

Specificity

The following substances were tested at 1000 mg/ml and exhibited no cross-reactivity: Mouse leptin, TNF-α, IL-2, II-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12, IL-16, GM-CSF, CSF and EGF.

Calculations

The mean optical density of each calibrator was calculated at 450 nm. A calibrator curve was drawn on a semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on X-axis. The mean optical density of each unknown was calculated. The values of the unknowns were read directly off the calibrator curve.

If a sample read more than100 ng/ml then it was diluted with assay buffer at a dilution of no more than 1:8. The results obtained were multiplied by the dilution factor.

Reference values:

Serum: < 14.4 pg/ml

Vitreous: < 2.0 pg/ml

2.6 Statistical Analyses

Data are presented as mean ± SD (standard deviation). Analyses were done using the SPSS version 14 (Chicago, Illinois, USA). Where required, data were corrected for age, sex and duration of diabetes. As the data were non-parametric group comparisons were made with Kruskal-Wallis one-way ANOVA on ranks. Students t-test was used to compare the between male and females the difference in mean values of parameters. Where t distribution failed, analysis was done with

Mann-Whitney U test. Post hoc Tukey's analysis or Dunn's tests were done to further verify the data. Spearman's correlation analysis was carried out to find correlations between serum parameters and of serum parameters with the VEGF, IL-6 and leptin. Since the VEGF, Leptin and IL-6 were determined one time, values were not subjected to multivariate analyses; hence simple linear or logistic regression analysis was carried out keeping conventional parameters dependent variables to determine if VEGF, Leptin and IL-6 act as predictors. $P < 0.05$ was considered significant difference.

RESULTS

RESULTS

3.1 Distribution of Subjects and Retinopathic Patients

Of 2000 male and female patients with type-II diabetes (hyperglycemics), 338 patients (16.9%) were confirmed diabetics. Of these diabetics, 38 (11.24%) were diabetic but non-retinopathic (DNR) and therefore taken as positive controls, 108 (31.95%) were diabetic having non-proliferative retinopathy (NPDR), and 192 (56.80%) were diabetic with proliferative retinopathy (PDR). Normal healthy subjects were taken as negative control (n=39, 17 males and 22 Females). Sex-wise, of 338 diabetic patients, 129 (38.16%) were males consisting of 15 (11.62%) DNR patients, 47 (36.43%) NPDR patients, and 67 (51.9%) were PDR patients. Of 209 (61.83%) female patients, 23 (11.00%) DNR patients, 61 (29.18%) NPDR patients, and 125 (59.8%) were PDR patients (Fig. 3.1a & b).

3.2 Age of subjects and Patients

Mean age of diabetic and diabetic retinopathic patients was 50 years ranging between 37-65 years, while of normal subjects was 53 ranging from 35-61 years. No difference in age was found among between each of these groups compared to normal subjects (Table 3.1).

Median ages of normal, DNR, NPDR and PDR male and female subjects are given in Table 3.2. Females of normal subjects and diabetic but non retinopathic group had significantly older age as compared to males ($P < 0.41$ and $P < 0.001$ respectively). In NPDR patients, males were of significantly older age ($P < 0.002$). In PDR patients, mean age of males and females did not differ significantly (Table 3.2).

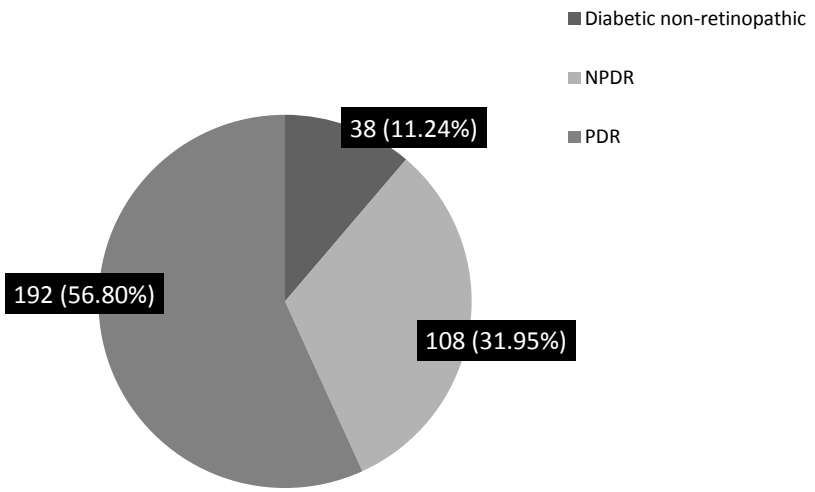


Fig. 3.1a Number of patients in each study group

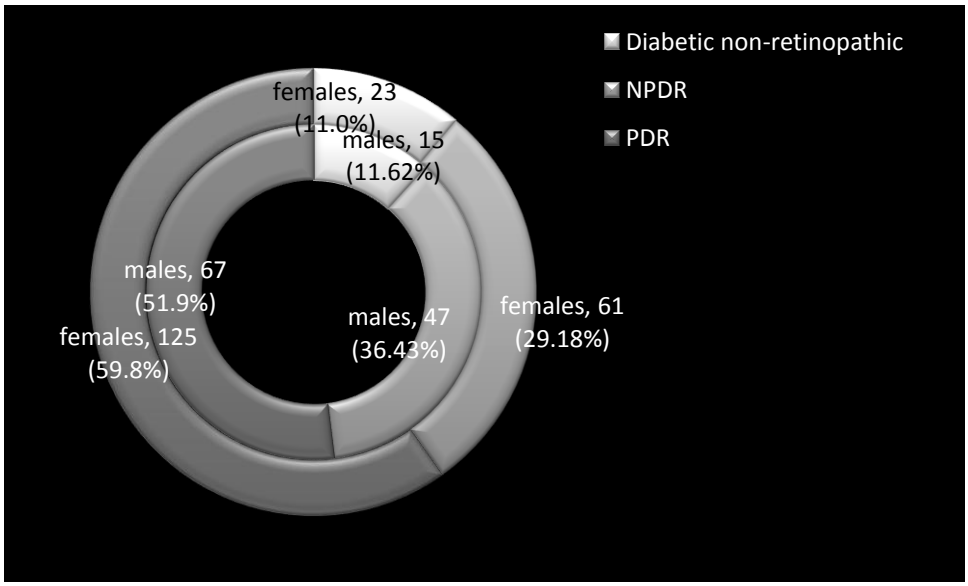


Fig. 3.1b Gender distribution in each study group

Fig. 3.2 Normal retina of human Eye shows fundoscopy (A) and flourescein fundus angiography (B)

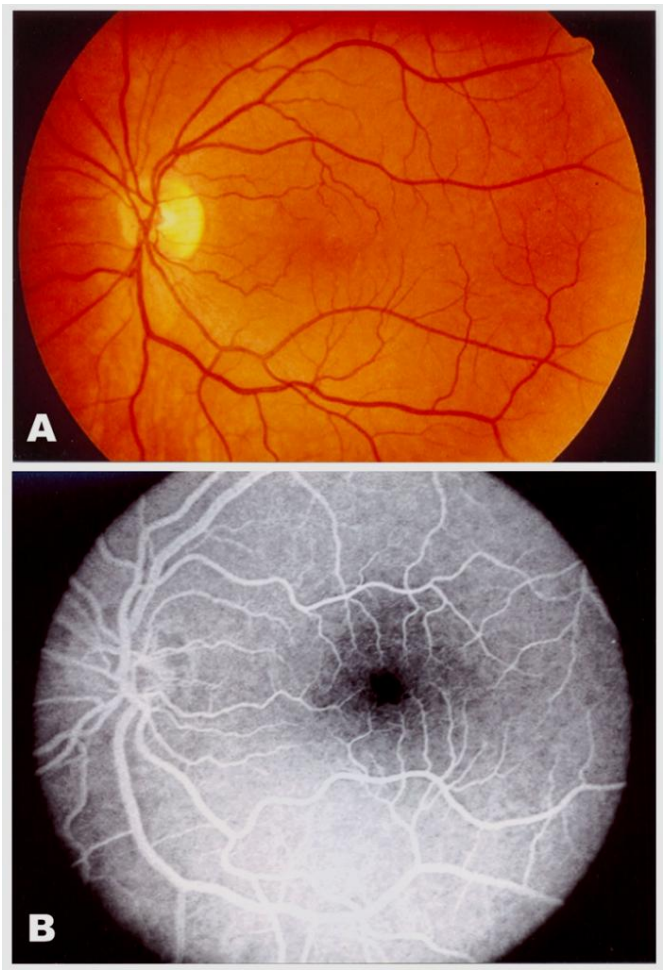

A
B

Fig. 3.3 Fundoscopic appearance if the retina showing NPDR

A: Mild NPDR shows dilated retinal veins branching off from the central retinal vein. There are microaneurysms appearing as focal spots. Hard exudates are few and scattered.

B: Moderrate NPDR shows dot and blot pattern of retinal hemorrhages. Cotton wool spot are interpoles between the two retinal vessles.

C: Severe NPDR shows flame shaped and dot/blot pattern of retinal hemorrhages. There is assocated increase in the retinal vein tortuusity.

Abbreve: Dilated vein (DV); Hard exudates (HE); Microaneurysms (MA); Multiple retinal hemorrhages (MRH); retinal hemorrhages (RH); Retinal venous tortuosity (RVT)

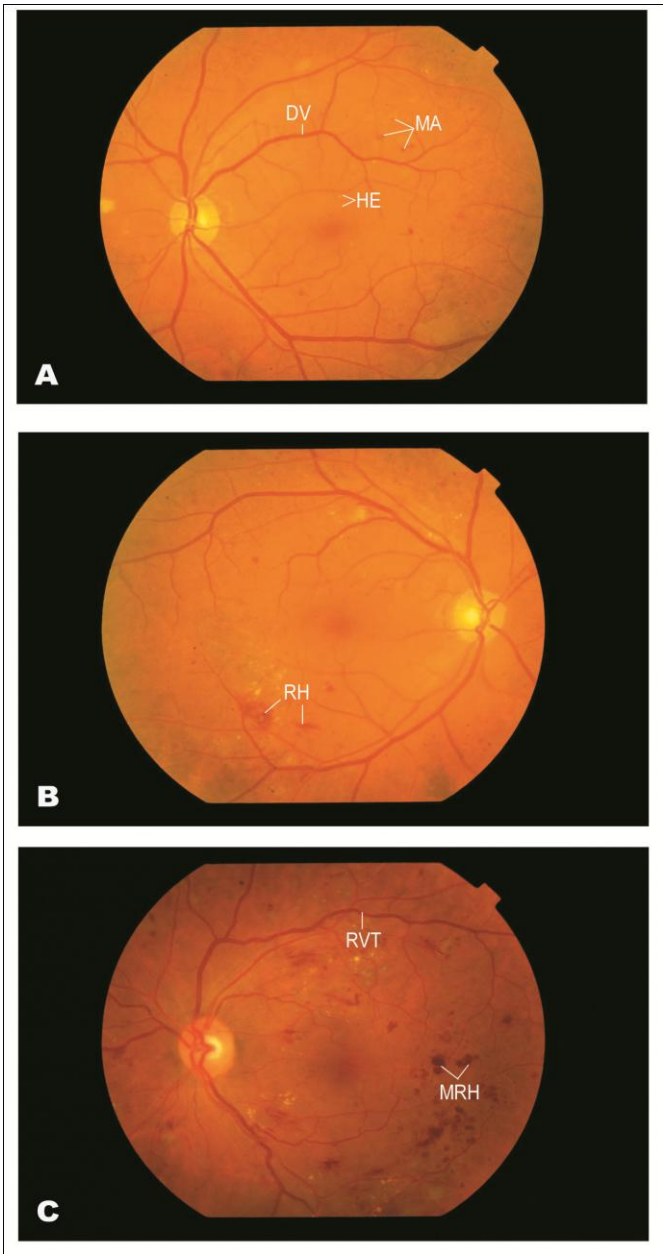

DV
MA
HE
A
RH
B
RVT
MRH
C

Fig. 3.4 FLuorescein Fundus Angiography in NPDR

A: shows mild NPDR with dilated reinal veins. Hard exudates appear as hypofluorescent zones surrounded by zone of hyperfluorescence (edema fluid). There are multiple discrete microaneurysms showing focal hyperfluorescence. Retinal ede,ma at this stage is more localized.

B: shows moderate NPDR with dilated retrinal veins showing increased tortuiosity and looping. Multiple retinal hard exudates are present along with retinal edema. Multiple venous aneurysms can also be seen along the retinal veins. The reinatl hemorrhages are dot/blot type as well as more spread out floame shaped and appear as zones of hypofluorescence.

C: severe NPDR with multiple microaneurysms appearing as areas of hyperfluoresence. The reinal hemorrhages both dot/blot and flames shaped types consists of hypofluorescent core surrounded by hyperfluoresncece due to edema.

Abbreve: Dilated retinal vein (DRV); Hard exudates (HE); Microaneurysms (MA); Multiple microaneurysms (MMA); Multiple retinal exudates (MRE); Multiple venous aneurysms (MVA); Retinal edema (RE); Retinal hemorrhage (RH)

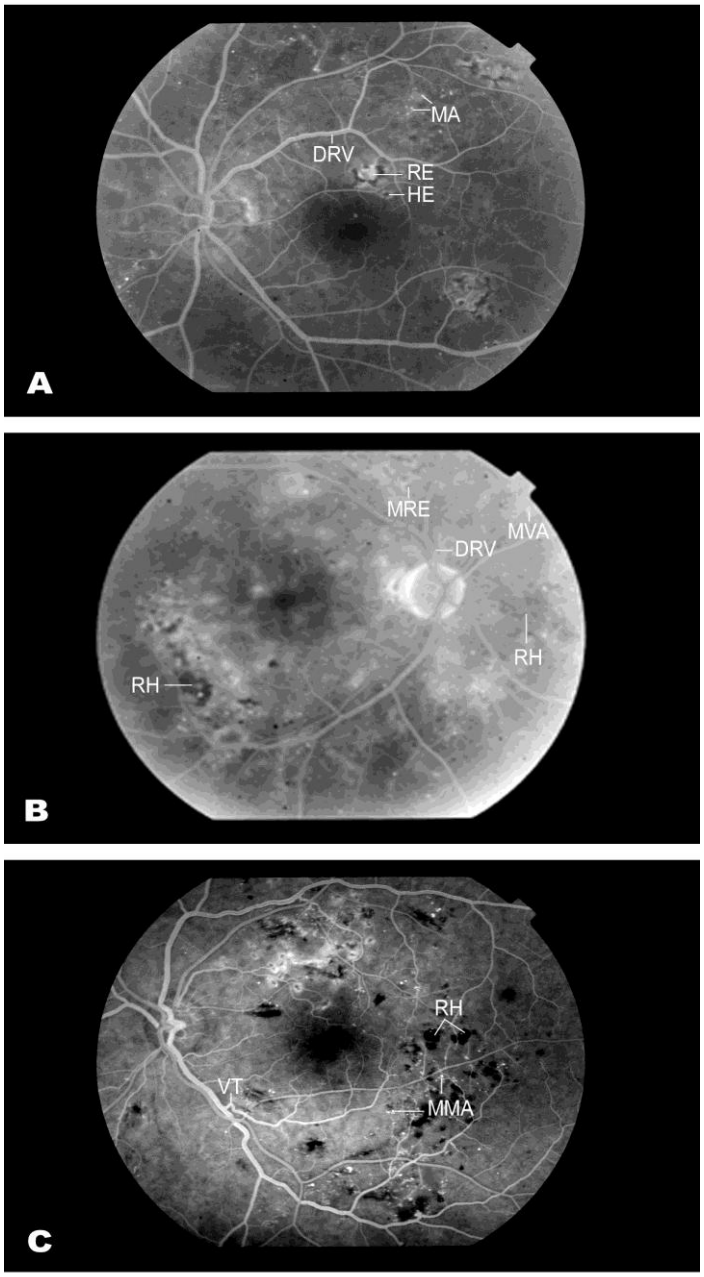

MA
DRV
RE
HE
A
MRE
MVA
DRV
RH
RH
B
RH
VT
MMA
C

Fig. 3.5: Fundoscopy in PDR

A: shows cotton wool spots with ill defined margins. There is associated diffuse retinal edema. Hard exudates are more prominent and numerous. There is also severe neovascularisation at the disc.

B: shows diffuse vitreous hemorrhage with a cloudy appearance. There is severe neovascularisation at the disc. Pre retinal hemorrhage is crescentic shaped and the edges mark the extent of posterior vitreous detachment.

Abbreve: Cotton wool spot (CWS); Diffuse retinal edema (DRE); Diffuse vitreous hemorrhage (DVH); Hard exudates (HE); Neo vascularisation (NV); Neo vascularitstion at the disc (NVD); Retinal hemorrhage (RH)

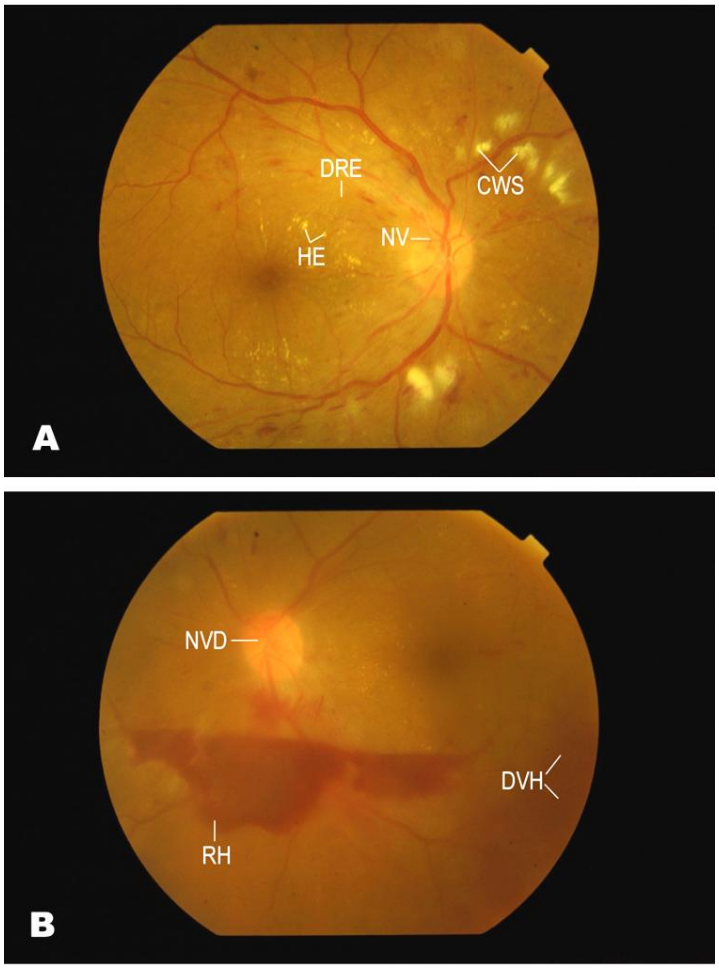

DRE
CWS
HE
NV
A
NVD
DVH
RH
B

Fig. 3.6: Flourescin fundus angiography in PDR.

A**:** shows diffuse retinal edema with numerous microaneurysms. Retinal hemorrhage in the form of dot/blot type can be appreciated. There is a zone of retinal ischemia corresponding with cotton wool spots. Venous tortuosity and segmentation along with beading are very prominent features at this stage.

B: shows diffuse vitreous hemorrhage. Shunts of intra retinal microvascular anomalies can be seen. Microaneurysms are numerous and severe neo vascularisation at the disc can be appreciated. The pre retinal hemorrhage appears hypoflourescent.

Abbreve: Diffuse vitreous hemorrhage (DVH); Diffuse retinal edema (DRE); Intra retinal microvascular anomaly (IRMA ; Micro aneurysm (MA); Neo vascularisation at the disc (NVD); Pre retinal hemorrhage (PRH); Retinal ischemia (RI);

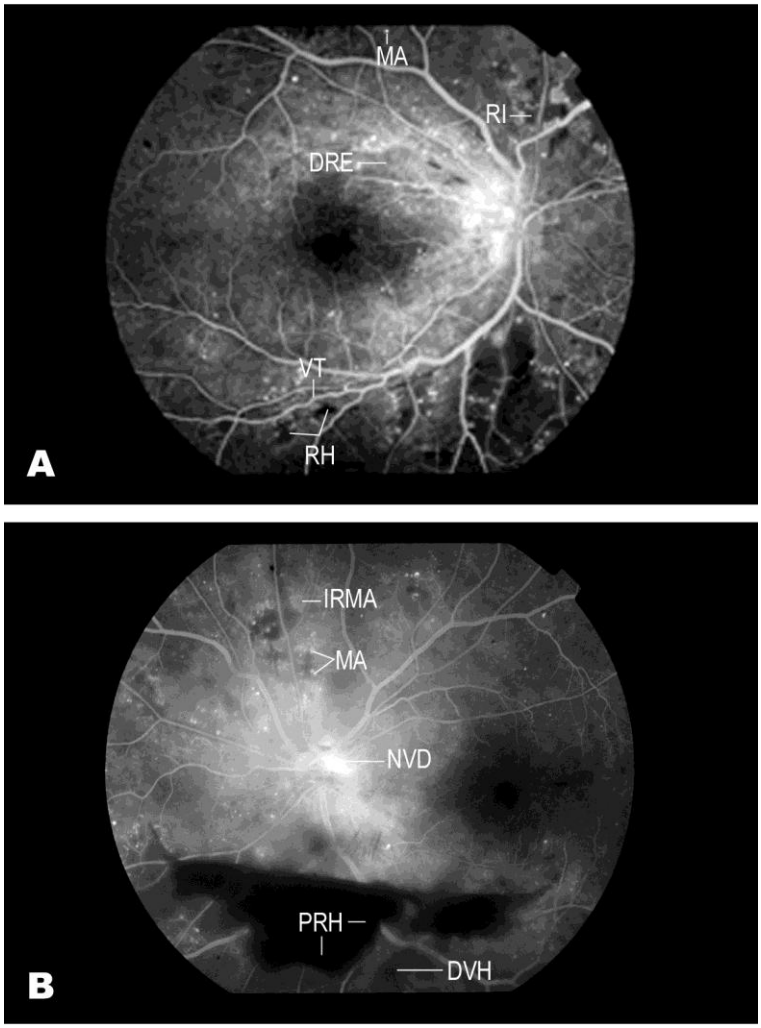

MA
RI
DRE
VT
RH
A
IRMA
MA
NVD
PRH
DVH
B

Table 3.1 Mean ages (years) of total male and female subjects and diabetic and/or retinopathic patients

	Mean ± SD (n)	Median	SE	Range Min -Max
Normal Subjects	49.82 ± 7.30 (39)	53.0	1.17	35-61
CDNPR	50.47 ± 6.40 (38)	50.5	1.04	40-60
NPDR	49.83 ± 8.11 (108)	50.0	0.78	37-65
PDR	50.41 ± 6.57 (192)	50.0	0.47	38-65
P Value F = 0.211	0.889			

Table 3.2 Sex-wise distribution of age of subjects and patients in different groups

Age	Mean ± SD	n	Median	Range Min -max	t value	df	*P* value	95 % CI Upper Lower
Normal Subjects								
Males	47.11±7.93	17	46.0	35-57	2.12	37	0.041*	-9.365 to -0.218
Females	51.90± 6.17	22	54.0	40-61				
CDNR								
Males	50.53±5.78	15	50.0	40-60	8.504	28	0.001*	12.045 to 19.689
Females	34.66±4.33	23	53.0	40-60				
NPDR								
Males	52.48±6.42	47	53.0	38-60	3.10	106	0.002*	1.699 to 7.706
Females	47.78±8.71	61	45.0	37-65				
PDR								
Males	49.85±5.83	67	50.0	39-60	0.87	190	0.384	-2.835 to 1.096
Females	50.72±6.94	125	50.0	38-65				

M represents males; F represents females; n sample size; SD = standard deviation.

3.3 Fundus Examination

The sample size consisted of 338 patients who underwent slit lamp biomicroscopy and flourescin fundus angiography. They were divided into two main groups i.e. NPDR and PDR. The NPDR group was further divided into three subgroups of mild, moderate and severe types. Figures 3.2a & b show normal fundus.

The mild NPDR subgroup was characterized by:

1) Retinal Artery microaneurysms: localized saccular outpouchings from the capillary wall at sites of pericyte loss. Their appearance at the temporal retina is one of the earliest signs of diabetic retinopathy. Initially they appear as tiny red dots. Later on they resemble dot hemorrhages due to leakage of blood that eventually surrounds the aneurysm.
2) Dilated Retinal Veins: the main temporal and nasal branches of the central retinal vein show dilatation and looping due to reduced venous blood flow.
3) Hard Exudates: localized collection of lipid and protein exudates along with infiltration of lipid filled macrophages from damaged blood vessels. They are refractile and have well defined margins.
4) Retinal edema: leakage from the microaneurysms is initially localized and later on becomes more diffuse as microvascular damage becomes more extensive (Fig. 3.3a and Fig. 3.4a).

The moderate NPDR subgroup was characterized by:

1) Features of mild NPDR.
2) Dilated main retinal veins where the tortuosity increased further.
3) Multiple Retinal Venous Aneurysms
4) Hard Exudates became more multiple and scattered as the microvascular damage extended further.
5) Retinal hemorrhages mainly of two types. Those arising from the venous end of the capillaries are located in the deeper and compact inner nuclear and plexiform layers adopt a dot and blot pattern. Those arising from pre-

capillary arterioles are situated in the nerve fiber layer and are more widespread and appear flame-shaped (Fig. 3.3b and Fig. 3. 4b).

In the severe NPDR subgroup we found:

1) Features of mild and moderate NPDR
2) Venous tortuosity became more prominent
3) Hard exudates became more numerous and there is additional presence of soft exudates in the form of cotton wool spots that appear at the zone of ischemic and non-ischemic retina due to disrupted axonal transport.
4) Retinal hemorrhages in the form of dot/blot and flame-shaped become more numerous as the microvascular damage extends.
5) Intra-retinal microvascular anomalies are shunts that open up in response to ischemia and communicate directly between the arteries and veins thereby bypassing the capillaries. They occur at sites of capillary occlusion where they appear as red streaks on fundoscopy (Fig. 3.3c and Fig. 3.4c).

