University of Kerbela

College of Science

Department of Biology

Association of B-reg Frequency and Some Immunological Parameters with Urinary Tract Infections in Type 1 Diabetes

A Dissertation

Submitted to the Council of the Collage of Science, University of Kerbela in Partial Fulfillment of the Requirements for the Degree of Doctorate of Philosophy in Biology

By

Noor Abdulameer Oudah Ismail

B.Sc. Biology/ Kerbela University,2011

M.Sc. Biology/Kerbela University,2014

Supervised by

Professor Dr. Haider H. Mohammed Ali

Assist.Pro.Dr. Kawkab A. Alsaadi

2023 A.D

1444 A.H

﴿ قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ ﴾

صدق الله العلي العظيم

سورة البقرة , آية ٣٢

Supervisors Certification

We certify that this dissertation was prepared under our supervision at the College of Science, University of Kerbela, as a partial fulfilment of the requirement for the Degree of Doctorate of Philosophy in Biology.

Signature:

Name: Dr. Haider H. Mohammed Ali

Scientific degree: Professor

Address: College of Science - University of Kerbela

Date: 15/5/ 2023

Signature:

Name: Dr. Kawkab A.H. Alsaadi

Scientific degree: Assistant professor

Address: College of Science - University of of Kerbela

Date: 15/5/ 2023

Chairman of Biological Department

In view of the available recommendations, I forward this dissertation for debate by examining committee.

Signature:

Name: Dr. Khalid Ali Hussein

Scientific degree: Assistant professor

Address: College of Science - University of Kerbela

Date: 15/5/ 2023

Committee Certification

We certify that we have read this dissertation, entitled " Association of B-reg frequency and some immunological parameters with urinary tract infections in type 1 diabetes " and as examining committee, examined the student " Noor Abdulameer Oudah Ismail " in its contents and that in our opinion it is adequate for the partial fulfilment of the requirements for the Degree of Doctorate of Philosophy (Ph.D) in Biology with (Excellent) estimation.

Signature:
Name: Dr. Abdulnabi Jwaid Abid Almaamori
Scientific degree: Professor
Address: College of Science for Women-University of Babylon
Date: 26/6/2023

Signature:
Name: Dr. Hadi Rasool Hassan
Scientific degree: Professor
Address: College of Applied Medical Science - University of Kerbela
Date: 26/6/2023

Signature:
Name: Dr. Dunya Fareed Salloom
Scientific degree: Professor
Address: College of Science - University of Baghdad
Date: 26/6/2023

Signature:
Name: Dr. Ali A. Al-Hisnawi
Scientific degree: Professor
Address: College of Science - University of Kerbela
Date: 18/7/2023

Signature:
Name: Dr. Suhad Hadi Mohammed
Scientific degree: Professor
Address: College of Applied Medical Science - University of Kerbela
Date: 18/7/2023

Signature:
Name: Dr. Haider H. Mohammed Ali
Scientific degree: Professor
Address: College of Science - University of Kerbela
Date: 26/6/2023

Signature:
Name: Dr. Kawkab A.H. Alsaadi
Scientific degree: Assistant professor
Address: College of Science - University of Kerbela
Date: 26/6/2023

Approved for the council of college

Signature:
Name: Dr. Jasem Hanoon Hashim Al-Awadi
Scientific degree: Professor
Address: Dean of College of Science / University of Kerbela
Date: 23/7/2023

Dedication

To my father's soul who gave me passion , tenderness and overflowed me with care and love ... god bless him , and to the one who carries the true meaning of love, my dear mother.

To my lovely husband Ibrahim for constantly being on my side, and for all his support, care and love, to my lovely daughter and son (Haider & Lamar).

To my All my wonderful sisters, especially Marwa, to all of my friends who supported me.

Noor

Acknowledgements

In the beginning, I have to mention and thank God for giving me wisdom and bliss. I know that without the mighty and sublime "Allah", I could not complete this work.

I would like to express my profound thanks and sincere gratitude to my supervisors Professor Dr. Haider H. Mohammed Ali and Asst.Pro.Dr. Kawkab A. Alsaadi for their valuable scientific guidance, encouragement and support that made it possible for me to accomplish this study.

I wish to express my sincere gratitude to all patients who participated in this study for their help and cooperation.

I should express my gratitude to the Head of the Department of Biology/College of Science for providing this opportunity to accomplish this research work. I would also like to thank all members of this department for giving me their valuable advice.

I would also like to express my very great appreciation to Dr.Mohammed Salih - Al-Hussein Teaching Hospital ,Holy Karbala-Iraq and Israa M. Al-Bayaa College of Medicine – University of Karbala for their valuable support and advice in this project and provide me with all the facilities to complete this work.

My special thanks extend to the staff and people at Imam AL- Hussein Diabetes Center and Dr.Mohammed Salih laboratory for their great help, support, and advice during the phase of data and blood sample collection.

Noor

Summary

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease characterized by lack of insulin and destruction of pancreatic β cells. Autoimmune reaction is a key step during the progression of T1DM, where T-cells play a vital role. However; B-cells also play important role in the occurrence and development of T1DM. The B-cells producing Interleukin-10 (IL-10) have been labeled as regulatory B-cells (B-reg). Diabetes and bacterial infections are linked in both directions. Chronic infections are associated with higher proinflammatory cytokines, which can exacerbate insulin resistance and compromise glycemic management, whereas diabetes increases susceptibility to bacterial infections and their consequences. There is growing proof that changes in the microbiota's makeup that may have a significant impact on how diabetes develops. The current study aimed to consolidate and update the current knowledge about the cellular and regulatory molecules that are exhibited by B-reg for T1DM patients and the role of the disease in the development of urinary tract infection (UTI).

The current investigation was carried out in the provinces of Kerbala. A total of 90 male children blood and urine samples were collected, whose ages ranged between 5-15 years. 60 patients with T1DM (30 T1DM patients with UTIs and 30 T1DM patients without UTIs) and 30 healthy individuals (control group). The frequency of B-cell subsets were measured using flow cytometry and cultivation urine on agar to indicate UTIs. The level of human serum of some immunological markers was measured by ELISA assay.

The results of the current study showed that a significant decreased the percentage of cluster of differentiation (CD)19+B cells on level $P \leq 0.0001$ for both patients groups of T1DM compared to control . CD19+IL10+ B-cell (B-reg) was significant decreased in T1DM with UTIs compared with healthy controls but increased in the group of T1DM without UTIs. T1DM with UTIs significantly increased CD80 expression on B-reg, whereas T1DM without UTIs only slightly decreased it when compared to the control group. Additionally, it demonstrated that in both groups of T1DM patients, the concentration of CD80 expression on B-reg has significantly decreased.

Summary

IL-35, macrophage migration inhibitory factor (MIF), Lipopolysaccharide binding protein (LBP) and Toll-like receptors (TLR4) were higher for both patients groups of T1DM compared to control group but more significant increase in barameters levels for T1DM with bacterial infection compared with both other groups. Transforming growth factor-β (TGF-β1) level was higher in T1DM with UTIs compared with patients without bacterial infection and healthy individuals.

The bacterial infections lead to an increase in the plasma level of total cholesterol (CHOL), total to high-density lipoprotein cholesterol (TC/HDL-C) , Low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL-C) and non-high-density lipoprotein cholesterol (Non-HDL-C) compared to diabetic patients without UTIs and healthy individuals. Triglyceride (TC) concentration was significantly higher in diabetic patients without bacterial infection compared to T1DM with UTIs and healthy individuals.

The concentration of serum aspartate transaminase (AST) and alanine transaminase (ALT) were significantly decreasing in T1DM in comparison with controls. The concentration of alkaline phosphatase (ALP) were significantly elevated in diabetic patients without UTIs compared to T1DM with UTIs and controls. A significant increases are in the levels of total serum bilirubin and creatinine in T1DM with UTIs when compared to controls and T1DM without UTIs.

The bacterial pathogens that cause UTI belong to gram negative 54% and gram-positive bacteria 46%. Among Gram negative pathogens, *Escherichia coli* is the principal etiology of UTI in diabetic individuals that estimated at 35% followed by *Pseudomonas stutzeria* while among Gram positives, *Kocuria rosea* is the major uropathogen estimated at 18.5 % followed by *Streptococcus thoraltensis* , *Streptococcus gallolyticus* , *Staphylococcus haemolyticus* , *Kocuria kristinae*.

Summary

These findings suggest that numerically deficient for CD19+B cells ,CD19+IL10+ B-cell and CD19+IL10+CD24+CD27+ B-reg percentage in B-cell and difference in the cytokines concentration for T1DM children with bacterial infection, that may involve in the loss of auto-tolerance of immunity system that ensuing death of pancreatic beta cells, making it a viable immunotherapy target.

Table of Contents

Table of Tables

Table of Figures

Table of Abbreviations

Abbreviation	Term
Ab	**A**ntibodies
ALP	**A**lkaline **P**hosphatase
ALT	**A**lanine **T**ransaminase
Anti-GAD	**G**lutamic **A**cid **D**ecarboxylase **A**ntibodies
APC	**A**ntigen-**P**resenting **C**ells
AST	**As**partate **T**ransaminase
BCR	**B C**ell **R**eceptor
B-reg	**R**egulatory B cells
CD	**C**luster of **D**ifferentiation
CHOL	**Ch**olester**ol**
C-peptide	**C**onnecting **P**eptide
CR2	**C**omplement **R**eceptor **2**
CTLA-4	**C**ytotoxic **T**-lymphocyte **A**ssociated **P**rotein **4**
DM	**D**iabetes **m**ellitus
ECL	**E**lectro**c**hemi**l**uminescence
EDTA	**E**thylene**d**iaminete**t**raacetic **a**cid
ELISA	**E**nzyme-**L**inked **I**mmunosorbent **A**ssay
HbA1c	**H**emoglo**b**in **A1**$_{\mathbf{C}}$
HDL-C	**H**igh-**D**ensity **L**ipoprotein **C**holesterol
HLA	**H**uman **L**eukocyte **A**ntigen
IgM	**I**mmuno**g**lobulin **m**
IL	**I**nter**l**eukin
IL-1R1	**I**nter**l**eukin -1 **R**eceptor 1
LBP	**L**ipopolysaccharide **B**inding **P**rotein

Abbreviation	Term
LDL-C	**L**ow-**D**ensity **L**ipoprotein **C**holesterol
LPS	**L**ipo**p**oly**s**accharide
MFI	**M**ean **F**luorescence **I**ntensity
MHC	**M**ajor **H**istocompatibility **C**omplex
MIF	**M**acrophage **M**igration **I**nhibitory **F**actor
MZ	**M**arginal **Z**one
NK cells	**N**atural **K**iller **C**ells
Non-HDL-C	**N**on-**H**igh-**D**ensity **L**ipoprotein **C**holesterol
OD	**O**ptical **D**ensity
SLE	**S**ystemic **L**upus **E**rythematosus
T1DM	**T**ype 1 **D**iabetes **m**ellitus
TC/HDL-C	**T**otal to **H**igh-**D**ensity **L**ipoprotein **C**holesterol
TG	**T**ri**g**lyceride
TGF-β1	**T**ransforming **G**rowth **F**actor-β
TLR4	**T**oll-**L**ike **R**eceptors
TNFα	**T**umor **N**ecrosis **F**actor **A**lpha
TSB	**T**otal **S**erum **B**ilirubin
UTIs	**U**rinary **T**ract **I**nfection
ZNT8	**Zi**n**c** **T**ransporter 8

Chapter One
Introduction

Chapter One : Introduction

1.1. Introduction

Diabetes mellitus (DM) is a disease of dysfunctional glucose homeostasis resulting from insufficient insulin secretion to meet the demand of peripheral insulin-responsive tissues. The two most common types of diabetes, type 1 diabetes mellitus and type 2 Diabetes mellitus In addition to these major classifications of DM, gestational DM , monogenic DM, latent autoimmune DM in adults, diseases of the exocrine pancreas, and drug- and chemically-induced DM have also been identified in individuals who present with hyperglycemic phenotypes distinct from type 1 or type 2 diabetes (Hillier *et al*., 2021).

Type 1 diabetes mellitus is an autoimmune disease in which the insulin-producing β-cells in the islets of Langerhans of the pancreas are gradually destroyed over months to years by autoreactive immune cells (Lowe *et al*., 2019). T-lymphocyte-mediated insulitis, followed by the presence of one or more type of autoantibody marks stage 1 of T1DM. Stage 2 is marked by dysglycaemia or glucose intolerance, both stages are asymptomatic; stage 3, however, is defined by the clinical presentation with symptoms of hyperglycaemia (polydipsia, polyuria, enuresis, blurred vision and weight loss), sometimes even with diabetic ketoacidosis or diabetic hyperosmolar syndrome.Individuals with T1DM are also more susceptible to other autoimmune diseases, such as coeliac disease, Hashimoto's thyroiditis, vitiligo, Addison's disease and myasthenia gravis, moreover, the association of T1DM with thyroid autoimmunity and coeliac disease may also be mechanistically determined (Zajec *et al*., 2022).

To maintain glycemic control, T1DM patients need lifelong insulin therapy. T1DM onset is on the rise and it is a significant global cause of healthcare resource utilization due to complications related to hyperglycemia-induced microvascular disease as well as rising morbidity and mortality related to insulin therapy-induced hypoglycemia unawareness (American Diabetes Association , 2021). Development of and persistence of pancreatic β-cells autoantibodies such antibodies (Ab) to glutamic acid decarboxylase , Ab to islet antigen 2, or Ab to zinc transporter 8 is a risk factor for clinical DM and may serve as an indication for

intervention in the setting of a screening for T1DM and undetectable levels of C-peptide as a marker of endogenous β-cell function (Care, 2022).

There is disagreement about which environmental factors are most crucial, despite the fact that the role of environmental factors in the pathogenesis of T1DM has been well recognized for many years. Although the evidence supporting viral infection, more crucially, the precise viral types responsible for T1DM are still up for debate. They continue to be one of the most likely choices (Akil *et al*., 2021).

The organizational defect in the immune system plays a vital role in T1DM pathogenesis if pancreatic beta cells are destroyed by T-cells but some research's results indicate that β cells have an active role for eliciting its own demise, instead of being an unwitting spectator to an autoimmune onslaught. Virtually all individuals who develop T1DM before the age of five years produce insulin-specific autoantibodies , suggesting an important role for peptides derived from the insulin molecule in disease pathogenesis (Atkinson, 2012; Pietropaolo *et al*., 2012; Thayer *et al*., 2011).

Regulatory B cells (B-reg), human B-reg cells in peripheral blood are contribute to the maintenance of tolerance, limiting on going immune responses and reestablishing immune homeostasis (De Giacomo *et al*., 2021). Recent studies have suggested that B-reg may also play a role in other immune-related conditions like infections, allergies, cancer, and chronic metabolic diseases. B-reg have a critical role also to play in preventing the pathology associated with exacerbated inflammatory responses in graft rejection and autoimmunity (Moore *et al*., 2019). Although IL-10 was initially recognized as the characteristic of B-reg function, other substances used by human and murine B-cells to control immunological responses have been discovered during the past ten years (Ferreira *et al*., 2019).This new arsenal includes other anti-inflammatory cytokines such interleukin-35 (IL-35), Macrophage migration inhibitory factor (MIF) and transforming growth factor-β (TGF-β), as well as cell surface molecules like cluster of differentiation 1d (CD1d) and Programmed death-ligand 1 (Catalán *et al.*, 2021).

In particular, TGF-β and IL-35 have the capacity to convert naïve CD4+T-cells into T-reg (Li *et al*., 2020). Considering that the insulitis process marks the beginning of the disease and is an autoimmune inflammatory process. MIF was found to play important roles in the processes of antigen presentation and inflammatory cell activation (Sánchez-Zamora and Rodriguez-Sosa, 2014).

T1DM inflammatory process depends on T-cell activation. This necessitates T-cell receptor activation in response to the MHC-peptide complex on the cell surface of APC, including B cells (Nandi *et al*., 2020). Second interaction involving a costimulatory molecule, CD80, CD86, and PD-L1, which express on the surface of B-reg and belong to the B7 family, is in addition to this antigen-specific stimulation crucial for preserving the equilibrium between a sufficient immune response, immunosuppression, and autoimmune disease (Salek-Ardakani *et al*., 2011 ; Hlela *et al*., 2009). Since both CD80 and CD86 interact differently with their ligands, CD28 and CTLA-4 (CD152), respectively, they each perform distinct roles in immunological regulation. As a result, their expression is only found on B-reg (Marcucci and Rumio,2021). A stronger affinity exists between CD80 and CTLA-4 (the negative regulatory) than between CD86 and CTLA-4, Conversely, CD28 has a stronger connection with CD86 (the positive regulatory) than CD80. A costimulatory-co-inhibitory system is formed by these interactions, which controls immunological reactions (Kennedy *et al*., 2022).

Diabetics have the risk of community-acquired bacterial infections, such as enterobacterial infections, streptococci and pneumococci , compared to non-diabetics. Some bacteria are harmful and can cause various diseases in humans (Nagendra *et al*., 2022). Uncontrolled hyperglycemia is associated with impaired innate and adaptive immune responses that predispose to bacterial infections (Unnikrishnan and Misra, 2020). In addition, chronic diabetic complications such as neuropathy (sensorimotor and autonomic neuropathy) and peripheral vascular disease can lead to skin ulcers with secondary bacterial infections. Diabetes also increases infection-related mortality (Rao and Lipsky, 2007). The relationship between diabetes and bacterial infections may be reciprocal, with certain infections such as periodontal disease exacerbating insulin resistance (Dhande *et al*., 2022). Abnormalities in the

gut microbiota may contribute to the development of diabetes. The most common sites of infection in diabetes are the urinary tract, respiratory tract, skin, and soft tissues (Dekaboruah *et al*., 2020). Certain bacterial infections are highly specific to diabetes, such as emphysematous pyelonephritis, emphysematous cholecystitis, and malignant otitis externa (Nagendra *et al*., 2022) .

There is a two-way relationship between diabetes and bacterial infections. Diabetes increases susceptibility to bacterial infections and their complications, while chronic infections such as periodontitis are associated with elevated proinflammatory cytokines, which can exacerbate insulin resistance and impair glycemic control (Mirza *et al*., 2021).

Pascale *et al*., (2019) mentioned that there is increasing evidence that abnormalities in microbiota composition may play an important role in the development of diabetes. Recognizing the complex interrelationships between diabetes and associated bacterial infections is critical for prevention and prompt treatment.

Despite a working knowledge of genetic risk factors associated with T1DM, determining specific β cell targets and preventing beta cell destruction by autoreactive immune cells remains elusive. To develop a successful approach to protect beta cells, we must understand how and why immune cells are directed to specifically destroy insulin-producing cells in the pancreas while sparing adjacent hormone-producing cells.

1.1. Aim of the study

The current study aimed to consolidate and update the current knowledge about the cellular and immune regulatory molecules mechanisms that are exhibited by B-reg for T1DM patients and the role of the disease in the development of some bacterial infections. This will be achieved by:

1- Phenotypic identification of CD19+CD24+CD27+ regulatory B-cells that produce interleukin 10 in human peripheral blood the study will include the following groups: control group, diabetic patients with bacterial infection group and diabetic patients without bacterial infection group using flow cytometric analysis.

2- Studying the role of the co-stimulatory molecule CD80+ expression on B- cells in healthy children, diabetic patients with UTIs group and diabetic patients without UTIs .

