1.1. Introduction:

Diabetic retinopathy may be the most common micro vascular complication of diabetes (Fowler, 2008). It is the most common cause of blindness during reproductive years (Frank RN, 1986). The risk of developing diabetic retinopathy or other microvascular complication of diabetes depends on both duration and these verities of hyperglycemia. Development of diabetic retinopathy in patients with type 2 diabetes was found to be related to both severity of hyperglycemia and presence of hyper tension, and most patients with type 1 diabetes develop evidence of retinopathy within 20 years of diagnosis. Retinopathy may begin to develop as early as 7 years before the diagnosis of diabetes in patients with type2 diabetes (Fowler, 2008).

Diabetic retinopathy is characterized by capillary cell reduction and increased vasopermeability which induce hypoxia and ischemia concluding to increased angiogenesis (Triebel J *et al*, 2011).

Disturbance in antiangiogenic protection mechanisms can accelerate neovascularization and retinopathy. Vasoinhibins are peptides derived from prolactin (PRL), growth hormone and placenta lactogen. Cathepsin-D or matrix met aloproteases proteolytically cleave PRL to 16 KD N-terminal fragments with antiangiogenic actions (Clapp C *et al*, 2008). Theses peptides decrease vasodilatation and angiogenesis (Clapp C *et al*, 2009). Animal studies have demonstrated that Vasoinhibins antagonize vascular endothelial growth factor (VEGF) and reduce NOS activation (Garcia C *et al*, 2008). Prolactin, which is secreted from pituitary gland provides other benefits when produced locally in multiple human tissue such as endothelial cells (Clapp C *et al*, 1998). Prolactin is expressed throughout retina (Clapp C *et al*, 2008). Study reported that PRL level is increased in diabetics and it is lower in patients with retinopathy than in diabetics without retinopathy (Arnold E *et al*, 2010).

Therefore, inflammation seems to be very important in the development of diabetic retinopathy. C-reactive protein (CRP). It is an acute phase protein and is mainly synthesized by the liver or an adipose tissue when microbial invasion or tissue injury occurs (Genest J, 2010).

The measurement of CRP is useful in clinical practice or the diagnosis and treatment of some acute or chronic inflammatory condition (Lim LS, 2010).

1.2. Rationale:

Diabetes mellitus is a medical condition, social and economic problem in Sudan it is fatal unless treated properly.

The global prevalence of diabetes mellitus for all age group was estimated to be 2.8% in the years 2000 and is projected to rise to 4.4% in 2030, corresponding to 366 million people with diabetes (Triebel J et al, 2011).

Increased retinal vasopermeability (RVP) occurs early in diabetes and is crucial for the development of sight-threatening proliferative diabetic retinopathy (DR).

Prolactin has protective role in diabetes and diabetic retinopathy, it is acts on pancreatic β-cell to stimulate proliferation, survival, synthesis and secretion of insulin (Brelje T *et al*, 1993).

Hyper prolactinemia lead to accumulation of vasoinhibins in retina that inhibit increased retinal vasopermeability.

The study conducted by Mooradian et al, 1985 reported that male patients with diabetes mellitus had elevated levels of mean serum prolactin which could not be attributed to diseases or medication known to elevate circulating prolactin levels.

Some studies suggest that C-reactive protein is associated with diabetic retinopathy (Budak, 2013 and Wang, 2010).

This study aimed to estimate serum prolactin level and C-reactive protein in type2 diabetic patients with retinopathy.

1.3. Objectives:

1.3.1. General objectives:

To study the serum prolactin level and C-reactive protein in type 2 diabetic male patients with retinopathy.

1.3.2. Specific objectives:

1- To measure and compare the level of prolactin hormone and C-reactive protein in diabetic male patients with retinopathy and control group.

2- To correlate the level of prolactin hormone with age and duration of diabetes.

2. Literature Review

2.1. Diabetes mellitus:

Diabetes is a family of disorders that is characterized by hyperglycemia. The disorders of diabetes differ in their etiology and symptoms and in the consequences of disease. The American Diabetes Association (ADA) estimates that approximately 70% of the population of America suffers from diabetes. Therefore, it is a serious public health threat and economic burden on health care funds. 1-3 timely, specific the rapeutic intervention may reduce the serious consequences of diabetes. To aid the physician in choosing a appropriate therapy, the laboratory plays role in diagnosis of the disease, identification of the type of the disorder, and assessment of progression of tissue damage (Arneson, 2007).

2.1.1. Classification of diabetes mellitus:

(A) Primary diabetes mellitus:

Which classify to two types:

I-Type 1 DM:

Insulin dependent diabetes mellitus (IDDM) is characterized by loss of beta cells functions which leading to insulin deficiency also can be classified as immune mediated or idiopathic. The majority of type 1 is the immune mediated nature, where beta cell loss is T-cell mediated autoimmune attack (Rother, 2007).

There is no known preventive measure against type 1 which causes approximately 10% of DM in North America and Europe, type 1 can affect children or adult but traditionally termed juvenile diabetes because it represents a majority of the diabetes cases in children (Lawrence *et al*, 2008).

Causes of type 1 DM:

(i) Genetics:

Susceptibility to type 1 diabetes is inherited, but the mode of inheritance is complex and has not been completely defined.it is a multigenic trait, and the major locus is themajor histocompatibility complex on chromosome (Lawrence *et al*, 2008).

Subjects most at risk are those with HLA- types DR3 and DR4 of the major histocompatibility complex (Mayne, 1998).

(ii) Environmental factors:

Environmental factors are thought to be involved in initiating diabetes, for example, viruses, such as: rubella, mumps and coxsackievirus B, have been implicated others

environmental factors that have been suggested include chemicals and cow s milk (Carl *et al*, 2008).

2-Type 2 DM:

Formerly called non-insulin-dependent diabetes mellitus (NIDDM) or adult onset diabetes is a disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency (Wild *et al*, 2004).

Type 2 constitutes the majority of diabetes cases, and represent more chronically in the middle aged and elderly with symptoms developing over months or even longer (Marshall, 2004).