In the PDR group following were the observations:

1) Features of mild, moderate and severe NPDR.
2) Soft exudates in the form of cotton wool spots.
3) Numerous hard exudates.
4) Diffuse retinal edema indicating the extent of microvascular damage.
5) Neovascularisation at the Disc (NVD) which can be defined as neovascularisation within one disc diameter (5 mm) of the optic nerve head. It was of severe type as it involved more the one third of the area.
6) Pre-retinal hemorrhage is a complication of the widespread neovascularisation as it extends to involve the subhyaloid space. The hemorrhage may extend to involve the vitreous as well known as diffuse vitreous hemorrhage as it spreads through the vitreous humor (Fig. 3.5a-b and Fig. 3.6a-b).

3.4 Comparison of male retinopathic patients with normal subjects and positive controls

3.4.1 Specific parameters

Serum IL-6

Serum IL-6 was significantly elevated in both NPDR and PDR patients than DNR patients and normal subjects. Median values were 60.03; 118.16 and 165.00 pg/ml respectively for CDNR, NPDR, and PDR versus normal healthy subjects 56.28 pg/ml. For PDR patients the values ranged between 101.67-380.00 pg/ml ($P < 0.001$). Mean value of serum IL-6 of CDNR patients was no different from normal subjects (Table 3.3).

Serum Leptin

Serum leptin concentration was significantly greater in NPDR and PDR patients than DNR patients and normal subjects ($P < 0.001$). Median values of serum leptin were 17.09, 25.80 and 36.40 for CDNR, NPDR and PDR patients respectively versus normal healthy subjects, 12.45 ng/ml. Mean value of serum leptin in PDR patients was 41.18 ng/ml and ranged from 34.00-68.10 ng/ml (Table 3.3). Intergroup comparison showed that serum leptin was significantly greater than both the NPDR and DNR patients (Table 3.3).

Serum VEGF

Serum VEGF concentration was significantly greater in DNR, NPDR and PDR groups as compared to normal subjects ($P < 0.001$). Median values of serum VEGF in CDNR, NPDR and PDR patients were: 75.0; 180.0 and 258.0 compared to normal subjects, 26.0 pg/ml. In PDR patients the values ranged between 110.00-412.00 pg/ml. Intergroup comparison showed serum VEGF in DNR patients was lower from both NPDR and PDR patients ($P < 0.05$) (Table 3.3).

Vitreous IL-6

Vitreous IL-6 levels of PDR patients were significantly higher than NPDR patients with a median value of 1045.0 pg/ml ranging from 391.67-1680.33 pg/ml ($P < 0.013$). For NPDR patients the median value was 600.0 pg/ml (Table 3.4).

Vitreous Leptin

Vitreous leptin concentration of PDR patients did not differ significantly from NPDR patients ($P = 0.125$). Median values for NPDR and PDR patients were 36.95 and 65.0 ng/ml (Table 3.4).

Vitreous VEGF

Vitreous VEGF concentration was found significantly greater in PDR compared to NPDR patients ($P < 0.001$). Median value was 1148 pg/ml, while the range was 480-1584 pg/ml. Median value for NPDR patients was 450.0 pg/ml (Table 3.4).

Table 3.3 Serum levels for specific parameters for male subjects and patients

	Mean ± SD	n	Median	SE	Range Min - Max	*F*/H value (df)	*P* Value
Serum IL-6 (*pg/ml*)							
Normal	57.72± 9.37	17	56.28	2.27	42.21-77.53	$H = 37.6_{(3)}$	$P < 0.001$
CDNR	63.91± 16.22a	15	60.03	4.19	39.71-97.53		
NPDR	126.83±45.36 *	12	118.16	13.09	67.33- 214.33		
PDR	181.30± 80.25*	11	165.00	24.19	101.67- 380.33		
Serum leptin (*ng/ml*)							
Normal	13.30±6.44	17	12.45	1.56	6.26-31.01	$H = 30.0_{(3)}$	$P < 0.001$
CDNR	19.87± 9.23	15	17.09	2.38	6.26-38.95		
NPDR	25.77±9.25*	12	25.80	2.67	13.10-38.90		
PDR	41.18±10.44*c	11	36.40	3.14	34.00-68.10		
Serum VEGF (*pg/ml*)							
Normal	28.05± 7.43	17	26.0	1.80	20.00-43.00	$H = 46.1_{(3)}$	$P < 0.001$
CDNR	76.03±24.29*a	15	75.0	6.27	42.50-120.50		
NPDR	185.16±70.25*	12	180.0	20.28	92.00-304.00		
PDR	243.09±85.25*	11	258.0	25.70	110.00-412.00		

Serum IL-6 * P < 0.05 vs normal, a vs b & c; Serum leptin * P < 0.05 vs normal, c vs a & b; Serum VEGF * P < 0.05 vs normal, a vs b & c

Table 3.4 Vitreous levels for specific parameters for male subjects and patients

	Mean ±SD	n	Median	SE	Range Min- Max	*F*/H value (df)	*P* Value
Vit IL-6 (*pg/ml*)							
Normal	–	–	–	–	–		
CDNR	–	–	–	–	–		
NPDR	622.69± 263.18	12	600.0	75.97	315.00-997.00	$t = 2.17_{(21)}$	$P < 0.013$
PDR	974.00± 354.15 *	11	1045.0	106.78	391.67-1680.33		
Vit Leptin (*ng/ml*)							
Normal	–	–	–	–	–		
CDNR	–	–	–	–	–		
NPDR	45.60±16.51	12	36.95	4.76	32.00-82.80	$t = 1.59\pm_{(21)}$	ns
PDR	59.02± 23.42	11	65.00	7.06	23.90-88.90		
Vit VEGF (*pg/ml*)							
Normal	–	–	–	–	–		
CDNR	–	–	–	–	–		
NPDR	421.75±167.05	12	450.0	48.22	189.00-696.00	$t = 5.81_{(21}$	$P < 0.001$
PDR	1038.98±323.86*	11	1148.0	97.64	480.00-1584.00		

Vitreous IL-6 * P < 0.013; Viterous leptin ns; Vitreous VEGF * P < 0.00

3.4.2 Conventional Parameters

Body Mass Index

BMI of DNRP, NPDR and PDR patients was found significantly greater than the normal subjects ($P < 0.001$). Maximum median value of BMI was found in PDR patients, while the range was 29-40 kg/m^2 (Appendix-II; Table 1).

Plasma Glucose

Two hours post prandial blood sugar was significantly greater in NPDR and PDR patients as compared to DNR and normal subjects ($P < 0.001$). Median value for random blood sugar was 362.76 mg/dl in PDR patients while the range was 190-520 mg/dl (Appendix-II; Table 1). No difference was found between DNR patients and normal subjects.

Fasting blood sugar was significantly greater in NPDR and PDR patients as compared to DNR patients and normal subjects ($P < 0.001$); however FBS of DNR patients and normal subjects did not differ significantly. PDR patients had greater FBS than the NPDR patients (Appendix-II; Table 1). Median value for PDR patients was 223.80 mg/dl while the range was 130-335 mg/dl.

Glycated Hemoglobin (HbA1c)

Glycated hemoglobin was significantly in both the NPDR and PDR patients as compared to DNR patients and normal subjects ($P < 0.001$). Glycated hemoglobin of diabetic but non-retinopathic patients did not differ significantly from normal subjects; however PDR patients had slightly greater Hb.A1c than NPDR patients. Median value for PDR patients was 8.40 % while the range was 6.2-12.0 % (Appendix-II; Table 1)

Lipid Profile

Total cholesterol concentration did not differ significantly when NPDR and PDR patients were compared with DNR patients and normal subjects (Appendix-II; Table 2).

High density lipoprotein of DNR, NPDR and PDR patients differed significantly from normal subjects ($P < 0.001$). Minimum median value was found for DNR patients which was 28 mg/dl, while the range was 21-42. Mean values also differed among the three groups (Appendix-II; Table 2).

Low density lipoprotein values were significantly greater in NPDR patients as compared to DNR patients, PDR patients and normal subjects ($P < 0.001$), with a median value of 100 mg/dl and the range was 35-190. Mean LDL concentration of DNR patients was lower as compared to NPDR and PDR patients both (Appendix-II; Table 2).

Serum triglyceride concentrations were significantly greater in all the three groups, DNR, NPDR, PDR patients than normal subjects ($P < 0.002$). TG concentration ranged from 70-446 mg/dl in the three groups' vs normal subjects whose TG level ranged between 56-178 mg/dl (Appendix-II; Table 2).

TC/HDL ratios of DNR, NPDR and PDR patients were significantly greater than normal subjects ($P < 0.001$). DNR and NPDR patients TG levels differed from PDR patients ($P < 0.05$). Median value was found greater in DNR patients while maximum range was obtained for PDR patients (Appendix-II; Table 1). HDL/LDL ratios of NPDR and PDR patients were significantly greater than DNR patients and normal subjects ($P < 0.001$) (Appendix-II; Table 3).

Renal Function

Serum creatinine values were found significantly greater in DNR, NPDR and PDR patients than normal subjects ($P < 0.001$). PDR patients had comparatively higher levels of serum creatinine than the other groups with a median value of 1.1 mg/dl and ranged from 07-6.2 mg/dl (Appendix-II; Table 4).

Urine creatinine values of DNR, NPDR and PDR patients were significantly greater than normal subjects ($P < 0.002$). Between comparisons showed urine creatinine levels of PDR were greater than both NPDR and DNR patients. Maximum concentration was found for PDR patients with a median value of 49 mg/kg/24hr ranging between 20-87 mg /24hr (Appendix-II; Table 4).

Urinary protein found significantly greater in PDR patients with a median value of 1040 mg/24hr ranging between 346-7500 mg/24 hr. All the three groups of patients, the DNR, NPDR and PDR had significantly elevated levels of urinary protein as compared to normal subjects ($P < 0.001$). Between comparisons showed significant differences of urinary protein values between DNR patients with NPDR and PDR patients and NPDR and PDR patients (Appendix-II; Table 4).

Blood Pressure

Systolic blood pressure was found significantly higher in both NPDR and PDR patients than DNR patients and normal subjects ($P < 0.001$). Maximum median value was 140 mmHg while the range was 110-160 mmHg (Appendix-II; Table 5).

Diastolic blood pressure was found higher in DNR and NPDR patients' vs normal subjects ($P < 0.05$). Median value was 90 mmHg (Appendix-II; Table 5).

3.5 Comparison of female retinopathic patients with normal subjects and diabetic but non-retinopathic patients

3.5.1 Specific parameters

Serum IL-6

Serum IL-6 concentration was significantly greater in NPDR and PDR patients than DNR patients and normal subjects with significantly elevated values found in PDR patients having a median value of 219.0 pg/ml and ranging from 71.33-356.00 pg/ml ($P < 0.001$). No difference was found between NDR patients and normal subjects as regards serum IL-6 concentration (Table 3.5).

Serum Leptin

Serum leptin concentration was found significantly elevated in NPDR and PDR patients than the DNR patients and normal subjects ($P < 0.001$). A greater median value (31.15 ng/ml) was found in PDR patients, while a greater upper limit was found in NPDR patients (54.20 ng/ml). The levels were significantly lower ($P < 0.05$) in DNR patients than the NPDR and PDR patients (Table 3.5).

Serum VEGF

Serum VEGF concentration was significantly greater in DNR, NPDR and PDR patients as compared to the normal subjects ($P < 0.001$). Highest median value and range was found in PDR patients (257pg/ml; 118-430 pg/ml). Serum VEGF in DNR patients was significantly lower than values found in NPDR and PDR patients ($P < 0.001$) (Table 3.5).

Vitreous IL-6

Concentration of IL-6 in the vitreous humor was found significantly greater in PDR patients than NPDR patients ($P < 0.006$). The median value was 1045 pg/ml with a range of 564-1969 pg/ml (Table 3.6).

Vitreous Leptin

Vitreous leptin concentration was non-significantly different between NPDR and PDR patients ($P = 0.086$). Median value of 59 ng/ml was greater in NPDR patients as compared to PDR patients (Table 3.6).

Vitreous VEGF

Vitreous VEGF concentration was found significantly elevated in PDR patients than the NPDR patients ($P < 0.001$). The median value was 1152 pg/ml with arrange of 400-1440 pg/ml (Table 3.6).

Table 3.5 Serum levels for specific parameters for female subjects and patients

	Mean ±SD	n	Median	SE	Range Min-Max	*F*/H Value (df)	*P* Value
Serum IL-6 (*pg/ml*)							
Normal	58.79± 13.57	22	57.5	2.96	37.80-88.47	H = 41.9	*P* < 0.001
CDNR	75.55± 16.73a	23	77.2	3.48	43.16-101.28		
NPDR	119.16±36.16*	08	119.1	12.78	72.00-187.67		
PDR	199.54±89.21*	14	219.0	23.84	71.33-356.00		
Serum leptin (*ng/ml*)							
Normal	11.42± 4.53	22	9.97	0.96	6.26-22.76	$H = 35.1_{(3)}$	*P* < 0.001
CDNR	13.28±4.64[a]	23	14.51	0.96	6.26-21.11		
NPDR	30.68±11.52*	08	26.35	4.07	18.60-54.20		
PDR	28.00±9.55*	14	31.15	2.55	9.70-41.00		
Serum VEGF (*pg/ml*)							
Normal	28.50± 6.55	22	28.50	1.39	20-40	$H = 55.6_{(3)}$	*P* < 0.05
CDNR	103.52±28.66*a	23	100.00	5.97	55-145		
NPDR	228.75±95.30*	08	191.00	33.69	136-390		
PDR	250.42±89.29*	14	257.00	23.86	118-430		

Serum IL-6 * P < 0.05 vs normal, a b & c; Serum Leptin * P < 0.05 vs normal, a vs b & c; Serum VEGF c P < 0.001 vs b

Table 3.6 **Serum levels for specific parameters for female subjects and patients**

	Mean ± SD	n	Median	SE	Range Min- Max	*F*/H Value (df)	*P* Value
Vit IL-6 (*pg/ml*)							
Normal	–	–	–	–	–		
CDNR	–	–	–	–	–		
NPDR	642.58 ±150.57	08	634.0	53.23	418.33-897.00	t = 51	*P* = 0.006
PDR	1236.57±539.22	14	1045.0	144.11	564.00-1969.00		
Vit Leptin (*ng/ml*)							
Normal	–	–	–	–	–		
CDNR	–	–	–	–	–		
NPDR	61.25±19.89	08	59.70	7.03	27.20-88.30	t = 1.809 $_{(20)}$	*P* = 0.086
PDR	47.35± 15.78	14	46.60	4.21	18.30-82.20		
Vit VEGF (*pg/ml*)							
Normal	–	–	–	–	–		
CDNR	–	–	–	–	–		
NPDR	515.62±239.29	08	424.00	84.60	296-96	t = 3.90 P $_{(20)}$	*P* < 0.001
PDR	1027.71±322.33c	14	1152.00	86.14	400-144		

Serum Leptin * P < 0.05 vs normal, a vs b & c; Vit IL-6 t ; Vit Leptin;

3.5.2 Conventional Parameters for Female Ptients

Body Mass Index

BMI of DNR, NPDR and PDR patients was significantly greater than normal subjects. Maximum BMI was found in NPDR patients with a median value of 38 kg/m^2 and ranged from 26-42 kg/m^2 ($P < 0.001$). BMI of DNR patients was significantly lower than NPDR and PDR patients ($P < 0.05$) (Appendix III; Table 1).

Plasma Glucose

Random blood sugar measured 2 h post prandial demonstrated significantly greater plasma glucose concentration in NPDR and PDR patients as compared to DNR patients and normal subjects ($P < 0.001$). Median value of 350 mg/dl ranging from 222-535 m/dl was found in NPDR patients. Mean fasting plasma glucose levels of DNR patients were significantly lower than NPDR and PDR patients ($P < 0.05$) (Appendix III; Table 1).

Fasting blood sugar levels were significantly greater in NPDR and PDR patients as compared to DNR patients and normal subjects ($P < 0.001$); however greater mean values were found for NPDR patients than PDR patients ($P < 0.05$). Median value was 215 mg/dl, but a greater range was found for PDR patients that ranged from 100-380 mg/dl (Appendix III; Table 1).

Glycated Hemoglobin (HbA1c)

Glycated hemoglobin concentration was significantly elevated in DNR, NPDR and PDR patients as compared to normal subjects ($P < 0.001$). Highest concentration was found in PDR patients with a median value of 8.40 % ranging from 5.8-14.1 % (Appendix III; Table 1).

Lipid Profile

Serum total cholesterol concentration of DNR, NPDR and PDR patients did not differ significantly when compared with normal subjects ($P = 0.90$) (Appendix III; Table 2).

High density lipoprotein values were significantly lowered in DNR, NPDR and DPR patients than normal subjects ($P < 0.001$). Lowest serum concentration was found for DNR patients with a median value of 31 mg/dl ranging from 16-46 mg/dl. Serum HDL of DNR patients was significantly lower than NPDR and PDR patients ($P < 0.05$) (Appendix III; Table 2).

Low density lipoprotein concentration was significantly greater in NPDR and PDR patients than DNR patients and normal subjects ($P < 0.001$). A greater median value (90 mg/dl) was found in NPDR patients while a greater range (35-210 mg/dl) was found in DNR patients. LDL values of DNR patients did not differ significantly from normal subjects (Appendix III; Table 2).

Serum triglyceride concentration was significantly in NDR, NPDR and PDR patients than normal subjects ($P < 0.001$). Intergroup comparison demonstrated greater serum TG concentrations in DNR patients as compared to NPDR and PDR patients ($P < 0.05$). Median value in DNR patients was 189 mg/dl ranging from 134-278 mg/dl. However, a higher upper limit was found in PDR patients (Appendix III; Table 2).

TC/HDL ratio was significantly greater in DNR, NPDR and PDR patients as compared to normal subjects ($P < 0.001$). Intergroup comparison showed that TC/HDL ratio of DNR patients was significantly higher than the NPDR and PDR patients ($P < 0.05$), with a median value of 5.2 and range from 2.98-13.25 (Appendix III; Table 3). HDL/LDL ratio was significantly greater in DNR, NPDR and PDR patients than normal subjects with a median value of 2.8 in PDR patients and an upper limit of 4.57 in DNR patients ($P < 0.001$) (Appendix III; Table 3). Intergroup comparison showed non significant difference between diabetic and diabetic retinopathic groups ($P = 0.19$).

Renal Profile

Serum creatinine levels of NPDR and PDR patients were significantly higher than DNR patients and normal subjects ($P < 0.001$). Mean levels were 1.10 mg/dl that ranged from 0.7-6.5 mg/dl. Intergroup comparison showed statistically non significant difference between NPDR and PDR, and DNR and normal subjects (Appendix III; Table 4).

Urine creatinine concentration was significantly greater in DNR, NPDR and PDR groups when compared with normal subjects ($P < 0.001$). A greater median value was found for DNR patients (46 mg/24 hr). The levels ranged from 23-98 mg/dl). The levels did not differ significantly between diabetic and diabetic retinopathic groups (Appendix III; Table 4).

Urinary protein concentration was found significantly greater in NDR, NPDR and PDR patients than the normal subjects ($P < 0.001$). Significantly greater concentration was found for NPDR patients with a median value of 973 mg/24 hr and ranging from 157-2045 mg/24 hr. Intergroup comparison showed DNR urinary protein values to be significantly lower from NPDR patient values, while NPDR urinary protein values to be significantly elevated from PDR patients ($P < 0.05$) (Appendix III; Table 4).

Blood Pressure

Systolic blood pressure was significantly higher in NPDR and PDR patients than DNR patients and normal subjects ($P < 0.001$). Highest median value was 130 mmHg with a range of 110-160 mmHg (Appendix III; Table 5).

Diastolic blood pressure was significantly greater only in PDR patients when compared with normal subjects and DNR and NPDR patients ($P < 0.05$). Median value was 90 mmHg with a range of 70-90 mmHg (Appendix III; Table 5).

3.6 Comparison of male and female retinopathic patients with normal subjects and diabetic but non-retinopathic patients (Combined population data)

3.6.1 Specific parameters

Serum IL-6

Serum IL-6 levels were significantly greater in NPDR and PDR patients as compared to the DNR patients and normal subjects ($P < 0.001$). Median levels were 170 pg/ml ranging from 71-380 pg/ml. DNR patients had lower levels of IL-6 as compared to NPDR and PDR patients ($P < 0.001$), and as such did not differ significantly from normal subjects (Table 3.7).

Serum Leptin

Serum leptin concentrations were also significantly greater in NPDR and PDR patients as compared to DNR patients and normal subjects ($P < 0.001$). DNR patients had lower levels of serum leptin than NPDR and PDR patients ($P < 0.001$), but no different from normal subjects when intergroup comparisons were made (Table 20). Highest median concentration and range were recorded in PDR patients (34.20 ng/ml; 10-68.1 ng/ml) (Table 3.7).

Serum VEGF

Serum VEGF values were elevated in DNR, NPDR and PDR patients as compared to the normal subjects ($P < 0.001$). Highest median concentration and range were found in PDR patients (258 pg/ml; 110-430 pg/ml). Levels of VEGF in DNR patients were significantly lower than the NPDR and PDR patients ($P < 0.001$) (Table 3.7).

Vitreous IL-6

IL-6 concnetration in the vitreous fluid was found significantly greater in PDR patients as compared to NPDR patients with a median value of 1045 pg/ml and range from 391-1969pg/ml ($P < 0.001$) (Table 3.8).

Vitreous Leptin

Vitreous leptin concentrations did not differ significantly between NPDR and PDR patients ($P = 0.915$) (Table 3.8).

Vitreous VEGF

VEGF concentration in the vitreous fluid was significantly greater in PDR patients when compared with NPDR patients ($P < 0.001$). The median value was 1148, while the range was 400-1584 pg/ml (Table 3.8).

Table 3.7 **Serum levels for specific parameters for male and female subjects and patients**

	Mean ± SD	n	Median	SE	Range Min -Max	*F*/H value	*P* Value
Serum IL-6 (*pg/ml*)							
NS	58.33 ±11.58	39	57.53	1.85	37.81- 88.47	F = 57.69	*P* < 0.001
CDNR	70.95 ±17.29a	38	68.03	2.80	39.71- 101.28		
NPDR	123.76 ± 41.08*	20	119.16	9.18	67.33- 214.33		
PDR	191.49 ± 84.19*	25	170.0	16.83	71.00- 380.33		
Serum leptin (*ng/ml*)							
NS	12.24 ±5.45	39	11.60	0.87	6.26- 31.01	F = 41.58	*P* < 0.001
CDNR	15.88 ± 7.47a	38	15.44	1.21	6.26- 38.95		
NPDR	27.74 ± 10.22*	20	25.80	2.28	13.10- 54.20		
PDR	33.88 ± 11.76*	25	34.20	2.35	10.00- 68.10		
Serum VEGF (*pg/ml*)							
NS	28.30 ±6.86	39	28.0	1.09	20.0-43.0	F = 104.16	*P* < 0.001
CDNR	92.67 ±29.95* a	38	87.5	4.86	42.5-145.0		
NPDR	202.60 ± 81.75*	20	189.0	18.28	92.0- 390.0		
PDR	247.20 ± 85.79*	25	258.0	17.15	110.0- 430.0		

Serum IL-6 * P < 0.001 vs normal Tukey's; pair-wise Dunn's $H_{(2)}$ = 51.96 a vs b & c P < 0.001
Serum leptin * P < 0.001 vs normal, Tukey's; pair-wise Dunn's $H_{(2)}$ = 35.06 a vs b & c P < 0.001
Serum VEGF * P < 0.001 vs normal, Tukey's; pair-wise Dunn's $H_{(2)}$ = 50.58 a vs b & c P < 0.001

Table 3.8 **Vitreous levels for specific parameters for male and female subjects and patients**

	Mean ± SD	n	Median	SE	Range Min -Max	*t* value	*P* Value
Vit IL-6 (*pg/ml*)							
NS	–	–	–	–	–		
CDNR	–	–	–	–	–		
NPDR	630.65 ± 220.35*	20	618.5	49.27	315.0-997.0	t = -4.24	*P* < 0.001
PDR	1121.08 ± 476.97	25	1045.0	95.39	391.6- 1969.0		
Vit Leptin (*ng/ml*)							
NS	–	–	–	–	–		
CDNR	–	–	–	–	–		
NPDR	51.86 ±19.12	20	50.6	4.27	27.20- 88.30	t = -0.107	*P* = 0.915
PDR	52.49 ±19.92	25	51.0	3.98	18.00- 88.90		
Vit VEGF (*pg/ml*)							
NS	–	–	–	–	–		
CDNR	–	–	–	–	–		
NPDR	459.30 ± 198.69	20	444.0	44.42	189.00- 961.00	t = -7.061	*P* < 0.001
PDR	1032.67 ± 316.25	25	1148.0	63.25	400.00- 1584.00		

Vitreous IL-6 * P < 0.001 between groups by Mann-Whitney Rank Sum Test
Viterous leptin ns between groups unpaired t-test
Vitreous VEGF * P < 0.001 between groups unpaired t-test

3.6.2 Conventional Parameters

Body Mass Index

BMI was significantly greater in DNR, NPDR and PDR patients compared to normal subjects ($P < 0.001$). A greater median value was found for NPDR patients ranging from 23-42 kg/m^2. BMI of DNR patients was significantly lower than the NPDR and PDR patients (Appendix IV; Table 1).

Plasma Glucose

Plasma glucose concentration determined 2 hr post prandial was found significantly greater in NPDR and PDR patients with a median value of 355 mg/dl in NPDR patients ($P < 0.001$), while a collective range for NPDR and PDR patients was 190-535 mg/dl. No difference was found between DNR patients and normal subjects and plasma glucose of diabetic patients was significantly lower than the diabetic retinopathic patients (Appendix IV; Table 1).

Fasting Blood Sugar was significantly greater ($P < 0.001$) in NPDR and PDR patients than DNR and patients and normal subjects with median values of 200 mg/dl and 185 mg/dl, while a collective range was 100-335 mg/dl. FBS of CNDR patients and normal subjects did not differ significantly; however, plasma glucose of DNR patients was significantly lower than both the NPDR and PDR patients ($P < 0.05$) (Appendix IV; Table 1).

Glycated Hemoglobin (HbA1c).

Glycated hemoglobin concentration was significantly greater in DNR, NPDR and PDR patients with PDR patients having the highest median concentration of 8.4 % ranging between 5.8-14.1 % as compared to normal subjects ($P < 0.001$). HbA1c concentration of DNR patients was lower than both the DNR and PDR patients ($P < 0.05$) (Appendix IV; Table 1).

Lipid Profile

Plasma cholesterol concentration of diabetic and diabetic retinopathic patients did not differ significantly than normal subjects ($F = 0.08$; $P < 0.969$). PDR patients had however highest upper limit of 275 mg/dl (Appendix IV; Table 2).