3-Assessing the level some of these are immunological markers you should be separated in serum of type 1 diabetes patients and comparing it with healthy children.

4. Detecting levels of C-peptide , anti-GAD , HbA1c and fasting blood glucose which can be used to guide diabetes diagnosis

5. Will be determined of lipid profile, liver function and kidney function parameters of all groups.

6- Will be studied the role of diabetes in the development of some bacterial infections and the effect of infection on a change in the immune system.

Chapter Two
Literature Review

Chapter Two: Literature Review

2.1. *Diabetes Mellitus*

Diabetes mellitus (DM) is a syndrome of impaired glucose metabolism caused by either lack of insulin secretion or decreased tissue sensitivity to insulin. The main clinical manifestation of the diabetic condition is hyperglycemia. However, insulin deficiency and/or insulin resistance are also associated with abnormalities in lipid and protein metabolism, and mineral and electrolyte imbalances (Thomas *et al*., 2019)**.** According to the American Diabetes Association , (2021) Diabetes can be classified into the following general categories:

1-Type 1 Diabetes mellitus: is due to destruction of autoimmune beta cells, usually leading to absolute insulin deficiency, including latent autoimmune diabetes in adulthood.

2-Type 2 Diabetes mellitus : is due to the gradual loss of adequate beta-cell insulin secretion, often in the context of insulin resistance.

3- Specific types of Diabetes mellitus: is due to other causes, e.g., monogenic Diabetes mellitus syndromes such as neonatal Diabetes mellitus and maturity-onset diabetes of the young, diseases of the exocrine pancreas (such as cystic fibrosis and pancreatitis), and drug- or chemical-induced diabetes such as with glucocorticoid use, in the treatment of human immunodeficiency virus, or after organ transplantation.

4-Gestational diabetes mellitus : Diabetes mellitus is diagnosed in the second or third trimester of pregnancy and not overt pre-pregnancy diabetes.

2.1.1. Type 1 Diabetes mellitus (T1DM)

Type 1 Diabetes mellitus is an autoimmune disease in which the insulin-producing β-cells in the islets of Langerhans of the pancreas are gradually destroyed over months to years by autoreactive T-cells (Hillier *et al.*, 2021). Damage to pancreatic beta cells or disease that affects insulin production can lead to T1DM. Genetics predisposition or autoimmune diseases may play a role in β-cell destruction, viral infections also play a pivotal role in determining susceptibility to β-cell destruction by these attacks (Kakleas, 2015).

The presence of high levels of glucose in blood and tissue fluids promotes nonenzymatic conversion of glucose to reactive carbohydrate derivatives such as glyoxal. These derivatives crosslink proteins and carbohydrates in the membranes and walls of blood vessels, neurons, and the extracellular matrix, leading to the well-known symptoms of diabetes (Schalkwijk and Stehouwer, 2020). There are three main consequences of Type 1 diabetes mellitus: raised blood sugar levels, increased utilization of fat for energy and hepatic cholesterol production, and cell depletion. (Chen *et al*., 2018).

2.1.2. Type 1 Diabetes mellitus as an autoimmune disease

Autoimmune diseases occur when an immune response is directed against a particular organ or set of organs. Genetic susceptibility and environmental factors are major contributors in the development of autoimmune processes that lead to clinically manifest disease (Caso *et al*., 2018). Most organ-specific autoimmune diseases are characterized by initial infiltration of organs by lymphocytes and macrophages with compromised organ activity, followed by atrophy. This ongoing autoimmune process is slow and mediated by T cells. Antibodies to specific antigens of affected glands are detectable in the blood prior to the clinical onset of a particular disease and thus represent risk markers, screening and follow-up of which can help identify genetically susceptible individuals. It enables to early diagnosis and treatment of autoimmune diseases in individuals (Burrack *et al*., 2017).

The relationship between the immune system and T1DM was first suggested in 1973 when Human leukocyte antigens (HLA) antigens were found to be associated with insulin-dependent diabetes, but not with non-insulin-dependent Diabetes mellitus. Because cells of the adaptive immune system kill cells of the islets of langerhans in the pancreas, insulin is completely deficient in diabetics that depend on exogenously supplied hormones for survival (Nerup *et al*., 1974; Lugovaya *et al*., 2022) (Figure1).

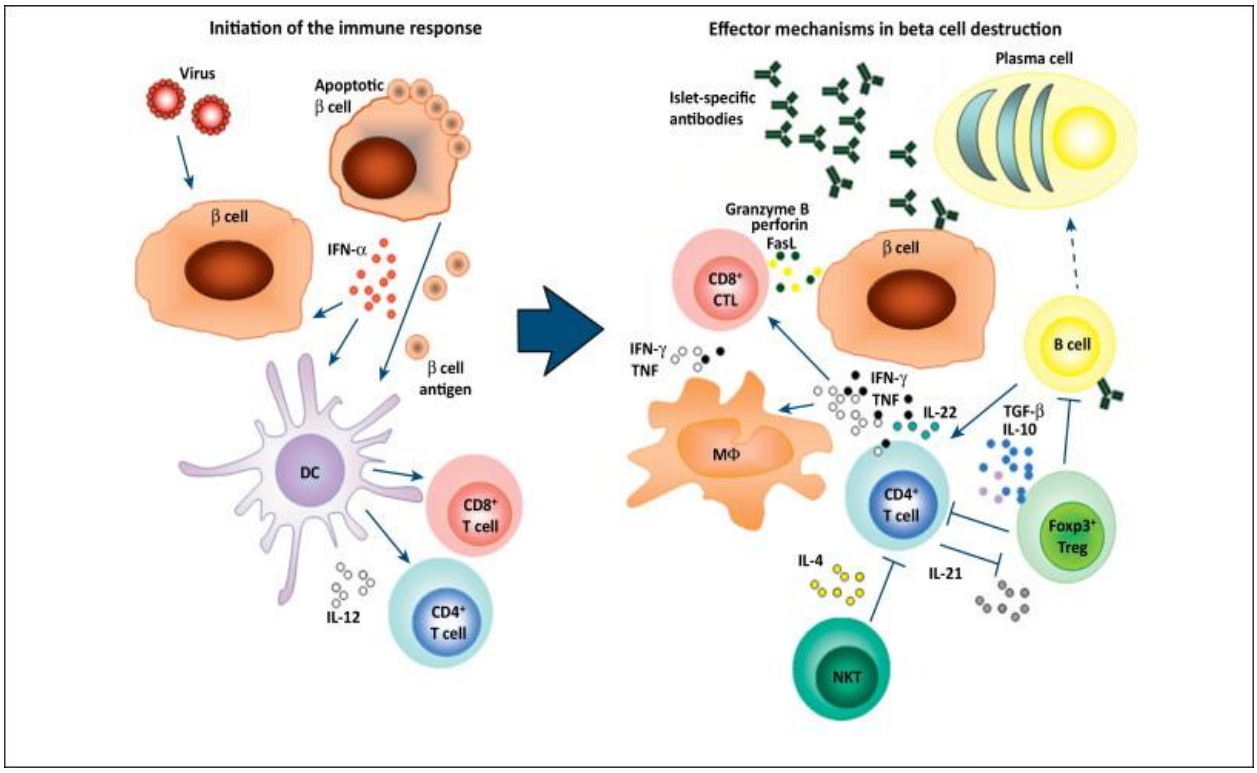

Figure 2.1: Molecules expressed by pancreatic β-cells involved in their destruction or protection (adapted from Wallberg and Cooke, 2013).

During the development of T1DM, seroconversion of islet autoantibodies against insulin, glutamate decarboxylase, insulinoma antigen 2, or zinc transporter 8 is the first hallmark of autoimmunity, and the presence of their combination in serum , remain the best predictors of loss of immune tolerance (i.e., induction of autoimmunity, although their role in β-cell destruction remains unclear) and clinical manifestations of T1DM **(**Thomas *et al*., 2019 ; Lugovaya *et al*., 2022**)**. During the course of the disease, immune cells that infiltrate the pancreas and attack insulin-producing cells induce and accelerate the development of T1DM by gradually presenting the immune system with islet antigens presented by HLA class I molecules,which creates an inflammatory environment characteristic of insulitis (Bloem and Roep, 2017).

2.2. B-lymphocyte

B-lymphocyte or B-cell is a key player of the adaptive immune response that is responsible for humoral immunity in mammals (Althuwaiqeb and Bordoni, 2022). B-cell production in humans is a lifelong process that starts in the fetal liver intrauterine and bone marrow after birth. Their development is from hematopoietic stem cells. B-cell development constitutes of all the stages of early differentiation in the absence of antigen interaction until the maturation, antigen interaction, and, ultimately, antibodies synthesis (Vale and Schroeder, 2010). Generally, B-cell is a key regulatory cell in the immune system; it acts by producing antibodies, antigen-presenting cells, supporting other mononuclear cells, and contributing to inflammatory pathways directly (Althuwaiqeb and Bordoni, 2022).

2.2.1. B lymphocytes subtypes

B lymphocytes play a crucial role in the immune system's defense against pathogens because they are long-lived lymphocytes that take part in the immune response by gathering and concentrating antigens for the presentation and manufacture of antibodies (Akkaya *et al*., 2020**)**. There are a number of transitional stages that must take place before a B lineage cell can mature into a mature B cell that expresses the B-cell receptor (BCR) on its membrane. B cells are steered toward negative and likely positive selection during this process, which results in the development of a mature B-cell repertoire (Wen *et al*., 2005**)** . According to where they first began to differentiate into B1 and B2 cells, B lymphocytes are split into two kinds. The first cells made during ontogenesis are B1- lymphocytes figure 2 (Herzenberg,2000). B2 cell precursors in the bone marrow give origin to B-cell populations of the marginal zone and the follicular zone. These B-cells are the main populations that respond to antigen contact then forming the germinal centers and therefore are long-term responders. Based on CD5 expression, B1 cells are further subdivided into B1-a and B1-b subsets; B1a-cells (CD5+) and B1-b cells (CD5) seem to share developmental ancestors. They are phenotypically identical, with only a few minor functional distinctions like the capacity to internalize bacterial pathogens, apart from the differential expression of CD5 (Cerutti *et al*., 2013**)** .

The primary role of B1-lymphocytes is the creation of significant amounts of IgM-isotype natural antibodies in response to encapsulated bacterial infections (among other bacterial challenges) and IgA-isotype natural antibodies in connection with mucosal defense against parasites (Scapigliati *et al*., 2018**)**. These cells can also spontaneously create IgG2 and IgG3 isotypes, and under some circumstances. They may also produce IgE. Although specific cytokines and T-cell independent antigens can trigger the production of antibodies by B1-lymphocytes, this process is characterized by being spontaneous (Dema and Charles, 2016).

B1-lymphocytes actively contribute to the bacterially induced immune response in addition to producing natural antibodies; various studies have examined B1 cell responses to pathogens including *Streptococcus pneumoniae*, *Salmonella* spp., *Francisella* spp., *Borrelia hermsii*, and influenza virus, among others .Each case's antibody response analysis revealed a rise in IgM generated by B1-cells in the spleen, local lymph nodes, or plasma (Castañeda-Sánchez *et al*., 2017).

B1 cell heterogeneity is supported by several investigations, although the underlying mechanisms are poorly understood (Dema and Charles, 2016). However, Baumgarth has considered three factors that can modulate the functions of B1 cells: (1) spontaneous IgM secretion; (2) efficient T cell stimulation; and, (3) chronic intracellular signaling. Some additional features that have been reported to distinguish B-1 cells in comparison to B-2 cells include larger overall size; enhanced survival ex vivo; resistance to Fas-mediated apoptosis; unique expression of gene transcripts and proteins; distinct signaling characteristics, including responsiveness to phorbol ester and increased intracellular Ca++; and, developmental independence from BAFF/BLys and IL-7 (Baumgarth , 2016).

B2 cell precursors in the bone marrow give origin to B-cell populations of the marginal zone and the follicular zone; these B cells are the main populations that respond to antigen contact then forming the germinal centers and therefore are long-term responders. These cell populations are the majority B lymphocytes in the host and are predominant in all lymphoid tissues. Marginal zone (MZ) B lymphocytes in the mouse are restricted to the splenic marginal

zone, while their human counterparts appear to be also in blood circulation (Castañeda-Sánchez et al., 2017).

The classical view of complement's role in the B2 cell response is as follows: B2 cell costimulation occurs as a result of ligation of B2 cell expressed CD21 [complement receptor 2 (CR2) which induces phosphorylation of closely associated CD19. C3dg is the ligand for CD21. It is generated from C3b that covalently associates with IgM Ab-antigen complexes (Ag-Ab) comprised of the BCR and the cognate Ag that triggers its activation. B2 cell activation that primes Ab production against most polypeptide antigens requires CD4+ cell help (Owen *et al.*, 2012).

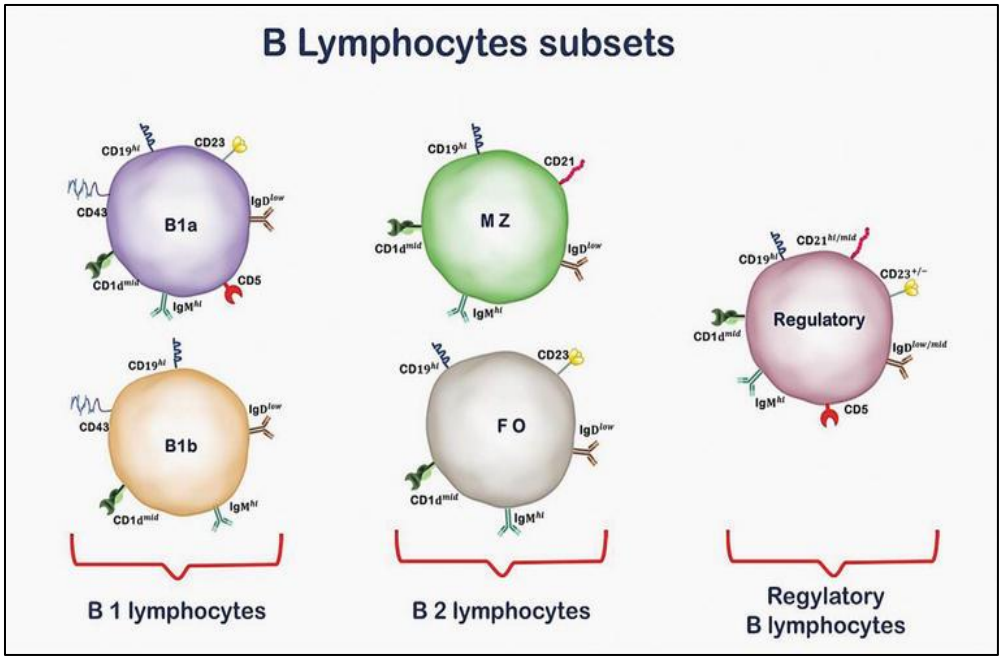

Figure 2.2: Immunophenotype of mature B-cells subpopulations. The B2-cell population constitutes the majority of spleen B cells formed by follicular cells (FZ) and marginal zone B cells (MZ). B1-a and B1-b cells are smaller populations in terms of frequency in the spleen; they can be distinguished based on CD5 expression: B1-a (CD5+) cells and B1-b (CD5−) cells. It appears that regulatory B cells have phenotypic markers of B1 and B2 cells. (adapted from Castañeda-Sánchez *et al.*, 2017).

2.2.2. Role of regulatory B cells (B-reg)

Regulatory B-cells are immunosuppressive cells that support immunological tolerance. Through the production of interleukin-10 (IL-10), IL-35, and transforming growth factor β (TGF-β), B-reg cells suppress immunopathology by prohibiting the expansion of pathogenic T cells and other pro-inflammatory lymphocytes (Rosser and Mauri, 2015).

2.2.3.The diversification of B-reg phenotypes

Different research teams have discovered B-cell populations with unique phenotypes that, when stimulated, demonstrate a higher level of regulatory competence. Normally, <1% of peripheral blood B-cells in humans are the IL-10-producing subgroup (Iwata *et al*.,2011). Peripheral blood B10-cells and B10PRO cells are highly enriched in the $CD24^{hi}$ CD27+ B cell subset, with approximately 60% also expressing CD38. Similar total numbers of IL-10+ B cells have been described in the $CD24^{hi}CD38^{hi}$ and $CD24^{int}CD38^{int}$ B cell subsets (Blair *et al*., 2010) . A separate study showed that B10 cells did not fall within any of the previously defined B cell subsets, but they were enriched in the CD27+ and the $CD38^{hi}$ compartments (Bouaziz *et al*,. 2010). The CD27+ B-cell subset can also expand during the course of autoimmunity and has been proposed as a marker for disease activity (Sanz *et al*., 2008).

The immunosuppressive function of regulatory B-cells has been shown in several murine models of chronic inflammation, including collagen-induced arthritis, inflammatory bowel disease, and experimental autoimmune encephalomyelitis (Wu *et al*., 2020). Despite interest in these cells, their relevance to the maintenance of peripheral tolerance in humans remains elusive (Blair *et al*,. 2010). The human $CD19+CD24^{hi}CD38^{hi}$ B-cells were shown possessed regulatory capacity ,after CD40+ stimulation,$CD19+CD24^{hi}CD38^{hi}$ B-cells suppressed the differentiation of T-helper 1 cells, partially via the provision of IL-10, but not transforming growth factor-β (TGF-β) (Li *et al*,. 2010), and their suppressive capacity was reversed by the addition of CD80+ and CD86+ mAbs (Ahmad *et al*,. 2015) .

In addition,$CD19+CD24^{hi}CD38^{hi}$ B-cells isolated from the peripheral blood of systemic lupus erythematosus (SLE) patients were refractory to further CD40+stimulation, produced

less IL-10, and lacked the suppressive capacity of their healthy counterparts. Altered cellular function within this compartment may impact effector immune responses in SLE and other autoimmune disorders (Blair *et al*., 2010).

2.2.3.Suppressive mechanisms of B-reg by soluble molecules

Recent years have witnessed a rise in interest in the potential of B-cells to release immunomodulatory substances. Although the production of anti-inflammatory cytokines is responsible for many B-reg functions, other soluble chemicals have recently been identified as mediating B-cell suppression (Catalán *et al*., 2021), such as granzyme B(GrB) (Hagn *et al*., 2009), adenosine (Kaku *et al*.,2014 ; Figueiro *et al*., 2016), IDO (indoleamine 2,3 dioxygenase is an enzyme that catalyzes the first step in the metabolism of tryptophan, which is needed for healthy functioning of CD8+ T-cells) (Nouel *et al*., 2015),progesterone-induced blocking factor1 (Huang *et al*,.2017),and heat shock protein-70 (Wang *et al*., 2021). Summary of the primary inhibitory strategies used by B-reg through the release of soluble substances in Figure 3.

