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"Comparative Study on the Cholecystokinin-Beta Receptor levels and IL-27 Gene SNP and some Biochemical Parameters in patients with Type1 Diabetes Mellitus in Babylon Province"

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By

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بسم الله الرحمن الرحيم

﴿ اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ ۚ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا
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لِلنَّاسِ ۗ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيم ﴾.

صدق الله العلي العظيم

الاية﴿ ٣٥ ﴾سورة النور

Supervisor Certification

We certify that this thesis entitled "**Comparative Study on the Cholecystokinin-Beta Receptor levels and IL-27 Gene SNP and some Biochemical Parameters in Patients with Type1 Diabetes Mellitus in Babylon Province"** Was carried under our supervision at the College of Medicine, University of Babylon, as partial fulfillment for the requirement of the degree of Master of Science in Clinical Biochemistry.

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Dedication

If the dedication is even part of the fulfillment

So the dedication

To....

My dear father, who always support me

My Mother…My mother’s love is all I have to complete my life Iam here thanks to her.

My husband Raed, son Muhammad and my daughters, Ola and Batool who have suffered a lot, and I love you all are very much.

My brother Hussain never left my side and are very special.

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Summary

Diabetes mellitus (DM) is a main public health problem affecting more than 400 million people in the world. This metabolic disease progressively drives to chronic microvascular, macrovascular and neuropathic life dangerous complications.

Cholecystokinin (CCK) is a peptide hormone joined to the gastro-intestinal (GI) system cholecystokinin, officially called pancreozymin, is synthesized and secreted by enter endocrine cells in the duodenum.

Interleukin-27 (IL-27) is a member of the IL-6/IL-12 cytokine superfamily. It is a heterodimer composed of two non- associated covalently subunits.

The study included 90 persons with type 1 DM were enrolled in the study, all of them were diagnosed with no other diseases , there ages ranged from 5 to 30 years old and the mean was (16.52 ± 7.575) years. This study is conducted in Marjan hospital ,diabetes and endocrine center , appears Ninety healthy people volunteered as control group, there ages ranged from 5 to 30 years old, and the mean was (15.43 ± 7.312) years, from 1st September 2021 to February 2022 in Babylon city, Iraq.

The study involved some measurements, including body mass index (BMI) and age, the markers parameter for T1DM are HbA1C, study the effect of CCK B-R on T1DM, study the effect of immune system by determining the IL-27 and the genetic study for IL-27 SNP gene rs153109 to find the genetic effect of gene on T1DM, study some related chemical parameters like lipid profile and renal function by using ELISA kits and spectrophotometric methods. The statistical comparisons performed by one-way analysis of variance (T test). Genetic study has been done by using allele frequency, genotype model and Hardy-Weinberg equilibrium.

The result obtained in the study are summarized as following:

- There are no significant differences in age among controls (15.43±7.312) and T1DM patients (16.52 ± 7.575) (*P* > 0.05) (see Fig 3-1) which desired for the study in order to exclude the age factor which could affects the clinical parameters of the study, and narrowing the possible effects to T1DM factors.
- The percentage of CCKBR was non-significant (p> 0.05) between patients (319.19±61.52) and healthy control (290.6±21.5).
- While other parameter of lipid profile (Tri, HDL, LDL, VLDL) where none significantly (p >0.05) difference between patient and control with T1DM.
- The levels of urea (patients 48.82±14.84; control 26.34±8.34) and creatinine (patients 1.53±0.65; control 0.86±019).
- Urea and creatinine were significantly (p <0.005) in the patients as compared with control,
- IL-27 was significantly (p <0.005) in the patients as compared with control,
- The allele frequencies and genotype of the g.-964A > G SNP of IL-27 p28 in T1DM patients were different from those significantly in the healthy control group. This result suggested that the g.-964A > G poly morphism of IL-27 p28 be connected with tendency to causes T1DM
- The genotype frequencies of TT, CT and CC in rs153109 poly morphism were 57.8%, 36.7% and 5.6% in T1DM patients and 34.4%, 51.1%, 14.4% in the healthy controls, respectively. It was significantly different among the T1DM studied patients and controls

The ORs of the associations of rs153109 of IL27 gene with T1DM before and after adjusting for confounders (age and gender) clearly show that no significant association between rs153109 of IL27 and T1DM was observed .(Table 3, P>0.05), a significant connection of IL27 rs153109 with T1DM was seen beneath the allele model (OR=2.124, 95% CI (1.349–3.345), P=0.00105),and under genotype model in dominant model (OR=1.00 , 95% CI , P=0.0016), recessive model (OR=0.35, 95% CI (0.12–1.02), P=0.043) and homozygous model (OR=1.00 , 95% , P=0.0037)

In the conclusion the result collected in this work shows that the having T1DM has a very significant increase in the level of HbA1C; Elevating in the level of IL-27 gives the evidence that there is a relation between T1DM and the effect of immune system. From the genetic study, it is clear that T1DM has an effect on the SNPgene used as a promoter to the excretion of IL-27 and increase its excretion. Lipid profile shows an effect on the level of glucose in blood and high level of cholesterol may cause a serious problem if it is combined with T1DM. The elevated level of glucose happened because of T1DM has an effect on renal and cause a serious conditions like renal failure and other renal dysfunction diseases. This is the first identification of the association of IL27 rs153109 with T1DM and the relation between CCKBR, lipid profile, urea and creatinine. Our findings added new data on the genetic contribution to T1DM susceptibility and support the crucial role of IL27 in T1DM pathogenesis. However, further researches paper are warranted to elucidate the molecular mechanisms underlying the relationship between IL27 and T1DM.

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List of Abbreviations

Abr.	Details
AIRE	Auto immune regulator
APCs	Anti-gene presenting cell
Arg	Arginine amino acid
BMI	Body mass index
BUN	Blood urea nitrogen
CVD	Cardio vascular disease
Cys	Cysteine amino acid
CCK	Cholecystokinin
CART	Cocaine amphetamine regulated transcript
DNA	Deoxyribonucleic Acid
DCs	Dendritic Cell
ELISA	Enzyme linked immune sorbent assay
EDTA	Ethylene di-amine tetra acetic acid
IDF	International Diabetes Federation
Gp	Glycol protein
HDL-C	High density lipoprotein-Cholesterol
HLA	Human leukocyte antigen
HbA1c	Hemoglobin A1C
ICAM	Inter cellular addition molecule

IDL-C	**Intermediate density lipoprotein-Cholesterol**
IFN-γ	**Interferon-gamma**
IL-27	**Interleukin – 27**
LDL-c	**Low density lipoprotein cholesterol**
MHC	**Major histocompatibility complex**
ML	**Milli Litter**
HRP	**Horse Radish Peroxidase**
NK	**Natural killer**
NADPH	**Nicotinamide adenine dinucleotide phosphate**
NADH	**Nicotinamide adenine dinucleotide**
PCR	**Polymerase chain reaction**
STAT1	**Signaling Primarily Activator Transcription 1**
SNP	**Single Nucleotide Polymorphism**
T1DM	**Type one Diabetic mellitus**
Taq Polymerase	**Thermus aquaticus polymerase**
TBE buffer	**Tris borate EDTA buffer**
TC	**Total cholesterol**
JAK	**Janus kinase**
TE	**Tries EDTA**
GR	**Gastrin receptor**
TG	**Triglyceride**
TGF	**Transforming growth factor**

Th	**T helper**
TNF-α	**Tumor Necrosis Factor alpha**
Treg	**T regulator cell**
TRLs	**Triglyceride rich lipoproteins**
VLDL-C	**Very low density lipoprotein cholesterol**
WHO	**World Health Organization**
μL	**Micro Litter**

CHAPTER ONE

INTRODUCTION

1. Diabetes Mellitus

It is a common health concern that impacts more than 400 million people over the world, according to the World Health Organization (WHO), Chronic microvascular, macrovascular, and neuropathic complication result from this metabolic disorder, which can be life-threatening(1). A lack of insulin production due to pancreatic cell damage, or insulin resistance are factors that contribute to diabetes mellitus, Diet, lack of activity might be key contributors to the global increase in diabetes, which is predictable to reach 366 million in the over-65population by 2030, according to the (WHO). Diabetes-related complications such as nephropathy, neuropathy, cardiovascular and retinopathy are just a few of the issues that might arise(2).

Type 1 DM occurs when an autoimmune illness affects pancreatic cells so that insulin production is reduced or impaired, Whereas type 2 diabetes mellitus is caused by decrease insulin secretion and insulin resistance, which impairs the individual's capacity to utilize insulin (3).

When the level of glucose in the blood rises, cells generate insulin to counteract the rise in glucose, Insulin reduces blood glucose in two ways ; firstly by lowering the production of glucose in the liver through glycogen lysis and gluconeogenesis; and by increasing the rate of glycogenesis in response to insulin stimulation(4). Additionally, cortisol and catecholamine, growth hormone in addition to glucagon, are known to raise plasma sugar levels(5).

1.1. Diabetes Mellitus Type 1:

It is a Type of diabetes that affects the pancreas, despite the fact that it only accounts for around 10% of all diabetes cases worldwide, it is becoming more frequent and present in earlier younger ages. It is believed that T1DM is caused by the autoimmune attacking and killing endocrine pancreatic cells(6). There is a very small ratio of patients (10%) who are distinguished as type 1B, which means they have no evidence of autoimmunity, and the pathophysiology is considered idiopathic in these cases(7).

1.1.1. The Effects of Genetics on Type 1 Diabetes:

Individuals with type 1 diabetes who have a family history of the disease are more likely to get it, However 10-15% of patients have a first- or second-degree relative according to the researchers(8).T1DM is more common in patients' families than in the general population, with around 6 percent of children, 5 percent of siblings, and 50 percent of monozygotic twins having the illness, as compared to the general.

Over 50 genetic risk loci for type 1 diabetes have been recognized via genome-wide connection studies and analyses(9). The major histocompatibility complex (MHC) region of chromosome 6 (also known as the HLA region) contains the majority of the genes related to type 1 diabetes (human leucocyte antigen)(10). At least 40% to 50% of the genetic risk of developing type 1 diabetes (T1DM) is due to HLA complex polymorphisms on chromosome 11(11).

In the insulin gene (Ins-VNTR, IDDM 2) and the cytotoxic T lymphocyte related antigen-4 gene can be found on (chromosome 11)(12).

1.1.2. The Environmental Factors:

Environmental variables have a substantial impact on the pathophysiology of type 1 diabetes(13). Several studies of monozygotic twins have provided compelling evidence of this effect, with illness frequency ranging from 50 to 100 percent in some cases(9).A variety of factors, including viruses (such as rubella, coxsackie virus B, or entero viruses), toxins, and nutrients (such as cow's milk and cereals), all have a role in the environment(14).

Despite the fact that the precise impact of these factors is unknown, it is vital to identify them since they may be changed and may result in preventative or therapeutic treatments(15).

1.1.3 Immunological Determinants:

The human immune system is exposed to large groups of antigens, its goal is to distinguish between unknown and native ones, and as a result of rearrangements, the repertoire of special recognition molecules is not genetically restricted and, as a result, can grow to be incredibly extensive (16). Because of this, many tissues, including pancreatic cells, acquire antigen receptors that interact with their cells (17).

1.1.3.1 Infection Resistance:

During the development of T lymphocytes in the thymus and B lymphocytes in the marrow of the bone, negative selection and elimination of potentially dangerous immune cells take place (central tolerance)(6). The immune system normally engages in procedures that neutralize or inhibit self-reactive lymphocytes that have escaped central tolerance mechanisms and have moved to the periphery (peripheral tolerance), As a result of a number of autoimmune disorders, these immune systems might be disrupted, resulting in various symptoms(18). Genetic abnormalities in the AIRE gene, as well as non-genetic factors, can decrease central tolerance in the thymus, ultimately leading to the development of type 1 diabetes(19).

The influence of viral infections on the human thymus is a fascinating subject to consider, According to experimental evidence, thymic epithelial cells and thymocytes are vulnerable to infection by coxsackie virus B4 and other enter viruses (20). Following this discovery, it was determined that the maturation and discrimination processes of T cells were abnormal. Recent years have seen an upsurge in the number of people who have the disease by this way(21).

1.1.3.2 Immunity at the Cellular Level:

In type 1 diabetes, the endocrine pancreas cells are most likely eliminated by apoptosis, a known as systemic cell death, that is characterized by the activation of a cascade of cysteine-asparaginase enzymes known as caspases (22).Animal studies and in vitro tests have confirmed that apoptosis is the most common mode of cell death; moreover, necrosis also be substantial in humans, according to some evidence(23).

T lymphocytes inside the islet microenvironment generate an inflammatory response with higher levels of pro-inflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor (TNF-α), and interferon (IFN-gamma) (40).The caspase cascade is activated as a result of the release of these cytokines, Apoptosis is thought to be caused directly by auto reactive T lymphocytes coming into contact with cells through the perforating mechanism or a link between Fas and its ligand(FasL), according to several theories of apoptosis(24).

Misregulation of cytokine secretion is required even in this situation, The T-reg subpopulation, as well as their quantitative and qualitative inadequacies in individuals with sickness, are of great interest to researchers(25).

Asymptomatic people with normal glucose levels may acquire this illness over an interval of months or years(26), but they do not experience any symptoms(27). Symptomatic hyperglycemia happens after a protracted latency interval, that corresponds to the big number of functioning cells which must be eliminated before the illness manifests itself in a clinically significant manner(28).

1.1.3.3 Pathogen-Specific Immunity:

Due to the impaired immune response, autoantibodies can accumulate in the humoral immunity arm of the body, which can persist for years before the development of clinical signs of type 1 diabetes(29). Patients with type 1 diabetes are more likely than control not to have autoantibodies against GAD65, tyrosyl phosphatase (IA-2), insulin (IAA) and zinc transporter, among other things (30).

In addition, the fact that insulin's gene deletion has a significant impact on disease progression , As well as the fact that insulin injection during the pre-diabetic phase may prevent or delay clinical manifestations of type 1 diabetes (T1DM), these demonstrate insulin's critical role as an autoantigen, The GAD enzyme, which is abundant in cells, the central nervous system, and the testicles, is another important autoantigen(31).

A total of around 70% of individuals with type 1 diabetes showed anti-GAD autoantibodies at the period of their diagnosis(32). IA-2 is even a prominent auto antigen, with positive autoantibodies found in around 60% of people with type 1 diabetes(33). IA-2 autoantibodies often emerge later in life than insulin or GAD auto antibodies, and they are substantially related to the development of the illness in most cases(34). The zinc transporter (ZnT8) was shown to be an autoantigen in the development of type1diabetes.ZnT8 autoantibodies are found in 60–80% of newly diagnosed people, which is a significant proportion(35).

In babies who have been monitored from birth through the development of T1DM, ZnT8 auto antibodies show later than insulin auto antibodies, and they frequently disappear before the onset of clinical signs of the illness(36). While the quantities of ZnT8 autoantibodies present at diagnosis are connected to age, HLA Geno type, and metabolic state at the time of diagnosis, A large Finnish cohort has demonstrated that these antibodies do not significantly assist in the detection of cell autoimmunity(21). When two or three autoantibodies from the list above are produced simultaneously, it is considered to be one of the most reliable predictors of progression to clinical T1DM(37).

According to a recent study, people who had previously acquired two or more autoantibodies were studied in the TEDDY (the environmental reasons of diabetes in the young people) study(38). Children at genetic risk are included in the TEDDY project, which is a prospective band study financed by the National Institutes of Health with the purpose of uncovering environmental causes of type 1 diabetes(32).

1.2. Type 2 Diabetes Mellitus:

Type 2 diabetes mellitus (T2DM) is one of the most common metabolic illnesses in the world, and it is generally caused by a combination of two factors: impaired insulin secretion by pancreatic cells and an inability of insulin-sensitive tissues to respond insulin and because the precise timing of insulin release required to fulfill metabolic demand (39) the molecular processes behind insulin production and release, as well as the insulin response in tissues, must be tightly controlled (40).

Type 2 diabetes (T2DM) accounts for more than 90% of all diabetes mellitus cases and is distinguished by insufficient insulin secretion by pancreatic islet cells, tissue insulin resistance (IR), and an insufficient compensatory insulin secretory response (41).As the illness worsens, insulin secretion becomes ineffective in maintaining glucose homeostasis, resulting in hyperglycemia (high blood sugar)(42).

Patients with type 2 diabetes are often obese or have a higher body fat percentage, with the majority of the fat being located in the abdominal region. Adipocytes contribute to IR in this circumstance through a range of inflammatory processes, including increased release of free fatty acids (FFAs) and dysregulation of adipokines

, among others (3). One of the key causes of the T2DM epidemic is the global rise in obesity, which is accompanied by sedentary lifestyles, high-calorie meals, and population aging together, these factors have more than quadrupled T2DM incidence and prevalence.

The pancreas (both beta cells and alpha cells), liver, skeletal muscle, kidneys, brain, small intestine, and adipose tissue are all implicated in the development of type 2 diabetes (T2DM) (43). Inflammation, changes in gut microbiota and immunological dysregulation have all been identified as important pathophysiological contributors in type 2 diabetes(44) . Furthermore, the full disease burden of type 2 diabetes is likely underestimated as one in every three diabetics is underdiagnosed, resulting in a total of 232 million people who are not diagnosed, Between the ages of 40 and 59, diabetes affects the largest proportion of adult patients(45). Geographic differences in T2DM incidence and prevalence exist, with more than 80% of patients living in low-to-middle-income countries, making significant challenges to effective treatment .When compared to those who do not have diabetes, patients with type 2 diabetes (T2DM) have a 15% higher risk of death from any cause, with cardiovascular disease (CVD) being the leading cause of morbidity and mortality in this population (46).

The prevalence of type 2 diabetes is determined by both heredity and the environment factors (47). While genome-wide association studies have discovered widespread glycemic genetic polymorphisms linked with type 2 diabetes, these variations account for just 10% of overall phenotypic variance,

Genetic, metabolic, and environmental risk factors for type 2 diabetes are complex interactions of elements that contribute to the disease's prevalence(1). Eventhough an individual's predisposition to type 2 diabetes is primarily genetic in nature due to non-modifiable risk factors (ethnicity and family history/genetic predisposition) (48).

1.3 Cholecystokinin:

is a peptide hormone secreted to the GI system, Endocrine cells create and release cholecystokinin (pancreozymin) in the duodenum, the first section of the small intestine(49). It works as an appetite suppressant , regulate gastric acid secretion and gallbladder emptying the receptors are found in the cerebral cortex, hippocampus, and striatum, It's in the nucleus of solitary tracts and the lower zone of the brain stem(50). Tissue and development-specific CCK expression is biphasic, falling just before delivery and rising promptly after delivery (51).

The number of CCK-manufacturing neurons in the brain increases with age(52). The pituitary corticotrophs and spermatogenic cells all have low levels of CCK, CCK-1 and 2 are G-protein-coupled receptors of class 1(50). The gallbladder's smooth muscles and D cells CCK1R, In the stomach (parietal and Enterochromaffin like cells of the gastric mucosa), human pancreas, and specific parts of the central and peripheral neurological systems, CCK2R/GR are detected, whereas CCK1R/GR are present (CNS)(53).

1.3.1 A brief history of CCK:

Joy Simcha Cohen, a British scientist, proposed CCK's existence in 1905(49). In 1928, Andrew Conway Ivy and Eric Oldberg of Northwestern University Medical School discovered pancreozymin as a hormone that promotes pancreatic enzyme synthesis(54). It is a peptide hormone that is structurally related to gastrin, another gastrointestinal hormone,The C-terminus of CCK and gastrin is identical, This is because the 150-amino acid precursor, preprocholecystokinin, is post-translationally changed(55).

As a result, the CCK peptide hormone comes in several forms, each named by the number of amino acids it contains, e.g., CCK58 is a helix-turn-helix(56). The peptide's biological activity is at its C-terminus, Most CCK peptides have a sulfate group attached to a tyrosine seven residues from the C-terminus (57).This modification allows CCK to operate as a cholecystokinin A receptor activator. Other unsulfated CCK peptides exist, but their biological role is unclear(48).

1.3.2 Cells contain cholecystokinin:

CCK has been discovered, in Peripheral neurons, including those in the gut mucosa, express CCK. Food intake and glucocorticoids all influence CCK expression(51). Dopamine, estrogen, and cell damage activate CCK-mRNA in neurons, The signaling pathways' transcription factors are unknown(48). The CCK-2 receptor subgroup (formerly known as CCK-B) is seen chiefly in the brain, whereas the CCK-1 receptor subtype (formerly known as CCK-A) is found primarily peripherally, such as in the skin and pancreas(55).

When Adenylyl cyclase is activated, increasing intracellular c-AMP and protein kinase A activity (PKA), This attributes to rise in c-AMP to a CCK-receptor-mediated mechanism(58). CAMP-dependent protein kinase activity is CCK dependent , PKA activity spikes and plateaus in the presence of high CCK doses(59).

Pancreatic acinar cells' CCK receptors have two unique affinities, prompting two separate responses, At low dosages, CCK enhances zymogen secretion, but inhibits both stimulation and secretion at high levels(60). The binding sites of the CCK-A and CCK-B receptors engage different CCK neuropeptide segments, Because CCK-A prefers the sulfated intermediate neuropeptide CCK-8 over CCK-4, its effects are mostly peripheral(58).

CCK-BR prefers CCK-4 over other CCK fragments, indicating that most CCK activity occurs in the brain(57). Stimulation of CCK-containing neuronal pathways in humans can cause panic, CCK activation might cause panic episodes(61). CCK antagonists can be anxiolytic by inhibiting CCK-2 receptors(49).