High density lipoprotein concentration was found significantly lower in DNR, NPDR and PDR patients as compared to the normal subjects ($P < 0.001$). Lowest

concentration was found in DNR patients with a median value of 31 mg/dl and differed significantly from levels in DNR and PDR patients (Appendix IV; Table 2).

Low density lipoprotein concentration was significantly elevated in NPDR and PDR patients when compared with DNR patients and normal subjects ($P < 0.001$). Highest median value of 95 mg/dl was found in NPDR patients, while a wider range was noticeable in DNR patients (34-215 mg/dl) (Appendix IV; Table 2).

Serum triglyceride concentration was significantly elevated in DNR, NPDR and PDR patients as compared to normal subjects ($P < 0.001$). Highest median value 189 mg/dl was found in DNR patients, while a wider range was observed in PDR patients (81-446 mg/dl). Intergroup comparison showed significantly greater mean TG concentration in DNR patients as compared to NPDR and PDR patients ($P < 0.008$) (Appendix IV; Table 2).

TC/HDL ratio was significantly greater in DNR, NPDR and PDR patients than the normal subjects ($P < 0.001$). NPDR patients had highest median value of 4.62 and an upper limit of 12.0. DNR patients had lowest ratio of 0.74 (Appendix IV; Table 3).

LDL/HDL ratio was significantly greater in DNR, NPDR and PDR patients than normal subjects ($P < 0.001$). Highest ratio of 2.7 with a range of 0.87-7.18 was found in NPDR patients. LDL/HDL ratio was comparatively lower in diabetic but non-retinopathic patients as compared to diabetic retinopathic patients (Appendix IV; Table 3).

Renal Profile

Serum creatinine concentration was significantly elevated ($P < 0.001$) in NPDR and PDR patients than DNR and patients and normal subjects with the highest median value of 1.15 mg/dl found in DNR patients but a higher upper limit of 6.5 mg/dl in PDR patients. Serum creatinine levels of DNR patients were significantly low ($P < 0.026$) in DNR patients than the PDR patients on Tukey's test but showed no significant difference on Kruskal-Wallis one way ANOVA on ranks ($P < 0.081$) (T Appendix IV; Table 4).

Urinary creatinine concentration of DNR, NPDR and PDR patients was significantly greater than the normal subjects ($P < 0.001$). Greatest concentration was found in DNR patients with a median value of 43 mg/kg/24 hr and a range of 23-98 mg/kg/24hr (Appendix II; Table 1). Values did not differ significantly among the diabetic non-retinopathic and diabetic retinopathic patients when inter group comparisons were made (Appendix IV; Table 4).

Urinary protein concentration was found significantly elevated in the NPDR and PDR patients as compared to DNR patients and normal subjects ($P < 0.001$). Highest median concentration of 633 mg/24 hr was found in NPDR patients, however maximum concentration of 7500 mg/24 hr was found in PDR patients (Appendix II; Table 1). DNR patients had significantly lower urinary protein values than both the NPDR and PDR patients (Appendix IV; Table 4).

Blood Pressure

Significantly higher ($P < 0.001$) systolic blood pressure was found in NPDR and PDR patients than DNR patients and normal subjects having a median value of 130 mmHg (Appendix IV; Table 5). Values between DNR and normal subjects however did not differ significantly.

Significantly higher diastolic blood pressure was found in NPDR and PDR patients as compared to DNR patients and normal subjects with a median value of 90 mmHg ($P < 0.001$). Diastolic blood pressure of DNR patients and normal subjects did not differ from each other (Appendix IV; Table 5).

3.7 Male vs Female Analyses

3.7.1 Normal Subjects

BMI, Plasma Glucose, HbA1c, Lipid Profile, Renal profile, Blood Pressure, Serum IL-6, leptin and VEGF values

In normal subjects, values for BMI, RBS, FBS, HbA1c, total cholesterol, HDL, LDL, TG, TC/HDL ratio, HDL/LDL ratio, urinary protein, systolic and diastolic blood pressure, serum IL-6, leptin and VEGF did not differ significantly between males and females. However glycated hemoglobin, serum creatinine and urine creatinine values differed significantly between males and females ($P = 0.004$, $P = 0.05$ and $P = 0.001$ respectively) (Appendix V; Table 1-5).

3.7.2 Diabetic Non-Retinopathic Patients (DNR)

BMI, Plasma Glucose, Hb.A1c, Lipid Profile, Renal profile, Blood Pressure, Serum IL-6, leptin and VEGF values

In DNR patients, no significant differences were found for RBS, FBS, Hb.A1c, total cholesterol, HDL, LDL, TGTC/HDL ratio, LDL/HDL ratio, serum creatinine, urine creatinine, urinary protein, systolic and diastolic blood pressures, visual acuity when males were compared with females. However, mean BMI was greater in males as compared to the females (P = 0.041). Similarly, mean concentrations of serum IL-6, and VEGF were significantly greater in male patients (P = 0.041 and P = 0.004 respectively), while serum leptin concentrations were significantly greater in female patients (P = 0.006) (Appendix V; Tables 1-5).

3.7.3 Diabetic Non-Proliferative Retinopathic Patients (NPDR)

BMI, Plasma Glucose, Hb.A1c, Lipid Profile, Renal profile, Blood Pressure, Serum IL-6, leptin and VEGF values

BMI, RBS, FBS, HbA1c total cholesterol, LDL, serum creatinine, urine creatinine, systolic blood pressure, visual acuity (Left eye), serum and vitreous IL-6, leptin and VEGF did not differ significantly between males and females. In contrast, significant differences were observed for HDL (P = 0.004), serum triglycerides (P = 0.048), TC/HDL ratio (P = 0.002), HDL/LDL ratio (P < 0.001), urinary protein concentration (P = 0.016 and diastolic blood pressure (P = 0.016) between males and female patients (Appendix V; Tables 1-5).

3.7.4 Diabetic Proliferative Retinopathic Patients (PDR)

BMI, Plasma Glucose, Hb.A1c, Lipid Profile, Renal profile, Blood Pressure, Serum IL-6, leptin and VEGF values

No significant differences were found between male and female patients for the following parameters: HbA1c, total cholesterol, HDL, LDL, TC/HDL ratio, HDL/LDL ratio, diastolic blood pressure, serum IL-6, serum VEGF, and vitreous IL-6, leptin and VEGF. In contrast BMI, RBS and FBS differed significantly between males and females (P = 0.005, P = 0.013, P = 0.006 respectively). Similarly, for lipid profile only serum triglyceride concentration was significantly different which was higher in males (P = 0.003). For renal profile, serum creatinine (higher in females), urine creatinine (higher in females) and urinary protein (higher in males) values differed significantly

between males and females (P = 0.015, P = 0.007, P < 0.001 respectively). Systolic blood pressure was significantly greater in male patients (P = 0.003) with a mean value of 138 mmHg. Only serum leptin levels were significantly greater in female PDR patients than the male patients (P = 0.003) (Appendix V; Tables 1-5).

Table 3.9 **Comparison of specific parameters between male and female subjects and patients (normal)**

Parameter	Mean ± SD	n	t -value	df	P value	95% CI Upper Lower
Serum IL-6 (*pg/ml*)						
Males	57.72 ±9.37	17	0.28	37	0.778	-8.752 to 6.600
Females	58.80 ± 13.25	22				
Serum Leptin (*ng/ml*)						
Males	13.30 ±6.44	17	1.06	37	0.292	-1.684 to 5.437
Females	11.42 ±4.53	22				
Serum VEGF (*pg/ml*)						
Males	28.05 ±7.43	17	0.19	37	0.845	-4.988 to 4.105
Females	28.50 ±6.55	22				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 3.10 Comparison of specific parameters between male and female subjects and patients (CDNR)

Parameter	Mean ± SD	n	t -value	df	*P* value	95% CI Upper Lower
Serum IL-6 (*pg/ml*)						
Males	63.91 ± 16.22	15	2.12	36	0.041*	-22.763 to -0.502
Females	75.55 ±16.73	23				
Serum Leptin (*ng/ml*)						
Males	19.87 ±9.23	15	2.91	36	0.006*	2.013 to 11.181
Females	13.28 ±4.64	23				
Serum VEGF (*pg/ml*)						
Males	76.03 ±24.29	15	3.06	36	0.004*	-45.698 to -9.279
Females	103.52 ± 28.66	23				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 3.11 Comparison of specific parameters between male and female subjects and patients (DNPDR)

Parameter	Mean ± SD	n	t -value	df	P value	95% CI Upper Lower
Serum IL-6 (*pg/ml*)						
Males	126.83 ± 45.36	12	0.40	18	0.694	-32.636 to 47.968
Females	119.16 ± 36.16	08				
Serum Leptin (*ng/ml*)						
Males	25.77 ±9.25	12	1.05	18	0.305	-14.689 to 4.864
Females	30.68 ± 11.52	08				
Serum VEGF (*pg/ml*)						
Males	185.16 ± 70.25	12	1.18	18	0.253	-121.181 to 34.014
Females	228.75 ± 95.30	08				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 3.12 Comparison of specific parameters between male and female subjects and patients (DNPDR)

Parameter	Mean ± SD	n	t -value	df	P value	95% CI Upper Lower
Vit IL-6 (*pg/ml*)						
Males	622.69 ± 263.18	12	0.19	18	0.849	-236.755 to 196.980
Females	642.58 ± 150.57	08				
Vit Leptin (*ng/ml*)						
Males	45.60 ±16.51	12	1.91	18	0.072	-32.813 to 1.530
Females	61.25 ±19.89	08				
Vit VEGF (*pg/ml*)						
Males	421.75 ± 167.05	12	1.03	18	0.313	-284.032 to 96.282
Females	515.62 ± 239.29	08				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 3.13 Comparison of specific parameters between male and female subjects and patients (PDR)

Parameter	Mean ± SD	n	t value	df	P value	95% CI Upper Lower
Serum IL-6 (*pg/ml*)						
Males	181.30 ± 80.25	11	0.53	23	0.601	-89.453 to 52.966
Females	199.54 ± 89.21	14				
Serum Leptin (*ng/ml*)						
Males	41.18 ±10.44	11	3.28	23	0.003*	4.883 to 21.466
Females	28.00 ±9.55	14				
Serum VEGF (*pg/ml*)						
Males	243.09 ± 85.25	11	0.20	23	0.837	-80.317 to 65.642
Females	250.42 ± 89.29	14				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 3.14 Comparison of specific parameters between male and female subjects and patients (PDR)

Parameter	Mean ± SD	n	t value	df	P value	95% CI Upper Lower
Vit IL-6 (*pg/ml*)						
Males	974.00 ± 354.15	11	1.39	23	0.177	-652.509 to 127.367
Females	1236.57 ± 539.22	14				
Vit Leptin (*ng/ml*)						
Males	59.02 ±23.42	11	1.48	23	0.151	-4.563 to 27.903
Females	47.35 ±15.78	14				
Vit VEGF (*pg/ml*)						
Males	1038.98 ± 323.86	11	0.086	23	0.932	-257.950 to 280.485
Females	1027.71 ± 322.33	14				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

3.8 Correlation Analyses

3.8.1 Normal Subjects Males

Age

Body Mass Index

BMI showed significant positive correlation with TC/HDL ratio (r= 0.534; $P < 0.027$) and significant negative correlation with systolic blood pressure (r = - 0.498; $P < 0.042$) (Table 3.15).

Plasma Glucose

Random blood sugar showed significant positive correlation with FBS, serum TG, systolic blood pressure (r = 0.483; r = 0.567; r = 0.658; $P < 0.05$; $P < 0.018$; $P < 0.004$ respectively), and significant negative correlation with serum creatinine (r = - 0.681; $P < 0.004$). Fasting blood sugar was also positively correlated with systolic blood pressure (r = 0.623; $P < 0.008$) (Table 3.15).

Lipid profile

HDL was negatively correlated with TC/HDL ratio and LDL/HDL ratio (r = - 0.770; $P < 0.0001$; r = - 0.781; $P < 0.0001$). Serum TG was negatively correlated with serum creatinine (r = - 0.524; $P < 0.031$). TC/HDL ratio was positively correlated with LDL/DHL ratio (r = 0.533; $P < 0.027$) (Table 3.15).

Renal profile

Serum creatinine was negatively correlated with diastolic blood pressure (r = - 0.493; $P < 0.044$), while urine creatinine was negatively correlated with systolic blood pressure (r = - 0.582; $P < 0.014$) (Table 3.15).

Blood Pressure

Diastolic blood pressure was negatively correlated with serum VEGF (r = - 0.486; $P < 0.048$) (Table 3.15).

Table 3.15 Correlation Table Normal Males

Spearman's Two Tailed correlation normal males

	Age	BMI	RBS	FBS	HB.A1C	Cholest	HDL	LDL	TG	TC/ HDL	LDL/HDL	Ser.Creatinine	Urine Creatinine	Urine Protein	SYS BP	DIAS BP	Ser IL-6	Ser Leptin	Ser VEGF
Age	1																		
BMI	-0.02	1																	
	0.94																		
RBS	0.393	-0.153	1																
	0.119	0.557																	
FBS	0.089	-0.378	**.483***	1															
	0.733	0.135	0.05																
HB.A1C	0.179	0.34	-0.224	-0.353	1														
	0.493	0.182	0.387	0.165															
Cholest	0.005	0	0.126	0.063	-0.36	1													
	0.985	1	0.63	0.809	0.156														
HDL	0.003	-0.403	-0.324	-0.401	0.122	-0.057	1												
	0.991	0.109	0.205	0.11	0.64	0.827													
LDL	-0.01	-0.47	0.144	0.363	0.123	-0.111	0.031	1											
	0.968	0.057	0.582	0.153	0.637	0.672	0.907												
TG	-0.084	0.071	**.567***	0.173	-0.007	0.236	-0.057	0.047	1										
	0.747	0.788	0.018	0.507	0.977	0.362	0.829	0.857											
TC/HDL	0.026	**.534***	0.034	0.064	0.016	0.398	**-0.770****	-0.309	-0.044	1									
	0.922	0.027	0.896	0.807	0.951	0.114	0.0001	0.227	0.866										
LDL/HDL	0.079	0.104	0.218	0.383	0.201	-0.194	**-.781***	0.356	-0.11	**.533***	1								
	0.764	0.692	0.4	0.13	0.44	0.455	0.0001	0.16	0.676	0.027									
Serum creatinine	0.035	-0.168	**-.681***	-0.364	0.074	-0.102	0.458	-0.23	**-.524***	-0.237	-0.256	1							
	0.894	0.519	0.008	0.151	0.777	0.696	0.065	0.375	0.031	0.36	0.321								
Urine creatinine	-0.343	0.326	-0.229	-0.309	0.092	-0.196	-0.12	0.055	0.038	0.119	0.275	0.091	1						
	0.178	0.201	0.376	0.227	0.726	0.452	0.647	0.835	0.886	0.649	0.286	0.728							
Urine protein	0.015	-0.193	0.197	-0.004	-0.349	0.183	0.112	-0.058	0.271	-0.258	-0.386	0.051	-0.305	1					
	0.955	0.458	0.449	0.989	0.17	0.481	0.668	0.825	0.292	0.317	0.126	0.845	0.233						
SYST BP	0.354	**-.498***	**.658****	**.623****	-0.321	-0.041	-0.249	0.296	0.166	-0.061	0.21	-0.409	**-.582***	0.178	1				
	0.163	0.042	0.004	0.008	0.209	0.875	0.335	0.248	0.525	0.815	0.417	0.103	0.014	0.494					
DIAST BP	-0.384	-0.034	0.271	0.407	-0.433	0.192	-0.126	-0.237	0.396	-0.056	-0.282	**-.493***	-0.419	0.351	0.277	1			
	0.128	0.897	0.293	0.104	0.083	0.46	0.63	0.359	0.115	0.83	0.272	0.044	0.095	0.168	0.282				
Serum IL-6	-0.28	-0.191	0.206	-0.022	-0.149	0.207	0.42	-0.108	0.299	-0.412	-0.445	-0.051	0.045	0.165	-0.084	0.357	1		
	0.276	0.462	0.428	0.933	0.569	0.424	0.094	0.68	0.244	0.101	0.073	0.846	0.862	0.528	0.75	0.16			
Serum Leptin	-0.257	-0.115	-0.144	0.002	-0.061	-0.462	0.026	-0.157	-0.142	-0.076	0.072	0.323	0.433	0.09	-0.065	-0.124	0.163	1	
	0.32	0.66	0.582	0.994	0.815	0.062	0.921	0.548	0.586	0.772	0.782	0.207	0.082	0.732	0.805	0.634	0.531		
Serum VEGF	-0.058	0.08	-0.361	-0.431	0.053	0.039	0.16	-0.09	-0.356	0.108	0.004	0.385	0.197	-0.36	-0.165	**-0.486***	0	0.107	1
	0.825	0.759	0.154	0.084	0.839	0.881	0.539	0.732	0.16	0.68	0.989	0.127	0.448	0.156	0.527	0.048	1	0.683	.

Correlation is significant at the 0.05 level (2-tailed).
Correlation is significant at the 0.01 level (2-tailed).

Females

Age

Age did not show any correlation with any of the parameters (Table 3.16).

Body Mass Index

BMI was positively correlated with random blood sugar and serum HDL (r = 0.469; $P < 0.028$ and r = 0.426; $P < 0.048$ respectively), (Table 3.16).

Plasma Glucose

FBS was positively correlated with serum creatinine (r = 0.448; $P < 0.037$), (Table 3.16).

Lipid Profile

Total cholesterol showed significant positive correlation with TC/HDL ratio (r = 0.690; $P < 0.0001$) but negative correlation with serum VEGF (r = -0.434; $P < 0.044$). HDL was negatively correlated with the TC/HDL ratio and LDL/HDL ratio (r = – 0.671; $P < 0.001$ and r = - 0.542; $P < 0.009$ respectively). LDL showed significant positive correlation with LDL/DHL ratio (r = 0.786; $P < 0.0001$), (Table 3.16).

Renal profile

Urinary protein was negatively correlated with serum leptin (r = - 0.484; $P < 0.022$), (Table 3.16).

Blood Pressure

Systolic blood pressure was negatively correlated with serum IL-6 (r = - 0.426; $P < 0.048$), (Table 3.16).

Table 3.16 Correlation Table Normal Females

Spearman's two-tailed correlation normal females

	Age	BMI	RBS	FBS	HB.A1C	Cholest	HDL	LDL	TG	TC/ HDL	LDL/HDL	Ser.Creatinine	Urine Creatinine	Urine Protein	SYS BP	DIAS BP	Ser IL-6	Ser Leptin	Ser VEGF
Age	1																		
BMI	0.272	1																	
	0.221																		
RBS	-0.045	**.469***	1																
	0.844	0.028																	
FBS	-0.262	0.2	0.222	1															
	0.239	0.372	0.322																
HB.A1C	-0.177	0.058	0.332	0.28	1														
	0.43	0.799	0.131	0.207															
Cholest	0.117	-0.042	-0.012	0.076	-0.153	1													
	0.603	0.852	0.959	0.738	0.496														
HDL	0.151	**.426***	0.197	0.224	-0.31	-0.003	1												
	0.504	0.048	0.38	0.317	0.16	0.991													
LDL	-0.14	0.296	0.266	0.128	0.246	0.017	0.031	1											
	0.533	0.182	0.232	0.571	0.269	0.939	0.889												
TG	0.116	-0.168	0.085	-0.106	0.067	-0.025	-0.109	-0.338	1										
	0.606	0.455	0.707	0.64	0.768	0.911	0.628	0.124											
TC/HDL	-0.04	-0.286	-0.053	-0.013	0.138	**.690****	**-0.671****	-0.089	0.108	1									
	0.861	0.197	0.816	0.954	0.54	0.0001	0.001	0.694	0.632										
LDL/HDL	-0.14	0.093	0.127	-0.09	0.378	0.02	**-0.542****	**.786****	-0.178	0.299	1								
	0.535	0.682	0.574	0.691	0.083	0.931	0.009	0.0001	0.428	0.177									
Ser Creatinine	0.36	0.202	-0.107	**.448***	-0.012	0.016	0.336	-0.066	-0.296	-0.224	-0.337	1							
	0.1	0.367	0.634	0.037	0.957	0.943	0.126	0.77	0.182	0.315	0.125								
Urine Creatinine	-0.021	0.174	-0.057	0.19	0.107	-0.218	0.066	-0.243	-0.294	-0.094	-0.227	0.052	1						
	0.926	0.439	0.8	0.398	0.635	0.329	0.771	0.276	0.184	0.678	0.309	0.818							
Urine Protein	-0.132	0.084	0.128	0.147	-0.202	0.134	0.28	0.018	-0.091	-0.085	-0.281	0.406	-0.045	1					
	0.558	0.711	0.571	0.514	0.367	0.552	0.206	0.936	0.687	0.706	0.205	0.061	0.841						
SYS BP	0.128	0.358	0.254	-0.221	0.06	0.177	-0.339	0.076	0.02	0.397	0.326	-0.207	0.123	0.016	1				
	0.569	0.101	0.254	0.324	0.791	0.429	0.123	0.735	0.928	0.067	0.139	0.355	0.586	0.944					
DIAST BP	0.134	0.257	0.206	-0.053	0.008	0.267	0.049	0.359	-0.248	0.045	0.275	0.203	-0.284	0.167	0.186	1			
	0.553	0.249	0.359	0.813	0.971	0.229	0.828	0.101	0.266	0.842	0.216	0.364	0.2	0.459	0.408				
Serum IL-6	-0.129	-0.208	-0.382	0.091	-0.133	-0.238	0.269	-0.186	0.099	-0.329	-0.353	0.299	-0.014	0.03	**-0.426***	-0.169	1		
	0.566	0.352	0.079	0.687	0.554	0.286	0.227	0.408	0.663	0.135	0.107	0.176	0.952	0.895	0.048	0.452			
Serum Leptin	0.227	-0.299	-0.196	-0.016	0.168	0.192	-0.221	-0.306	0.239	0.257	-0.082	-0.078	0.03	**-0.484***	-0.048	-0.062	0.125	1	
	0.309	0.176	0.382	0.942	0.454	0.393	0.324	0.165	0.285	0.248	0.718	0.731	0.895	0.022	0.832	0.785	0.579		
Serum VEGF	-0.047	-0.105	0.127	-0.096	0.276	**-0.434***	-0.087	0.116	-0.334	-0.324	0.099	0.051	-0.168	-0.057	-0.257	0.114	-0.114	-0.214	1
	0.837	0.643	0.574	0.67	0.213	0.044	0.701	0.608	0.129	0.142	0.661	0.822	0.454	0.799	0.249	0.613	0.612	0.34	

Correlation is significant at the 0.05 level (2-tailed).
Correlation is significant at the 0.01 level (2-tailed).

3.8.2 Diabetic Non-Retinopathic Patients

Males

Age

In these patients, age was found to be positively correlated with RBS (r = 0.724; *P* < 0.002), (Table 3.17).

Body Mass Index

BMI was negatively correlated with LDL/HDL ratio (r = - 0.521; P < 0.046), while it was positively correlated with systolic blood pressure, serum leptin and serum VEGF (r = 0.560; r = 0.519; r = 0.720; *P* < 0.03; *P* < 0.048; *P* < 0.002 respectively), (Table 3.17).

Plasma Glucose

RBS was negatively correlated with serum IL-6 (r = - 0.616; *P* < 0.015). FBS was positively correlated with urinary protein (r = 0.601; *P* < 0.018), while it was negatively correlated with serum VEGF (r = - 0.659; *P* < 0.008), (Table 3.17).

Lipid Profile

Cholesterol was negatively correlated with TC/HDL ratio (r = - 0.603; *P* < 0.017) and positively correlated with diastolic blood pressure (r = 0.525; *P* < 0.044) (Table 17).

LDL was positively correlated with TC/HDL ratio and LDL/HDL ratio (r = 0.722; *P* < 0.002 and r = 0.894; *P* < 0.0001 respectively), but negatively correlated with systolic blood pressure (r = - 0.641; *P* < 0.01), (Table 3.17).

Serum TG was positively correlated with urine creatinine (r = 0.519; *P* < 0.047). TC/HDL ratio was positively correlated with LDL/HDL ratio (r = 0.561; *P* < 0.03), (Table 3.17).

LDL/DL ratio was negatively correlated with systolic blood pressure (r = - 0.732; *P* < 0.002), (Table 3.17).

Renal Profile

Urinary protein was positively correlated with systolic blood pressure (r = 0.562; *P* < 0.029), (Table 3.17).

Serum Il-6, Serum Leptin and Serum VEGF

Serum IL-6 and serum leptin were positively correlated with serum VEGF (r = 0.540; $P < 0.038$ and r = 0.573 and $P < 0.026$ respectively), (Table 3.17).