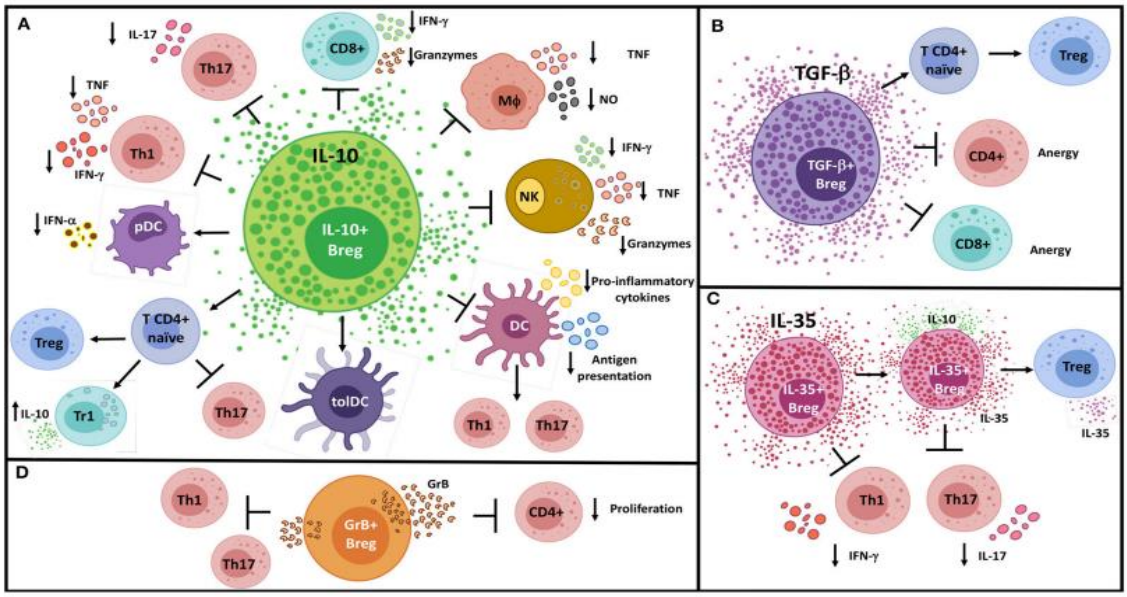

Figure 2.3: Suppressive mechanisms of B-reg by soluble molecules (adapted from Catalán *et al*., 2021).

2.2.4. Interleukin-10 (IL-10)

Interleukin-10, a cytokine with anti-inflammatory properties, has a central role in infection by limiting the immune response to pathogens and thereby preventing damage to the host. Recently, an increasing interest in how IL-10 expression is regulated in different immune cells has revealed some of the molecular mechanisms involved at the levels of signal transduction, epigenetics, transcription factor binding and gene activation (Althuwaiqeb and Bordoni, 2022).

Understanding the specific molecular events that regulate the production of IL-10 will help to answer the remaining questions that are important for the design of new strategies of immune intervention (Saraiva *et al*., 2010) .

2.2.5.The role of IL-10 during infection

During infection it inhibits the activity of Th1 cells, NK cells, and macrophages, all of which are required for optimal pathogen clearance but also contribute to tissue damage. The role of IL-10 can prevent pathogen clearance and improve immunopath . Many different types of cells can produce IL-10 , with the major source of IL-10 varying in different tissues or during acute or chronic stages of the same infection. The priming of these various IL-10-producing populations during infections is not well understood .It is not clear whether the cellular source of IL-10 during infection dictates its cellular target and thus its outcome (Couper *et al*., 2008).

2.2.6.The transforming growth factor (TGF-β)

The transforming growth factor family of growth factors controls an immense number of cellular responses and figures prominently in development and homeostasis of most human tissues. Work over the past decades has revealed significant insight into the TGF-β signal transduction network, such as activation of serine/threonine receptors through ligand binding, activation of SMAD proteins through phosphorylation, regulation of target genes expression in

association with DNA-binding partners and regulation of SMAD activity and degradation (Li and Flavell, 2008).

At different phases of their development, B-cells are regulated by TGF, and it plays a significant role in the regulation of autoimmunity and self-tolerance (Tamayo *et al*., 2018). Contrarily, data support the assertion that human B cells at rest express TGF-β and TGF-β receptors, the expression of which increases in response to stimulation. Numerous murine and human IL-10+ Breg populations have been extensively documented as having the ability to release TGF-β; nevertheless, in many instances, the importance of TGF-β suppression mediated by these cells has been disregarded (Huai *et al*., 2021). Nevertheless, in other cases, TGF-β has been described to exert a dominant part in B-reg functions. In particular, TGF-β has the capacity to convert naïve CD4+T-cells into T-reg. Consequently, it was not surprising to find out that TGF-β+B-reg are able to induce T-reg in healthy mice and humans, as well as in inflammatory conditions such as transplantation, allergy, and cancer (Tamayo *et al*., 2018).

2.2.7. Interleukin-35 (IL-35)

Interleukin-35, a potent anti-inflammatory cytokine, is the newest member of the IL-12 family of heterodimeric cytokines and is composed of the Ebi3 and the IL-12p35 chains. It has been shown that mouse and human T-reg produce substantial amounts of IL-35, which is a key component of their suppressive functions. Similar to this, it has been suggested that IL-35 plays a role in the induction of T-reg, and that these T-reg use IL-35 to mediate suppression (Collison *et al*., 2007).

In this context, Yu *et al*., (2018) have demonstrated that the addition of IL-35 to LPS-stimulated human or murine B-cells causes both an expansion of IL-10+ B-reg as well as the development of IL-35-producing B cells. Additionally, IL-35 injection causes IL-35+ B-reg to grow in vivo. This expansion is linked to an increase in T-reg and a decrease in Th1 and Th17 cells via the production of IL-10 and IL-35, which lessens the severity of experimental autoimmune uveitis but impairs protective immunity in a model of mycobacterial infection. In parallel, Shen *et al*.,(2014) described the ability of CD40 and TLR4 stimulation to induce IL-

35 production by murine B cells. They also reported that a population of CD138+ plasma cells were the main producers of IL-35 in EAE mice and mice infected with *Salmonella* (Chen *et al*., 2020) .

2.2.8. Macrophage migration inhibitory factor (MIF)

Cytokines are essential effectors molecules of innate immunity that initiate and coordinate the cellular and humoral responses aimed at the eradication of microbial pathogens (Calandra and Roger, 2003). Initially, it was believed that the immune system's primary cellular source of MIF was T-cells. However, it has been demonstrated that MIF is expressed by monocytes, macrophages, blood dendritic cells, B-cells, neutrophils, eosinophils, mast cells, and basophils (Lue *et al*., 2002; Baugh and Bucala, 2003).

MIF is rapidly released following exposure to microbial products and pro-inflammatory mediators, as well as in reaction to stress. It is constitutively expressed by a wide variety of cells and tissues, including monocytes and macrophages. (Sumaiya *et al*., 2022). After being released, MIF triggers biological processes that are pro-inflammatory and regulate immune responses. MIF reduces the activity of Jun activation domain-binding protein 1 (JAB1), a co-activator of activator protein 1 (AP1), and stimulates the extracellular signal-regulated kinase 1 (ERK1)/ERK2-mitogen-activated protein kinase pathway. -increases the expression of Toll-like receptor 4 to aid in the identification of bacteria that are pathogenic and express endotoxins, maintains pro-inflammatory activity by preventing macrophage p53-dependent apoptosis, and regulates the immunosuppressive effects of glucocorticoids on immune cells (Xu *et al*., 2013). MIF has been demonstrated to play a role in the pathogenesis of septic shock, severe sepsis, acute respiratory distress syndrome, and a number of other inflammatory and autoimmune illnesses, such as rheumatoid arthritis, glomerulonephritis, and inflammatory bowel diseases (Calandra and Roger, 2003).

2.3. Role of CD80 expression in the regulation of the activation of B cells

Immune cell functions are regulated by co-inhibitory and co-stimulatory receptors (*Mayes et al.,* 2018). Since the original proposal of a two-signal model for T-cell activation, there has been a continuous discovery of new costimulatory molecules on antigen-presenting cells (APCs) and their receptors on T-cells, but till date, CD80 and CD86 remain the best-defined costimulatory molecules (Podojil and Miller, 2009). The ability of APC to deliver the costimulatory signal to T-cells by CD80 and CD86 molecules is very well established (Cederbom *et al.*, 2000). Contrarily, there is very little information on whether the engagement of CD80 and CD86 molecules by CD28 and CTLA-4 also results in bidirectional costimulation that may impact how APCs function explain in figure 4 (Ovcinnikovs *et al*., 2019).

However, it has been shown shown that the costimulatory signals not only influence the activation of T-cells but can also affect the activity of APCs (Bcells) through reverse/bidirectional costimulation. This is because the concept of reverse costimulation or bidirectional costimulation (Halliday, 2019). Despite the complex roles and interactions within the CD28 and B7 costimulatory families, that's why he expected that novel approaches targeting these families will yield new therapies for the treatment of inflammation, autoimmunity, cancer, and infectious diseases (Podojil and Miller, 2009) .

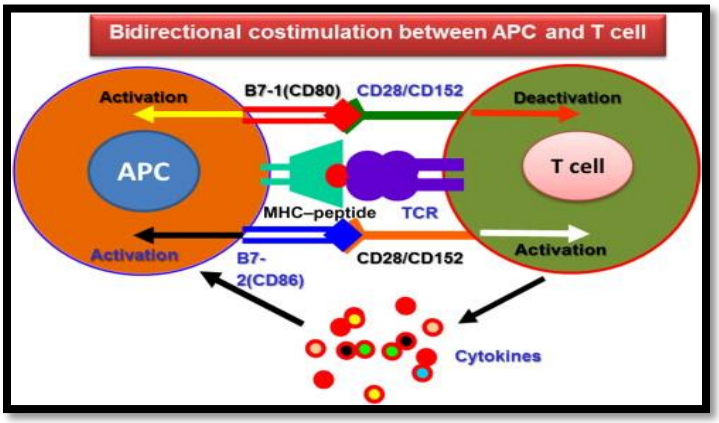

Figure 2.4: Innate and adaptive arms of immune responses against pathogens (adapted from Mir, 2015)

2.4.Urinary tract infections (UTIs)

A urinary tract infection (UTI) is an infection of any part of the urinary system, including kidneys (kidney infection),urethra (urethritis) and the bladder (cystitis). Urinary tract infections are one of the most common bacterial infections (Tullus and Shaikh, 2020). The clinical manifestations of UTI vary with age, stage of infection, host response, and type of bacteria causing the infection. Infants often present with nonspecific symptoms such as irritability, fever, lethargy, vomiting, and malnutrition (Chaurasia and Jain, 2019) .

As children and adults age, symptoms such as pain during urination and an increased incidence of lower urinary tract infections become more prominent gain (Paudel *et al*., 2022). However, the clinical phenotype of UTI is heterogeneous, ranging from fairly benign, uncomplicated infection to complex UTI (cUTI), pyelonephritis, and severe urosepsis (Tullus and Shaikh, 2020).

The etiology of community-acquired and hospital-acquired UTIs is different. Enteric bacteria, especially *E. coli*, continue to be the most common cause of UTIs, but there is evidence that the rate of UTIs caused by *E. coli* is declining (Paudel *et al*., 2022). The rates of urinary tract infections caused by *E. coli*, *Proteus* spp. and *Pseudomonas* spp. decreased, and those caused by yeasts, group B streptococci and *Klebsiella pneumoniae* increased. Variation in the causative agents of UTIs, with a decreasing proportion of UTIs caused by *Enterobacter* spp. but an increasing proportion of UTIs caused by *Acinetobacter* spp. and *Pseudomonas aeruginosa*. Candida albicans is the most common cause of mycoses, followed by *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei* . Some studies have shown that other types of bacteria are responsible for urinary tract infections (Chaurasia and Jain, 2019; Cunningham *et al*., 2014).

2.5. Lipopolysaccharide

Uropathogenic bacteria have evolved many virulence factors that facilitate colonization and infection of the urinary tract. The virulence factors most commonly associated with these organisms include the adhesin-tipped pilus, protectins, bacterial capsules containing lipopolysaccharide (LPS), and the production of toxins such as hemolysin and colony necrosis factor (Shah *et al*., 2019).

Lipopolysaccharide, also called endotoxin, is a major component of the outer membrane of gram-negative bacteria that activates TLRs (Vijay, 2018). LPS causes endotoxemia when released into blood. Release of high levels of LPS into the systemic circulation (or release of lower levels in case of LPS-sensitization), induces a systemic inflammatory response syndrome, also called SIRS (Abraham and Miao, 2015). The onset of SIRS exacerbates any other concurrent inflammatory illness, such as sepsis, and can lead to the emergence of septic shock (Shah *et al*., 2019) .

LPS is composed of three covalently linked domains, lipid A (endotoxin), a core region, and an O antigen polymer (Vijay,2018). LPS is the most potent stimulator of macrophage-derived cytokine secretion(Cardoso *et al*., 2015). This overstimulation can lead to septic shock. Septic shock is the leading cause of death in hospitalized patients. Macrophage-derived cytokines, particularly tumor necrosis alpha (TNFα) and interleukin-1 (IL-1), have been shown to be involved in septic shock . At the outermost bacterial membrane, the core region that confers endotoxic properties to LPS is a phosphorylated, non-repeating oligosaccharide that links lipid A to the hypervariable O-antigen polymer (Kucheria *et al*., 2005).

2.6. Lipopolysaccharide binding protein (LBP)

Lipopolysaccharide (LPS)-binding protein (LBP) is mainly produced in hepatocytes, functions as a secretory class I acute-phase protein and plays a pivotal role in the innate immune response. Initially, LPS binds to LBP forming the LPS-LBP complex, which subsequently binds to CD14 and to the MD-4/MD-2 complex, ultimately results in the activation of signal transduction pathways and the production of cytokines and other pro-inflammatory mediators (Meng *et al*., 2021). Circulating LBP has a concentration-dependent immunologic function. Subnormal levels of LBP protein enhance phagocytosis and clearance of LPS from blood. In contrast, at high concentrations, LBP protein attenuates the release of pro-inflammatory cytokines (Mohr *et al*., 2022). Additionally, LBP transfers LPS to very-low-density lipoproteins, low-density lipoproteins, high-density lipoproteins or chylomicrons, contributing to immune reaction against the infection . LBP also contributes to the immune response to gram-positive bacterial and fungal infection (Shah *et al*., 2019).

The physiological concentration of LBP in the serum of healthy human individuals is 5 to 10 μg/mL. In contrast, during sepsis, up to seven-fold higher levels were observed within 24h (Sakura *et al*., 2017). Increased LBP levels were also induced by a diet rich in fat and carbohydrates (Meng *et al*., 2021). Therefore, individuals suffering from obesity, diabetes, and related metabolic disorders also present with highly elevated LBP-levels , which was probably attributed to chronic low-grade inflammation induced by alterations of the intestinal flora together with an increased gut permeability to LPS (Ha *et al*., 2021).

Chapter Three

Materials and Methods

Chapter Three : Materials and Methods

3.1 Methodology of study

This is, a case - control study, which is also known as " case ─ referent study " is a type of observational study (Tenny *et al*., 2022). In this type of study, three groups, two for patients called a Cases and one for control were compared. First group, children who expect to be healthy (apparently healthy) that it is called " control subject group " Second group, a children patients who has a T1DM with bacterial infection, third group, a children patients who has a T1DM without bacterial infection. The practical side of the study was performed at the center of Imam AL- Hussein Medical city of Kerbala, Iraq. All samples were collected in the peroid between February 2022 till June, 2022. These samples were selected from the patients attending the Diabetic Consultation Unit, questionnaires were designed to obtain the information of control subject and cases group.

3.1.1 Control Subject and Cases Groups

The study was executed on 30 control subjects, 30 patients in first group and 30 in third group, age ranged between 5-16 years (All of them are male). The selection of achildren patients depends on a number of criteria that will be discussed in the following subsections.

3.1.2. Patients Criteria

All patients in this study diagnosed of T1D.M with bacterial infection and/or T1DM without bacterial infection. The medical history of each patient that was taken regarding, age, type of treatment, history of any other diseases, have any other bacterial infections Appendix 2.

3.1.3. Exclusion criteria

There were a number of patients' conditions considered as exclusion criteria of this study. The exclusion criteria were included one or more of the following conditions:

patient with anyother autoimmune disease, patient with chronic liver disease, patient with thyroid problem, all subjects ages up 15 years, patient with kidney failure .

3.1.4. Official and ethical approvals

The dissertation steps have been approved by the comprehensive examination committee referred to in paper official address (no.ع/6/3735- in 28 /10/2021).Appendix 3

As a mandatory step to take samples from patients, is to get this study approved by Ethical Committees, which include: committee of Imam AL- Hussein Diabetes and Endocrinology Medical Center (no.240 - in 14/2/2022), Kerbala Health Directorate /Holly Kerbala governorate –Iraq Appendix 4.

3.2 Materials

3.2.1.Instruments

All the instruments and tools which were used in the current study are shown below in Table 3.1.

Table 3.1: Instruments used in this study

Instruments and tools	Supplier
Autoclave	Labtech (Korea)
Centrifuge	HUMAN Humax4K - Germany
Chemistry analyzer	HROCHE COBAS Integra400 plus - Switzerland
Cobas c 111	Roche/Germany
Compound Light microscope	Olympus(Japan)
Cooling bag	Tinka-India
Deep Freezer	Aruide-China
Distillator	GFL (Germany)
EDTA Tube	Xinel - China
ELISA reader	HUMAN Humareader HS - Germany
ELISA washer and Incubator	HUMAN COMBI WASH - Germany

Flow cytometer	BD-USA
Gel tube	ALS-UK
loop	Writeg- Germany
Micropipette (1-100) μL	HUMAN Humapette - Germany
Multichannel pipette	HUMAN Humapette - Germany
Petri dish	Sun- China
Sensitive electronic balance	Sartorius -Germany
Spectrophotometer	Human Humalyzer 2000 - Germany
VITEK-2	Biomerieux -France
Vortex mixer	Bohemia / Germany

3.2.2. Kits

Kits with their suppliers which were used in this study are listed in table 3.2.

Table 3.2: Kits used in this study

Kit type	Company-Origin
CD19 PE-CY7 MAB	BD - USA
CD24 FITC MAB	
CD27 PE (LEU-27)	
IL-10 APC-R700 JES3-1	
CD80 APC-H7 MAB	
BD IntraSure kit (permeabilizing solution)	
Human LBP(Lipopolysaccharide Binding Protein)- ELISA Kit	Elabscience - Chine
TGF-β1(Transforming Growth Factor Beta 1) -ELISA Kit	
Human MIF(Macrophage Migration Inhibitory Factor) -ELISA Kit	
Human IL-35 - ELISA Kit	

Human Toll - like receptor 4 -ELISA Kit	
Human anti-GAD	MyBioSource - USA
Cobas Fasting blood glucose kit	Roche - Switzerland
Cobas C-peptide kit	Roche - Switzerland
Cobas HbA1c kit	
Cobas CHOL kit	
Cobas TG kit	
Cobas LDL-C kit	
Cobas HDL-C kit	
Cobas ALT kit	
Cobas AST kit	
Cobas ALP kit	Roche – Switzerland
Cobas TSB kit	
Cobas blood urea kit	
Cobas serum creatinine kit	
Gram-stain kit	HiMedia- India
VITEK-2 system	Biomerieux -France

3.2.3. Culture media

Culture media used in this study and their origins are given in Table 3.3.

Table 3.3 Culture media

Chemicals	Manufacturing - Origion
Blood agar	Oxoid - UK
MacConkey agar	

3.3. Methods

3.3.1. Study Design :-

Study design was illustrated in figure (3-1).