The prevalence of type 2 diabetes increases with increasing age and reaches over 10% in people over the age of 75 years. It has become apparent that some young patients with diabetes are not insulin dependent, while approximately 10% of patients developing diabetes over the age of 25 have Latent Autoimmune Diabetes of Adulthood (LADA) patient with LADA may be misclassified as having type 2 diabetes (Marshall, 2004).

Causes of type 2 DM:

(i) Genetics:

Genetic factor contribute to the development of type 2 diabetes. For example, the concordance rate for type 2 diabetes in identical twins approaches 100%. In addition, type 2 diabetes it is 10 times more likely to occur in obese individual without a diabetic family history. The mode of inheritance however is unknown (Carl *et al*, 2008).

A variety of approaches have identified several genes that are associated with type 2 diabetes. Therefore the gene or genes causing the common forms of type 2 diabetes remain unknown (Carl *et al*, 2008).

(ii) Environmental factors such as diet and exercise are important determinants in the pathogenesis of type 2 diabetes. Although 60% to 80% of those with type 2 diabetes are obese, diabetes develops in fewer than 15% of obese individuals. In contrast, virtually all obese people even those with normal carbohydrate tolerance have hyperinsulinemia and are insulin resistant (Carl *et al*, 2008).

Other factors such as:

- Family history of type 2 diabetes.
- The duration of obesity

- The distribution of fats. (Carl *et al*, 2008)

(B) Secondary DM:0

May be caused by:

- Absolute insulin deficiency due to pancreatic disease (chronic pancreatitis, haemochromatosis, cystic fibrosis).
- Relative insulin deficiency due to excessive growth hormone (acromegaly), glucocorticoid secretion (Cushing syndrome), or increased plasma glucocorticoid concentrations due to administration of steroids.
- Drugs such as thiazide diuretics (Mayne, 1998).

(C) Gestational DM:

Gestational DM is any degree of glucose intolerance with onset or first recognition during pregnancy (Bishop *et al*, 2005).

2.1.2. Pathophysiology of diabetes mellitus:

In both type 1 and type 2 diabetes, the individual will be hyperglycemic which can be severe, glucosuria can also occur after the renal tubular transporter system for glucose becomes saturated. (Bishop *et al*, 2005)

As hepatic glucose overproduction continues, the plasma glucose concentration continues, the plasma glucose concentration reaches a plateau around 300-500 mg/dl (17-28mmol/L) provided output is maintained, glucose excretion will match the overproduction, causing the plateau (Bishop *et al*, 2005).

The individual with type 1 diabetes has a higher tendency to produce ketones. Patient with type 2 diabetes seldom generate ketones, but instead have a greater tendency to develop hyperosmolar nonketotic states (Bishop *et al*, 2005).

The difference in glucagons and insulin concentrations in these two groups appears to be responsible for the generation of ketones through increased B-oxidation. In type 1, there is an absence of insulin with an excess of glucagons. This permits gluconeogenesis and lipolysis to occur (Bishop *et al*, 2005).

In type 2, insulin is present as (at times) hyperinsulinemia, therefore, glucagons is attenuated. Fatty acid oxidation is inhibited in type 2. This causes fatty acids to be incorporated into triglycerides for release as very low-density lipoprotein (Bishop *et al*, 2005).

2.1.3. Diagnosis of diabetes:

Symptoms of hyperglycemia (e.g. polyuria, polydipsia, unexplained weight loss, visual blurring, genital thrush, lethargy) and raised venous glucose detected once. Fasting ≥ 126 mg/dL (7 mmoL/L) or random ≥ 200 mg/dL (11.1 mmoL/L) or oral glucose tolerance test-2h value ≥ 200 mg/dL (11.1 mmoL/L) (Bishop, 2005).

The diagnostic criteria for diabetes mellitus were modified by the expert committee to allow for earlier detection of the disease. According to ADA recommendations, all adults older than age 45 years should have a measurement of fasting blood glucose every 3 years unless the individual is otherwise diagnosed with diabetes. The criteria suggested three methods of diagnosis, each of which must be confirmed on a subsequent day by any one of the three methods. These methods are 1) symptoms of diabetes plus a random plasma glucose level of ≥ 200 mg/dL (11.1 mmoL/L),2) a fasting plasma glucose of ≥ 126 mg/dL (7 mmoL/L), or 3) an oral glucose tolerance test (OGTT) with a 2 hour post-load (75-g glucose load) level ≥ 200 mg/dL (11.1 mmoL/L). The preferred test for diagnosing diabetes is the measurement of fasting plasma glucose level (Longmore, 2007).

2.1.4. Complications of DM:

(A) Acute metabolic complications:

Patients with diabetes mellitus may develop one of several metabolic complications. These include:

- Diabetic ketoacidosis.
- Hyperosmolar non ketotic coma (Mayne, 1998).

(B) Late complications:

Vascular disease is a common complication of DM:

1. Macrovascular disease:

Is due to abnormalities of large vessels, may present as coronary artery, cerebrovascular or peripheral vascular insufficiency. The condition is probably related to alterations in lipid metabolism (Mayne, 1998).

2. Microvascular disease:

Is due to abnormalities of small blood vessels, particularly affects the retina and the kidney, the incidence of both may be related to inadequate glucose control (Mayne, 1998).

(i) Retinopathy: may lead to blindness because of vitreous haemorrhage from proliferating retinal vessels, and maculopathy as a result of exudates from vessels or oedema affecting the macula (Allan *et al,* 2004).

(ii) Nephropathy: leads ultimately to renal failure. In the early stage there is kidney hyperfunction, associated with an increased glomerular filtration rate (GFR), increased glomerular size and microalbuminuria.

In the late stage there is increasing proteinuria and a marked decline in renal function, resulting in ureamia (Allan *et al*, 2004).

(iii) Neuropathy may become evident as diarrhea, postural hypotension, impotence, neurogenic bladder and neuropathic food ulcers due to microangiopathy of nerve blood vessels and abnormal glucose metabolism in nerve cells (Allan *et al*, 2004).