Table 3.17 Correlation Table Diabetic Non Retinopathic Males

Diabetic non-retinopathic males Spearman's correlation

	Age	BMI	RBS	FBS	HB.A1C	Cholest	HDL	LDL	TG	TC/ HDL	LDL/HDL	Ser.Creatinine	Urine Creatinine	Urine Protein	SYS BP	DIAS BP	Ser IL-6	Ser Leptin	Ser VEGF
Age	1																		
BMI	-0.23	1																	
	0.411																		
RBS	**.724****	0.105	1																
	0.002	0.71																	
FBS	0.006	-0.439	-0.193	1															
	0.982	0.102	0.491																
HB.A1C	-0.061	-0.173	0.038	-0.302	1														
	0.828	0.537	0.893	0.274															
Cholest	0.298	-0.213	-0.116	0.282	0.348	1													
	0.281	0.447	0.682	0.308	0.203														
HDL	0.127	0.455	0.305	-0.008	-0.389	-0.126	1												
	0.651	0.088	0.269	0.977	0.152	0.654													
LDL	-0.17	-0.267	0.07	-0.006	-0.184	-0.501	0.029	1											
	0.545	0.336	0.803	0.982	0.512	0.057	0.919												
TG	-0.168	0.114	-0.03	0.42	-0.093	-0.089	0.314	-0.012	1										
	0.549	0.686	0.916	0.119	0.741	0.753	0.255	0.967											
TC/HDL	-0.311	-0.15	0.104	0.125	-0.177	**-0.603***	0.336	**.722****	0.313	1									
	0.26	0.593	0.711	0.657	0.527	0.017	0.221	0.002	0.256										
LDL/HDL	-0.197	**-0.521***	-0.095	0.034	-0.077	-0.365	-0.291	**.894****	-0.116	**.561***	1								
	0.48	0.046	0.735	0.904	0.785	0.181	0.293	0.0001	0.68	0.03									
Serum creatinine	-0.305	0.049	0.009	0.214	0.278	0.013	0.284	-0.103	0.351	0.427	-0.149	1							
	0.269	0.864	0.974	0.444	0.316	0.964	0.305	0.714	0.199	0.113	0.596								
Urine creatinine	-0.441	0.084	-0.479	0.136	-0.15	-0.183	0.194	0.091	**.519***	0.105	0.136	-0.061	1						
	0.1	0.765	0.071	0.629	0.595	0.513	0.488	0.746	0.047	0.708	0.629	0.829							
Urine protein	0.013	0.135	-0.076	**.601***	-0.328	0.354	0.076	-0.22	0.297	-0.113	-0.208	0.318	-0.047	1					
	0.962	0.633	0.788	0.018	0.232	0.195	0.787	0.43	0.283	0.689	0.458	0.248	0.867						
SYST BP	0.126	**.560***	-0.021	0.002	-0.168	0.433	0.395	**-0.641***	0.082	-0.404	**-0.732****	0.107	-0.033	**.562***	1				
	0.654	0.03	0.94	0.995	0.549	0.107	0.145	0.01	0.771	0.135	0.002	0.703	0.906	0.029					
DIAST BP	-0.091	-0.087	-0.391	-0.153	0.418	**.525***	0.058	-0.405	-0.041	-0.334	-0.252	0.269	0.223	-0.105	0.213	1			
	0.746	0.758	0.15	0.587	0.121	0.044	0.837	0.135	0.884	0.224	0.366	0.332	0.425	0.709	0.446				
Serum IL-6	-0.483	0.306	**-0.616***	-0.365	-0.027	0.029	-0.219	-0.025	-0.492	-0.311	0.004	-0.371	0.132	-0.206	0.1	0.256	1		
	0.068	0.267	0.015	0.181	0.924	0.919	0.433	0.929	0.063	0.26	0.99	0.173	0.638	0.462	0.724	0.358			
Serum Leptin	0.042	**.519***	0.29	-0.299	-0.004	-0.295	0.242	-0.039	-0.29	-0.097	-0.256	-0.085	-0.01	-0.155	0.104	-0.209	0.303	1	
	0.881	0.048	0.295	0.28	0.99	0.286	0.385	0.889	0.295	0.732	0.357	0.765	0.972	0.581	0.711	0.455	0.273		
Serum VEGF	-0.139	**.720****	0.041	**-0.659****	-0.211	-0.363	0.34	-0.197	-0.27	-0.091	-0.367	-0.155	-0.033	-0.323	0.297	0.004	**.540***	**.573***	1
	0.62	0.002	0.886	0.008	0.451	0.184	0.215	0.482	0.331	0.746	0.179	0.582	0.907	0.241	0.283	0.988	0.038	0.026	

Correlation is significant at the 0.01 level (2-tailed).
Correlation is significant at the 0.05 level (2-tailed).

Females

Age

Age was positively correlated with urinary protein, serum IL-6 and Serum VEGF (r = 0.414; r = 0.425 and r = 0.416; $P < 0.049$; $P < 0.043$ and $P < 0.049$ respectively), (Table 3.18).

Body Mass Index

BMI was positively correlated with LDL/HDL ratio and urinary protein (r = 0.461; $P < 0.027$ and r = 0.496; $P < 0.016$ respectively), (Table 3.18).

Plasma Glucose

RBS was also positively correlated with urinary protein (r = 0.433; $P < 0.039$), (Table 3.18).

Lipid Profile

HDL was positively correlated with serum creatinine (r = 0.446; $P < 0.033$) (Table 3.18).

LDL was positively correlated with TC/HDL ratio and LDL/HDL ratio (r = 0.839; $P < 0.0001$ and r = 0.895; $P < 0.0001$ respectively), (Table 3.18).

TC/HDL ratio was positively correlated with LDL/HDL ratio (r = 0.733; $P < 0.0001$) and diastolic blood pressure (r = 0.478; $P < 0.021$), (Table 3.18).

LDL/HDL ratio was positively correlated with serum VEGF (r = 0.482; $P < 0.02$), (Table 3.18).

Renal Profile

Serum creatinine was negatively correlated with serum VEGF (r = 0.447; $P < 0.033$). Urinary protein was positively correlated with serum VEGF (r = 0.667; $P < 0.001$), (Table 3.18).

Blood Pressure

Systolic blood pressure was positively correlated with diastolic blood pressure (r = 0.483; $P < 0.019$), (Table 3.18).

Table 3.18 Correlation Table Diabetic Non Retinopathic Females

Control diabetic non-retinopathic females Spearman's correlation

	Age	BMI	RBS	FBS	HB.A1C	Cholest	HDL	LDL	TG	TC/ HDL	LDL/HDL	Ser.Creatinine	Urine Creatinine	Urine Protein	SYS BP	DIAS BP	Ser IL-6	Ser Leptin	Ser VEGF
Age	1																		
BMI	0.221	1																	
	0.31																		
RBS	0.119	0.26	1																
	0.589	0.231																	
FBS	0.095	-0.111	-0.097	1															
	0.666	0.614	0.66																
HB.A1C	-0.184	-0.081	0.017	-0.207	1														
	0.4	0.713	0.937	0.343															
Cholest	0.318	-0.009	-0.219	-0.096	0.216	1													
	0.139	0.966	0.314	0.664	0.322														
HDL	-0.067	-0.049	-0.11	0.161	-0.044	-0.321	1												
	0.762	0.825	0.616	0.463	0.841	0.136													
LDL	0.237	0.324	0.133	0.316	-0.025	0.061	0.371	1											
	0.277	0.132	0.546	0.142	0.909	0.783	0.081												
TG	0.271	-0.02	0.122	-0.168	0.117	0.197	0.123	0.068	1										
	0.212	0.928	0.58	0.444	0.594	0.369	0.577	0.759											
TC/HDL	-0.088	0.335	0.211	0.171	-0.097	-0.304	0.35	**.839****	0.051	1									
	0.688	0.118	0.333	0.434	0.661	0.159	0.102	0.0001	0.817										
LDL/HDL	0.287	**.461***	0.255	0.184	-0.084	0.218	0.006	**.895****	0.055	**.733****	1								
	0.184	0.027	0.24	0.401	0.703	0.319	0.977	0.0001	0.804	0.0001									
Serum Creatine	-0.089	-0.343	-0.14	0.262	0.156	-0.115	**.446***	0.19	0.09	0.148	0.074	1							
	0.688	0.109	0.523	0.227	0.477	0.603	0.033	0.385	0.684	0.5	0.738								
Urine Creatinine	-0.006	0.025	0.349	-0.273	-0.119	-0.201	0.202	0.211	-0.036	0.258	0.161	-0.087	1						
	0.979	0.909	0.103	0.207	0.588	0.357	0.355	0.333	0.87	0.234	0.463	0.692							
Urine Protein	**.414***	**.496***	**.433***	0.167	-0.244	-0.072	-0.08	0.359	0.039	0.352	0.382	-0.343	-0.006	1					
	0.049	0.016	0.039	0.446	0.263	0.743	0.718	0.093	0.861	0.1	0.072	0.109	0.979						
SYST BP	0.058	0.363	-0.102	-0.01	-0.278	-0.318	-0.14	-0.057	0.006	0.177	0.01	-0.236	-0.087	0.292	1				
	0.794	0.088	0.644	0.965	0.2	0.139	0.525	0.796	0.978	0.419	0.963	0.279	0.692	0.177					
DIAST BP	-0.17	0.252	-0.003	-0.127	-0.071	-0.412	0.248	0.228	0.079	**.478***	0.073	-0.267	0.175	0.348	**.483***	1			
	0.438	0.246	0.987	0.564	0.747	0.051	0.254	0.295	0.721	0.021	0.742	0.217	0.424	0.104	0.019				
Serum IL6	**.425***	-0.089	-0.293	0.123	-0.22	0.136	-0.285	-0.058	-0.097	-0.177	0.019	-0.007	-0.197	0.136	0.174	-0.257	1		
	0.043	0.688	0.175	0.577	0.314	0.537	0.187	0.794	0.659	0.42	0.93	0.973	0.367	0.537	0.427	0.236			
Serum Leptin	-0.266	-0.211	0.153	0.081	0.12	-0.059	-0.391	-0.366	0.304	-0.242	-0.318	-0.313	-0.04	0.044	0.11	0.045	0.051	1	
	0.22	0.333	0.487	0.714	0.585	0.79	0.065	0.086	0.158	0.265	0.139	0.146	0.857	0.843	0.618	0.838	0.817		
Serum VEGF	**.416***	0.407	0.336	0.202	-0.341	0.276	-0.139	0.371	-0.097	0.197	**.482***	**.447***	0.077	**.667****	0.15	0.045	0.302	-0.048	1
	0.049	0.054	0.117	0.355	0.112	0.202	0.526	0.081	0.66	0.368	0.02	0.033	0.725	0.001	0.495	0.838	0.161	0.828	

Correlation is significant at the 0.05 level (2-tailed).
Correlation is significant at the 0.01 level (2-tailed).

3.8.3 Diabetic Non-Proliferative Retinopathic Patients (NPDR) Males

Age Age was negatively correlated with RBS, FBS, HbA1c and urinary protein (r = - 0.348; r = - 0.676; r = - 0. 305; r = - 0.313; $P < 0.016$; $P < 0.0001$; $P < 0.037$ and $P < 0.032$ respectively), while it was positively correlated with serum TG, serum creatinine, urine creatinine (r = 0.354; r = 0.492 and r = 0.358; $P < 0.015$; $P < 0.0001$ and $P < 0.013$ respectively), and also with systolic and diastolic blood pressure (r = 0.721; $P < 0.0001$ and r = 0.662; $P < 0.0001$ respectively), (Table 3.19).

Body Mass Index

BMI showed significant negative correlations with RBS, FBS, HbA1c and HDL (r = - 0.775; r = - 0.377; r = - 0.717 and r = - 0.442; $P < 0.0001$; $P < 0.009$; $P < 0.0001$ and $P < 0.002$ respectively), but showed significant positive correlations with total cholesterol, TC/HDL ratio, and urinary protein (r = 0.550; r = 0.435; r = 0.391; $P < 0.0001$; $P < 0.002$; and $P < 0.007$ respectively), (Table 3.19).

Plasma Glucose

RBS was was positively correlated with FBS, HbA1c and HDL (r = 0.587; r = 0.860 and r = 0.334; $P < 0.0001$; $P < 0.0001$ and $P < 0.022$ respectively) and negatively correlated with serum total cholesterol and TC/HDL ratio (r = 0.423; $P < 0.003$ and r = - 0.377; $P < 0.009$). Fasting blood sugar was positively correlated with Hb.A1c and urinary protein concentration (r = 0.463; $P < 0.001$ and r = 0.403; $P < 0.005$), while it showed significant negative correlation with serum creatinine, systolic blood pressure, serum leptin and vitreous VEGF levels (r = - 0.429; r = - 0.515; r = - 0.826 and r = - 0.798; $P < 0.003$; $P < 0.005$; $P < 0.0001$; $P < 0.001$ and $P < 0.002$ respectively), (Table 3.19).

HbA1c HbA1c was positively correlated with HDL (r = 0.527; $P < 0.0001$) and negatively correlated with TC/HDL ratio (r = - 0.399; $P < 0.005$), (Table 3.19).

Lipid Profile

Total cholesterol showed positive correlations with LDL, TC/HDL ratio, LDL/HDL ratio, serum creatinine, urine creatinine, and urinary protein (r = 0.337; r = 0.602; r = 0.390; r = 0.391; r = 0.297 and r = 0.480; $P < 0.021$; $P < 0.0001$; $P < 0.007$; $P < 0.042$ and $P < 0.001$ respectively), and negative correlations with HDL, serum leptin and vitreous VEGF (r = - 0.377; r = - 0.651 and r = - 0.683; $P < 0.009$; P < 0.022 and $P < 0.014$), (Table 3.19).

HDL was positively correlated with diastolic blood pressure (r = 0.360; *P* < 0.013) but negatively correlated with TC/HDL ratio and LDL/HDL ratio (r = - 0.912; *P* < 0.0001 and r = - 0.655; *P* < 0.0001) but. LDL was positively correlated with TC/HDL and LDL/HDL ratio (r = 0.303; *P* < 0.038 and r = 0.811; *P* < 0.0001) but negatively correlated with serum TG, and diastolic blood pressure (r = - 0.450; P < 0.002 and r = - 0.355; *P* < 0.014 respectively). Serum TG was positively correlated with serum creatinine, urine creatinine, systolic blood pressure, and diastolic blood pressure (r = 0.584; r = 0.462; r = 0.412 and r = 0.428; *P* < 0.0001; *P* < 0.001; *P* < 0.004 and *P* < 0.003 respectively) but negatively correlated with LDL/HDL ratio (r = - 0.333; *P* < 0.022). TC/HDL ratio was positively correlated with LDL/HDL ratio and serum creatinine (r = 0.732; *P* < 0.0001 and r = 0.357; *P* < 0.014), and was negatively correlated with diastolic blood pressure (r = - 0.358; *P* < 0.013). LDL/HDL ratio was negatively correlated with diastolic blood pressure (r = - 513; *P* < 0.0001), (Table 3.19). *Renal Profile*

Serum creatinine was positively correlated with urine creatinine and systolic blood pressure (r = 0.322; *P* < 0.027 and r = 0.366; *P* < 0.011). Urinary creatinine was positively correlated with systolic and diastolic blood pressures (r = 0.492; *P* < 0.0001; r = 0.390; *P* < 0.007 respectively). Urinary protein was negatively correlated with systolic blood pressure and vitreous VEGF (r = - 0.352; *P* < 0.015 and r = - 0.616; *P* < 0.033), (Table 3.19).

Blood Pressure

Systolic blood pressure was positively correlated with diastolic blood pressure (r = 0.668; *P* < 0.0001), (Table 3.19).

Serum and Vitreous IL-6, Leptin and VEGF

Serum leptin was positively correlated with vitreous VEGF (r = 0.664; *P* < 0.018). Serum VEGF showed positive correlation with vitreous VEGF (r = 0.816; *P* < 0.001) and negative correlation with vitreous leptin (r = - 0.805; *P* < 0.002), while vitreous leptin was negatively correlated with vitreous VEGF (r = - 680; *P* < 0.015), (Table 3.19).

Table 3.19 Correlation Table NPDR males

NPDR males Spearman's two-tailed correlation

	Age	BMI	RBS	FBS	HB.A1C	Cholest	HDL	LDL	TG	TC/ HDL	LDL/HDL	Ser.Creatinine	Urine Creatinine	Urine Protein	SYS BP	DIAS BP	Ser IL-6	Ser Leptin	Ser VEGF	VITIL6	VITLEP	VITVEGF
Age	1																					
BMI	0.277	1																				
	0.06																					
RBS	**-.348***	**-.775****	1																			
	0.016	0.0001																				
FBS	**-.676****	**-.377****	**.587****	1																		
	0.0001	0.009	0.0001																			
HB.A1C	**-0.305**	**-.717****	**.860****	**.463****	1																	
	0.037	0.0001	0.0001	0.001																		
Cholest	0.218	.550(**)	**-.423****	-0.15	-0.24	1																
	0.142	0.0001	0.003	0.313	0.105																	
HDL	-0.072	**-.442****	**.334***	0.268	**.527****	**-.377****	1															
	0.63	0.002	0.022	0.069	0.0001	0.009																
LDL	-0.081	-0.046	-0.161	-0.132	-0.126	**.337***	-0.195	1														
	0.587	0.758	0.279	0.375	0.398	0.021	0.189															
TG	**.354***	-0.044	0.147	-0.221	0.222	0.082	0.209	**-.450****	1													
	0.015	0.767	0.325	0.136	0.134	0.582	0.159	0.002														
TC/HDL	-0.014	**.435****	**-.377****	-0.266	**-.399****	**.602****	**-.912****	**.303***	-0.115	1												
	0.926	0.002	0.009	0.071	0.005	0.0001	0.0001	0.038	0.44													
LDL/HDL	-0.152	0.063	-0.145	-0.176	-0.204	**.390****	**-.655****	**.811****	**-.333***	**.732****	1											
	0.306	0.675	0.331	0.236	0.169	0.007	0.0001	0.0001	0.022	0.0001												
Serum creatinine	**.492****	0.166	-0.156	**-.429****	-0.016	**.391****	-0.237	-0.035	**.584****	**.357***	0.148	1										
	0.0001	0.264	0.295	0.003	0.915	0.007	0.109	0.814	0.0001	0.014	0.32											
Urine creatinine	**.358***	-0.118	0.126	0.047	0.189	**.297***	0.075	-0.218	**.462****	0.014	-0.145	**.322***	1									
	0.013	0.431	0.398	0.756	0.202	0.042	0.617	0.141	0.001	0.925	0.33	0.027										
Urine protein	**-.313****	**.391****	-0.112	**.403****	-0.121	**.480****	0.051	0.063	0.101	0.057	-0.001	0.105	-0.016	1								
	0.032	0.007	0.452	0.005	0.417	0.001	0.733	0.675	0.498	0.703	0.994	0.482	0.917									
SYST BP	**.721****	-0.045	-0.012	**-.515***	0.24	0.247	0.262	0.001	**.412****	-0.168	-0.134	**.366***	**.492****	**-.352***	1							
	0.0001	0.762	0.935	0.0001	0.104	0.094	0.076	0.994	0.004	0.259	0.368	0.011	0.0001	0.015								
DIAST BP	**.662****	0.176	-0.125	-0.27	0.01	0.17	**.360***	**-.355***	**.428****	**-.358***	**-.513****	0.227	**.390****	0.019	**.668****	1						
	0.0001	0.237	0.402	0.067	0.948	0.254	0.013	0.014	0.003	0.013	0.0001	0.125	0.007	0.898	0.0001							
Serum IL-6	-0.273	0.202	0.092	0.34	0.28	0.184	0.138	0	0.127	0.007	0	0.553	-0.106	0.451	-0.15	0.024	1					
	0.391	0.529	0.777	0.279	0.378	0.567	0.67	1	0.693	0.983	1	0.062	0.743	0.141	0.641	0.94						
Serum Leptin	0.177	-0.301	-0.099	**-0.826**	-0.122	**-.651***	0.236	-0.113	0.149	-0.275	-0.148	-0.195	-0.004	-0.542	0.073	0.171	-0.028	1				
	0.582	0.341	0.76	0.001	0.705	0.022	0.46	0.727	0.645	0.388	0.646	0.544	0.991	0.069	0.821	0.594	0.931					
Serum VEGF	0.044	-0.108	0.25	-0.528	0	-0.383	-0.244	0.042	-0.011	0.141	0.18	-0.057	-0.207	-0.37	-0.233	-0.221	-0.231	0.375	1			
	0.891	0.737	0.432	0.078	1	0.219	0.445	0.896	0.974	0.662	0.576	0.861	0.519	0.236	0.466	0.491	0.47	0.23				
Vitreous IL-6	0.025	-0.192	-0.493	-0.067	-0.205	0.23	0.367	0.141	0.23	-0.12	-0.106	-0.128	0.18	0.035	0.337	0.367	0.042	0.063	-0.55	1		
	0.939	0.551	0.103	0.835	0.523	0.472	0.241	0.662	0.472	0.711	0.744	0.693	0.576	0.913	0.284	0.24	0.897	0.846	0.064			
Vitreous Leptin	-0.009	0.052	-0.113	0.528	0.279	0.179	0.329	-0.049	-0.002	-0.268	-0.169	0.339	0.177	0.321	0.312	0.147	0.186	-0.487	**-.805***	0.186	1	
	0.978	0.874	0.727	0.078	0.38	0.578	0.297	0.879	0.996	0.4	0.599	0.281	0.583	0.309	0.323	0.648	0.564	0.108	0.002	0.564		
Vitreous VEGF	0.05	-0.33	0.032	**-.798***	-0.032	**-.683***	-0.032	0.088	-0.081	-0.032	0.137	-0.121	-0.303	**-.616***	-0.088	-0.073	-0.336	**.664***	**.816****	-0.196	**-.680***	1
	0.878	0.295	0.922	0.002	0.921	0.014	0.922	0.786	0.801	0.922	0.67	0.709	0.338	0.033	0.786	0.821	0.286	0.018	0.001	0.542	0.015	.

Correlation is significant at the 0.05 level (2-tailed).
Correlation is significant at the 0.01 level (2-tailed).

Females

Age

Age was positively correlated with BMI, HDL, urine creatinine, and diastolic blood pressure (r = 0.358; r = 0.369; r = 0.423 and r = 0.324; $P < 0.005$; $P < 0.003$; $P < 0.001$ and $P < 0.011$ respectively). In contrast, age was found negatively correlated with serum TG, urinary protein and vitreous leptin (r = - 0.351; r = - 0.257; r = - 0.773; $P < 0.006$; $P < 0.046$; $P < 0.024$), (Table 3.20).

Body Mass Index

BMI was positively correlated with RBS (r = 0.337; $P < 0.008$), whereas BMI was negatively correlated with HDL, serum creatinine and systolic blood pressure (r = - 0.264; r = - 0.494 and r = - 0.312; $P < 0.04$; $P < 0.0001$ and $P < 0.014$ respectively), (Table 3.20).

Plasma Glucose

Random blood sugar was found positively correlated with FBS, HbA1c, TG, TC/HDL ratio and urinary protein (r = 0.657; r = 0.516; r = 0.400; r = 0.338 and r = 0.573; $P < 0.0001$; $P < 0.0001$; $P < 0.001$; $P < 0.008$ and $P < 0.0001$ respectively). Fasting blood glucose was positively correlated with HbA1c, cholesterol, and urinary protein (r = 0.311; r = 0.264 and r = 0.592; $P < 0.015$; $P < 0.04$ and $P < 0.0001$), (Table 3.20).

HbA1c

Glycated hemoglobin was positively correlated with TC/HDL ratio and LDL/HDL ratio (r = 0.264; $P < 0.04$; r = 0.277; $P < 0.031$), (Table 3.20).

Lipid Profile

Serum total cholesterol was positively correlated with HDL, LDL, TC/HDL ratio, urinary protein and diastolic blood pressure (r = 0.383; r = 0.754; r = 0.338; r = 0.386 and r = 0.625; $P < 0.002$; $P < 0.0001$; $P < 0.008$; $P < 0.002$ and $P < 0.0001$ respectively). HDL was positively correlated with urine creatinine, systolic and diastolic blood pressure (r = 0.384; r = 0.313 and r = 0.391; $P < 0.002$; $P < 0.014$ and $P < 0.002$ respectively); while it was negatively correlated with TC/HDL ratio and LDL/HDL ratio (r = - 0.618; $P < 0.0001$ and r = - 0.640; $P < 0.0001$). LDL was found positively correlated with TC/HDL ratio, LDL/HDL ratio and diastolic blood pressure (r = 0.498; r = 0.632 and r = 0.501; $P < 0.0001$ respectively for all parameters). Serum

TG was positively correlated with urinary protein (r = 0.400; *P* < 0.001). TC/HDL ratio was positively correlated with LDL/HDL ratio (r = 0.851 *P* < 0.0001), (Table 3.20).

Renal Profile

Serum creatinine was positively correlated with urinary creatinine and systolic blood pressure (r = 0.459; *P* < 0.0001 and r = 0.415; *P* < 0.001). Urinary creatinine was positively correlated with urinary protein and systolic blood pressure (r = 0.280; *P* < 0.029 and r = 0.342; *P* < 0.007), (Table 3.20).

Blood Pressure

Systolic blood pressure with positively correlated with diastolic blood pressure (r = 0.428; *P* < 0.001). Diastolic pressure was positively correlated with serum leptin (r = 0.769; *P* < 0.026), (Table 3.20).

Serum IL-6, VEGF

Serum IL-6 was negatively correlated with vitreous IL-6 (r = - 0.810; *P* < 0.015), while serum VEGF was positively correlated with vitreous VEGF (r = 0.738; *P* < 0.037), (Table 3.20).