1.3.3 Affinity for cholecystokinin:

CCK and gastrin's actions are determined by their affinity for target cell membrane receptors(53). CCK-1 and CCK-2 are G-protein-coupled receptors of class 1(56). It has 7 trans membrane domains joined by intra cellular and extra cellular loops and N- and C-terminal tails(62).

Classification is depend on affinity for CCK or gastrin(62). The CCK-1 receptor binds 500 times more CCK than gastrin(63). The CCK2 receptor binds to CCK like gastrin does, Also CCK-1 receptors preferentially prefer sulfated CCK over non-sulfated CCK, whereas CCK-2 receptors cannot tell the difference(64). Five amino acids that are necessary for gastrin sensitivity are found in the CCK-2 extracellular loop, The CCK-2 receptor loses CCK binding when His207 is deleted(50). The CCK-1 receptor binding site contains His207, and substituting another amino acid for the Asp area of CCK results in a lack or profit of affinity. As a result, CCK-1 and CCK-2 receptor interacts with the CCK-1 receptor via the asp of CCK(53).

1.3.4 The function of cholecystokinin:

The work performed by cholecystokinin is classified as follows(65):

A- in gastric tract:

1. Aids digestion by modulating pancreatic exocrine enzymes involved in fat, protein, and glucose breakdown(61).

2. CCK adjusts the release of bile acid to relief in further fat breakdown in the small intestine movement regulation (48).

3. It slows gastric drainage to regulate chyme flow inside the duodenum(60).

4. Controls gastrin production via somatostatin, preventing gastric acid release after meals(46).

5. As a result, it lowers post-meal stomach H_2^+ secretion, It assists in protein absorption in the intestines ,stimulating cell growth, energy production, expression of genes and protein synthesis(66).

B- The brain:

1. Anxiety management

2. Perception of pain

3. Memory(67)

1.3.5 Cholecystokinin Action:

Fatty acids and proteins directly stimulate I-cell CCK release(68). Inflammatory cells express GPR, which binds to long-chain fatty acids(46). CCK first stimulates the small intestine's afferent fibers via a paracrine process(62).CCK-1 receptor activation increases vagal efferent neuron firing by raising intracellular calcium(61). This restrains the excitatory vagal efferent pathway to the distal stomach(40). Gastric vagal afferents are stimulated by hormonal activity and the inhibitory vagal efferent pathway(106).

CCK reduces gastric emptying by relaxing the proximal stomach and raising tension in the pyloric sphincter(69). High levels of CCK can speed up gastric emptying by stimulating the small and large intestines, causing bowel movement or by relaxing the pyloric sphincter(68). Afferent and vagal efferent pathways influencing gastric motility are activated by CCK, regulating the reflex regulation of stomach emptying(42).

1.3.6 Cholecystokinin Clinical Value:

Obesity lowers CCK function, indicating insensitive vagal afferent neurons(44). This low expression of CCK explains the diminished influence on satiety and why most obesity people are always hungry, High-fat meals with low CCK-1 receptor expression elevate plasma ghrelin(41). Suppression of CART in vagal afferent neurons increases food intake by suppressing the satiety peptide CART(61). CCK also regulates metabolism and fat absorption, They show that blocking the CCK signaling pathway reduces weight profit(63). Inactivation raises energy consumption and lowers energy extraction(64). CCK is a vasovagal hormone that is activated by distension of the stomach wall(53).

In obesity, reduced islet cell mass correlates with increased CCK expression and insulin release sensitivity(62). A mediator of compensatory mechanisms inside the Langerhans islets (65). Dietary changes that reduce fat and calorie consumption enhance CCK and PP secretion, resulting in weight loss and developed glucose homeostasis(62). The presence of CCK in several midbrain locations suggests it is involved in behavioral processes like anxiety(69).

CCK is found in solitary tract nuclei and area postrema , They link these zones to Parkinson's disease are often hypersensitive to physical stimuli(70).

brainstem nuclei that contains 5-hydroxytryptamine receptors reacted with these zones, committing them in PD pathogenesis, Gastrin and CCK-B receptors are found in medullary thyroid carcinoma and small cell lung cancer(66).

Gastrin and the CCK2R/gastrin receptor (GR) regulate cell proliferation and adhesion loss in addition to epithelial cell motility and invasion(5).When the CCK2R/GR is active intracellularly, it might cause cancer(79). As a result, these receptors are vital in the development of pre-neoplastic lesions and cancer(68).

1.4 Cholecystokinin receptors:

CCK and gastrin bind to CCK receptors, which are G protein-coupled receptors that bind the peptide hormones CCK and gastrin, The CCKA and CCKB subtypes are 50% identical(65).

1.4.1 Subtypes of CCK receptors:

Physiological CCK-AR stimulation induces exocrine pancreatic enzyme secretion and endocrine pancreatic polypeptide secretion, CCK-AR limits acid secretion by releasing pepsin from gastric chief cells and somatostatin from gastric mucosa D cells(66). A decrease in small bowel transit time and an increase in colonic transit time are all caused by CCK-AR on smooth muscle cells(52).

They are present in the Oddi sphincter and lower esophageal sphincter inhibitory postganglionic neurons, where they relax the sphincter(71). In parts of the central nervous system and peripheral nervous system (vagus nerve), CCK-AR colocalizes with CCK-like immune reactive nerve fibers and mediates the satiating action of CCK produced by small intestinal I cells or enteric neurons following a meal(66). CCK-AR activates anterior pituitary corticotrophs to produce ACTH (72).

CCK-B receptor Similar to CCK immune reactivity and mRNA distribution, CCK-BR is widely distributed throughout the CNS, Co localization of CCK and dopamine in mesolimbic (dopaminergic pathway), as well as dopaminergic-related movement and behavioral diseases such as Parkinson's and schizophrenia, may be caused by CCK receptors found in these dopaminergic pathways(40).

Unlike the posterior nucleus accumbens CCK-AR, the anterior nucleus accumbens CCK-BR inhibits dopamine release. Anxiety is regulated by peripheral and central CCK, possibly through interactions with other co localizing neurotransmitters such as 5-hydroxytryptamine(42). CCK-BR is present on monocytes and T lymphocytes, although its function is unknown(67). A human leiomyosarcoma cell line contain CCK-BR, which may promote proliferation(53).

1.4.2 Protein Structure Deduction and cDNA Cloning:

Both CCK1R and CCK2R feature seven hydrophobic regions, most likely transmembrane domains, with exterior NH2-and intra cellular COOH-terminal ends(48). These structures are similar to the G protein-coupled receptor rhodopsin's high-resolution three-dimensional structure (GPCRs)(73).

Cloning of CCK1R and CCK2R cDNAs in Guinea pig gallbladder and pancreas, mouse pancreas, rabbit stomach, and human gallbladder revealed that both proteins had 3 - 4 N-linked glycosylation portions in their amino termini, corresponding to a high and variable of glycosylation for example, endoglycosidase-F reduces native pancreatic CCK1R from 85,000 to 100,000 Da to 38,000 to 42,000 Da(28). The level of glycosylation of affinity tagged CCK2R changes depending on the cell kind(46).

A 76,000 Da component was found in isolated parietal cells, while a 47,000 Da component was found in pancreatic membranes, The carbohydrate moieties' role in these receptors is unknown; The CCK1R gene is located on chromosome 4p15.1-p15.2. CCK2R is found on chromosome 11p15.4 in humans and on chromosome 7 in mice(74).

The human CCK1R transcriptional start portion is 206 base pairs up stream of the translation start portion. The CCKR gene's regulation is unknown at this time. However, variations in the CCKR gene's promoter region have been linked to illnesses including panic disorder, alcoholism, and obesity(75).

Exon/intron arrangement may drive to protein variety, The existence of two CCK2R mRNA isoforms caused by alternative splicing of exon 4 in the human stomach was even found(68).

Humans have a little variety; mice, rats, dogs, and calves have a long variant, A clone expressing a receptor with detention of intron IV in the third intracellular loop was also discovered, In the absence of a ligand, this misplaced variation appears to generate spontaneous rhythmic increases in intracellular Ca2(76).

1.4.3 Cholecystokinin and signal transduction:

CCK receptors are seven-transmembrane G-protein coupled receptors present on the plasma membrane of CCK-regulated cells(46). Previously known as CCKA (alimentary) and CCKB (brain), CCK1R and CCK2R are high-affinity CCK receptors(77). A sulfated tyrosine residue 7 amino acids in the C terminal reduces the affinity of CCK1R for the homologous hormone gastrin(56).

There are vagal afferent nerve terminals in numerous species of pancreatic and gallbladder smooth muscle(48). Moreover, the entity of CCK1R in human pancreatic acinar cells is controversial, with mixed results. In the absence of protease, a significant response was seen in non-treated slices, suggesting that human CCK1R is sensitive to collagenase(45). CCK2R interacts with CCK and gastrin similarly, but does not require a sulfated tyrosine. It is located in the stomach and is a wide distributed in the brain, where it is known as the gastrin receptor(41).the mature receptor is glycosylated on the amino terminal and an extracellular loop and phosphorylated on Ser and Thr residues in the third intracellular loop and the carboxyl terminal (40).

This phosphorylation is expected to contribute to receptor desensitization and perhaps internalization, The carboxyl terminus also contains two palmitoylated cysteine that function as membrane anchors(78). Many articles have been written to explain how CCK interacts with the receptor, They use techniques such as photoaffinity labeling of receptor residues within a calibrated distance using a probe containing a ligand that includes a photoactivatable ligand in an amino acid three-dimensional receptor models were included(79).

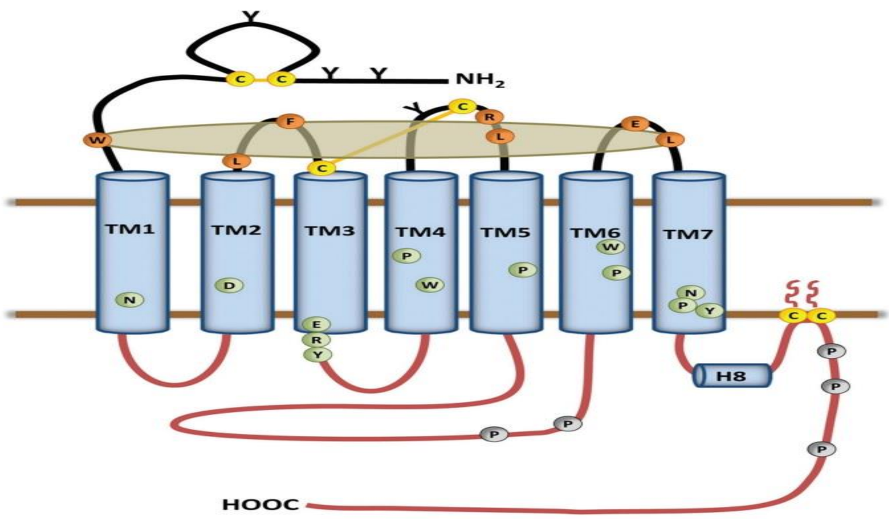


Figure 1-1: the membrane-spanning topology and posttranslational modification sites are highlighted(46).

Two receptor models are currently being considered, Its amino terminus spans the receptor's first transmembrane region, The CCK1R has several small-molecule antagonists and agonists(79). It's not always clear if they get into the CCK binding pocket or not(38).

A small chemical agonist or antagonist can bind to the CCK1R allosterically(42). Benzodiazepines like devazepide and JMV-antibodies are examples of CCK1R antagonists employed in physiological research(84).

A recent research has shown that the CCK1R is regulated by the plasma membrane's lipid composition, initially shown to be cholesterol, but now recognized to be phospholipids that can fluctuate in metabolic disease(52). Obesity and diabetes cause increased membrane cholesterol, which reduces CCK sensitivity(82). The CCK1R is activated by heterotrimeric G proteins on the cytoplasmic face of the plasma membrane, Contrary to popular belief, the major activation region of the G receptor is the third cytoplasmic loop(80).

As soon as a heterotrimeric G protein subunit is active, it binds GTP and releases it. The active subunits mediate most activities, The human genome has 16, 5, and 12-subunits (77). Ca2+ signaling and digestive enzyme production are mediated by G protein subunits q and 13 (78). Microinjection of a blocking antibody reduces Ca2+ signaling in acinar cells (110). Toxins from bacteria like cholera, pertussis, and pasturella target G proteins.(75).

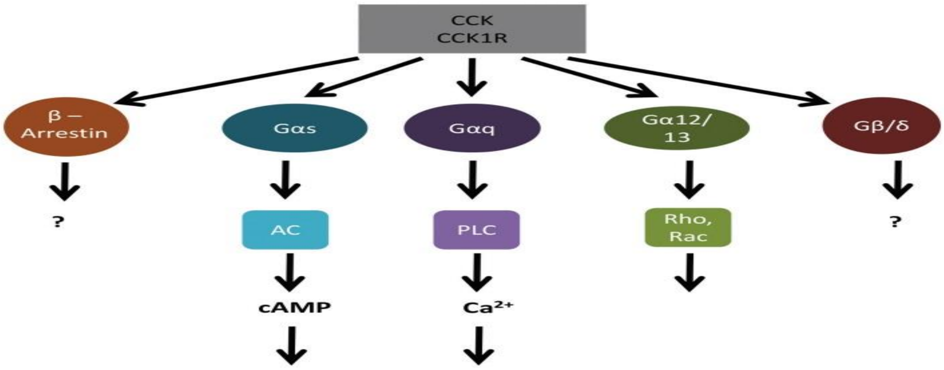


Figure 1-2: CCK1R signals through multiple G proteins to activate different signaling cascades(81).

1.5 Interleukin-27:

Interleukin-27 (IL-27), an IL-6/IL-12 cytokine , is made up of two noncovalently linked subunits; EBI3 and IL-27p28, The IL-27 receptor is a heterodimer made up of the IL-27 receptor subunit alpha (IL-27Ra) and glycoprotein 130 (gp130), The receptor complex is expressed on numerous immune cells, including T cells, B cells, DCs, macrophages, and natural killer cells(82).

Signaling by IL-27 stimulates STAT1 and STAT3, as well as the MAPK pathway, IL-27 has been demonstrated to have pro-and anti-inflammatory properties in autoimmune illnesses such as rheumatoid arthritis, MS, and IBD, IL-27 also promotes Th1 cell growth by activating the STAT1-mediated T-box expression in T cells pathway(83). In a mouse colitis model induced by CD4+ T cell transfer, It is found that IL-27 suppresses differentiation of inducible T-regs and that IL-27R deficiency enhances FOX3 expression (84).

1.5.1 Structure and Mechanism of Interleukin-27 Production:

Ebi3, an IL-27 component, was discovered in 1996, Since EBI3 lacks a membrane anchoring domain, like IL-12 p40, and is not successfully secreted from transfected human B-lymphoma cells, it was assumed that another subunit was required for EBI3 secretion(85). Computational studies revealed the other component, IL-27p28, as a member of the four-helix bundle cytokine receptor class I(86).

Due to its low secretion and lack of biological activity, IL-27p28 is dependent on its companion molecule, IL-27p28 secretion was determined to be the most effective molecule that binds to Ebi3, the cytokine was named IL-27, and its receptors were IL-27R and gp130(87). Diverse cell types manufacture these components, Antigen-presenting cells in immune cells create IL-27 (APCs)(88). Toll-like receptor (TLR) activation by LPS, CpG, and Poly (I:C), following IL-27p28 synthesis, NF- κB is required in innate immunity for early TLR-mediated induction(89).

1.5.2 Interlukine-27 genes:

The IL-27 gene has five exons and is found on chromosome 16p11(90). These polymorphisms were recently linked to a number of illnesses, Inflammatory illnesses such as ulcerative colitis, chronic obstructive pulmonary disease (COPD), and rheumatoid arthritis have been linked to the rs153109 (964 A > G) polymorphism (91).

As a result, rs178855750 (2905 T > G) in exon 2 of the IL-27 gene has been associated with allergic rhinitis and cervical cancer(92). Unclear mechanisms through which these differences alter systemic inflammatory responses during sepsis remain unknown(86).

T lymphocytes attack insulin-producing pancreatic b cells, causing type 1 diabetes. The p28 subunit is encoded by IL27 (encoding the p28 subunit) and has been linked to T1D in humans by many genome-wide association studies(13).

It was then reported that there was a missense mutation in IL27 (rs153109), that was linked to elevated STAT1 in human CD4 T cells(81). Interestingly, the same rs181206 variant improved IL-27 function, These findings suggest that IL27 allelic variations may directly affect the downstream signaling pathway and therefore the etiology of type 1 diabetes(7).

Inflammatory illnesses such as ulcerative colitis, chronic obstructive pulmonary disease (COPD), and rheumatoid arthritis have been linked to the rs153109 (964 A > G) polymorphism. As a result, rs178855750 (2905 T > G) in exon 2 of the IL-27 gene has been associated with allergic rhinitis and cervical cancer(81). Unclear mechanisms through which these differences alter systemic inflammatory responses during T1DM remain unknown(87).

1.6 Chemical parameter of T1DM:

1.6.1 Profile of Lipids:

The lipid profile or lipid panel is a collection of blood tests that acts as an initial screening tool for lipid disorders. It includes tests for cholesterol, triglycerides, high-density lipoproteins (HDL), low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), and chylomicrons. This test can detect some hereditary illnesses and estimate the risk of developing cardiovascular disease, certain types of pancreatitis, and other diseases(88).

1.6.1.1 Cholesterol:

Cholesterol is a waxy molecule found in all cells. It performs a variety of essential tasks, including assisting in the formation of new cells in the body(89). It is transported through the circulation in conjunction with proteins. It is found in tissue and plasma lipoproteins as a free fatty acid or as a cholesterol ester when coupled with a long-chain fatty acid. Cholesterol is found in two forms: dietary cholesterol and in biosynthesis in vivo from acetyl-CoA.

It is produced in a variety of tissues from acetyl-co A and excreted in the bile as cholesterol or bile salts(90). Cholesterol is a precursor to several other steroid hormones found in the body, including corticosteroids, sex hormones, bile acid, and vitamin D. It is generally an animal metabolic product and so appears in animal-derived foods such as egg yolk, meat, liver, and brain. (91).

1.6.1.2 Triglyceride:

Triglyceride (TG) is a compound composed of glycerol and three long chain fatty acids(92). It is found in dietary fat and may be produced in the liver and adipose tissue to serve as a reservoir of stored energy that can be mobilized when needed. For example, triacylglycerol, which contains both saturated and unsaturated fatty acids, is a critical component of cell membranes during hunger(93).

It accounts for approximately 90% of the lipid in the chylomicron(94). It is degraded from chylomicrons by the enzyme lipoprotein lipase (LPL), which is found on the luminal surface of the capillary endothelium of adipose tissue, skeletal muscle, cardiac muscle, and lactating breast. This results in the delivery of free fatty acids to these tissues for use as energy sources or for storage as triacylglycerol. Apo C-II activates LPL. Triacylglycerol produced in the liver is also accessible to tissues as a stored energy source via VLDL(95).

1.6.1.3 Lipoproteins:

It is defined as any material that contains both lipid (fat) and protein. They are found in both soluble and insoluble complexes. Due to the hydrophobic nature of lipids and their insoluble nature in blood water, cholesterol, cholesterol esters, TGs, and phospholipids must be carried through the circulation packed as lipoproteins. These macromolecules are water soluble; they aid in the transportation of lipid molecules by transporting them through the bloodstream; lipoproteins are even classified into several categories(96).

A- Lipoproteins with a high-density lipoprotein (HDL):

A lipoprotein found in blood plasma that is heavy in protein and low in triglycerides and cholesterol, it is even referred to as "paradox" cholesterol since it aids in the removal of other types of cholesterol from the bloodstream. Increased HDL cholesterol levels are related with a decreased risk of heart disease(97).

B- Lipoproteins with a low density:

A blood plasma lipoprotein having a low protein content and a high triglyceride and cholesterol content (98). Increased amounts of (LDL) can ultimately accumulate inside the walls of blood vessels, obstructing their pathways. Occasionally, a clot will develop and become trapped in the restricted area, resulting in a heart attack or stroke (99).

C- Chylomicrons:

The type of fat that is carried from the stomach to the rest of the body; it is composed of a lipid core surrounded by a protein, cholesterol, and phospholipid envelope(100). Chylomicrons are around 100 nm in diameter and are generated in the intestinal mucosa before entering the lacteals and ultimately the circulation(101).

1.6.1.4 Lipids' biochemical functions:

Lipids leave their tissue of origin via this carrier mechanism, enter the circulation, and are delivered to tissues, where their components are either employed in synthetic or oxidative processes or stored for later use. The Apo proteins ("Apo" refers to the protein contained within the particle's shell in its lipid-free form) not only contribute to the particle's hydrophilicity and structural stability, but also act as activators of certain enzymes necessary for normal lipoprotein metabolism and as ligands on the lipoprotein's surface that target specific receptors on peripheral tissues that require lipoprotein delivery for their innate cellular function(102).

1.6.2 Renal function tests (RFTs):

Renal function tests (RFTs) are used to determine the renal function of it is a collection of tests that may be used in conjunction to assess kidney (renal) function(103). The tests assess the levels of a variety of chemicals in the blood, including many minerals, electrolytes, proteins, and glucose, in order to identify the present state of the kidneys' health. They are also related with diabetic nephropathy as a cause of late-stage diabetes (blood urea nitrogen and creatinine)(104).