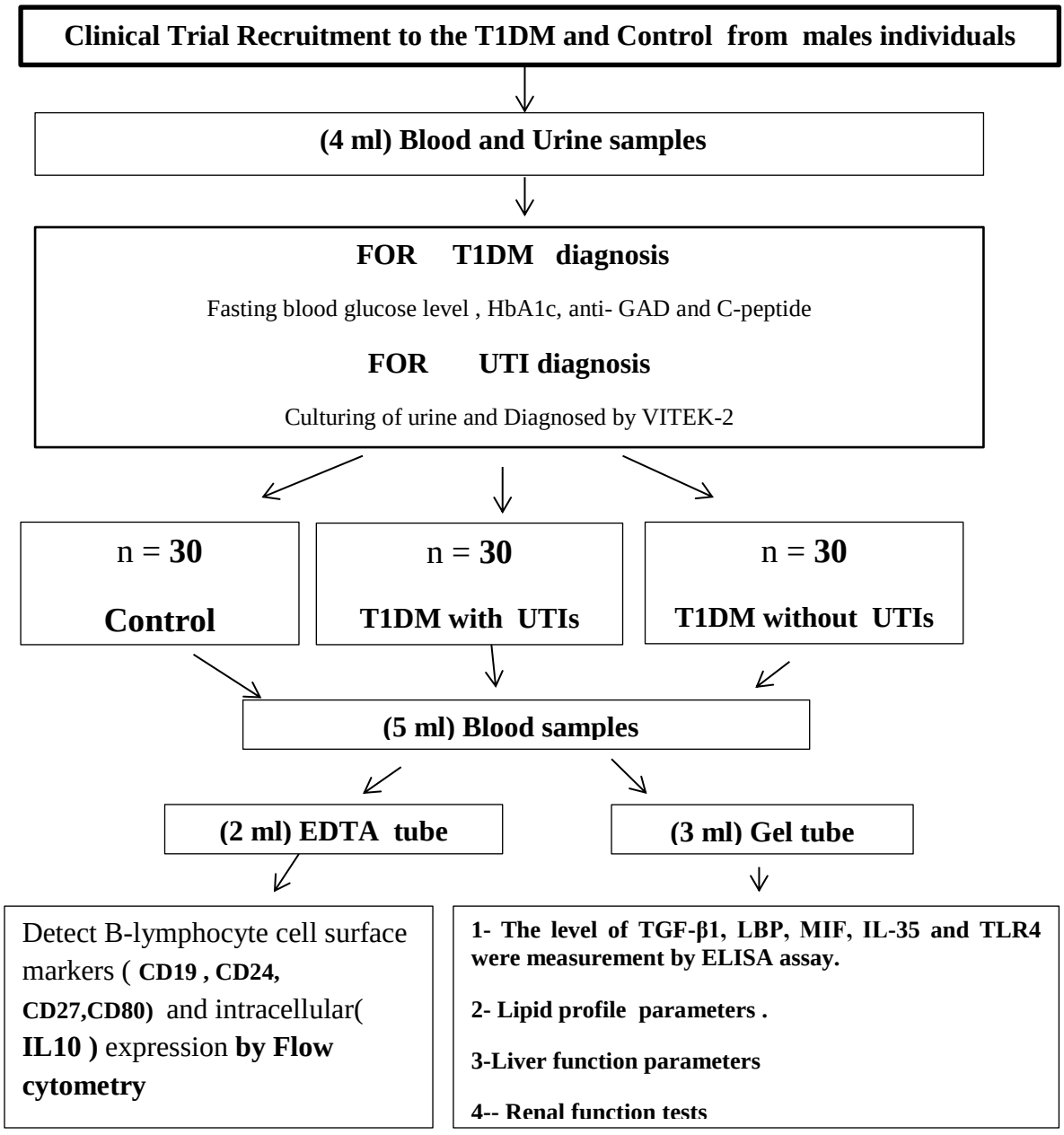

Figure 3.1: The study design.

3.3.2. Samples collection

3.3.2.1. Collected data using a questionnaire form

This study analyzed a number of clinical parameters. Some of parameters were collected from a number of blood tests, while others were collected from the case directly (either a patient or control subject). In the following subdivisions, these parameters were discussed. Furthermore, Appendix A includes the questionnaire form on which all collected data were registered for each case. Demonstrates the distribution of three groups for this study by age periods explain in table 3.4 and distribution of the diabetic patients by the duration of the disease explain in Table 3.5 .

Table 3.4. Distribution of all three groups (n=90) by the age.

Age periods (Year)	C		A		B	
	N.	%	N.	%	N.	%
5-10	13	43	8	27	8	27
11-16	17	57	22	73	22	73

C: Control group

A D1TM patients with bacterial infections group .

B: D1TM patients without bacterial infections group.

N. : Number

Table 3.5. Distribution of D1TM patients (n=60) by the duration of the disease.

Duration of diabetes (Year)	A		B	
	N.	%	N.	%
1-5	17	57	23	77
6-10	13	43	7	23

A: D1TM patients with bacterial infections group.

B: D1TM patients without bacterial infections group .

N.: number.

3.3.2.2. Blood Collection and processing

Blood samples were collected from each case after being fast for 8 -12 hours. The collected blood volume was 8 ml, which withdrawn by disposable syringes in the sitting position. The collected blood stored in two types of tubes: one is EDTA tube for Flow , CBC , HbA1c , and the other one is gel tube (contain a special gel that separates blood cells from serum to cause blood to clot quickly). The samples were collected between 8:30 am – 1:30 pm. Blood was allowed to clot at 37°C for 15-20 minutes and then centrifuged at 3000xg for approximately 12 -15 minutes and the separated serum were stored in deep freezer -80°C. The collected serum from patients and control subjects were used for the measurements of the following parameters (Human Toll-like receptor 4 , Human LBP (LPS Binding Protein) , Macrophage migration inhibitory factor (MIF) , IL 35 , TGF beta and anti- GAD).

3.3.3. Urine samples collection and storing

Ten ml urine samples were collected from all patient and control groups . Patients with bacterial infections were separated according to result of inoculated the sample on the culture media.

3.4. Measurements

The measurements in the current study were divided into four sections:-

1-Measurement of the immunological markers by Flow cytometry.

2-Measurement of the immunological parameters by Enzyme-Linked Immunosorbent Assay (ELISA)

3-Measurement of some physiological parameter concentration by electrochemiluminescence (ECL) immunoassay.

4- Cultivation of urine samples to identify bacteria causing a urinary tract infection (UTI).

3.4.1. Measurement of the immunological markers by Flow cytometry

3.4.1.1. Identification of B reg and CD80

Percentage of B reg and the expression of co-stimulatory molecule CD80 on B reg were identified by flow cytometry. The following monoclonal antibodies were used : anti CD19 PE-CY7 MAB (HIB19) , anti-IL-10 /APC-R700 (cloneJES3-19F1) , anti-CD24/FITC (clone ML5), CD27 PE (clone LEU-27) and anti-CD80/APC-H7 (clone L307). Test was conducted at Imam Zain El-Abidine Hospital .

3.4.1.2. Blood sampling and flow cytometey staining procedures in human

To detect B-lymphocyte cells surface markers and intracellular IL10+ expression by flow cytometry (Cognasse *et al.*,2008 ; Rico *et al.*,2021). The staining was performed in a darkened room and according to following steps:

https://www.bdbiosciences.com/en-us/resources/protocols/immunofluorescence.

1- For each sample,100 µL of whole blood was transferred into 2 mL polypropylene collection tubes (Biozym Scientific GmbH).

2- Five µl of anti- (CD19,CD24,CD27 and CD80) monoclonal antibody (WT.5, phycoerythrin) was added to the tube prepared in the first step.

3- The tube containing blood with anti- monoclonal antibody was mixed by Vortex for 3 sec and incubated for 20 minutes on 4^{o}C in the dark .

Note: For IL-10 intracellular detection in B-reg , it is necessary to block cytokines' secretion by inhibiting protein transport inside the cell. Therefore, we added BD IntraSure™ kit (intra A and intra B) , containing ready-to-use reagents for fixation and permeabilization that enable optimal staining of intracellular markers while maintaining cell surface staining.

4- The tubes was removed from dark chamber and mix each tube well. 100 µl of intra A was added to each tube and incubate for 5 minutes.

5- Two ml of lysing solution was added to each tube. Each tube was vortexed and incubated them in the dark chamber for 10 minutes.

6- The solution was sedimentation by centrifugation for 5 minutes at 1000 rpm (200 x g) and crefully rmoved supernatant by aspiration.

7- Fifty µl intra B and 20 µl of Anti- IL-10 was added and gentle vortex for 3-5 seconds and incubate for 20 minutes on 4^{o}C in the dark chamber.

8- Afterward,cell solutions were twice washed by added 2 ml of washing buffer to each tube .

9- Resuspened by 500 µl was added of wash buffer to each tube, vortex, and analyze.

10- The stained cells were analyzed within 24 hours (The only 50’000 cells gated events were acquired for each compensation control) in a BD Fortessa System. Samples were anlyzed using the gating strategy as shown in Figure3.1 and 3.2.

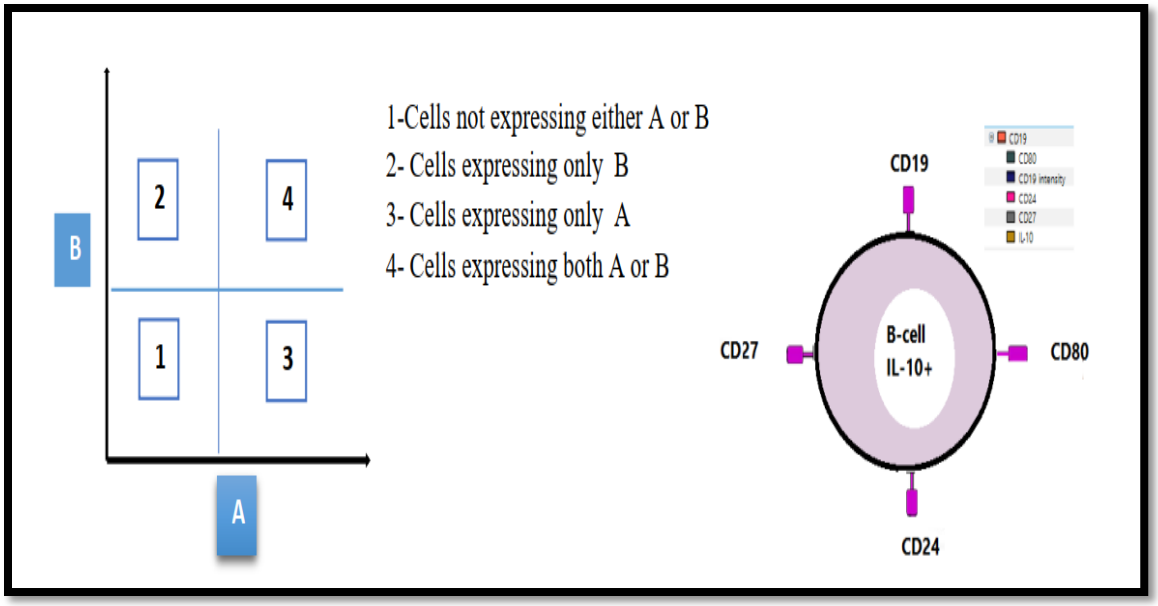

Figure 3.2: The approach used to analyze the results of flow cytometry and the study's target cell.

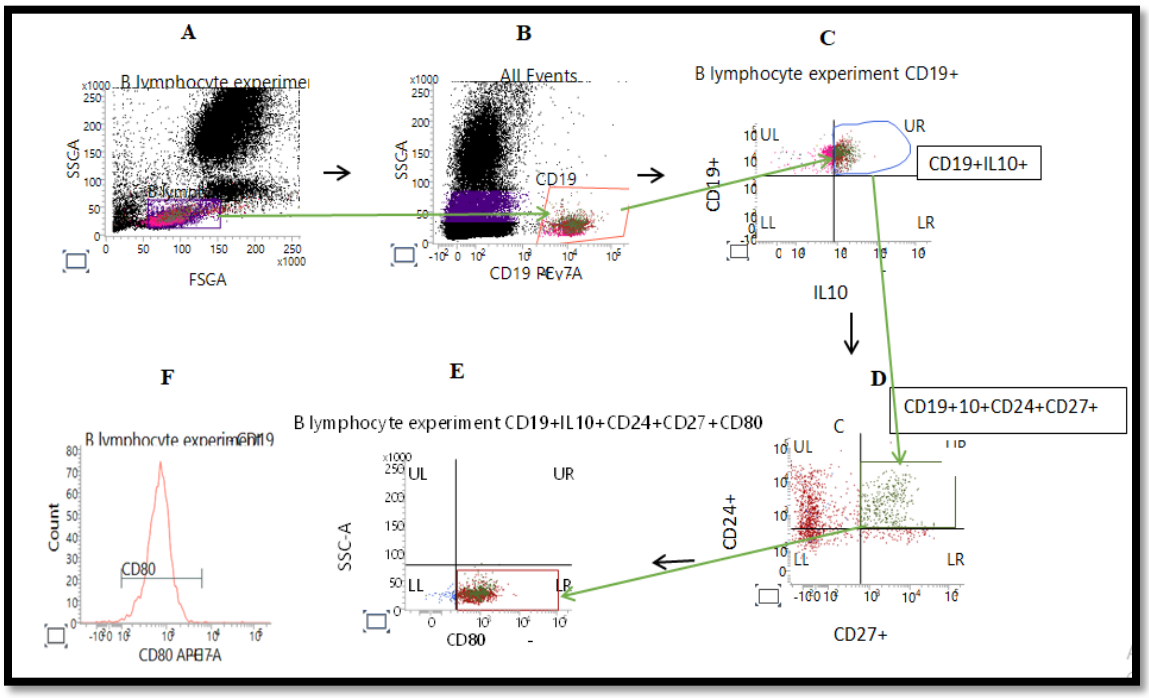

Figure 3.3: Gating strategy for CD80+ quantification in B-reg . (A) Within singlets of the examined PBMC population, every lymphocyte was identified based on its FSC and SSC characteristics (B) CD19+B-cells were gated (C) IL-10-producing B-cells were gated (D) CD19+IL10+CD24+CD27+B-reg were calculated (E) CD19+IL10+CD24+CD27+ CD80+B-reg were calculated (F) Mean fluorescence intensity (MFI) of CD19+IL10+CD24+CD27+CD80+B-reg.

3.4.2. Measurement of the immunological parameters by Enzyme-Linked Immunosorbent Assay (ELISA)

Measurements performed in main places, namely: Dr.Mohammed Salih laboratory and Imam AL- Hussein Diabetes and Endocrinology Medical Center ,Holy Kerbala-Iraq. ELISA test was conducted in the period from February to August 2022 . The following subsections discusses briefly the procedures of different measurements. The level of human TGF-β1, LBP, MIF, IL-35 ,TLR4 and GAD/IA2 combined autoantibody were measured using ELISA assay .

3.4.2.1. Principle of kit of (TGF-β1, LBP , MIF, IL-35 and TLR4) serum concentration

ELISA kits were use the Sandwich-ELISA principle . The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to human immunological parameters. Samples (or standards) were added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human Immunological Parameters and Avidin-Horseradish Peroxidase (HRP) conjugate were added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human Immunological Parameters , biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. concentration of parameters in the samples was calculated by comparing the OD of the samples to the standard curve.

3.4.2.2. Procedures

Measurements of studied were performed using specific kit following the manufacture's protocol by following steps which mentioned in the instructions attached to each kit, described in the links included.

Table 3.6: Catalogue link of immunological parameters kits used in this study.

No.	kits	Catalogue link
1	Human LBP (Lipopolysaccharide Binding Protein) ELISA Kit	https://www.elabscience.com/p-human_lbp_lipopolysaccharide_binding_protein_elisa_kit-356233.html
2	TGF-β1(Transforming Growth Factor Beta 1) ELISA Kit	https://www.elabscience.com/search-keywords=tgf-%CE%B21(transforming%20growth%20factor%20beta%201)%20elisa%20kit.html
3	Human MIF(Macrophage Migration Inhibitory Factor) ELISA Kit	https://www.elabscience.com/search-keywords=human%20mif(macrophage%20migration%20inhibitory%20factor)%20elisa%20kit.html
4	Human IL-35 ELISA Kit	https://www.elabscience.com/p-human_il_35_interleukin_35_elisa_kit-19828.html
5	Human Toll - like receptor 4 ELISA Kit	https://www.elabscience.com/p-human_tlr4_toll_like_receptor_4_elisa_kit-356245.html
6	Human anti-GAD	https://www.mybiosource.com/human-elisa-kits/gad-ia2-combined-autoantibody/2602893#QLAPP_MBS2602893_SC

3.4.2.3. Preparation of standard calculation

The concentrations were calculated using a standard curve fitting equations for human immunological Figure 3.4., 3.5., 3.6. ,3.7,3.8 and 3.9.

