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Biochemical and Genetics Study of Preptin Hormone in Thyroid Diabetic Dysfunction Patient

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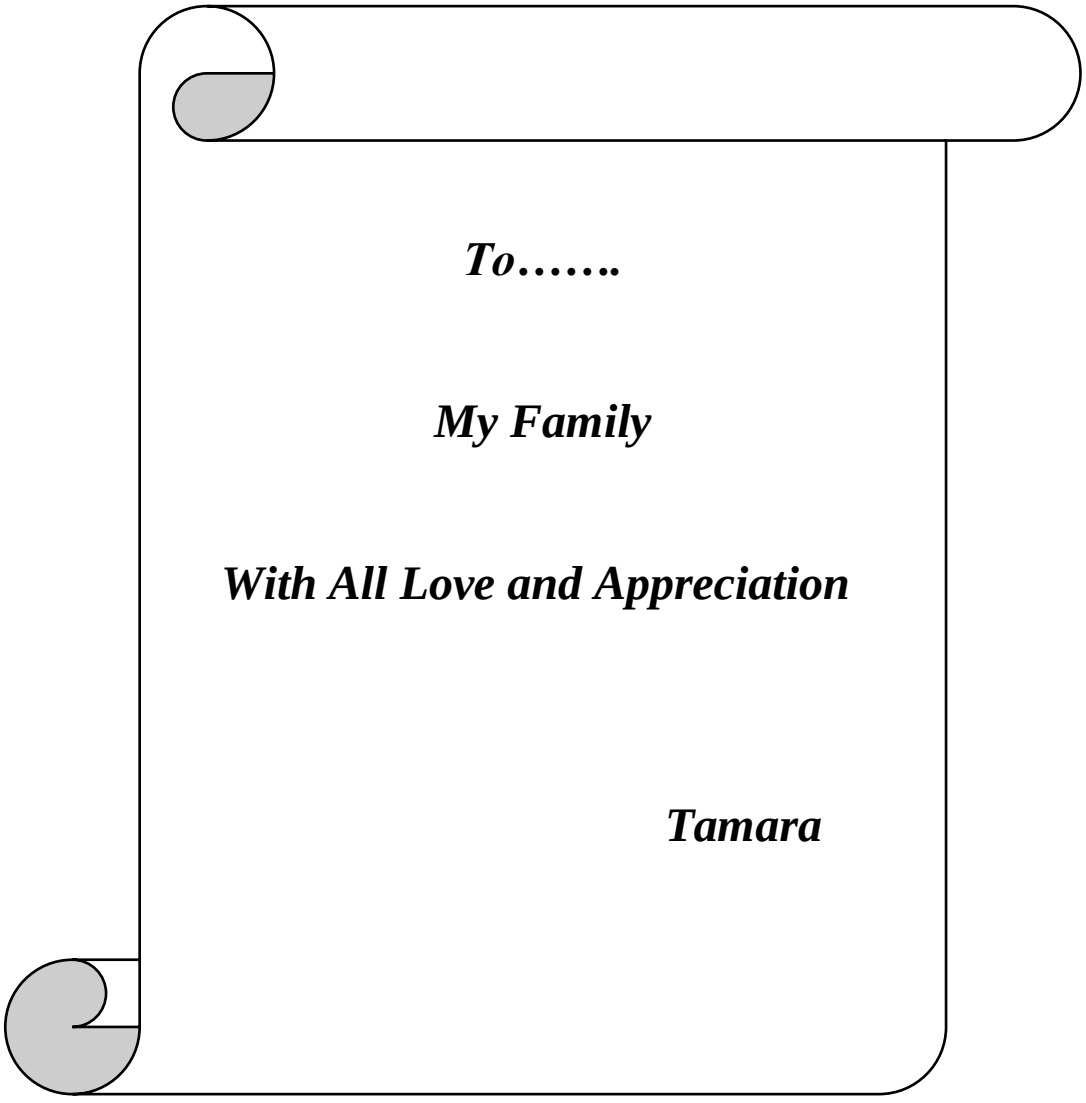
Dedication



To…….

My Family

With All Love and Appreciation

Tamara

acknowledgment

Praises to my God almighty for enabling to me finish my study, which cannot be truly without the bounty almighty.

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List of Abbreviations

Code	***Word***
ALS	Amyotrophic lateral sclerosis
BMD	Bone mineral density
BMI	Body mass index
BWS	Beck with-Weidman syndrome
DMR	Differentially methylated region
DMRS	Differentially methylated regions
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
ELSA	Enzyme-linked immunosorbent assay
EP	Enzyme product

FBG	Fasting blood glucose
FSH	Follicle-stimulating hormone
G_0	Glucose
GD	Graves' disease
GDM	Gestational diabetes mellitus
GH	Growth hormone
GO	Graves' ophthalmopathy
GOD	Glucose oxidase
HbA1c	Hemoglobin A1c
HOMA-IR	Homeostasis model assessment for insulin resistance
HRP	Horseradish peroxidase
ICR	Imprint Control Region
IGF-I	Insulin-like growth factor-I
IGF-II	Insulin-like growth factor-II
IGF-1R	Insulin-like growth factor-1 receptor
IGF-2R	Insulin-like growth factor-2 receptor
IGFBS	Insulin-like growth factor binding proteins
IGFBP-1	Insulin-like growth factor binding protein-1
IGFBP-2	Insulin-like growth factor binding protein-2
IGFBP-3	Insulin-like growth factor binding protein-3
IR	Insulin resistance
Kb	kilobases
KD	Kilo Dalton
LH	Luteinizing hormone

MODY	Maturity Onset Diabetes young
OD	Optical density
OCC	One cresolftatein complex
OOC	O- cresolftalien complexone
PC4	Proprotein convertase 4
PCOS	Polycystic ovarian syndrome
PCR	Polymerase chain reaction
POD	Peroxidase
POMC	Pro-opio melanocortin
PM	Pretibial Myxedema
QUICKI	Quantitative insulin-sensitivity check index
REE	Resting Free Expenditure
RFV	Relative Feed Value
RFLP	Restriction fragment length polymorphism
SPR	Surface Plasma resonance
STR	Short Tandem report
T3	Tri iodothyronine
T4	Tetra iodothyronine
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TH	Thyroid hormone
TSH	Thyroid stimulating hormone
TT3	Total tri iodothyronine
TT4	Total thyroxine
V.D3	Vitamin D3
IBM- SPSS	Integration Bus Manufacturing Pack Statistical Package for the Social Science

CHPTER ONE

INTRODUCTION AND LITERATURES REVIEW

1.1- Thyroid gland:

The thyroid gland is an endocrine gland located in the anterior part of the neck. It plays a major role in the basal metabolic rate, stimulates somatic and physical growth, and plays an important role in calcium metabolism [(1)], and figure(1.1) show the thyroid gland.

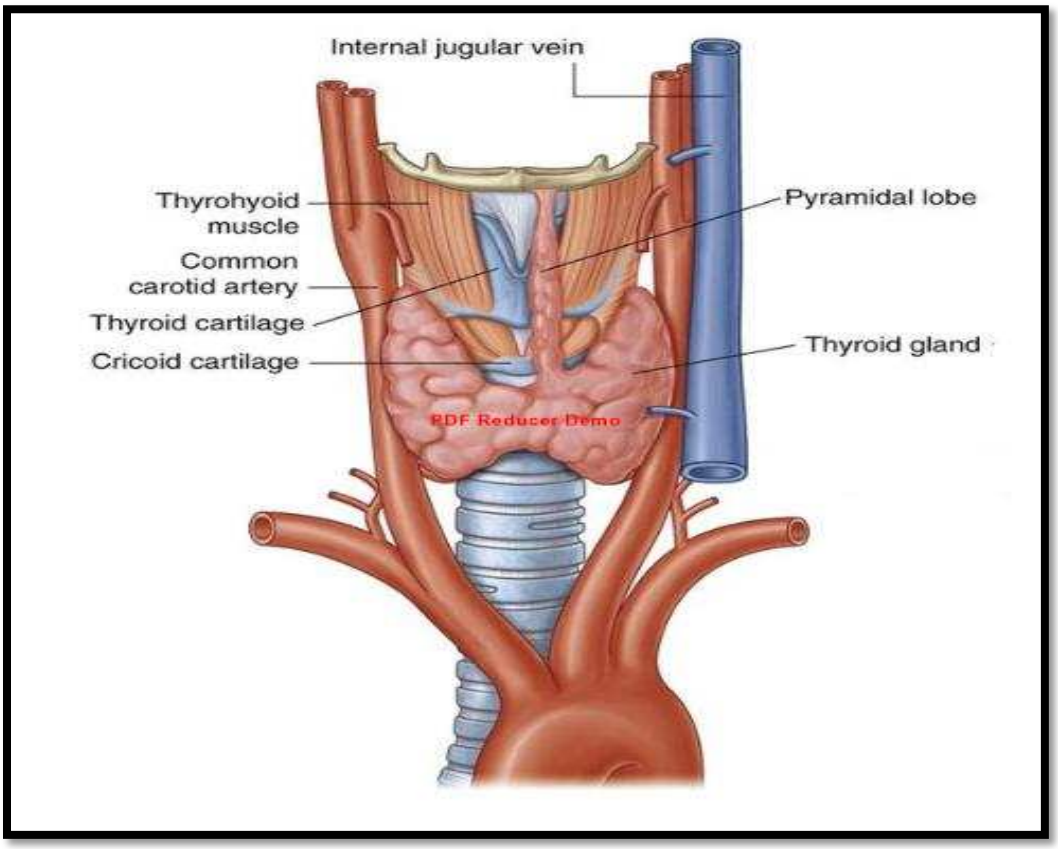


figure (1.1): Thyroid gland [(2)].

The ordinary thyroid is consisting of two lobes attached by weak band of tissue, the isthmus, which is 0.5 cm thick, 2 cm broad, and 1 to 2 cm high. The person lobes normally have a pointed superior pole and poorly explain blunt inferior pole that merges medially with the isthmus. Every one lobe is approximately 2.0 to 2.5 cm in thickness and width at its sizable diameter and is approximately 4.0 cm in

length[4]. Whereas the thyroid gland is evolving, the hypothalamus and pituitary are as well creating. The hypothalamus starts to send signals to the pituitary, which in its turn encourages the thyroid gland to mature and produce the thyroid hormones (TH) which includes3,5,3`-triiodothyronine ($T_3$) and 3,5,3`,5`-tetra iodothyronine (T_4) are single in that iodine (as iodide) is a necessary part of both [5].

In the most compounds of the world, iodine is a rather rare element of soil, and for that because there is few in food. A composite mechanism has developed to again and keep this critical part and to mutate it into a shape proper for internalization into organic compounds [6].

The main function of the thyroid organ is excreting thyroid hormones (TH), (T_3) and (T_4) which assist the body to utilize energy, keep warm and maintain the capability action of vital organ (brain, heart, muscles, and other organs) [3,4,7].

TH is main in controlling body's metabolic average during life, so that when there is not enough TH, the metabolism slow up, and when there is too much, the metabolism quickens [5]. Main factors contributing to human difference in resting free expenditure (REE)contain: age, sex, body size, body composition, ethnicity, physical fitness level, hormonal case, and a range of genetic and environmental influences [6].

TH are recognized to impact energy metabolism. Many patients of metabolic syndrome have subclinical hypothyroidism and conversely [7].

Fatness, a key component of metabolic syndrome, happens due to elevated energy expenditure, or blend of both, thus leading to positive energy balance.TH inadequacy damage learning and memory, which rely on the structural integrity of hippocampus [10].

1.2-Disorder of Thyroid Gland:

Disorder of thyroid gland can be classified into [11] hypothyroidism and hyperthyroidism figure (1.2) show the thyroid disorder.

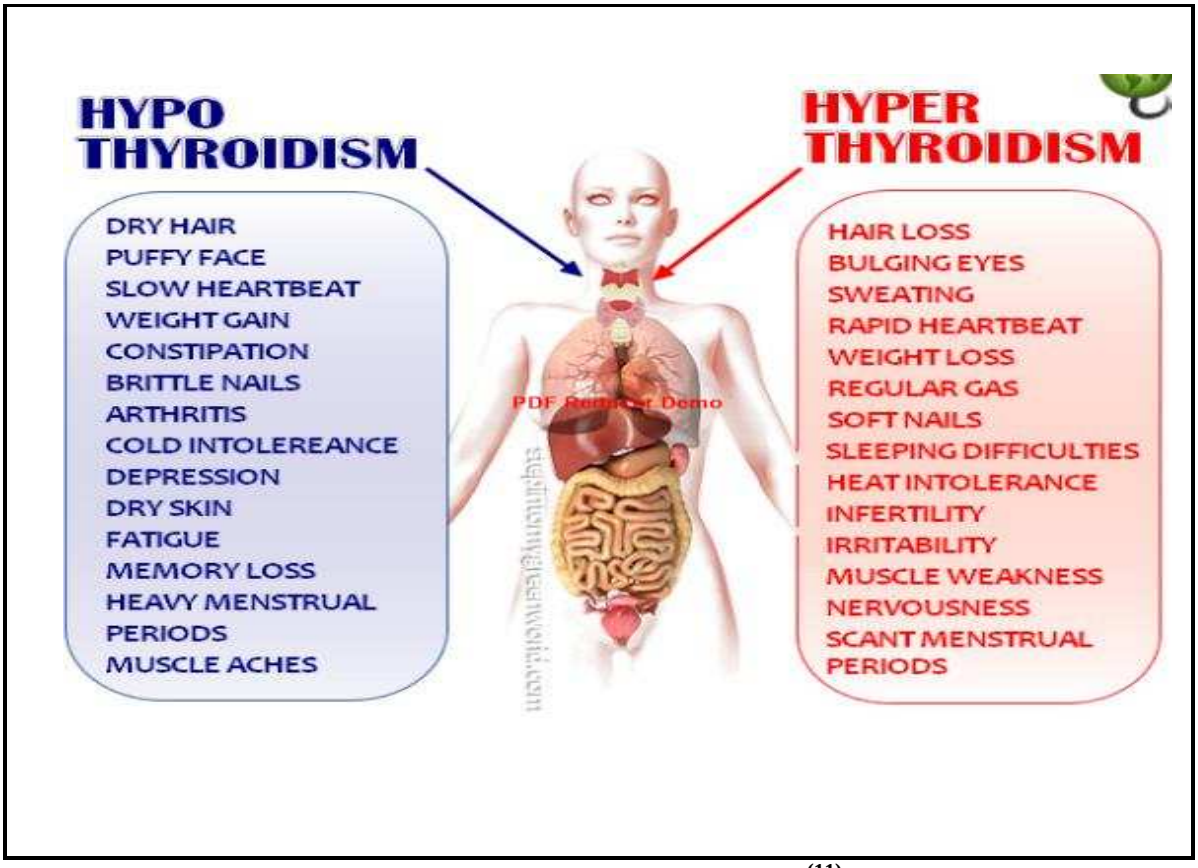


Figure (1.2): Thyroid Disorder [11].

1.2.1-Hypothyroidism:

Hypothyroidism is a common pathological condition of TH (T3, T4) inadequacy. If untreated, it can lead to serious adverse health effect and ultimately death [12]. Hypothyroidism can be classified as:

(1) Primary (due to TH inadequacy).

(2) Secondary (due to thyroid stimulating hormone (TSH) inadequacy)

(3) Tertiary (due to thyrotropin (TRH)-releasing hormone inadequacy)

(4) Peripheral (extra-thyroidal; panel)

(5) Central hypothyroidism (including both secondary and tertiary)

Peripheral hypothyroidism is rare and account for less than 1% cases. The most usual signs of hypothyroidism in adults are fatigue, cold intolerance, weight gain, constipation, change in voice, and dry skin, but the clinical presentation can involve a wide change of marks that differ with age, sex, and time between start and diagnosis [13].

The signs for diagnosis hypothyroidism are non-specific, principally in elderly patients who present with fewer classic signs symptoms than younger humans. Hypothyroidism has clinical system is the almost strictly studied. Hypothyroidism produce elevated vascular resistance, reduced cardiac output, reduced left ventricular function, and change several other symptoms of cardiovascular contractility [14]. There are many diseases that have resulted from hypothyroidism:

1.2.1.1-Cretinism:

The most usual reason of inherent hypothyroidism is iodine inadequacy. Cretinism is thus most likely due to a diet insufficient in iodine. Iodine is a necessary trace element, essential primarily for synthesis of TH. Iodine adequacy is the most usual preventable cause of brain harm worldwide [15].

Inherited hypothyroidism can be endemic, genetic, or intermittent. If untreated, it produce mild to moderate damage of both physical and intellectual growth and evolvement poor length growth which is visible as early as the first year of life. Mature states without handling ranges from 100 to 160 cm, rely on severity, sex,

and other genetic factors [16]. It can be corrected by giving thyroxine if started early enough [15].

1.2.1.2-Myxedema coma:

Myxedema coma is rare life- threatened clinical state that represents acute hypothyroidism with physiological decompensation. Myxedema coma is a typically severe TH lack and is characterized by impairment of cerebral states, hypothermia, hypotension, hyponatremia and hyporentilation[17].

Myxedema crisis is one of the most acute possible results of pro-found, long-standing hypothyroidism. Myxedema crisis may be a suitable word as quit a few patients are obtunded, rather than candidly comatose. It is a rare and unrecognized. Accurate prevalence of myxedema crisis is obscure [18].

Even with detection and suitable handling death rate ranges from 30 to 60% where most collapse due to respiratory failure, sepsis and gastrointestinal bleeding. Myxedema coma occur mostly in individuals 60 years or older and nearly 80% of cases happen in females [19].

1.2.1.3-Goiter:

Goiter mentions to an enlarged thyroid gland. Status of goiter involve autoimmune illness, the formation of one or more thyroid nodules. Goiter happens when there is decreased TH synthesis secondary to biosynthesis defects and or iodine lack, leading to elevated thyroid stimulating hormone (TSH) [20].

Enlargement of thyroid gland can be caused by: insufficient iodine in the diet producing little levels of T_3 and T_4, or an autoimmune attack against elements of the thyroid gland (called Hashimoto's thyroiditis), the synthesis of TSH is normally prevented as the levels of T_3 and T_4 increase in the blood [21], and figure (1.3) show goiter shape.

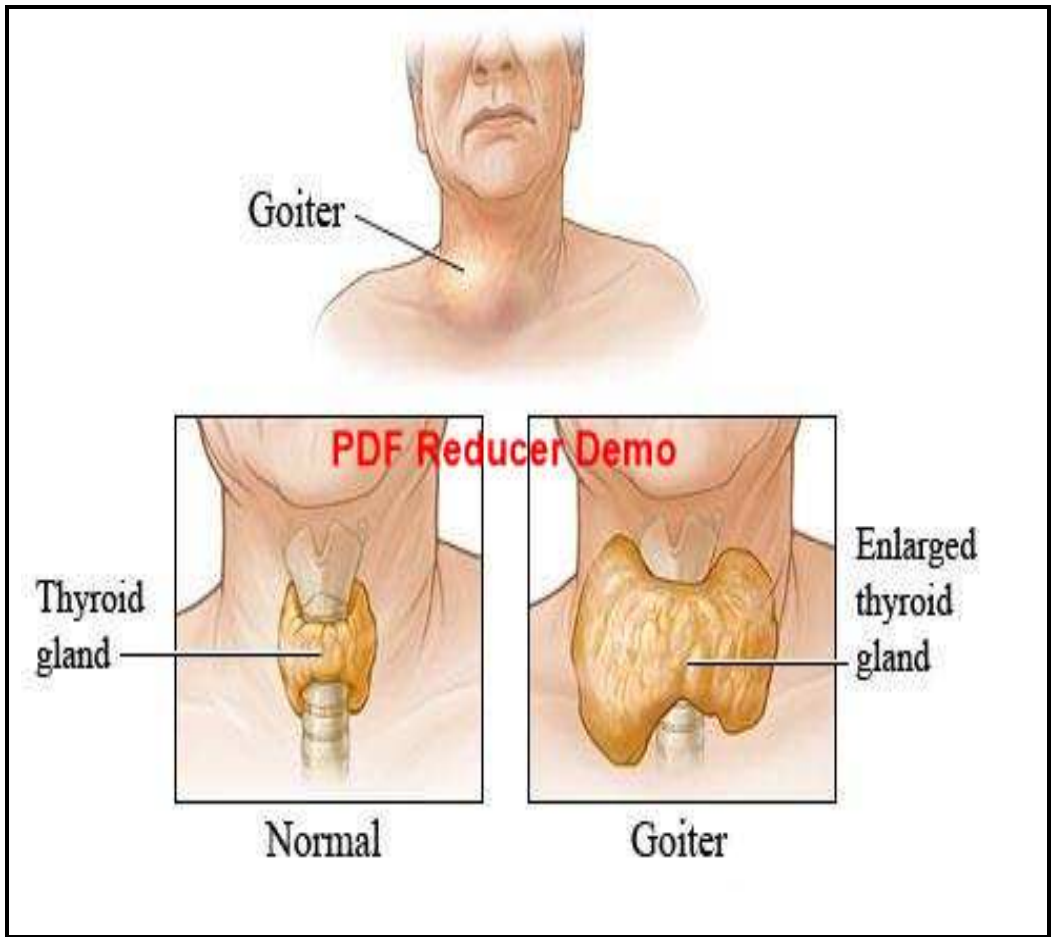


Figure (1-3): Goiter shape [22].

1.3-Hypothyroidism and Brain Development in Humans:

The neonatal phase of evolvement in an individual in known to be responsive to thyroid hormone, principally as disclosed in the disorder familiar as congenital hypothyroidism. Congenital hypothyroidism happens at average of approximately 3,500 live childbirths [(23)].

The congenital hypothyroidism babies do not give a particular clinical picture early, their diagnosis based just on clinical signs was delayed before neonatal screening for TH. In reality, only 10% congenital hypothyroidism infants were determined within the initial year, and 100% only after age 3 [(24)].

The mental deficits as a result of this delayed diagnosis and handling were difficult. TSH are discovered in an individual coelom and amniotic fluids as early as 8 weeks of pregnancy, before the start of fetal thyroid function at 10-12 weeks. In addition, individual fetal brain tissues express TH receptors, and receptor occupancy by TH is in the range familiar to result physiological impacts as early as 9 weeks of pregnancy [(25)].

Eventually, the messenger RNAs (mRNAs) encoding the two familiar TR classes display complex temporal design of expression throughout individual pregnancy, and the mRNAs encoding these TR isoforms are expressed in the individual oocyte. Two types of pathological situations reveal the functional results of defects in TH throughout fetal evolvement [(18)].

The first is that of cretinism, a case generally linked with severe iodine insufficiency in the diet. There are two forms of cretinism based on clinical presentation: neurological cretinism and myx-edematous cretinism. Neurological cretinism is characterized by maximal intellectual retardation, deaf-mutism,

impaired voluntary motor activity, and hypertonia. In disparity, myxedematous cretinism is distinguished by less acute intellectual backwardness and all the main clinical signs of persistent hypothyroidism [26].

1.2.2-Hyperthyroidism:

Hyperthyroidism occurs mainly due to increased concentration of circulating thyroid hormones (T3 or T4 or both) in the body tissues which can be treated with anti-thyroid medications (methimazole and propylthiouracil)[26].

The clinical features of hyperthyroidism ranges from a symptomatic to thyroid storm. Increased TH levels raise catecholamine signaling through elevated numbers of cell surface beta- adrenergic receptors. The adrenergic symptoms (e.g., palpitation, heart intolerance, diaphoresis, tremor, stare [an appearance of a fixed look due to retraction of eyelids, hyper-defecation] are the most common manifestation of hyperthyroidism [27].

Subtotal or total thyroidectomy may have done depending on the involvement of thyroid gland. Radioactive iodine treatment is reserved for elderly humans who represent a poor surgical risk. The complications of thyroid operation are hemorrhage, respiratory obstruction, vocal cord paralysis, hypoparathyroidism thyroid deficiency, thyrotoxic storm, and wound infection. [7,28] The common disease in the case of hyperthyroidism is:

1.2.2.1-Graves' Disease:

Graves illness is an autoimmune thyroid disorder caused by stimulating antibodies to the thyrotropin TSH receptor on thyroid follicular cells. It is the much

usual cause of hyperthyroidism with 20 to 30 cases per 100 000 individuals each year [29].