Table 3.20 Correlation Table NPDR Females

NPDR females Spearman's two tailed correlation

	Age	BMI	RBS	FBS	HB.A1C	Cholest	HDL	LDL	TG	TC/ HDL	LDL/HDL	Ser Creatinine	Urine Creatinine	Urine Protein	SYS BP	DIAS BP	Ser IL-6	Ser Leptin	Ser VEGF	Vit IL6	Vit Leptin	Vit VEGF
Age	1																					
BMI	**.358****	1																				
	0.005																					
RBS	0.052	**.337****	1																			
	0.689	0.008																				
FBS	0.091	-0.041	**.657****	1																		
	0.487	0.757	0.0001																			
HB.A1C	0.019	0.23	**.516****	**.311***	1																	
	0.884	0.074	0.0001	0.015																		
Cholest	0.179	-0.069	0.14	**.264***	0.117	1																
	0.168	0.596	0.282	0.04	0.37																	
HDL	**.369****	**-.264***	-0.118	0.164	-0.241	**.383****	1															
	0.003	0.04	0.365	0.207	0.061	0.002																
LDL	-0.014	-0.03	0.001	0.151	0.119	**.754****	0.015	1														
	0.918	0.817	0.996	0.244	0.36	0.0001	0.912															
TG	**-.351****	-0.241	**.400****	0.187	0.114	0.214	0.191	-0.142	1													
	0.006	0.062	0.001	0.148	0.383	0.098	0.141	0.274														
TC/HDL	-0.221	0.083	**.338****	0.062	**.264***	**.338****	**-.618****	**.498****	0.079	1												
	0.087	0.526	0.008	0.638	0.04	0.008	0.0001	0.0001	0.546													
LDL/HDL	-0.159	0.045	0.244	0.166	**.277***	0.231	**-.640****	**.632****	-0.194	**.851****	1											
	0.221	0.729	0.058	0.2	0.031	0.074	0.0001	0.0001	0.134	0.0001												
Serum creatinir	0.229	**-.494***	0.106	0.181	0.07	0.042	0.209	-0.157	0.186	0.044	-0.086	1										
	0.075	0.0001	0.414	0.164	0.59	0.751	0.107	0.227	0.151	0.735	0.508											
Urine creatinine	**.423****	-0.032	0.186	0.251	0.131	0.21	**.384****	-0.08	0.004	-0.195	-0.244	**.459****	1									
	0.001	0.805	0.15	0.051	0.313	0.103	0.002	0.542	0.974	0.132	0.058	0										
Urine protein	**-.257***	-0.146	**.573****	**.592****	0.196	**.386****	0.172	0.177	**.400****	0.127	0.04	0.069	**.280***	1								
	0.046	0.262	0.0001	0.0001	0.129	0.002	0.185	0.171	0.001	0.33	0.759	0.595	0.029									
SYST BP	0.104	**-.312***	-0.07	0.025	-0.09	0.164	**.313***	-0.148	-0.013	-0.039	-0.184	**.415****	**.342****	0.196	1							
	0.425	0.014	0.59	0.85	0.492	0.206	0.014	0.256	0.922	0.763	0.156	0.001	0.007	0.13								
DIAST BP	**.324***	-0.184	-0.04	0.187	-0.122	**.625****	**.391****	**.501****	-0.172	0.157	0.164	0.174	0.19	0.239	**.428****	1						
	0.011	0.156	0.759	0.15	0.348	0.0001	0.002	0.0001	0.185	0.226	0.206	0.18	0.142	0.064	0.001							
Serum IL-6	0.171	-0.356	0.048	-0.024	0.275	0.619	0.301	0.452	0.262	0.119	0.036	0.602	0.19	-0.214	0.136	0	1					
	0.686	0.387	0.911	0.955	0.509	0.102	0.468	0.26	0.531	0.779	0.933	0.115	0.651	0.61	0.748	1						
Serum Leptin	-0.586	0.049	0.214	0.452	-0.443	0.143	0.313	0.238	-0.452	-0.548	-0.108	0.172	0.143	0.619	0.605	**.769***	0.119	1				
	0.127	0.908	0.61	0.26	0.272	0.736	0.45	0.57	0.26	0.16	0.799	0.684	0.736	0.102	0.112	0.026	0.779					
Serum VEGF	-0.439	-0.061	0.619	0.31	-0.275	0.143	-0.145	-0.238	0.5	0.524	0.096	-0.11	0.071	0.619	0.148	-0.026	0.333	0.071	1			
	0.276	0.885	0.102	0.456	0.509	0.736	0.733	0.57	0.207	0.183	0.821	0.795	0.867	0.102	0.726	0.951	0.42	0.867				
Vitreous IL-6	-0.293	0.012	-0.333	-0.024	-0.323	-0.143	0.193	0.095	-0.214	-0.571	-0.311	-0.233	-0.31	0.143	-0.148	0.183	**-.810***	0.167	-0.524	1		
	0.482	0.977	0.42	0.955	0.435	0.736	0.647	0.823	0.61	0.139	0.453	0.578	0.456	0.736	0.726	0.665	0.015	0.693	0.183			
Vitreous Leptin	**-.773***	0.142	-0.108	-0.156	-0.602	0.06	0.455	0.144	-0.06	-0.503	-0.452	-0.222	0.036	0.635	0.659	0.643	-0.263	0.551	0.275	0.371	1	
	0.024	0.737	0.799	0.713	0.114	0.888	0.258	0.734	0.888	0.204	0.261	0.597	0.933	0.091	0.076	0.086	0.528	0.157	0.509	0.365		
Vitreous VEGF	-0.61	-0.025	0.357	0.333	0.072	0.333	0.084	-0.048	0.476	0.071	-0.431	0.012	-0.476	0.524	0	-0.17	0.429	0.286	**.738***	-0.357	0.275	1
	0.108	0.954	0.385	0.42	0.866	0.42	0.843	0.911	0.233	0.867	0.286	0.977	0.233	0.183	1	0.688	0.289	0.493	0.037	0.385	0.509	.

Correlation is significant at the 0.01 level (2-tailed).
Correlation is significant at the 0.05 level (2-tailed).

3.8.4 Diabetic Proliferative Retinopathic Patients (PDR)

Males

Age

Age was positively correlated with BMI, LDL, systolic and diastolic blood pressure (r = 0.310; r = 0.297; r = 0.296 and r = 0.436; $P < 0.011$; $P < 0.015$; $P < 0.015$ and $P < 0.0001$) and negatively correlated with HDL (r = - 0.258; $P < 0.035$), (Table 3.21).

Body Mass Index

Body mass index was negatively correlated with urinary protein (r = - 0.289; $P < 0.018$), (Table 3.21).

Plasma Glucose

Radom blood glucose was positively correlated with FBS, HbA1c and serum creatinine (r = 0.572; r = 0.605 and r = 0.366; $P < 0.0001$; $P < 0.0001$ and $P < 0.002$ respectively). It was negatively correlated with cholesterol (r = - 0.244; $P < 0.047$). Fasting blood glucose was positively correlated with HbA1c, HDL (r = 0.860; r = 0.616; $P < 0.0001$) but correlated negatively with cholesterol, LDL, TG, TC/HDL ratio and LDL/HDL ratio, systolic and diastolic blood pressure (r = - 0.528; r = - 0.513; r = - 0.,354; r = - 0.529; r = - 0.551; r = - 0.396 and r = - 0.279; $P < 0.0001$; $P < 0.0001$; $P < 0.0001$; $P < 0.0001$; $P < 0.0001$; $P < 0.001$ and $P < 0.022$ respectively), (Table 3.21).

HbA1c

Hb.A1c was found positively correlated with HDL, serum creatinine and urinary protein (r = 0.520; r = 0.444 and r = 0.392; $P < 0.0001$; $P < 0.0001$ and $P < 0.001$), while it was negatively correlated with cholesterol, LDL, TC/HDL ratio, LDL/HDL ratio, systolic and diastolic blood pressure (r = - 0.339; r = - 0.501; r = - 0.379; r = - 0.457; r = - 0.316 and r = - 0.286; $P < 0.005$; $P < 0.0001$; $P < 0.002$; $P < 0.0001$; $P < 0.009$ and $P < 0.019$ respectively), (Table 3.21).

Lipid Profile

Serum total cholesterol was positively correlated with LDL, TG, TC/HDL ratio, LDL/HDL ratio, systolic and diastolic blood pressure (r = 0.697; r = 0.391; r = 0.890; r = 0.796; r = 0.492 and r = 0.437; $P < 0.0001$; $P < 0.001$; $P < 0.0001$; $P < 0.0001$; $P < 0.0001$ and $P < 0.0001$ respectively), but correlated negatively with HDL (r = - 0.625;

P < 0.0001). HDL cholesterol was positively correlated with serum creatinine and urinary protein (r = 0.271; *P* < 0.027 and r = 0.243; *P* < 0.047), whereas negative correlations of HDL were found with LDL, TG, TC/HDL ratio, LDL/HDL ratio, systolic and diastolic blood pressures (r = - 0.540; r = - 0.570; r = - 0.875; r = - 0.833; r = - 0.654 and r = - 0.652; *P* < 0.0001 for all parameters). LDL cholesterol correlated positively with TG, TC/HDL ratio, systolic and diastolic blood pressures (r = 0.386; r = 0.641; r = 0.850; r = 0.534 and r = 0.624; *P* < 0.001; *P* < 0.0001; *P* < 0.0001; *P* < 0.0001; *P* < 0.0001). Serum triglyceride levels were positively correlated with TC//HDL ratio, LDL/HDL ratio, systolic and diastolic blood pressure (r = 0.539; r = 0.550; r = 0.746; r = 0.482; *P* < 0.0001; *P* < 0.0001; *P* < 0.0001; *P* < 0.0001 respectively), but correlated negatively with vitreous leptin (r = - 0.775; *P* < 0.007). TC/HDL ratio was positively correlated with LDL/HDL ratio, systolic and diastolic blood pressure (r = 0.899; r = 0.627 and r = 0.604; *P* < 0.0001 for all parameters respectively). LDL/HDL ratio correlated positively with systolic and diastolic blood pressure (r = 0.660; r = 0.693; *P* < 0.0001 for both), and correlated negatively with vitreous leptin (r = - 0.664; *P* < 0.026), (Table 3.21).

Renal Profile

Serum creatinine was positively correlated with urinary protein only (r = 0.464; *P* < 0.0001); Urinary protein was negatively correlated with systolic blood pressure (r = - 0.249; *P* < 0.042), (Table 3.21).

Blood Pressure

Systolic blood pressure was positively correlated with diastolic blood pressure, and serum IL-6 (r = 0.780; *P* < 0.0001 and r = 0.720; *P* < 0.012), while it was negatively correlated with vitreous leptin and vitreous VEGF (r = - 0.796; *P* < 0.003 and r = - 0.668; *P* < 0.025). Diastolic blood pressure was correlated positively with vitreous IL-6 (r = 0.671; *P* < 0.024), and negatively correlated with serum leptin and vitreous VEGF (r = - 0.679; *P* < 0.022 and r = - 0. 672; *P* < 0.023), (Table 3.21).

Table 3.21 Correlation Table PDR Males

PDR males Spearman's two-tailed correlation

	Age	BMI	RBS	FBS	HB.A1C	Cholest	HDL	LDL	TG	TC/ HDL	LDL/HDL	Ser.Creatinine	Urine Creatinine	Urine Protein	SYS BP	DIAS BP	SerIL-6	Ser Leptin	Ser VEGF	VITIL6	VITLEP	VITVEGF
Age	1																					
BMI	**.310***	1																				
	0.011																					
RBS	0.168	-0.177	1																			
	0.175	0.152																				
FBS	-0.124	-0.131	**.572****	1																		
	0.319	0.292	0.0001																			
HB.A1C	-0.237	-0.228	**.605****	**.860****	1																	
	0.053	0.063	0.0001	0.0001																		
Cholest	-0.105	0.033	**-.244***	**-.528****	**-.339****	1																
	0.398	0.789	0.047	0.0001	0.005																	
HDL	**-.258***	0.072	0.174	**.616****	**.520****	**-.625****	1															
	0.035	0.563	0.16	0.0001	0.0001	0.0001																
LDL	**.297***	0.127	-0.206	**-.513****	**-.501****	**.697****	**-.540****	1														
	0.015	0.308	0.094	0.0001	0.0001	0.0001	0.0001	.														
TG	0.105	-0.23	0.194	**-.354****	-0.157	**.391****	**-.570****	**.386****	1													
	0.396	0.062	0.117	0.003	0.204	0.001	0.0001	0.001														
TC/HDL	0.035	-0.061	-0.16	**-.529****	**-.379****	**.890****	**-.875****	**.641****	**.539****	1												
	0.78	0.623	0.197	0.0001	0.002	0.0001	0.0001	0.0001	0.0001													
LDL/HDL	0.155	-0.098	-0.171	**-.551****	**-.457****	**.796****	**-.833****	**.850****	**.550****	**.899****	1											
	0.21	0.431	0.166	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001												
Serum creatinine	-0.021	-0.182	**.366****	**.540****	**.444****	-0.015	**.271***	-0.101	-0.214	-0.127	-0.148	1										
	0.868	0.14	0.002	0.0001	0.0001	0.904	0.027	0.417	0.082	0.306	0.232											
Urine creatinine	0.145	-0.18	0.114	0.073	0.105	-0.015	-0.015	0.089	0.016	0.039	0.104	-0.115	1									
	0.243	0.144	0.36	0.559	0.397	0.903	0.904	0.472	0.9	0.752	0.402	0.356										
Urine protein	-0.215	**-.289***	0.111	**.360****	**.392****	-0.128	**.243***	-0.19	-0.17	-0.128	-0.047	**.464****	0.097	1								
	0.081	0.018	0.37	0.003	0.001	0.303	0.047	0.125	0.169	0.303	0.706	0.0001	0.435									
SYST BP	**.296***	-0.013	0.077	**-.396****	**-.316****	**.492****	**-.654****	**.534****	**.746****	**.627****	**.660****	-0.155	0.096	**-.249***	1							
	0.015	0.916	0.538	0.001	0.009	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.211	0.437	0.042								
DIAST BP	**.436****	0.187	0.112	**-.279***	**-.286***	**.437****	**-.652****	**.624****	**.482****	**.604****	**.693****	0.015	0.015	-0.225	**.780****	1						
	0.0001	0.13	0.368	0.022	0.019	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.903	0.904	0.067	0.0001							
Serum IL-6	0.257	-0.111	0.082	-0.018	-0.073	0.009	-0.251	-0.228	0.582	0.073	0.082	0.029	-0.173	-0.218	**.720***	0.224	1					
	0.445	0.744	0.811	0.957	0.831	0.979	0.457	0.501	0.06	0.832	0.811	0.933	0.612	0.519	0.012	0.509						
Serum Leptin	-0.256	-0.455	0.097	0.056	-0.106	-0.346	0.456	0.002	-0.184	-0.506	-0.377	0.058	-0.06	0.547	-0.364	**-.679***	0.064	1				
	0.448	0.159	0.778	0.871	0.756	0.298	0.158	0.995	0.588	0.112	0.253	0.866	0.861	0.082	0.271	0.022	0.851					
Serum VEGF	-0.014	-0.237	-0.391	-0.321	-0.333	0.023	-0.333	-0.342	0.318	0.191	0	-0.143	-0.509	-0.009	0.273	-0.149	0.591	0.253	1			
	0.968	0.483	0.235	0.336	0.318	0.947	0.318	0.304	0.34	0.574	1	0.675	0.11	0.979	0.416	0.662	0.056	0.453				
Vitreous IL-6	0.547	0.52	0.336	0.119	-0.014	-0.328	0.141	-0.05	0.264	-0.264	0.036	-0.086	-0.009	-0.245	0.537	**.671***	0.427	-0.451	-0.209	1		
	0.082	0.101	0.312	0.727	0.968	0.325	0.679	0.884	0.433	0.433	0.915	0.802	0.979	0.467	0.089	0.024	0.19	0.164	0.537			
Vitreous Leptin	-0.285	0.339	0.145	0.477	0.501	-0.159	0.36	-0.483	**-.775****	-0.245	**-.664****	0.124	-0.209	-0.164	**-.796****	-0.522	-0.445	0.124	-0.245	-0.391	1	
	0.395	0.308	0.67	0.138	0.116	0.64	0.277	0.132	0.007	0.467	0.026	0.717	0.537	0.631	0.003	0.1	0.17	0.716	0.467	0.235		
Vitreous VEGF	-0.376	0.156	-0.305	-0.17	-0.37	-0.413	0.461	-0.171	-0.424	-0.46	-0.492	-0.349	-0.405	0.191	**-.668***	**-.672***	-0.36	0.539	0.159	-0.31	0.342	1
	0.255	0.647	0.361	0.617	0.263	0.206	0.153	0.615	0.194	0.154	0.124	0.293	0.216	0.573	0.025	0.023	0.277	0.087	0.64	0.354	0.304	.

Correlation is significant at the 0.05 level (2-tailed).

Correlation is significant at the 0.01 level (2-tailed).

Females

Age

Age was positively correlated with HDL and LDL (r = 0. 178; *P* < 0.047 and r = 0. 299; *P* < 0.001), and negatively correlated with urinary creatinine and urinary protein (r = - 0.266; *P* < 0.003 and r = - 0.224 and *P* < 0.012), (Table 3.22).

Body Mass Index

Body mass index was positively correlated with RBS, FBS, Hb.A1c, cholesterol, TG, urinary creatinine, diastolic blood pressure and serum VEGF (r = 0.280; r = 0.215; r = 0.191; r = 0.182; r = 0.220; r = 0.257; r = 0.272 and r = 0.553; *P* < 0.002; *P* < 0.016; *P* < 0.032; *P* < 0.042; *P* < 0.014; *P* < 0.004; *P* < 0.002 and *P* < 0.04 respectively). BMI was negatively correlated serum creatinine (r = - 0.321; *P* < 0.0001), (Table 3.22).

Plasma Glucose

Random blood sugar was positively correlated with FBS, Hb.A1c, TG and urinary protein (r = 0.755; r = 0.849; r = 0.283 and r = 0.367; *P* < 0.0001; *P* < 0.0001; *P* < 0.001 and *P* < 0.0001 for all parameters). Fasting blood sugar was positively correlated with HB.A1c, HDL, TG, urinary creatinine and urinary protein (r = 0.630; r = 0.378; r = 0.190; r = 0.215 and r = 0.603; *P* < 0.0001; *P* < 0.0001; *P* < 0.016 and *P* < 0.0001 respectively), and negatively correlated with TC/HDL ratio and LDL/HDL ratio (r = - 0.242; *P* < 0.007 and r = - 0.190; *P* < 0.034), (Table 3.22).

HbA1c

Glycated hemoglobin was positively correlated with HDL, TG and urinary protein (r = 0.180; r = 0.380 and r = 0.453; *P* < 0.044; *P* < 0.0001 and *P* < 0.0001), but correlated negatively with urine creatinine (r = - 0.199; *P* < 0.026), (Table 3.22).

Lipid Profile

Serum total cholesterol was positively correlated with LDL, TG, TC/HDL ratio, LDL/HDL ratio, systolic and diastolic blood pressure (r = 0.541; r = 0.300; r = 0.434; r = 0.273; r = 0.317 and r = 0.236; *P* < 0.0001; *P* < 0.001; *P* < 0.0001; *P* < 0.002; *P* < 0.0001 and *P* < 0.008 respectively). It correlated negatively with serum creatinine and urinary protein (r = - 0.209; *P* < 0.019 and r = - 0.184; *P* < 0.04). HDL was positively correlated with urinary protein, systolic and diastolic blood pressure, vitreous IL-6 and vitreous VEGF (r = 0.501; r = 0.492; r = 0.355; r = 0.574 and r = 0.824; *P* < 0.0001; *P* < 0.0001; *P* < 0.0001; *P* < 0.032 and *P* < 0.0001 respectively). HDL

was negatively correlated with TC/HDL ratio, LDL/HDL ratio and serum creatinine (r = - 0.785; r = - 0.664 and r = - 0.218; $P < 0.0001$; $P < 0.0001$ and $P < 0.015$). LDL correlated positively with TC/HDL ratio, LDL/HDL ratio and serum leptin concentrations (r = 0.249; r = 0.635 and r = 0.732; $P < 0.005$; $P < 0.0001$ and $P < 0.003$), while it correlated negatively with serum creatinine, and urinary protein (r = - 0.318; $P < 0.0001$ and r = - 0.182; $P < 0.042$). Serum triglyceride levels were positively correlated only with TC/HDL ratio (r = 0.256; $P < 0.004$) and correlated negatively with urinary creatinine (r = - 0.213; $P < 0.017$). TC/HDL ratio was positively correlated with LDL/HDL ratio and urinary creatinine (r = 0.780; $P < 0.0001$ and r = 0.207; $P < 0.02$), but correlated negatively with urinary protein, systolic and diastolic blood pressure, vitreous IL-6 and vitreous VEGF (r = - 0.528; r = - 0.286; r = - 0.232; r = - 0.607 and r = - 0.630; $P < 0.0001$; $P < 0.001$; $P < 0.009$; $P < 0.021$ and $P < 0.016$ respectively). LDL/HDL ratio was positively correlated only with urinary creatinine (r = 0.228; $P < 0.011$) and correlated negatively with serum creatinine, urinary protein, systolic and diastolic blood pressures, vitreous IL-6 and vitreous VEGF (r = - 0.197; r = - 0.431; r = - 0.290; r = - 0.211; r = - 0.572 and r = - 0.551; $P < 0.028$; $P < 0.0001$; $P < 0.001$; $P < 0.018$; $P < 0.021$; $P < 0.033$ and $P < 0.041$ respectively), (Table 3.22).

Renal Profile

Serum creatinine was positively correlated with urinary protein (r = 0.248; $P < 0.005$), but correlated negatively with urinary creatinine and systolic and diastolic blood pressures (r = - 0.220; r = - 0.190 and r = - 0.409; $P < 0.014$; $P < 0.034$ and $P < 0.0001$). Urine creatinine was positively correlated with vitreous VEGF (r = 0.606; $P < 0.022$) and negatively correlated with systolic blood pressure (r = - 0.181; $P < 0.044$). Urinary protein was positively correlated with systolic and diastolic blood pressures (r = 0.229; $P < 0.01$ and r = 0.179; $P < 0.046$), (Table 3.22).

Blood Pressure

Systolic blood pressure was positively correlated with diastolic blood pressure (r = 0.561; $P < 0.0001$). Serum-IL-6 was positively correlated with vitreous leptin (r = 0.563; $P < 0.036$) and vitreous IL-6 was positively correlated with vitreous VEGF (r = 0.684; $P < 0.007$), (Table 3.22).

Table 3.22 Correlation Table PDR Females

PDR females Spearman's two-tailed correlation

	Age	BMI	RBS	FBS	HB.A1C	Cholest	HDL	LDL	TG	TC/ HDL	LDL/HDL	Ser.Creatinine	Urine Creatinine	Urine Protein	SYS BP	DIAS BP	Ser IL-6	Ser Leptin	Ser VEGF	Vit IL6	Vit Leptin	Vit VEGF
Age	1																					
BMI	0.154	1																				
	0.087																					
RBS	0.09	**.280****	1																			
	0.318	0.002																				
FBS	-0.165	**.215***	**.755****	1																		
	0.066	0.016	0.0001																			
HB.A1C	0.034	**.191***	**.849****	**.630****	1																	
	0.707	0.032	0.0001	0.0001																		
Cholest	0.171	**.182***	0.15	0.155	0.046	1																
	0.057	0.042	0.095	0.084	0.614																	
HDL	**.178***	0.174	0.058	**.378****	**.180***	0.11	1															
	0.047	0.052	0.518	0.0001	0.044	0.222																
LDL	**.299****	0.095	-0.018	-0.001	-0.106	**.541****	0.047	1														
	0.001	0.292	0.839	0.988	0.238	0.0001	0.601															
TG	-0.087	**.220***	**.283****	**.190***	**.380****	**.300****	-0.072	0.044	1													
	0.337	0.014	0.001	0.034	0.0001	0.001	0.423	0.629														
TC/HDL	-0.089	0.015	-0.029	**-.242****	-0.155	**.434****	**-.785****	**.249****	**.256****	1												
	0.325	0.87	0.747	0.007	0.084	0.0001	0.0001	0.005	0.004													
LDL/HDL	0.018	-0.065	-0.037	**-.190***	-0.128	**.273****	**-.664****	**.635****	0.165	**.780****	1											
	0.843	0.47	0.684	0.034	0.153	0.002	0.0001	0.0001	0.066	0.0001												
Serum creatinin	0.113	**-.321****	0.008	0.02	0.076	**-.209***	0.001	**-.318***	-0.039	-0.067	**-.197***	1										
	0.211	0.0001	0.93	0.822	0.399	0.019	0.994	0.0001	0.665	0.459	0.028											
Urine creatinine	**-.266****	**.257****	0.071	**.215***	**-.199****	0.012	**-.218***	0.077	**-.213***	**.207***	**.228***	**-.220***	1									
	0.003	0.004	0.433	0.016	0.026	0.892	0.015	0.392	0.017	0.02	0.011	0.014										
Urine protein	**-.224***	0.148	**.367****	**.603****	**.453****	**-.184***	**.501****	**-.182***	0.052	**-.528****	**-.431****	**.248****	0.083	1								
	0.012	0.1	0.0001	0.0001	0.0001	0.04	0.0001	0.042	0.566	0.0001	0.0001	0.005	0.355									
SYST BP	-0.119	0.09	-0.007	0.135	0.085	**.317****	**.492****	0.13	-0.108	**-.286****	**-.290****	**-.190***	**-.181***	**.229***	1							
	0.188	0.317	0.938	0.133	0.347	0.0001	0.0001	0.149	0.229	0.001	0.001	0.034	0.044	0.01								
DIAST BP	0.047	**.272****	-0.055	0.111	0.028	**.236****	**.355****	0.093	0.012	**-.232****	**-.211***	**-.409****	-0.083	**.179***	**.561****	1						
	0.603	0.002	0.54	0.217	0.755	0.008	0.0001	0.301	0.894	0.009	0.018	0.0001	0.356	0.046	0.0001							
Serum IL-6	0.015	-0.143	-0.01	-0.315	0.186	0.172	-0.027	-0.135	-0.257	-0.066	-0.071	0.174	0.023	-0.379	-0.058	-0.107	1					
	0.961	0.627	0.973	0.272	0.524	0.557	0.928	0.647	0.376	0.822	0.811	0.552	0.937	0.182	0.845	0.717						
Serum Leptin	0.165	-0.037	-0.261	-0.35	-0.444	0.243	-0.107	**.732****	-0.342	0.23	0.449	-0.327	0.117	0.053	0.322	-0.036	0.112	1				
	0.573	0.9	0.367	0.221	0.111	0.402	0.717	0.003	0.232	0.429	0.107	0.254	0.69	0.857	0.261	0.904	0.703					
Serum VEGF	-0.132	**.553***	-0.073	0.237	-0.116	0.464	0.122	0.303	0.223	0.171	0.191	0.301	0.388	-0.037	0.175	0	0.049	-0.108	1			
	0.654	0.04	0.805	0.415	0.693	0.095	0.679	0.293	0.443	0.56	0.514	0.296	0.171	0.899	0.549	1	0.869	0.712				
Vitreous IL-6	-0.223	-0.164	0.403	0.361	0.358	-0.009	**.574***	-0.295	0.166	**-.607***	**-.572***	0.04	0.383	-0.178	-0.155	-0.089	0.432	-0.108	0.002	1		
	0.444	0.574	0.153	0.205	0.209	0.976	0.032	0.306	0.572	0.021	0.033	0.893	0.177	0.543	0.597	0.763	0.123	0.713	0.994			
Vitreous Leptin	0.508	-0.176	0.002	-0.279	0.096	-0.05	-0.119	-0.067	-0.46	-0.032	-0.05	-0.025	-0.251	-0.363	-0.267	0.036	**.563***	0.113	-0.034	0.253	1	
	0.063	0.547	0.994	0.334	0.743	0.866	0.684	0.819	0.098	0.914	0.866	0.931	0.386	0.201	0.356	0.904	0.036	0.701	0.908	0.382		
Vitreous VEGF	-0.231	0.125	0.155	0.335	0.042	0.222	**.824****	-0.15	0.057	**-.630***	**-.551***	0.012	**.606***	-0.158	0.164	-0.071	0.357	0.03	0.218	**.684****	0.03	1
	0.427	0.671	0.596	0.242	0.887	0.445	0.0001	0.609	0.845	0.016	0.041	0.967	0.022	0.589	0.574	0.81	0.21	0.919	0.454	0.007	0.92	.

Correlation is significant at the 0.05 level (2-tailed).
Correlation is significant at the 0.01 level (2-tailed).