1.6.2.1 Urea:

The simplest way to monitor kidney function is through blood tests for blood urea and creatinine, these are common metabolic waste products that the kidneys eliminate. Urea is a result of the breakdown of proteins. A blood test can be performed to determine the level of urea nitrogen in the blood. These chemicals (along with countless others) are not eliminated regularly in renal disease, and consequently accumulate in the body, resulting an increase in urea levels in the blood. BUN levels should be between 7 and 20 mg/dL (105).

1.6.2.2 Creatinine: Creatinine is a non-essential amino acid

Serum creatinine is largely a creatine metabolite, with virtually all of it being found in skeletal muscle. Creatinine levels should be between 0.8 and 1.4 mg/dL. Females often have a lower creatinine level (0.6 to 1.2 mg/dL) than males, owing to their lower muscle mass. The concentration of creatine per unit of skeletal muscle mass remains constant, as is the rate of creatine breakdown.

As a result, plasma creatinine content is extremely constant and serves as a direct indicator of skeletal muscle mass. Reduced blood creatinine levels are connected with an increased risk of type 2 diabetes, which may be due to a decreased skeletal muscle volume. Skeletal muscle is a significant insulin target tissue, and a decreased amount of skeletal muscle results in fewer insulin target sites, resulting in an increase in insulin resistance. As a result, type 2 diabetes develops (106).

1.6.3 Glycosylated hemoglobin:

Rahbar and colleagues published the first description of glycated hemoglobin (HbA1c) in 1969(107).A significant link was found between HbA1c levels and glycemic control over a two-to three-month period as a consequence of further research, which led to the test's gradual integration into clinical practice beginning in the 1980s(108). HbA1c estimation has been a cornerstone of diabetes therapy since studies such as the Diabetic Control and complications (109).

The United Kingdom Prospective Diabetic Study established a correlation between high blood glucose levels and the development of diabetes problems(110). The tetramer hemoglobin (Hb) molecule is made up of two alpha and two beta globin chains and carries oxygen throughout the body, In the presence of high blood glucose levels, hemoglobin gets non-enzymatically glycated at a variety of sites across the structure of the molecule(111). When glucose is added to the beta chain's N-terminal valine residue, the hemoglobin A1c level increases(112). HbA1c levels in blood represent the glucose concentrations to which the erythrocyte was exposed during its life (roughly 117 days in men and 106 days in women)(113).

As a result, the HbA1c test is a measure of glycemic control throughout the preceding two-to-three-month period. HbA1c is determined by the 30 days immediately before this period, which accounts for 50% of the total(106). When determining HbA1c, a number of different techniques are employed, When it comes to laboratory tests, high-performance liquid chromatography is by far the most commonly utilized technique (HPLC)(104).

Boronate affinity tests and immunoassays are also employed in addition to these methods, The assay is being standardized on a worldwide basis, and efforts are being made to achieve this goal(114). In patients with diabetes, the HbA1c level is primarily used to monitor their condition in order to be considered optimal diabetes treatment, several organizations have endorsed HbA1c goals of less than 6.5 to 7% as the gold standard(101).

The capacity of anti-diabetic medications to lower HbA1c levels is also a factor in determining their effectiveness, Hemoglobin A1c (HbA1c) is used by a variety of algorithms for diabetes management to make important therapeutic decisions(115). The hemoglobin A1C (HbA1c) test has recently been presented as a tool for detecting diabetes. HbA1c is a very excellent indication of glucose levels in most cases, but it can be inaccurate in some situations(116). It has been demonstrated that high doses of antioxidants such as Vitamin C and Vitamin E can reduce hemoglobin glycation and thereby lower HbA1c levels, although it is unclear to what degree this occurs with medicinal quantities of these antioxidants(117).

When individuals with diabetes were given pharmaceutical amounts of vitamin C or vitamin E for four months, discovered that there was no effect on their HbA1c levels(118).

Agents that cause erythropoiesis to be impaired Anemia caused by iron and vitamin B12 deficiency results in higher hemoglobin A1c levels in patients who are anemic(105)

Aims of Study:

1- Study the genetic variations of IL-27 (−964 A > G)
2- Study the relationship between the levels of IL-27 and CCKBR.
3- Study the effect of other parameters like (lipid profile, renal function and study correlation with age, and body max index) with T1DM.

Chapter Two

Materials and Methods

2. Materials and Methods

2.1. Tools and Instruments

The tools that had been used in the study, as well as instruments are listed in Table 2-1, along with their provenance of manufacturing:

No.	Material	Produced Company	Origin
1.	100 bp step DNA ladder	Cyntol	Russia
2.	Agarose	Pronadisa	Spain
3.	EDTA	Scr	USA
4.	Ethanol	Scr	Chia
5.	Ethidium bromide	Intron	Korea
6.	master mix kit	Cyntol	Russia
7.	Primers	Bioneer	Korea
8.	Gel loading dye purple DNA	Biolabs	New England
9.	Nuclease free water	Cyntol	Russian
10.	Ethanol (70%).	Fluka Chimeka	Switzerland
11.	Mgcl2	Cyntol	Russia
12.	Tris- base	Thomas Baker	India
13.	Boric acid	CDH	Spain
14.	Pcr Master mix	Cyntol	Russia
15.	CCKBR kit	Al-shkairate	Jordon
16.	IL -27 kit	BT Lab	China
17.	HBA1C	Roche	Germany
18.	Triglyceride kit	BIOLABO	France

19.	Cholesterol kit	BIOLABO	France
20.	HDL kit	BIOLABO	France
21.	Urea kit	Linear	Spain
22.	Creatinine kit	BIOLABO	France

2.2 Devices

Table (2.2); List the used devices and their produced company and origin.

Table (2.2) Devices used in the study.

No	Device and tools	company	Origin
1.	Cobas C111	Roche	Germany
2.	Different size Mechanical pipettes	Slamed	Germany
3.	Distillatory	Shin saeng	Korea
4.	Digital camera	Canon	China
5.	Horizontal gel electrophoresis	Cleaver scientific	UK
6.	Hot plate	Medico	USA
7.	Deep freezer	Almateen	China
8.	Centrifuge for Eppendorf tube	Hittch	Germany
9.	Microwave oven	Argos	Germany
10.	Autoclave	Hirayama	Jaban
11.	Shaker-centrifuge	Bioneer	Koria
12.	Spectrophotometer	BIOTECH	UK
13.	Thermocycler	Biometra	Germany
14.	Vortex	Griffin and George Ltd	UK
15.	Water bath	Mermert	Germany
16.	Different size tips	Biobaseic	Canada
17.	ELIZA	Biotek	USA

2.3 Subjects

Ninety patients with type 1 DM were enrolled in the study. All of them were diagnosed by HbA1C and with no other diseases, there ages ranged from 5 to 30 years old and the mean was (16.52 ± 7.575) years. This study is conducted in Marjan Hospital diabetic and endocrine center , Ninety healthy people volunteered as control group, there ages ranged from 5 to 30 years old, and the mean was (15.43 ± 7.312) years, the study whene conducted from 1 September 2021 to February 2022 in Babylon city, Iraq the project proposal and sampling method were approved by research Ethics Committee of Babylon Health directorate according to the directorate administrative order no 9274 , date 1/11/2021 Also the project achieve the permission of research ethics in Marjan medical city at Marjan medical city the no 1012 date 1/11/2021.

Inclusion and exclusion criteria are:

A) Inclusion Criteria:

1. Persons with Type1DM.
2. Age from 5 to 30 years

B) Exclusion Criteria:

1. Pregnancy
2. Other autoimmune disease
3. Malignancies
4. Hemoglobinopathys

2.4. Samples Collection

Plastic syringe were used to pull 8mL of blood from the vein. The blood were divided in to two tubes. First were the EDTA tube and the second were transferred to gel tube, and then the serum were separated by the centrifuge instrument, centrifugation instruction were 2500 g for 10 min. The process proceed under room temperature. The serum then divided on three Eppendorf tubes, and stored in a deep freezer (-20 $^{\circ}$C) until analysis.

2.5. Study Design

Volunteers were under through anthropometric measurements at the beginning, which include waist, height, and weight measurements. Serums of each sample were running under both ELISA technology to get the levels of CCKBR and IL-27 , as well as genetic analysis technology such as PCR and RFLP-technique for detect the target gene (*SNP* gene) and also used molecular absorption technology to analyze serum fasting 12 hours triglyceride, cholesterol, high-density lipoprotein, low-density lipoprotein, very low-density lipoprotein and urea, creatinine concentrations ,were HBA1C has been done in EDTA tube by the use of Cobas C111 chemoluminescence.

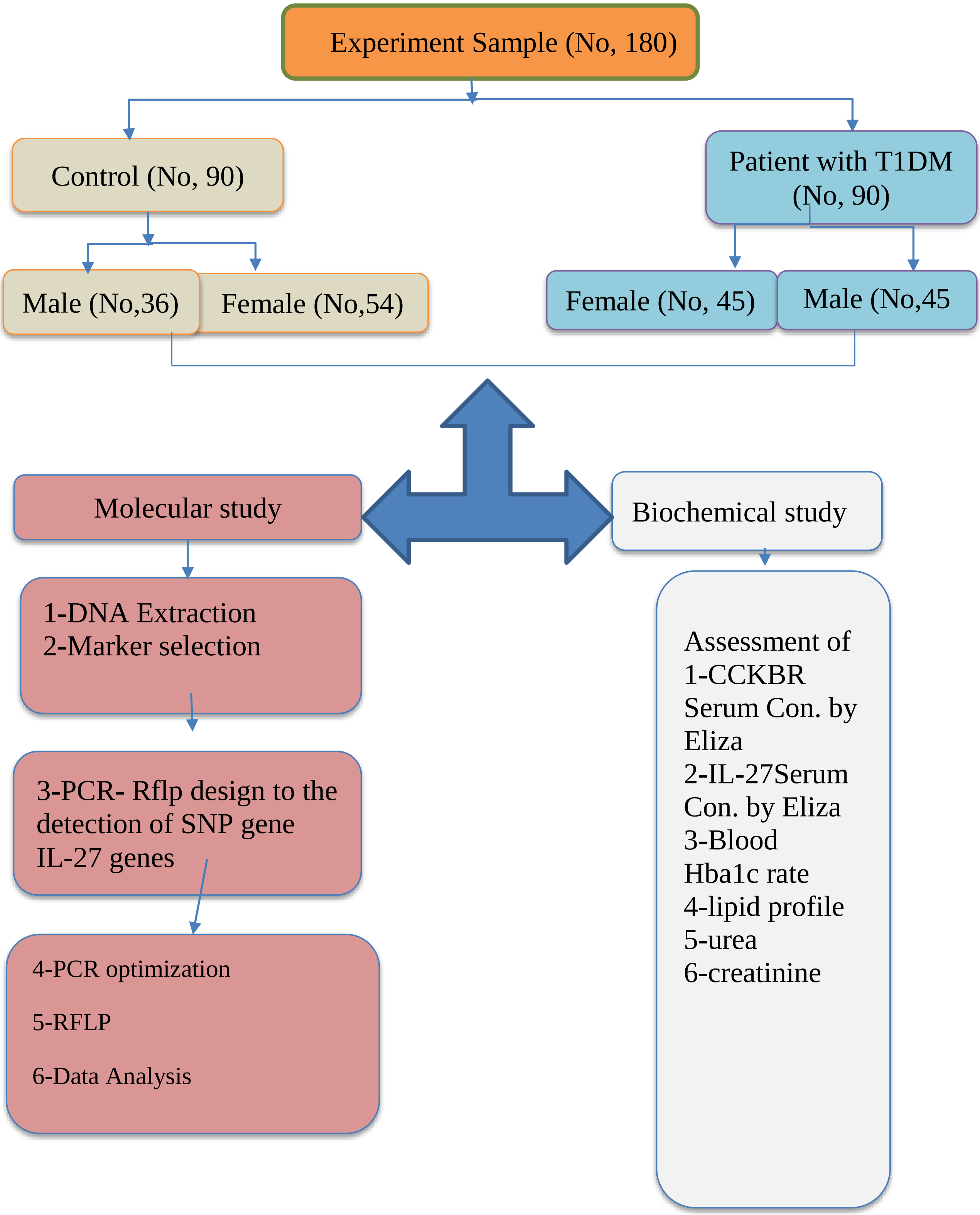


Figure 2.1 Experimental Design

2.6.Measurement of Body Mass Index (BMI)

The BMI is defined as the body weight divided the square of the height of the body in meters and is expressed worldwide in kg/m^2 units, resulting from kilograms of mass and meters of height (119).

The BMI number and classifications are listed below according WHO.

Severely underweight - BMI less than 16.5k kg / m^2

Underweight - BMI under 18.5 kg/ m^2

Normal weight - BMI greater than or equal to 18.5 to 24.9 kg/ m^2

Overweight – BMI greater than or equal to 25 to 29.9 kg/ m^2

Obesity – BMI greater than or equal to 30 kg/ m^2

Obesity class I – BMI 30 to 34.9 kg/ m^2

Obesity class II – BMI 35 to 39.9 kg/ m^2

Obesity class III – BMI greater than or equal to 40 kg/ m^2 (also referred to as severe, extreme ,or massive obesity) (120).

2.7-Biochemical study

2.7.1 Human Interleukin 27,

2.7.1.1 Assay Principle Sandwich

The kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human IL27 antibody. IL27 present in the sample is added and binds to antibodies coated on the wells. Then biotinylated Human IL27 Antibody is added and binded to IL27 in the sample. Then Streptavidin-HRP is added and binded to the Biotinylated IL27 antibody. After incubation, unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human IL27. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Table 2.3 Reagent provided of IL-27:

Components	Quantity (96T)	Quantity (48T)
Standard solution (2400ng/L)	0.5ml x1	0.5ml x1
Pre-coated ELISA plate	12 * 8 well strips x1	12 * 4 well strips x1
Standard diluent	3ml x1	3ml x1
Streptavidin-HRP	6ml x1	3ml x1
Stop solution	6ml x1	3ml x1
Substrate solution A	6ml x1	3ml x1
Substrate solution B	6ml x1	3ml x1
Wash buffer Concentrate (25x)	20ml x1	20ml x1
Biotinylated Human IL27 antibody	1ml x1	1ml x1

User instruction	1	1
Plate sealer	2 pics	2 pics
Zipper bag	1 pics	1 pics

2.7.1.2. Assay Procedure

1. Reagents all standard solutions and samples prepared, as instructed. Bring all reagents to room temperature before use. The assay was they were all brought to room temperature.
2. The number was determined of strips required for the assay, the stripsin was inserted the frames for use. The unused strips was stored at 2-8°C.
3. Standard solution 50ul was added to standard well.
4. **Note:** the antibody hasn't added to standard well because the standard solution contains biotinylated antibody.
5. Sample 40ul was added to sample wells and then 10ul Human IL27 antibody was added too, then 50ul of streptavidin-HRP was added to sample wells and standard wells (Not blank control well). After that, the mixture should mix well and covered the plate with a sealer and Incubated 60 minutes at 37°C.
6. The sealer changed into removed and the plate washed five instances with wash buffer. The wells Soaked with 300ul wash buffer for 30 seconds to one minute for every wash. For automatic washing, it should be aspirated or decanted very nicely, and wash five instances with wash buffer. The plate changed into bloated onto paper towels or different absorbent material.

7. Substrate solution A 50ul and equal volume from substrate solution B was added to each well and the plate was incubated and covered with a new sealer for 10 minutes at 37°C in the dark.
8. Stop Solution 50ul was added to each well, the blue color of the plate was changed to yellow immediately after adding.
9. The optical density (OD value) was determined for each well directly by using a microplate reader, which is set to 450 nm within 10 minutes after adding the stop solution.

2.7.2 Human CCKBR (Gastrin/cholecystokinin type B receptor)ELISA Kit

Principle: Sandwich

Table 2.4 Kit Component

Item	Storage	Specifications(48T/96T)
Elisa Microplate(Dismountable)	8×6/8×12	2-8 ˚c/-20 ˚c
Lyophilized standard	1vial/2vial	2-8 ˚c/-20 ˚c
Sample /standard dilution Buffer	10ml/20ml	2-8 ˚c
Biotin-labeled Antibody (concentrated)	60µl/120 µl	2-8 ˚c (Avoid direct light)
Antibody dilution buffer	5ml/10ml	2-8 ˚c
HRP-streptavidin conjugate (SABC)	60µl/120 µl	2-8 ˚c (Avoid direct light)
SABC Dilution buffer	5ml/10ml	2-8 ˚c
TMB substrate	5ml/10ml	2-8 ˚c (Avoid direct light)

Stop solution	5ml/10ml	2-8 ˚c
Wash buffer (25x)	15ml/30ml	2-8 ˚c
Plate sealer	3/5 pieces	
Product description	1 copy	

2.7.2.1Principle of the Assay:

This package became primarily based totally on sandwich enzyme-related immune-sorbent assay technology. Capture antibody has been pre-lined onto 96-nicely plates. In addition, the biotin-conjugated antibody became used as detection antibodies. The standards, biotin conjugated detection antibody have been introduced to the wells subsequently, and washed with wash buffer. HRP-Streptavidin has introduced and unbound conjugates have been washed away with wash buffer. TMB substrates have been used to visualise HRP enzymatic reaction. TMB has been catalyzed through means of HRP to provide a blue color product that modified into yellow after including acidic forestall solution. The density of yellow is proportional to the goal quantity of pattern captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, after which the attention of goal may be calculated

2.7.2.2 Assay Procedure:

The procedure has been done by the instruction of Al-shkairate establishment for medical supply, when diluting samples and reagents; they should be combined absolutely and evenly. Before including TMB into wells, equilibrate TMB Substrate for half-hour at 37°C. It is suggested to plan a widespread curve for every test.

1. Standard and check samples turned into set (diluted at the least 1/2 with Sample Dilution Buffer), control (blank) wells at the pre-lined plate respectively, and then, recorded their positions.

2. Standards was prepared by added 100ul of zero tube, 1st tube, 2nd tube, 3rd tube, 4th tube, 5th tube, 6th tube and sample dilution buffer (blank) into the standard wells.

3. The plate was incubated with a cover after sealed at 37°C for 90 minutes.

4. The cover was removed and discarded the plate content, the plate was washed 2 times with Washing buffer. The wells should not left to dried completely at any time.

5. From Biotin-labeled antibody working solution 100ul were added into above wells (standard, test sample and blank wells). The solution were added at the bottom of each well without touching the sidewall; the plate was covered and incubated at 37°C for 60 minutes.

6. The covered was removed, and washed plate 3 times with washing buffer, the Washing buffer was stayed in the wells for 1-2 minute each time.

7. HRP-streptavidin working solution 100 ul was added into each well; the plate was covered and incubated at 37°C for 30 minutes.

8. The covered was removed and plate was washed 5 times with washing buffer, the washing buffer was stayed in the wells for 1-2 minute each time.

9. TMB Substrate 90ul was added into each well; the plate was covered and incubated at 37°C in dark within 10-20 minutes.

10. Stop Solution 50ul was added into each well. The color was changed to yellow directly after adding.

11. The O.D. absorbance was read at 450nm in Micro plate Reader immediately after added the stop solution.

2.7.3 HbA1C Test by Cobas C111:

2.7.3.1 Test Principle:

The blood pattern changed into diluted and blended with TRIS buffer to launch hemoglobin from the erythrocytes. A fraction of the pattern changed into conveyed right into a response chamber in which its miles blended with sodium lauryl sulfate (SLS). SLS is used to shape the SLS-hemoglobin complicated. The awareness of overall hemoglobin is calculated via way of means of measuring SLS-hemoglobin complicated with a wavelength of 525 nm. Hemoglobin A1C (HbA1c) in every other fraction of the pattern is first denatured via way of means of potassium ferricyanide and sucrose laurate. The denatured HbA1c bonds with HbA1c antibody at the latex particle. Latex agglutination inhibition response then happens through reacting the agglutinator that has artificial antigen that can bond with HbA1c antibody (121). The attention of HbA1c is calculated through measuring the latex agglutination inhibition response with a wavelength of 625 nm. % hemoglobin A1c cost is measured the use of a ratio of concentrations of HbA1c to general hemoglobin.

Reagents of one test contains

1. Dilution buffer TRIS (hydroxyl methyl amino methane) 0.94 mg
2. Erythrocyte Hemolysis: Sodium Lauryl Sulfate, 0.15 mg
3. Sodium chloride: 0.21 mg
4. Denaturation: Potassium ferricyanide 60 µg,
5. Sucrose laurate: 40 µg
6. HbA1c antibody-latex conjugate 85 µg
7. Agglutination Glycopeptide-globulin conjugate 2µg(122)

Expected values

Concentrations were between 4.2 -9.9 % HbA1c National Glycohemoglobin standardization Program(NGSP) Diabetes complication Trial (DCCT).

2.7.4. Determination of Lipid Profile

Lipid profile measured by spectrophotometric analysis using commercial kits supplied by BIOLABO, France.

A. Triglyceride

Principle

Fossati and Prencipe method were associated with Trinder reaction used for the determination of TG. Lipase converts triglycerides to free fatty acids and glycerol molecules, the glycerol then used along with ATP by glycerol kinase enzyme, yielding ADP and glycerol-3-phosphate. The following step involved the enzyme glycerol-3-phosphate oxidase which catalysis the reaction of glycerol-3-phosphate and O_2 to produce H_2O_2 and dihydroxyacetone-phosphate. The last step is the reaction of H_2O_2 with chloro-4-phenol and 4-amino-antipyrine in the presence of peroxidase leading to colored complex of quinoneimine. The absorbance of the colored complex measured at wave length of 500 nm using spectrophotometer device (123).