The majority of symptoms of graves illness are caused by the extravagant production of TH by the thyroid gland. These may involve, but are not limited to, racing heart, hand tremors, sleeping disturbance, weight loss, muscle weakness, neuropsychiatric marks and heat intolerance [30].

*Graves' ophthalmopathy (GO): an autoimmune state linked with Graves' disease (GD), occurs at a prevalence of nearly 40% in patients diagnosed with GD. It is commonly seen in people who have had GD for some time, and thus are also probably to be seen in a more advanced age group than those who do not present with visual defect. GO is more common in women, often linked with autoimmune illness, and has an incidence of 16.3 per 100,000 people/year [31].

*Graves dermopathy: The most usual manifestation of dermopathy in Graves' disease is Pretibial Myxedema (PM). Clinical features are nonbiting scaly thickening and induration of the skin [32].

1.4- Diabetes Mellitus:

Diabetes mellitus is a group of metabolic disorders with a usual phenotype of elevated blood glucose level (hyperglycemia). Many factors contribute to this widely disease, like obesity, sedentary life-style and high blood pressure [33,34].

The pathogenic pathway involved in hyperglycemia involve autoimmune demolition of pancreatic insulin excreting cells (β-cells) leading to insulin lack and anomalies that give to insulin resistance [33,34].

Diabetes is a complex, chronic disease needing continuous medical support with multifactorial danger-reduction strategies behind glycemic control. Ongoing patient

self-management teaching and clinical care are clinical to stop severe complications and reducing the danger of long-term complications [35].

Diabetes mellitus (diabetes) is a heterogeneous group of multifactorial, polygenic syndrome distinguished by an increased fasting blood glucose (FBG). Caused by a proportionate or complete lack in insulin. Diabetes is linked with elevated danger of cancers of the liver, pancreas, endometrium colon/ rectum, breast, and bladder [18]. Diabetes can be classified into the following common classes:

(1) Type 1 diabetes (T_1D):

Due autoimmune β-cell dysfunction, generally leading to absolute insulin lack. It is characterized by an absolute lack of insulin caused by an autoimmune attack on the β-cells of pancreas. previously called insulin-a dependent diabetes. In this case pancreatic beta cells secrete deficient quantity of insulin or no insulin [36].

T_1D may also happen with other autoimmune illness in the cortex of specific genetic disorder or polyglandular autoimmune abrupt appearance of polyuria (frequent urination), polydipsia (excessive thirst), and polyphagia (excessive hunger), often triggered by physiologic stress such as a disease poly cystic ovaries (PCOS). These symptoms are generally a companied by fatigue and weight loss [18], and figure (1.4) show a diagram diabetes type 1

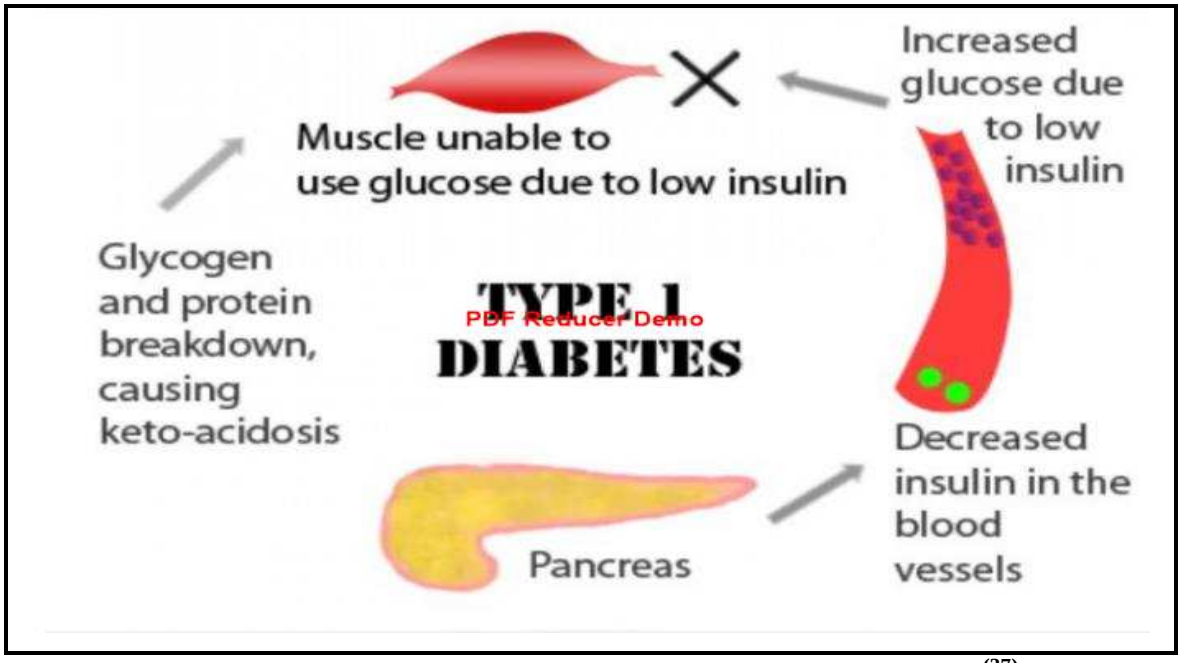


Figure (1.4): A diagram showing diabetes type 1[37].

(2) Type 2 diabetes (T_2D):

Due to progressive loss of β-cell insulin excretion capacity on the background of insulin resistance is a major kind of diabetes previously called insulin non-dependent diabetes or type 2 diabetes which is the more common from diabetes mellitus [(90-95) % of all diabetes cases] [34].

This type produce from integration of resistance of insulin action in body cell and insufficient insulin excretion from pancreatic β-cell. T2D is generally connected with dyslipidemia, which elevate the danger of cardiovascular disorder [38].

Patient with diabetes type 2 should be encourage to undergo recommended age and gender-suitable cancer viewing and to decrease their modifiable cancer danger factors (fatness, physical inactivity, and smoking) [37], and figure (1.5) show a diagram diabetes type 2.

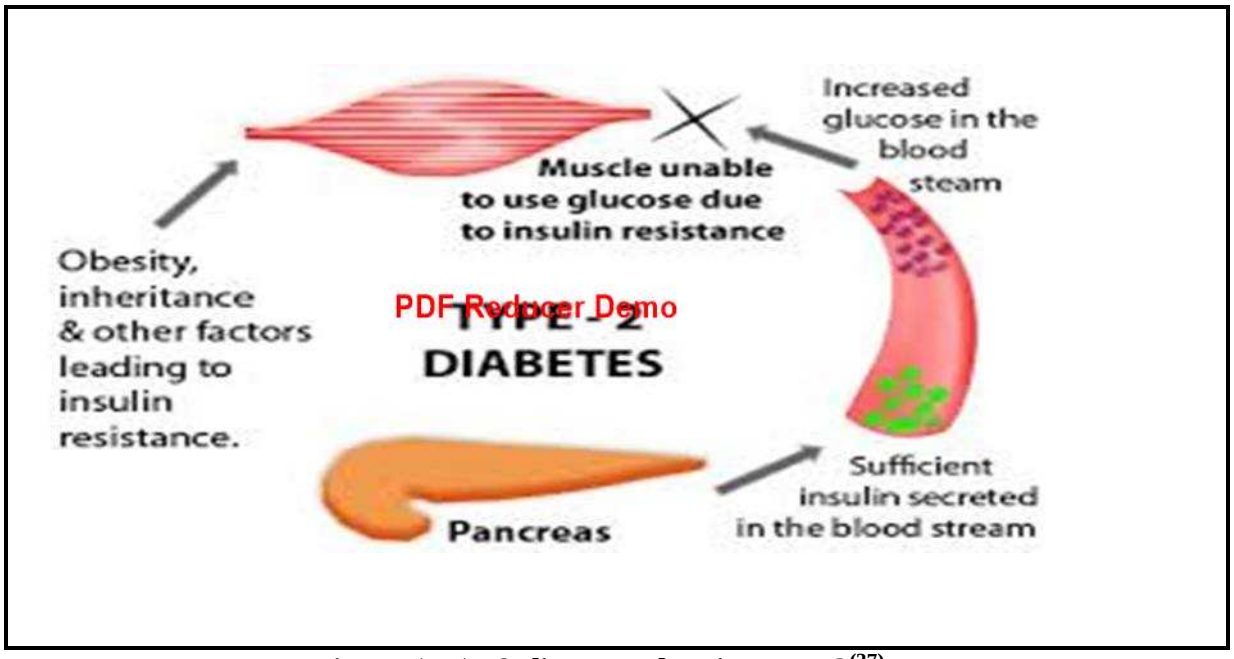


Figure (1.5): A diagram showing type 2[37].

(3) Gestational diabetes mellitus (GDM):

Diabetes determined in the second or third trimester of pregnancy that was not distinctly overt diabetes phase pregnancy [38].

Insulin resistance and danger for the evolvement of T2D in the further is usual observed in women who are overweight, physically inert, and in the (3-5) % of pregnant woman who evolve gestational diabetes [18].

(4) Particular types of diabetes:

Due to other reasons, e.g., monogenic diabetes syndrome (such as neonatal diabetes and maturity- onset diabetes of young (MODY), illness of the exocrine pancreas (such as cystic fibrosis and drug- or chemical diabetes (such as with glucocorticoids, in the handling of HIV/ADIS, or after organ transplantation [39].

1.5-Preptin Hormone:

Preptin is peptide hormone critical for controlling energy metabolism. Preptin is synthesized primarily in the pancreas, salivary gland, mammary tissue, and kidney. The first roles of preptin is controlling carbohydrate metabolism by moderating glucose-mediated insulin free. [(40)]

Preptin was first isolated from the pancreatic beta-TC6-F7 cell lines of rats by Bucham and colleagues in 2001. Some peptide hormones show a high degree of similarity. Scientists have classified certain hormones with similar structures and characteristics in hormone families (e.g., the pro-opio melanocortin (POMC) hormone family) [(41)].

Insulin, insulin-like growth factor-II (IGF-II, as well called somatomedin), pro-insulin-like growth factor-II and relax in hormones family have high grade of homology and are suppose to be in the insulin family. Being a derivate of pro-insulin-like growth factor II (pro IGFII), preptin is considered to be the latest member of insulin family as well [(42)].

Buchanan and *et al.* identified a 34-amino acid peptide hormone that is consecrated with insulin and amylin from the pancreatic β-cells, peptide (3948 Da) crossponding to ASP (69)-Leu (102) of the proinsulin-like growth factor IIE–peptide (pro-IGF-IIE), which they have called preptin[(4)], figure (1.6) show the amino acid serious of preptin.

Figure (1.6): the amino acid series of preptin [(40)].

The amino acid series of preptin has been mainly preserved through the evolutionary operation in both mice and individuals. Individual preptin is 79.41% analogue with mouse preptin and 75.53% alike with rat preptin, while the mouse and rat preptin are 94.12% analogue preptin is cleaved by proteases at the 21st phenylalanine amino acid piece [(41)].

The portion of preptin peptide that results from this segmentation (preptin 1-16) does not affect insulin excretion full length (34-amino acid) preptin, however, physiologically elevate-mediated insulin excretion. The half-life of preptin in circulation is shorter than 5 min and the elevation or reduction in the circulatory preptin quantity linked with insulin levels an individual's [(4,41)].

Preptin has an anabolic impact on bone, which is synergetic with the impacts of insulin hormone. Of it is one molecule, the agent that most strongly affect cell differentiation and cell activity of osteoblasts and osteoclasts is insulin-like growth factor-I (IGF-I) [(42)].

The authors concluded that preptin was included in the pathogenesis of osteoporosis, probably through bone formation, rather bone resorption. As the examples show, preptin is included in the etiopathology of wide array of illnesses from fatness and polycystic ovary syndrome (PCOS) to diabetes, osteoporosis and

osteopenia [42], and figure (1.7) show the present a summary of the major synthesis regions and the principle biochemical effect of preptin.

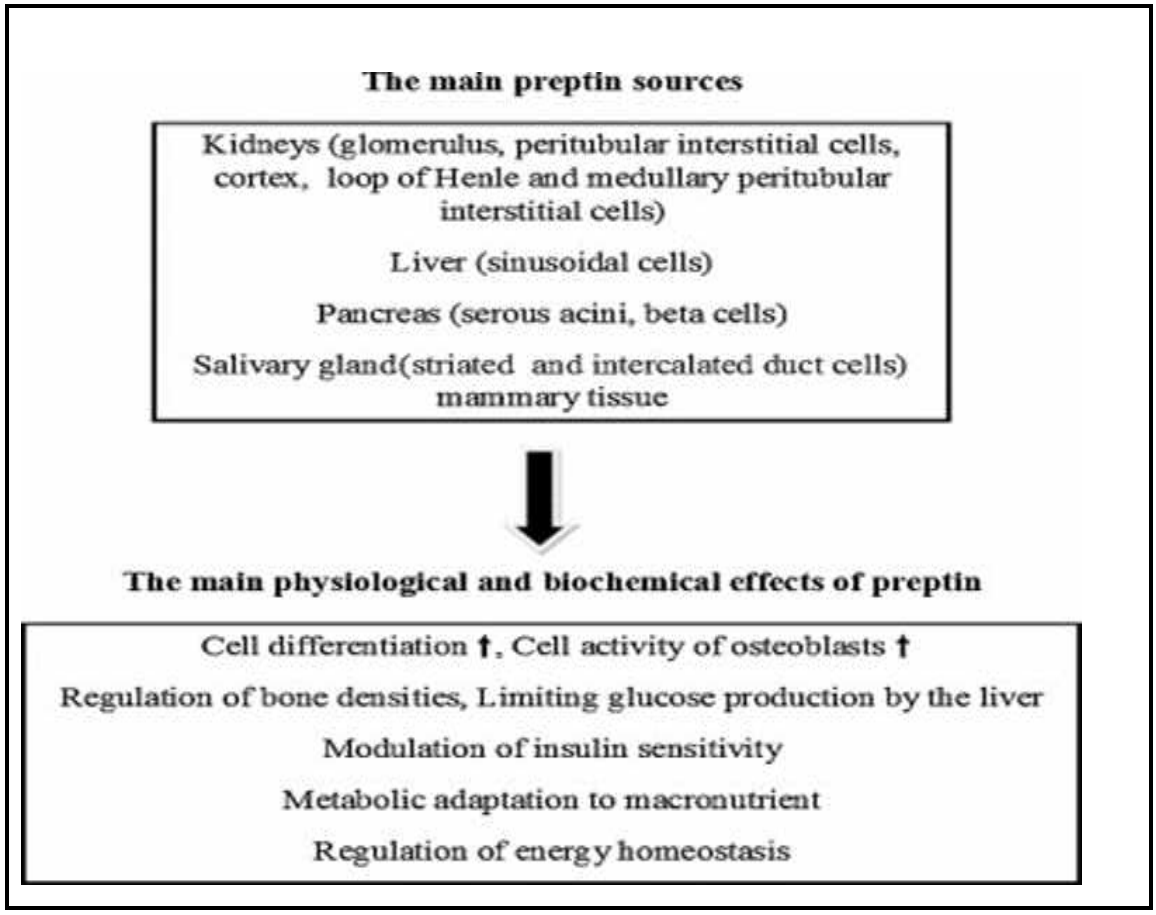


Figure (1.7): Present a summary of the major synthesis regions and the principle biochemical effect of preptin [4].

1.6-Mechanism Preptin of action:

The carbohydrate metabolism is controlled by a host of enzymes and hormones. A recently evolved hormone that is included in the carbohydrate mechanism is preptin. Rely on the nutritional case, the hypothalamus has two choices, either there is sufficient energy or a feeling of satiety, it elevates the synthesis and excretion of anorexigenic peptide (like leptin) from the peripheral tissues to limit food intake [4,42].

If there is a lack of energy in the organism, this causes a sense of hunger, in which status the synthesis and excretion of orexigenic peptide (like preptin) from the peripheral organs is elevated. These orexigenic and anorexigenic peptides excreted from the peripheral tissues. All living things in nature must feed in order to grow and maintain their life [43].

If they take higher (hypercaloric) or fewer (hypocaloric diet) calories than the optimal (isocaloric diet) quantity, their metabolic equilibrium is disrupted. If energy intake is higher than energy expenditure, a positive energy equilibrium emerge and the buildup of fat in the body leads to fatness. The reverse state of negative energy equilibrium, on the other hand lead to weight loss [45,46].

Energy metabolism in living things should run smoothly to stop all possible disruption. Protein, lipids and carbohydrates are the main nutritional origin of energy. In order for these molecules to be used by the body, they must be cracked down into their subunits [4].

All carbohydrate that enter the organism are finally transformed to glucose, irrespective of their kind. Glucose is either formed from dietary origin, the collapsed of the glycogen through glycogenesis in the liver, or from other carbon origins

(lactate, pyruvate, amino acids and glycerol) through gluconeogenesis in the renal and hepatic tissues [45], and mechanism preptin hormone show in figure (1.8).

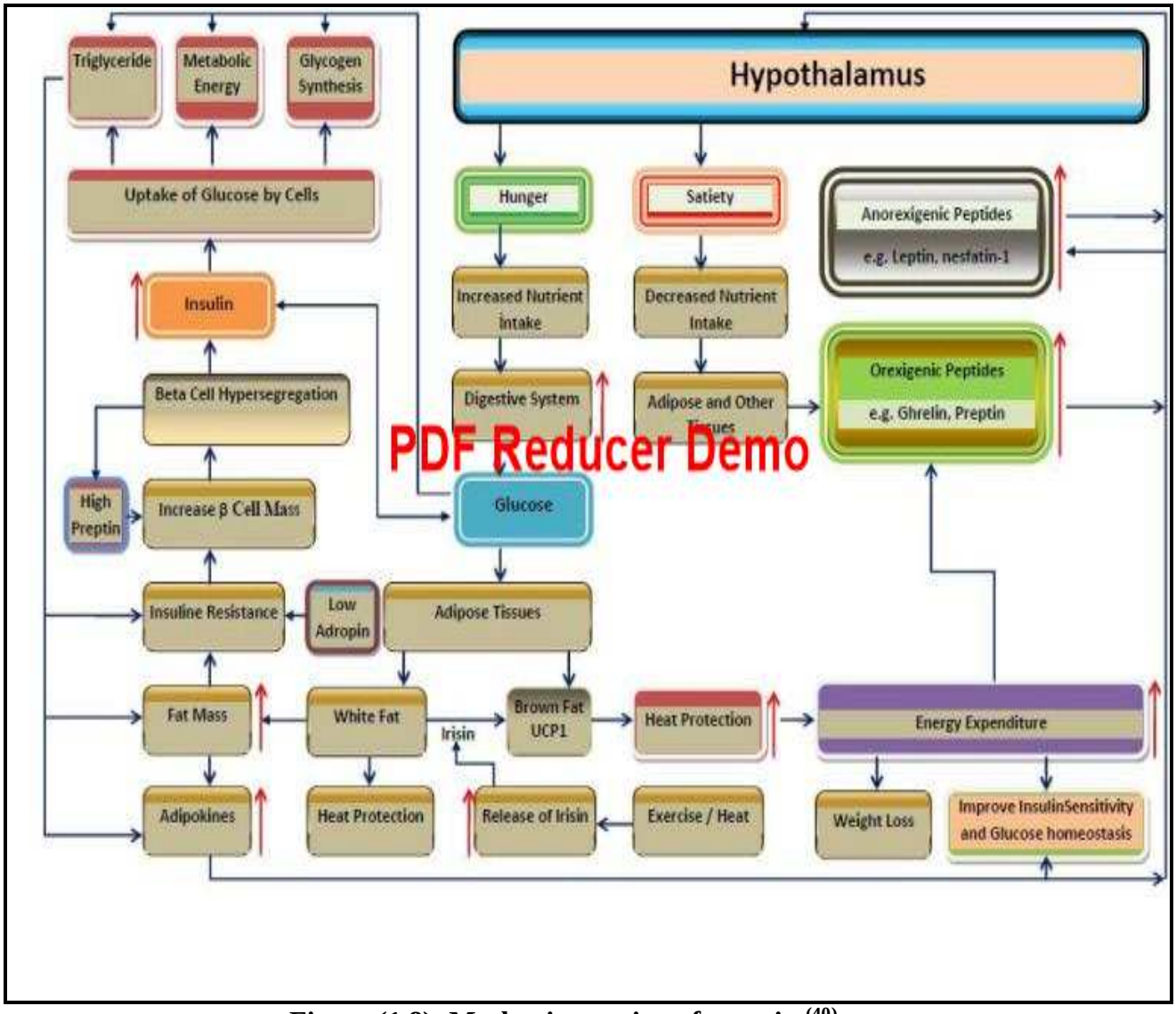


Figure (1.8): Mechanism action of preptin [40].

1.7-Insulin resistance (IR):

Insulin resistance (IR) is defined as reduced allergy or responsiveness to metabolic works of insulin. It should be recognized that *in vivo* insulin resistance is tethered to hyperinsulinemia. Hyperinsulinemia is deemed indicative of IR. It is play a main part in the pathophysiology of diabetes, and it has exhibit to be feature predictor of many health connected adverse outcomes including coronary artery disease and stroke[46]. Rarely, hyperinsulinemia is caused by:

* A tumor of the insulin-producing cells of the pancreas.

* Excessive numbers of insulin-producing cells in the pancreas (nesidioblastosis). Hyperinsulinemia source no signs or mark unless it causes hypoglycemia. IR is also connected with fatness as well as hypertension, coronary artery disease, and dyslipidemia [47].

1.7.1- Measurement Tools Assessing Insulin Resistance:

There are many ways to measure insulin resistance, for example; homeostasis model assessment for insulin resistance (HOMA-IR) and quantitative insulin-sensitivity check index(QUICKI), hyperinsulinemic euglycemic clamp tests and insulin suppression tests [48]. HOMA has been widely utilize in clinical research to ass's insulin sensitivity. Rather than using fasting values of glucose (G_0) (expressed as mg/dl) and insulin (expressed as µu/ml) is divided by a constant:

$$\text{HOMA} = \frac{I_0 X\ G_0}{405}$$

The constant 405 should be changed in S.I. units. Unlike $I_0$and the G/I ratio, the HOMA calculation compensates for fasting hyperglycemia. HOMA and I_0 values elevate in the insulin resistant patient while the G/I ratio reduces. The HOMA vale relates to well with clamp techniques and has been frequently used to asses vary in insulin sensitivity after therapy. Like HOMA, QUICKI can be applied to norm-glycemic and hyperglycemic patients [49,50]. It is derived by calculating the inverse of the sum of logarithmically expressed values of fasting glucose and insulin:

$$\text{QUICKI} = \frac{1}{[\log (I_0) + \log (G_0)]}$$

1.8-Insulin-like growth factor-II:

Insulin-like Growth Factor-2 (IGF-II), also familiar as Somatomedin-A, is a glycosylated 8 kDa Insulin family peptide hormone. It is portion of a composite system of growth and metabolic adjust proteins that is particularly essential throughout evolvement in the nervous system, adrenal cortex, and skeletal system. [51]

Somatomedin-A is peptide including of 67 amino acid residues which has 67% homology with IGF-I. It is expressed from the 30-kb IGF-2 gene found on 11p 15.5

one of a group of genes interested with growth. Liver is the major origin of IGF-II in the mature, but it is also synthesized by many other tissues, from which it is freed into parvocellular fluid [45], and the structure of IGF-II shown in figure (1.9).