2.2. Prolactin:

Prolactin is a polypeptide hormone that is synthesized in and secreted from specialized cells of the anterior pituitary gland, the lacto trophs. The hormone was given its name based on the fact that an extract of bovine pituitary gland would cause growth of the crop sac and stimulate the elaboration of crop milk in pigeons or promote lactation in rabbits (Riddle, *et al* 1993). However, we now appreciate that prolactin has over 300 separate biological activities (bole-feysot C *et al*, 1998).prolactin is also a highly versatile hormone whose functions are related to reproduction, growth and development, metabolism, immune regulation, brain function, and (Freeman ME *et al*, 2000 and Ben-Jonathan N *et al*, 2006) PRL plays an important role in milk production in the mammary glands of lactating females. It binds to intra celluler protiens (Genuth, 1998).The phosphorylated protein's produce the response in the cell. Prolactin can also increase the number of receptor molecules for FSH and LH in the ovaries (up regulation), and it they for has a permissive effect for FSH and LH on the ovary. Prolactin also can enhance progestron secretion of the ovary after ovulation. Several hypothalamic neuro hormones can be involved in the complex regulation of prolactin secretion. One neuro hormone is prolactin –Releasing Hormone (PRH) and another is prolactin Inhibiting Hormone (PIH) (Ganong WF, 2001).

2.3. Diabetic Retinopathy

Among the risk factors in diabetic retinopathy is chronic hyperglycemia which, by producing reactive oxygen species, activates multiple biochemical pathways that lead

to retinal micro vascular dysfunction (Brownlee M, 2001). An early event of vascular damage is the loss of pericytes and endothelial cells resulting in acellular and ischemic capillaries. Reduced perfusion increases vaso permeability and the accumulation of extracellular fluid and hard exudates that impair vision when the macula is affected. Over time, intra retinal hemorrhages and capillary occlusion create areas of ischemia, and the resulting hypoxia upregulates the production of proangiogenic factors, such as vascular endothelial growth factor (VEGF). In the more severe stages, the new blood vessels invade and bleed into the vitreous, producing a fibrovascular tissue that may result in retinal detachment and blindness (for reviews on the pathogenesis of diabetic retinopathy, the reader is referred to references (Cheung N *et al*, 2010 and Hammes H *et al*, 2008). Among the current treatments for diabetic retinopathy, laser photocoagulation remains the most effective for preventing visual loss. However, despite of its efficacy, the destructive nature of the laser damages neural tissue and has other significant side effects (Cheung N *et al*, 2010).

In contrast to the tremendous efforts concentrated on investigating the effect of factors thought to be specific for vascular function, the role of ‘‘broadly acting agents’’ such as hormones in diabetic retinopathy remains obscure (Clap C *et al*, 2009).

2.4. PRL and vasoinhibin actions against Diabetic retinopathy:

PRL, the hormone fundamental for lactation, is known to exert a wide variety of actions in reproduction, osmoregulation, immune response, brain function, behavior, energy metabolism, and angiogenesis (Clap C *et al*, 2009). (Freeman M *et al*, 2000). Among these effects, the last two could have protective value against diabetes and diabetic retinopathy. PRL acts on pancreatic β-cells to stimulate proliferation, survival, synthesis and secretion of insulin (B-relje T *et al*, 1993). These effects occur at least in part through the classic janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathway that leads to the activation of cyclin D2 (Friedrichsen B *et al*, 2003) and theup regulation of glucose transporter 2, gluco kinase (Petryk A *et al*, 2000) (Weinhaus A *et al*, 2007), and pyruvate dehydrogenase activities (Arumugam *et al*, 2010). PRL also triggers β-cell expansion by up regulating the expression of tryptophan hydroxylase-1, which increases serotonin levels (Kim *et al*, 2010).These actions on β-cells are essential for maintaining glucose homeostasis during pregnancy (Huang *et al*, 2009), when the PRL receptor serves

both PRL and placental lacto gens, but they are not restricted to pregnancy, as both male and female PRL receptor null mice have reduced islet density, β-cell mass, and insulin synthesis and are glucose intolerant (Freemark *et al*, 2002). Of relevance, by increasing islet number and reducing mononuclear cell infiltration, PRL is able to protect against the development of hyperglycemia in diabetic rats (Holstad M and Sandler S, 1999). Although protecting β-cells from dysfunction could lower diabetes progression and the appear-ance of long-term complications such as retinopathy, there is another potent mechanism by which PRL could prevent progression and promote regression of diabetic retinopathy, namely by its proteolytic conversion to vasoinhibins. PRL is cleaved by cathepsin D, matrix met alo proteases (MMP), and bone morphogenetic protein-1 to yield vasoinhibins (Clapp C *et al*, 2009). Cathepsin D appears to be the primary protease cleaving PRL in the pituitary gland during the secretory process (Cruz-Soto M, 2009), whereas MMP generate vasoinhibins in the extracellular space and at the target tissue level (Macotela Y *et al*, 2006). The vascular vascular effects and signaling mechanisms of vasoinhibins have been recently reviewed (Clap C *et al*, 2009). The following summarizes some of the relevant findings. Vasoinhibins act directly on endothelial cells to inhibit vasopermeability, vasodilation, and angiogenesis induced by several vasoactive substances, including: VEGF, basic fibroblast growth factor (b FGF), interleukin 1-b, bradykinin, and acetylcholine. Also, vasoinhibins promote the apoptosis-mediated regression of blood vessels. Vasoinhibins signal through a still-unidentified receptor distinct from the PRL receptor to block activation of the Ras-Raf-MAPK pathway, theRas-Tiam1-Rac1-Pak1 pathway, and the Ca21/calmodulin-activation of endothelial nitric oxide synthase (eNOS). They also promote protein phosphatase 2A-induced dephosphorylation/inactivation of eNOS, the activation of pro apoptotic proteins of the Bcl-2 family, and the NFjB-mediated activation of caspases. Vasoinhibins decrease angiogenesis in the chick embryo chorioallantoic membrane, in coronary vessels, and in several tumor models. Vasoinhibins are potent inhibitors of blood vessels in the eye. The local administration of vasoinhibins reduces the stimulation of corneal angiogenesis induced by b FGF (Duenas Z *et al*, 1999), and gene transfer of vasoinhibins via an adenoviral vector inhibits ischemia-induced retinal angiogenesis (Pan H *et al*, 2004). Furthermore, vasoinhibins block increased retinal vasopermeability in diabetic rats and in response to intra vitreous injection of VEGF