Reagents

Materials supplied in the test kit that used in the study are listed in **Table 2-5:**

Table 2-5: TG kit components.

Components		**Concentration**
Reagent 1	PIPES	100 mmol/L
	Magnesium chloride	9.8 mmol/L
	Chloro-4-phenol	3.5 mmol/L
Reagent 2	Lipase	≥ 1000 IU/L
	Peroxidase	≥ 1700 IU/L
	Glycerol-3-phosphate oxidase	≥ 3000 IU/L
	4-Amino-antipyrine	0.5 mmol/L
	Adenosine triphosphate Na	1.3 mmol/L
Reagent 3	Standard solution	200 mg/dL

Procedure

1. The samples were extracted from the Freezer and placed at room temperature for 30 min.
2. Reagents were extracted from the refrigerator and placed at room temperature for 30 min.

3. In a container, reagent 1 mixed gently with reagent 2, allowed to stand for 2 minutes, and the new reagent is termed reagent 0.

4. A volume of 1 mL from reagent 0 added to sample test tubes, standard tube, and blank tube.

5. A volume of 10 µL from distal water added to blank tube. Followed by the addition of 10 µL from reagent 3 to the standard tube.

6. A volume of 10 µL from serums added to corresponding sample test tubes.

7-Tubes allowed standing for 5 minutes at 37 ºC. Then absorbance of solutions measured by spectrophotometer at wavelength equal to 500 nm.

Calculations

The concentration of TG was obtained from the following mathematical equation:

$$TG\ (mg/dL) = Abs\ T/AbsS \times S\ (mg/dL)$$

Concentration.

Abs_T: Sample absorbance.

Abs_S: Standard absorbance.

S: Standard concentration.

B: Blank absorbance.

B. Cholesterol

Principle

Allain et al. method used for the determination of cholesterol. The steps of the enzymatic method are as following equations:

$$\text{Cholesterol esters} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{Free fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{Chloro-4-phenol} + \text{PAP} \xrightarrow{\text{Peroxidase}} \text{Quinone mine} + 4H_2O$$

The absorbance of the colored complex measured at wave length of 500 nm using spectrophotometer device (124).

Reagents

Materials supplied in the test kit that used in the study are listed in **Table 2-6:**

Table 2-6: Cholesterol kit components.		
Components		**Concentration**
Reagent 1	Phosphate buffer	100 mmol/L
	Sodium cholate	9.8 mmol/L
	Chloro-4-phenol	5 mmol/L
	Triton x 100	1.5 mmol/L
Reagent 2	Cholesterol oxidase	≥ 100 IU/L
	Peroxidase	≥ 1200 IU/L

	Cholesterol esterase	≥ 170 IU/L
	4-Amino-antipyrine	0.25 mmol/L
	PEG 6000	167 µmol/L
Reagent 3	Standard solution	200 mg/dL

Procedure

1) The samples were extracted from the Freezer and placed at room temperature for 30 min.

2) Reagents were extracted from the refrigerator and placed at room temperature for 30 min.

3) In a container, reagent 1 mixed gently with reagent 2, allowed to stand for 2 minutes, and the new reagent is termed reagent 0.

4) A volume of 1 mL from reagent 0 added to sample test tubes, standard tube, and blank tube.

5) A volume of 10 µL from distal water added to blank tube. Followed by the addition of 10 µL from reagent 3 to the standard tube.

6) A volume of 10 µL from serums added to corresponding sample test tubes.

7) Tubes allowed standing for 5 minutes at 37 ºC. Then absorbance of solutions measured by spectrophotometer at wavelength equal to 500 nm.

Calculations

The concentration of cholesterol was obtained from the following mathematical equation:

$$T\ (mg/dL) = \text{Abs T}/\text{AbsS} \times S\ (mg/dL)$$

Abs_T: Sample absorbance.

Abs_S: Standard absorbance.

S: Standard concentration.

B: Blank absorbance.

C. High-Density Lipoprotein (HDL)

Principle

The determination of HDL-cholesterol proceeded in an accelerator detergent methodology. A direct method of two phases, in the first one LDL, VLDL, and chylomicrons generate free cholesterol, which through an enzymatic reaction produce H_2O_2. The generated H_2O_2 was consumed by a peroxidase reaction with DSBmT yielding a colorless product. In the second one, specific detergent solubilizes HDL-cholesterol.

In conjunction with cholesterol oxidase and cholesterol esterase action, peroxidase + 4amino-antipyrine develop a colored reaction, which is proportional to HDL cholesterol concentration. The absorbance is measured at 600 nm (125, 126).

Reagents

Materials supplied in the test kit that used in the study are listed in **Table 2-7:**

Table 2-7: HDL kit components.		
Components		**Concentration**
Reagent 1	Good's buffer	-
	Cholesterol oxidase	< 1000 UI/L
	Peroxidase	< 1300 UI/L
	DSBmT	< 1 mmol/L
	Accelerator	< 1 mmol/L
Reagent 2	Good's buffer	-
	Cholesterol esterase	< 1500 UI/L
	Ascorbate oxidase	< 3000 UI/L
	4-Amino-antipyrine	< 1 mmol/L
	Detergent	< 2 %
	Stabilizer	< 0.15 %

Procedure

1) The samples were extracted from the Freezer and placed at room temperature for 30 min.

2) Reagents were extracted from the refrigerator and placed at room temperature for 30 min.

3) A volume of 300 µL from reagent 1 added to sample test tubes, calibrator tube, and blank tube.

4) A volume of 3 µL from calibrator added to calibrator tube. Then 3 µL from serums added to corresponding sample test tubes.

5) Tubes mixed well, and let stand for 5 minutes at 37 ºC. Then the first absorbance (A1) against blank measured at 600 nm.

6) A volume of 100 µL from reagent 2 added to all tubes.

7) Tubes mixed well, and let stand for 5 minutes at 37 ºC. Then the second absorbance (A2) against blank measured at 600 nm.

Calculations

The concentration of HDL wasobtained from the following mathematical equation:

$$T\ (mg/dL) = \text{Abs T/AbsS} \times S\ (mg/dL)$$

Abs_T: Sample absorbance.

Abs_S: Calibrator absorbance.

S: Calibrator concentration.

$\Delta Abs = A2 - A1$.

D. Low- and Very Low-Density Lipoproteins (LDL, VLDL)

For the calculation of low-density lipoprotein (LDL), and very lowdensity lipoprotein (VLDL) Friedewald equation is used (127).

VLDL = TG / 5

LDL = Total Cholesterol – (HDL + VLDL)

2.7.5. Determination of Urea

Urea measured by spectrophotometric analysis using commercial kits supplied by Linear, Spain

$$Urea + H_2O \xrightarrow{\text{Urease}} 2\,NH_3 + CO_2$$

$$NH_4 + \text{Salicylate} + NaClO \xrightarrow{\text{NITROPRUSSIDE}} \text{Indophenol} + NaCl$$

Urea is hydrolyzed by urease1, 2 into ammonia and carbon dioxide. The ammonia generated reacts with alkaline hypochlorite and sodium salicylate in presence of sodium nitroprusside as coupling agent to yield a green cromophore. The intensity of the color formed is proportional to the concentration of urea in the sample. When it measured over a fixed time interval at 600 nm, is proportional to urea concentration in serum (121).

Reagents

Materials supplied in the test kit that used in the study are listed in **Table 2-8:**

Table 2-8: Urea kit components.		
Components		**Concentration**
Reagent 1	Urease	500 > U/mL
Reagent 2	Buffered chromogen: Phosphate buffer	20 mmol/L
	EDTA 2 mmol/L	2 mmol/L
	sodium salycilate 60 mmol/L	60 mmol/L,
	sodium nitroprusside	3.4 mmol/L
Reagent 3	Sodium hypochlorite NaOH	10 mmol/L, 150 mmol/L
CAL	Urea standard	50 mg/dL (8.3 mmol/L)

Procedure

1) The samples were extracted from the Freezer and placed at room temperature for 30 min.

2) Reagents were extracted from the refrigerator and placed at room temperature for 30 min.

3) In a container, reagent 1 mixed gently with reagent 2, allowed to stand for 2 minutes, and the new reagent is termed reagent 0.

4) A volume of 1 mL from reagent 0 added to sample test tubes, and standard tube.

5) A volume of 10 µL from standard added to the standard tube. Then followed by the addition of 10 µL from serums to the corresponding sample test tubes.

6) Tubes gently shacked and incubated at 37 ºC for 5 minutes.

7) A volume of 1 mL from reagent 3 added to sample test tubes, and standard tube. Then, Tubes gently shacked and incubated at 37 ºC for another 5 minutes.

8) After the end of waiting time, the standard and test tubes read at the absorbance of 600 nm.

Calculations

The concentration of urea was obtained from the following mathematical equation:

T (mg/dL) = Abs T/AbsS × S c (mg/dL)

T: Sample concentration.

Abs_T: Sample absorbance.

Abs_S: Standard absorbance.

S: Standard concentration.

2.7.6. Determination of Creatinine

Creatinine measured by spectrophotometric analysis using commercial kits supplied by BIOLABO, France.

Jaffe reaction used for the determination of serum creatinine. Creatinine reacts with alkaline picrate and produce a colored product, which measured spectrophotometry at wavelength equal to 490 nm. The reaction has been improved by the development of an initial-rate method (128).

Reagents

Materials supplied in the test kit that used are listed in **Table 2-9**:

Table 2-9: Creatinine kit components.

Components		Concentration
Reagent 1	Disodium phosphate	6.4 mmol/L
	Sodium hydroxide	5 mmol/L
Reagent 2	Sodium dodecyl sulfate	0.75 mmol/L
	Picric acid	4 mmol/L
Reagent 3	Standard solution	2 mg/dL

Procedure

1) The samples were extracted from the Freezer and placed at room temperature for 30 min.

2) Reagents were extracted from the refrigerator and placed at room temperature for 30 min.

3) In a container, reagent 1 mixed gently with reagent 2, allowed to stand for 2 minutes, and the new reagent is termed reagent 0.

4) A volume of 1 mL from reagent 0 added to sample test tubes, standard tube, and blank tube.

5) A volume of 100 µL from distal water added to blank tube. Followed by the addition of 100 µL from reagent 3 to the standard tube.

6) A volume of 100 µL from serums added to corresponding sample test tubes.

7) Tubes gently shacked and incubated at 37 ºC for 30 seconds, then the first absorbance (A1) measured at wavelength of 490 nm.

8) Tubes then went a second incubation for two minutes at 37 ºC and the second absorbance (A2) measured at wavelength of to 490 nm.

Calculations

The concentration of creatinine was obtained from the following mathematical equation:

$$T\ (mg/dL) = Abs\ T / AbsS \times S\ (mg/dL)$$

Sample concentration.

Abs_T: Sample absorbance.

Abs_S: Standard absorbance.

S: Standard concentration.

$\Delta Abs = A2 - A1$.

2.8. Genetic study:

2.8.1 rs153109 IL-27 gene design:

The selection of genotyping technique according to(129)
The design of PCR primers according to the protocol of (130) The primers had been designed via way of means of the resource of NCBI-primer BLAST on-line software , on the identical time the produced primers turned into checked for specificity for his or her goal sequences via way of means of appearing the BLAST towards the human genome ,
Then the primers pair turned into decided on consistent with the call for standards such as product length, the similarity of melting temperature, primers length, specificity, etc. Then the mutations turned into interred consistent with the layout demands .The primer capacity to shape secondary shape turned into checked via way of means of the resource of Oligo Calc on-line software, the primer might be rejected if it had 5 bases or greater capable of shape self-dimerization and/or it had 4 bases capable of shape hairpin.

Each primers pair became checked for dimer formation via way of means of the resource of "Multiple Primer Analyzer" on line software program from Thermo Fisher Scientific Inc, the sensitivity of the software program became adjusted to the value 2, the primer pair might be rejected if it made any dimers on this diploma of sensitivity.

2.8.2- Restriction Enzyme Selection

The selection of the suitable restriction enzyme was performed by the aid of Wat Cut online software, selection of restriction enzyme was done according to several criteria such as: the lesser primer mutations needed, the distance of mutation from the variant compatibility of the produced primers, cost and availability.

CAAGGTGCTGACTGGGACTGGGACTCAGCAGGGGGTCCAGGCTT
GGGCTGGGATGAGAGCTCAGTCAGTGACCAGGATCGGGGCTCAG
CCTGTGGCCAGGCT/CGAGTTGAGTGAGGTCAGGATCAGGGCTCA
ACCAGGTCCAAGAATAATCTTGGTGTTTCTGCTCTGTAGAGGCCA
TGGTCAGGGCTGGGCCACATGGACCACAGGTCTCCCTTCCAGCAC
AGCCCCACCCTCAGCCCCGCCCTGGATTTCCCATCTTCAGGGAGA
GGATGGGGT

Figure (2.2) show the Amplicon of selected Primers Pairs for rs153109 genotype (gray labeled sequence represent primers site, blue shaded represent the restriction site of (Taq 1), back slash represent the cut site, the red C represent the C allele of rs153109, (Taq 1) restriction enzyme recognize the C allele of rs153109and cut the amplicon to 102+174 fragments while the T allele produce 276 bp fragment

Table (2-10) primers pairs employed for rs153109 genotyping

	Sequence (5'->3')	**Template strand**	**Length**	**Start**	**Stop**	**Tm**	**GC %**
NR09 f	CAAGGTGCTGACTGGGACTG	Plus	20	28507673	28507692	60.60	60.00
NR09 r	ACCCCATCCTCTCCCTGAAG	Minus	20	28507948	28507929	60.33	60.00
Product length 276 bp							

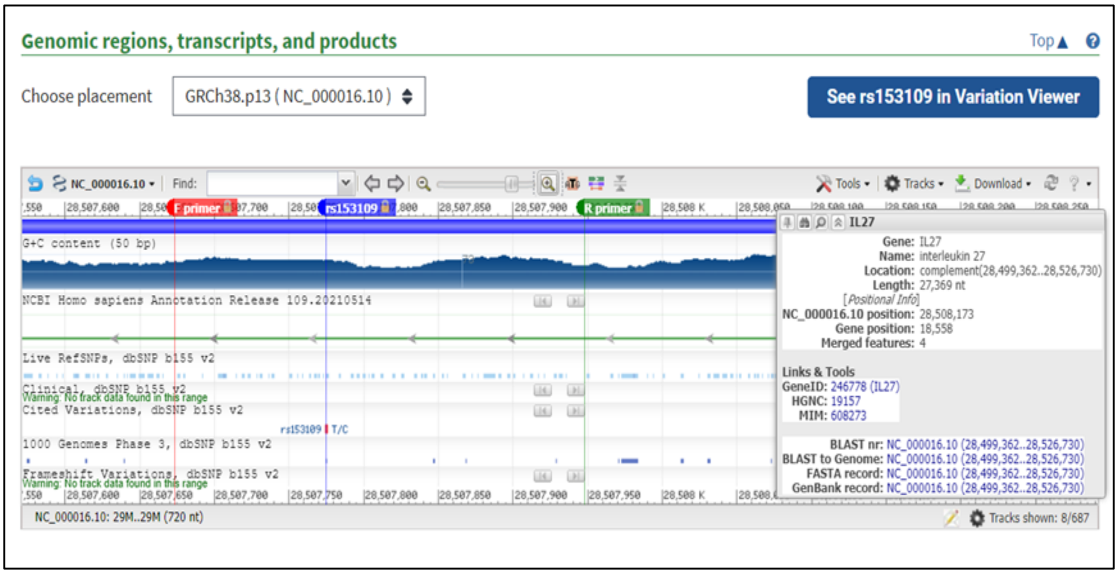


Figure (2.3) the exact forward and revers primers position on Il27 gene and around rs153109 polymorphisms

2.8.3. DNA extraction protocol

DNA was extracted from frozen blood sample according to protocol described by (Hashim and AL-shuhaib2020)

Procedure

1-Cells washing; the frozen blood pattern that became located in a vacutainer tube became thawed at room temperature and blended lightly.

2-Then, simplest 500 µl of the blood becam transferred to an Eppendorf tube of 1.5 ml capacity. Up to at least 1 ml of washing buffer(methanol10% +80% Tris+normal saline 10%) became brought to the blood pattern, blended, and incubated at temperature for 10 min in a rotating mixer

3- The combination became centrifuged at 10000xg for 1 min. After discarding the supernatant, 1 ml of washing buffer became brought again, and the tube became inverted numerous instances to clean the pellet.

4-The precipitate was suspended with 200 µl of EDTA solution.

5-Lysis solution are added 400 µl and suspended well until complete diffusion are placed at temperature of 60°C for 15 minute.

6 -then cool to room temperature, and then added 100 µl of sodium acetate, PH 4.5, then vortex for 1 minute.

7-Centerifuge 10000xg for 10 minute the sediment is discarded and the filter is taken and placed in the column.

8- Centerifuge10000xg for 30 second.

9-Washed twice with 600 µl of 70% ethanol +30% Tris (PH8) add 600 µl of column wash solution, then centrifuge 10000 for 30 second, repeat the process twice.

10 - After getting rid of the filter, the column is dried by placing centrifuge 10000xg for 3 minutes to get rid of the largest amount of washing solution.

11 - The collection tube is disposed of and the column is transferred to a sterile 1.5 ml.

12-Added 100 µl of elution buffer to the center of the column and left for 5 minutes after addition, it is ensured that all the solution is absorbed from the column.

13-Then centrifuge 10000xg for 1 minute takes the filter that represents pure DNA and neglects the column.

14-Extracted DNA became checked for its integrity and purity through agarose electrophoresis as proven in figure (2.8) and through spectrophotometry.

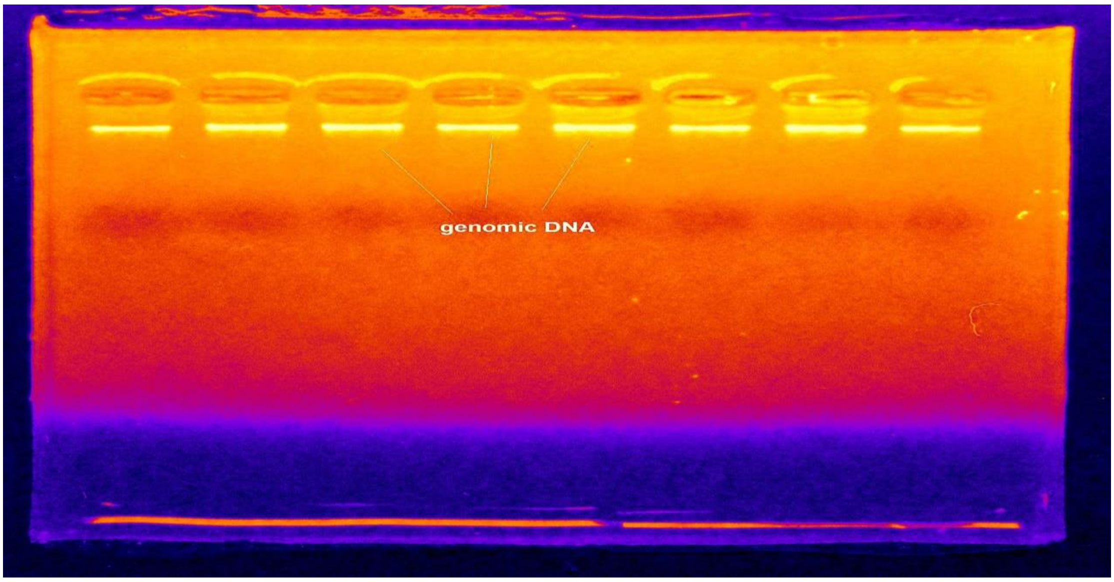


Figure (2.4) extracted genomic DNA agarose gel electrophoresis, the electrophoresis was conducted on 1% agarose, 80 volts for 20 min as described in (2.13).

2.8.4 Qualitative and Quantitative Evaluation of the Isolated DNA

The concentration and purity of the isolated DNA were analyzed by a Nano drop spectrophotometer. DNA concentration measurements were recorded as µg/ml, while DNA purity was taken from the ratio obtained from the A260/280 absorbance formula. The 260/280 and 260/230 ratios were calculated to determine both the quantity and quality of the DNA. Whenever the 260/280 ratio was less than 1.7 and/or the 260/230 ratio was less than 1.7, the sample was re-extracted(131)

.

2.8.5 Optimization of PCR Conditions

Started the optimization of PCR reaction for rs153109 genotyping with the thermo- cycling condition listed in the (2-14) and with reaction, ingredients as listed in table (2-15).

Table (2-11) optimized reaction mixture for PCR

	Composition	Concentration	Volume
1	Master mix	2.5 X	8
2	$Mgcl_2$	25 MM	0.5
3	Forward primer	10 PM	1
4	Revers primer	10 PM	1
5	DNA sample	10-20 ng/µl	2
6	Nucleases free water		7.5
7	Total volume	20	

Table (2-12): the starting thermo-cycle condition for rs153109 optimization processes

2.8.6 Electrophoresis

No	Stage	Temperature	Time	Number of cycle
1	Initial denaturation	94 °C	5 min	1
2	Denaturation	94 °C	30 sec	35
3	Annealing	Gradient from 55 °C to the 66 °C	30 sec	
4	Elongation	72 °C	30 sec	
5	Final elongation	72 °C	5 min	1

The agarose electrophoresis was carried out according to (Sambook and Russell, 2001) as describe below in brief:

1- The gel (1%) for extract DNA and 2% for optimize pcr was prepared by dissolving 0.8 gram of agarose in 40 ml of 0.5x TBE buffer and heat by microwave oven for 2 minutes.