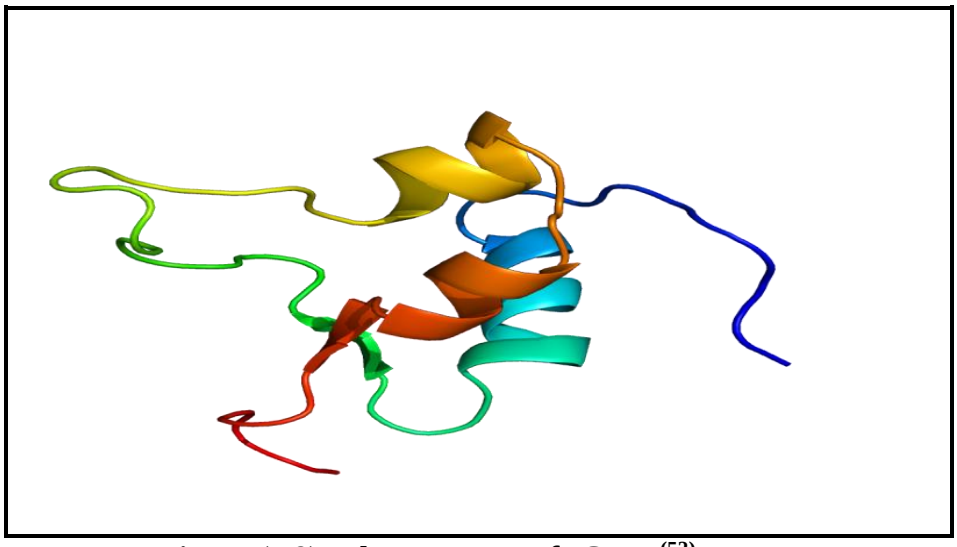


Figure (1.9): The structure of IGF-II[52].

The embryo and placenta are also copious origins. Its precursor molecule is pre pro-IGF-II, which includes a 24-residue N-terminal signal peptide and A-E domains. Segmentation of the signal peptide yields pro-IGF-II [1-156]. The E domain which proteolysis by prohormone convertase proprotein convertase 4 (PC4) to give mature IGF-II (1-67). [50]

This posttranslational operation is insufficient, outcome in a diversity of pro-IGF-II peptides, 10-18 KDa, processing all or portion of the E domain. Collectively these are called "big" IGF-II. They are excreted into blood, quantity to (10-20) % to total circulating IGF-II. The physiological function of big IGF-II is uncertain. A 34-amino acid E domain [69-102] piece called "preptin" was identified in β-cells. [53]

It is co-secreted with insulin and considered a physiological amplifier of insulin excretion. The structure of IGF-II and its post-translational operation are shown in figure (1.10) [45].

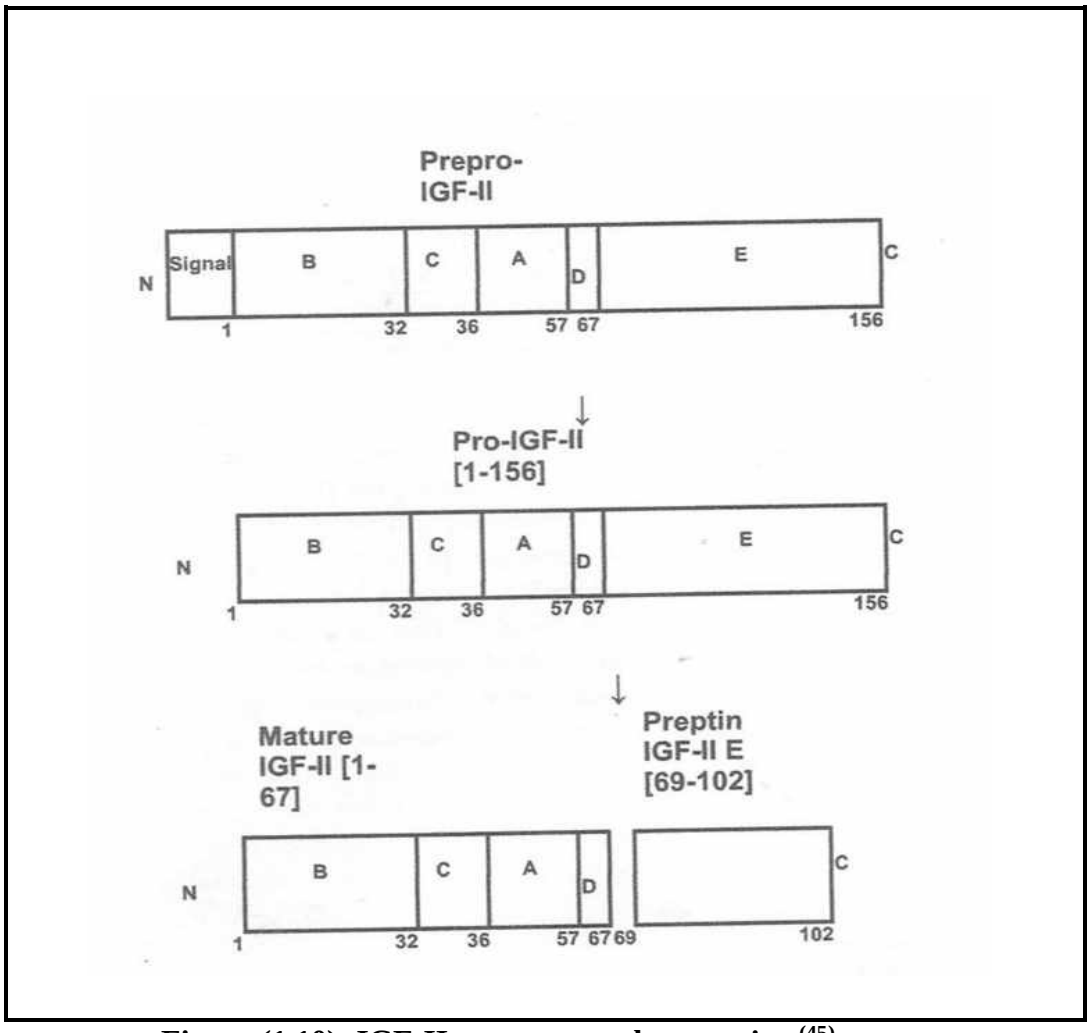


Figure (1.10): IGF-II structure and processing [45].

Serum IGF-II raises through early babyhood, remains stable through mature life and then decreases in old age [45].

1.9-Regulation of IGF-II:

The rule of IGF-II is composite. This complication may enable fine tuning of its impacts and eschew over-expression which could lead to illness. Transcription of the IGF-2 gene is regulated by a epigenetic mechanism called stamping which limits expression to the fatherly allele in the most tissue. (52)

This is achieved by methylation of the differentially methylated region (DMR) on the maternal allele stopping its transcription. Stamping can be deemed a form of control on expression without which IGF-II concentrations would be immoderate. There are four stimulates position (1-4) from which IGF 2 is transcribed. Position (2-4) governs IGF2 transcription in the embryo, whereas transcription happens from all four stimulates in the liver of mature individuals. (52)

Insulin-like growth factor binding proteins (IGFBs) antagonize the biological impacts of IGF-II by binding it in serum. This stops much release of IGF-II which could reason tumor expansion or hypoglycemia. After insulin-like growth factor binding protein-3 (IGFBP-3). Insulin-like growth factor binding protein-2 (IGFBP-2) accounts for most of the residual IGF-II binding in the circulation. (52)

1An elevate in the circulating IGFBP-1 concentration repress free IGF-II. The impacts of IGF-II on a tissue depend on the number and kind of receptors expressed. (54,55)

IGF-II also binds the kind 2 IGF receptor (IGF-2R) which terminates its work, thereby acting as tumor suppressor. IGF-II is regulated nutritionally. This is to be explicit because both IGFs are portion of a mechanism connect nutrition to growth. Their protrusion signals the existence of enough substrate to meet the protein and energy requirements of growth. (56)

IGF-II is down-regulated through under nutrition to eschew hypoglycemia. It is also regulated hormonally. Growth hormone (GH) elevates hepatic synthesis of IGFBP-3 and amyotrophic lateral sclerosis (ALS) thereby elevating triple composite formation and total serum IGF-II. Insulin promotes translocation of insulin –like growth factor receptor-1 (IGF-1R) to cell surface, stimulating IGF-II cleans [50], the regulation of IGF-II is shown in figure (1.11).

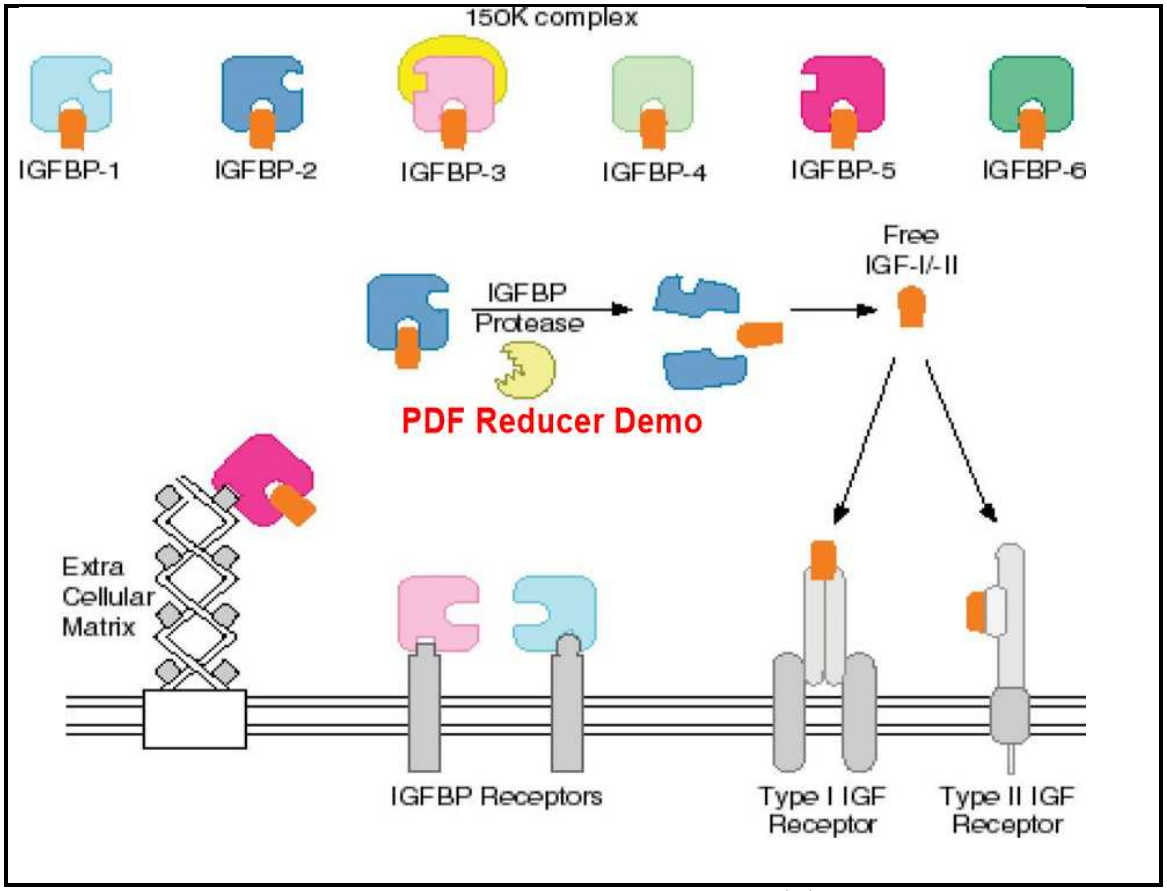


Figure (1.11): Regulation of IGf-II[57].

1.10-Dysregulation of IGF-II in metabolic diseases:

(A) Thyroid Disease:

TH are familiar to elevate IGF-I output in the liver through the enhancement of GH excretion. In situation of IGF-II however, knowledge has been rather boundary. (56)

In situation of IGF-II, knowledge is rather boundary than in IGF-I. IGF-II in patients with graves thyrotoxicosis, hypothyroidism or thyroid adenoma with specific and sensible radioimmunoassay of serum IGF-II. (56)

(B) Growth disease:

IGF-2 and growth stems from the clinical cases of Beckwith-Wiedemann syndrome (BWS) and Russell-Silver syndrome (RSS). In BWS, there is a gain of methylation at the motherly ICR leading to biallelic IGF2 gene expression andfetal excessive growth. In reverse, RSS fetuses have a phenotype of growth restriction, caused by a loss of methylation on the paternal ICR leading to biallelic loss of IGF2 expression (58).

Pro-IGF-II concentrations were normal in GH lack, consistent with its rule being detached from that of IGF-II (59).

(C) Obesity:

Obesity is a composite metabolic unrest that is characterized by an elevation in white adipose tissue mass. Clinically it is defined as a BMI of ≥30 kg/m^2, however

this definition fails to take into account sex, differences in muscle mass, and the proportional quantities of peripheral and central fat in a person topic. [58,60]

(D) Diabetes mellitus:

It is well recognized that IGF-II is dysregulated in diabetes. Overweight (25-29.9) Kg/m^2 topics that elevated serum IGF-II and IGF-2R compared with normal controls. The concentrations were often higher in topics with both states and reduced upon weight decrease. These changes may in portion reflect nutritional regulation of these proteins. [61]

Although the reason of the diabetes-related elevate in IGF-II is unknown, it could be caused by elevated excretion from adipose tissue in response to hyperglycemia.[62]

1.11-IGF-II Gene:

The IGf-II gene supplies directives for produce a protein called IGF-II. This protein playa necessary function in growth and evolution before birth. Studies propose that IGF-II helps that growth and proliferation of cells in many change tissues. Although the IGF-II gene is highly active through fetal evolution, it is much reduce active after birth. [65]

This sort of parent-specific change in gene activation is cause by a phenomenon called genomic stamping. IGF-II is portion of a cluster of genes on the short (P) arm of chromosome 11 that undergo genomic imprinting. The stamped gene IGF-II has been reported to be paternally expressed and it is a growth factor in mammals. [66]

Numerous studies have focused on analysis of Deoxyribonucleic acid (DNA) methylation at a region found upstream of the three main imprinted promoters of IGF-II individuals. This area is one of the differentially methylated regions (DMRS) contributing to the regulation of IGF-II, an imprinted gene that is expressed from paternally-derived chromosome. IGF-II encodes for a small protein that is portion of the insulin family and roles as a signaling molecule through binding to the IGF-I and insulin receptors. (67)

IGF-II protein also binds to the membrane-bound and soluble forms of the IGF-II receptor, but this leads to internalization and degradation of IGF-II in the lysosome. IGF-II has been shown to be deregulated in ueuro evolution disorders fatness and cancer and is also familiar to have a critical function in memory consolidation in the brain. (68,69)

1.12-Mutations and IGF-II:

IGF-II is a peptide hormone and member of IGF family. IGF-I and IGF-II control body growth and cell proliferation by bonding to the IGF-I receptor (IGF-1 R). Although both (IGF-I and IGF-II) are evident through fetal evolution, IGF-II has a main impact on embryonic growth, and after birth its action together with IGF-I became essential. (70)

Studies on mice declare the main function of IGF receptor pathway in growth and give a result of overgrowth due to over-expression of IGF-II. (71)

An impact on postpartum growth has also been noticed in mice where interruption of the fatherly IGF-II allele cause acute slowing of antenatal growth while disruption of the motherly allele of IGF-II has been found to have no impact on growth. (71)

Aims of the Study

The present study aims to:

Aim: The study and genetic of preptin hormone in thyroid dysfunction patients with diabetes.

Objectives:

- Studying the effect of thyroid disorder on serum preptin level.
- Know whether a person's sex affects the rise of the preptin hormone in the serum of patients with thyroid dysfunction.
- A comparative study of preptin hormone among patients with thyroid dysfunction and diabetes.
- Measurement of insulin hormone for both patients with thyroid and diabetes for the purpose of knowing which of the most influential effects on insulin sensitivity.
- clarify of the prevailing allele in Iraqi society for patients with thyroid dysfunction.
- Measurement of LH, FSH for exclusion PCOS.
- Measurement of vitamin D and calcium to exclude osteoporosis.

CHAPTER TWO

SUBJECTS AND METHODS

2.1- Chemicals, Subjects and Methods:

The Chemicals and their Suppliers which used in the study were summarized in Table (2.1):

Chemicals	Suppliers
Agarose	Conda / USA
Calcium	Spectrum, Germany
FBG	Randox,France
FSH	ichroma
HbA1c	Biotech
Insulin (Kit)	Elabscience
Ladder 100bp	Intron / Korea
LH	ichroma
Preptin (Kit)	Elabscience
Primer	IDT/Canada
Pre mixpcr	Intron / Korea
Red safe staining solution	Kapa /USA
TT3 TT4 TSH	Biomeruix
TBE buffer 10 X	Conda / USA
Vit. D3	Boditech

2.2-Instruments and Manufactures:

The instrument and manufactures which used in the study were summarized in table (2.2):

Instruments	manufactures
AURA TM PCR Cabinet	Italy
Balance	Germany/ Kernpfb
Biopette Variable Volume 2-20 ul	Germany
Bio TDB-100, Dry block thermostatbuilt	Germany/ Bio San
Combi-spin	Lative /Biosan
Electroporation	USA / CBS, Scientific
Freezer (-20ºC)	Euron, France
i-chroma laser fluoresce reader	Germany
Incubation	China / Jrad
Micro Elisa System	Thermo, Germany
Microspin 12, High-speed Mini-centrifuge	Germany/ Bio San
Microwave	China /Gosonic
Microspin	Lativa/ Biosan
Mini-Power Supply 300V, 2200V	Chain
Multi-Gene-Opti-Max Gradient Thermal Cycler	USA / Labnet
Promega Quants fluorimeter	USA
Spectrophotometer	CEIL, CE-1011 Germany
UV.transmission	Farance/Vilberlourmat
Vidas	Biomerax
V-1 plus, Personal Vortex for tubes	Germany/ Digsystem
Water distilater	China

2.3- Subjects:

2.3.1-Analytical Methods and Procedures for Characterized:

Study design:

One hundred fifty individuals with age ranged between (20-45) years were including in this study. They were divided into two groups: first part of the study was divided into five groups; group one (G1) consist of 30 patients with hypothyroidism. Group two (G2) consist of 30 patients with hyperthyroidism. Group three (G3) consist of 30 patients with diabetes. Group four (G4) consist of 30 patients with diabetes and hypothyroidism. Group five (G5) consist 0f 30 healthy individuals as a control group.

Parts two of the study (genetic study) was divided into three groups, group one (G1) consist of 30 patients with hypothyroidism. The group two (G2) consist of 30 patients with hyperthyroidism. Group three (G3) consist of 30 healthy individuals as a control group. The study was done at the specialized center for Endocrinology and Diabetes during June 2017 to August 2017. Information was collected according to the questionnaire information. People with osteoporosis, heart disease and women with polycystic ovarian cyst syndrome (PCOS) were excluded

Samples collection:

Ten milliliters of blood were divided into two parts, first part for study of biochemistry and the second part for genetic study. Blood were collected from all fasting subjects, serum obtained was used for determination of Preptin, thyroid hormones [Thyroid-stimulating hormone (TSH), Total

triiodothyronine(TT3), Total tetra iodothyronine (TT4)], and glucose, two ml of blood was put in EDTA tube for HbA1c assay, Vitamin D3 (V. D_3), Insulin, Insulin resistance, Follicle-stimulating hormone (FSH), Luteinizing hormone(LH), and Calcium.

2.3.2-Calculation of body Mass Index (BMI):

The BMI (weight in kg)/ (height in meters) ^{2} supplies a measure of proportional weight, modify for height. This allows comparisons both within and between inhabitants. The healthy range for the BMI between (18.5-24.9) Kg/ m^2, those with a BMI equal to or greater than 30 Kg/ m^2 are considered overweight, and a BMI over 35 Kg/ m^2 describe as obese is consider. BMI over 40 Kg/ m^2 considered as morbid (extreme) obesity.

2.3.3-Determination of Preptin:

Principle:

This ELISA kit uses Sandwich-ELISA as the method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human Preptin. Standards or samples are added to appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibodies specific for Human Preptin and Avidin-Horseradish Peroxidase (HRP) conjugate were added to each micro plate well successively and incubated. After incubation, free components were washed away. Then the Substrate Reagent is added to each well, only those wells that contain Human Preptin, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme substrate reaction will be terminated by adding Stop

Solution and appears yellow in color. The optical density (OD) was measured with spectrophotometry at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human Preptin. The concentration of Human Preptin in samples was calculated by comparing the OD of the samples with the standard curve.

Reagent preparation

1. All reagents was brought at room temperature (18~25℃) before use.
2. Thirty ml of Concentrated Wash Buffer was diluted with deionized or distilled water to prepare 750 ml Wash Buffer.
3. Standard working solution was centrifuged at 10,000×g for 1 min. and 1.0 ml of Reference Standard and Sample Diluent was added and let it standfor10 min and turn it upside down for several times. After it dissolves fully, we mix it thoroughly with a pipette. This reconstitution produces a stock solution of 4000 pg/ml. Then we make serial dilutions as needed. The recommended dilution gradient is as follows: 4000, 2000, 1000, 500, 250, 125, 62.50, 0 pg/ml.
4. Calculation of the required amount Biotinylated Detection Ab working solution the required amount before experiment (100 µl/well) was done in actual preparation, more account of 100~200 µl should be prepared. Centrifuge the stock tube before use, dilute the 100× Concentrated Biotinylated Detection Ab to 1×working solution with Biotinylated Detection Ab Diluent.
5. Concentrated HRP Conjugate working solution: The required amount was calculated before experiment (100 µl/well), the 100× Concentrated HRP

was diluted and Conjugated to 1× working solution with Concentrated HRP Conjugate Diluent.

Procedure:

1. Standard working solution of different concentrations was added to the first two columns: Each concentration of the solution is added into two wells side by side (100 µl for each well). Moreover, and the samples were added to other wells (100 µl for each well). Moreover, and the plate was covered with sealer provided in the kit. And incubated for 90 min at 37℃.
2. The liquid of each well was removed and was not washed and immediately 100 µl of Biotinylated Detection Ab working solution was added to each well and covered with the Plate sealer. Gently mixed up and incubated for 1 hour at 37°C.
3. The solution from each well was aspirated and 350 µl of wash buffer was added to each well and soaked for 1~2 min and we aspirate the solution from each well and let it dry against clean absorbent paper. And this wash step was repeated 3times.
4. Hundred µl of HRP Conjugate working solution was added and covered to each well with the Plate sealer and incubated for 30 min at 37℃.
5. The solution from each well was aspirated and the wash process was repeated for five times as conducted in step 3
6. Ninety µl of Substrate Reagent was added to each well and covered to each well with a new plate sealer. And incubated for about 15 min at 37°C.The plate was protected from light.
7. Fifty µl of Stop Solution to each well was added

8. The optical density (OD value) was determined of each well at once with a micro-plate reader set to 450 nm.

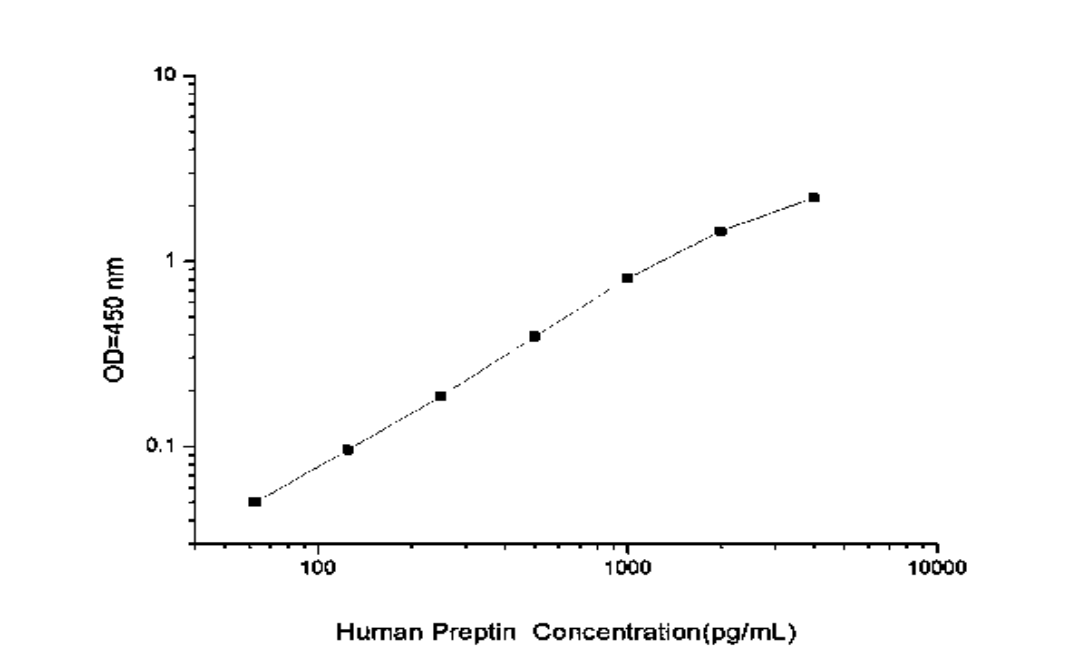


Figure (2.1): Standard curve of preptin.