or of vitreous from patients with diabetic retinopathy (Garcia C, *et al* 2008).Besides having therapeutic potential for controlling blood vessel dysfunction, vasoinhibins have emerged as endogenous inhibitors of ocular blood vessels. PRL and vasoinhibins are present in the retina (Aranda J, *et al* 2005) and may derive from the local synthesis synthesis of PRL by different glial and neuronal cell types (Rivera J.C *et al*, 2008). In addition, retinal PRL and vasoinhibins may also originate from systemic PRL. Radioactive PRL injected intracardially is incorporated in to the retina, the choroids, and the ciliary body (O'steen, W.K and Sundberg D.K,1982).Also, hyperprolactinemia leads to the accumulation of vasoinhibins in the retina, and lowering PRL levels with the dopamineD2 receptor agonist bromocriptine, an inhibitor of pituitary PRL secretion, blocks this effect (Amold E *et al*, 2010). In this regard, the ciliary body, which is responsible for the transport of plasma proteins to ocular fluids (Mesteriner A.C and Haddad A, 1997), expresses the PRL receptor, and the genetic deletion of the PRL receptor prevents the hyperprolactinemia-induced accumulation of retinal vasoinhibins (Amold E *et al*, 2010). Therefore, it is concluded that the PRL receptor mediates the incorporation into the eye of systemic PRL, which can then be cleaved to vasoinhibins. In support of a functional role for endogenous vasoinhibins, antibodies sequestering these peptides stimulate angiogenesis in the cornea (Duenas Z *et al*, 1999) and in the retina (Aranda J *et al*, 2005), and the intraocular transfection of small interfering RNA to block thelocal expression of PRL stimulates retinal angiogenesis and vasodilation (Aranda J *et al*, 2005). Moreover, immunodepletion of vasoinhibins in neonatal rats reduces the apoptosis of the hyaloid vasculature suggesting that vasoinhibins stimulate the physiological regression of intraocular blood vessels after birth (Duenas Z *et al*, 2004).These findings implicate vasoinhibins as major inhibitors of ocular blood vessels and raise the possibility that altering their levels could influence the progression of diabetic retinopathy.

2.5. C-reactive protein :(CRP)

C-reactive protein is an acute phase reactant that has long been considered a classic marker for inflammation. Al though normally circulating at low level, acute inflammation, infection, or tissue injury induces. A marked increase in hepatic synthesis of CRP, which can raise the serum level a hundred fold or more (Ross R, 1999).

C-reactive protein was first discovered by William S. Tillett and Thomas Francis at the Rockefeller Institute for medical Research in 1930, while studing serum in the immune response of pneumonia. They tested "Fraction C"with a soluble extract Known as c- poly saccharide, for a possible relationship response to strep to coccus pneumonia. A positive result occured between "Fraction C" and C-poly saccharide while the patients were ill put immediately dis appeared once the pneumonia had been resolved. The term acute- phase response is used to describe the greatly increased synthesis and secretion of certain plasma proteins including CRP, principally by the liver in response to the cytokine interleukin-6.C-reactive protein (CRP) is a serum marker for inflammation. The function of CRP is felt to be related to its role in the innate immune system. Similar to immune globin IgG, it activates complement, binds to Fc receptors and acts as an opsonin for various pathogens. Interaction of CRP with Fc receptors leads to the response. Unlike IgG, which specifically recognizes distinct antigenic epitopes, CRP recognizes altered self and foreign molecules based on pattern recognition. Thus CRP is thought to act as surveillance molecule defense and leads to a pro inflammatory signal and activation of the humoral, adaptive immune system (pepys and Hirs chfield, 2003).

2.5.1. Genetic and biochemistry:

The CRP gene is located on the first chromosome (Iq21-q23).CRP is a224 residue protein with a monomer molar mass of 25106 Da. The protein is an annular pentameric disc in Shape. Proteins with this type of configuration are known as pentraxins. Native CRP is a bit different as it has10- subunits making two pentameric discs, with an over all molecular mass of 25 106 Dalton (pepys and Hirs chfield, 2003).

2.5.2. Conditions Causing elevation of C- reactive protein:

There are many conditions can increase the level of CRP which include: Bacterial infections (i.e. meningitis),hyper sensitivity, inflammatory diseases (i.e. rheumatoid arthritis), renal transplantation, cancer (i.e. lymphoma and Sarcoma and Necrosis (i.e. myocardial infraction).In some inflammatory disease like: systemic lupus erythematous, dermatomyositis sclerosis, ulcerative colitis and sjogren's syndrome, there is no elevation of CRP level (Ridker *et al*, 1997).

2.5.3 CRP and diabetic retinopathy:

The role of CRP in the pathogenesis of diabetic retinopathy is still unknown. Some studies suggest that CRP level is associated with diabetic retinopathy and with the severity of the disease (Budak, 2013 and Wang, 2010).

3. Material and Methods

3.1. Materials:

3.1.1. Study approach:

A quantitative method was used to measure the level of serum prolactin hormone and C-reactive protein in type2 diabetic Sudanese male patients with diabetic retinopathy during the period from April to May 2016.

3.1.2. Study design:

This is case control study.

3.1.3. Study area:

The study was conducted in Jabber Abo Aleiz Hospital.

3.1.4. Study population:

The study included male patients with type 2 diabetes mellitus.

3.1.5. Sample size:

This study included 50 diabetic patients with retinopathy (cases) and 25 diabetic patients without retinopathy (control).

Inclusion Criteria:

Sudanese male patients type 2 diabetes mellitus with diabetic retinopathy and diabetic patients without retinopathy were included in this study.

Exclusion Criteria:

This study excluded female patients, prolactinoma, hypothyroidism, chronic renal failure, liver cirrhosis and treatment with drugs that increase prolactin level and acute inflammatory disease.

3.1.6. Ethical consideration:

Consent was taken regarding acceptance to participate in the study and reassurance of confidentiality. Before the specimen was collected, the donors knew that this specimen was collected for research purpose.