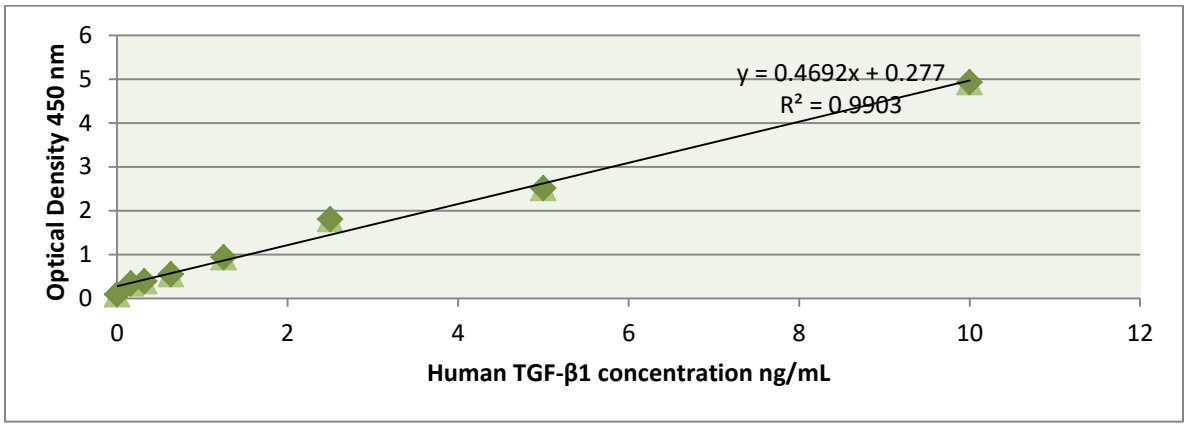

Figure 3.4: The standard curve of TGF-β1

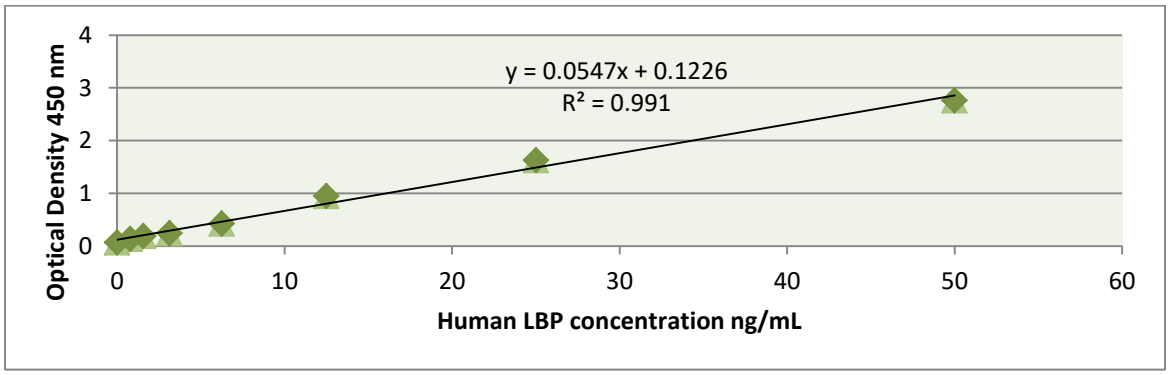

Figure 3.5: The standard curve of LBP

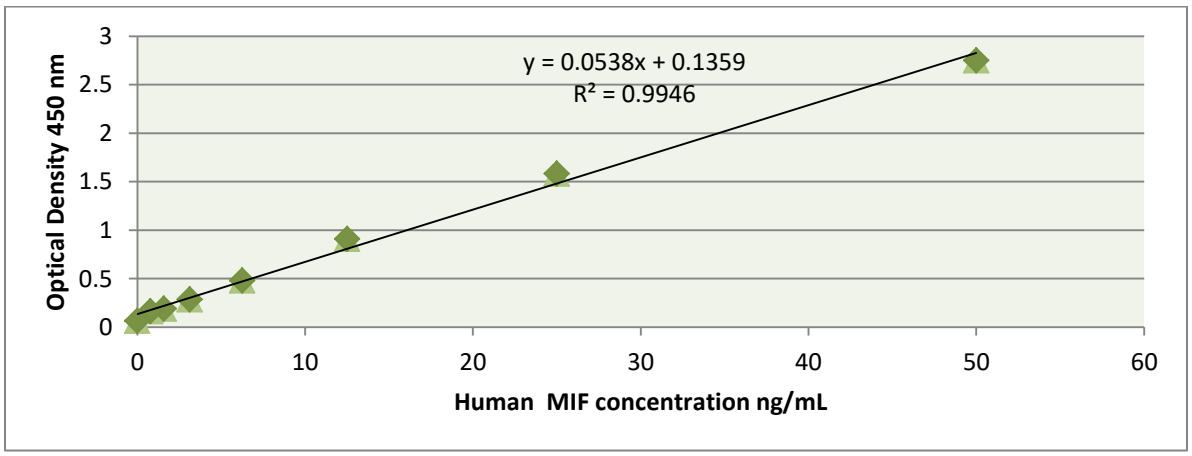

Figure 3.6: The standard curve of MIF.

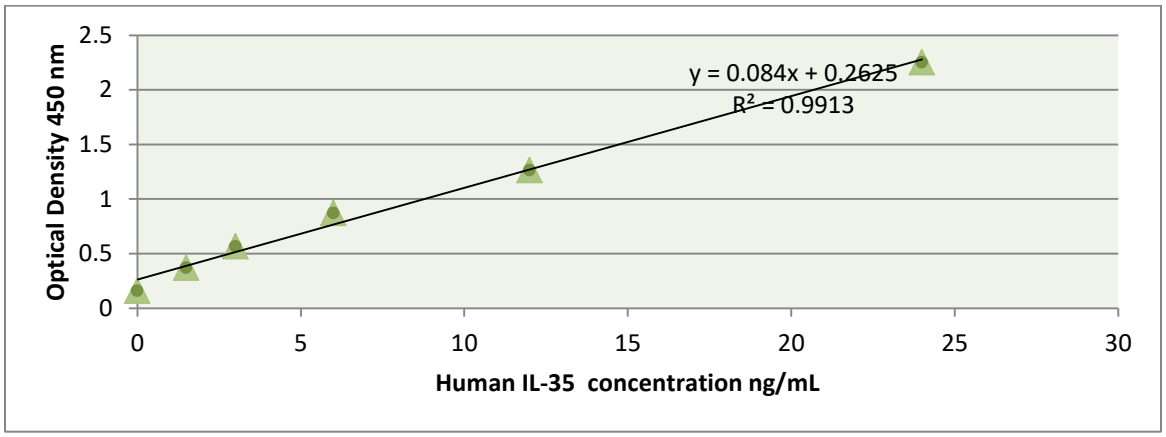

Figure3.7: The standard curve of IL-35

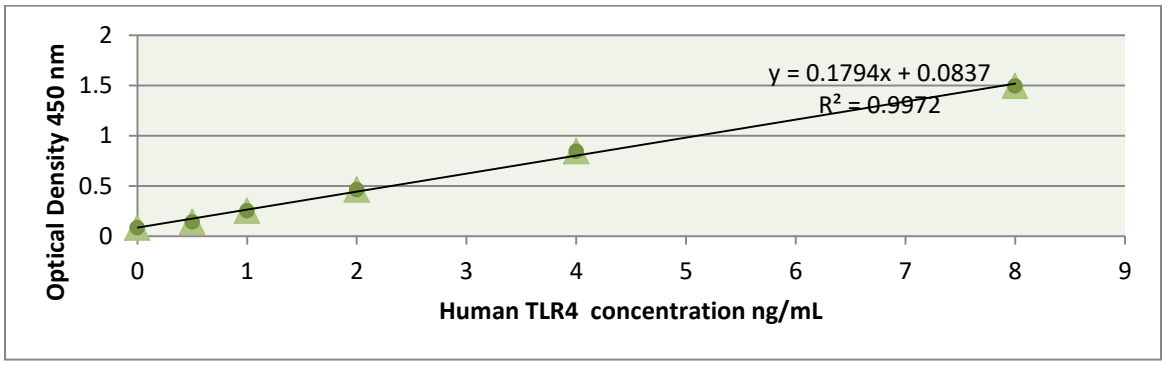

Figure3.8: The standard curve of TLR4

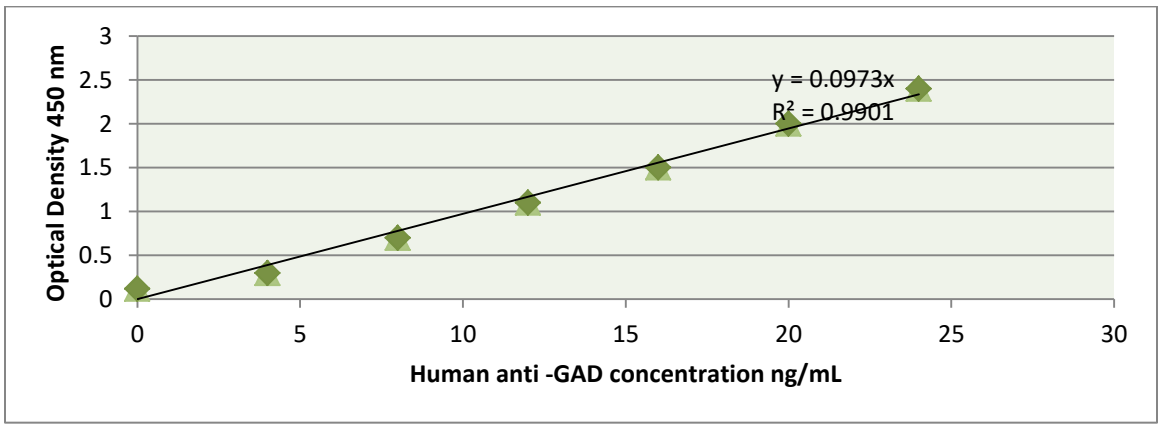

Figure3.9: The standard curve of Human anti -GAD

3.5. Measurement of some physiological parametes concentration by electrochemiluminescence (ECL) immunoassay

The cobas- e - 411 analyzer is a fully automated analyzer electrochemiluminescence (ECL) technology for immunoassay analysis. It is designed for quantitative and qualitative in vitro test measurements for a wide range of applications (Syme *et al*., 2011; Sasano *et al*., 2017; Dahman *et al*., 2022). This technique was used in the measurement the following parameters (1-5 in serum but 6 in blood):

1-Fasting blood glucose level.

2- C-peptide.

3- Lipid profile parameters [Total cholesterol(CHOL), Triglyceride (TG), Total to high-density lipoprotein cholesterol(TC/HDL-C) , Low-density lipoprotein cholesterol(LDL-C), High-density lipoprotein cholesterol(HDL-C), Non-high-density lipoprotein cholesterol (Non-HDL-C)].

4-Liver function parameters Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP) , and total serum bilirubin (TSB).

5- Renal function tests [blood urea and serum creatinine].

6- Hemoglobin A1C (HbA1c) Test.

3.5.1. Principle of assay

Specific antigen is mixed together with an antigen-specific biotinylated monoclonal antibody and an antigen-specific monoclonal antibody labeled with tris (2,2′-bipyridine) ruthenium (II) (Ru2+ in red) to form a sandwich complex which bind to streptavidin-coated. The reaction mechanism is shown in Figure 3.9.

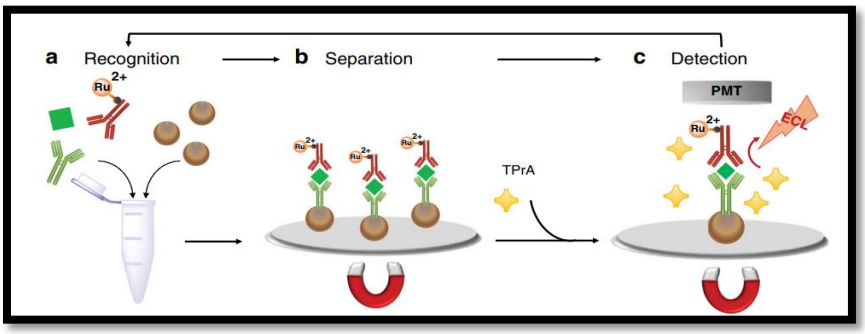

Figure 3.10 : Schematic representation of the commercial electrochemiluminescence (ECL) immunoassay (sandwich assay).

3.5.2. General working methods

1-In the first step, the patient sample was combined in an Assay Cup with a reagent containing biotinylated parameter antibody and a ruthenium-labeled parameter –specific antibody in an assay cup. During a 9-minute incubation step, antibodies capture of any markers are present in the sample.

2-In the second step, streptavidin- was coated paramagnetic microbeads were added. During a second 9-minute incubation, the biotinylated antibody attaches to the streptavidin-coated surface of the microbeads.

3-After the second incubation, the reaction was mixtured containing the immune complexes is transported into the measuring cell; the immune complexes are magnetically entrapped on the working electrode.The unbound reagent and sample are washed away by ProCell.

4-In the ECL reaction was conjugated a ruthenium-based derivative and the chemiluminescent reaction is electrically stimulated to produce light. The amount of light produced is directly proportional to the amount of parameter in the sample.

5-The concentration of the antigen or analyte was evaluated and calculated by means of a calibration curve using standards of known antigen concentration.

Table 3.7: Catalogue link of physiological parameter kits used in this study.

No.	Test	Catalogue link
1	Fasting blood glucose level	https://e-katalog.lkpp.go.id/jcommon.blob.filedownloader/download? id =f94a3815ea57cc6cd630b6a4930033b4d507a74610fb5e2b4695871bbc949bdbb9bf403283df87df437df9de8d9ed513bfb239967825064fbd4bb19c1182711afdb2133091462aa0a171fcee2bef9467686035380e3545e7243fca0c85c08677
2	C-peptide	https://labogids.sintmaria.be/sites/default/files/files/c-peptide_2018-08_v10.pdf
3	HbA1c	https://www.rochecanada.com/content/dam/rochexx/roche-ca/products/docs/package_inserts/06378676190CobasHbA1c-CanEnVers3-a.pdf
4	CHOL	https://www.rochecanada.com/content/dam/rochexx/roche-ca/products/docs/package_inserts/06380115190CobasLipidPanel-CanEnVers4.pdf
5	TG	https://repository.sustech.edu/bitstream/handle/123456789/12435/TRIG.pdf?sequence=6&isAllowed=y
6	LDL-C	https://labogids.sintmaria.be/sites/default/files/files/ldlc3_2017-06_v3.pdf
7	HDL-C	https://labogids.sintmaria.be/sites/default/files/files/hdlc4_2017-07_v2.pdf
8	ALT	https://labogids.sintmaria.be/sites/default/files/files/altl_2017-01_v12.pdf
9	AST	https://labogids.sintmaria.be/sites/default/files/astl_2018-09_v14.pdf
10	ALP	https://labogids.sintmaria.be/sites/default/files/files/alp2_2017-08_v11.pdf
11	TSB	https://labogids.sintmaria.be/sites/default/files/files/bilt3_2017-08_v7.pdf
12	blood urea	https://labogids.sintmaria.be/sites/default/files/files/ureal_2020-01_v13_0.pdf
13	serum creatinine	https://labogids.sintmaria.be/sites/default/files/files/crej2_2018-12_v19.pdf

3.6. Cultivation of urine samples to identify bacteria causing the urinary tract infection (UTI):

All urine samples directly after receiving from the patients were inoculated on MacConkey agar and Blood agar base aerobically at 37°C for 24 hours. Bacterial colonies appeared on the plates after the incubation period were enumerated to determine urine samples with significant bacteriuria. Plates with growth of ≥100 colonies (10^5 cfu/ml) were considered positive bacteriuria and involved in patient group, while ≤ 100 colonies were excluded (Ness and Olsburg, 2019).

3.6.1. Preparation of media

MacConkey agar and Blood agar base agar were prepared according to manufacture information. All media were sterilized by autoclave at 121°C and pressure 15 pound/Inch for 15 min (Paul, 2019).

3.6.2. Diagnosis of bacterial isolates traditionally

The bacterial isolates were diagnosed according to cultural and morphological properties tests by observing the shape and color of the colonies on the selective media. The isolates were also stained by Gram stain to identify Gram positive or Gram negative bacteria (Nimer *et al*., 2016).

3.6.3. Diagnosis of bacterial isolates by VITEK-2 apparatus system

All bacterial isolates present in diabetic patients with urinary tract infections were diagnosed by VITEK-2 .The bacterial suspension was adjusted to McFarland standard of 0.5 in 2.5ml of a 0.45% sodium chloride solution with a VITEK-2 instrument (bioMérieux, France). The time between preparation of the inoculum and the card filling was always less than 30 min , the identification card is a fully closed system to which no reagents have to be added. The card was put on the cassette designed for use with the VITEK-2 system, placed in the instrument, automatically fill in a vacuum chamber, sealed, incubated at 37^{o}C, and automatically subjected to colorimetric measurement (with a new reading head) every 15 min for a maximum incubation period of 8 hours.

Data were analyzed using VITEK-2 database, which allows organism identification in a kinetic mode beginning 180 min after the start of incubation (Nimer *et al*., 2016).

3.7. Statistical Analysis

The statistical analysis of the present study was carried out using the software statistical package for social sciences (SPSS) version 22, where data were expressed as the Mean ± standard error of the mean, independent-sample T-test with their 95% confidence interval (CI), was used to find the association between the categorical variables, $P \leq 0.05$ was considered statistically significant.The significance value was indicated as * between the groups . The level of probability was indicated as *$P \leq 0.05$, **$P \leq 0.01$ *** $P \leq 0.001$ and **** $P \leq 0.0001$.

Chapter Four

Results

Chapter Four : Results

4.1. Patient's samples

The current study included the collection of 60 Iraqi children males with type 1 diabetes (30 with bacterial infections and 30 without bacterial infections) and 30 healthy match as control which were distributed according to the several parameters.

4.2. Routine tests for the diagnosis of the disease

4.2.1. Fasting blood glucose serum level (BGL)

The study revealed that a significant rising ($P \leq 0.0001$) in the serum levels of BGL in diabetic patients with bacterial infections and diabetic patients without bacterial infections compared to the control group. The mean was 250.00 ± 19.76 mg/dl for diabetic patients with bacterial infections, 245.93 ± 25.98 mg/dl for diabetic patients without bacterial infections and 92.30 ± 0.66 mg/dl for control (Figure 4.1).

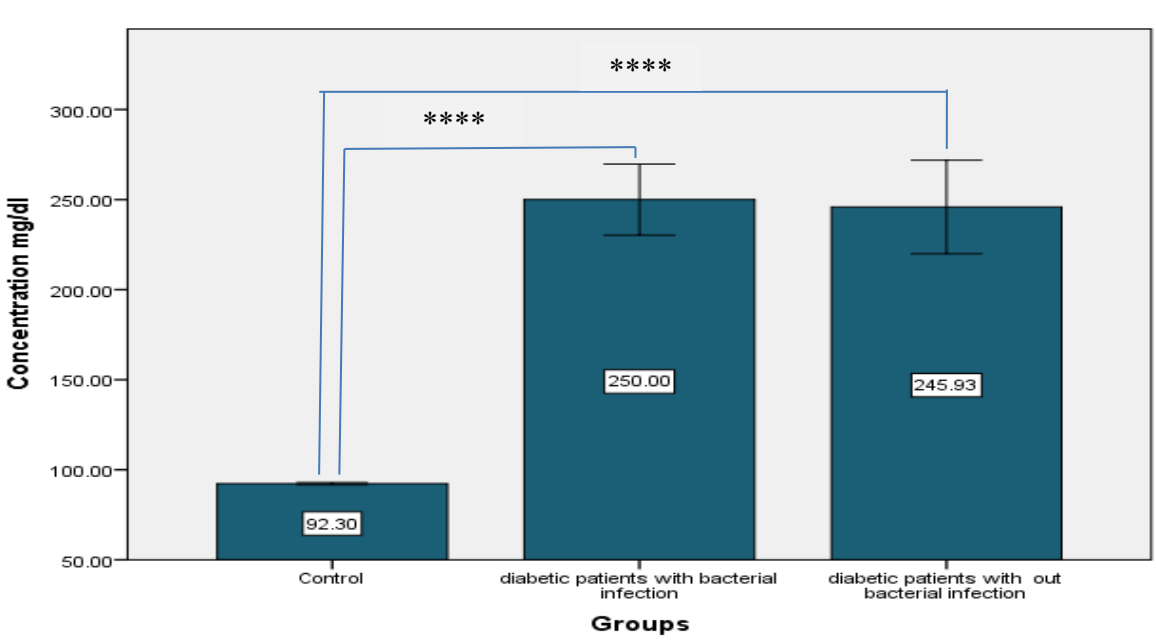

Figure 4.1: Concentration of BGL in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections, the significance value was indicated as * between the groups. The level of probability was indicated as **** $P \leq 0.0001$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.2.2. Hemoglobin A1C (HbA1c) Test

According to the presented data which explain by Figure 4.2, showed that a significant rising $P \leq 0.0001$ in the blood mean levels of HbA1c (%) increased for both patients groups of T1DM. The mean was 10.29 ± 0.42 for diabetic patients with bacterial infections group, 10.43 ± 0.40 for diabetic patients without bacterial infections group and 4.94 ± 0.06 for the control group.