2.3.4-Determination of total triiodothyronine (TT3):

Principle:

The assay principle combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA). The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and predisposed in the sealed reagent strips. All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times. The sample was taken

and transferred into the well containing the T3 antigen labeled with alkaline phosphatase (conjugate). Competition occurs between the antigen present in the sample and the labeled antigen for the specific anti-T3antibodies (sheep) coated on the interior of the SPR. Unbound components are eliminated during washing steps. During the final detection step, the substrate (4-Methylumbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methylumbelliferone) the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is inversely proportional to the concentration of antigen present in the sample. At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

Required equipment and Reagent:

The SPR

The interior of the SPR is coated during production with anti-T3 monoclonal antibodies (sheep). Each SPR s identified by the T3 code.Only remove the required number of SPRs from the pouch and reseal the pouch correctly after opening.

The strip

The strip consists of 10 wells covered with a labeled, foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the

fluorometric reading is performed. The wells in the center section of the strip contain the various reagents required for the assay.

Description of the T_3 strip

Wells	Reagents
1	Sample well.
2 - 3 - 4 - 5	Empty wells.
6	Conjugate: alkaline phosphatase labeled T3 derivative + ANS (0.95 mmol/l) + sodium salicylate (11.9 mmol/l) + 1 g/l sodium azide (400 µl).
7-8-9	Wash buffer: Tris, NaCl (0.05 mol/l) pH 7.4 + 1 g/l of sodium azide (600 µl).
10	Cuvette with substrate: 4-methyl-umbelliferyl phosphate (0.6 mmol/l) + diethanolamine (0.62 mol/l or 6.6%, pH 9.2) + 1 g/l sodium azide (300 µl)

Procedure:

1. We removed the required reagents from the refrigerator and allow them to come to room temperature for at least 30 minutes.
2. One T3 strip and one T3 SPR was used for each sample, control or calibrator to be tested.
3. "T3" was selected on the instrument to enter the test code. The calibrator was identified by "S1", and tested in triplicate. For the control to be

tested, it was identified by "C1".

4. The calibrator, control and samples was mixed using a Vortex type mixer.
5. Hundred µl of calibrator, sample or control was pipetted into the sample well.
6. The surface plasma resonance (SPRs) and strips was inserted into the instrument.
7. All the assay steps was performed automatically by the instrument. The assay was completed within approximately 40 minutes.
8. After the assay is completed, we remove the SPRs and strips from the instrument.
9. The used SPRs and strips were disposed into an appropriate recipient.

2.3.5- Determination of Total thyroxine (TT4):

Principle:

The assay principle combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA). The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and predisposed in the sealed reagent strips. All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times. The sample is taken and transferred into the well containing the T4 antigen labeled with alkaline phosphatase (conjugate). Competition occurs between the antigen present in the sample and the labeled antigen for the specific anti-T4 antibodies coated on the interior of the SPR. During the final detection step, the substrate (4-Methylumbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-

Methylumbelliferone) the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is inversely proportional to the concentration of antigen present in the sample.

Required equipment and Reagent:

The SPR

The interior of the SPR is coated during production with anti-T4 monoclonal antibodies (mouse). Each SPR s identified by the T4 code. Only remove the required number of SPRs from the pouch and reseal the pouch correctly after opening**.**

The strip

The strip consists of 10 wells covered with a labeled, foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the fluorometric reading is performed. The wells in the center section of the strip contain the various reagents required for the assay.

Description of the T_4 strip:

Wells	Reagents
1	Sample well.
2 - 3 - 4 - 5	Empty wells.
6	Conjugate: alkaline phosphatase labeled T4 derivative + ANS (0.8 mmol/l) + sodium salicylate (9.3 mmol/l) + 1 g/l sodium azide (400 µl).
7	Wash buffer: Tris, NaCl (0.05 mol/l) pH 7.4 + 1 g/l of sodium azide (600 µl).
8	Wash buffer: Tris-Tween, NaCl (0.05 mol/l) pH 7.4 + 1 g/l sodium azide

	(600 µl).
9	Wash buffer: diethanolamine (1.1 mol/l or 11.5%) pH 9.8 + 1 g/l sodium azide (600 µl).
10	Cuvette with substrate: 4-methyl-umbelliferyl phosphate (0.6 mmol/l) + diethanolamine (0.62 mol/l or 6.6%, pH 9.2) + 1 g/l sodium azide (300 µl)

Procedure:

1. The required reagents was removed from the refrigerator and allowed to come to room temperature for at least 30 minutes.
2. One T4 strip and one T4 SPR was used for each sample, control or calibrator to be tested.
3. "T4" was typed on the instrument to enter the test code. The calibrator was identified by "S1", and tested in triplicate. For the control to be tested, it was identified by "C1".
4. The calibrator, control and sampleswas mixed using a Vortex type mixer.
5. Two hundred µl of calibrator, sample and control were pipetted into the sample well.
6. The SPRs and strips were inserted into the instrument.
7. All the assay steps were performed automatically by the instrument. The assay was completed within approximately 40 minutes.
8. After the assay was completed the SPRs and strips was removed from the instrument.

2.3.6-Determination of Thyroid Stimulating Hormone:

Principle:

The VIDAS _ Thyroid Stimulating Hormone assay is ELFA that is performed in an automated VIDAS _ instrument. All assay steps and assay temperature are controlled by the instrument. A pipette tip-like disposable device, the Solid Phase Receptacle (SPR), serves as a solid phase for the assay as well as a pipetting device. The SPR is coated at the time of manufacture with mouse monoclonal anti-TSH antibodies. The VIDAS _ TSH assay configuration prevents nonspecific reactions with the SPR. Reagents for the assay are located in the sealed Reagent Strips. The sample is transferred into the well containing anti-TSH antibody conjugated with alkaline phosphatase. The sample/conjugate mixture is cycled in and out of the SPR and the TSH will bind to antibodies coated on the SPR and to the conjugate forming a "sandwich". Wash steps remove unbound conjugate. A fluorescent substrate, 4-methylumbelliferyl phosphate, is cycled through the SPR. Enzyme remaining on the SPR wall will catalyze the conversion of the substrate to the fluorescent product 4-methylumbelliferone. The intensity of fluorescence is measured by the optical scanner in the instrument; it is proportional to the TSH concentration present in the sample. When the VIDAS _ TSH assay is completed, the results are analyzed automatically by the computer, and a report is printed for each sample [(74)].

Procedure:

1. The necessary components from the kit was removed and all unused components were returned to storage at 2-8°C.
2. The components were allowed to reach room temperature (approximately 30 minutes).
3. The TSH Reagent Strips were labeled with the appropriatesample identification numbers.
4. The Calibrator, Control and sera werevortexed.
5. Two hundered µl of sera, Control or Calibrator were pepitted into the sample well of each TSH Reagent Strip.
6. The TSH Reagent Strips and SPRs were loaded into appropriate instrument section positions.
7. All theassay steps are performed automatically by the instrument. The assay are completed in approximately 40 minutes.

2.3.7- Determination of serum glucose:

Principle:

Glucose was determined after enzymatic oxidation in the presence of glucose oxidase (GOD) [75]. The formed hydrogen peroxide reacts under catalysis of peroxidase (POD), with phenol and 4-aminophenazone to form a red –violet quinoneimine dye as indicator.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{POD}} \text{quinoneimine} + 4H_2O$$

Reagents:

Reagent type	Material	concentration
Reagent(1) Buffer	**Phosphate buffer** **Phenol**	**0.1 mol/L, PH7** **11 mmol/L**
Reagent(2)GOD-POD	**4-aminophenazone** **Glucose oxidase** **Peroxidase**	**0.77 mmol/L** **>1.5KU/L** **>1.5KU/L**
Standard	**Glucose**	**5.55 mmol/L** **(100mg/dl)**

Procedure:

Working solution: The contents of one vial of reagent 2 was reconstituted with a portion of buffer 1 and then transfer entire contents to bottle 1, with rinsing several times.

The series of following tubes are prepared as follows:

	Blank	Standard	Test
Standard	--	10µl	-
Serum	--	--	10µl
Distilled water	10µl	--	--
Working solution	1000 µl	1000 µl	1000 µl

The tubes were mixed and incubated for 10 min at 37 ºC, the absorbance was measured for the standard (A standard) and the test (A test) against the blank at 500nm.

Calculations:

$$\text{Concentration of glucose (mg/dl)} = \frac{\Delta \text{ A test}}{\Delta \text{ A Standard}} \text{ x Standard Conc. (100)mg/dL}$$

Normal value (fasting) =70-120 mg/dl.

2.3.8- Determination of calcium:

Principle:

The method is based on the specific binding of o-cresolftalein complexone (OCC), a metal-chromic indicator, and calcium at alkaline PH with the resulting shift in the absorption wavelength of the complex. The intensity of the chromophore formed is proportional to the concentration of total calcium in the sample [(76)].

$$\text{OCC + Calcium} \xrightarrow{\text{PH 10.7}} \text{OOC-Calcium complex}$$

Reagent composition:

R1	OCC indicator. O-Crespolphtaleincomplexone0.16 mmol/L, HCl 60mmol/L, 8-quinolinol 7 mmol/L.
R2	OCC buffer. AMP 0.35 mol/L, PH 10.7.
CAL	Calcium/ Maggnesium standard. Calcium 10 mg/dL / Magnesium 2 mg/dL. Organic matrix based primary standard. Concentration value is traceable to standard Reference material 099b.

Procedure:

1. Reagents and samples was brought at room temperature, and pipetted into labelled test tubes and mixed.

	Blank	Standard	Test
Working reagent	1.0 mL	1.0 mL	1.0 mL
Sample (serum)	--	--	10µl
CAL. Standard	--	10µl	--

2. The tubes were let to stand 2 minutes at room temperature.
3. The absorbance (A) of the samples and the standard were read at 570 nm against the reagent blank.
4. The color was stable for at least 1 hour.

Calculations:

$$\frac{\text{A sample}}{\text{A standard}} \times \text{C standard (10)} = \text{mg/dl Total calcium}$$

2.3.9- Determination of Vitamin D3:

Principle:

This ELISA kit uses Competitive-ELISA as the method. The micro ELISA plate provided in this kit has been pre-coated with VD3. During the reaction, VD3 in the sample or standard competes with a fixed amount of VD3 on the solid phase supporter for sites on the Biotinylated Detection Ab specific to VD3. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each

microplate well and incubated. Then the Substrate Reagent is added to each well. The enzyme-substrate reaction is terminated by adding Stop Solution and the color change can be measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of VD3 in samples can be calculated by comparing the OD of the samples with the standard curve.

Reagent preparation:

1. All reagents was brought to room temperature (18~25℃) before use. Preheat the Microplate reader was heated for 15 min before OD measurement.
2. Thirty ml of Concentrated Wash Buffer was diluted with distilled water to prepare 750 ml Wash Buffer.
3. Standard working solution was centrifuged at 10,000×g for 1 min. and 1.0 ml of Reference Standard and Sample Diluent was added and let to stand for 10 min and turned upside down for several times. After it dissolves fully, we mix it thoroughly with a pipette. This reconstitution produces a stock solution of 100 ng/ml. Serial dilutions were med as needed. The recommended dilution gradient is as follows: 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0 ng/ml.
4. The required amount Biotinylated Detection Ab working solution was calculated before experiment (50 µl/well). The stock was centrifuged tube before use, dilute the 100× Concentrated Biotinylated Detection Ab was diluted to 1×working solution with Biotinylated Detection Ab Diluent.
5. The required amount of Concentrated HRP Conjugate working solution was

calculated the required amount before experiment (100 µl/well). The 100× Concentrated HRP Conjugate was diluted to 1× working solution with Concentrated HRP Conjugate Diluent.

procedure:

1. Standard working solution of different concentrations was added to the first two columns: Each concentration of the solution was added into two wells side by side (50 µL for each well). Immediately added 50µl of Biotinylated Detection Ab working solution to each well. The plate was covered with sealer provided in the kit. Incubated for 45 min at 37℃.
2. The solution from each well was Aspirated, 350 µl of wash buffer was added to each well. And soaked for 1~2 min the solution was aspirated from each well and let to dry against clean absorbent paper. This wash step 3 was repeated times.
3. Hundred µl of HRP Conjugate working solution was added to each well. And cover with the Plate sealer and incubated for 30 min at 37°C.
4. The solution was Aspirated from each well, the wash process for five times as conducted in step 3.
5. Ninety µl of Substrate Reagent was added to each well and covered with a new plate sealer. Incubated for about 15 min at 37°C. The plate was protected from light.
6. Fifty µl of Stop Solution was added to each well.
7. The optical density (OD value) of each well was determined at once, using a micro-plate reader set to 450 nm.

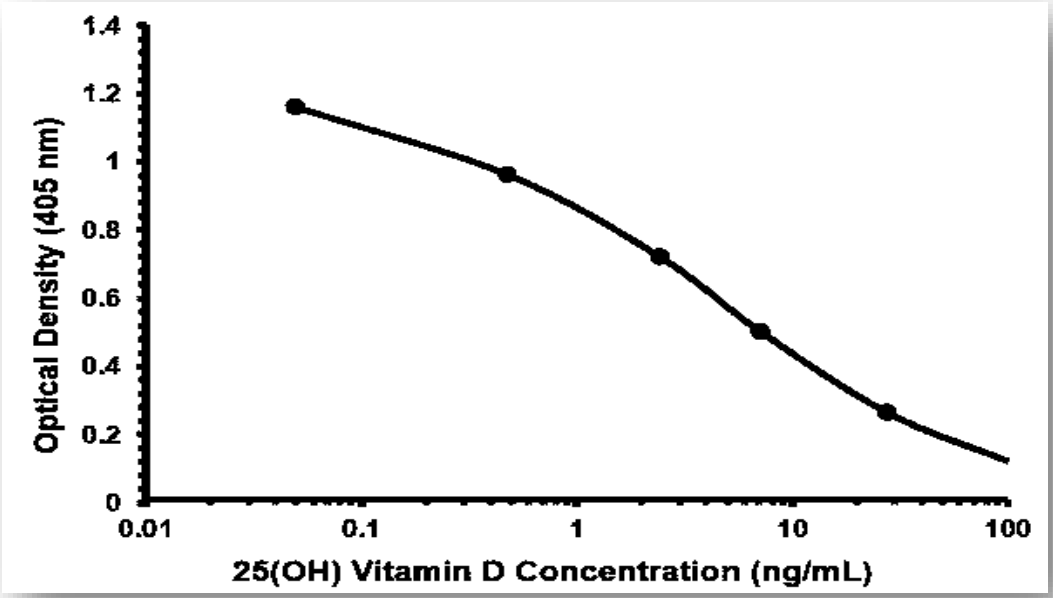


Figure (2.3): Standard curve of vitamin D3.

2.3.10- Determination of Insulin:

Principle:

This ELISA kit uses Sandwich-ELISA as the method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human insulin. Standards or samples are added to appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibodies specific for Human INS and Avidin Horseradish Peroxidase (HRP) conjugate are added to each micro plate well successively and incubated. After incubation, free components are washed away. Then the Substrate Reagent is added to each well, only those wells that contain Human

INS, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme substrate reaction will be terminated by adding Stop Solution and appears yellow in color. The optical density (OD) can be measured with spectrophotometry at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human INS. The concentration of Human INS in samples can be calculated by comparing the OD of the samples with the standard curve.

Reagent preparation:

1. All reagents was brought to room temperature (18~25℃) before use. The Microplate reader was heated for 15 min before OD measurement.
2. Thirty ml of Concentrated Wash Buffer was diluted with or distilled water to prepare 750 ml Wash Buffer.
3. Standard working solution was centrifuged the standard at 10,000×g for 1 min. 1.0 ml of Reference Standard and Sample Diluent was added and, let stand for 10 min and turned upside down for several times. After it dissolves fully, we mixed it thoroughly with a pipette. This reconstitution produces a stock solution of 20 ng/ml. Serial dilutions were med as needed. The recommended dilution gradient is as follows: 20, 10, 5, 2.5, 1.25, 0.63, 0.31, 0 ng/ml.
4. The required amount Biotinylated Detection Ab working solution was calculated before experiment (100 µl/well). The stock tube was centrifuged before use, dilute the 100× Concentrated Biotinylated Detection Ab was diluted to 1×working solution with Biotinylated Detection Ab Diluent.

5. The required amount Concentrated HRP Conjugate working solution was calculated before experiment (100 µl/well). The 100× Concentrated HRP Conjugate was diluted to 1× working solution with Concentrated HRP Conjugate Diluent.

Procedure:

1. Standard working solution of different concentrations was added to the first two columns: Each concentration of the solution is added into two wells side by side (100 µl for each well). And the samples was added to other wells (100 µL for each well). And the plate was covered with sealer provided in the kit and incubated for 90 min at 37℃.
2. The liquid of each well was removed and washed and immediately 100 µl of Biotinylated Detection Ab working solution was added to each well and covered with the Plate sealer. Gently mixed up and incubated for 1 hour at 37°C.
2. The solution from each well was aspirated, and 350 µl of wash buffer was added to each well and soaked for 1~2 min and we aspirate the solution from each well and let it dry against clean absorbent paperand this wash step 3 times.
3. Hundred µl of HRP Conjugate working solution to each well and covered with the Plate sealer and incubated for 30 min at 37°C.
4. The solution was from each well was Aspirated, the wash process was repeated for five times as conducted in step 3.

5. Ninety µl of Substrate Reagent was added to each well. And covered with a new plate sealer. Incubated for about 15 min at 37°C. The plate was protected from light.

7. Fifty µl of Stop Solution to each well was added.

8. The optical density (OD value) was determined of each well at once with a micro-plate reader set to 450 nm.

2.3.11-Determination of Hemoglobin A1c (HbA1c):

Principle

Glycated protein is formed post-translationally through the slow, non-enzymatic reaction between glucose and amino groups on proteins. HbA1c is a clinically useful index of mean glycemia during the preceding 120 days, the average life span of erythrocytes. Carefully controlled studies have documented a close relationship between the concentrations of HbA1c and mean glycemia. HbA1c is considered as a more reliable parameter in monitoring glycemia over the glycemic reading with the conventional glucometer. i-CHROMATM HbA1c is based on the fluorescence immunoassay technology, specifically the competition immune-detection method. Whole blood is added to the mixture of hemolysis buffer and detection buffer, which results in hemolysis of red blood cells. The mixture containing HbA1c from the hemolyzed red blood cells and fluorescence-labeled HbA1c peptides from detection buffer is loaded onto the sample well of the Cartridge. The mixture then migrates through the nitrocellulose matrix of the test strip by capillary action. HbA1c from the blood competes with fluorescence-labeled HbA1c peptides for binding sites on HbA1c antibodies fixed on the nitrocellulose matrix. As a result, the higher

concentration of HbA1c produces a lower fluorescence signal from HbA1c-peptides. The signal is interpreted and the result displayed on i-CHROMATM Reader in units of percentage.

Materials Supplied:

Box contains:

Cartridge 25 pouches

ID Chip 1 each

Insert 1 sheet

Detection Buffer 1 vial

Hemolysis Buffer 25 tubes

Procedure:

1. One hundred µl detection buffer was drawn and it was added into hemolysis buffer tube.
2. Five µl whole blood was drawn, the blood was mixed before drawing.
3. The hemolysis buffer tube was shake for 15 times.
4. The cartridge was taken half way out after 5 minutes.
5. The sample mixture was drawn.
6. The sample was loaded:
   * One hundred fifteen µl was applied in the Hb well.
   * Seventy-five µl was applied in the A_1c well.
7. The cartridge was inserted into i-chumber, and it was leaved in i-chumber for 12 minutes before removing.
8. The cartridge was inserted into i-chroma reader.
9. Button was pressed.
10. The result was read

2.3.12-Determination of Follicle-stimulating hormone (FSH):

Principle:

The test uses a sandwich immune detection method; the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip.

The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody, which is processed by instrument for ichroma tests to show FSH concentration in sample[77].

Materials Supplied:

Components of ichroma FSH

- Cartridge Box:
- Cartridges 25
- ID Chip 1
- Instruction for Use 1
- Box containing Detection Buffer Tubes
- Detection Buffer tubes 25

Procedure:

1. Transfer 150 µl (Human serum/ plasma/ control) of sample using a transfer pipette to a tube containing the detection buffer.
2. Close the lid of the detection buffer tube and mix the sample thoroughly by shaking it about 10 times.
3. Pipette out 75 µl of a sample mixture and load it into the sample well on the cartridge.

4. Leave the sample-loaded cartridge at room temperature for 15 minutes.
5. To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.
6. Press "select" button on the instrument for ichroma tests to start the scanning process.
7. Instrument for ichroma tests will start scanning the sample-loaded cartridge immediately.
8. Read the test result on the display screen of the instrument for ichroma tests.

2.3.13- Determination of Luteinizing hormone (LH):

Principle:

The test uses a sandwich immune detection method; the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip.

The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody, which is processed by instrument for ichroma tests to show LH concentration in sample[(78)].

Materials Supplied:

Components of ichroma LH

- Cartridge Box:
- Cartridges 25
- ID Chip 1
- Instruction for Use 1
- Box containing Detection Buffer Tubes
- Detection Buffer tubes 25

Procedure:

1. Transfer 150 µl (Human serum/ plasma/ control) of sample using a transfer pipette to a tube containing the detection buffer.
2. Close the lid of the detection buffer tube and mix the sample thoroughly by shaking it about 10 times.
3. Pipette out 75 µl of a sample mixture and load it into the sample well on the cartridge.
4. Leave the sample-loaded cartridge at room temperature for 15 minutes.
5. To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.
6. Press “select” button on the instrument for ichroma tests to start the scanning process.
7. Instrument for ichroma tests will start scanning the sample-loaded cartridge immediately.

8. Read the test result on the display screen of the instrument for ichroma tests.

2.3.14-Molecular Biological Studies:

2.3.14.1- DNA Extraction:

Principle:

DNA was extracted from the samples by wizard genomic (DNA purification kit) according to the isolating genomic DNA from 200 µl whole blood in each case. The volume of the extracted DNA solution was usually 100 µl were stored at -20ºC.

Kit Contents of wiz Prep™ gDNA, korea biotechnology and cat.No.

Contents	Contents 200 Columns
Buffer BL	90 ml
Buffer WA^1	160 ml
Buffer WA^2	40ml
Elution Buffer	10 ml
Proteinase K (lyophilized)	3 mg X 4 vials
DNA spin columns	200 pcs
Collection Tubes	200 pcs

procedure

1. Two hundred µl was Pipetted of whole blood into a 1.5 ml micro centrifuge tube.
2. Twenty µl was added of Proteinase K and 5 µl of RNase a Solution into sample tube and gently mix.

3. Two hundred µl was added of Buffer BL into upper sample tube and mix thoroughly3.
4. The lysate was incubated at 56°C for 10 min.
5. The 1.5 ml tube was centrifuged briefly to remove drops from the inside of the rid.
6. Two hundred of absolute ethanol was added into the lysate, and the well was mixed by gently inverting for 5 - 6 times or by pipetting.
7. The mixture from step 6 was applied to the Spin Column (in a 2 ml Collection Tube) without wetting the rim, close the cap, and centrifuged at 13,000 rpm for 1 min.
8. Seven hundred µl of Buffer WA was added to the Spin Column without wetting the rim, and centrifuged for 1 min at 13,000 rpm.
9. Seven hundred was added of Buffer WB to the Spin Column without wetting the rim, and centrifuge for 1 min at 13,000 rpm.
10. The Spin Column was placed into a new 1.5 ml tube.

2.3.14.2 Determination of DNA Concentration:

Procedure:

1. Two hundred µl of Tris-EDTA (TE) was added to 3,800µl of D. water the 4000 µl was mixed, the10 µl pulled ignore and 10 µl of dye (DNA Di) was added.
2. Two hundred µl of each sample was Pulled of the mix for each sample.
3. The series of the following tubes were prepared as follows:

	Blank	Standard	Sample
Standard	----	2µl	----
DNA Extraction	----	----	2µl
Mix	200 µl	200 µl	200 µl

4. Vortex was made for second to mix.
5. The samples were leaved on rake at room temperature for 5 min.
6. The value was extracted from the device immediately.