Table 3.23 Summary of Correlations for Normal Males

	+ ive Correl	-ive Correl
Age	-	-
BMI	TC/HDL ratio	Systolic BP
RBS	FBS TG Systolic BP	Serum creatinine -
FBS	Systolic BP	-
HbA1c	-	-
Cholesterol	-	-
HDL	-	TC/HDL ratio LDL/HDL ratio
LDL	-	-
TG	-	Serum creatinine
TC/HDL ratio	LDL/HDL ratio	-
LDL/HDL ratio	-	-
Serum creatinine	-	Diastolic BP
Urinary creatinine	-	Systolic BP
Urinary protein	-	-
Systolic BP	-	-
Diastolic BP	-	Serum VEGF
Serum IL-6	-	-
Serum leptin	-	-
Serum VEGF	-	-

Table 3.24 Summary of Correlations for Normal Females

	+ ive Correl	-ive Correl
Age	-	-
BMI	RBS HDL	-
RBS	-	-
FBS	Serum creatinine	-
HbA1c	-	-
Cholesterol	TC/HDL ratio	Serum VEGF
HDL	-	TC/HDL ratio LDL/HDL ratio
LDL	LDL/HDL ratio-	-
TG	-	-
TC/HDL ratio	-	-
LDL/HDL ratio	-	-
Serum creatinine	-	-
Urinary creatinine	-	-
Urinary protein	-	Serum leptin
Systolic BP	-	Serum IL-6
Diastolic BP	-	-
Serum IL-6	-	-
Serum leptin	-	-
Serum VEGF	-	-

Table 3.25 Summary of Correlations for CDNR Males

	+ ive Correl	-ive Correl
Age	RBS	-
BMI	Systolic BP Serum leptin Serum VEGF	LDL/HDL ratio
RBS	-	Serum IL-6
FBS	Urinary protein	Serum VEGF
HbA1c	-	-
Cholesterol	Diastolic BP	TC/HDL ratio
HDL	-	-
LDL	TC/HDL ratio LDL.HDL ratio	Systolic BP -
TG	Urinary creatinine	-
TC/HDL ratio	LDL/HDL ratio	-
LDL/HDL ratio	-	Systolic BP
Serum creatinine	-	-
Urinary creatinine	-	-
Urinary protein	Systolic BP	-
Systolic BP	-	-
Diastolic BP	-	-
Serum IL-6	Serum VEGF	-
Serum leptin	Serum VEGF	-
Serum VEGF	-	-

Table 3.26 Summary of Correlations for CDNR Females

	+ ive Correl	-ive Correl
Age	Urinary protein	-
	Serum IL-6	-
	Serum VEGF	-
BMI	LDL/HDL ratio	-
	Urinary protein	
RBS	Urinary protein	-
FBS	-	-
HbA.1c	-	-
Cholesterol	-	-
HDL	Serum creatinine	-
LDL	TC/HDL ratio	-
	LDL/HDL ratio	-
TG	-	-
TC/HDL ratio	LDL/HDL ratio	-
	Diastolic BP	
LDL/HDL ratio	-	Serum VEGF
Serum creatinine	-	Serum VEGF
Urinary creatinine	-	-
Urinary protein	Serum VEGF	-
Systolic BP	Diastolic BP	-
Diastolic BP	-	-
Serum IL-6	-	-
Serum leptin	-	-
Serum VEGF	-	-

Table 3.27 Summary of Correlations for NPDR Males

	+ ive Correl	-ive Correl
Age	TG Serum creatinine Urinary creatinine Systolic BP Diastolic BP	RBS FBS HbA1c Urinary protein
BMI	Cholesterol TC/HDL ratio Urinary protein	RBS FBS HbA1c
RBS	FBS HbA.1c HDL	Cholesterol TC/HDL ratio
FBS	HbA.1c Urinary protein	Serum creatinine Serum creatinine Systolic BP Serum leptin Vitreous VEGF
HbA.1c	HDL	TC/HDL ratio
Cholesterol	LDL TC/HDL LDL/HDL ratio Serum creatinine Urinary creatinine Urinary protein	HDL Serum leptin Vitreous VEGF
HDL	Diastolic BP	TC/HDL ratio LDL/HDL ratio
LDL	LDL/HDL ratio	TG Diastolic BP

Continued……

Summary of Correlations for NPDR Males

	+ ive Correl	-ive Correl
TG	Serum creatinine Urinary creatinine Systolic BP Diastolic BP	LDL/HDL ratio
TC/HDL ratio	LDL/HDL ratio Serum creatinine	Diastolic BP
LDL/HDL ratio	-	Diastolic BP
Serum creatinine	Systolic BP	-
Urinary creatinine	Systolic BP Diastolic BP	-
Urinary protein	-	Systolic BP Vitreous VEGF
Systolic BP	Diastolic BP	-
Diastolic BP	-	-
Serum IL-6	-	-
Serum Leptin	Vitreous VEGF	-
Serum VEGF	Vitreous VEGF	Vitreous leptin
Vitreous IL-6	-	-
Vitreous leptin	-	vitreous VEGF
Vitreous VEGF	-	-

Table 3.28 Summary of Correlations for NPDR Females

	+ ive Correl	-ive Correl
Age	BMI	TG
	HDL	Urinary protein
	Urinary creatinine	Urinary protein
	Diastolic BP	Vitreous leptin
BMI	RBS	HDL
		Serum creatinine
		Systolic BP
RBS	FBS	-
	HbA.1c	
	TG	
	TC/HDL ratio	
	Urinary protein	
FBS	HbA.1c	-
	Cholesterol	
	Urinary protein	
HbA.1c	TC/HDL ratio	-
	LDL/HDL ratio	
Cholesterol	HDL	-
	LDL	
	TC/HDL ratio	
	Urinary protein	
	Daistolic BP	
HDL	Urinary creatinine	TC/HDL ratio
	Diastolic BP	LDL/HDL ratio
LDL	TC/HDL ratio	-
	LDL/HDL ratio	
	Diastolic BP	
TG	Urinary protein	-

Continued…….

Summary of Correlations for NPDR Females

	+ ive Correl	-ive Correl
TC/HDL ratio	LDL/HDL ratio	-
Serum creatinine	Urinary creatinine Systolic BP	-
Urinary creatinine	Urinary protein Systolic BP	-
Systolic BP	Diastolic BP	-
Serum IL-6	-	Vitreous IL-6
Serum leptin	-	-
Serum VEGF	Vitreous VEGF	-
Vitreous IL-6	-	-
Vitreous leptin	-	-
Vitreous VEGF	-	-

Table 3.29 Summary of Correlations for PDR Males

	+ ive Correl	-ive Correl
Age	BMI LDL Systolic BP Diastolic BP	HDL
BMI	-	RBS Urinary Protein
RBS	FBS HbA1c Serum creatinine	Cholesterol
FBS	HbA1c HDL Serum creatinine Urinary Protein	Cholesterol LDL TG TC/HDL ratio LDL/HDL ratio Systolic BP Diastolic BP
HbA1c	HDL Serum creatinine Urinary protein	Cholesterol LDL TC/HDL ratio LDL/HDL ratio Systolic BP Diastolic BP
Cholesterol	LDL TG TC/HDL ratio LDL/HDL ratio Serum creatinine Urinary protein Systolic BP Diastolic BP	HDL

Continued …….

Summary of Correlations for PDR Males

	+ ive Correl	-ive Correl
HDL	Serum creatinine Urinary protein	LDL TG TC/HDL ratio LDL/HDL ratio Systolic BP Diastolic BP
LDL	TG TC/HDL ratio LDL/HDL ratio Systolic BP Diastolic BP	Vitreous leptin
TG	TC/HDL ratio LDL/HDL ratio Systolic BP Diastolic BP	Vitreous leptin
Serum creatinine	Urinary protein	-
Urinary protein	-	-
Systolic BP	Diastolic BP Serum IL-6	Vitreous leptin Vitreous VEGF
Diastolic BP	Vitreous IL-6	Serum leptin Vitreous VEGF

Continued……..

Table 3.30 Summary of Correlations for PDR Females

	+ ive Correl	-ive Correl
Age	HDL LDL	Serum creatinine Urinary protein
BMI	RBS FBS HbA1c Cholesterol TG Urinary creatinine Diastolic BP Serum VEGF	Serum creatinine
RBS	FBS HbA1c TG Urinary protein	-
FBS	HbA1c HDL TG Urinary creatinine Urinary protein	TC/HDL ratio LDL/HDL ratio
HbA1c	HDL TG Urinary protein	Serum creatinine
Cholesterol	LDL TG TC/HDL ratio LDL/HDL ratio Systolic BP Diastolic BP	Serum creatinine Urinary creatinine

Continued……..

Summary of Correlations for PDR Females

	+ ive Correl	-ive Correl
HDL	Urinary protein Systolic BP Diastolic BP Vitreous IL-6 Vitreous VEGF	TC/HDL ratio LDL/HDL Urinary creatinine
LDL	TC/HDL ratio LDL/HDL Serum leptin	Serum creatinine Urinary protein
TG	TC/HDL ratio	Urinary creatinine
TC/HDL ratio	LDL/HDL Urinary protein	Urinary protein Systolic BP Diastolic BP Vitreous IL-6 Vitreous VEGF
Serum creatinine	Urinary protein	Urinary creatinine Systolic BP Diastolic BP
Urinary Creatinine	Vitreous VEGF	Systolic BP
Urinary protein	Systolic BP Diastolic BP	-
Systolic BP	Diastolic BP	-
Serum IL-6	Vitreous leptin	-
Vitreous IL-6	Vitreous VEGF	-

3.9 Serum versus Vitreous Analyses for Specific Factors in NPDR and PDR

3.10.1 Comparison of serum versus vitreous IL-6, leptin and VEGF concentrations in NPDR and PDR male patients

Mean concentrations of vitreous IL-6, leptin and VEGF in NPDR patients showed significant elevations as compared to serum concentrations ($P < 0.001$; $P = 0.002$; $P < 0.001$ respectively) (Fig.3.7A). In PDR patients, vitreous concentrations of IL-6 and VEGF showed several fold significant increase as compared to the serum concentrations ($P < 0.001$; $P < 0.001$). Serum leptin was also elevated ($P < 0.088$) (Fig 3.7A).

3.10.2 Comparison of serum versus vitreous IL-6, leptin and VEGF concentrations in NPDR and PDR female patients

Vitreous IL-6, leptin and VEGF concentrations were significantly elevated in NPDR females than their levels in the serum ($P < 0.001$; $P = 0.002$; $P < 0.003$) (Fig. 3.7B). In the case of PDR females also, the vitreous IL-6 and VEGF showed several fold increased levels as compared to their concentrations in the serum ($P < 0.001$; 0.001), while serum leptin also showed significantly greater concentrations (Fig. 3.7B).

3.10.3 Comparison of serum versus vitreous IL-6, leptin and VEGF concentrations in NPDR and PDR male and female patients combined

To get an estimate on combined population, male and female patients were combined. Results showed significantly elevated concentrations of vitreous IL-6, leptin and VEGF were noticeable in NPDR patients as compared to serum concentrations ($P < 0.001$ for all three) (Fig. 3.7C). In PDR patients, the concentration of vitreous IL-6, leptin and Vitreous VEGF showed significant increase in their levels than the serum ($P < 0.001$; $P < 0.001$; $P < 0.001$) (Fig. 3.7C).

Prevalence or risk distribution in the diabetic population, non-retinopathic and retinopathic without or with proliferation is shown in Table 3.31.

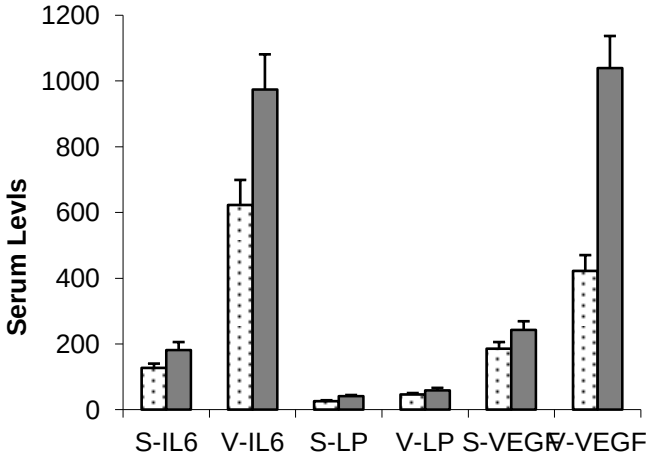


Fig. 3.7A Serum levels for NPDR and PDR males

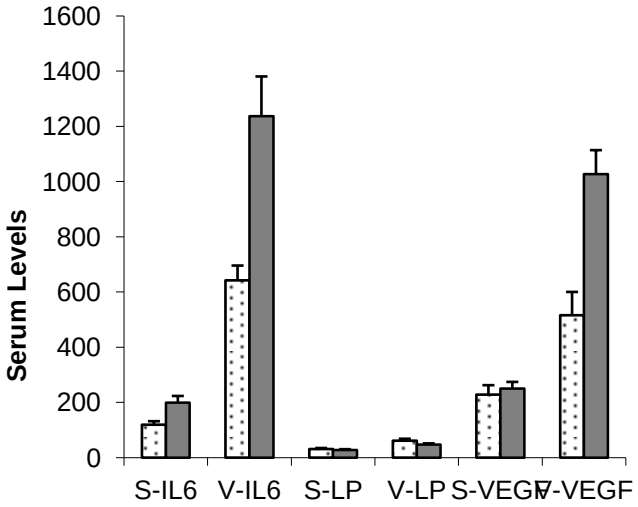


Fig. 3.7B Serum levels for NPDR and PDR females

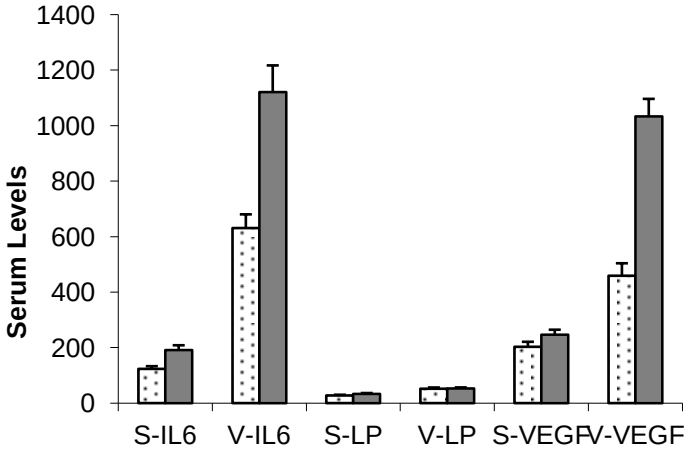


Fig. 3.7C Serum levels for NPDR and PDR total patients

Table 3.31 Prevalence or risk in the diabetic population (diabetics non-retinopathic and retinopathic NPDR and PDR) (*n* =338)

Parameters	Standrad values	Risk Population (%)
Body mass index (BMI)	> 26 kg/m^2	97 %
Plasma Glucose		
Random	> 200 mg/dl	88.46 %
Fasting	>126 mg/dl	87.2 %
Glycated Hemoglobin (HbA1c)	> 7%	66.8 %
Lipid Profile		
Cholesterol	> 200 mg/dl	13.9 %
HDL	< 60 mg/dl	98.5%
LDL	> 100 mg/dl	37.2 %
TG	> 150 mg/dl	68 %
Renal Profile		
Serum Creatinine	> 1.2 mg/dl	37.57 %
Urine Creatinine	> 26 mg/24 hr	25.7 %
Urinary protein	> 225 mg/24 hr	12.7 %
Blood Pressure		
Systolic BP	> 130 mmHg	40.53 %
Diastolic BP	> 90 mmHg	1.18 %
Cytokines and Growth Factors		
In Serum		
IL-6	> 60.0 pg/ml	70.0 %
Leptin	> 14.4 ng/ml	63.9%
VEGF	> 86 pg/ml	54.9 %
In Vitreous		
IL-6	> 100 pg/ml	100%
Leptin	> 25 ng/ml	93.3 %
VEGF	> 138 pg/ml	100 %

DISCUSSION

DISCUSSION

Diabetic retinopathy is a chronic progressive microvascular disorder and is the consequence of a sequential series of pathological changes in the retina because of metabolic disturbances of diabetes (Frank, 2004; Antonetti et al., 2012). The most feared complication is progressive vision loss and blindness. The common findings in diabetic retinopathy that appear on fundus examination are capillary occlusion followed by retinal ischemia and neovascularisation. The earliest phase of diabetic retinopathy is characterized by capillary leakage with capillary microaneurysm formation, dot and blot intraretinal hemorrhages and lipid exudation. Extensive capillary leakage can lead to macular edema (Cheung et al., 2010). In the next stage appearance of cotton wool spots marks the beginning of severe capillary closure with resulting ischemia. Associated morphological abnormalities include venous bleeding and irregular segmental dilatation of retinal capillaries, so called intraretinal microvascular abnormalities or IRMA. The hallmark of proliferative diabetic retinopathy is noevascularization or growth of new fragile blood vessels from the optic nerve head (Neovascularisation at the disc, NVD) or along the retinal venules elsewhere (Neovascularisation Elsewhere, NVE) (Aiello, 2005). The new blood vessels extend through the internal limiting membrane of the retina along the surface of and into the vitreous body producing vitreous hemorrhage leading to extensive fibrous tissue formation resulting in severe retinal distortion and detachment (Wong and Aiello, 2000).

The present study showed the prevalence of retinopathy to be 38% and majority of the cases were females making a total of 61 cases of NPDR in contrast to 47 in males. As regard the PDR the number of females seen were 125 while male cases with PDR numbered 67, and this whole profile shows the number of both NPDR and PDR to be double in females as has shown by many other studies as well (Basit et al., 2005; Hallman et al., 2005). Beside genetic factors, females seem to be a victim of retinopathy and other diabetic complications due to factors like ignorance, gender preferences etc. playing a major role in the alarming rise of blindness in females' especifically of the Frontier Province of Pakistan.

Increase in the BMI appears to be the main contributing factor and reflects the pattern of food intake which mostly constitutes carbohydrates and fats (Hallman et al., 2005; Feldstein et al., 2008). Moreover, it also indicates lack of exercise and stress.

Presently, both fasting random glucose levels were found to be higher in both NPDR and PDR cases with a parallel rise of HbA1c. A similar pattern of high levels of these patterns is seen in studies done in different parts of the world and specifically the neighbouring countries (Krishanamurti and Staffes, 2001; Agrawal et al., 2003; Basit et al., 2005; Manaviat et al., 2008).

High levels of cholesterol is a particularly important risk factor in the progression of retinopathy and visual loss (Haddad and Saad, 1998), however, currently these levels appeared to be in control and did not show significant differences among various groups (Uçgun et al., 2007).

Serum triglyceride levels were raised in NPDR and PDR groups. Serum TG is a very important and independent risk factor for progression of retinopathy (Hadjadj et al., 2004).

Significant elevations in the levels of all conventional parameters account for the marked retinal changes observed currently which in turn most likely led to the leading angiogenic growth factors causing abnormal vessel growth as was seen in the various stages of retinopathy.

The duration of the diabetes in the present study sample of patients was 5-20 years. Intensive treatment of patients can bring the conventional parameters within normal range in the initial stages of diabetes; however, damage done to retina cannot be reversed in long standing history of diabetes, a situation which is evident in the present study (Frank, 2004; Schiffelers et al., 2007).

During the course of present study regular follow ups of the patients were made possible by educating them about the disease. Many patients who presented in the hospitals came with severe retinopathy and a long history of diabetes. They were undergoing photocoagulation for extensive neovascularisation and worsening vision. Follow up of these patients showed a transient halt in the progression of retinopathy but serious side effects as impaired peripheral vision, destruction of

pigment epithelium, and fibrosis of the eye layers were seen (DCCT, 1998; Joussen and Jores, 2007).

Current invasive treatments include laser photocoagulation and anti-VEGF therapy. With anti-VEGF therapy some visual improvement is seen in the retina, however this remains for 3-4 months and repeated Anti-VEGF is advised 3-4 times in a year (Loughery, 2008).

The present study was conducted on diabetic non-retinopathic and diabetic retinopathic (non-proliferative and proliferative) patients. The study was designed to evaluate the serum and vitreous levels of VEGF, IL-6 and Leptin and to correlate these with the conventional clinical parameters. The present is the first study from Pakistan that evaluated the levels of these growth factors and cytokines and simultaneously along with the other very well known predictor parameters of the disease. Different statistical approaches were used to evaluate and analyze the data. Males and females data were analyzed separately and also in combined form to get an overview of the population. The results demonstrated an overall linear correlation of serum VEGF, IL-6 and leptin levels with their levels in the vitreous, and with BMI, fasting and random blood sugar, glycosylated hemoglobin, serum cholesterol, low-density lipoprotein and triglyceride concentrations, blood pressure and with visual acuity. HDL showed inverse correlation with the disease. Most importantly, levels of VEGF, IL-6 and leptin were found significantly elevated in NPDR and PDR patients as compared to diabetic but non-retinopathic patients. The negative correleation of serum and vitreous both with the systeolic and diastolic blood pressure remained unknown. Again reverse correlation of IL-6 with blood pressure could have been because of proliferation of extra retinal blood vessels, but it is not clear why exactly this was so. Further studies are required to address the relationship of blood pressure with cytokines and growth factors.

The involvement of VEGF in neovascularization and worsening of retinopathy is already very well established (Duh and Aiello, 1999; Citirik et al., 2012), however, the involvement of IL-6 as indicated by significantly higher levels in both the vitreous and serum is worth mentioning (Omori et al., 2004; Mocan et al., 2006; Funatsu et al., 2002). Similarly, Mocan et al. (2006) reported significantly elevated intravitreal IL-6 levels in PDR patients (775 ± 177 pg/ml, mean ±SD) compared with control

subjects (93 ± 151 pg/ml, mean ±SD). They could not find any association in intravitreal IL-6 levels with patient age, duration of diabetes or vitreous hemorrhage, hyperglycemia, biochemical indicators of renal function and panretinal photocoagulation.

It is to be noted that all of the patients had the disease duration of 5-20 years and all were receiving medications against diabetes, which included metformin, a biguanide, thiazolidones, glyburide and others in combination or alone according to the disease status. Also, they were taking medicines for hypertension and nephropathic complicacies. It is therefore alarming that the levels of these growth factors and cytokines and hormones have been found elevated.

Once the process of angiogenesis that is initiated in the retina due to the activation of enzyme pathways, continuous release of growth factors and cytokines leading to the progression of newer vessel growth. Early in the onset of diabetes, control of blood sugar with oral hypoglycemic agents is possible; however, these therapies are unable to control the process of vasculogenesis after a certain critical stage is reached (Aiello, 2005). This ultimately leads to worsening retinopathic changes that may culminate in total blindness. Blindness is the most feared complication of diabetes if retinopathy develops. Pathophysiologically, this is due to the severe hypoxia and ischemia that are produced in the retina. These two factors by themselves act as promoters for the synthesis and release of VEGF, IL-6 and leptin, mainly to initiate abnormal blood vessel growth circumvent the hypoxia and ischemia. These fragile vessels may rupture leading to hemorrhagic retina. The viscious cycle of generation of even more blood vessels continues irrespective of therapeutically controlled blood sugar level (Fong et al., 2003; Aiello et al., 2005; Al Maaskari and El Sadig, 2007; Singh et al., 2008). The process of angiogenesis in the retina can be controlled in diabetics, with intensive therapy in the initial NPDR and even late NPDR stages. Not only this, damage to the kidneys and cardiovascular system can also be controlled (Mitamura et al., 2005).

The present study revealed rising VEGF and IL-6 levels in diabetic patients. Higher concentrations were observed in PDR patients both in the vitreous and serum. Current strategies focus on the application of anti-VEGF treatment to prevent angiogenesis. Improvement of vision has been shown in patients who underwent

anti-VEGF treatment (Knudsen, 2007). Sadly, the effect has been reported to be transient, and recurring vision loss occur 3-4 after the anti-VEGF therapy, necessitating repeated application of the anti-VEGF. Interestingly, an equal rise is serum and vitreous IL-6 was observed presently, making IL-6 another very significant marker for diagnosing or understanding at least the status of the disease. Although there are as such no reports on clinical trials of anti-IL-6, application of anti-IL-6 along with anti-VEGF may help in better control of retinopathic changes. Notably, that IL-6 acts as an inducer of the VEGF (Omori et al., 2004). Thus IL-6 levels along with VEGF in the serum may indicate the process of neovascularization and ultimate sever damage to the retina (Funatsu et al., 2002).

For current patients, serum and vitreous leptin levels were also found significantly elevated in both NPDR and PDR patients. Leptin is an adipose tissue derived hormone that informs the brain about appetite and satiety (Hernandez et al., 2004). It stimulates angiogenesis in conditions of oxidative stress; hence play profound role in the development of retinopathic retina (Rahmouni and Haynes, 2005).

Most importantly, the angiographic data presented in the results section clearly demonstrate growth of new blood vessels, hard and soft exudates, excessive hemorrhages, cotton wool spots. These changes in the retina are are all very well established in patients with NPDR or PDR retinopathy, however, the current study indicates that specifically the vitreous and serum levels of VEGF and IL-6 correlate well with changes in the fundus that were observed in these patients. Neovascularization appears to have been due to the increased levels of VEGF.

Since in the present study, although clear cut stronger associations of growth factors with known risk factors like BMI, plasma glucose, HbA1c, cholesterol, TG, LDL, indicators of renal impairment and blood pressure were not observed, even though significant positive correlations were evident. High level expression of IL-6, VEGF and leptin may possibly act as independent risk factors. Although significantly greater concentrations of IL-6, leptin and VEGF were found in the vitreous, significantly elevated concentrations in the serum of NPDR and PDR patients as compared to diabetic non-retinopathic patients and alarmingly high concentrations in the sera of NPDR and PDR patients than normal healthy subjects and non-retinopathic diabetic

patients highlights the diagnostic significance of these factors during routine clinical examination. While it is not the common practice in most hospitals, evaluation of serum samples of patients at the time when these patients present with diabetes related complaints, may prove to be very helpful in indicating the onset of retinopathy to an ophthalmologist or a diabetologist to take and suggest better preventive measures in the benefit of the patients long before the occurrence of retinopathy.

As plethora of past studies indicate; currently also, statistical analysis of the data identified the conventional parameters as BMI, HbA1c, Fasting or postprandial glucose levels, serum triglycerides serum creatinine and blood pressure as independent risk factors for the development of pathologic retina. The data were analyzed separately for male and female patients and then these were combined to get estimates of overall population. Apart from few minor differences no significant differences were found. The disease appears to strike male and female population equally as evidenced by the conventional parameters and growth factors and cytokines. Levels of IL-6, leptin and VEGF were found significantly elevated in both the serum and vitreous fluid irrespective of sex. It is not certain though as to why this was so, it might have been due to genetic or racial difference, especially VEGF in the vitreous, as possible speculation could be stimulation of endothelial lining as result of worsening disease in PDR patients.