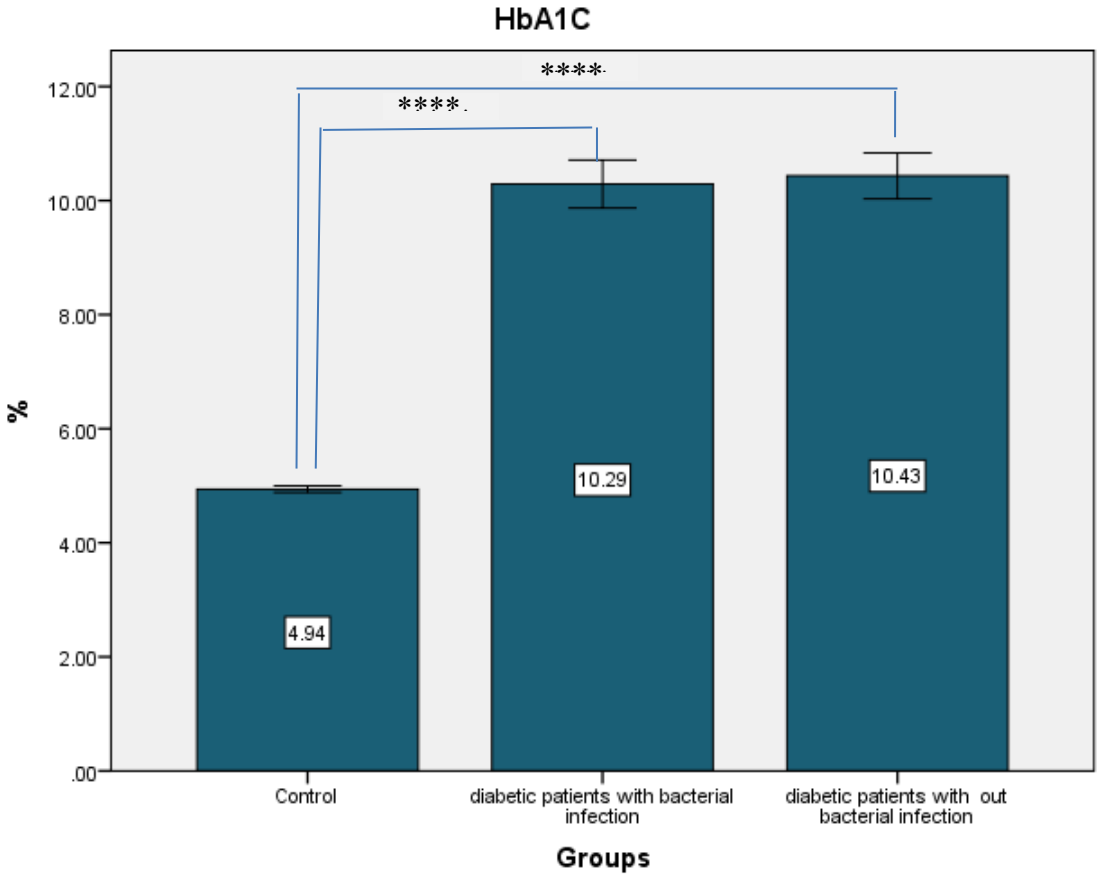

Figure 4.2. The percentage of HbA1C in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups . The level of probability was indicated as **** $P \leq 0.0001$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.2.3. Human glutamic acid decarboxylase antibodies (Anti-GAD)

Similarly to the levels of serum glucose and blood HbA1c, the mean levels of serum anti-GAD was significantly higher in both patients groups compared to the control group (Figure 4.3).

Anti-GAD were significant high $P \leq 0.0001$ for D1TM without bacterial infections group and D1TM with bacterial infections group which reached to 364.49 ± 122.72 U/ml and 226.94 ±109.93 U/ml, respectively compared to the control group.

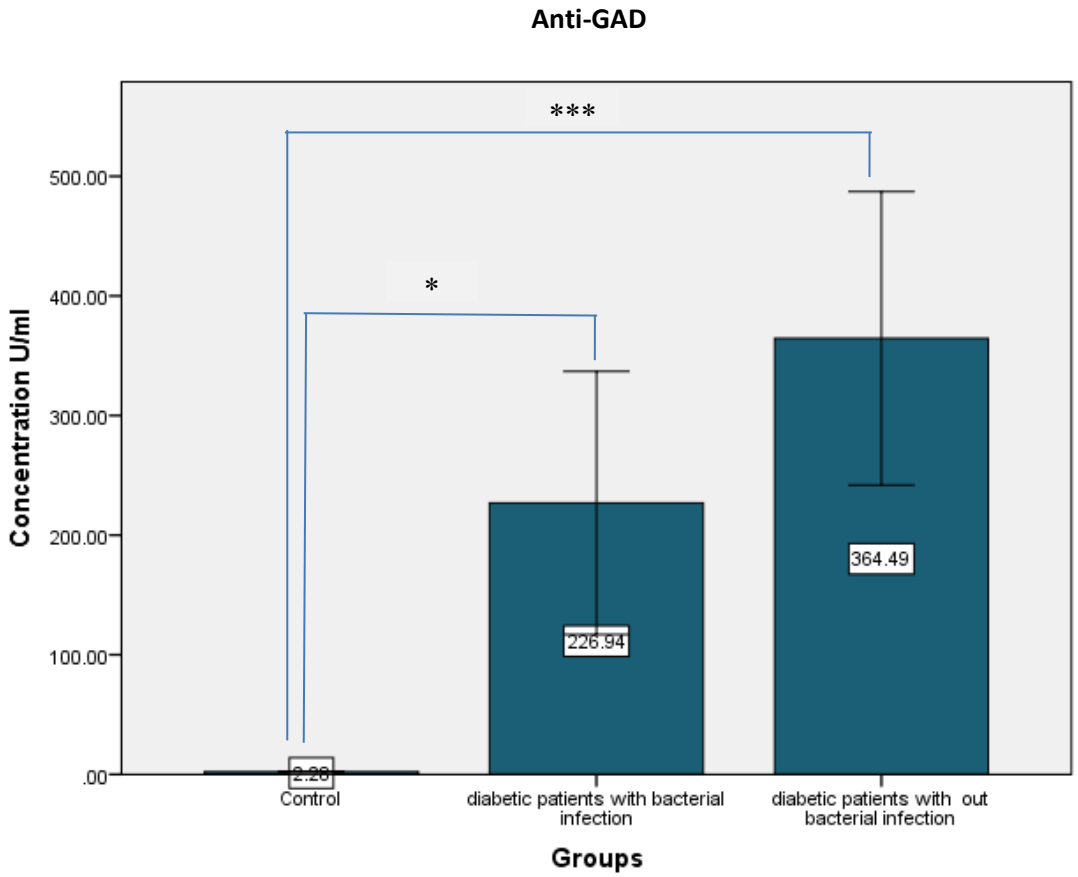

Figure 4.3: Concentration of A Anti-GAD/IA2 in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups . The level of probability was indicated as *$P \leq 0.05$ and *** $P \leq 0.001$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.2.4. The connecting peptide (C-peptide) serum level

As shown in Figure 4.4, the results revealed that a significant decrease in C-peptide level for both patients groups of T1DM compared to the control group was showed at the level $P \leq 0.001$. The mean was 0.225 ± 0.12 ng/ml for diabetic patients with bacterial infections group, 0.176 ± 0.07 ng/ml for diabetic patients without bacterial infections group compared with 2.458 ± 0.16 ng/ml for the control group.

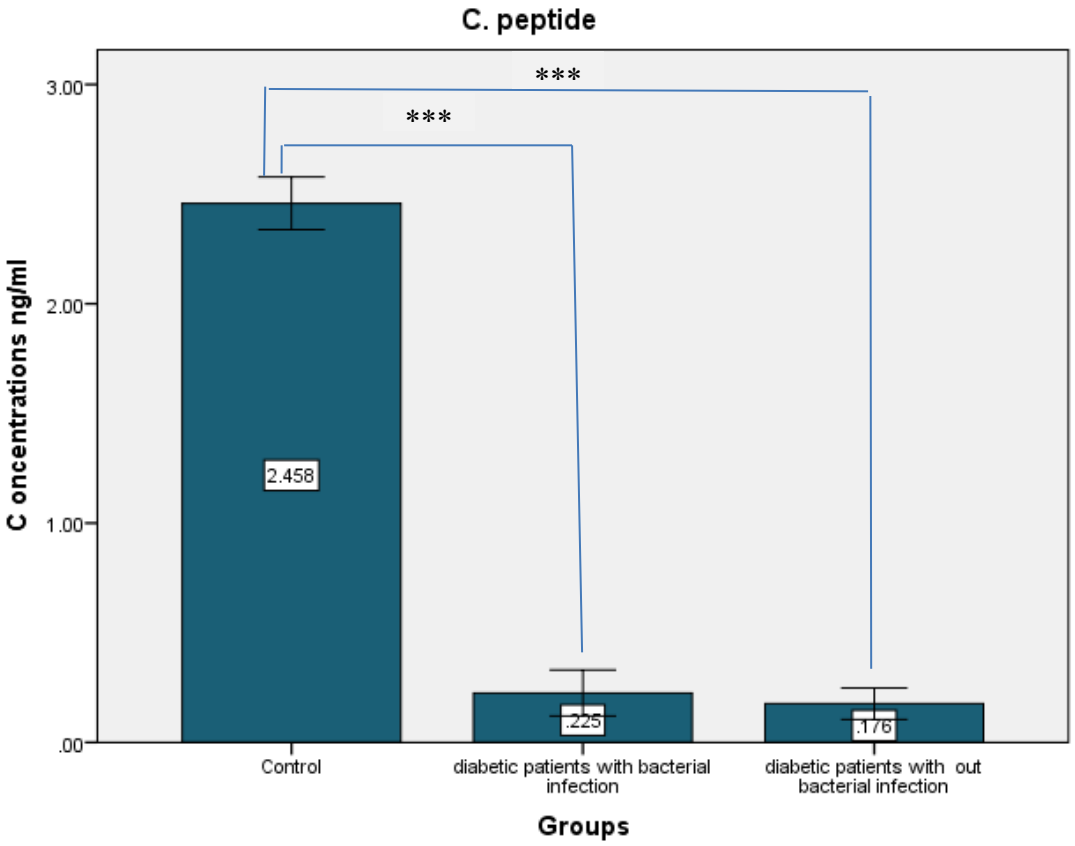

Figure 4.4 : Concentration of C.peptide in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups. The level of probability was indicated as *** $P \leq 0.001$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.3. Flow cytometry analysis of B regulatory B cells

4.3.1. CD19+B cells in human peripheral blood

The results of the current study showed that the percentage of CD19+B cells, for both T1DM groups were significantly lower compared to the control group on level $P \leq 0.0001$ (Figure 4.5). The percentage of CD19+B cells decreased in the group of T1DM with bacterial infection, as it reached the mean of percentage was 3.077 ± 0.26 for compared to D1TM without bacterial infections group 3.428 ± 0.46, if bacterial infections led to a decrease in the expression rate of regulated B cells.

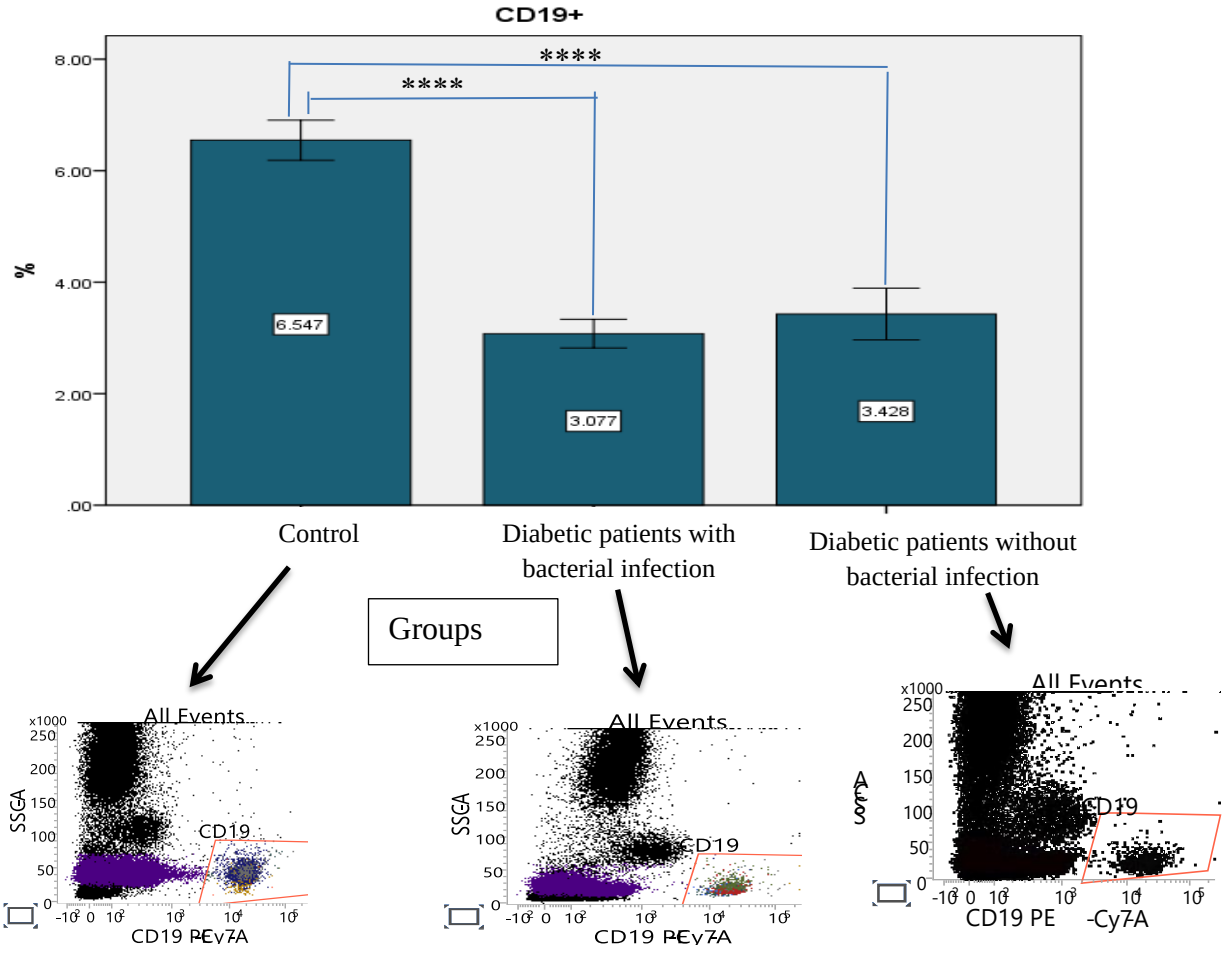

Figure 4.5: The percentage of CD19+ cells in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups . The level of probability was indicated as **** $P \leq 0.0001$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.3.2. Expression of B-reg cellsin B-lymphocyt

According to the presented data which explain by Figure 4.6, a significant decreased for percentage to CD19+IL10+ B-cells (B-reg) for T1DM with bacterial infection group compared to control group on level ($P \leq 0.05$). B-reg increased in the group of T1DM without bacterial infection, as it reached the mean of percentage was 33.46 ± 5.26 for compared to infected patients, if bacterial infections led to a decrease in the expression rate of regulated B cells.

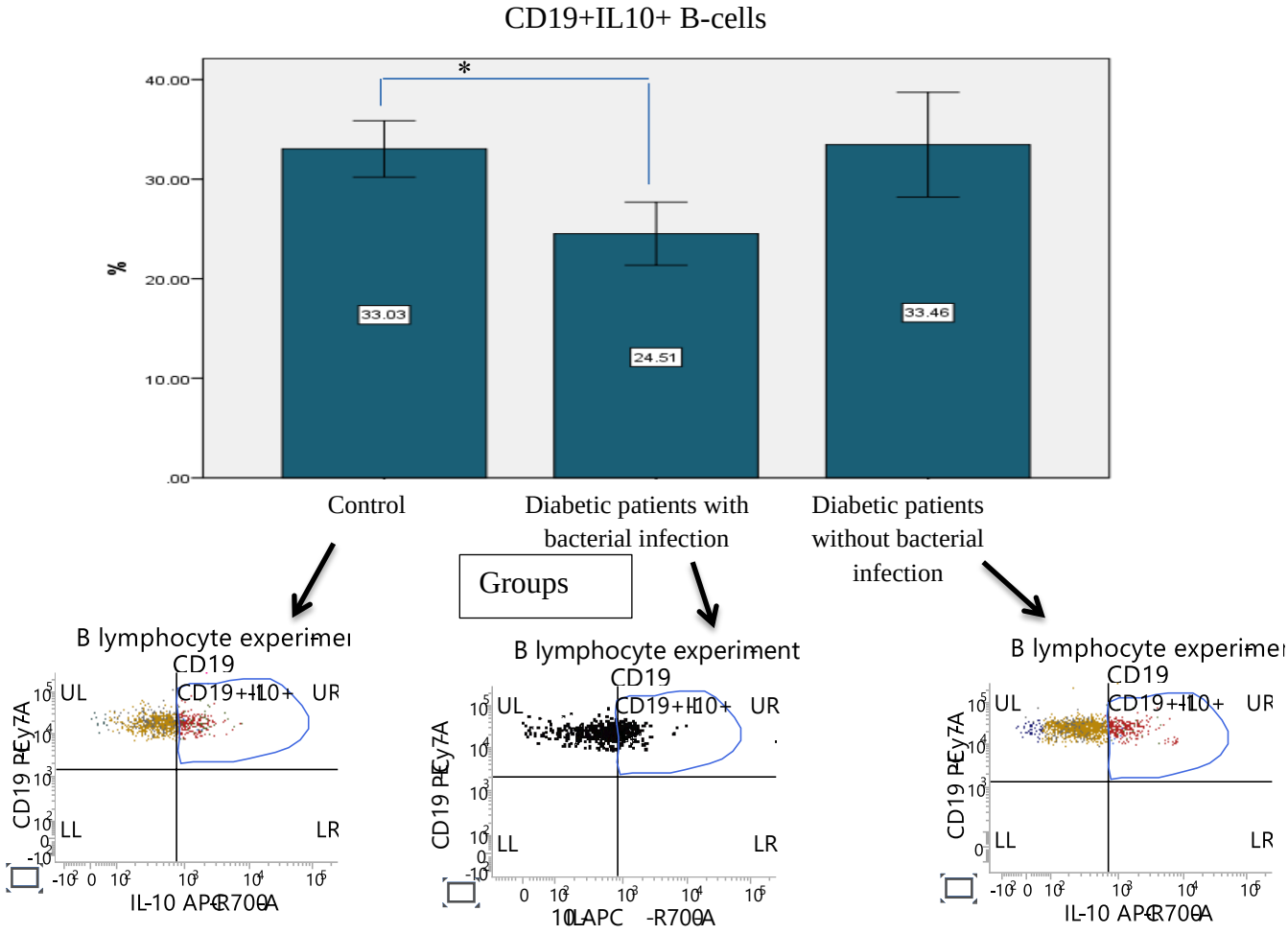

Figure 4.6 : The percentage of CD19+IL10+ cells in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups. The level of probability was indicated as *$P \leq 0.05$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.3.3. Expression of CD19+IL10 +CD24+CD27+ B-reg cells

As shown in Figure 4.7, the results revealed a significant decrease in CD19+IL10 + CD24+ CD27+ B-reg cells percentage between both patients groups of T1DM compared to the control group. The mean was 47.07 ± 2.78 , 26.98 ± 2.36 and 26.97 ± 2.59 for the control group, diabetic patients with bacterial infections group and diabetic patients without bacterial infections group, respectively .