2.3.14.2- Polymerase Chain Reaction (PCR):

A. principle:

The polymerase chain reaction (PCR)[79],is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. PCR technique often requires extensive optimization produced because primer dimer and other non- specific produced may interfere with the amplification of specific products. (Denaturation, annealing and extension). PCR is useful in the investigation and diagnosis of a growing number of diseases. In this study Qualitative PCR, protocol was used to detect the presence IGF-II gene.

B. Preparation of Primers:

The primer used to identify and amplify the PCR-RFLP method as described by Henrik Berg Rasmussen *et.al*[80]. IGF-2 (rs 680) polymorphism. A fragment with the size of 292 bp and 229bp of IGF-II gene was amplified with a specific

primer. The lyophilized primers were, dissolved in free DdH_2O to give a final concentration of (100 pmol/µl) as stock solution and the stock was kept at (-20 ºC) to prepare (10 pmol/µl) concentration as work primer suspended, (10 µl) of the stock solution (90µl) of the free DdH_2O water was added to reach a final volume (100 µl).

C. Primers Selection:

Materials of polymerase chain reaction for IGF-II (OLIGO POOLS) as shown in table (2-1):

Table (3- 3): Primers used in study.

Primer	IGF2 Sequence	Tm (ºC)	GC (%)	Product size
Forward	**5'-CTT GGA CTT TGA AGT CAA ATT GG - 3'**	**52.6**	**39.1**	**229bp and 292 bp**
Reverse	**5'-GGT CGT GCC AAT TAC ATT TCA- 3'**	**53.6**	**42.9**	

A. Working Principle of PCR:

Polymerase chain reaction (PCR) is chain reaction which is a small fragment of the DNA section of interest, identified which serves as the template for producing the primers that initiate the reaction. One DNA molecule is used to produce two copies, then four, then eight and so forth. This continuous doubling is accomplished by specific proteins known as polymerases enzymes that are able to string together individual DNA building blocks to form long molecular strands. To do their job polymerases require a supply of DNA building blocks, i.e. the nucleotides consisting of the four bases adenine (A), thymine (T),

cytosine (C) and guanine (G). They also need a small fragment of DNA, known as the primer, to which they attach the building blocks as well as a longer DNA molecule to serve as a template for constructing the new strand. If these three ingredients are supplied, the enzymes will construct exact copies of the templates. PCR contained 2µl genomic DNA, 5µl Master Mix,1µl of primer forward, 1µl of primer reverse and 16distilled water. Thermal cycling condition for the IGF-IIwas: initial denaturation 1 step for 3 minutes at95°C, followed by 1 cycles and {denaturation 2 step for 45 seconds at 95°C, annealing step for 45 seconds at 54°C and extension 1 step for 45 seconds at 72 °C} followed by 35 cycle. The final extension 2 step was performed at 72°C for 7 minutes. The essential components of polymerase chain reaction were adopted as seen in table (2-6).

Table (2-4): The mixture of working solution.

PCR components	Amount
PCR Master Mix	5µl
Primer Forward(10 pmol / µl)	1µl F
Primer Reverse(10 pmol / µl)	1µl R
Distilled water (D.W)	16µl
DNA template	2µl
Final volume	25µl

A. PCR Program:

Table (2-5): PCR program that was applied in the thermocyler devices.

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	3 min.	35 cycle
2-	Denaturation -2	95°C	45sec	
3-	Annealing	54°C	45sec	
4-	Extension-1	72°C	45sec	
5-	Extension -2	72°C	7min.	

Gradient Annealing	48	50	52	54	56	58

The products of PCR from amplification of IGF-IIwas then electrophoreses on2% agarose gel stained with Red stain. The presence of bands of 292bpand 229 bp were indicatives of the IGF-II genotypes.

2.3.14.3-Preparation of Tris-Borate EDTA Buffer(TBE10X) (TBE buffer):

1X Tris Boric EDTA buffer prepared by dilution the stock solution (TBE10X) buffer by using 100 ml of 10X TBE to 900 ml of distilled water to prepared 1 litter.

2.3.14.4-PCR-RFLP and genotyping:

Rs680/IGF-II genes:

PCR-restriction fragment length polymorphism (RFLP)-based analysis, is a common technique for genetic analysis. It has been utilized for the detection of intra species as well as interspecies variation. Simple PCR-RFLP was used to detect the rs 680 variant, because the C to G Trans version creates a new Apa-1 restriction site. The primers used for amplification of IGF-II, was performed by polymerase chain reaction (PCR), with the use of specific published primers. The primer used for amplification of IGF-II gene 3 exon Forward primer: 5'-CTTGGACTTTGAAGTCAAATTGG-3'; Reverse primer: 5'GGTCGTGC ATT ACATTTCA-3' synthesized from sigma - Aldrich Chemical Pvt Limited (Bangalore, India), followed by restriction fragment length polymorphism (RFLP) analysis. A three-step PCR was performed using XP thermal cycler as described by us earlier.11 Briefly the PCR conditions included an initial denaturation at 95ºC for 3 minutes, followed by 35 cycles of denaturation at 95ºC for 45 seconds, annealing at 54ºC for 45 seconds and extension at 72ºC for 45 seconds, final extension at 72ºC for 7 minutes. The 292 bp amplified PCR product was digested with Apa1 enzyme at 37ºC for 2 hours and electrophoresed on 2% agarose gel with ethidium bromide. Bands of 229 bp were observed in case of GG genotype, 292 bp and 229 bp in AG genotype and an undigested 292 bp band in AA genotype. Restriction enzyme digested PCR products were imaged and analyzed by documentation in UVI Tech gel documentation system (UVI Tech Ltd., Cambridge, United Kingdom). Restriction fragment length polymorphism (RFLP) cut a DNA sequence by using restriction enzymes (Apa 1)in to pieces; the action of this enzyme is

specific [63]. After PCR, amplification product was digested the reaction was conducted in 10µl final volume; at (37°C for 30 hours), for genotyping of studied samples, (table 2.6), the digested fragments were electrophoresed on 2% agarose gel mixed with red stain. Table (2.6): Cut Smarter with Restriction Enzymes(Apal).

Table (2-6): Cut Smarter with Restriction Enzyme (Apa 1).

Component	Reaction size
Restriction Enzyme (Apa 1)	0.5 µl
DNA.	5 µl
10X NE Buffer	4.5 µl
Total Volume	10 µl
Temperature of reaction and time	37°C ,30 min

2.3.15-Statistical analysis:

The data were statistically analyzed by using the computer IBM SPSS program version 25. The data were expressed as mean ± SE, ANOVA table and Duncan test were used to express the significant differences among the studied groups. While, in genotype and allele frequency, the Hardy-Weinberg equilibrium was used to find the observed and expected frequencies, chi-square and the probability value [81].

CHAPTER THREE

RESULTS AND DISCUSSION

3. Results and Discussion:

3.1. Part 1 (Biochemistry Study)

3-1-1-Body Mass Index (BMI):

The mean value ± SE of BMI for (G3) hypothyroidism, (G2) hyperthyroidism, (G4) diabetes, (G5) (diabetes and hypothyroidism), and (G1) control are respectively shown in table (3-1), and figure (3-1).

Table (3-1): Mean ± SE BMI values studied groups according to the gender

Gender	Mean ± SE of BMI (Kg/ m^2)				
	G1	G2	G3	G4	G5
Male	37.9 ± 0.5 a	21.4 ± 0.5 a	34.9 ± 0.8 a	38.3 ± 0.4 a	25.0 ± 0.8 a
Female	38.1 ± 0.5 a	22.2 ± 0.5 d	35.9 ± 1.0 b	38.9 ± 0.3 a	24.9 ± 1.0 c

Similar letters: No significant difference (p> 0.05) between means
Different letters: Significant difference ($p \leq 0.05$) between means

The results show a non-significant change of all groups (p> 0.05) in male but in female show significant decrease in hyperthyroidism and diabetes groups compared to hypothyroidism and diabetes with hypothyroidism groups ($p \leq 0.05$). Obesity represent energy inlet from foods that exceed energy spending in physical activity. The two most popular endocrine problems diagnosed and found in clinical practice in different ages are diabetes mellitus (DM) and thyroid dysfunction (TD). The higher BMI levels in females than in males have previously been reported in population studies (40). The hormones of thyroid gland control metabolism processes of carbohydrate in the body and on the other hand, DM impacts thyroid profile [82]. The results of BMI effect for diabetic patients in this study have been consistent with the results reached by the study done by Yingying S., *et al* which concluded that BMI is a major risk factor for diabetes [83].

Figure (3-1): BMI in studied groups according to the gender.

3-1-2-Preptin Hormone:

The mean value ± SE of Preptin for hypothyroidism (G1), hyperthyroidism (G2), diabetes (G3), diabetes & hypothyroidism (G4), and control (G5) are respectively shown in table (3-2), and figure (3-2).

Table (3-2): Mean± SE of preptin concentration in groups under investigation according to the gender

Gender	Mean ± SE of Preptin (Pg/ mL)				
	G1	G2	G3	G4	G5
Male	2638.4 ± 280.0 b	589.0 ± 90.1 d	2085.5 ± 282.8c	3314.3 ± 177.3 a	384.7 ± 62.4 d
Female	2960.4 ± 256.6 b	993.2 ± 103.9 d	2465.6 ± 282.4 c	3179.4 ± 265.7 a	427.8 ± 60.4 d

Similar letters: No significant difference ($p > 0.05$) between means
Different letters: Significant difference ($p \leq 0.05$) between means

The results show a non- significant change in hyperthyroidism as compared with groups ($p > 0.05$) with a significant increased in hypothyroidism, diabetes, and (diabetes with hypothyroidism) ($p \leq 0.05$), for males and females, there was no literature to explain the relationship between the hormone preptin and thyroid dysfunction, but the interpretation can be predicted the mechanism of hormone preptin that in the case of hunger will need the body to energy and this energy comes from glucose and when eating a meal will rise blood sugar ratio, which leads to rise of insulin hormone and therefore increases the hormone preptin also in the case of hypothyroidism suffers from slow metabolism, high insulin hormones well as preptin hormone, vice versa in case of hyperthyroidism. Results were matched with studies Gangyi Y., *et al* But diabetes mellitus show a significant this is confirmed by literature where it was shown the concentration of preptin levels were higher in patients with diabetes mellitus [84].

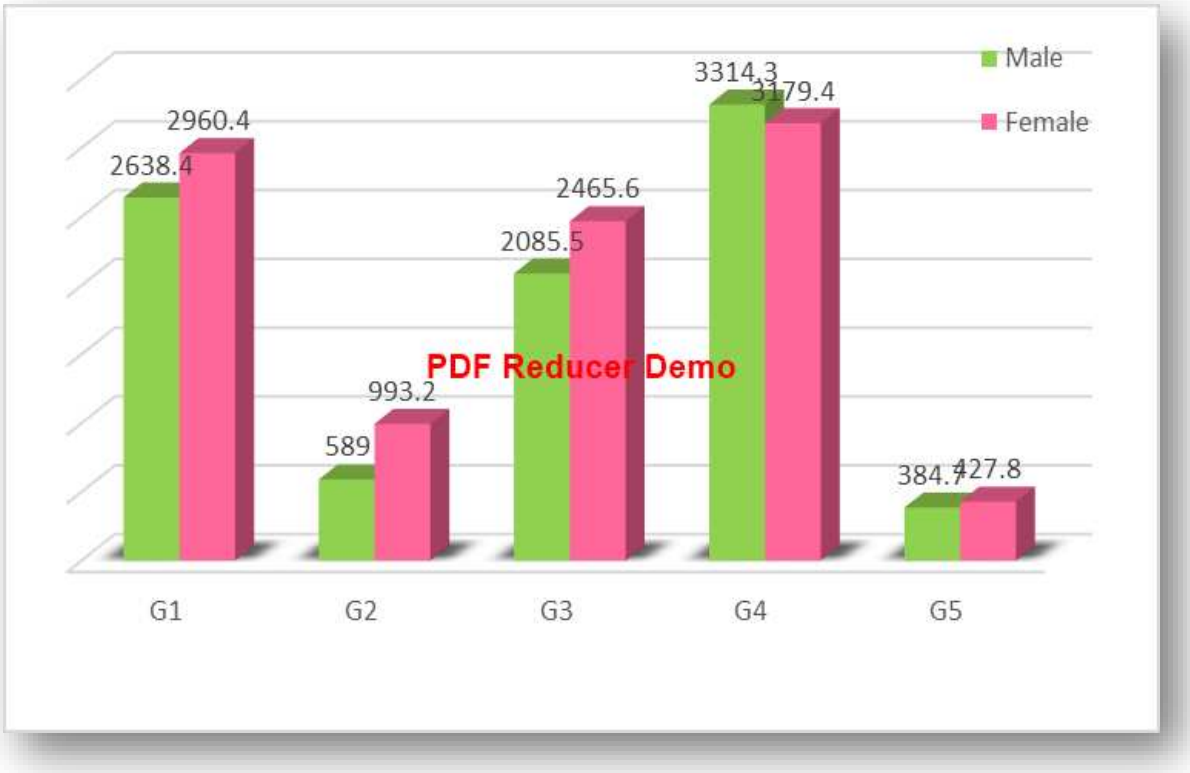


Figure (3-2): Preptin concentration in studied groups according the gender

3-1-3-Serum glucose:

The mean value ± SE of serum glucose for hypothyroidism (G1), hyperthyroidism (G2), diabetes (G3), (diabetes with hypothyroidism) (G4), and control (G5) are respectively shown in table (3-3), and figure (3-3).

Table (3-3): Serum glucose concentration in groups under investigation according to the gender

Gender	Mean ± SE of Serum glucose conc. (mg/dl)				
Male	G1	G2	G3	G4	G5
	88.0 ± 2.8 b	83.0 ± 2.1 b	332.8 ± 16.6 a	342.7 ± 25.5 a	89.9 ± 2.7 b
Female	88.9 ± 2.7 b	88.4 ± 1.8 b	329.4 ± 17.6 a	343.0 ± 14.2a	88.9 ± 2.7 b

Similar letters: No significant difference ($p > 0.05$) between means
Different letters: Significant difference ($p \leq 0.05$) between means

The results show a significant change of hypothyroidism and hyperthyroidism groups compared to diabetes with hypothyroidism and diabetes ($p \leq 0.05$) in male and female. The results coincided with Hassan M., 2017 study which declared that glucose is fundamentally utilize in screening for pre-diabetes or diabetes. [85] It was observed that the results were identical with the study Daly M., *et al* which showed that diabetes mellitus is a chronic heterogeneous illness in which there was dysregulation of carbohydrates, protein and lipid metabolism, leading to increased blood glucose levels [86].

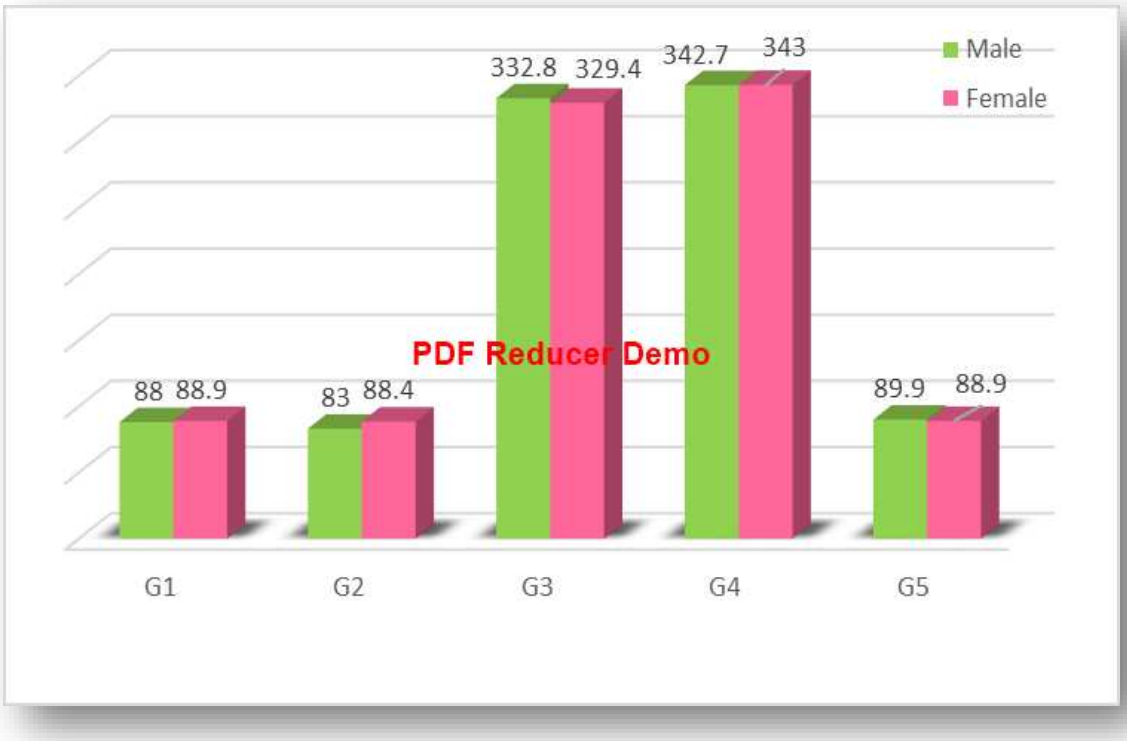


Figure (3-3): FG concentration in studied groups according the gender.

3-1-4-Hemoglobin A1c (HbA1c):

The mean value ± SE of HbA1c for hypothyroidism (G1), hyperthyroidism (G2), diabetes (G3), diabetes with hypothyroidism (G4), and control (G5) are respectively shown in table (3-4), and figure (3-4).

Table (3-4): HbA1c concentration in groups under investigation according to the gender

Gender	Mean ± SE of Serum glucose conc. (mg/dl)				
Male	G1	G2	G3	G4	G5
	6.2 ± 0.1 b	4.1 ± 0.1 b	12.4 ± 0.6 a	12.2 ± 0.8 a	5.5 ± 0.1 b
Female	5.0 ± 0.1b	5.0 ± 0.1b	10.5 ± 0.4a	10.9 ± 0.8a	4.9 ± 1.5b

Similar letters: No significant difference ($p > 0.05$) between means
Different letters: Significant difference ($p \leq 0.05$) between means

The results show a significant change of diabetes and diabetes & hypothyroidism compared with hypothyroidism, hyperthyroidism and control ($p \leq 0.05$) in male and female. HbA1c measurements are typically minimal change than fasting plasma glucose (FPG) for diagnosing diabetes, and for estimate of advance on glucose control treatment. However, HbA1c reaches fixed-state proportional to average plasma over about 120 days [84]. The results coincided with Anjali G., *et al* that HbA1c is a helpful foreteller of diabetes danger in childhood and can be applied to identify prediabetes in childhood with other type 2 diabetes danger factor with the same predictive value as serum glucose [87].

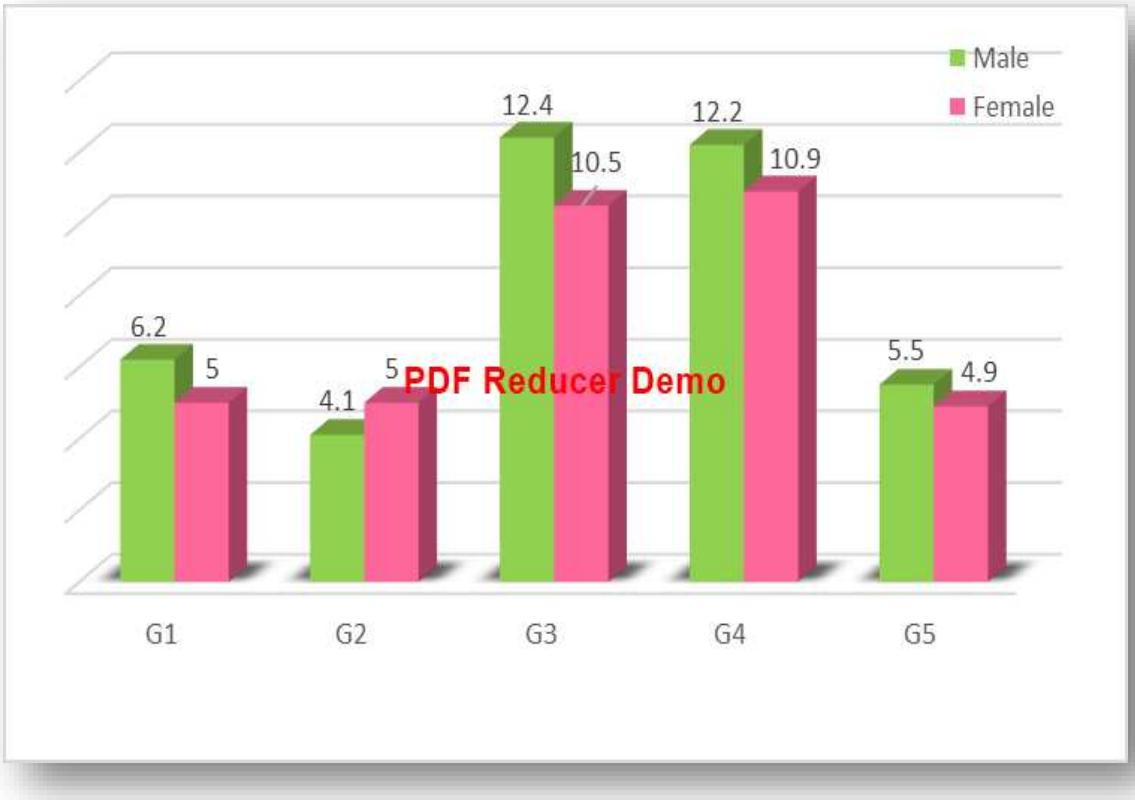


Figure (3-4): HbA1c concentration in studied groups according to the gender.

3-1-5- Insulin:

The mean value ± SE of insulin for hypothyroidism (G1), hyperthyroidism (G2), diabetes (G3), diabetes with hypothyroidism (G4), and control (G5) are respectively shown in table (3-5), and figure (3-5).

Table (3-5): Insulin concentration in groups under investigation according to the gender

Gender	Mean ± SE of insulin conc. (µl/ml)				
Male	G1	G2	G3	G4	G5
	40.0 ± 1.1 a	41.4 ± 1.4 a	42.7 ± 1.4 a	43.8 ± 1.0 a	6.1 ± 0.3 b
Female	40.7 ± 1.2 b	39.2 ± 1.8 b	44.3 ± 1.0 a	45.5 ± 1.0 a	6.2 ± 0.5 c

Similar letters: No significant difference ($p > 0.05$) between means
Different letters: Significant difference ($p \leq 0.05$) between means

The results show a significant difference in control group compared with other groups in male, but in female show a significant difference in control group compared with hypothyroidism and hyperthyroidism, and also show a significant difference in control group compared with diabetes and diabetes & hypothyroidism.

Insulin (from Latin insula, island) is a peptide hormone produced by beta cells of the pancreatic islets, and it is deemed to be the major anabolic hormone of the body [88]. The results were identical with study Pavithra V., *et al* that emphasizes that thyroid hormone also effect carbohydrate metabolism in skeletal muscle and adipose tissue by positive transcriptional organizing of the muscle/ fat particular GLUT4, and catalyze lipolysis. All these step react with insulin work. So newly, society of insulin resistance with TSH levels in fatness topics have been reported [89].

Show results similar to study Voet D., *et al* that a very high rise in the results of hypothyroidism, hyperthyroidism, diabetes, diabetes & hypothyroidism. In type 2 diabetes mellitus includes β-cells dysfunction and insulin resistance [90], results showed high insulin injection, this is because thyroid dysfunction and diabetes are considered of metabolic disorder.

Figure (3-5): Insulin concentration in studied groups according the gender

3-1-6- Insulin Resistance (IR):

The mean value ± SE of IR for hypothyroidism (G1), hyperthyroidism (G2), diabetes (G3), (diabetes with hypothyroidism) (G4), and control (G5) are respectively shown in table (3-6), and figure (3-6).