2- The homogenized agarose then cooled to55°C by water bath.

3- Ethidium bromide 50 µl of stock (1mg/ml) solution was added to the gel and mixed by swirling.

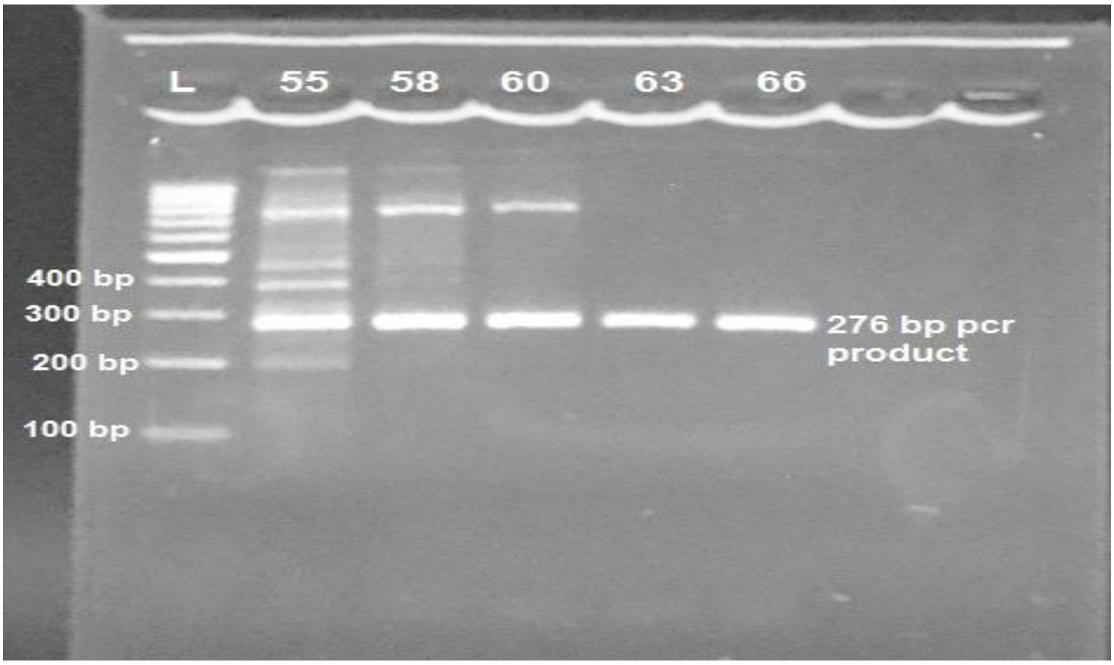
4- The gel then poured to the gel try and let to polymerize for 30 minutes.

5- The polymerized gel then transferred to the electrophoresis devise and submerged with 0.5x TBE running buffer.

6- Extracted DNA 5 microliters and 5 microliters of pcr product was mixed with 1 µl of lauding buffer and loaded carefully by mechanical pipet to the gel wells.

7- The electrophoresis was carried out by setting the device on 50 volts and 40 AM for 60 minutes.

8- The gel then imaged and the image analyzed by Cs analyzer® software to determine the extracted DNA molecular weight.



Optimization image (2.5)

After optimization, found that 66°C for annealing temperature produce the best and efficient PCR product. For this, we choose this annealing temperature for further PCR reactions(132).

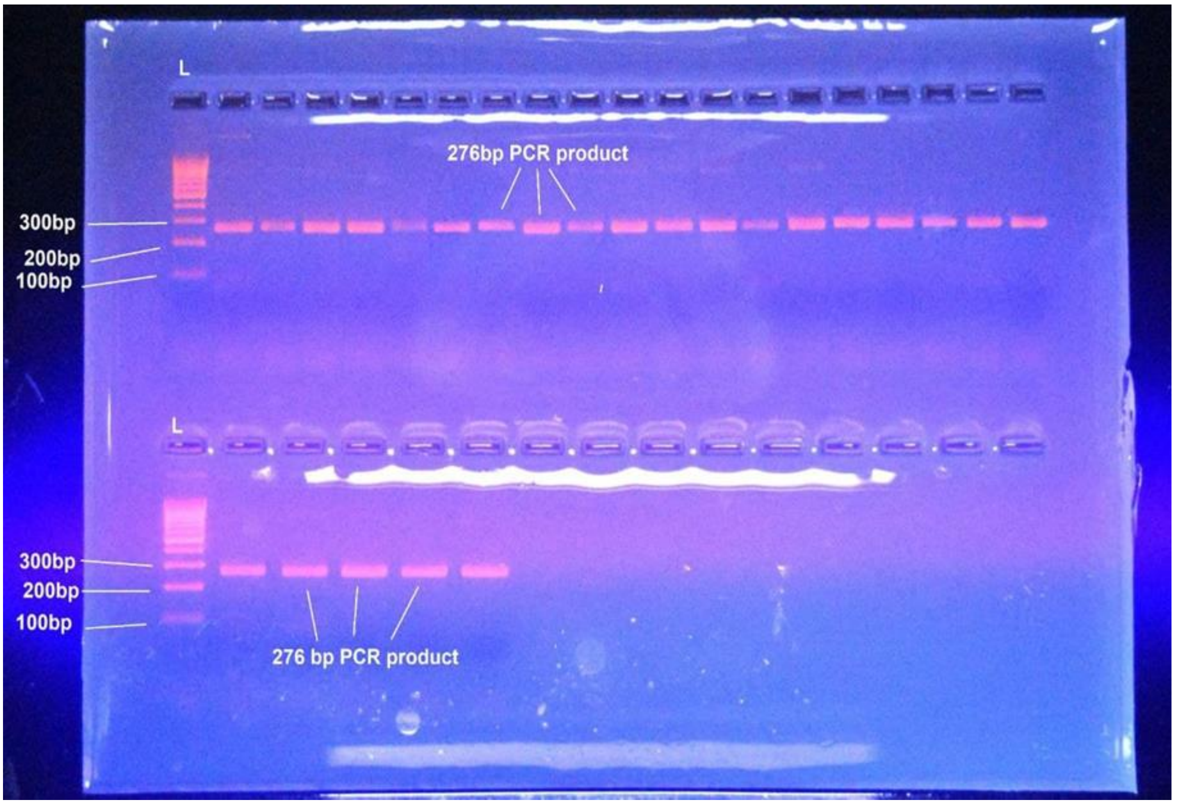


Figure (2.6). the electrophoresis of 276bp PCR product that employed for rs153109 genotyping by PCR-RFLP technique , lane L represent 100bp DNA ladder , other lane represent 276 bp of PCR product

2.8.7 PCR-RFLP: Restrictive Digestive Digestion for the Rs153109 IL-27 Gene PCR-RFLP

PCR-RFLP is a two-part method as indicated by its name. The first part consists of a traditional PCR with primers surrounding the possibly mutated area. The PCR product is then subjected to restriction enzymes which have the ability to cut DNA at specific sequences, also called restriction sites, thus creating DNA fragments. The restriction enzymes can either be used as positive or negative markers meaning that if the DNA is mutated the enzyme can either gain a restriction site, creating more fragments and of different sizes than with normal DNA, or lose a site that normally exists in un mutated DNA.

The latter will then result in less fragments and of different sizes than with normal DNA. After restriction, the fragments are analyzed by gel electrophoresis, which creates visible bands, and by assessing; how far the bands have travelled through the gel in comparison to a DNA ladder with known band sizes the restriction fragments' sizes can be estimated. The number of fragments combined with their sizes allows the original sequence to be identified as mutated(133).

The following ingredients are combined to produce the restriction reaction:

1) An amount of (5 µl) of PCR product

2- The restriction enzyme (Taq 1) 0.25 µl

3- Restriction buffer 1.5 µl (each restriction enzyme has its own restriction buffer, which is given by the manufacturer).

4-Bovine serum albumin (BSA) 0.15 g/L add 0.1 µl

5- Using molecular grade water, the reaction mixture was brought to an end at 15 µl add 20 µl of mineral oil

6- The reaction mixture was incubated for 24 hours at 65°C in a water bath.

7- The RFLP reaction product was resolved on 2% Agarose gel electrophoresis as described in (2.7) as figure of RFLP.

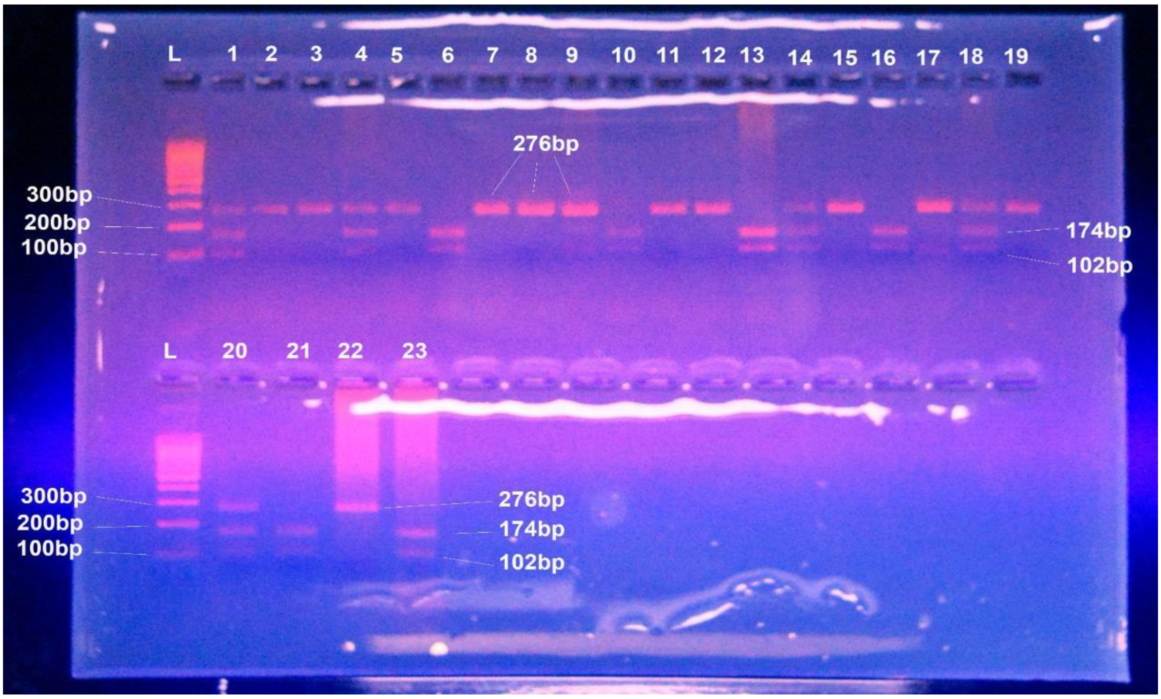


Figure (2.7) : PCR-RFLP genotyping of rs153109, lane L 100bp DNA ladder , lanes 2,3,5,7,8,9,11,12,15,19 and 22 TT genotype ; lanes 1,4,14,18 and 20 TC genotype ; lanes 6,10,13,16,21 and 23 CC genotype.

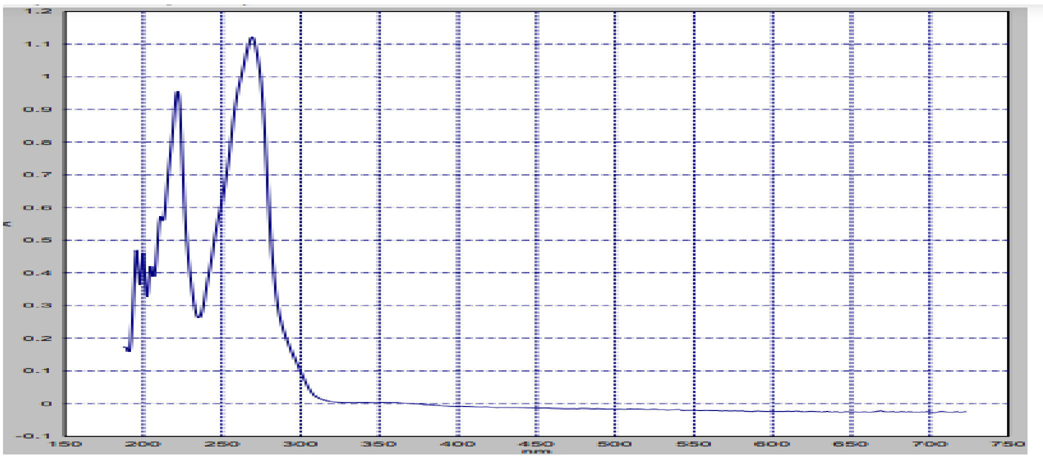


Figure 2-8: DNA purification.

2.9.Statistical Analysis

The statistical analysis of this prospective study performed with the statistical package for social sciences(SPSS) and Microsoft Excel 2013. The data described as mean and standard deviation and independent sample t-test used for comparison between two groups. The lower level of accepted statistically significant difference is bellow or equal to 0.05 odd ratio was calculated to estimate the potential risk of allele among their carrier (134).

CHAPTER 3

RESULTS AND DISCUTION

3.1. Age and Anthropometric Measurements:

The results of age and anthropometric measurements for controls and T1DM, both healthy and associated-T1DM, are listed in **Table 3-1**, in the form of mean ± standard deviation (SD).

Table 3-1 Age and Anthropometric Measurements

Parameters	Control mean±SD	Patients mean± SD	P-value
Age	**15.43±7.31**	**16.52±7.57**	**0.328 (NS)**
BMI	**22.15±3.78**	**21.29±4.55**	**0.17 (NS)**

NS: Non-significant, *: Significant at *P* ≤ 0.01

There are no significant differences (P > 0.05) in age (see Fig 3-1) among controls (15.43±7.31) and T1DM patients (16.52 ± 7.57) which desired for the study in order to exclude the age factor whom could affects the clinical parameters of the study, and narrowing the possible effects to T1DM factors. Control's highest percentage was the 8-22 years old category.

The body mass index in $Kg.m^{-2}$ (see Table 3-1) shows no significant differences (P > 0.05) between controls (22.15±3.78) and T1DM patients (21.292±4.55).this results showed that there was no effect of age and BMI on the T1DM and it is even confirm that the disease is associated with other parameter not correlated with age and BMI (123).

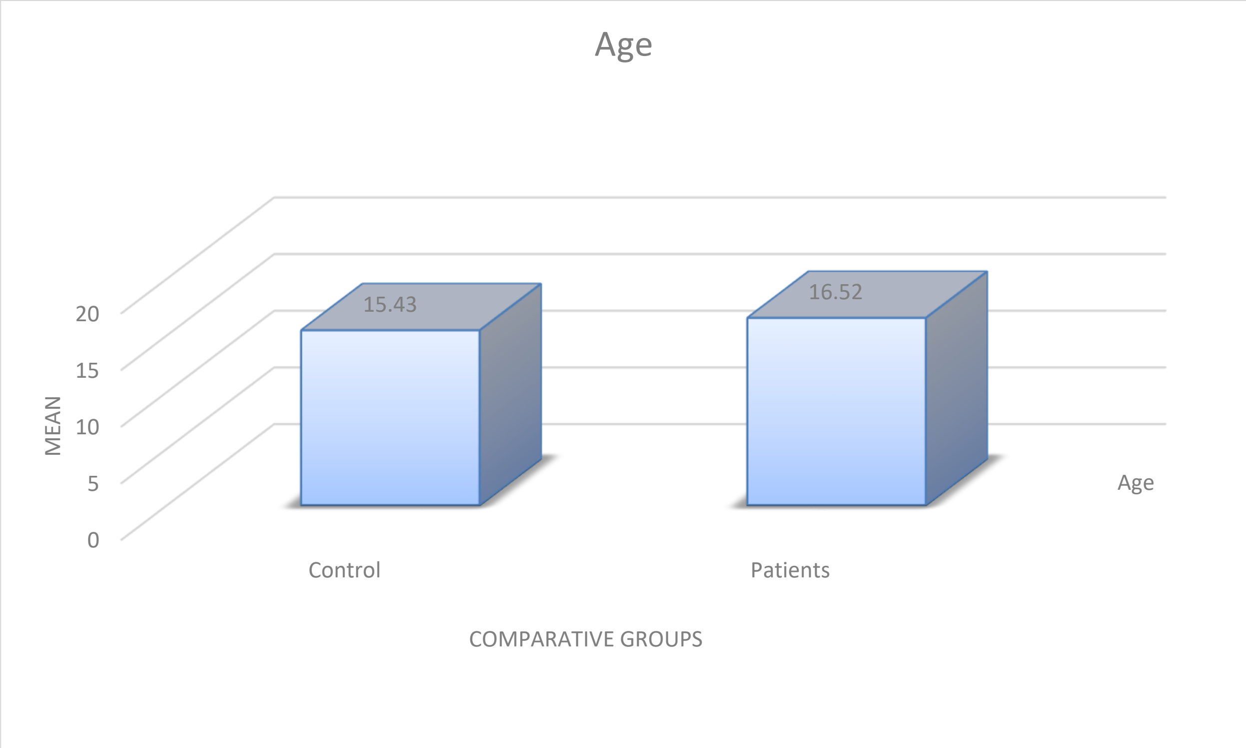


Figure 3-1: The Mean, Median and Range Of Age in Patients and Control.

Other parameters like (W.C, WHpR and WHtR) does not been used because the result collected from the BMI gave the hole information we need to detect the effect of weight on the studied case.

3.1.1 Comparison among controls and T1DM patients

Table 3-2: distribution of patient among age study

Age (Binned)					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	<= 18	112	62.2	62.2	62.2
	19+	68	37.8	37.8	100.0
	Total	180	100.0	100.0	

In **Table (3-2),** the result obtained shows that the most number of T1DM patients has been diagnosed in the age down to 18 years old and that confirm that T1DM disease effect people from an early stage of life generally from the birth and start to damage the human body and pancreas from a very small age.

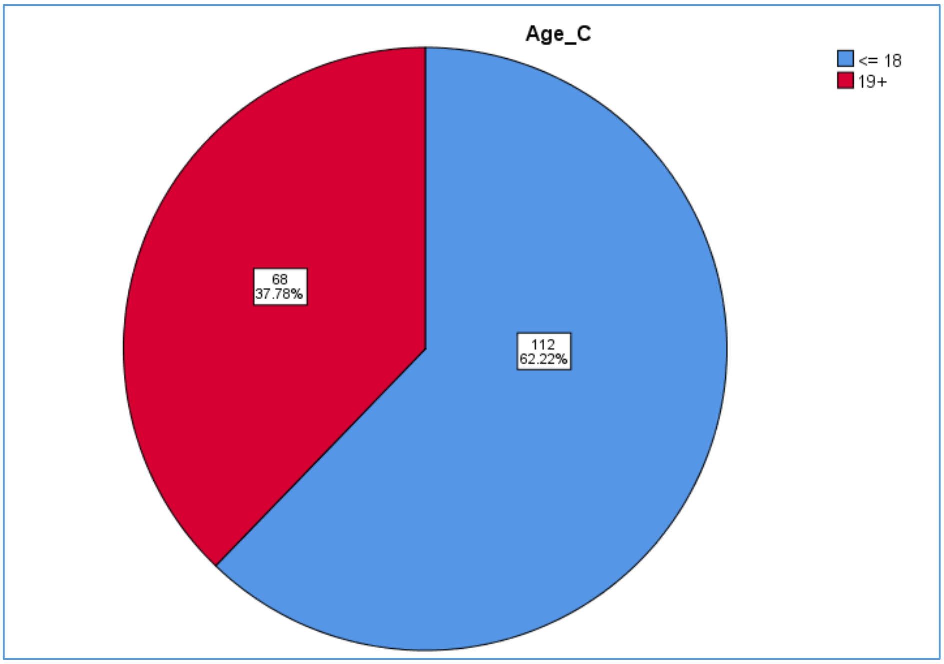


Figure 3-2: shows the effect of age upon the disease

3.2. Diagnosed Parameters For T1DM:

The results of the parameters that have been used in the study for diabetes diagnosis are listed in **Table 3-3.**

Table 3-3: The Levels of Diabetes Parameters in Patients and Control.

Parameters	Control mean±SD	Patients mean±SD	P-value
HbA1c (%)	5.06±0.5	9.88±5.98	0.000
CCK B-R(pg/ml)	290.6±21.5	319.19±61.5	0.23
*** Significant at *P* ≤0.05 by using independent sample t-test.**			

The percentage of CCKBR was non-significant (p> 0.05) between patients (319.19±61.5) and healthy control (290.6±21.5)

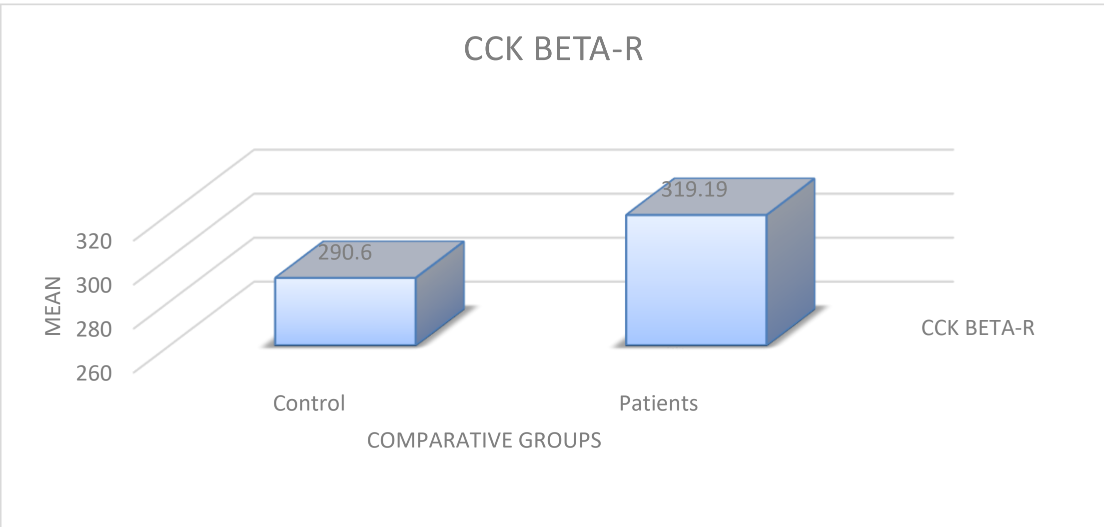


Figure 3-3: The mean, median and range of CCK BETA-R in patients and control.