3.1.7. Data Collection:

Data were collected using a structural interviewing questionnaire, which was designed to collect and maintain all valuable information concerning each case examined.

3.1.8. Sample collection and processing:

About 2.5 ml of venous blood were collected by safe aseptic procedures. In serum sample, blood should be allowed to clot at room temperature. The sample should be centrifuged, and the serum separated.

Serum sample should be stored frozen below-20^{o}C. Samples should be thawed and mixed before assay.

3.2. Methods:

3.2.1. Estimation of prolactin level using Immuno Enzymometric Assayusing ELISA method: (Appendix II)

3.2.1.1. Principle of method: immunoenzymetric assay:

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme labeled and immobilized). With different and distinct epitope recognition, In excess, and native antigen. In this procedure, the immobilization takes place during the assay of the surface of a microplate well through and exogenously added biotinylated monocloned anti-PRL antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme labeled antibody and a serum containing the native antigen results between the native antigen and the antibodies, without competition or steric hindrance, to from a soluble sandwich complex.

3.2.2. Estimation of C-reactive protein using qualitative method: (Appendix III)

Principle of Method:

The CRP latex test is a rapid slide agglutination test for the qualitative and semi-quantitative detection of C-reactive protein in serum. The reagent containing particles coated with specific anti-human C-reactive protein antibodies, agglutinates in the presence of CRP in the patients serum.

3.3. Quality Control:

The precision and accuracy of all methods used in this study were checked by commercially prepared control sample before it is application for the measurement of test and control samples.

3.4. Statistical analysis:

Data obtained from this study was analyzed using statistical package for the social science (SPSS).

4. Results

The result of the biochemical determinant serum of prolactin and C-reactive protein in diabetic patients with retinopathy (cases) and diabetic patients without retinopathy (control) are given in tables and figures:

Table (4-1): Represent the C-reactive protein frequency in both of the study groups.

Show that (36%) of diabetic patients with retinopathy have positive (+ve) results of C-reactive protein and 64% have negative results of C-reactive protein.

Table (4-2): Represent the mean of level of serum prolactin hormone in both of the study groups.

The level of prolactin was significantly decreased in diabetic patients with retinopathy compared to control group.

Mean (± SD cases versus control:

(3.156 ± 1.352 versus 4.979 ± 2.438).

Figure (4-1): show correlation between prolactin level and duration of diabetic patients with retinopathy.

The scatter showed that no correlation between prolactin level and duration of diabetic patients with retinopathy (r=0.034, p-value=0.774).

Figure (4-2): show correlation between prolactin level and age of diabetic patients with retinopathy.

The scatter showed that no correlation between prolactin level and age of diabetic patients with retinopathy (r=0.020, p-value=0.865).

Table (4.1) Qualitative method of the C-reactive protein in diabetic patients with retinopathy group and control group:

Variable	C-reactive protein		Total
	+ve	-ve	
Case	18(36%)	32(64%)	50
Control	2(8%)	23(92%)	25
Total	20	55	75

Table (4.2) Comparison of prolactin level in diabetic patients with retinopathy group and control group:

Variable	Case Mean ± SD	Control Mean ± SD	p-value
Prolactin(ng/ml)	3.156±1.352	4.979±2.438	0.000

. Results given in mean ± SD .

. P-value ≤ 0.05 consider significant.

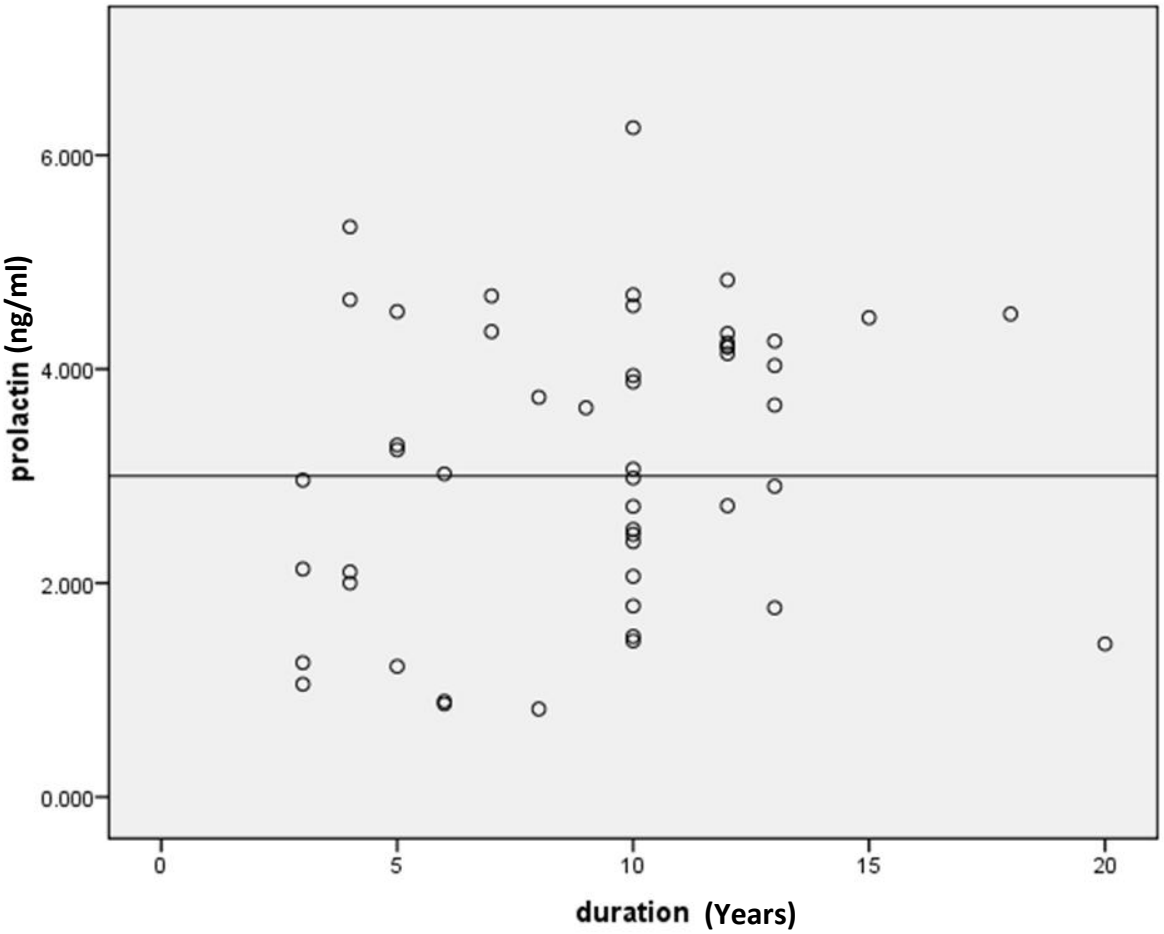

Figure (4.1) Correlation between prolactin level and duration of diabetic patients with retinopathy (r=0.034, P-value = 0.774).