Table (3-6): Insulin resistance concentration in groups under investigation according to the gender

Gender	Mean ± SE of insulin resistance conc. (µU/ml)				
Male	G1	G2	G3	G4	G5
	8.6 ± 0.3 c	9.0 ± 0.4 c	33.6 ± 1.7 b	38.2 ± 2.3 a	1.7 ± 0.4 d
Female	8.9 ± 0.3c	8.6 ± 0.5c	35.2 ± 1.8b	40.1 ± 1.2a	1.3 ± 0.1d

Similar letters: No significant difference ($p > 0.05$) between means
Different letters: Significant difference ($p \leq 0.05$) between means

The results show a significant difference in diabetes, diabetes & hypothyroidism and control ($p \leq 0.05$), but show non-significant in hypothyroidism and hyperthyroidism ($p > 0.05$) of male and female. Insulin Resistance (IR) is a state in which given concentration of insulin produces biological impact minimal than prospective [91]. One of the characteristics of a big number of patients with diabetes mellitus is the existence of IR within target tissues and the response of hyperinsulinemia [92,93].

The results were identical with study Marcin G., *et al* insulin resistance is one of the key factors in the pathogenesis of diabetes mellitus. The spread of thyroid disorders has elevated [94] along with the elevated spread of diabetes mellitus and IR globally in recent years.

Figure (3-6): Insulin resistance concentration in studied groups according to the gender.

3-1-7- Calcium:

The mean value ± SE of calcium for hypothyroidism (G1), hyperthyroidism (G2), diabetes (G3), diabetes with hypothyroidism (G4), and control (G5) are respectively shown in table (3-7), and figure (3-7).

Table (3-7): Calcium concentration in groups under investigation according to the gender.

Gender	Mean ± SE of Calcium conc. (mg/dl)				
Male	G1	G2	G3	G4	G5
	9.6 ± 0.1 a	9.6 ± 0.2 a	9.7 ± 0.2 a	9.6 ± 0.1 a	9.5 ± 0.2 a
Female	9.4 ± 0.2a	9.6 ± 0.2a	9.5 ± 0.2a	9.4 ± 0.2a	9.4 ± 0.2a

Similar letters: No significant difference ($p > 0.05$) between means
Different letters: Significant difference ($p \leq 0.05$) between means

The results show a non-significant change of all groups ($p > 0.05$) in male and female. Because the conditions of the study were that the samples were non- osteoporosis and calcium is considered with vitamin D3 proof of the free sample from the disease of osteoporosis.

Calcium is the most spread mineral in the body. Bones and teeth consist approximately 99% of calcium subsist in both plant and animal food. The better source of calcium is milk. Calcium deficiency can be therapy with difference calcium preparations, organic salts like tricalcium citrate, calcium lactate and et al. An enough calcium intake through proper chosen of calcium salt is one of the measure to ensure a healthy skeleton [(94)].

Figure (3-7): Calcium concentration in studied groups according the gender.

3-1-8- Vitamin D3:

The mean value ± SE of vitamin D3 for hypothyroidism (G1), hyperthyroidism (G2), diabetes (G3), diabetes with hypothyroidism (G4), and control (G5) are respectively shown in table (3-8), and figure (3-8).

Table (3-8): Vitamin D_3 concentration in studied groups under investigation according to the gender

Gender	Mean ± SE of Vitamin D3 conc. (µU/ml)				
Male	G1	G2	G3	G4	G5
	42.6 ± 0.6 a	44.3 ± 1.6 a	43.7 ± 1.0 a	43.4 ± 0.7 a	43.5 ± 1.6 a
Female	43.9 ± 1.0a	42.3 ± 1.5a	42.9 ± 0.8a	43.1 ± 0.8a	43.0 ± 1.5a

Similar letters: No significant difference ($p > 0.05$) between means
Different letters: Significant difference ($p \leq 0.05$) between means

The results show a non-significant change of all groups ($p > 0.05$) in male and female. Because the samples should be free from osteoporosis and the appearance of a non- significant change is evidence that people are healthy and without osteoporosis.

Vitamin D is a fat-soluble vitamin, the term "vitamin D" refers to both ergocalciferol (vitamin D2) and cholecaliferol (vitamin D3), which are formed from particular pro-vitamins, ergosterol and 7-dehydrocholesterol (7-DHC). The dominant natural source of vitamin D3 in persons is output in the skin where 7-DHC follows a two step-reaction including ultraviolet-B (UV-B) irradiation to from previtamin D3 followed by a subsequent thermal isomerization to vitamin D3[95].

Figure (3-8): Vitamin D_3 concentration in studied groups according to the gender.

3-1-9- Total Triiodothyronine(TT3):

The mean value ± SE of TT3 for hypothyroidism (G1), hyperthyroidism (G2), diabetes (G3), diabetes with hypothyroidism (G4), and control (G5) are respectively shown in table (3-8), and figure (3-8).

Table (3-9): TT3 concentration in studied groups under investigation according to the gender

Gender	Mean ± SE of TT3 conc. (nmol/l)				
Male	G1	G2	G3	G4	G5
	0.3 ± 0.09 c	11.9 ± 0.5 a	1.7 ± 0.1 b	0.3 ± 0.07 c	1.7 ± 0.1 b
Female	3.5± 0.09c	9.7± 0.5a	1.6 ± 0.1b	0.2± 0.07c	1.0± 0.1b

Similar letters: No significant difference ($p > 0.05$) between means
Different letters: Significant difference ($p \leq 0.05$) between means

The results show a significant change of hyperthyroidism compared with other groups ($p \leq 0.05$) in male and female. Triiodothyronine, also famous as T3 is a thyroid hormone. It impacts nearly all physical process in the body, including growth and expansion, metabolism, body heat and heart average [96]. The results were matched with study Ibrahim M., *et al* that in the case of hypothyroidism there is not enough production of T3, it is considered the most common condition in puberty. While in the case of hyperthyroidism the thyroid gland increased amounts of T3[33].

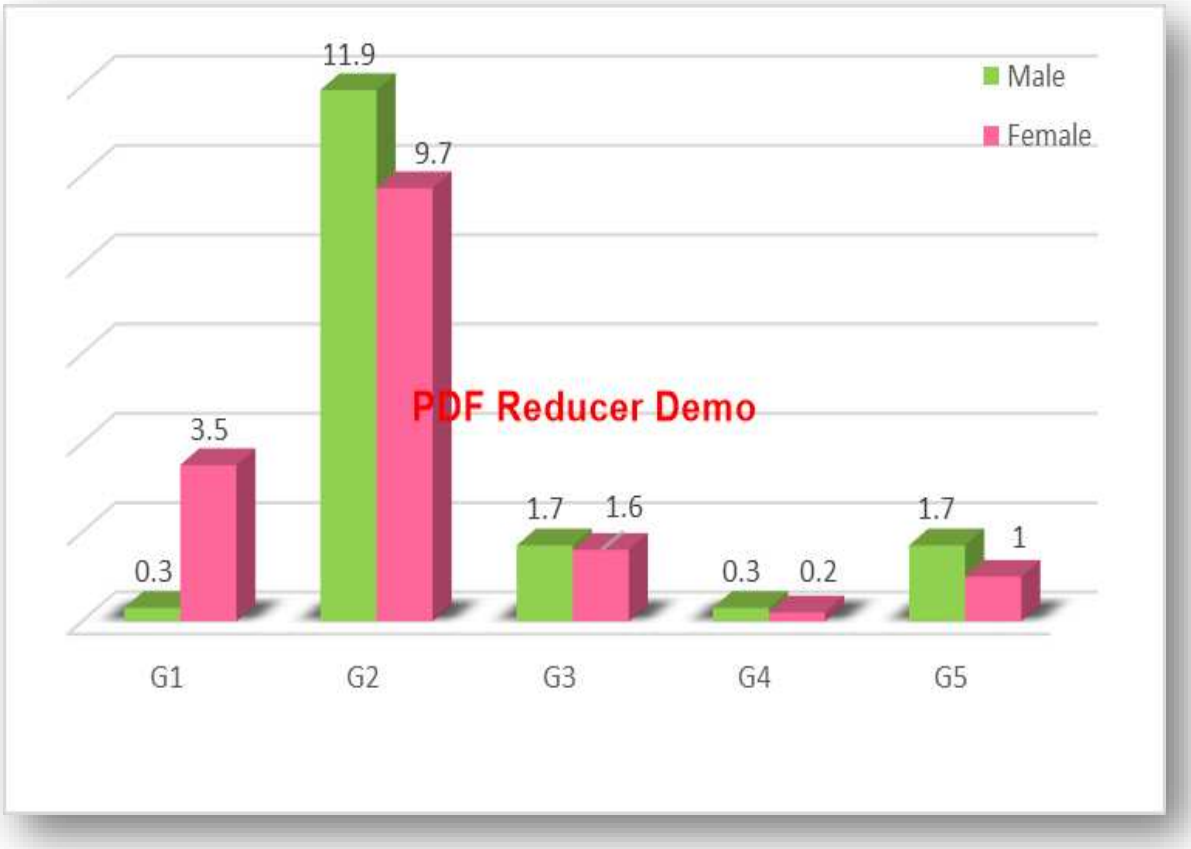


Figure (3-9): TT3 concentration in studied groups according to the gender.

3-1-10-TotalThyroxine TT4:

The mean value ± SE of TT4 for hypothyroidism (G1), hyperthyroidism (G2), diabetes (G3), diabetes with hypothyroidism (G4), and control (G5) were respectively shown in table (3-10) and figure (3-10).

Table (3-10): TT4 concentration in studied groups under investigation according to the gender

Gender	Mean ± SE of TT4 conc. (nmol/l)				
Male	G1	G2	G3	G4	G5
	33.5 ± 2.1 c	142.6 ± 3.0 a	88.4 ± 1.0 b	34.4 ± 2.7 c	90.5 ± 3.3 b
Female	36.0 ± 2.8c	145.8 ± 3.5a	88.9 ± 4.0b	33.0 ± 2.2c	92.5 ± 3.7b

Similar letters: No significant difference ($p > 0.05$) between means
Different letters: Significant difference ($p \leq 0.05$) between means

The results show a significant change of hyperthyroidism compared with other groups ($p \leq 0.05$) in male and female. Throxine (T4) is maked by thyroid-stimulating hormone (TSH), which free from the anterior pituitary gland [97]. The results were matched with study Ibrahim M., *et al* that the main reason for the emergence of a result T3 high in the case of hyperthyroidism was the thyroid gland free increased amounts of T4, but hypothyroidism there is not enough production of T4 [33].

Figure (3-10): TT4 concentration in studied groups according to the gender.

3-1-11- Thyroid-Stimulating Hormone (TSH):

The mean value ± SE of TSH for hypothyroidism (G1), hyperthyroidism (G2), diabetes (G3), diabetes with hypothyroidism (G4), and control (G5) were respectively shown in table (3-11), and figure (3-11).

Table (3-11): TSH concentration in studied groups under investigation according to the gender

Gender	Mean ± SE of TSH conc. (mlU/L)				
Male	G1	G2	G3	G4	G5
	38.8 ± 3.0 b	0.1 ± 0.02 d	1.8 ± 0.4 c	47.6 ± 2.8 a	1.6 ± 0.3 c
Female	32.6 ± 3.4b	0.1 ± 0.02d	1.9 ± 0.3c	38.0 ± 4.2a	2.2 ± 0.4c

Similar letters: No significant difference ($p > 0.05$) between means
Different letters: Significant difference ($p \leq 0.05$) between means

The results show a significant difference in control and diabetes compared with other groups, the metabolic homeostasis in an individual is preserved by thyroid gland which is achieved during excretion of two hormones thyroxine (T4) and triiodothyronine (T3); a adjusted by thyroid stimulating hormone (TSH), excreted by the anterior pituitary. TSH is the main adjusted for the growth and rules of the thyroid. In healthy adults, the rate of the production of TSH was within 100 and 200 mu/day [(4)]. Shlomo M., *et al* that the first test to know that the patient was infected with hypothyroidism is that test TSH is high this is confirmed by the results in the case of hypothyroidism and (diabetes and hypothyroidism) [(4)].

Figure (3-11): TSH concentration in studied groups according to the gender.

3-1-12-Luteinizing hormone (LH):

The mean value ± SE of LH for hypothyroidism (G1), hyperthyroidism (G2), diabetes (G3), diabetes with hypothyroidism (G4), and control (G5) are respectively shown in table (3-12), and figure (3-12).

Table (3-12): LH concentration in studied groups under investigation according to the gender

Gender	Mean ± SE of LH conc. (mlU/ml)				
Male	G1	G2	G3	G4	G5
	3.7 ± 0.5 a	3.2 ± 0.6 a	3.2 ± 0.6 a	4.2 ± 0.8 a	2.6 ± 0.5 a
Female	3.5 ± 0.6 a	4.3 ± 0.6 a	4.0 ± 0.6 a	3.8 ± 0.6 a	4.1 ± 0.7 a

Similar letters: No significant difference ($p > 0.05$) between means
Different letters: Significant difference ($p \leq 0.05$) between means

The results show a non-significant change of all groups ($p > 0.05$) in male and female, the main reason for show a non-significant change is that the samples should be uninfected with multiple polycystic ovaries in females, and males should be infertile. Luteinizing hormone (LH) move an opener function in gonadal role [98]. It structure is alike to that of the other glycoprotein hormone [90]. it catalyzes leydig cell production of testerone it acts synergistically with FSH.

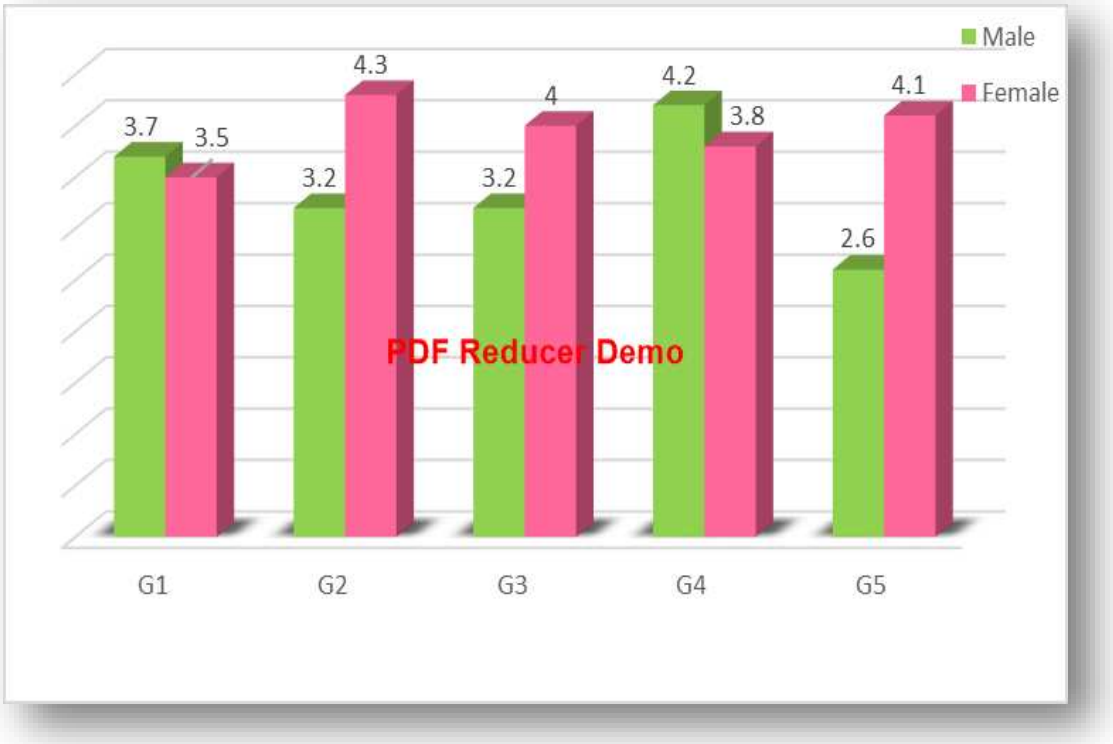


Figure (3-12): LH concentration in studied groups according to the gender.

3-1-13-Follicle-Stimulating Hormone (FSH):

The mean value ± SE of FSH for hypothyroidism, hyperthyroidism, diabetes, diabetes & hypothyroidism, and control are respectively shown in table (3-13), and figure (3-13).

Table (3-13): FSH concentration in studied groups under investigation according to the gender

Gender	Mean ± SE of FSH conc. (mlU/ml)				
Male	G1	G2	G3	G4	G5
	5.8 ± 0.6 a	5.2 ± 0.6 a	5.5 ± 0.6 a	5.1 ± 0.6 a	5.5 ± 0.7 a
Female	5.7 ± 0.6a	6.3 ± 0.6a	5.9 ± 0.5a	6.3 ± 0.4a	6.0 ± 0.6a

Similar letters: No significant difference ($p > 0.05$) between means
Different letters: Significant difference ($p \leq 0.05$) between means

The results show a non-significant change of all groups ($p > 0.05$) in male and female, the main reason for show a non-significant change is that the samples should be uninfected with multiple polycystic ovaries in females, and males should be infertile.

Follicle-Stimulating Hormone (FSH) is a gonadotropin, a glycoprotein polypeptide hormone. FSH is synthesized and excreted by the gonadotropic cells of the anterior pituitary gland [99], and regulates the development, growth, pubertal ripeness, and reproductive processes of the body. FSH and LH work together in the reproductive system.

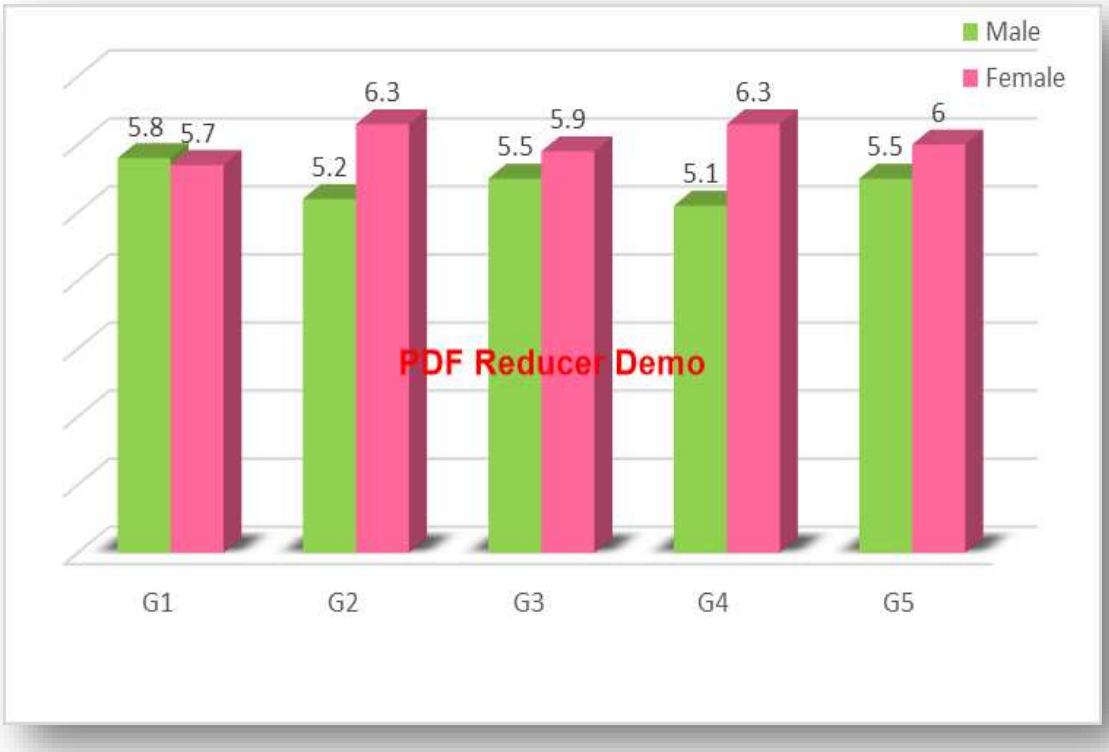


Figure (3-13): FSH concentration in studied groups according to the gender

3-1-14- Correlation of preptin and other parameter:

From the table (3-15) it was observed that the preptin hormone correlation with calcium, TT3, FG, and FSH showed a negative relationship, while showed a positive relationship in TT4, TSH, vitamin D3, LH, insulin resistance, and BMI in hypothyroidism group. For the group of hyperthyroidism, the preptin correlation with calcium, TT4, FG, HbA1c, vitamin D3, and BMI showed a positive relationship, while TT3, TSH, insulin, FSH, LH, and insulin resistance showed a negative relationship.

In diabetes group the preptin correlation with calcium, FSH, LH, and BMI showed a positive relationship, while TT3, TT4, TSH, FG, insulin, HbA1c, vitamin D3, and insulin resistance showed a negative relationship. In diabetes & hypothyroidism group the preptin correlation with TT4, TSH, insulin, and vitamin D3 showed a negative relationship, while the calcium, TT3, HbA1c,

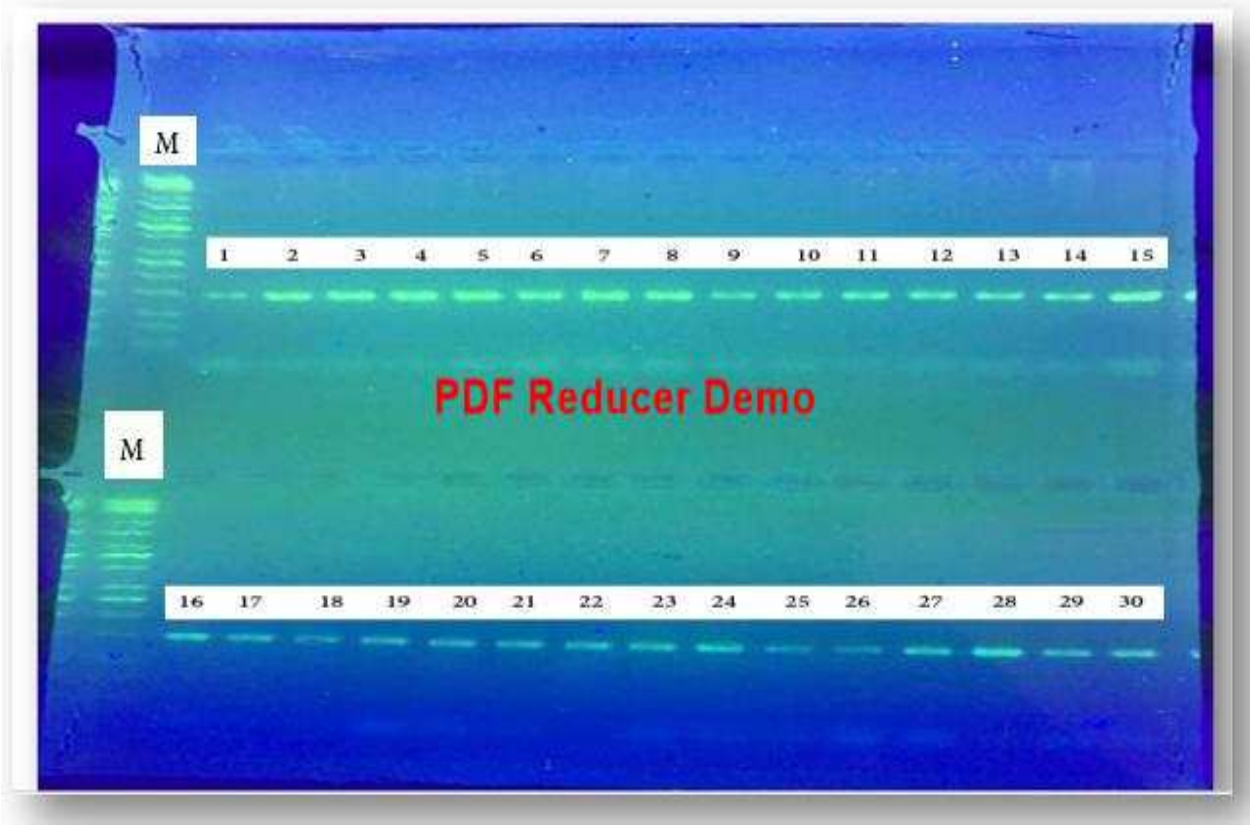
FSH, LH, and insulin resistance showed a positive relationship, but FG showed a significant adverse relationship (0.05).