The percentage of HbA1c was significant in T1DM patients (9.88±5.98%) compared to healthy control (5.06±0.5 %) (P<0.01).

The HbA1c results in this study is also agreed with the results of a study done by adrekani, et al(135)., which showed that the level of glycated hemoglobin were significantly higher in patient with T1DM. The increase in the levels of HbA1c in this research means that people with diabetes have impaired blood glucose regulation. This study also agreed with a study done by Sohal, et al(136). Which showed a significant increase in HbA1c levels in hypothyroidism and hyperthyroidism patient when compared to the controls this due to insulin antagonists that also affect the action of insulin indirectly(131). This study was also agrees with a study done by Satyanarayana, et al(137) which showed that high level of FBG and HbA1c in diabetic group with hyperthyrodism compared to the healthy control which appeared due to the changes of carbohydrate metabolism.

Recent glycaemia has the largest influence on the HbA1c value, with 50% of HbA1c formed in the month prior to sampling and 25% in the month before that It therefore seems logical that such a test would be appropriate in diagnosing a disease characterized by chronic hyperglycemia and a gradual progression to complication, In individuals with type 1 diabetes who have insulin resistance, the insulin secretory response can initially compensate for the insulin resistance. However, eventually, first-phase insulin secretion is lost, and second-phase secretion is impaired, causing postprandial hyperglycemia, one of the earliest markers of disease progression. Abnormalities in hepatic, pancreatic and muscle metabolism all result from long-standing hyperglycemia.

By the time most patients experience symptoms significant enough to cause them to seek medical attention, type 1 diabetes has often been present ,unrecognized for months or may be years, hence the presence of complications at diagnosis in some subjects(121). HbA1c can therefore be used for assessing the risk of complications of diabetes as well as for monitoring glycemic control in patients with T1DM, elevated HbA1c variability was significantly higher, The HbA1c results in this study is also agreed with.

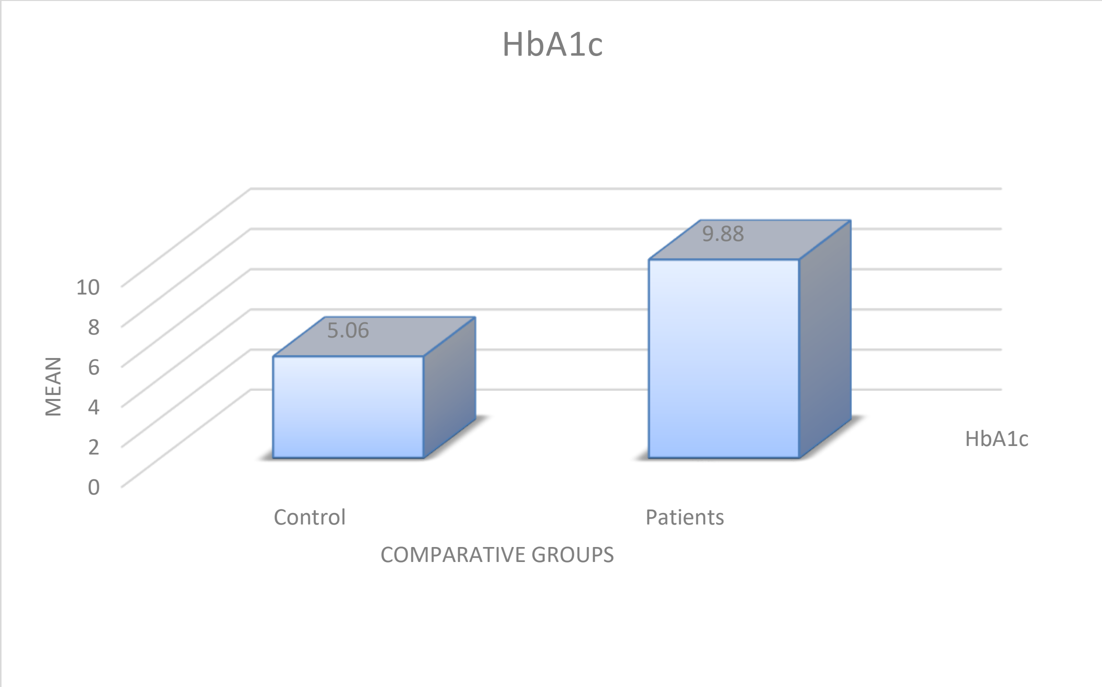


Figure 3-4: The mean, median and range of HbA1c in patients and control.

3.3. Chemical parameters:

3.3.1 Lipid profile:

The results of the lipid profile parameters that have been used in the study for diabetes diagnosis are listed in Table 3-5.

Table 3-4: The levels of lipid profile parameters in patients and control.

Parameters	Control mean±SD	Patients mean±SD	P-value
Cholesterol(mg/dl)	189.42±25.5	198.64±35.37	0.046
TGs(mg/dl)	97.7±50.4	108.05±64.04	0.23
HDL(mg/dl)	50.22±8.99	52.54±13.32	0.174

LDL(mg/dl)	121±28.94	124.49±25.03	0.388
VLDL(mg/dl)	19.54±10.09	21.61±12.81	0.23

The levels of TGs (control 97.7±50.4; patients 108.05±64.04), cholesterol (control 189.42±25.5; patients 198.64±35.37), HDL (control50.22±8.99 patients 52.54±13.32) LDL-C (patients 124.49±25.03; control 121±28.94), and VLDL-C (patients 21.61±12.81; control 19.54±10.09)

Cholesterol were significantly ($P<0.05$) higher in the serum of T1DM patients compared to healthy control subjects. The reason of high cholesterol levels in patients with T1DM due to Type1 DM has emerged one of the most common causes of dyslipidemia vascular complications are believed to be critical for prognosis of DM and there development, in turn, is believed to depend on several factors such as duration, degree of control, and dyslipidemia in diabetes(92).

The study showed that obesity is strongly associated with elevated plasma lipid levels. Hyperlipidemia was not associated with exercise, smoking or alcohol consumption. Except for LDL levels, which tend to be higher in female DM patients(158) .Hyperlipidemia in females may be due to the effects of estrogen on body fat distribution, which results in differences in altered lipoproteins, lower HDL levels and high total and LDL cholesterol levels in T1DM patients Different types of dyslipidemia in patients with T1DM was attributed to the influence of hyper androgenism and insulin resistance that is commonly seen in patients with T1DM along with diet, exercise and genetic predisposition(97). The atherogenic dyslipidemia (low HDL cholesterol along with raised LDL cholesterol and triglycerides) that was observed in this study was also seen in various other studies conducted across the world.

This associated dyslipidemia may predispose these women to increased risk of premature atherosclerosis, and early preventive interventions may be required to halt the progress of atherosclerosis in these patient(43). The result of this study are agreement with (Verma V, Mengesha AY, and Shahwan MJ).

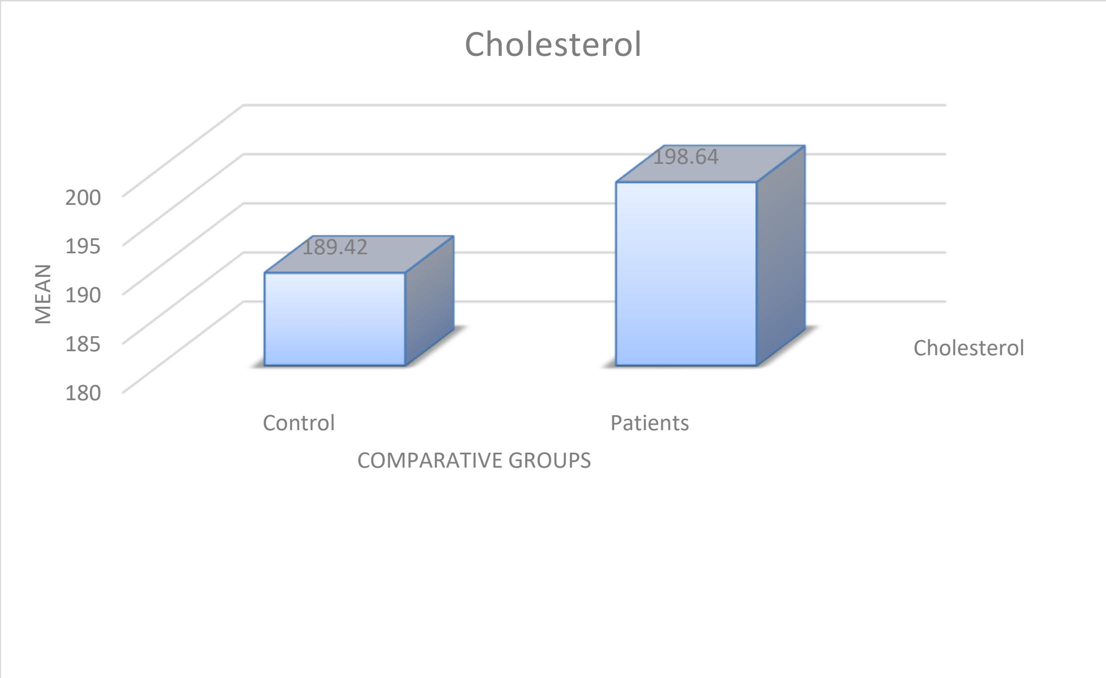


Figure 3-5: The mean, median and range of cholesterol in patients and control.

Other parameter where none significantly (p >0.05) difference between patient and control with T1DM.

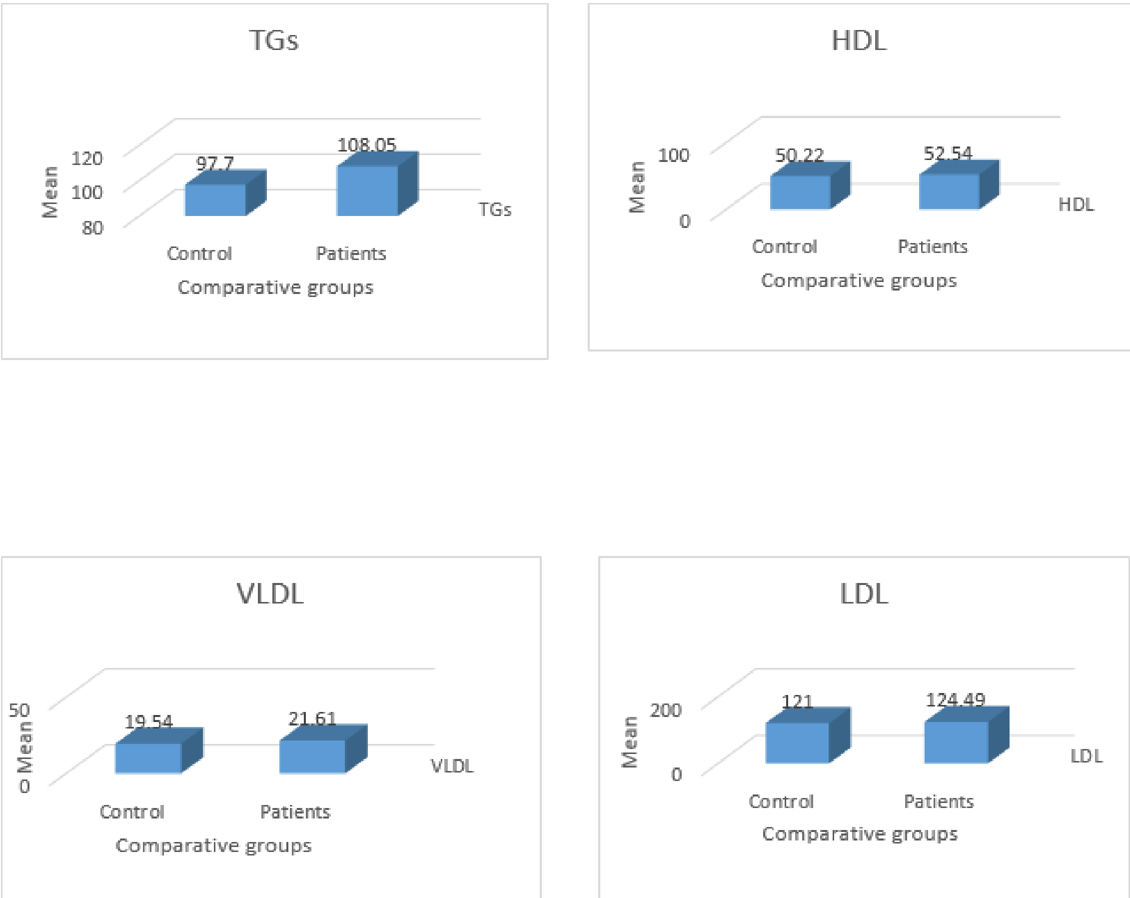


Figure 3-6: The mean, median and range of (TGS, HDL, LDL, and VLDL) in patients and control.

3.3.2 Renal Functions Test:

The results of the renal function parameters that have been used in the study for diabetes diagnosis are listed in Table 3-6.

Table 3-5: The levels of renal function parameters in patients and control.

Parameters	Control mean±SD	Patients mean±SD	P-value
Urea (mg/dl)	26.34±8.34	46.82±14.84	0.000
Creatinine (mg/dl)	0.86±0.19	1.53±0.65	0.000

The levels of urea (patients 46.82±14.84; control 26.34±8.34) and creatinine (patients 1.53±0.65; control 0.86±019).

Urea and creatinine were significantly higher (p <0.005) in the patients as compared with control,

Diabetes mellitus is a slow progressive disease characterized by hyperglycemia. Over time, high blood glucose levels causes damage to millions of nephrons (tiny filtering units within each kidney)(138). As a result, kidneys cannot maintain the electrolyte and fluid homeostasis. Creatinine is filtered by the glomerulus; thus, serum creatinine levels are used to indirectly measure the glomerular filtrations. When glomerular filtration rate (GFR) diminishes, there is an increase in of serum urea and creatinine. In addition, the increase indicates progression of kidney disorder and serum creatinine determination has greater prognostic capacity compared to urea to predict adverse outcome(102).

In humans, urea persists in blood at levels close to maximum solubility due to the lack of urease enzyme, that oxidizes uric acid to allantois. Normally, urea is completely filtered in the renal glomeruli and almost totally reabsorbed in the proximal tubules, whereas glucose competitively inhibits urea reabsorption and enhances its excretion at the same position, with normal kidney function(101).

Blood urea and creatinine have a better guiding value to treat AMI patients clinically. Higher values of urea and creatinine are associated with improved risks of long-term mortality in T1DM patients. Both the renin angiotensin aldosterone system (RAAS) and the sympathetic nervous system (SNS) are activated in CVD patients, which promotes sodium and water absorption and causes passive blood urea reabsorption in the kidney tubules(102).

Activation of the neuro-hormonal system may also result in kidney vasoconstriction and lowered glomerular filtration rates as well as excretion of BUN(61, 132). Moreover, insufficient blood volume secondary to low cardiac output causes stimulation arginine vasopressin (AVP) release, which can accelerate urea reabsorption in the collecting ducts. For the causes why creatinine is freely filtered via the glomeruli and not reabsorbed. Studies by revealed the possibility of using uric acid as an indicator for prognosis of many diseases such as cardiovascular disease, renal insufficiency, metabolic syndrome and Diabetes mellitus(6).

The present study showed a linear correlation between serum urea and creatinine, suggesting that serum urea level was affected by many factors like protein metabolism and kidney functions. The present study also showed that the distribution of renal function parameters showed remarkable alterations in hypertensive patients, DM, cerebral infarctions, compromised kidney functions, central nervous system infections and trauma(131).

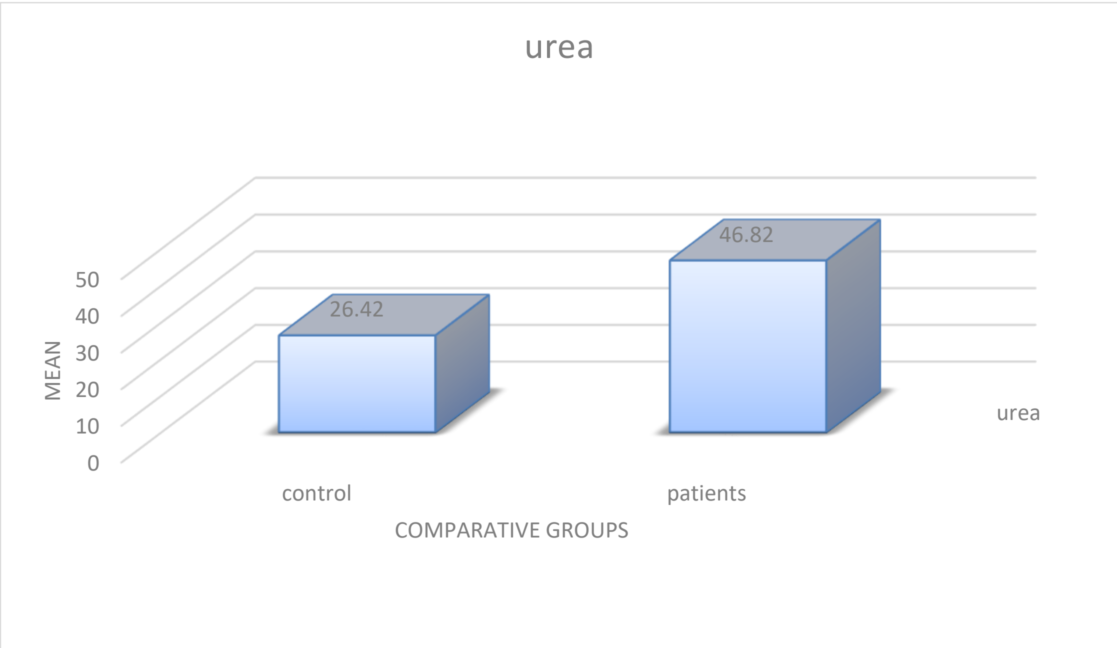


Figure 3-7: The mean, median and range of Urea in patients and control.

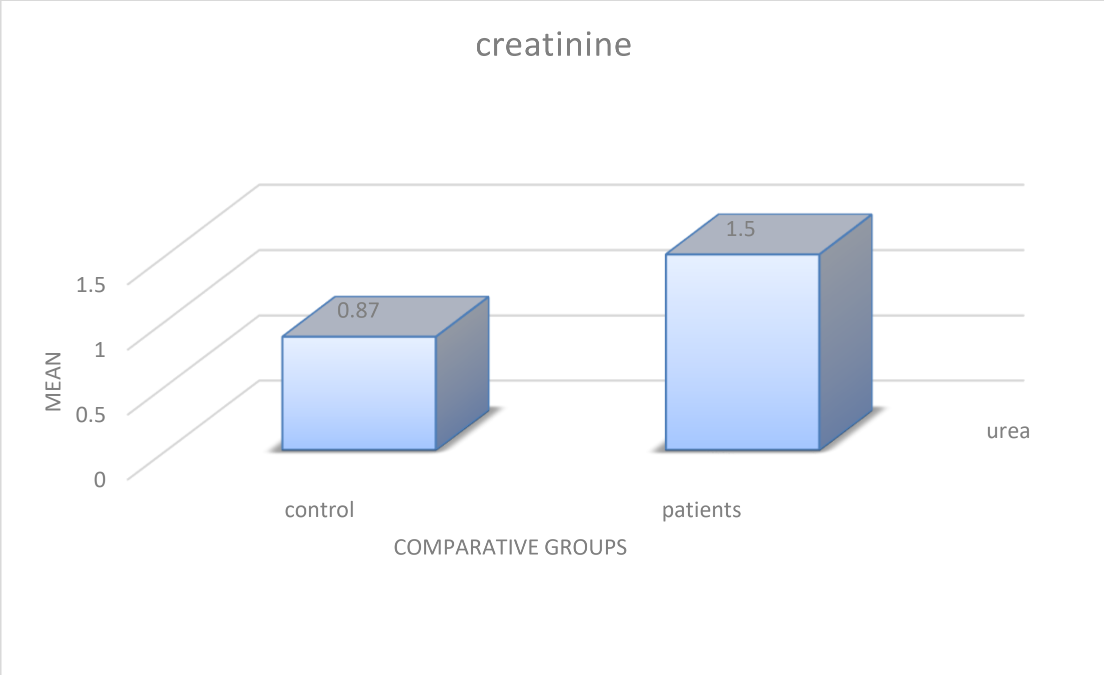


Figure 3-8: The mean, median and range of creatinine in patients and control

3.4. Interlukin-27 with T1DM:

The results of the IL-27 have been used in the study for diabetes diagnosis are listed in **Table 3-6**.