Since the current study dealt with a small sample size due to certain limitations as, patients' ignorance, illiteracy, low economic groups, unawareness, non-participating attitude of the general public in such studies, absence of regular follow ups, the data cannot be considered as reflective of huge population of Pakistan. Taking into consideration the importance of cytokines and growth factors and increase of diabetes at an alarming rate, there is a need to conduct large scale studies to gather first hand data if one is to opt for treatments like anti-VEGF or anti-IL-6 application to the retina in order to stop neovascularization and related changes.

This does not however undermine the importance of conventional parameters as first hand diagnostic tools. Regular measurements of BMI, plasma sugar, cholesterol, HbA1c, serum and urine creatinine levels and monitoring of blood pressure along with fundus examination certainly helps in early monitoring of the

disease and its control with oral hypoglycemics and stringent control on glucose concentration.

Presently, none of the patients was receiving insulin; instead they were taking oral hypoglycemics (biguanides, sulphonylureas, thiazolidones, metformin etc in combination or separate according to the condition of the disease. Looking at the data, except the cytokines, most serum parameters fall in almost near normal reference ranges, which apparently, is due to the strict glycemic control with oral hypoglycemics. However, it is pertinent that the IL-6, leptin and VEGF levels show marked elevations despite controlled blood sugar and other parameters. This indicates that conventional diabetes therapies have been unable to stop worsening of retinal changes and blood vessel growth. Fundus examination and angiography indicated severe retinal changes especially in the PDR patients. Lowering lipids as well as diastolic blood pressure in hypertensive patients may be effective in lowering the incidence of retinopathy. In Omani diabetics, factors significantly related to occurrence of retinopathy wer age, duration, ischemic heart disease, hypertension, fasting blood sugar, random blood sugar, elevated serum urea creatinine, cholesterol, and triglycerides. Duration was the only risk factor associated with mild NPR, while increased diastolic blood pressure, increased serum creatinine, cholesterol and triglycerides were significantly associated with the occurrence of proliferative retinopathy (El-Haddad and EL-Saad, 1998). Diastolic blood pressure is related more to the progression of rather than to the occurrence of retinopathy (Klein et al., 1989). It should be noted that high vitreous VEGF levels observed in diabetic patients with PDR were not due to diffusion through the blood retinal barrier, and instead may possibly be contributed by intraocular synthesis (Burgos et al., 1997).

VEGF is an endothelial cell-specific mitogen and angiogenic inducer in vivo. Its expression is upregulated by hypoxia in many retinal cell types ssuch as endothelial cells, retinal pigment epithelial cells, Muller cells, and pericytes (Pe'er et al., 1995). Expression of VEGF is induced by chronic exposure to a high glucose environment. Significantly high intravitreous levels of VEGF reported by Burgos et al., (1997) appear similar to the present study. However Burgos et al. (1997) found no

relationship between serum and vitreous VEGF concentration. VEGF may contribute to blood ocular barrier in a variety of ocular disorders.

Gariano et al. (2000) also found higher levels of leptin in the serum and vitreous samples of patients with diabetes than those without, while vitreous leptin was specifically elevated in patients with PDR or retinal detachment. Leptin is an angiogenic cytokine. In advanced diabetic retinopathy, retinal angiogenesis and growth of fibrotic tissue may result in vitreous hemorrhage and traction retinal detachment, the principal causes of severe vision loss in diabetics.

In the same study by Gariano et al. (2000), BMI was shown to be significantly higher in diabetics than non-diabetics. Serum leptin correlated positively with BMI, its levels were higher in females than males, but there was no relationship with age. Leptin levels were shown to higher in PDR, intermediate in NPDR and lower in DNR patients.

Similarly, Uckaya et al. (2000) also showed higher plasma leptin levels in advanced diabetic retinopathy. Leptin is known to induce promotion of angiogenesis and neovascularization. Funatsu et al. (2002) have reported increased levels of VEGF and IL-6 in aqueous humor of diabetics with macular edema. Also, these levels were significantly higher than plasma VEGF levels and also correlated with IL-6.

Excepting cholesterol, all other parameters viz, BMI, RBS, FBS, HbA1c, LDL, TG, serum creartinine, urine creatinine, urinary protein, systolic and diastolic blood pressures were found significantly elevated in NPDR and PDR patients and irrespective of sex. HDL levels were severly compromised and showed significant decrease in DNR patients as compared to NPDR and PDR patients.

Univariate analyses demonstrated significant independent association of each risk factor with the response variables, the growth factors. Marked hyperglycemia was encountered despite glucose control.

Males had comparatively greater HbA1c and BMI in normal subjects. In DNR patients' serum IL-6 and VEGF were higher in females but leptin levels were higher in males. Analysis of BMI shows that 97% of the diabetic population was obese. 88.46% and 87.2% diabetics had higher levels of random and fasting blood sugar respectively. 66.86% diabetics had > 7% HbA1c levels. However, only 13.9 % diabetics had total cholesterol concentration greater than 200 mg/dl. 98.5%

diabetics had HDL levels < 60 mg/dl. 68% diabetics had serum TG levels > 15 mg/dl. 37.57% diabetics had serum creatinine levels greater than 1.2 mg/dl.

In NPDR male patients, TG, TC/HDL ratio, LDL/HDL ratio were found to be greater, but on the other in female NPDR patients; HDL, urinary protein level and diastolic blood pressure were higher. In PDR patients, BMI, RBS, FBS, TG urinary protein and serum leptin levels were higher, while in females, serum creatinine and urine creatinine were higher. Higher BMI in not only NPDR and PDR but DNR patients indicates trend towards obesity.

The present study showed a linear increase in the levels of serum IL-6, leptin and VEGF in DNR, NPDR and PDR patients. This data also correlated with the ophthalmoscopic observations and fundus examination. PDR patients frequently showed retinal hemorrhage, ischemia, excessive blood vessel growth and a tendency toward blindness. However, in none of the patients visual acuity of both eyes was more than 6/36.

Currently, regression analysis showed weak associations with conventional risk factors and BMI. This could have been due to the reason that all of these patients were taking anti-diabetic oral hypoglycemics which included biguanide metformin, thiazolidones, sulphonylureas singly or in combination according to the severity of diabetes.

Despite therapy, higher levels of cytokines in the serum of NPDR and PDR patients were observed in comparison to diabetic non-retinopathic patients. Furthermore, IL-6 and VEGF levels were found significantly greater in the vitreous fluid obtained from NPDR and PDR patients. In contrast, leptin levels did not differ between the two groups. Although leptin levels show increase in NPDR and PDR patients, as compared to DNR patients, a drastic upregulation was not noticeable.

Similar results were obtained when males and females samples were analyzed separately and when they were combined to get population data. In male versus female comparisons, serum IL-6 and serum VEGF were levels were higher in females in DNR patients, while serum leptin levels were significantly lower in females than the male patients.

In diabetic patients with non proliferative retinopathy, levels of all the three cytokines IL-6, leptin and VEGF were non-significantly different in both the serum

and vitreous samples. In diabetic patients with proliferative retinopathy, levels of IL-6, VEGF in the serum and levels of IL-6, VEGF in vitreous were non-significantly different. However, only serum leptin levels in PDR females were significantly lower as compared to PDR males. Irrespective of the cause of ischemia, sustained overproduction of VEGF by ischemic retinal cells may promote retinal and iris neovascularization (Pe'er et al., 1995).

Presently, higher levels of VEGF encountered in the vitreous of PDR patients, indicate an intraocular synthesis as has been proposed previously (Burgos et al., 1997), since raised VEGF levels cannot be attributed to serum diffusion across the blood-retinal barrier. This appears similar to Burgos et al. (1997) who did not find any relationship between serum parameters and vitreous VEGF concentrations in PDR patients.

Systolic blood pressure is a very well known independent risk factor for diabetes mellitus (Adler et al., 2000; Estacio et al., 2000; Liew et al., 2009). Presently however, only serum IL-6 was found positively correlated with systolic blood pressure, and vitreous IL-6 was found positively correlated with diastolic blood pressure in male PDR patients. In female PDR patients, vitreous IL-6 and VEGF showed positive relationship with serum HDL, and serum leptin with LDL, vitreous VEGF with urinary creatinine, vitreous leptin with serum IL-6 and vitreous VEGF with vitreous IL-6. Thus these cytokines showed associations with some of the known risk factors. A positive association of vitreous VEGF and vitreous IL-6 appears significant from the view point of blood vessel growth in the retina. Notably, the mechanism by which neovascularization is induced is although not clear at present but some studies (Funatsu et al., 2002; Caldwell et al., 2003; Mocan et al., 2006; Crawford et al., 2009) indicate that IL-6 induces the upregulation of VEGF.

In NPDR males and females, a significant positive correlation of vitreous VEGF was obtained with serum VEGF. As to why a similar correlation in PDR was not found is unclear. Strikingly, in non-retinopathic male patients, serum VEGF showed association with serum IL-6 and serum VEGF, but no such associations were found in normal healthy subjects. Thus it can be hypothesized that when the retina gets hypoxic or severely ischemic, the vessel growth occurs due to interplay between IL-6 and VEGF.

Olmos et al. (2009) showed no difference between serum and vitreous leptin concentrations in PDR patients. Further they did not observe any relationship between intravitreous leptin levels and PDR activity. In contrast presently, vitreous leptin concentrations were found significantly elevated in vitreous fluid of both NPDR and PDR patients.

Present results show that the population is obese, having high levels of LDL and TG concentrations and very low HDL concentration. They also indicate sever nephropathic changes due to the elevated creatinine concentration. Taking into consideration the outcome of the present study restriction on carbohydrate intake can only improve the metabolic syndrome of diabetes (Accurso et al., 2008).

Future Directions

Currently application of ranibizumab (Arevalo and Garcia-Amaris; 2009; Rodriguez-Fontal et al., 2009; Elman et al., 2010) and photcoagulation (Akduman and Olk, 1998; Chew et al., 2003) are effective therapies however application of anti-VEGF and anti-IL-6 antibodies may play important roles against progressing retinopathic conditions (Mchiffelers et al., 2007; Andreoli and Miller, 2007; Nicholson and Schachat, 2010; Lacono et al., 2010). Moreover opticin, another extracellular matrix glycoprotein is localized to the vitreous humor. Opticin has been shown to possess anti-angiogeneic properties and inhibits the action of both the VEGF and FGF-2. Possibly via direct binding to the growth factors, it may be provide protection against proliferative diabetic retinopathy (Le Goff et al., 2004; 2005).

CONCLUSION

The present study was conducted on male and female diabetic patients with microvascular complications, only diabetic retinopathy was the focus of the present study. The investigated the the levels of VEGF, IL-6 and leptin in the serum of all diabetic patients (these included retinopathic patients and non-retinopathic patients), while the vitreous levels were possible to determine only in patients having non-proliferative or proliferative retinopathy. The study although was done for the first time in Pakistan with reference to measuring the levels of above growth factors whereby all three were attempted to determine together. However, there were few obvious limitations; the study population was although random but this needs to be done on a large scale. Ethnicity, dietary habits should also be taken into consideration. The levels were determined only one with no follow-ups, this has to be tackeled appropriately in future studies. Only VEGF and IL-6 showed strong positive correlatiuons with retinopathy, wheras leptin levels were least correlated. Additional up-coming factors like opticin and other angiogenic factors are also required to be done thoroughly. Correletions of these specific factors showed interesting results with conventional parameters like glucose levels, HbA1c levels, cholesterol and lipid levels, creatinine, urea and blood pressure. Again there is a need to collect more data but the study nonetheless shows that atleast VEGF and IL-6 are independent risk factors for diabetic retinopathy. IIt would be more appropriate if these factors are determined in the serum of diabetic patients at very early stage to save patients from possible blindness as the disease worsens.

Papers/Abstracts Published out of this Thesis

One full length paper out of this thesis has been accepted for publication in Journal of College of Physicians and Surgeons Pakistan. Impact factor 0.75.

Parveen, N and Qureshi, IZ. (2012). Expression of vascular endothelium growth factor, interleukin 6 and leptin in the serum and vitreous fluid of patients with diabetic retinopathy in type 2 diabetic patients. JCPSP.

APPENDICES

QUAID-I-AZAM UNIVERSITY

Department of Animal Sciences

Laboratory of Human and Animal Physiology

Suject Serial No ☐ Date ☐

Address ☐

Name ☐

Age ☐

Sex: Male ☐ Female ☐

Marital Satus: Married ☐

Unmarried ☐

Widow/Widower ☐

Separated ☐

Occupation: ☐

Educational Status:

Primary ☐

Middle ☐

Matric ☐

Intermediate ☐

Graduate ☐

Any Other ☐

No Education ☐

Socioeconomic Status:

Poor ☐

Satisfactory ☐

Good ☐

Body Weight (kg) ☐

Body Height (m) ☐

Body mass index (kg/m^2) ☐

CLINICAL HISTORY

Type of Diabetes:

Type-I ☐

Type-II ☐

Duration (yrs) ☐

Type of Diabetic Treatment:

Only Diet ☐

Oral Hypoglycemics ☐

Insulin ☐

Combined ☐

History of Smoking: Yes ☐ No ☐

History of Ischemic Heart Disease: Yes ☐ No ☐

History of Hypertension: Yes ☐ No ☐

History of Dyslipidemia: Yes ☐ No ☐

CLINICAL EXAMINATION

Blood Pressure (mmHg): Systolic: ☐ Disstolic: ☐

Visual Acuity: Right Eye: ☐ Left Eye: ☐

Fundoscopy Findings:

MA>Present ☐	Absent ☐		NVD >Present ☐	Absent ☐	
RH >Present ☐	Absent ☐		NVE > Present ☐	Absent ☐	
HE >Present ☐	Absent ☐		ME > Present ☐	Absent ☐	
SE > Present ☐	Absent ☐		VH > Present ☐	Absent ☐	

Diabetic Retnopathy Diagnosed by:

1. Fundoscopy: ☐
2. Fundus Photography: ☐
3: Fluorescein Angiography: ☐

Grade of Retinopathy:

1. No Retinopathy ☐
2. NPDR ☐
3. PDR ☐

Laboratroy Investigations

1. Hemoglobin (g/dl): ☐
2. HbA1c (%). ☐
3. Fassting Blood Glucose (mg/dl): ☐
4. Random Blood Glucose (mg/dl): ☐
5. Serum Cholesterol (mg/dl): ☐
6. Serum Triglycerides (mg/dl): ☐
7. LDL (mg/dl): ☐
8: HDL (mg/dl): ☐
9. Serum Creatinine (mg/dl): ☐
10. 24 HR Urinary protein (mg): ☐

Sepecific Laboratory Investigations

1. Serum VEGF	☐	2. Vitreous VEGF	☐
3. Serum Leptin	☐	4. Vitreous Leptin	☐
5. Serum Interleukin-6	☐	6. Vitreous Interleukin-6	☐

Abbreviations: DM=Diabetes mellitus; MA=microneurysm; RH=Retinal hemorrhage; HE=Hard exudate; SE=Soft exudate; NVD=New vessels on disc; NVE= New vessels elsewhere; ME=Maculaer edema; VH=Vitreous hemorrhage; DR=Diabetic retinopathy; NPDR=Non-proliferative diabetic retinopathy; PDR=Proliferative diabetic retinopathy; HbA1c=Glycosylated hemoglobin; LDL=Low density lipoprotein; HDL=High density lipoprotein; VEGF=Vascular endothelium growth factor

Table 1 **Comparison of BMI, Random and Fasting Blod Sugar and Glycated Hb between male subjects and patients**

	Mean ± SD	**n**	**Median**	**SE**	**Range**	***F*/H value (df)**	***P* Value**
BMI (kg/m^2)							
Normal	26.8± 3.08	17	27.0	0.74	22-35	$H = 42.1_{(3)}$	$P < 0.001$
CDNR	34.6 ± 4.33 *	15	35.0	1.12	27-41		
NPDR	36.3 ± 4.99 *	47	37.0	0.72	28-42		
PDR	37.1 ± 3.41 *	67	39.0	0.41	29-40		
RBS (mg/dl)							
Normal	152.11± 22.06	17	154.0	5.35	113-189	$H = 74.3_{(3)}$	$P < 0.0\ 01$
CDNR	156.53 ± 27.90	15	154.0	8.18	128-245		
NPDR	345.51 ± 79.24 *b	47	355.0	11.55	200-490		
PDR	362.76 ± 90.77 *c	67	370.0	11.09	190-520		
FBS (mg/dl)							
Normal	97.64 ± 2.93	17	98.0	2.93	78-121	$H = 72.5_{(3)}$	$P < 0.001$
CDNR	109.73 ± 4.48	15	104.0	4.48	87-145		
NPDR	214.04± 9.51*b	47	200.0	9.51	130-335		
PDR	223.80 ± 7.59*c	67	240.0	7.59	130-335		
Hb A1c (%)							
Normal	5.65±0.58	17	5.50	0.14	4.7-7.1	$H = 54.5_{(3)}$	$P < 0.001$
CDNR	7.09±0.72	15	6.90	0.18	6.0-8.5		
NPDR	8.06±1.35*	47	7.40	0.19	6.0-10.0		
PDR	8.58±1.45*c	67	8.40	0.17	6.2-12.0		

BMI *P < 0.05 from normal, ns in between; RBS * P < 0.05 with normal, P < 0.05 a with b & c; FBS * P < 0.05 a between 10 & 11, b between 11 & 12; Hb. AIC * P < 0.05, c between 14 & 16 One-Way Kruskal-Wallis ANOVA

Table 2 **Comparison of Total Cholesterol and Serum Lipids between male subjects and patients**

	Mean ± SD	**n**	**Median**	**SE**	**Range** Min -Max	**F/H value (df)**	***P* Value**
Cholesterol (mg/dl)							
Normal	169.94 ±26.33	17	176.0	6.38	115-203	$H = 4.7_{(3)}$	P = 0.19
CDNR	158.86 ±28.92	15	154.0	7.46	127-231		
NPDR	166.59±33.24	47	160.0	4.84	124-274		
PDR	173.94 ±37.22	67	170.0	4.54	120-275		
HDL (mg/dl)							
Normal	53.35± 10.17	17	57.0	2.46	36-67	$H = 42.4_{(3)}$	*P* < 0.001
CDNR	29.46±6.33*	15	28.0	1.63	21-42		
NPDR	33.04±10.54*	47	36.0	1.53	15-54		
PDR	40.95±10.214 *c,b	67	40.0	1.24	20-58		
LDL (mg/dl)							
Normal	75.94± 17.85	17	68.0	4.33	53-112	$H = 18.1_{(3)}$	*P* < 0.001
CDNR	71.33±44.76*a	15	64.0	11.55	34-215		
NPDR	99.59±35.50*	47	100.0	5.17	35-190		
PDR	95.62±35.00*	67	96.0	4.27	39-197		
TG (mg/dl)							
Normal	121.70±48.94	17	145.0	11.87	56-178	$H = 15.2_{(3)}$	*P* < 0.002
CDNR	197.93 ±59.83*	15	189.0	15.45	89-321		
NPDR	184.80 ±84.52*	47	175.0	12.32	79-447		
PDR	205.28±107.92*	67	187.0	13.18	86-446		

Cholesterol ns; HDL * P < 0.05 vs normal, c vs a, b vs a; LDL * P < 0.05 vs normal, a vs b and c; TG * P < 0.05 vs normal

Table 3 Comparison of Total cholesterol, HDL and LDL Ratio between male subjects and patients

	Mean ± SD	**n**	**Median**	**SE**	**Range Min - Max**	***F*/H value(df)**	***P* Value**
TC/HDL ratio							
Normal	3.34± 1.02	17	3.2	0.25	1.98-5.58	$H = 24.3_{(3)}$	$P < 0.001$
CDNR	5.62± 1.57*	15	5.6	0.40	3.71-9.24		
NPDR	5.91± 2.89* a	47	5.0	0.42	2.00-12.00		
PDR	4.62± 2.31* b	67	4.0	0.28	2.00-10.00		
HDL/LDL ratio							
Normal	1.50±0.61	17	1.3	0.14	0.86-3.11	H = 25.5(3)	$P < 0.001$
CDNR	2.42±1.17	15	2.2	0.30	0.81-5.37		
NPDR	3.40±1.72*	47	3.0	0.25	1.00-7.00		
PDR	2.73±1.30*	67	3.0	0.16	1.00-5.00		

TC/HDL ratio * $P < 0.05$ vs normal, a & b vs c; HDL/LDL ratio * $P < 0.05$ vs normal, $P = 0.072$ ns between groups

Table 4 Comparison of Serum and Urine Creatinine and, Urinary Protein values between male subjects and patients

	Mean ± SD	n	Median	SE	Range Min -Max	*F*/H value (df)	*P* Value
Serum Creatinine (mg/dl)							
Normal	0.82±0.41	17	0.7	0.10	0.5-2.3	$H = 23.5_{(3)}$	$P < 0.001$
CDNR	1.34±0.64*	15	1.3	0.16	0.6.2.6		
NPDR	1.321±0.99*	47	1.0	0.14	0.6-4.3		
PDR	1.386±0.76*	67	1.1	0.09	0.7-6.2		
Urine Creatinine (mg/24 hr)							
Normal	24.29 ± 4.31	17	25.0	1.04	25-30	$H = 30.1_{(3)}$	$P < 0.001$
CDNR	37.13 ± 10.21* a	15	34.0	2.63	25-55		
NPDR	38.14 ± 16.27 *	47	34.0	2.37	15-87		
PDR	48.47 ± 17.54 *	67	49.0	2.14	20-87		
Urinary Protein (mg/24 hr)							
Normal	158.29±50.81	17	145.0	12.32	79-256	$H = 54.7_{(3)}$	$P < 0.001$
CDNR	427.20±229.02 *a	15	324.0	59.13	156-900		
NPDR	655.55±609.99 *b	47	567.0	88.97	230-3118		
PDR	1519.32±1791.63*	67	1040.0	218.88	346-7500		

Creatinine * $P < 0.05$ vs normal; Urine Creatinine * $P < 0.002$ vs normal, a vs c, b vs c; Urinary protein * $P < 0.05$ vs normal, a vs b &c, b vs c;

Table 5 Comparison of Systolic and Diastolic Blood pressure between male subjects and patients

	Mean ±SD	n	Median	SE	Range Min -Max	*F*/H value(df)	*P* Value
Systolic BP (mmHg)							
Normal	124.706±5.14	17	125.0	1.24	120-135	$H = 19.0_{(3)}$	$P < 0.001$
CDNR	128.333±6.45	15	130.0	1.66	120-140		
NPDR	135.532±10.38*	47	140.0	1.51	120-150		
PDR	137.910±15.02*c	67	140.0	1.83	110-160		
Diastolic BP (mmHg)							
Normal	84.11±5.073	17	80.0	1.230	80-90	H = 8.35 (3)	$P < 0.05$
CDNR	86.33±4.806*	15	90.0	1.241	80-90		
NPDR	87.87±4.137*	47	90.0	0.603	80-90		
PDR	86.86±4.674	67	90.0	0.571	80-90		

Systolic * P < 0.05 vs normal, c vs a ; Diastolic *P < 0.039 vs Normal

Table 1 Comparison of BMI, Random and Fasting Blood Sugar and Glycated Hb between female subjects and patients

	Mean ±SD	n	Median	SE	Range	*F*/H Value (df)	*P* Value
BMI (kg/m^2)							
Normal	23.36± 4.56	22	25.0	0.97	16-30	$H = 55.2_{(3)}$	$P < 0.001$
CDNR	31.39± 4.83*a	23	34.0	1.00	23-40		
NPDR	35.21± 5.39 *	61	38.0	0.69	26-42		
PDR	35.09± 5.29 *	125	37.0	0.47	23-42		
RBS (mg/dl)							
Normal	166.09±30.10	22	167.5	6.41	121.0-242.0	$H = 103.63_{(3)}$	$P < 0.001$
CDNR	172.04±33.99a	23	165.0	7.08	132.0-234.0		
NPDR	354.21±93.95*	61	350.0	12.03	222.0-535.0		
PDR	329.46±85.56*	125	340.0	7.65	210.0-530.0		
FBS (mg/dl)							
Normal	93.77±14.74	22	95.0	3.14	67-121	$H = 109.3_{(3)}$	$P < 0.001$
CDNR	111.91±12.33a	23	112.0	2.57	89-131		
NPDR	225.44±64.53*b	61	215.0	8.26	110-365		
PDR	197.20±62.91*	125	180.0	5.62	100-380		
HbA1c (%)							
Normal	5.04±0.63	22	5.05	0.13	4.0-6.2	$H = 62.9_{(3)}$	$P < 0.001$
CDNR	7.30±0.75*	23	7.30	0.15	6.1-8.5		
NPDR	7.30±0.75*	61	7.30	0.15	6.1-8.5		
PDR	8.16±1.72*	125	8.40	0.15	5.8-14.1		

BMI * $P < 0.05$ vs normal, $P < 0.05$ a vs b & c; RBS * $P < 0.05$ vs normal, a vs b & c; FBS * $P < 0.05$ vs normal, a vs b & c, b vs c; HB.A1C * $P < 0.05$ vs normal.