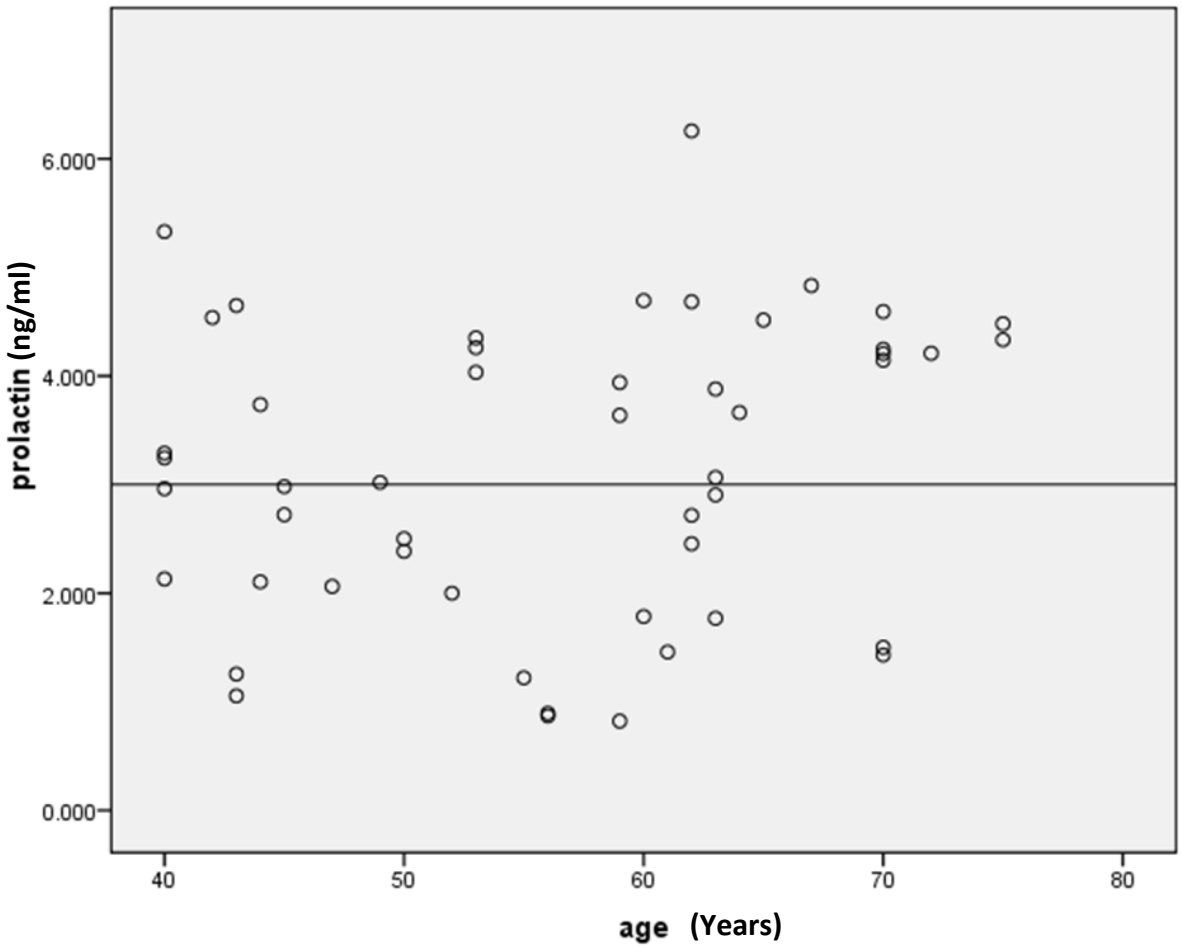

Figure (4.2) Correlation between prolactin level and age of diabetic patients with retinopathy (r=0.020, P-value = 0.865).

5.1 Discussion

Diabetic retinopathy (DR), a very common complication of diabetes mellitus, is the leading cause of visual deficits and blindness around the world (Yau *et al*, 2012).

High levels of serum prolactin protect against diabetic retinopathy by increasing ocular vasoinhibins (Arnold E *et al*, 2010). That inhibits increased retinal vasopermability which occur early in diabetes and lead to development of diabetic retinopathy.

C-reactive protein (CRP) is an inflammatory bio marker associated with diabetic retinopathy (DR). it is secreted from the liver when microbial invasion or tissue injury occurs (Genest J, 2010).

This study conducted to estimate prolactin level and C-reactive protein in diabetic patients with retinopathy.

Preliminary investigated and findings obtained from specially designed questionnaire revealed that (36%) of diabetic patients with retinopathy have (+ve) results of C-reactive protein and 64% have negative results of C-reactive protein. This result agreed with another result carried by (Song J *et al*, 2015) which showed, (40%) of cases have +ve result of C-reactive protein.

From the findings of this study it appears that serum level of prolactin was significantly decreased at (P-value= 0.000) in the Sudanese diabetic patients with retinopathy versus control subjects (3.156 ± 1.352 versus 4.979 ± 2.438). This result agreed with another result of study carried by (Arnold E *et al*, 2010), showed a significantly decreased in prolactin level in patients with retinopathy group when compared to control group.

Also the findings of this study showed, there were no correlation between duration, age of diabetic patients with retinopathy and concentration of prolactin ($r = 0.034$, P-value = 0.774), ($r = 0.020$, P-value = 0.865) respectively as appeared in figure (4-1) (4-2).