Parameters	Control mean±SD	Patients mean±SD	P-value
Il-27(ng/L)	195.658±173.6	275.99±111.76	0.000

Table 3-6: The levels of IL-27 in patients and control.

The levels of IL-27 (patients 275.99±111.76; control 195.658±173.6) IL-27 was significantly higher ($p < 0.005$) in the patients as compared with control, Interleukin 27 (IL-27) is a group of the IL-12 family of cytokines with proclaimed pro- and anti-inflammatory effect. It is a heterogeneous cytokine which is take shapes of two special genes(126).

Th1/Th2-mobileular stability and Th17-mobileular homeostasis take part with inside the improvement of diabetic complications, together with diabetic macular edema, diabetic nephropathy, and macro vascular complications, thru numerous pathways(.30–32) Cytokines related to Th2 and Th17 cells are tested to be concerned with inside the improvement of DR(.16,18) IL-27 is a pleiotropic cytokine that has been recognized as an immune regulatory cytokine concerned in inflammatory disorders.(33,34) Our consequences determined that the extent of IL-27 in serum become remarkably excessive in T1DM sufferers as compared with controls increased level of IL-27 may lead to an decreased Th17 response down regulated serum levels of IL-27 were associated with disease activity in multiple sclerosis, which implies that IL-27 may have anti-inflammatory effects on the process of multiple sclerosis. found that IL-27 in serum was lower in patients with systemic lupus erythematous that the effects were inversely related to the levels of Th17 cells(133).

Risk factors such as diabetes and hypertension do not influence the serum levels of IL-27 in patients with ischemic heart disease, elevated IL-27 expression in serum and lymphoid tissues in autoimmune diabetes fashions as compared with controls, These discrepancies can be because of variations withinside the form of diabetes, the length of diabetes, and distinctive immunologic mechanisms among scientific diabetes and animal fashions(84).

Regulating IL-27value, there was significant higher value of diabetic children compared to healthy children this result agreement with Ciecko et al. (146) found that IL-27 was increased in T1D. According to prior study, mice transgenically overexpressing IL27 showed increased islet inflammation and CD8 T-cell responses, which supports the significance of IL-27 in T1D Łukawska-Tatarczuk M et al. (147) found that levels of IL-27 in were non –significantly higher of IL-27 in adult T1D compared to controls.

3.5 Genetic Study of IL-27:

The results of the IL-27 gene study for allele counting have been used in the study for diabetes diagnosis are listed in Table 3-8.

Table 3-7: allele count.

	Control		Case		OR (95% CI)	P-value
Allele	**Count**	**Proportion**	**Count**	**Proportion**		
T	**108**	**0.6**	**137**	**0.76**	**2.124 (1.349-3.345)**	**0.00105**
C	**72**	**0.4**	**43**	**0.24**	**0.471 (0.299-0.741)**	

The results of the IL-27 rs153109 exact test for Hardy-Weinberg equilibrium have been used in the study for diabetes diagnosis are listed in Table 3-9.

Table 3-8: Hardy-Weinberg equilibrium.

rs153109 exact test for Hardy-Weinberg equilibrium (n=180)						
	TT	CT	CC	T	C	P-value
All subjects	83	79	18	245	115	1
Control	31	46	13	108	72	0.66
Case	52	33	5	137	43	1

The study of genotype model in IL-27 gene have been used in the study for diabetes diagnosis are listed in Table 3-9.

Table 3-9: Genotype Model.

Model	Genotype	Control	Case	OR (95% CI)	P-value
Codominant	T/T	31 (34.4%)	52 (57.8%)	1.00	0.0037
	C/T	46 (51.1%)	33 (36.7%)	0.43 (0.23-0.80)	
	C/C	13 (14.4%)	5 (5.6%)	0.23 (0.07-0.71)	
Dominant	T/T	31 (34.4%)	52 (57.8%)	1.00	0.0016
	C/T-C/C	59 (65.6%)	38 (42.2%)	0.38 (0.21-0.70)	
Recessive	T/T-C/T	77 (85.6%)	85 (94.4%)	1.00	0.043

	C/C	**13 (14.4%)**	**5 (5.6%)**	**0.35 (0.12-1.02)**	
Overdominant	**T/T-C/C**	**44 (48.9%)**	**57 (63.3%)**	**1.00**	**0.05**
	C/T	**46 (51.1%)**	**33 (36.7%)**	**0.55 (0.31-1.00)**	

The genotype and allele frequencies of the G.-964A > G SNP of IL-27p28 in T1DM patients were significantly different from those in the control group (Table 3-7).

This result suggests that the G.-964A > G polymorphism of IL-27p28 may be associated with susceptibility to T1DM(126). The polymorphisms with inside the binding web web page of the promoter vicinity probable impact the expression of IL-27p28 thru a discounted affinity of binding among the transcription thing and its binding, despite the fact that we cannot rule out the opportunity that more than one genetic changes additionally result in the aggravation of T1DM.

The distribution of the genotype frequencies of TT, CT and CC in rs153109 polymorphism had been 57.8%, 36.7% and 5.6% in T1DM sufferers and 34.4%, 51.1% ,14.4% within side the healthful controls respectively it was significantly different among the T1DM patients and healthy controls (Table 3-9). These results showed that there was a statistically significant difference between T1DM patients and healthy controls in both genotype distribution (P=0.0016) and allele frequencies of rs153109 (P=0.00105)(196).

The ORs of the associations of rs153109 of IL27 gene with T1DM before and after adjusting for confounders (age and gender) clearly show that (1) significant association between rs153109 of IL27 and T1DM was observed.

(Table 3-8, P>0.05), (2) a significant connection of IL27 rs153109 with T1DM was observed under the allele model (OR=2.124, 95% CI (1.349–3.345), P=0.00105),and under genotype model in dominant model (OR=1.00 , 95% CI , P=0.0016), recessive model (OR=0.35, 95% CI (0.12–1.02), P=0.043) and homozygous model (OR=1.00 , 95% , P=0.0037) **(Table 3-9)**. Multivariate logistic regression analyses determinate that rs153109 of IL27 gene is significantly associated with T1DM .It is most likely that those people with the allele frequencies TT in G.-964A > G of IL27p28 are more prone to T1DM than those with the other alleles.

Conversely, those people with the allele frequencies GG in g.-964A > G of IL-27p28 would be more resistant to T1DM. the study explored the association of one SNP of IL27 in autoimmune type 1 DM disease and found that both genotype frequency and allele frequency of rs153109 has a strong correlation with T1DM.the analysis of alleles done by Hardy-Weinberg Principle (HWE) equilibrium which is a law for alleles analysis in different genes, the simplest definition of this law explained through in the absence of other evolutionary factors the allele and genotypic frequencies remain constant from generation to generation (135, 136).

this means if considered a single locus with two alleles denoted ‘A’ and ‘a’, whose genotypic frequencies are ‘p’ and ‘q’ respectively, then the frequencies will add up to unity (i.e., p + q = 1). In the F1 generation, the predicted genotypic frequency of AA, Aa & aa (i.e p2 ,2pq & q2) parental generation will match with the experimental F1 frequency. there are clearly in the table (3-9) the evaluated in T allele as compared with C allele in rs153109 but it is not significant for this genotype frequencies .

To the best of our knowledge, the present study is the first on the association of IL27 polymorphism with T1DM and CCKBR relationship predisposition. The results suggest that these polymorphisms are possibly correlated with susceptibility to autoimmune responses. With the advancement of modern medicine, it is generally believed that interactions between environment and genes play a crucial role in the pathogenesis of T1DM. Although many genes have been reported to be associated with the susceptibility of T1DM, there is no well-recognized report to date.

The polymorphisms of IL27 gene has been substantially studied in lots of autoimmune illnesses because of IL27, that is a crucial cytokine that features as a mediator among the innate and adaptive immune system (126) . As we all know, Type 1 diabetes is the result of an autoimmune reaction to proteins of the islets cells of the pancreas (137). The autoimmune destruction of pancreatic β-cells, results in a deficiency of insulin secretion, which ends up withinside the metabolic derangements related to IDDM. In addition to the lack of insulin secretion, the characteristic of pancreatic α-cells is likewise unusual and there is immoderate secretion of glucagon in IDDM .

Normally, hyperglycemia results in decreased glucagon secretion, however, in sufferers with IDDM, glucagon secretion isn't always suppressed through hyperglycemia (138). The resultant inappropriately expanded glucagon stages exacerbate the metabolic defects because of insulin deficiency. The maximum said instance of this metabolic disruption is that sufferers with IDDM swiftly broaden diabetic ketoacidosis with inside the absence of insulin management. Although insulin deficiency is the number one illness in IDDM, there is additionally an illness with inside the management of insulin.

There are a couple of biochemical mechanisms that account for impairment of tissue's reaction to insulin. Deficiency in insulin results in out of control lipolysis and accelerated degrees of unfastened fatty acids with inside the plasma, which suppresses glucose metabolism in peripheral tissues which include skeletal muscle (138). This impairs glucose usage and insulin deficiency additionally decreases the expression of some of genes important for goal tissues to reply typically to insulin together with glucokinase in liver and the GLUT 4 elegance of glucose transporters in adipose tissue. IL27 is a seasoned inflammatory cytokine that performs an crucial function in transcriptional activation and law of T lymphocytes (139).

IL27 changed into considered as a pro-inflammatory cytokine before, because of its help to the improvement of interferon (IFN) - secreting T helper cells (Th). However, IL27 level changed into evaluated in T1DM patient's due to the fact pro-inflammatory cytokine manufacturing as an end result for hyper transcriptional activation of rs153109 (82) Gene as an autoimmune reaction mechanism Therefore, examination the affiliation of IL27 gene with numerous autoimmune and inflammatory diseases, analyzed the contribution of probably purposeful SNP of IL27 and tested a clean affiliation among IL27 polymorphisms and T1DM susceptibility.

Although our look at confirms that IL27 has a great affiliation with T1DM, there are nonetheless obstacles on this look at. First, our look at discovered for the primary time that the genetic susceptibility of IL27 is sincerely related to T1DM in Iraqi patient's populations, however whether or not it's also real in different ethnic groups wishes to be similarly explored with massive pattern size. Second, this examine handiest evaluated the affiliation among IL27 gene SNPs and T1DM susceptibility, and did now no longer examine the function of IL27 gene SNPs in predicting remedy outcomes. Third, our examine examined handiest one capacity SNP of IL27 in T1DM sufferers and wholesome controls. The affiliation of different SNP with T1DM desires to be in addition evaluated(126).

3.6. Correlation Study:

3.6.1 Correlation between Age and Other Parameters:

The results displayed in figures 3-9 and 3-10 as well as those in Table 3-10 demonstrate the correlation of age with other parameters of the study in T1DM patients These results indicate a significant positive correlation of Age with Duration year and negative correlation with BMI, no other correlation has been find with other parameters(140)

Table 3-10: The correlation of Age with other parameters in the patients group with T1DM.

Parameters	**r**	***P*-value**
Age	1	
BMI	-0.463**	0.00

HBa1c(%)	-0.099	0.187
Duration year	0.535**	0.00
IL-27(ng\L)	-0.065	0.389
CCKBR (pg/mL)	0.075	0.321
Cholesterol(mg/dl)	0.108	0.150
Triglyceride(mg\dl)	0.074	0.326
HDL(mg/dl)	-0.08	0.286
LDL(mg/dl)	0.105	0.160
VLDL(mg/dl)	0.074	0.326
Urea(mg\dl)	0.08	0.288
Creatinine(mg/dl)	-0.005	0.944
*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed)		

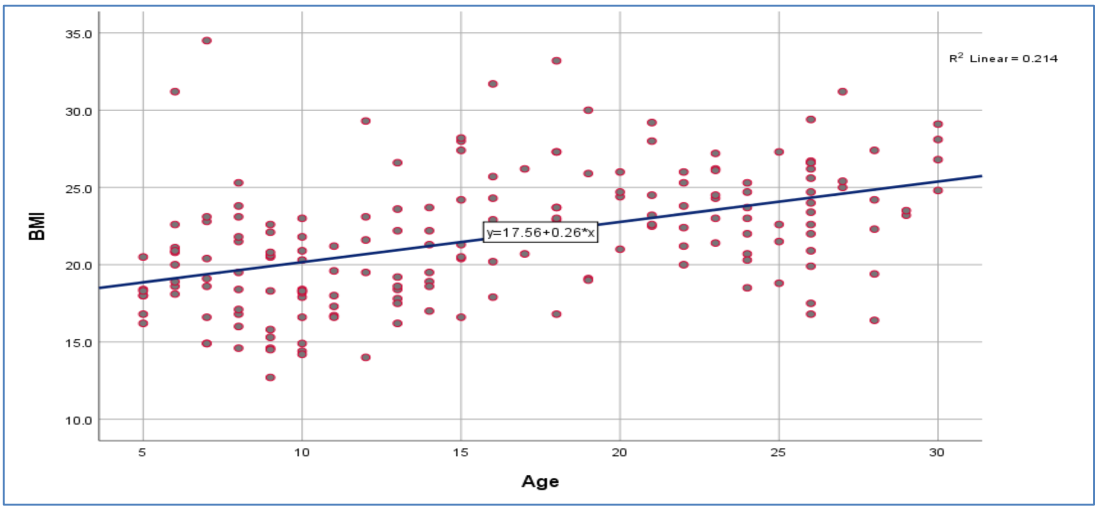


Figure 3-9: the correlation between Age and BMI.

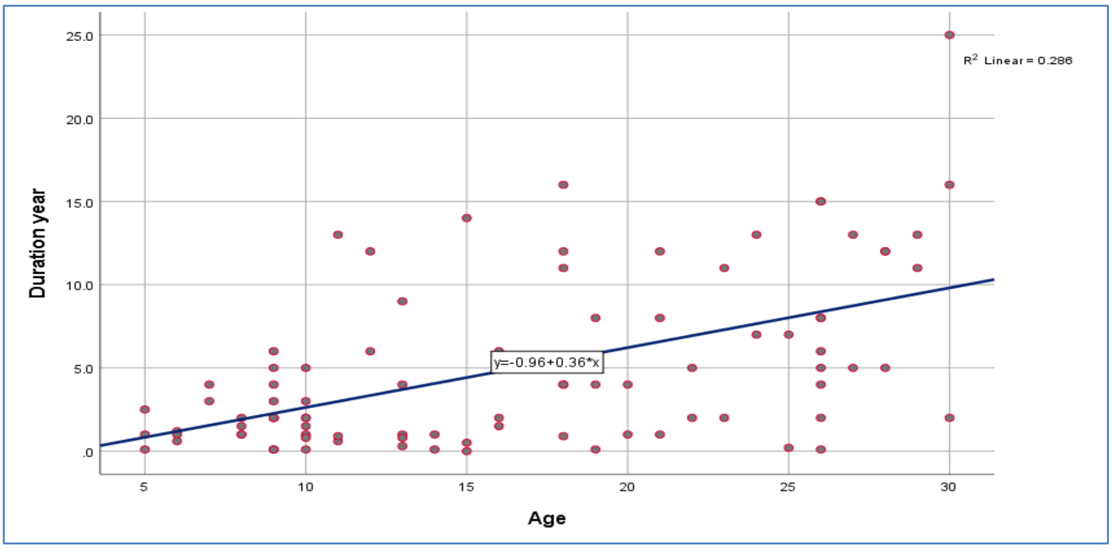


Figure 3-10: the correlation between Age and duration year:

BMI and changes in weight are inversely related to age at diagnosis of type 1 diabetes in a large cohort of children with type 1 diabetes, concluded that a higher BMI was associated with a younger age at diagnosis of type 1 diabetes and that an increased weight gain could be considered a risk factor for early manifestation of the disease increasing BMI is associated with younger age at diagnosis only in subjects with a reduced β-cell function and hypothesized that obesity is accelerating the onset of type 1 diabetes at a higher stage in the natural history of the disease, after substantial autoimmune destruction of the β-cell has occurred(141).

3.6.2 Correlation between BMI and other parameters:

The results displayed in figures 3-11 to 3-13 as well as those in Table 3-11 demonstrate the correlation of BMI with other parameters of the study in T1DM patients These results indicate a significant positive correlation of

BMI with duration year, LDL and negative correlation with HDL and age, no other correlation has been find with other parameters(142).

Table 3-11: The correlation of BMI with other parameters in the patients group with T1DM.

Parameters	r	*P*-value
Age	-0.463**	0.000
BMI	1	
HBa1c(%)	-0.143	0.05
Duration year	0.214*	0.042
IL-27(ng\L)	-0.009	0.904
CCKB R (pg/mL)	0.110	0.143
Cholesterol(mg/dl)	0.087	0.247
Triglyceride(mg\dl)	-0.012	0.878
HDL(mg/dl)	-0.171*	0.021
LDL(mg/dl)	0.164*	0.028
VLDL(mg/dl)	-0.012	0.878
Urea(mg\dl)	0.119	0.111
Creatinine(mg/dl)	0.031	0.683

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

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

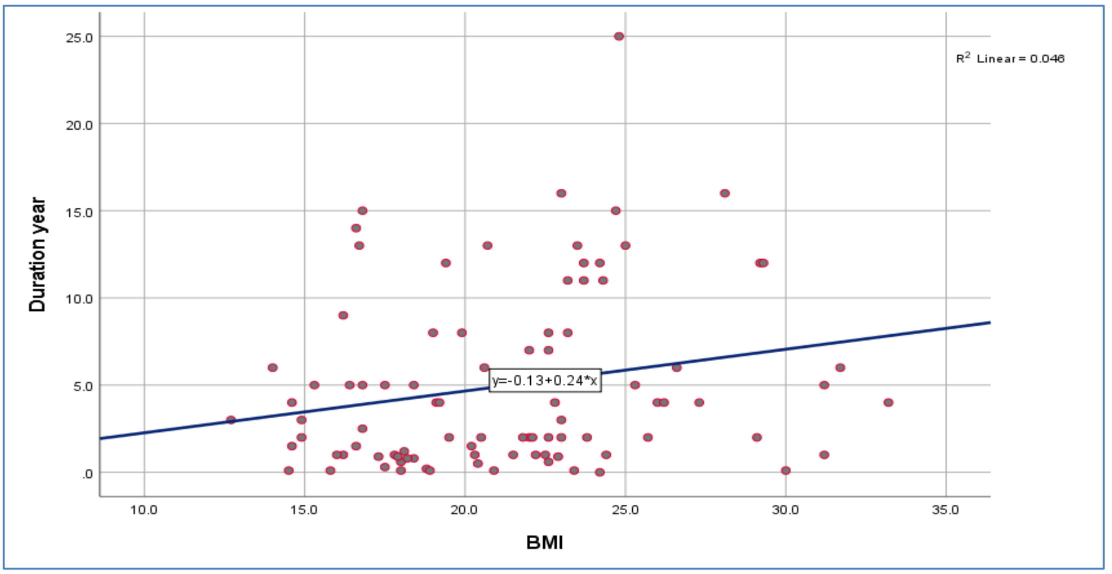


Figure 3-11: correlation between BMI and duration year.

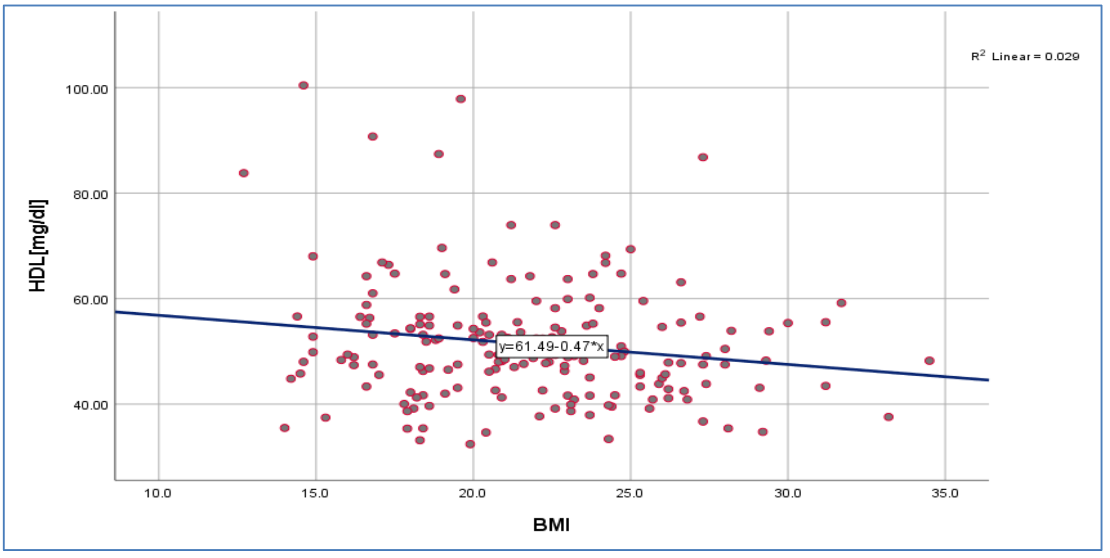


Figure 3-12; correlation between BMI and HDL.