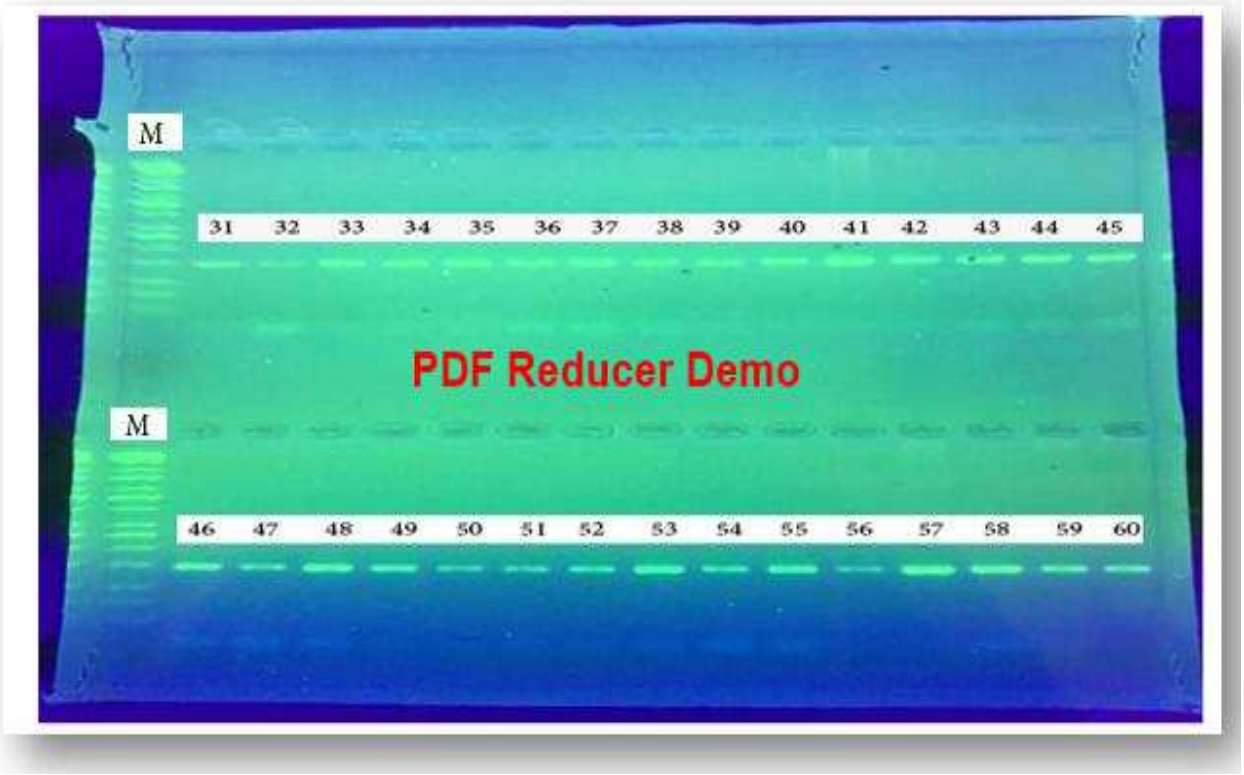
In control group the preptin correlation with calcium, TT3, vitamin D3, and insulin resistance showed a positive relationship, but TT4, TSH, FG, insulin, HbA1c, FSH and BMI showed a negative relationship.

3-2-2-Amplification of IGF-2 gene and the (rs680) polymorphisms:

Polymerase Chain Reaction (PCR) was used to amplify special region of the genome for millions time *in vitro*. PCR technique does not require very purified DNA outfit as it actions well with half purified DNA samples [(103)]. Genomic DNA of white blood cells was utilize for an expansion of IGF-II gene utilizing PCR specific primers for IGF-II gene.

The expand fragment which is yielded of single band of the coveted output with a molecular weight of 292 and 229 base pair evidence sharp in agarose gel during gel electrophoreses technique and loaded with 100bp DNA ladder, IGF-II (rs680) polymorphisms were genotyped with a PCR method by confronting primers briefly, rs 680 genotyping was also performed by PCR-restriction fragment length polymorphism (PCR-RFLP). Genomic DNA was amplified by up strand,5'-CTT GGA CTT TGA AGT CAA ATT GG - 3'and down strand, 5'-GGT CGT GCC AAT TAC ATT TCA- 3', followed by digestion with restriction enzyme (Apa 1). The AA and GG genotype (Homozygous) was indicated by a single band of (292bp) and (229bp) respectively, the AG genotype (Heterozygous) was indicated by two band of (292/229bp), as shown in figure (3-15) were studied by PCR-RFLP.


M
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
PDF Reducer Demo
M
16 17 18 19 20 21 22 23 24 25 26 27 28 29 30


M
31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
PDF Reducer Demo
M
46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

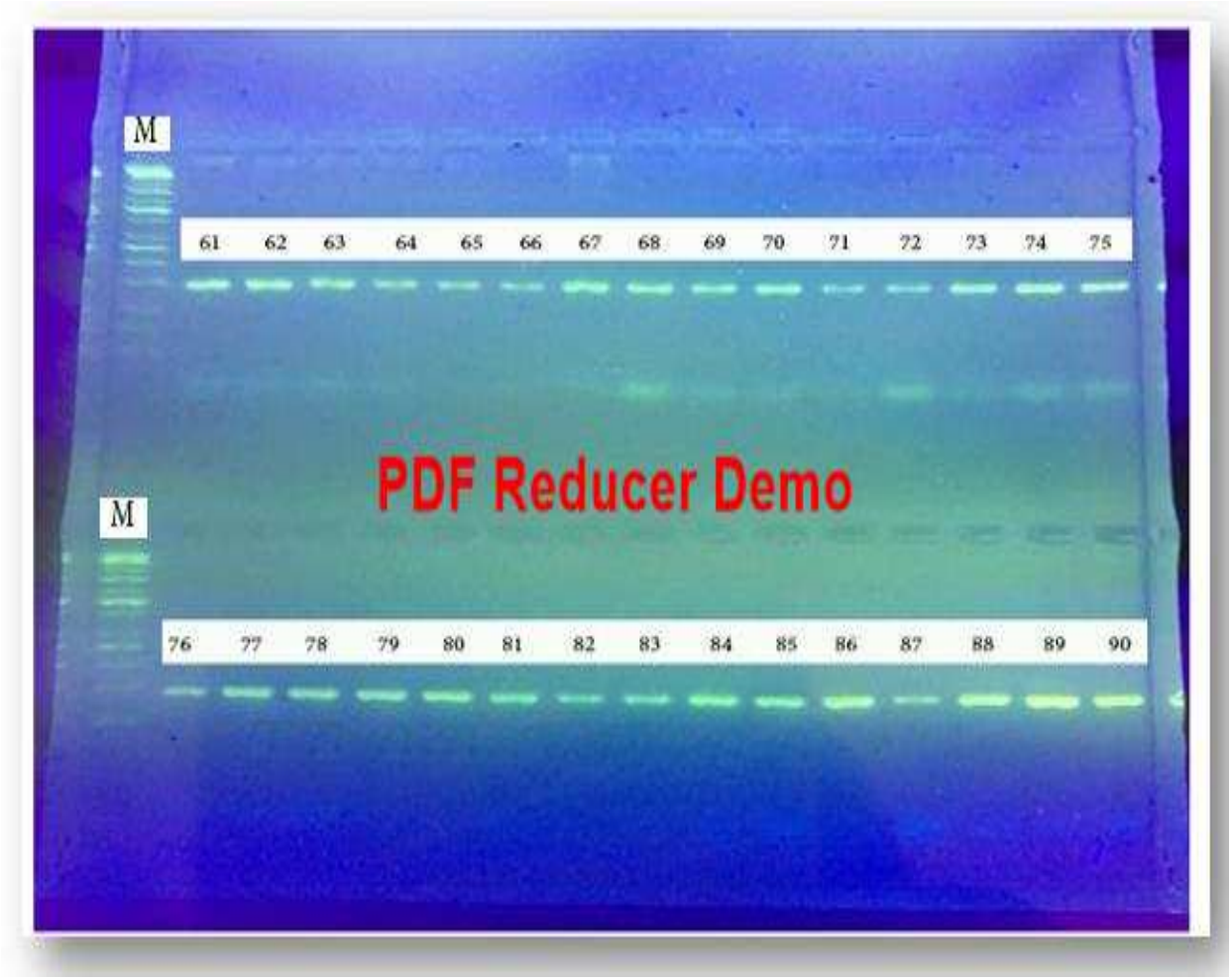


Figure (3-15) PCR product the band 292 bp. The product was electrophoresis on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours. N: DNA ladder (100).

3-2-3- Hypothyroidism group of IGF-II polymorphisms of Iraqi patients:

The frequency division of IGF-II gene polymorphism of hypothyroidism group was represented in table (3-16) and figure (3-16).

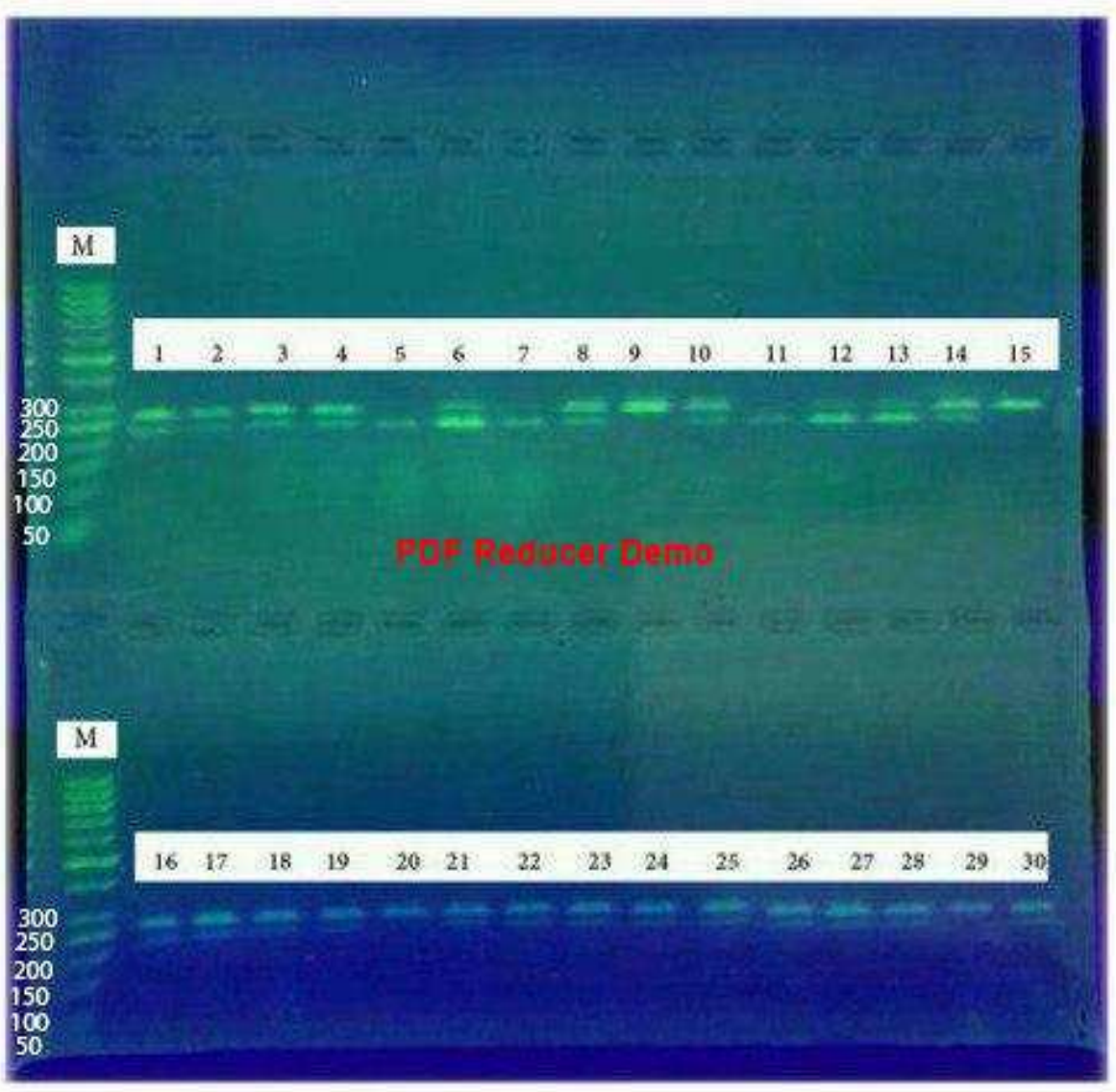


Figure (3-16): Electrophoresis pattern of 292bp PCR product (hypothyroidism) Apa 1 restriction enzyme (2.5 % agarose gel). Line's1, 2, 3, 4, 6, 8, 10, 12, 13, 14, 16, 17, 18, 19, 21, 22, 23, 24, 25, 26, 27, 28, 30 heterogeneous: AG genotype (229+292)bp. Line's 5, 7, 11 homogenous: GG genotype (229bp) and line's 9, 15, 20, 29 homogenous: AA genotype (292 bp). M: DNA molecular marker 100bp size. By redstain stained bands in the gel.

Patients with an allele AG (heterogeneous) genotype with risk factor (22%) and GG (homogenous) with risk factor (7%) were more likely to have a disease compared to patient who carry allele AA (homogenous)genotype. The ratio was allele A for the patient (52%), while allele G (48%), whilst control compared to patients.

A significant change was observed in the comparison between hypothyroidism group and the control group. The allele AA showed (0.20), while allele GG shows (0.30) on the other hand, the allele AG shows a non-significant (0.56). To determine odds ratios and 95% confidence intervals for hypothyroidism, various comparisons analyzed as shown in table (3-16).

The odds ratio for compared hypothyroidism with control for AA subjects was (0.42) (95%, CI, 0.11-1.56), AG subjects was (1.4) (95%, CI, 0.45-4.37) and GG subjects was 3.2 (95%, CI, 0.33-31.63). There is no literature linking hypothyroidism with IGF-II gene.

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3-2-4-Hyperthyroidism of IGF-II polymorphisms of Iraqi patients:

The frequency distribution of IGF-II gene polymorphism of hyperthyroidism with healthy control was represented in table (3-17) and (3-18) and figures (3-17) and (3-18).

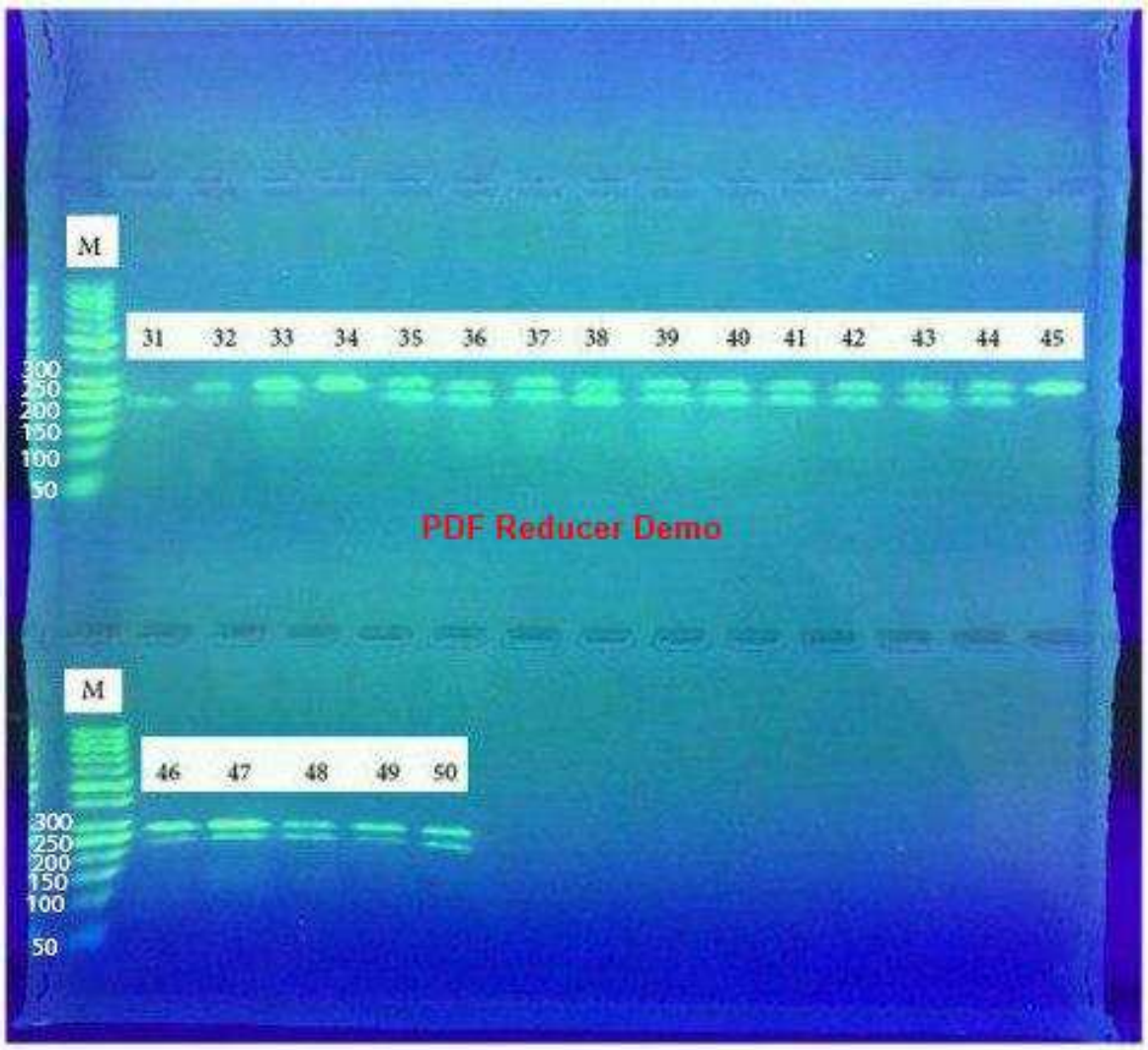

Figure (3-17): Electrophoresis pattern of 292bp PCR product (hypothyroidism) Apa 1 restriction enzyme (2.5 % agarose gel). Line's32, 33, 36, 37, 38, 39, 40, 41, 42, 43, 44, 46, 47, 48, 49,50 heterogeneous: AG genotype (229+292) bp. Line's 31 homogenous: GG genotype (229bp) and line's 34,45 homogenous: AA genotype (292 bp). M: DNA molecular marker 100bp size. By redstain stained bands in the gel.

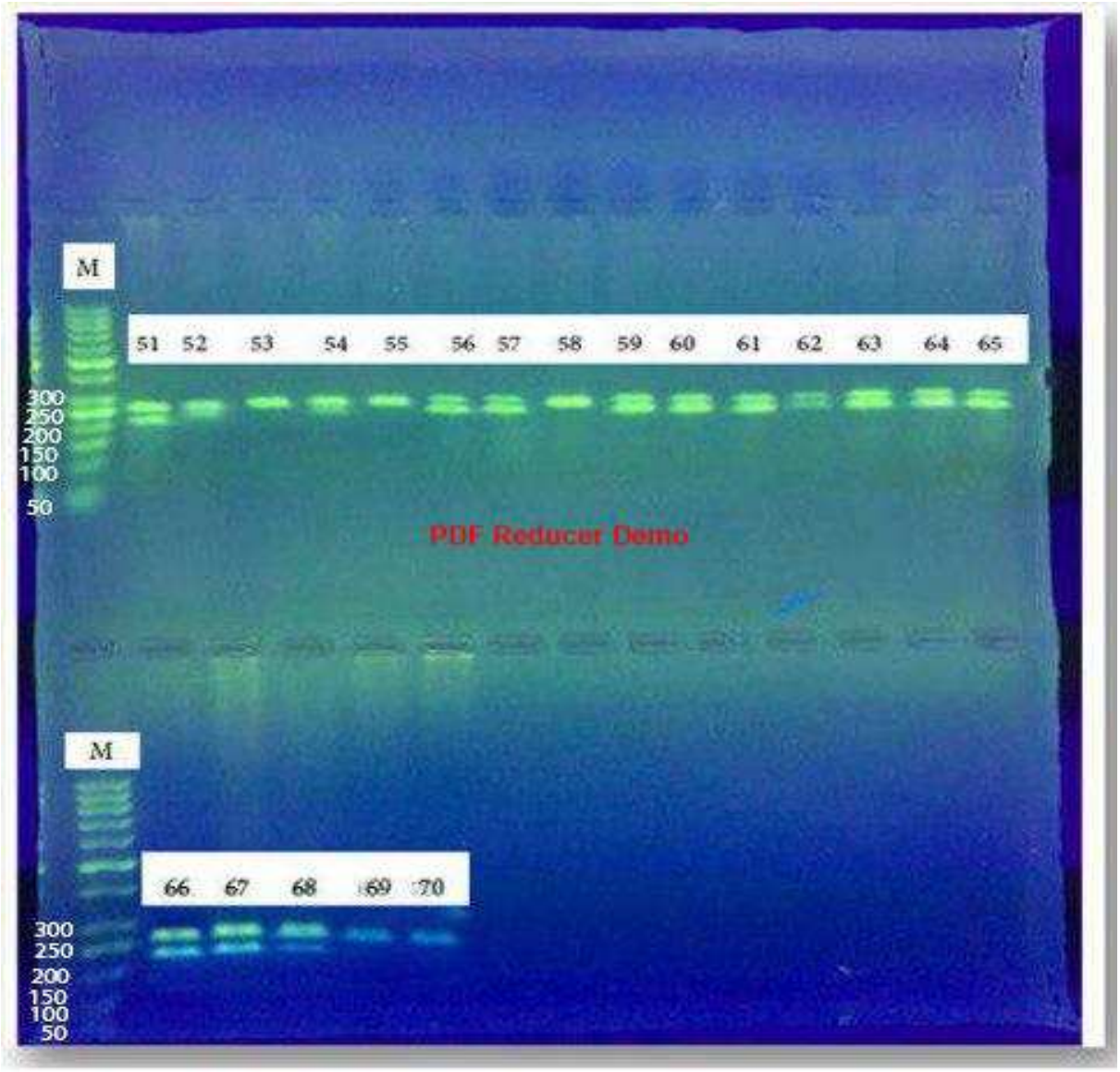

Figure (3-18): Electrophoresis pattern of 292bp PCR product (hyperthyroidism) Apa 1 restriction enzyme (2.5 % agarose gel). Lane's51, 52,54, 56, 57,59, 60, 61, 62, 63, 64, 65, 66, 67, 68 heterogeneous: AG genotype (229+292) bp. Line's 31 homogenous: GG genotype (229bp) and line's 53, 55, 58, 69, 70. M: DNA molecular marker 100bp size. By redstain stained bands in the gel.

Patients with an allele GG (homogenous) genotype with a risk factor of (3%) and AG (heterogeneous) with a risk factor of (67%) were more likely to have a disease compared to patients who carry allele AA (homogenous) genotype. The ratio was allele A for patient (45%), while allele G (52%), whilst control allele A (62%), allele G (38%). The allele G is common in control compared to hyperthyroidism. The non-significant change in comparison between the hyperthyroidism group and the control group.

The allele AG (0.05) on the other hand, GG (0.22) was non-significant. To determine odds ratios and 95% confidence intervals for hyperthyroidism, various comparisons of genotypes as well as A vs. G allele were analyzed as shown in table (3-17).

The odds ratio for compared hyperthyroidism with control AA subjects was 0.09 (0.01-0.79), AG subjects was 3.9 (0.95-15.67), GG subjects was 2.07 (0.19-23.17). There is no literature linking hyperthyroidism with IGF-II gene.

3-2-4-Hypothyroidism and Hyperthyroidism of IGF-II polymorphisms of Iraqi patients:

The frequency distribution of IGF-II gene polymorphism of hypothyroidism with hyperthyroidism was represented in table (3-19) and figure (3-19).