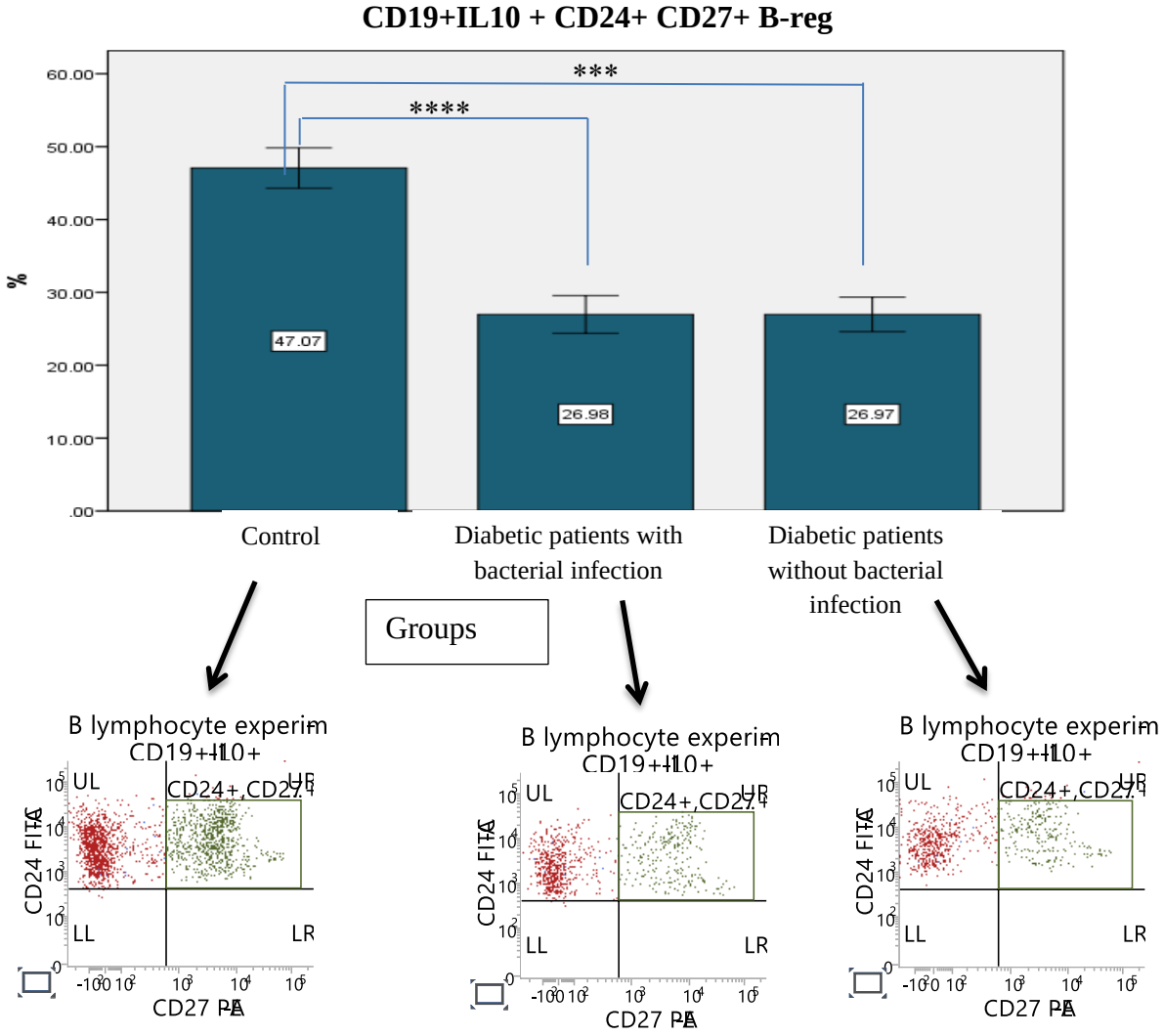

Figure 4.7: The percentage of CD19+IL10+CD24+CD27+ B-reg cells in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups . The level of probability was indicated as *** $P \leq 0.001$ and **** $P \leq 0.0001$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.3.4. Expression of co-stimulatory molecules CD80 on B-reg

Figure 4.8 shows the CD80 expression on B-reg in two groups of diabetic patients and control group. The frequency of CD80-expressing B cells a significant increase for T1DM with bacterial infection compared to control group, If the mean percentage are 92.46 ± 1.25 % vs. 89.16 ±1.13 % in $P \leq 0.05$.

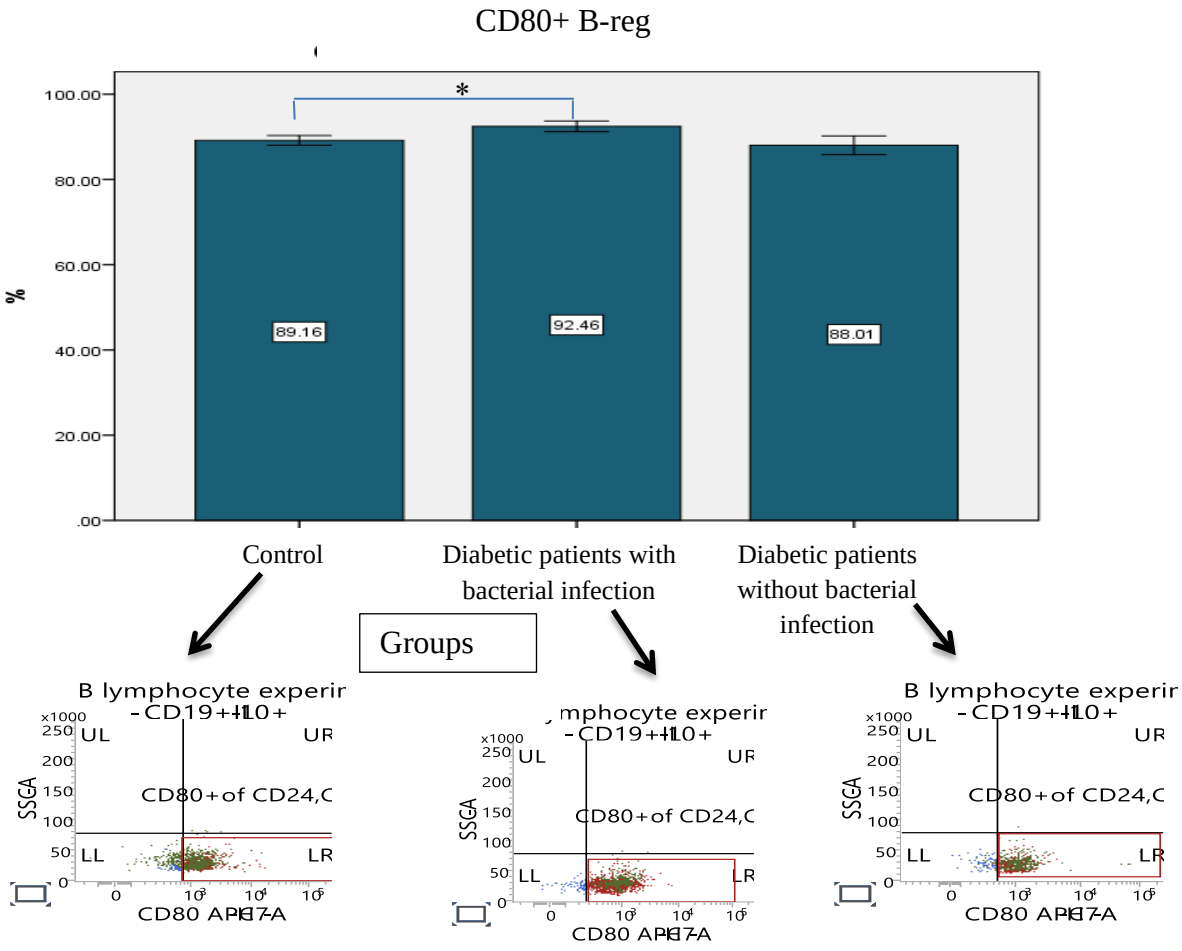

Figure 4.8:The percentage of CD80+ B-reg in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups . The level of probability was indicated as *$P \leq 0.05$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30 .

4.3.5. Mean fluorescence intensity (MFI) of CD80 expression on B-reg

The results of this part of the study revealed the decreased expression of CD80 on the surface of B-reg in T1DM patient for two groups compared to the control (Figure4.9).

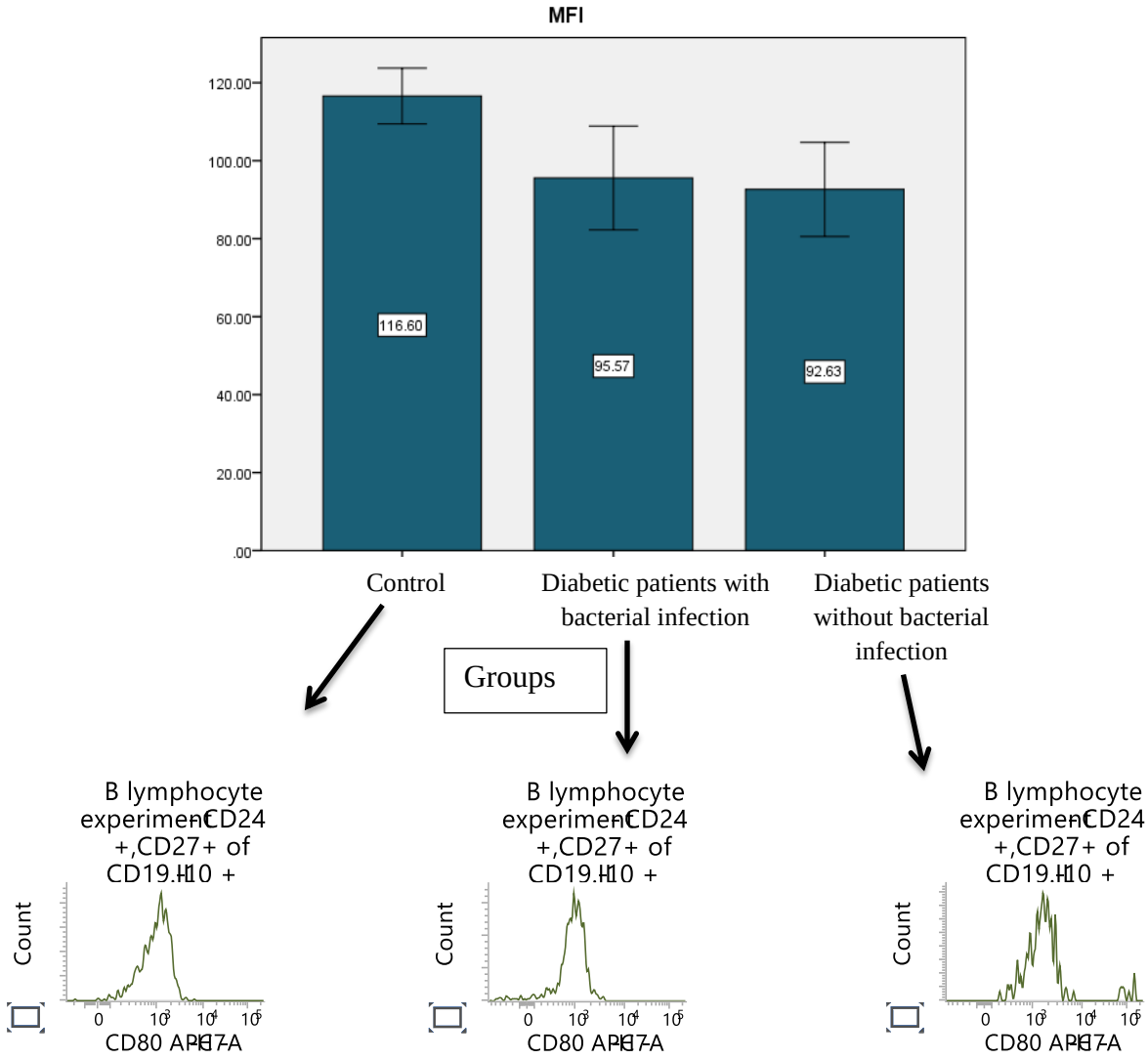

Figure 4.9 : MFI of CD80 expression on B-reg in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections. Data was presented as mean ± SE,T-test one-way ANOVA, n =30 .

4.4. Measurement of the immunological parameters in the serum by ELISA

4.4.1. Transforming growth factor beta 1 (TGF-β1) concentration

Transforming growth factor beta 1 serum levels were higher in diabetic patients with bacterial infections group (8576.91 ± 8276.6) ng/ml compared with patients without bacterial infection group (230.01 ±11.50) ng/ml and control group (278.91 ±15.01) ng/ml (Figure 4.10) .In addition showed that a significant decrease for T1DM with bacterial infections group compared to the control group on level $P \leq 0.01$.

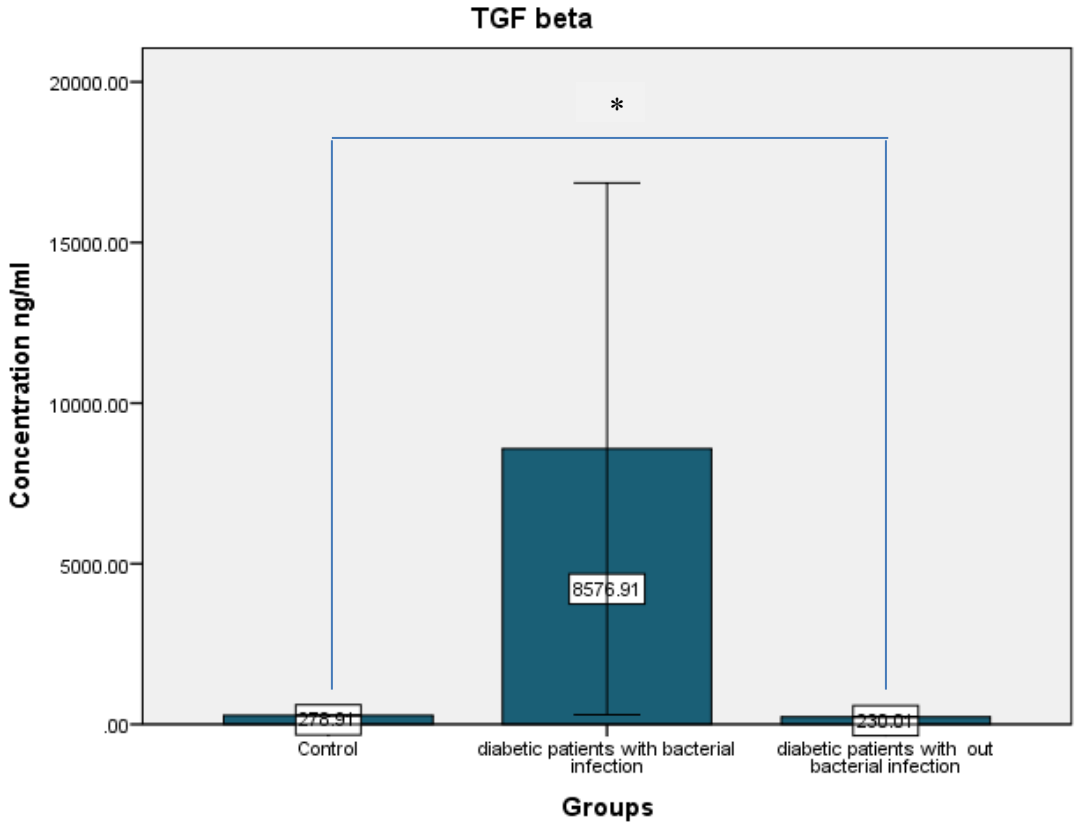

Figure 4.10 : The concentration of TGF-β1 in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups . The level of probability was indicated as *$P \leq 0.05$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30 .

4.4.2. Interleukin 35 (IL-35) concentration

As shown in Figure 4.11, serum IL-35 levels were higher for both patients groups of T1DM compared to the control group. The results also showed a significant increase in IL-35 levels for T1DM without bacterial infection and the mean was 7.091 ±1.85 and with bacterial infection group the mean was 10.42±1.56 ng/ml on level $P \leq 0.0001$ and $P \leq 0.001$)respectively compared to the control group 5.514 ±0.20 ng/ml. IL-35 levels were significantly elevated in diabetic patients with bacterial infections group compared with the patients without bacterial infection group on level $P \leq 0.05$.

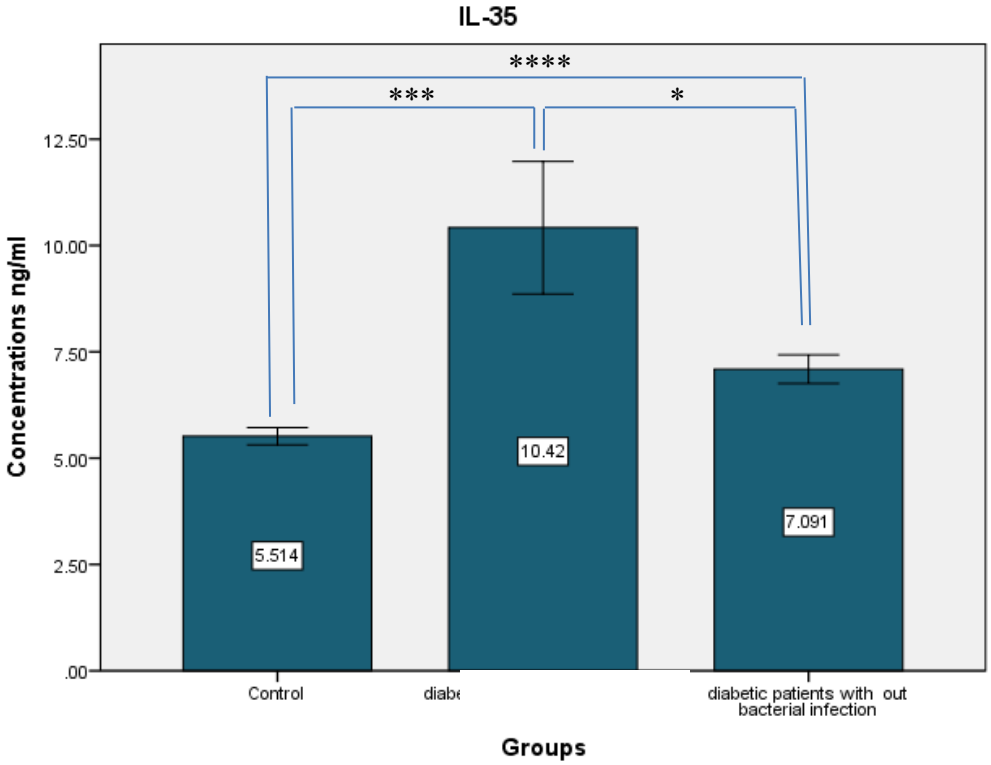

Figure 4.11 : The concentration of IL-35 in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups . The level of probability was indicated as *$P \leq 0.05$, *** $P \leq 0.001$ and **** $P \leq 0.0001$. Data was presented as mean ± SE , T-test one-way ANOVA, n =30.

4.4.3. Macrophage Migration Inhibitory Factor (MIF) concentration

The results showed a significant increase in MIF levels for T1DM with bacterial infection the mean was 29.68 ±7.18 ng/ml and T1DM without bacterial infection group the mean was 9.047 ±1.47 ng/ml on level ($P \leq 0.001$) compared to the control group 4.685 ±0.52 ng/ml. MIF levels were significantly elevated for diabetic patients with bacterial infections group compared with patients without bacterial infection group on level ($P \leq 0.001$) (Figure 4.12).