This result agreed with another result of study carried by (Arnold E *et al*, 2010), showed there were no correlation between duration, age of diabetic patients with retinopathy and concentration of prolactin.

5.2 Conclusions:

According to the results of this study it is concluded that:

1- Serum prolactin level significantly decreased in diabetic patients with retinopathy compared to control group.

2- The results from this study indicate that the C-reactive protein might be used as an inflammatory biomarker of diabetic retinopathy.

3- No correlation between age, duration of diabetic patients with retinopathy and prolactin concentration.

5.3 Recommendations:

From the findings of this study it is recommended that:

1. Further studies should be used quantitative methods to measure and compare C-reactive protein level in both group (diabetic patients with retinopathy and diabetic patients without retinopathy)
2. Further study to estimate vasoinhibins may be needed.

References

Allan G, Michael J, Robert A, Denis ST, Michael J and James S. (2004). Clinical biochemistry. 3th ed; 59.

Aranda J, Rivera JC, Jeziorski MC, Riesgo-Escovar J, Nava G, López-Barrera F, Quiróz-Mercado H, Berger P, Martínez de la Escalera G and Clapp C. (2005). Prolactins are natural inhibitors of angiogenesis in the retina. Invest Ophthalmol;46:2947–295

Arneson W, Brickell J. (2007). Clinical Chemistry a Laboratory perspective, laboratory of Congress Cataloging in Publication Data.

Arnold E, Rivera JCm, Thebault S, Moreno-Paramo D and Quiroz-Mercado H. (2010). High level serum prolactin protect against diabetic retinopathy by increasing ocular vasoinhibins. Diabetes;59:3192-3197.

Arumugam R, Horowitz E, Noland RC, Lu D, Fleenor D and Freemark M. (2010). Regulation of islet beta-cell pyruvate metabolism: interactions of prolactin, glucose, and dexamethasone. Endocrinology;151:3074–3083.

Ben-Jonathan N, Hugo ER, Brandebourg TD and LaPensee CR. (2006). Focus on prolactin as a metabolic hormone. Trends Endocrinol Metab;17:110–116

Bishop MS and Edward P. (2005). Clinical chemistry principles, procedures, correlation. 5th ed. Lippincott;267-286.

Bole-Feysot C, Goffin V, Edery M, Binart N and Kelly PA. (1998). Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocr Rev;19:225–268.

Brelje T C, Scharp D W, Lacy P E, Ogren L and Talamantes F. (1993). Effect of homologous placental lactogens, prolactins, and growth hormones on islet β-cell division and insulin secretion in rat, mouse, and human islets: implication for placental lactogen regulation of islet functionduring pregnancy. Endocrinology;132:879–887.

Brownlee M. (2001). Biochemistry and molecular cell biology of diabetic complications. *Nature*;414:813-820.

Budak YU, Akdogan M and Huysal K. (2013). Relationship of PON1 activity and hsCRP concentration with disease status in patients with type 2 diabetes

mellitus with and without retinopathy. International Journal of Diabetes in Developing Countries;33:40–45.

Carl A and David E. (2008). Fundamentals of clinical chemistry. 6 th ed saunders ; 381-382.

Cheung N, Mitchell P and Wong TY. (2010). Diabetic retinopathy. Lancet; 376:124–136.

Clapp C, Lopez-Gomez FJ, Nava G, Corbacho A and Torner L. (1998). Expression of prolactin mRNA and of prolactin-like proteins in endothelial cells: evidence for autocrine effects. J Endocrinol;158: 137-144.

Clapp C, Thebault S, Arnold E, Garcia C and Rivera JC. (2008) Vasoinhibins: novel inhibitors of ocular angiogenesis. Am J Physiol Endocrinol Metab;295:772-778.

Clapp C, Thebault S, Jeziorski MC, Martínez De La and Escalera G. (2009). Peptide hormone regulation of angiogenesis. Physiol Rev;89:1177–1215

Cruz-Soto M E, Cosio G, Jeziorski M C, Vargas-Barroso V and Aguilar M B. (2009). Cathepsin D is the primary protease for the generation of adenohypophyseal vasoinhibins: cleavage occurs within the prolactin secretory granules. Endocrinology;150:5446–5454.

Duenas Z, Rivera J C, Quiroz-Mercado H, Aranda J and Macotela Y. (2004). Prolactin in eyes of patients with retinopathy of prematurity: implications for vascular regression. Invest. Ophthalmol;45:2049–2055.

Duenas Z, Torner L, Corbacho A M, Ochoa A and Gutierrez-Ospina G. (1999). Inhibition of rat corneal angiogenesis by 16-kDa prolactin and by endogenous prolactin-like molecules. Invest. Ophthalmol;40:2498–2505.

Fowler MJ, (2008). Clinical diabetes;26:77-81.

Frank RN. (1986). Diabetic retinopathy: current concepts of evaluation and treatment. Clin Endocrinol Metab;15: 933-969.

Freeman M E, Kanyicska B, Lerant A and Nagy G. (2000). Prolactin: structure, function, and regulation of secretion. Physiol. Rev;80:1523–1631.

Laway B, Bashir M, Ganie M, Mir S and Gojwari T. (2010). Hyperprolactinemia in a patient with Sheehan's syndrome. Turk. JEM;14:47–49.

Freemark M, Avril I, Fleenor D, Driscoll P, Petro A and Opara E. (2002). Targeted deletion of the PRL receptor: effects on islet development, insulin production, and glucose tolerance. Endocrinology;143:1378–1385.

Friedrichsen B N, Richter H E, Hansen J A, Rhodes C J and Nielsen J H. (2003). Signal transducer and activator of transcription 5 activation is sufficient to drive transcriptional induction of cyclin D2gene and proliferation of rat pancreatic beta-cells. Mol. Endocrinol;17:945–958.

Ganong WF. (2001). Review of Medical physiology. 20th ed, Newyork: Mc Graw-Hill:383-97.