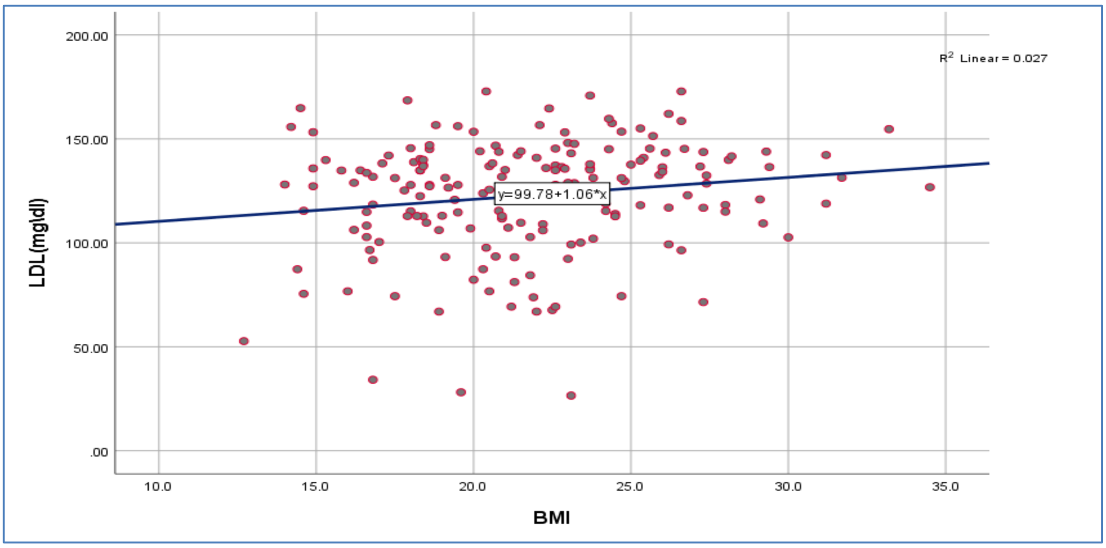


Figure 3-13: the correlation between BMI and LDL.

People with T1DM have an increased cardiovascular morbidity and mortality, and are affected more by CVD compared with non-diabetics. Prompt recognition and management of DM associated dyslipidemia might be one-step in controlling the risk of CVD both body fat and lipid parameters have been revealed the significant predictors for metabolic disturbances including diabetes, dyslipidemia, hypertension, hyperinsulinemia, and cardiovascular diseases.

Lipid profiles association is reported with lifestyle, age, and intra-abdominal adiposity, Obesity and BMI, BMI increased due to increase in adiposity characterized by decreased HDL-C and increased triglycerides. In some other studies, a significant relationship was found between cardio respiratory fitness, BMI and LDL-C, aerobic fitness is related to metabolic parameters and to body fat distribution in the patients, BMI and waist circumference independently contributed to the prediction of subcutaneous and visceral fat(142).

3.6.3 Correlation between HbA1C and other parameters:

The results displayed in figures 3-14 and 3-15 as well as those in Table 3-12 demonstrate the correlation of HbA1C with other parameters of the study in T1DM patients These results indicate a significant positive correlation of HbA1C with TG and VLDL, no other correlation has been find with other parameters(143).

Table 3-12: The correlation of HbA1C with other parameters in the patients group with T1DM.		
Parameters	**r**	***P*-value**
Age	-0.099	0.187
BMI	-0.143	0.55
HbA1c (%)	1	
Duration year	-0.140	0.188
IL-27(ng\L)	0.120	0.109
CCKB R (pg/mL)	0.060	0.426
Cholesterol(mg/dl)	0.087	0.247
Triglyceride(mg\dl)	0.155*	0.037
HDL(mg/dl)	0.001	0.986
LDL(mg/dl)	0.022	0.767
VLDL(mg/dl)	0.155*	0.037

Urea(mg\dl)	-0.106	0.158
Creatinine(mg/dl)	-0.031	0.683
*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed)		

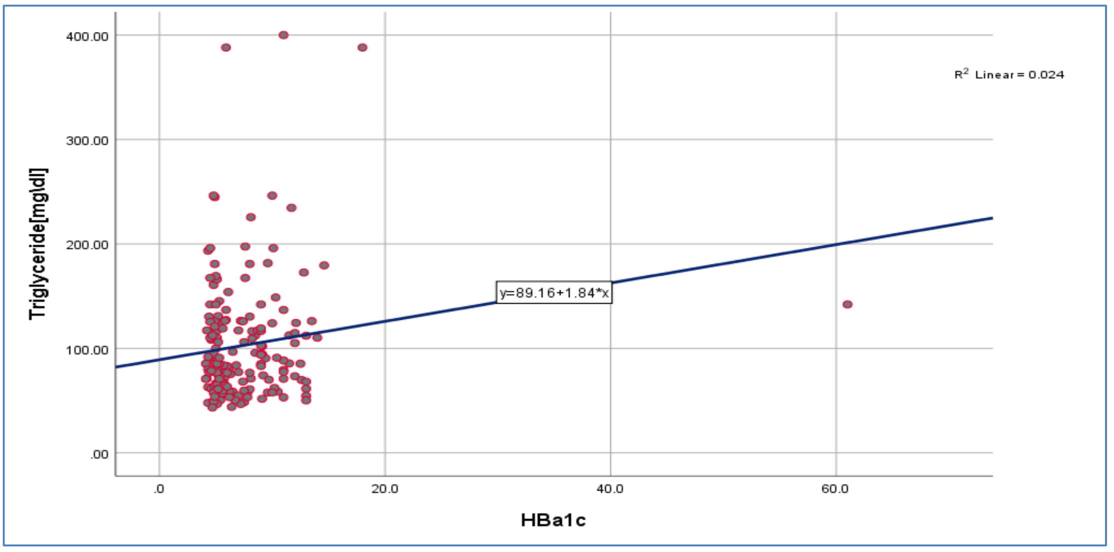


Figure 3-14: the correlation between HbA1C and TG.

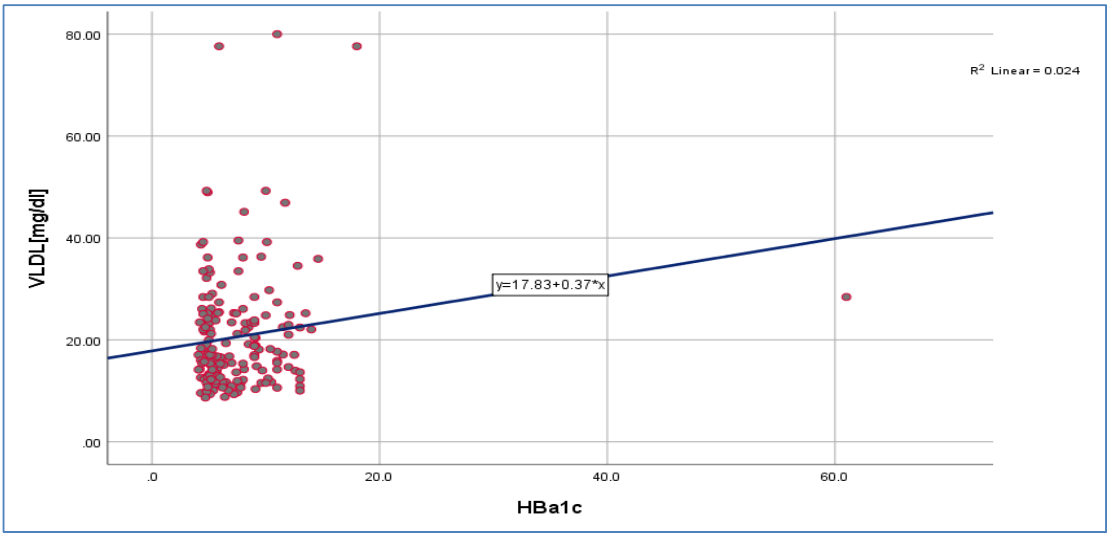


Figure 3-15: the correlation between HbA1C and VLDL.

HbA1c is characterized as the gold standard of glycemic control. In this study, HbA1c can be used as a potential biomarker for predicting dyslipidemia in patients with T1DM in addition to glycemic control. The results obtained in this study shows that those with HbA1c greater than 7.0% exhibit a significant increase in VLDL-C and TG comparative to those with HbA1c within target. This result is in agreement with the findings of several other investigations, which reported significant correlation between HbA1c and lipid profile. The diabetes mellitus patients with poor glycemic control exhibit a significant rise in TG and VLDL without any significant alteration in LDL, Hence, early diagnosis can be accomplished through relatively inexpensive blood testing and may be utilized for screening high-risk patients with DM for timely intervention with lipid-lowering drugs(144).

3.6.4 Correlation between CCKRB and other parameters:

The results displayed in figures 3-16 as well as those in Table 3-13 demonstrate the correlation of CCKRB with other parameters of the study in T1DM patients These results indicate a significant negative correlation of CCKRB with TG and VLDL, no other correlation has been find with other parameters(145, 146).

Table 3-13: The correlation of CCKRB with other parameters in the patients group with T1DM.		
Parameters	**r**	***P*-value**
Age	0.075	0.321
BMI	0.101	0.143
HbA1c (%)	0.006	0.426
Duration year	-0.059	0.580
IL-27(ng\L)	0.071	0.348
CCKBR (pg/mL)	1	
Cholesterol(mg/dl)	0.025	0.740
Triglyceride(mg\dl)	-0.164*	0.028
HDL(mg/dl)	-0.038	0.613
LDL(mg/dl)	0.099	0.186
VLDL(mg/dl)	-0.164*	0.028
Urea(mg\dl)	0.033	0.665
Creatinine(mg/dl)	0.005	0.944

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

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

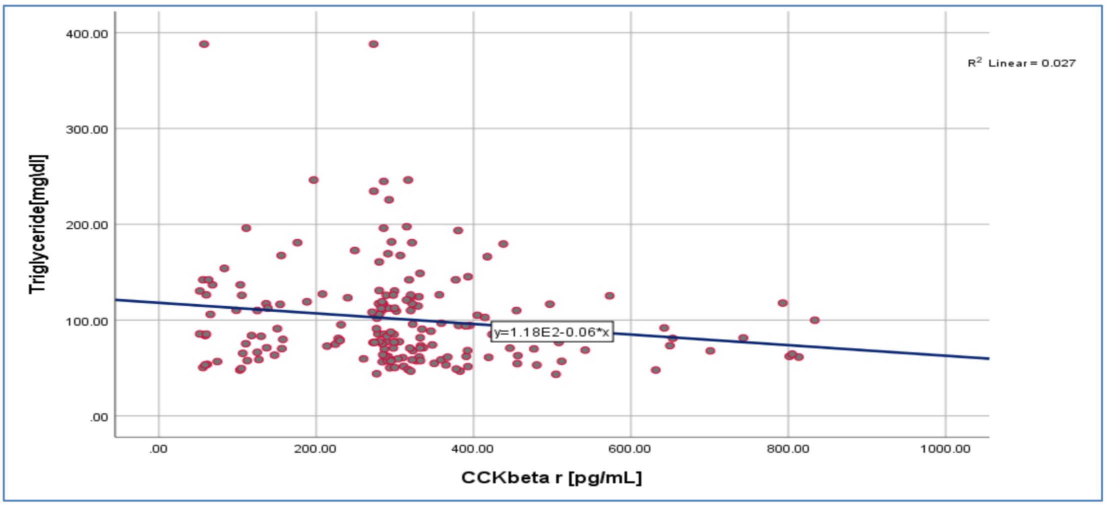


Figure: 3-16: the correlation between CCKRB and TG.

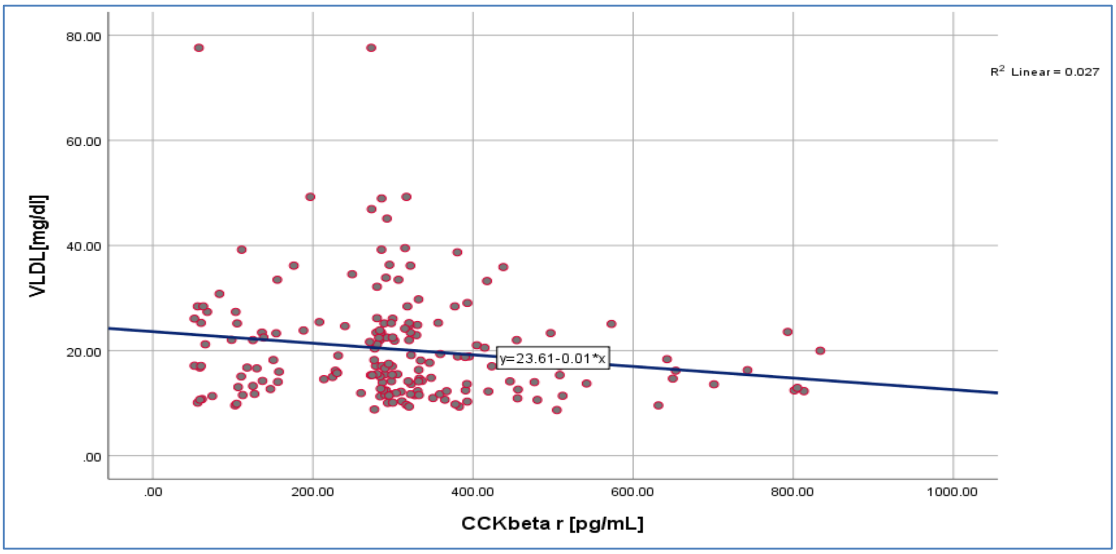


Figure 3-17: the correlation between CCKRB and VLDL.

Conclusion:

1- The study it is showed that T1DM is has an effect on the SNP gene used as a promoter to the excretion of IL-27 and increase its excretion,

2- This is the first identification of the association of IL27 rs153109 gene with T1DM and the relation between CCKBR, lipid profile, urea and creatinine.

3- The study revealed new data on the genetic contribution to T1DM susceptibility and support the crucial role of IL27 in T1DM pathogenesis. However, further researches are warranted to elucidate the molecular mechanisms underlying the relationship between IL27 and T1DM.

Recommendations:

1. Determination of new interleukins associated with IL-27 family and study it is effect on T1DM.
2. Study the effect of other SNP genes of IL-27 and other ILS family.
3. Study the effect of other Gastric enzymes and metabolic hormones on T1DM.
4. Study the effect of other receptors on T1DM.

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ملخص

مرض السكري (DM) هو مشكلة صحية عامة رئيسية تؤثر على أكثر من 400 مليون شخص في جميع أنحاء العالم. يؤدي هذا الاضطراب الأيضي بشكل تدريجي إلى مضاعفات الأوعية الدموية الدقيقة المزمنة والأوعية الدموية الكبيرة والاعتلال العصبي التي تهدد الحياة.

كوليسستوكينيين هو هرمون ببتيدي مرتبط بنظام كوليسيستوكينين المعدي المعوي، والذي يطلق عليه رسميًا بانكريوزيمين، يتم تصنيعه وإفرازه عن طريق دخول خلايا الغدد الصماء في الاثني عشري، الجزء الأول من الأمعاء الدقيقة. يتسبب وجوده في إطلاق الإنزيمات الهضمية من البنكرياس والصفراء من المرارة.

انترلوكين 27 وهو عبارة عن عضو في عائلة السيتوكين الفائقة عبارة IL-6 / IL-12 عن مغاير م يتكون من وحدتين تساهميتين غير مرتبطين.

اشتملت الدراسة على 90 شخصاً مصابين بالنوع السكر وتشخيصهم بعدم وجود أمراض أخرى، وتراوحت أعمارهم بين 5 إلى 30 سنة. أجريت هذه الأول من الدراسة في مستشفى مرجان، مركز السكري والغدد الصماء، تطوع تسعون شخصًا من 1 سبتمبر 2021 إلى فبراير 2022 سليمًا كمجموعة اصحاء، 5 إلى 30عاما في مدينة بابل، العراق.

اشتملت الدراسة على بعض القياسات بما في ذلك مؤشر كتلة الجسم والعمر وفحص سكر الدم التراكمي ودراسة تأثير مستقبلات كوليسستوكينين بيتا على مرضى السكر النوع الأول ودراسة تأثير الجهاز المناعي والجيني عن طريق تحديد انترلوكين 27 لمعرفة التأثير الوراثي للجين على مرضى السكر من النوع الأول ودراسة بعض المتغيرات الكيميائية ذات الصلة مثل قياس الدهون ووظائف الكلى باستخدام مجموعات طرق قياس الطيفي وتقنية الاليزا.

اجراء الدراسة والجينية المقارنات الإحصائية عن طريق تحليل التباين احادي الاتجاه باستخدام تردد الاليل ونموذج التركيب الوراثي وتوازن هاردي وينبرك.

النتيجة التي تم الحصول عليها في الدراسة

- **لا توجد فروق ذات دلالة إحصائية في العمر بين مجموعة الاصحاء (15.43 ± 7.312) ومرضى (7.575 ± 16.52) T1DM(p< 5 0.0) (انظر الشكل 3-1) الذي يرغب في الدراسة من أجل استبعاد عامل العمر الذي يمكن أن يؤثر المعلمات السريرية للدراسة، وتضييق الآثار المحتملة لسكر النوع الاول.**
- **كانت النسبة المئوية لـ CCKBR غير معنوية (P< 0.05) بين المرضى (319.19 ± 61.2) والأشخاص الاصحاء (290.6 ± 21.5)**
- **بينما النتائج الأخرى لملف الدهون (ثلاثي، HDL ،LDL ،VLDL) حيث لا يوجد فرق كبير (P< 0.05) بين المرضى ومجموعة الاصحاء باستخدام.**
- **مستويات اليوريا (المرضى 46.82 ± 14.84؛ الاصحاء 26.34 ± 8.34) والكرياتينين (المرضى 1.53 ± 0.65؛ الاصحاء 0.86 ± 019).**
- **كانت اليوريا والكرياتينين اعلى معنويا (P< 0.005) في المرضى مقارنة مع مجموعة الاصحاء.**
- **كان IL-27 معنويا (P< 0.005) في المرضى مقارنة مع مجموعة الاصحاء،**
- **كانت ترددات الأليل والنمط الجيني لـ G.-964A> G SNP لـ IL-27 P28 في مرضى T1DM مختلفة بشكل كبير عن تلك الموجودة في المجموعة الصحية.**

تشير هذه النتيجة إلى أن التشكل متعدد G.-964A> G IL-27 يكون مرتبطًا بالميل إلى التسبب للإصابة بمرض السكر من النوع الاول.

- **كانت ترددات التركيب الوراثي لـ TT وCT وCC في RS153109 57.8٪، 36.7٪ و5.6٪ في مرضى السكر النوع الاول و34.4٪، 51.1٪، 14.4٪ في الضوابط الصحية، على التوالي، كانت مختلفة بشكل كبير بين الأشخاص**

المصابين بمرض لسكر النوع الاول والاصحاء.

• أظهرت نسب الأرجحية لرابطات RS153109 لجين IL27 مع مرض السكر النوع الاول قبل وبعد ضبط عوامل الإرباك (العمر والجنس) بوضوح عدم وجود ارتباط كبير بين RS153109 لـ IL27 وDM1T. (الجدول 3، P> 0.05)، شوهد اتصال RS153109 IL27 مع مرض السكر النوع الاول أسفل نموذج الأليل (OR = 2.124، 95٪(1.349-3.345) CI ، P = 0.00105)، وتحت نموذج النمط الجيني في النموذج السائد (OR = 1.00، 95٪CI ، P = 0.0016)، نموذج متنحي (OR = 0.35، 95٪(0.12-1.02) CI ، P= 0.04ونموذج متماثل (OR = 1.00، 95٪، P = 0.0037)

في الختام، تظهر النتيجة التي تم جمعها في هذا العمل أن وجود السكر من النوع الاول له زيادة كبيرة جدًا في مستوىHBA1C، والارتفاع في مستوى IL-27 يعطي دليلًا على وجود علاقة قوية جدًا بين السكر النوع الاول وتأثير جهاز المناعة. النظام من الدراسة الجينية، من الواضح أن مرض السكر النوع الاول له تأثير على SNP جين المستخدم كمحفز لإفراز IL-27 وزيادة إفرازه. تظهر نتائج فحص الدهون تأثيرًا على مستوى الجلوكوز في الدم وقد يتسبب ارتفاع مستوى الكوليسترول في حدوث مشكلة خطيرة إذا تم دمجه مع السكر من النوع الاول. حدث ارتفاع مستوى الجلوكوز بسبب مرض السكر النوع الاول له تأثير على الكلى ويسبب حالات خطيرة مثل أمراض الفشل الكلوي. هذا هو أول تعريف لعلاقة IL27 RS153109 مع والعلاقة مرض السكر النوع الاول بين CCKBR والدهون واليوريا والكرياتينين. أضافت النتائج التي توصلنا إليها بيانات جديدة حول المساهمة الجينية في قابلية مرض السكر النوع الاول ودعم الدور الحاسم لـ IL27 في التسبب في المرض السكر النوع الاول. ومع ذلك، هناك ما يبرر المزيد من الأوراق البحثية لتوضيح الآليات الجزيئية الكامنة وراء العلاقة بين IL27 ومرض السكر النوع الاول.

جمهورية العراق
وزارة التعليم العالي والبحث علمي
جامعة بابل / كلية الطب
قسم الكيمياء والكيمياء الحياتية

"دراسة مقارنة لمستويات مستقبلات الكوليسيستوكينين بيتا و الانترلوكين 27 جين SNP وبعض المتغيرات البيوكيميائية في مرضى السكري من النوع الأول في محافظة بابل "

رسالة
مقدمة الى عمادة كلية الطب في جامعة بابل وهي جزء من متطلبات نيل درجة الماجستير في الكيمياء الحياتية السريرية

من قبل
نسرين رياض مهدي
بكالوريوس تقنيات التحليلات المرضية
2008

إشراف

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1443 هـ 2022 م