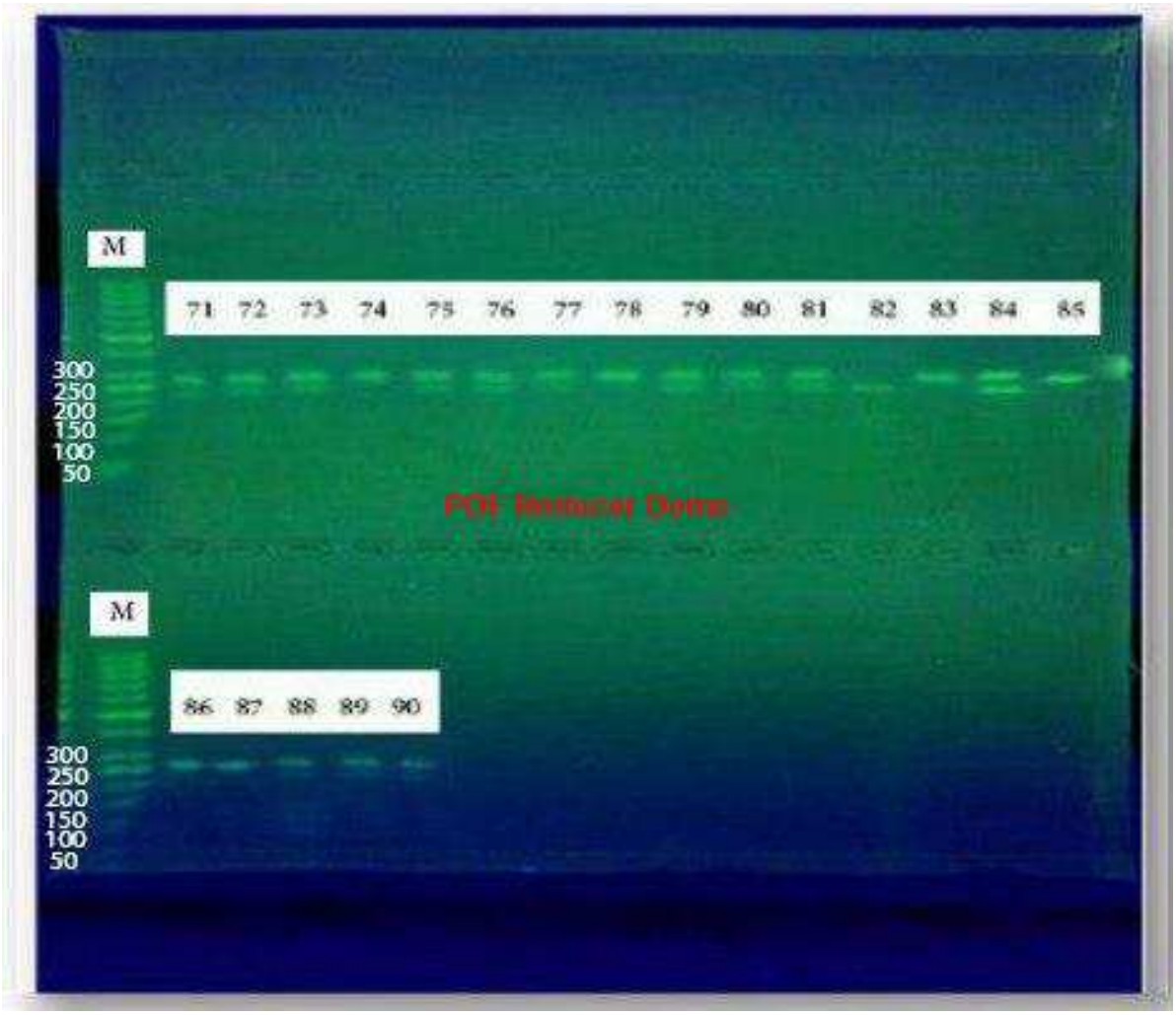

Figure (3-19): Electrophoresis pattern of 292bp PCR product (hypothyroidism) Apa 1 restriction enzyme (2.5 % agarose gel). Lane's 74, 78, 83, 85, 86, 87 homogenious(

292) pb. Line's71, 72, 73, 75, 76, 77, 79, 80, 81, 84, 88, 89, 90 heterogeneous: AG genotype (229+292) bp. Line's 31. M: DNA molecular marker 100bp size. By redstain stained bands in the gel.

Patients with an allele AA (homogenous) genotype with a risk factor of (10%) and GG (homogeneous) with a risk factor of (4%) were more likely to have a disease compared to patients who carry allele AG (heterogeneous) genotype. The ratio was allele A for patient hypothyroidism (52%), while allele G (48%), whilst hyperthyroidism allele A (48%), allele G (52%).

The allele G is common in hyperthyroidism compared to hypothyroidism. A non-significant change in comparison between the hyperthyroidism group and the hypothyroidism group. The allele AG (0.166) on the other hand, GG (0.64) was non-significant. To determine odds ratios and 95% confidence intervals for hyperthyroidism, various comparisons of genotypes as well as A vs. G allele were analyzed as shown in table (3-18).

The odds ratio for compared hyperthyroidism with hypothyroidism AA subjects was 4.5 (0.49-40.93), AG subjects was0.37 (0.09-1.54), GG subjects was 1.6(0.25-9.47). There is no literature linking hypothyroidism and hyperthyroidism with IGF-II gene.

CONCLUSIONS

Conclusions:

1. Preptin hormone was highly in patients with hypothyroidism compared with hyperthyroidism.
2. Preptin hormone was low in patients with hyperthyroidism.
3. Preptin hormone was high in patients with diabetes and (diabetes and hypothyroidism).
4. The results showed that there was a significant change for preptin hormone and patients with hypothyroidism
5. The study showed that individuals with non-homozygous allele AG had impaired thyroid dysfunction.
6. The results showed a significant change when compared with the results of hypothyroidism with hyperthyroidism. While in the healthy group, the laboratory results showed the safety of individuals from any thyroid gland disease, but the genetic study showed that the number is not small also carrying the allele AG heterogeneous and that if anything indicates that these individuals with the allele AG will be vulnerable to thyroid disease during next year.

Recommendation for Future Work:

1. To study of the relationship between Preptin hormone and thyroid hormones in hypothyroid and hyperthyroid patients before and after treatment.
2. Studying of the relationship of the preptin hormone with other hormones such as milk and sex hormone.
3. Studying the relationship preptin with other disease such as renal and heart disease.
4. Studying of the relationship of the preptin hormone with lipid profile.

Table (3-15): preprtin correlation with the studied parameter in the studied groups

Variables		Calcium	TT3	TT4	TSH	FG	Insulin	HbA1c	VD3	FSH	LH	BMI	Insulin resistance
Preptin	Hypothyroidism	-0.035	-0.205	0.110	0.069	-0.013	0.038	0.000	0.008	-0.216	0.189	0.096	0.055
	Hyperthyroidism	0.241	-0.246	0.044	-0.127	0.061	-0.141	0.159	0.038	-0.138	-0.204	0.144	-0.066
	Diabetes	0.204	-0.039	-0.308	-0.215	-0.005	-0.001	-0.103	-0.147	0.086	0.101	0.249	-0.017
	Diabetes & Hypothyroidism	0.198	0.013	-0.043	-0.062	-0.476*	-0.023	0.272	-0.074	0.096	0.182	-0.168	0.036
	Control	0.125	0.156	-0.159	-0.196	-0.038	-0.316	-0.122	0.165	-0.015	0.077	-0.191	0.142

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

Table (3-16): genotyping and alleles frequency between hypothyroidism and control

Polymorphisms	Groups								X^2	P-value	OR (95% CI)	RF	PF
	Hypothyroidism N=30				Control N=30								
	H-W observed frequency	%	H-W expected frequency	%	H-W observed frequency	%	H-W Expected frequency	%					
AA	4	13.33	8.01	26.69	8	26.67	11.41	38.03	1.7	0.20	0.42 (0.11-1.56)		0.15
AG	23	76.67	14.98	49.94	21	70.00	14.18	47.28	0.3	0.56	1.4 (0.45-4.37)	0.22	
GG	3	10.00	7.01	23.36	1	3.33	4.41	14.69	1.1	0.30	3.2 (0.33-31.63)	0.07	
Total	30	100.00	30	100.00	30	100.00	30	100.00					
X^2	8.6				6.9								
HWP	0.0034				0.0085								
Allele frequency													
A	0.52				0.62								
G	0.48				0.38								

H-W: Hardy-Weinberg; %: Percentage; X^2: Chi-square test; HWP: Hardy-Weinberg probability; P: probability; OR: Odd ratio; CI: Confidence interval; RF: risk fraction; PF: Protective fraction.

Table (3-17): genotyping and alleles frequency between hyperthyroidism and control

Polymorphisms	Groups								X^2	P-value	OR (95% CI)	RF	PF
	Hyperthyroidism N=30				Control N=30								
	H-W observed frequency	%	H-W expected frequency	%	H-W observed frequency	%	H-W expected frequency	%					
AA	1	3.33	7.01	23.36	8	26.67	11.41	38.03	6.4	0.011	0.09 (0.01-0.79)		0.24
AG	27	90.00	14.98	49.94	21	70.00	14.18	47.28	3.8	0.05	3.9 (0.95-15.67)	0.67	
GG	2	6.67	8.01	26.69	1	3.33	4.41	14.69	0.4	0.55	2.07 (0.19-23.17)	0.03	
Total	30	100.00	30	100.00	30	100.00	30	100.00					
X^2	19.3				6.9								
HWP	0.0000				0.0085								
Allele frequency													
A	0.48				0.62								
G	0.52				0.38								

H-W: Hardy-Weinberg; %: Percentage; X^2: Chi-square test; HWP: Hardy-Weinberg probability; P: probability; OR: Odd ratio; CI: Confidence interval; RF: risk fraction; PF: Protective fraction.

Table (3-18): genotyping and alleles frequency between hypothyroidism and hyperthyroidism

Polymorphisms	Groups								X^2	P-value	OR (95% CI)	RF	PF
	Hypothyroidism N=30				Hyperthyroidism N=30								
	H-W observed frequency	%	H-W expected frequency	%	H-W observed frequency	%	H-W expected frequency	%					
AA	4	13.33	8.01	26.69	1	3.33	7.01	23.36	2.0	0.161	4.5 (0.49-40.93)	0.10	
AG	23	76.67	14.98	49.94	27	90.00	14.98	49.94	1.9	0.166	0.37 (0.09-1.54)		0.57
GG	3	10.00	7.01	23.36	2	6.67	8.01	26.69	0.22	0.64	1.6 (0.25-9.74)	0.04	
Total	30	100.00	30	100.00	30	100.00	30	100.00					
X^2	8.6				19.3								
HWP	0.0034				0.0000								
Allele frequency													
A	0.52				0.48								
G	0.48				0.52								

H-W: Hardy-Weinberg; %: Percentage; X^2: Chi-square test; HWP: Hardy-Weinberg probability; P: probability; OR: Odd ratio; CI: Confidence interval; RF: risk fraction; PF: Protective fraction.

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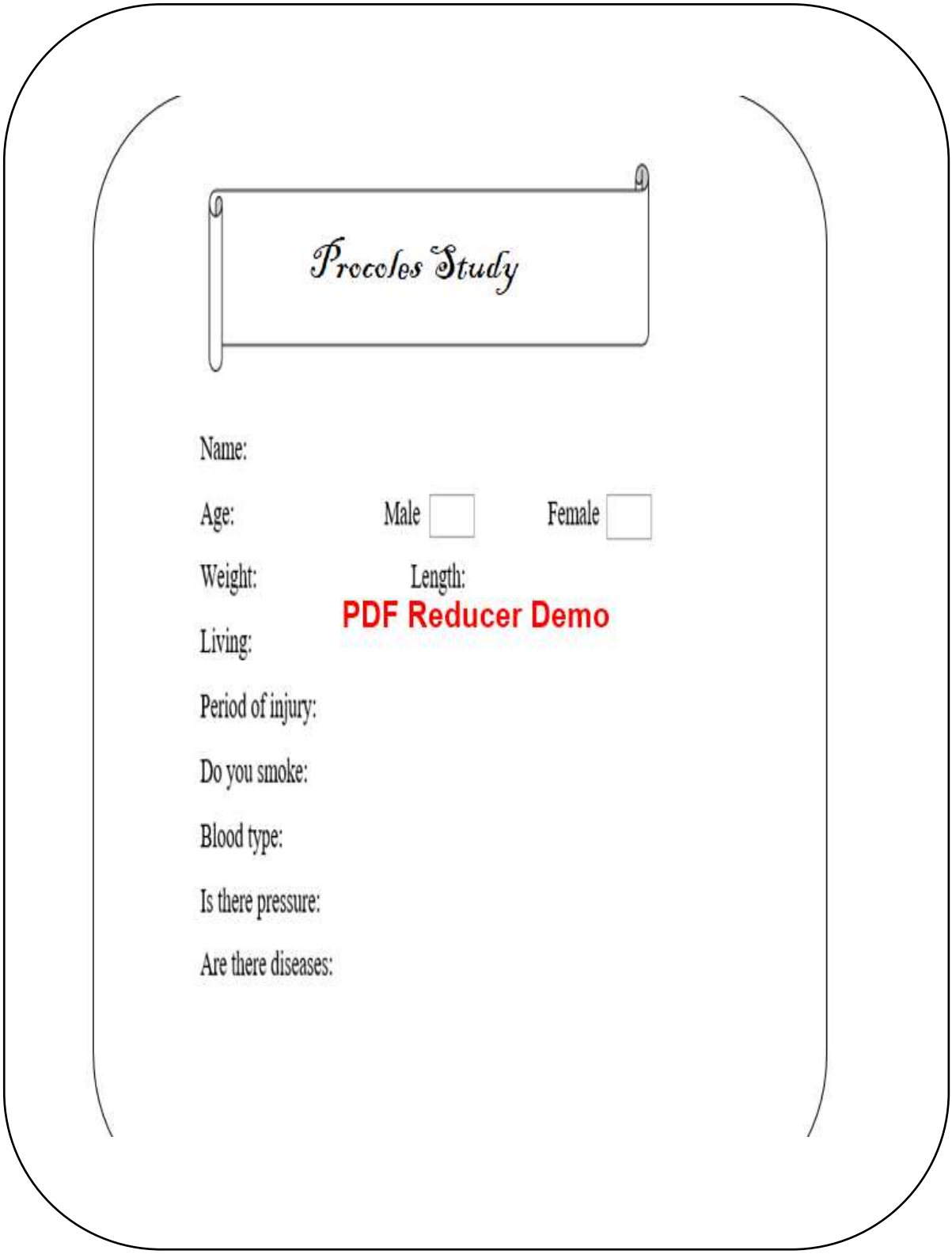
APPENDIXES


Procoles Study
Name:
Age:
Male
Female
Weight:
Length:
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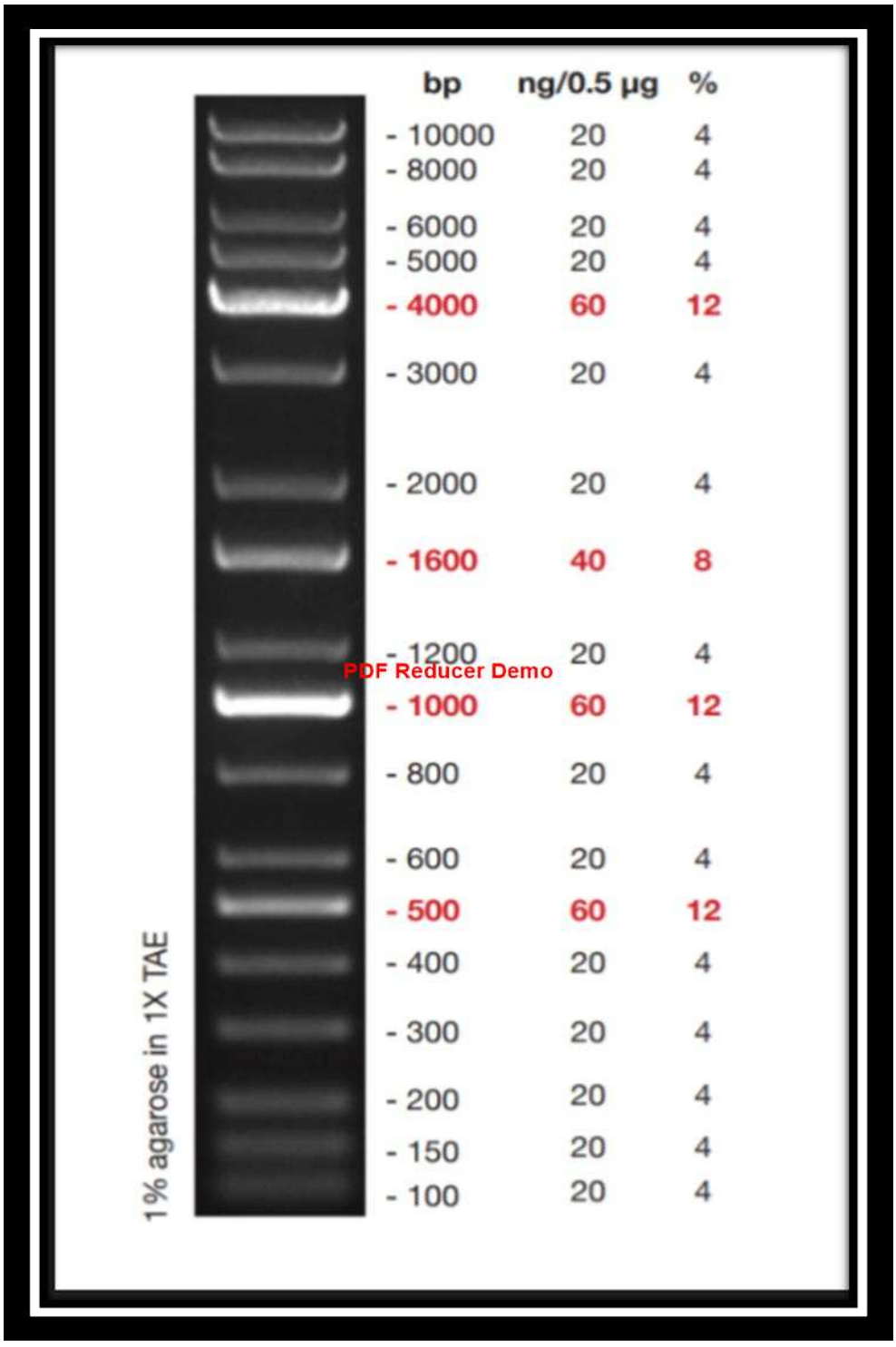
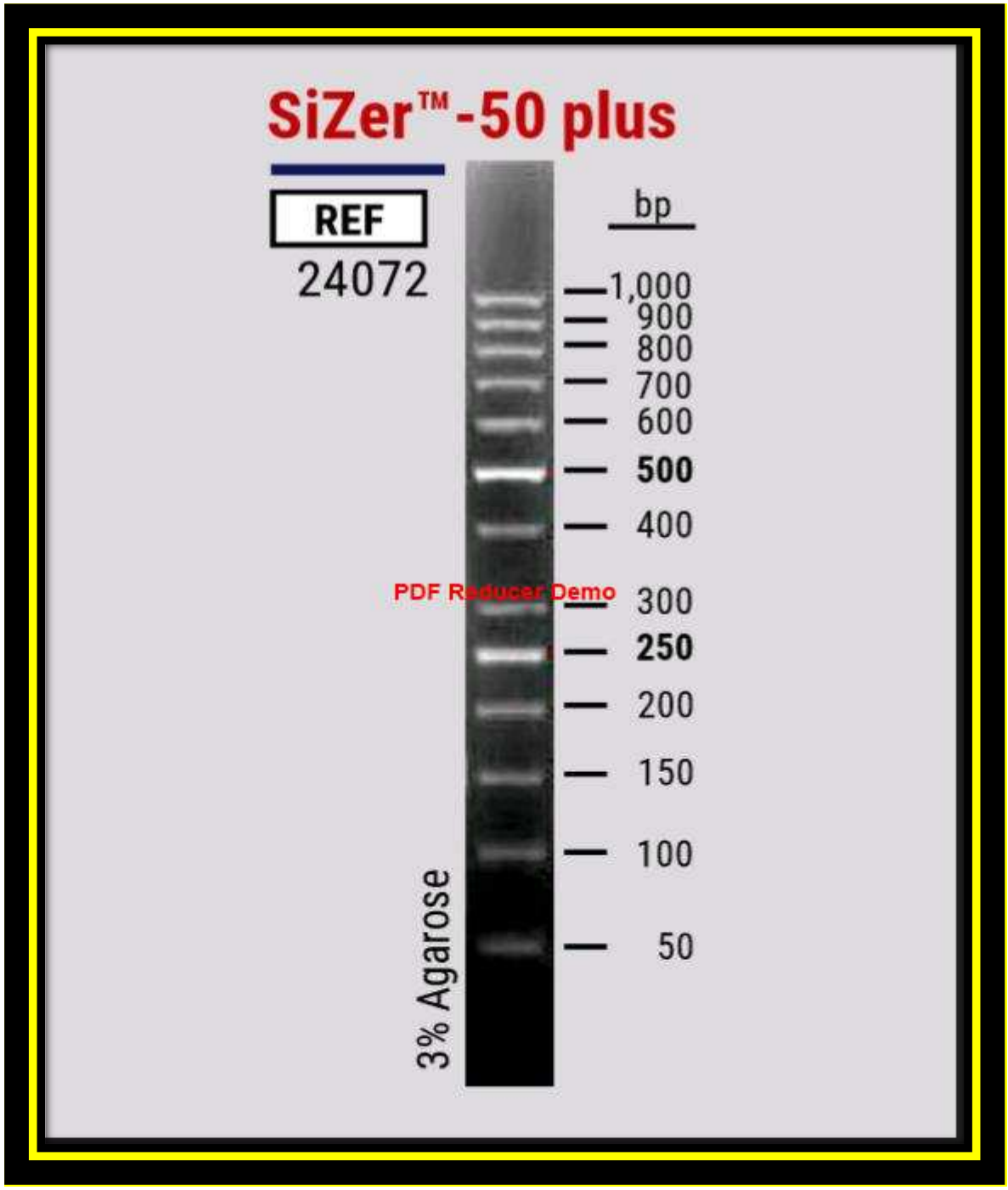
Living:
Period of injury:
Do you smoke:
Blood type:
Is there pressure:
Are there diseases:



Ladder use in PCR



Ladder use in RFLP

Nanodrop of DNA

260/280 purity	Nucleic acid conc (ng/ml)	sample ID
1.77	57	1
1.7	63	2
1.78	83	3
1.61	108	4
1.7 2	84	5
1.84	160	6
2.02	104	7
1.54	142	8
2.07	230	9
1.7 3	300	10
1.6	54	11
1.83	71	12
1.93	126	13
1.97	133	14
1.7	132	15
1.50	60	16
1.87	170	17
1. 71	156	18
1.32	231	19
2.09	143	20
1.45	345	21
1.34	99.8	22
1.8 7	88.7	23
0.76	197	24
1.19	234.8	25
1.87	63	26
1. 54	90	27
1. 69	141.8	28
1.77	156.8	29
1. 75	88.4	30
1.75	217. 4	31
1. 76	284	32
1.85	155.8	33
1.8	189.5	34
1.77	337.5	35

1.75	295	36
1.88	278.1	37
1.9	237.9	38
2.08	252.7	39
2.01	115.8	40
1.8	278	41
1.74	161.2	42
1.81	189	43
1.74	160.5	44
1.7	127	45
1.8	188.8	46
1.77	195.3	47
1.73	138	48
1.69	158	49
1. 87	192.9	50
1.71	155	51
1.9	282.1	52
1.85	339	53
2.00	166	54
1.65	161.5	55
2.09	347.7	56
1.83	256.8	57
1.9	296.8	58
1.83	534.8	59
1.83	321	60
1.78	498	61
1.75	110	62
1.75	270	63
2.03	518	64
1. 4	468	65
1.83	307	66
1.71	393	67
1.85	196	68
1.9	297	69
1.74	284	70
1.6	370	71
0.84	172	72
0.9	176	73
1.53	354	74
0.84	170	75

1.19	112	76
1.7	137	77
0.79	354	78
0.85	170	79
1.84	217	80
1.5	245	81
1.0	63.5	82
0.77	77.3	83
1. 71	172.4	84
1.7	98.9	85
2.09	86.9	86
1.7	94	87
0.94	106	88
1.77	272	89
1.46	144	90