Garcia C, Aranda J, Arnold E, Thebault S and Macotela Y. (2008). Vasoinhibins prevent retinal vasopermeability associated with diabetic retinopathy in rats via protein phosphatase 2A-dependent eNOS inactivation. J Clin Invest;118: 2291-300.

Genest J. (2010). C-reactive protein: risk factor, biomarker and/or therapeutic target? Can J Cardiol;26 Suppl A:41–44.

Genuth SM, Berne RA, Levy MN and Koepen BM. (1998). The hypothalamus and pituitary gland. 4th edition st. Louis: Mosby;872-909.

Hammes H P, Feng Y, Pfister F and Brownlee M. (2008). Diabetic retinopathy: targeting vasoregression. Diabetes;60: 9–16.

Holstad M and Sandler S. (1999). Prolactin protects against diabetes induced by multiple low doses of streptozotocin in mice. J. Endocrinol;163: 229–234.

Huang C, Snider F and Cross J C. (2009). Prolactin receptor is required for normal glucose homeostasis and modulation of beta-cell mass during pregnancy. Endocrinology;150: 1618–1626

Kim H, Toyofuku Y, Lynn F C, Chak E and Uchida T. (2010). Serotonin regulates pancreatic beta cell mass during pregnancy. Nat. Med;16:804–808.

Lawrence JM, Contreras R, Chen W and Sacks DA. (2008). Trends in the prevalence of preexisting diabetes and gestation diabetes mellitus among a racially/ ethnically diverse population of pregnant woman. Diabetes care:899-904.

Lim LS, Tai ES, Mitchell P, Wang JJ, Tay WT and Lamoureux E. (2010). C-reactive Protein, Body Mass Index, and Diabetic Retinopathy. Investigative Ophthalmology & Visual Science ;51:4458–4463.

Longmore, Wilkinson, Turmezei and Cheung. (2007). Oxford hand book of clinical medicine, 7th ed.

Macotela Y, Aguilar M B, Guzman-Morales J, Rivera J C and Zermeno C. (2006). Matrix metalloproteases from chondrocytes generate an antiangiogenic 16 kDa prolactin. J. Cell Sci;119:1790–1800.

Marshal WJ. (2004). Clinical chemistry. 5th ed; 194.

Mayne P D. (1998). Clinical chemistry in diagnosis and treatment. 6 th ed; 197-235.

Mestriner AC and Haddad A. (1997). Horseradish peroxidase: a reliable or a misleading tool for the investigations on the origin of the proteins of the aqueous humor? Cell Tissue Res;289:85–96

Mooradian AD, Morley JE, Billington CJ, Slag MF and Elson MK. (1985). Hyperprolactinaemia in male diabetics. Postgrad Med J; 61: 11-24.

O'Steen WK and Sundberg DK. (1982). Patterns of radioactivity in the eyes of rats after injection of iodinated prolactin. Ophthalmic Res;14: 54-62.

Pan H, Nguyen NQ, Yoshida H, Bentzien F, Shaw LC, Rentier-Delrue F, Martial JA, Weiner R, Struman I and Grant MB. (2004). Molecular targeting of antiangiogenic factor 16K hPRL inhibits oxygen-induced retinopathy in mice. Invest Ophthalmol;45:2413–2419

Pepys MB and Hirschield GM. (2003). C. reactive protein: critical update. J. Clin . Invest; 111 (12): 1805 – 12.

Petryk A, Fleenor D, Driscoll P and Freemark M. (2000). Prolactin induction of insulin gene expression: the roles of glucose and glucose transporter-2. J. Endocrinol;164: 277–286.

Riddle O, Bates RW and Dykshorn Sw. (1993). The preparation, identification and assay of prolactin—a hormone of anterior pituitary. Am J Physiol;105: 191–216.

Ridker PM, Cushman M, Stampfer MJ, Tracy RP and Hennekens CH. (1997) .inflammation , aspirin, and the risk of cardiovascular disease in apparently healthyment . NEngl J med; 336:973 – 9.

Rivera J C, Aranda J, Riesgo J, Nava G and Thebault S. (2008). Expression and cellular localization of prolactin and the prolactin receptor in mammalian retina. Exp. Eye Res;86: 314–321.

Ross R. (1999). Atherosclerosis: An inflammatory disease. N. Engl J med; 340 (2): 115 – 126.

Rother KI. (2007). Diabetes treatment-bridging the divide. England journal of medicine; 1499-50.

Song J, Chen S, Liu X, Duan H, Kong J and Li Z. (2015). Relationship between C-Reactive Protein level and Diabetic Retinopathy: A Systematic Review and Meta-Analysis;10(12).

Triebel J, Macotela Y, Martinez de la Escalera G and Clapp C. (2011) Prolactin and Vasoinhibins: Endogenous Players in Diabetic Retinopathy. Iubmb Life;63: 806-810.

Wang XL, Dai Y and Chen XH. (2010). Changes of the concentration of serum adiponectin and high sensitivity C-reactive protein in type 2 diabetes mellitus patients with retinopathy. International Journal of Ophthalmology;10:1699–1701.

Weinhaus A J, Stout L E, Bhagroo N V, Brelje T C and Sorenson R L. (2007). Regulation of glucokinase in pancreatic islets by prolactin: a mechanism for increasing glucose-stimulated insulin secretion during pregnancy. J. Endocrinol;193:367–381.

Wild S, Roglic G, Green A, Sicree R and King H. (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care ;27: 1047–1053.

Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW and Bek T. (2012). Global prevalence and major risk factors of diabetic retinopathy. Diabetes care; 35:556-564.

Appendix (I)

Questionnaire

Sudan University of Science and technology

College of Graduate studies

Estimation of Prolactin and C-Reactive Protein in Type 2 Diabetic Patients with Retinopathy in Khartoum State

Number ( )

A. General Information:

1. Name…… 2. Age……..……

3. Gender: ……. 4. Hospital ……..…..

B. Type of diabetes: ……..…….……

C. Duration of disease :(In years) …… …….....

D. History of other disease:

1. Hypertension: ( ) 2. Renal disease: ( )

3. Hepatic disease: ( ) 4. Others: ( )

E. Investigation:

1. Serum prolactin: ..…….ng/ml

2. Serum C-reactive protein: …… (+ve/-ve)