Table 2 Comparison of Total Cholesterol and Serum Lipids between female subjects and patients

	Mean ±SD	n	Median	SE	Range	*F*/H Value (df)	*P* Value
Cholesterol (mg/dl)							
Normal	162.50±25.18	22	161.0	5.37	119-210	$H = 0.57_{(3)}$	$P = 0.90$
CDNR	175.91±42.86	23	165.0	8.93	121-257		
NPDR	169.65 ±36.92	61	164.0	4.72	90-288		
PDR	165.37±29.45	125	165.0	2.63	90-221		
HDL (mg/dl)							
Normal	50.22±7.23	22	48.0	1.54	37-64	$H = 43.3_{(3)}$	$P < 0.001$
CDNR	31.21±7.00*a	23	31.0	1.46	16-46		
NPDR	38.88±9.78*	61	35.0	1.25	17-56		
PDR	38.25±9.14*	125	36.0	0.81	18-64		
LDL (mg/dl)							
Normal	67.72±13.40	22	67.0	2.85	46-94	$H = 32.9_{(3)}$	$P < 0.001$
CDNR	70.56±40.54a	23	56.0	8.45	35-210		
NPDR	92.03±21.22*	61	90.0	2.71	49-127		
PDR	89.64±24.70*	125	85.0	2.21	49-155		
TG (mg/dl)							
Normal	112.40±48.99	22	123.0	10.44	34-213	$H = 27.1_{(3)}$	$P < 0.001$
CDNR	197.04±37.81*a	23	189.0	7.88	134-278		
NPDR	158.72±50.33*	61	159.0	6.44	81-290		
PDR	169.09±61.05*	125	159.0	5.46	81-295		

Cholesterol n.s vs normal & between groups; HDL * P < 0.05 vs normal, a vs b & c; LDL * P < 0.05 vs normal, a vs b & c;TG * P < 0.05 vs normal, a vs b & c

Table 3 **Comaprison of Total cholesterol, HDL and LDL Ratio between female subjects and patients**

	Mean ± SD	**n**	**Median**	**SE**	**Range Min -Max**	***F*/H value(df)**	***P* Value**
TC/HDL ratio							
Normal	3.30± 0.75	22	3.2	0.16	2.29-5.68	$H = 31.8_{(3)}$	$P < 0.001$
CDNR	6.06± 2.55*a	23	5.2	0.53	2.98-13.25		
NPDR	4.58± 1.36*	61	4.3	0.17	2.60-9.00		
PDR	4.54± 1.28*	125	4.3	0.11	1.85-7.74		
HDL/LDL ratio							
Normal	1.37± 0.32	22	1.3	0.06	0.84- 2.16	$H = 38.3_{(3)}$	$P < 0.001$
CDNR	2.25± 1.02*	23	1.9	0.21	0.88- 4.57		
NPDR	2.50± 0.75*	61	2.5	0.09	0.88- 3.82		
PDR	2.46± 0.82*	125	2.4	0.07	0.88- 4.31		

TC/HDL ratio * P < 0.05 vs normal, a vs c, P = 0.028 between groups; HDL/LDL ratio * P < 0.05 vs normal, P = 0.19 ns between groups

Table 4 Comparison of Serum and Urine Creatinine and, Urinary Protein values between female subjects and patients

	Mean ±SD	n	Median	SE	Range Min -Max	*F*/H Value (df)	*P* Value
Serum Creatinine (mg/dl)							
Normal	0.95±0.31	22	0.85	0.06	0.6-1.6	$H = 13.7_{(3)}$	$P < 0.001$
CDNR	1.13±0.31	23	1.10	0.06	0.6-1.8		
NPDR	1.52±1.05*	66	1.10	0.13	0.7-4.5		
PDR	1.86±1.47*	125	1.10	0.13	0.7-6.5		
Urine Creatinine (mg/24 hr)							
Normal	17.40±4.49	22	17.5	0.95	11-26	$H = 29.13_{(3)}$	$P < 0.001$
CDNR	49.87±20.94*	23	46.0	4.36	23-98		
NPDR	38.53±21.07*	61	38.0	2.69	10-80		
PDR	41.79±23.91*	125	38.0	2.13	6-93		
Urinary Protein (mg/24 hr)							
Normal	142.95±13.77	22	134.0	13.77	67-320	$H = 73.87_{(3)}$	$P < 0.001$
CDNR	347.30±116.81*a	23	342.0	24.35	212-645		
NPDR	1005.65±584.73*b	61	973.0	74.86	157-2045		
PDR	662.17±486.32*	125	554.0	43.49	105-2375		

Serum creatinine * P < 0.05 vs normal, ns between groups; Urine creatinine * P < 0.05 vs normal; Urinary protein * P < 0.05 vs normal, a vs b, b vs c; Systolic BP * P < 0.05, ns between goups

Table 5 **Comparison of Systolic and Diastolic Blood Pressures between female subjects and patients**

	Mean ±SD	**n**	**Median**	**SE**	**Range Min -Max**	***F*/H Value (df)**	***P* Value**
Systolic (mmHg)							
Normal	126.36 ±4.41	22	127.5	0.94	115-130	H = 8.8 $_{(3)}$	$P < 0.001$
CDNR	130.21±8.32	23	130.0	1.73	120-150		
NPDR	133.11±11.90*	61	130.0	1.52	120-160		
PDR	132.48±10.13*	125	130.0	0.90	110-160		
Diastolic (mmHg)							
Normal	83.86±5.75	22	80.0	1.22	70-90	H = 11.1 $_{(3)}$	$P < 0.05$
CDNR	86.30±4.81	23	90.0	1.00	80-90		
NPDR	85.57±5.33	61	90.0	0.68	70-90		
PDR	87.20±5.40*	125	90.0	0.48	70-90		

Systolic BP * P < 0.05, ns between goups; Diastolic BP * P < 0.05 vs normal, ns between groups

Table 1 Comparison of BMI, Random and Fasting Blood Sugar and Glycated Hb between Subjects and Total Patients

		Mean ± SD	n	Median	SE	Range Min -Max	*F*/H value	*P* Value
BMI (kg/m^2)								
	Normal	24.87 ± 4.30	39	29.0	0.68	16-35	F = 91.62	<0.001
	CDNR	32.68 ± 4.86*a	38	34.0	0.78	23-41		
	NPDR	35.70 ± 5.23*	108	38.0	0.50	26-42		
	PDR	35.80 ± 4.81*	192	37.5	0.34	23-42		
RBS (mg/dl)	Normal	160.0 ± 27.48	39	160.0	4.40	113-242	F = 105.4	<0.001
	CDNR	165.92 ± 32.26 a	38	156.0	5.23	128-245		
	NPDR	350.42 ± 87.57*	108	350.0	8.42	200-535		
	PDR	341.08 ± 88.62*	192	352.5	6.39	190-530		
FBS (mg/dl)	Normal	95.46 ± 13.61	39	95.0	2.18	67-121	F = 73.94	<0.001
	CDNR	111.05 ± 14.34 a	38	112.0	2.32	87-145		
	NPDR	220.48 ± 64.77*	108	200.0	6.23	110-365		
	PDR	206.48 ± 63.77*	192	185.0	4.60	100-380		
Hb.A1c (%)	Normal	5.30 ± 0.67	39	5.3	0.10	4.0-7.1	F = 48.55	<0.001
	CDNR	7.22 ± 0.74*a	38	7.1	0.12	6.0-8.5		
	NPDR	8.16 ± 1.59*	108	7.6	0.15	6.1-12.2		
	PDR	8.31 ± 1.62*	192	8.4	0.11	5.8-14.1		

BMI * P < 0.001 vs normal Tukey's; *pair wise Dunn's, $H_{(2)} = 16.78$, a vs b & c P < 0.05
RBS *P < 0.001 vs normal Tukey's; *pair-wise Dunn's, $H_{(2)} = 94.99$, a vs b & c P < 0.05
FBS *P < 0.001 vs normal Tukey's**; ***pair-wise Dunn's $H_{(2)} = 96.26$, a vs b & c P < 0.05
HB.A1C * P < 0.001 vs normal Tukey's; * pair-wise Dunn's H(2) = 15.512 a vs b & c P < 0.05

Table 2 Comparison of Total Choesletrol and Serum Lipds between subjects and Total Patients

		Mean ± SD	n	Median	SE	Range Min -Max	F/H value	*P* Value
Cholesterol (mg/dl)	Normal	165.74 ± 25.62	39	167.0	4.10	115-210	F = 0.08	0.969
	CDNR	169.18 ± 38.47	38	156.0	6.24	121-257		
	NPDR	168.32 ± 35.24	108	164.0	3.39	90-198		
	PDR	168.36 ± 32.54	192	166.0	2.34	90-275		
HDL (mg/dl)	Normal	51.59 ± 8.65	39	52.0	1.38	36-67	F = 35.48	<0.001
	CDNR	30.52 ± 6.71*a	38	31.0	1.08	16-46		
	NPDR	36.34 ± 10.48*	108	35.0	1.00	15-56		
	PDR	39.19 ± 9.59*	192	39.0	0.69	18-64		
LDL (mg/dl)	Normal	71.30 ± 15.83	39	67.0	2.53	46-112	F = 11.86	<0.001
	CDNR	70.86 ± 41.66	38	61.0	6.75	34-215		
	NPDR	95.32 ± 28.43*	108	95.5	2.73	35-190		
	PDR	91.73 ± 28.76*	192	90.0	2.07	39-197		
TG (mg/dl)	Normal	116.46 ± 48.54	39	123.0	7.77	34-213	F = 10.33	<0.001
	CDNR	197.39 ± 46.95*a	38	189.0	7.61	89-321		
	NPDR	170.04 ± 68.30*	108	175.0	6.57	79-447		
	PDR	181.72 ± 82.12*	192	160.0	5.92	81-446		

Cholesterol ns vs normal and pair-wise
HDL * $P < 0.001$ vs normal Tukey's; pair-wise Dunn's $H = 30.59_{(2)}$ a vs b & c $P < 0.05$
LDL * $P < 0.001$ vs normal Tukey's; pair-wise Dunn's $H = 28.43_{(2)}$ a vs b & c $P < 0.05$
TG * $P < 0.001$ vs normal Tukey's; pair-wise Dunn's $H = 9.74_{(2)}$ a vs b & c $P < 0008$

Table 3 Comparison of Total cholesterol, HDL and LDL ratio between subjects and Total Patients

		Mean ± SD	n	Median	SE	Range Min- Max	*F*/H value	*P* Value
TC/HDL ratio	Normal	3.32 ± 0.87	39	3.22	0.14	1.98-5.68	F = 67.95	<0.001
	CDNR	0.74 ± 0.51*a	38	0.74	0.08	0.17-1.80		
	NPDR	5.13 ± 2.20*	108	5.13	0.21	2.30-12.0		
	PDR	4.62 ± 1.71*	192	4.62	0.12	1.85-10.3		
LDL/HDL ratio	Normal	1.43 ± 0.47	39	1.34	0.07	0.84-3.11	F = 17.27	<0.001
	CDNR	2.32 ± 1.07*a	38	2.06	0.17	0.81-5.38		
	NPDR	2.88 ± 1.36*	108	2.70	0.13	0.87-7.18		
	PDR	2.51 ± 1.00*	192	2.43	0.07	0.78-4.93		

TC/HDL ratio * $P < 0.001$ vs normal Tukey's; pair-wise Dunn's $H = 103.51_{(2)}$ a vs b & c $P < 0.05$
LDL/HDL ratio *$P < 0.001$ vs normal Tukey's; pair-wise Dunn's $H = 8.37_{(2)}$ a vs b $P < 0.015$

Table 4 Comparison of Serum and Urinary Creatinine and, Urinary Protein values bewteen subjects and Total Patients

	Mean (n)	n	Median	SE	Range Min -Max	*F*/H value	*P* Value
Serum Creatinine (mg/dl)							
Normal	0.87 ± 0.28	39	0.70	0.04	0.5-1.6	F = 7.33	<0.001
CDNR	1.21 ± 0.47a	38	1.15	0.07	0.6-2.6		
NPDR	1.43 ± 1.03*	108	1.06	0.09	0.6-4.5		
PDR	1.69 ± 1.29*	192	1.10	0.09	0.7-6.5		
Urine Creatinine (mg/24 hr)							
Normal	20.41 ± 5.56	39	20.0	0.89	11-30	F = 16.56	<0.001
CDNR	44.84 ± 18.44*	38	43.0	2.99	23-98		
NPDR	38.37 ± 19.00*	108	34.8	1.82	10-87		
PDR	44.12 ± 22.09*	192	40.0	1.59	6-93		
Urinary Protein (mg/24 hr)							
Normal	149.64 ± 58.75	39	143.0	9.40	47-320	F = 9.69	<0.001
CDNR	378.84 ± 171.82a	38	333.0	27.87	156-900		
NPDR	842.78 ± 628.90*	108	633.0	60.51	81-3118		
PDR	913.29 ± 1212.57*	193	587.0	87.51	82-7500		

Serum creatinine * P < 0.001 vs normal Tukey's; pair-wise Dunn's H(2) = 5.01 P < 0.081 ns, P < 0.026 a vs c on Tukey's
Urine creatininw * P < 0.001 vs normal Tukey's; pair-wise Dunn's H (2) = 4.84 ns
Urinary protein * P < 0.001 vs normal Tukey's; pair-wise Dunn's H (2) = 17.63 a vs b & c P < 0.001

Table 5 Comparison of Systolic and Diastolic Blood pressures between subjects andTotal Patients

	Mean ± SD (n)	n	Median	SE	Range Min -Max	*F*/H value	*P* Value
Systolic BP (mmHg)							
Normal	125.64 ± 4.75	39	125.0	0.76	115-135	F = 8.44	*P* < 0.001
CDNR	129.47 ± 7.60	38	130.0	1.23	120-150		
NPDR	134.16 ± 11.28*	108	130.0	1.08	120-160		
PDR	134.37 ± 12.30*	192	130.0	0.88	110-160		
Diastolic BP (mmHg)							
Normal	83.97 ±5.40	39	80.0	0.86	70-90	F = 4.06	*P* < 0.007
CDNR	86.31 ±4.74	38	90.0	0.77	80-90		
NPDR	86.57 ±4.96*	108	90.0	0.47	70-90		
PDR	87.08 ±5.15*	192	90.0	0.37	70-95		

Systolic * P < 0.001 vs normal Tukey's ; pair-wise ns on Kruskal-Wallis One Way Analysis of Variance on Ranks
Diastolic *P < 0.007 vs normal Tukey's; pair-wise ns on Kruskal-Wallis One Way Analysis of Variance on Ranks

Table 1 Comparaison of BMI, Random and Fasting sugar, and glycated Hb between male and female subjects (normal)

Parameter	Mean ± SD	n	t-value	df	P-value	95% CI Upper -Lower
BMI (kg/m^2)						
Males	26.82 ± 3.08	17	2.68	37	0.011	0.848 to 6.072
Females	23.36 ± 4.56	22				
RBS (mg/dl)						
Males	152.11 ± 22.06	17	1.60	37	0.11	-31.592 to 3.645
Females	166.09 ± 30.10	22				
FBS (mg/dl)						
Males	97.647 ± 12.08	17	0.87	37	0.385	-5.061 to 12.809
Females	93.773 ± 14.74	22				
Hb.A1c (%)						
Males	5.65 ± 0.58	17	3.09	37	0.004*	0.211 to 1.013
Females	5.04 ± 0.63	22				

* shows significant difference, M represents males; F represents females; n sample size; SD = standard deviation

Table 2 Comparaison of Total cholesterol and Serum lipid between male and female subjects (normal)

Parameter	Mean ± SD	n	t -value	df	P -value	95% CI Upper -Lower
Cholesterol (mg/dl)						
Males	169.94 ± 26.33	17	0.89	37	0.376	-9.368 to 24.250
Females	162.50 ± 25.18	22				
HDL (mg/dl)						
Males	53.35 ±10.17	17	1.12	37	0.269	-2.519 to 8.770
Females	50.22 ±7.23	22				
LDL (mg/dl)						
Males	75.94 ±18	17	1.64	37	0.109	-1.920 to 18.348
Females	67.72 ±23	22				
TG (mg/dl)						
Males	121.70 ± 48.94	17	0.588	37	0.560	-22.745 to 41.338
Females	112.40 ± 48.99	22				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 3 Comparison of Total Cholesterol, HDL and LDL ratio between male and female subjects (normal)

Parameter	Mean ± SD	n	t- value	df	P -value	95% CI Upper Lower
TC/HDL ratio						
Males	3.33 ± 1.02	17	0.12	37	0.900	-0.542 to 0.614
Females	3.30 ± 0.75	22				
HDL/LDL ratio						
Males	1.50 ± 0.61	17	0.84	37	0.405	-0.181 to 0.439
Females	1.37 ± 0.32	22				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 4 Comparison of renal function parameters between male and female subjects (normal)

Parameter	Mean ± SD	n	t- value	df	P- value	95% CI Upper -Lower
Serum creatinine (mg/dl)						
Males	0.77 ± 0.22	17	2.00	37	0.052	-0.360 to 0.001
Females	0.95 ± 0.31	22				
Urine Creatinine (mg/24 hr)						
Males	24.29 ±4.31	17	4.83	37	0.001	3.997 to 9.773
Females	17.40 ±4.49	22				
Urinary protein (mg/24 hr)						
Males	158.29 ± 50.81	17	0.80	37	0.426	-23.283 to 53.962
Females	142.95 ± 64.58	22				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 5 Comparison of Systolic and Diastolic blood pressures between male and female subjects and patients (normal)

Parameter	Mean ± SD	n	t-value	df	P- value	95% CI Upper Lower
Systolic BP (mmHg)						
Males	124.70 ± 5.14	17	1.08	37	0.286	-4.762 to 1.446
Females	126.36 ± 4.41	22				
Diastolic BP (mmHg)						
Males	84.11 ±5.07	17	0.14	37	0.887	-3.327 to 3.835
Females	83.86 ±5.75	22				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 1 **Comparison of BMI, Random and Fasting Sugar and Glycated Hb between male and female subjects and patients (CDNR)**

Parameter	Mean ± SD	n	t -value	df	P -value	95% CI Upper Lower
BMI (kg/m^2)						
Males	34.66 ± 4.33	15	2.12	36	0.041*	0.149 to 6.402
Females	31.39 ± 4.83	23				
RBS (mg/dl)						
Males	156.53 ± 27.90	15	1.47	36	0.150	-36.893 to 5.872
Females	172.04 ± 33.99	23				
FBS (mg/dl)						
Males	109.73 ± 17.38	15	0.453	36	0.653	-11.943 to 7.584
Females	111.91 ± 12.33	23				
Hb.A1c (%)						
Males	7.09 ± 0.72	15	0.390	36	0.390	-0.717 to 0.286
Females	7.30 ± 0.75	23				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 2 Comparison of Total Cholesterol and Serum Lipids between male and female subjects and patients (CDNR)

Parameter	Mean ± SD	n	t- value	df	P- value	95% CI Upper Lower
Cholesterol (mg/dl)						
Males	158.86 ± 28.92	15	1.35	36	0.186	-42.661 to 8.568
Females	175.91 ± 42.86	23				
HDL (mg/dl)						
Males	29.46 ±6.33	15	0.78	36	0.440	-6.293 to 2.792
Females	31.21 ±7.00	23				
LDL (mg/dl)						
Males	71.33 ±44.76	15	0.05	36	0.957	-27.662 to 29.198
Females	70.56 ±40.54	23				
TG (mg/dl)						
Males	197.93 ± 59.83	15	0.05	36	0.955	-31.151 to 32.931
Females	197.04 ± 37.81	23				

* Shows significant difference ; M represents males; F represents females; n sample size; SD = standard deviation

Table 3 Comparison of total cholesterol, HDL and LDL ratio between male and female subjects and patients (CDNR)

Parameter	Mean ± SD	n	t- value	df	P -value	95% CI Upper Lower
TC/HDL ratio						
Males	5.62 ± 1.57	15	0.59	36	0.556	-1.936 to 1.058
Females	6.06 ± 2.55	23				
HDL/LDL ratio						
Males	2.42 ± 1.17	15	0.47	36	0.639	-0.562 to 0.903
Females	2.25 ± 1.02	23				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 4 Comparison of Renal Function parameters between male and female subjects and patients (CDNR)

Parameter	Mean ± SD	n	t -value	df	P- value	95% CI Upper Lower
Serum Creatinine (mg/dl)						
Males	1.34 ± 0.64	15	1.38	36	0.174	-0.0996 to 0.532
Females	1.13 ± 0.31	23				
Urine Creatinine (mg/24 hr)						
Males	37.13 ± 10.21	15	2.18	36	0.036	-24.563 to -0.910
Females	49.87 ± 20.94	23				
Urinary Protein (mg/24 hr)						
Males	427.20 ± 229.02	15	1.42	36	0.164	-34.203 to 193.994
Females	347.30 ± 116.81	23				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 5 Comparison of Systolic and Diastolic Blood Pressures between male and female subjects and patients (CDNR)

Parameter	Mean ± SD	n	t- value	df	P -value	95% CI Upper Lower
Systolic BP (mmHg)						
Males	128.33 ± 6.45	15	0.74	36	0.463	-7.034 to 3.266
Females	130.21±8.32	23				
Diastolic BP (mmHg)						
Males	86.33 ± 4.80	15	0.01	36	0.986	-3.211 to 3.269
Females	86.30 ±4.81	23				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 1 Comparison of BMI, Random and Fasting Sugar and Glycated Hb between male and female subjects and patients (NPDR)

Parameter	Mean ± SD	n	t- value	df	P -value	95% CI Upper Lower
BMI (kg/m^2)						
Males	36.34 ±4.99	47	1.11	106	0.269	-0.883 to 3.138
Females	35.21 ±5.39	61				
RBS (mg/dl)						
Males	345.51 ± 79.24	47	0.51	106	0.611	-42.517 to 25.112
Females	354.21 ± 93.95	61				
FBS (mg/dl)						
Males	214.04 ± 65.20	47	0.90	106	0.367	-36.346 to 13.546
Females	225.44 ± 64.53	61				
Hb.A1c (%)						
Males	8.06 ± 1.35	47	0.54	106	0.591	-0.780 to 0.446
Females	8.22 ± 1.75	61				

* shows significant difference difference M represents males; F represents females; n sample size; SD = standard deviation

Table 2 Comparison of total Cholesterol and serum lipids between male and female subjects and patients (NPDR)

Parameter	Mean ± SD	n	t- value	df	P -value	95% CI Upper Lower
Cholesterol (mg/dl)						
Males	166.59 ± 33.24	47	0.44	106	0.657	-16.672 to 10.552
Females	169.65 ± 36.92	61				
HDL(mg/dl)						
Males	33.04 ±10.54	47	2.97	106	0.004*	-9.736 to -1.949
Females	38.88 ±9.78	61				
LDL (mg/dl)						
Males	99.59 ±35.50	47	1.37	106	0.172	-3.334 to 18.460
Females	92.03 ±21.22	61				
TG (mg/dl)						
Males	184.80 ± 84.52	47	1.99	106	0.048*	0.176 to 51.998
Females	158.72 ± 50.33	61				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 3 Comparison of total cholesterol, HDL and LDL ratio between male and female subjects and patients (NPDR)

Parameter	Mean ± SD	n	t-value	df	P -value	95% CI Upper Lower
TC/HDL ratio						
Males	5.85 ± 2.83	47	3.11	106	0.002*	0.466 to 2.104
Females	4.57 ± 1.36	61				
HDL/LDL ratio						
Males	3.36 ± 1.76	47	3.42	106	0.001*	0.362 to 1.358
Females	2.50 ± 0.75	61				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 4 Comparison of renal function parameters between male and female subjects and patients (NPDR)

Parameter	Mean ± SD	n	t- value	df	P -value	95% CI Upper Lower
Serum Creatinine (mg/dl)						
Males	1.32 ± 0.99	47	1.02	106	0.307	-0.603 to 0.191
Females	1.52 ± 1.05	61				
Urine Creatinine (mg/24 hr)						
Males	38.14 ±16.27	47	1.67	106	0.916	-7.741 to 6.957
Females	38.54 ± 21.00	61				
Urinary Protein (mg/24 hr)						
Males	690.06 ± 609.45	47	2.20	106	0.029*	-504.708 to -27.033
Females	955.93 ± 629.15	61				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 5 Systolic and Diastolic blood Pressures between male and female subjects and patients (NPDR)

Parameter	Mean ± SD	n	t- value	df	P- value	95% CI Upper Lower
Systolic BP (mmHg)						
Males	135.53 ± 10.38	47	1.10	106	0.272	-1.921 to 6.755
Females	133.11 ± 11.90	61				
Diastolic BP (mmHg)						
Males	87.87 ± 4.13	47	2.44	106	0.016*	0.433 to 4.164
Females	85.57 ± 5.33	61				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 1 Comparison of BMI, Random and Fasting sugar and Glycated Hb between male and female subjects and patients (PDR)

Parameter	Mean ± SD	n	t- value	df	P -value	95% CI Upper Lower
BMI (kg/m^2)						
Males	37.11 ±3.41	67	2.82	190	0.005*	0.611 to 3.436
Females	35.09 ±5.29	125				
RBS (mg/dl)						
Males	362.76 ± 90.77	67	2.51	190	0.013*	7.192 to 59.403
Females	329.46 ± 85.56	125				
FBS (mg/dl)						
Males	223.80 ± 62.18	67	2.80	190	0.006*	7.891 to 45.321
Females	197.20 ± 62.91	125				
Hb.A1c (%)						
Males	8.58 ± 1.45	67	1.71	190	0.088	-0.0639 to 0.911
Females	8.16 ± 1.72	125				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 2 Comparison of total Colesterol and serum lipids between male and female subjects and patients (PDR)

Parameter	Mean ± SD	n	t -value	df	P- value	95% CI Upper Lower
Cholesterol (mg/dl)						
Males	173.94 ± 19.1	67	1.74	190	0.082	-1.102 to 18.231
Females	165.37 ±28.6	125				
HDL (mg/dl)						
Males	40.95 ±10.21	67	1.87	190	0.063	-0.148 to 5.546
Females	38.25 ±9.14	125				
LDL (mg/dl)						
Males	95.62 ±35.00	67	1.37	190	0.171	-2.593 to 14.551
Females	89.64 ± 24.70	125				
TG (mg/dl)						
Males	205.28 ± 107.92	67	2.96	190	0.003*	12.147 to 60.228
Females	169.09 ± 61.05	125				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 3 Comparison of total cholesterol, HDL and LDL between male and female subjects and patients (PDR)

Parameter	Mean ± SD	n	t- value	df	P- value	95% CI Upper Lower
TC/HDL ratio						
Males	4.75 ± 2.31	67	0.80	190	0.420	-0.302 to 0.721
Females	4.54 ± 1.28	125				
HDL/LDL ratio						
Males	2.59 ± 1.29	67	0.86	190	0.391	-0.170 to 0.433
Females	2.46 ± 0.82	125				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 4 Comparison of renal function parameters between male and female subjects and patients (PDR)

Parameter	Mean ± SD	n	t -value	df	P- value	95% CI Upper Lower
Serum Creatinine (mg/dl)						
Males	1.38 ± 0.76	67	2.46	190	0.015*	-0.857 to -0.0950
Females	1.86 ± 1.47	125				
Urine Creatinine (mg/24 hr)						
Males	48.47 ±17.54	67	2.01	190	0.045*	0.140 to 13.231
Females	41.79 ±23.91	125				
Urinary Protein (mg/24 hr)						
Males	1468.89 ± 1818.38	67	4.92	190	0.001*	511.466 to 1195.366
Females	615.48 ± 498.68	125				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

Table 5 Systolic and Diastolic blood Pressures between male and female subjects and patients (PDR)

Parameter	Mean ± SD	n	t- value	df	P- value	95% CI Upper Lower
Systolic BP (mmHg)						
Males	137.91 ± 15.02	67	2.97	190	0.003*	1.828 to 9.033
Females	132.48 ± 10.13	125				
Diastolic BP (mmHg)						
Males	86.86 ±4.67	67	0.42	190	0.669	-1.876 to 1.207
Females	87.20 ±5.40	125				

* shows significant difference M represents males; F represents females; n sample size; SD = standard deviation

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