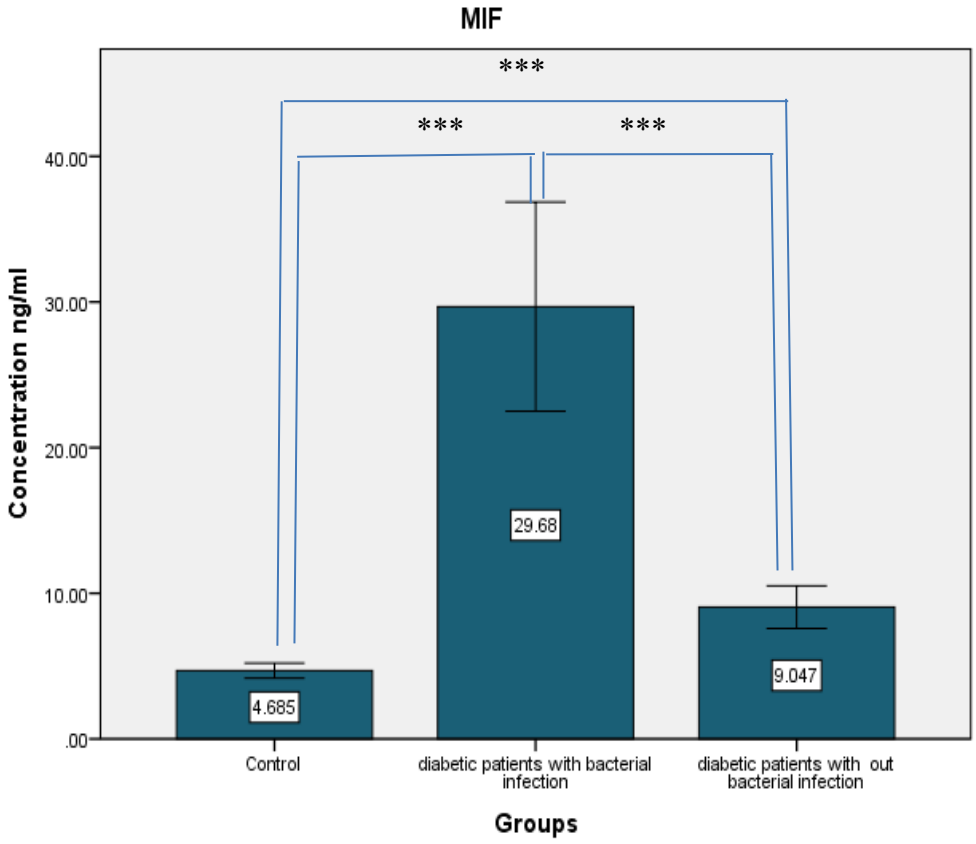

Figure 4.12 : The concentration of MIF in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups . The level of probability was indicated as *** $P \leq 0.001$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.4.4. Lipopolysaccharide Binding Protein (LBP) concentration

Figure 4.13, shows the serum LBP levels were higher for both patients groups of T1DM compared to control group.The results also showed a significant increase in LBP levels for T1DM with bacterial infection group and the mean was 3620.80 ± 456.24 ng/ml and T1DM without bacterial infections group the mean was 1230.89 ±138.96 ng/ml on level $P \leq 0.001$ compared to control 540.29 ±54.89 ng/ml. LBP levels were significantly elevated in diabetic patients with bacterial infections compared with patients without bacterial infection on level $P \leq 0.001$.

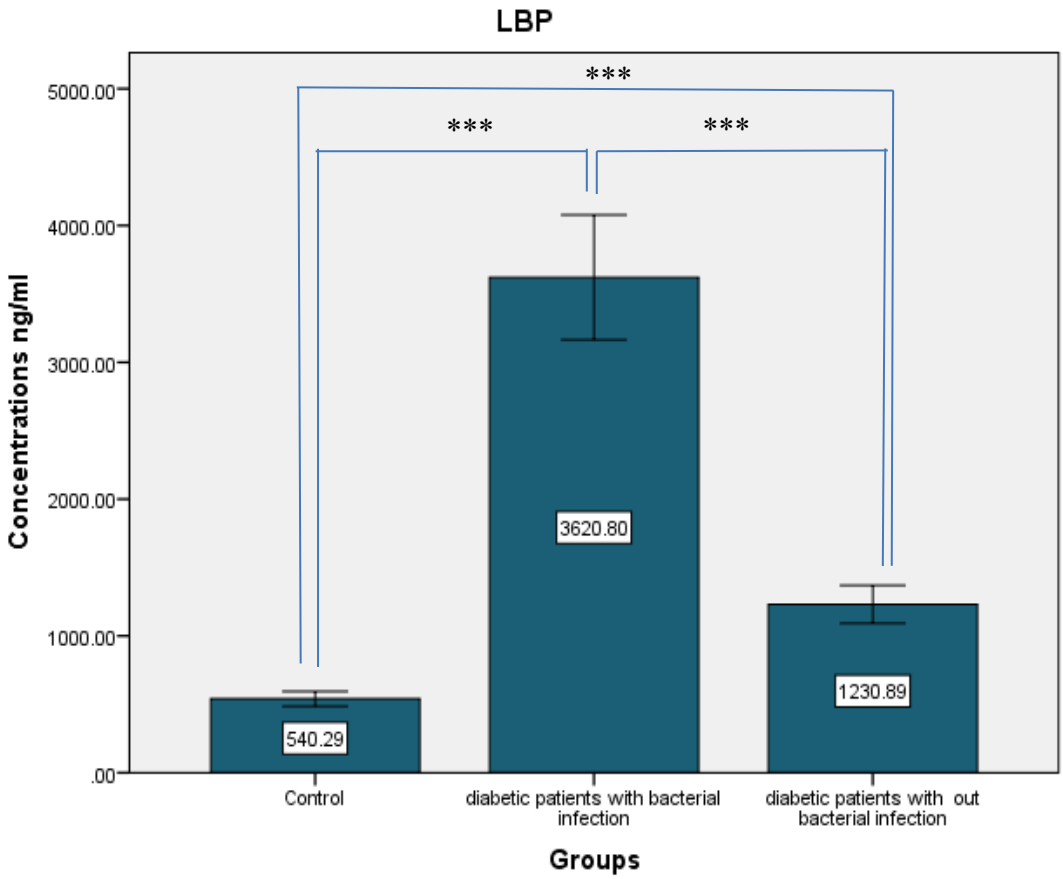

Figure 4.13 : Concentrations of LBP in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups . The level of probability was indicated as *** $P \leq 0.001$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.4.5. Toll - like receptor 4 (TLR4) concentration

The study revealed that a significant rising in the serum levels of TLR4 in diabetic patients with bacterial infections group and diabetic patients without bacterial infections group in compared to the control group.The results also showed a significant increase in TLR4 levels for T1DM with bacterial infection and the mean was 4.584 ± 0.50 ng/ml and T1DM without bacterial infection the mean was 2.849 ± 0.12 ng/ml on level $P \leq 0.0001$ and $P \leq 0.001$)respectively, compared to control group 2.524 ± 0.95 ng/ml. TLR4 levels were significantly elevated in diabetic patients with bacterial infections group compared with patients without bacterial infection group on level $P \leq 0.05$ (Figure 4.14).

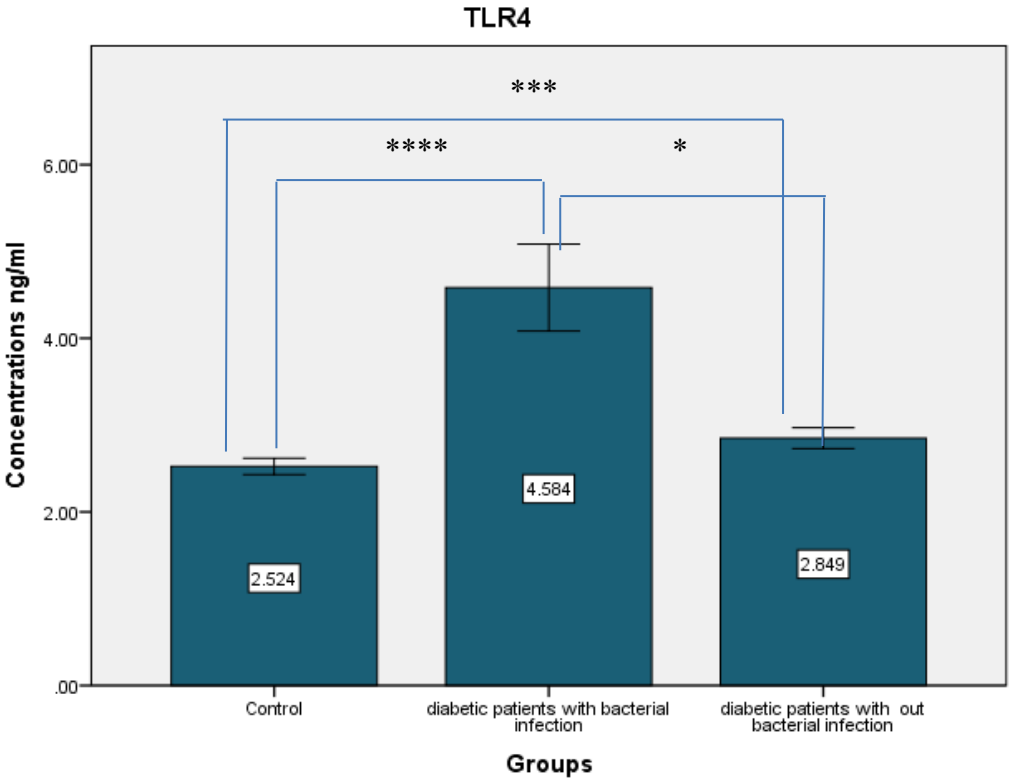

Figure 4.14 : The concentration of TLR4 in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups. The level of probability was indicated as *$P \leq 0.05$, *** $P \leq 0.001$ and **** $P \leq 0.0001$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.5. Measurement of some physiological parameter levels of the study population

4.5.1. Total cholesterol (CHOL) concentration

Data analysis indicated that there was revealed that a significant rising $P \leq 0.001$ in the serum levels of CHOL in diabetic patients with bacterial infections group compared to the control group and a significant rising $P \leq 0.05$ when compared to diabetic patients without bacterial infections group. The mean was 179.57 ± 9.08 mg/dl in diabetic patients with bacterial infections, 155.97 ± 4.61 mg/dl in diabetic patients without bacterial infections group and 147.60 ± 2.40 mg/dl in control group (Figure 4.15).

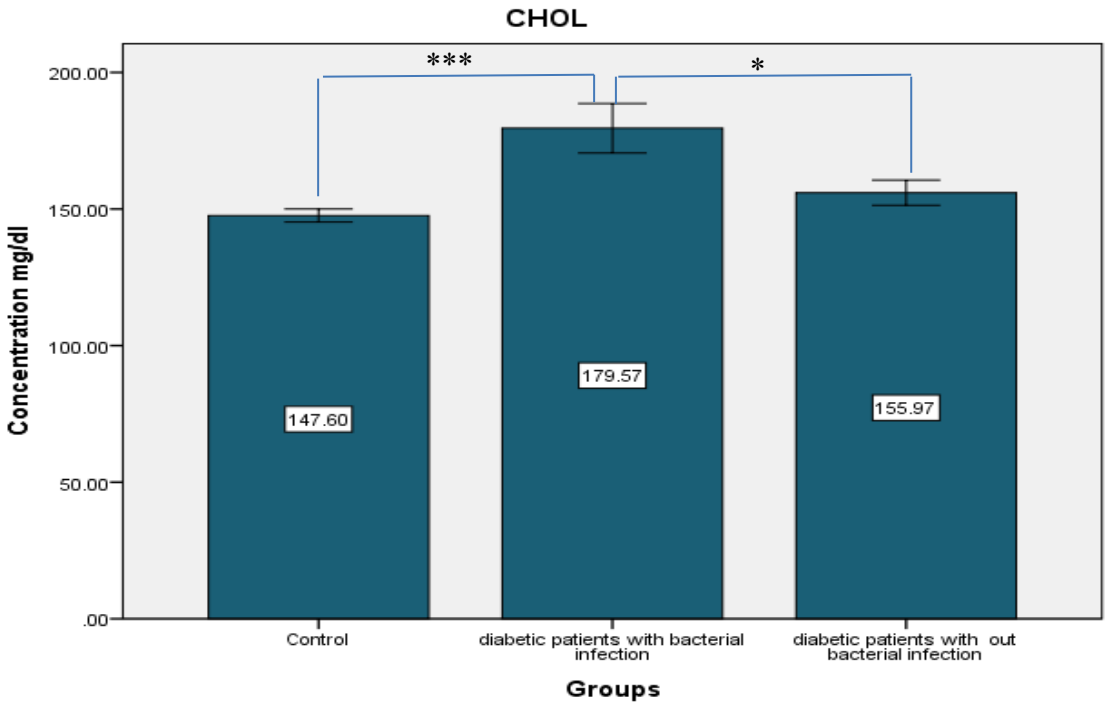

Figure 4.15: Concentration of CHOL in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups . The level of probability was indicated as *$P \leq 0.05$ and *** $P \leq 0.001$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.5.2. Triglyceride (TG) concentration

The results showed highly significant differences in the serum levels of TG between diabetic patients groups and control group.There was high increase in the mean level of TG in diabetic patients without bacterial infection group compared to diabetic patients with bacterial infections and control groups 125.55 ±14.02 mg/dl , 111.50 ±11.39 mg/dl and 72.60 ±2.50mg/dl respectively (Figure 4.16).

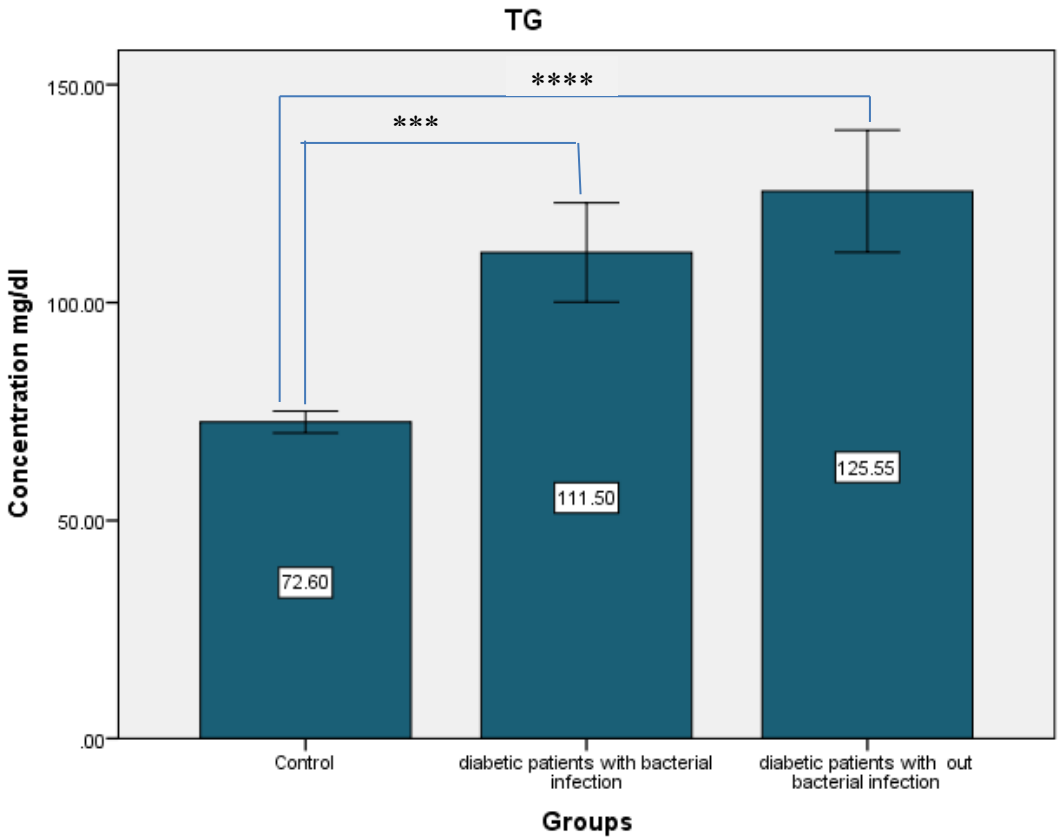

Figure 4.16 : Concentration of TG in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups . The level of probability was indicated as *** $P \leq 0.001$ and **** $P \leq 0.0001$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.5.3. Total to high-density lipoprotein cholesterol (TC/HDL-C) concentration

Present study showed that there was no significant differences between the diabetic patients with bacterial infection group, diabetic patients without bacterial infection and control groups with concentration of TC/HDL-C (Figure4.17).

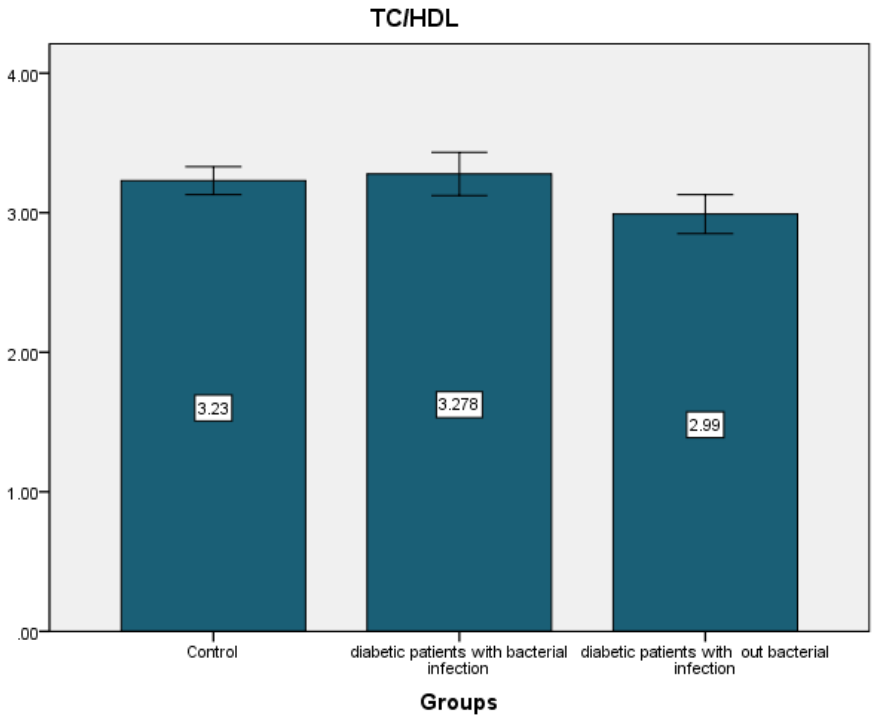

Figure 4.17 : TC/HDL in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.5.4. Low-density lipoprotein cholesterol (LDL-C) concentration

Figure 4.18 shows the average of LDL-C level in control group and two groups of diabetic patients. There was high significant increase in the mean level of LDL-C for diabetic patients with bacterial infection compared to diabetic patients group without bacterial infection group 102.87 ± 6.84 mg/dl and 81.30 ±3.92mg/dl , respectively in ($P \leq 0.001$).

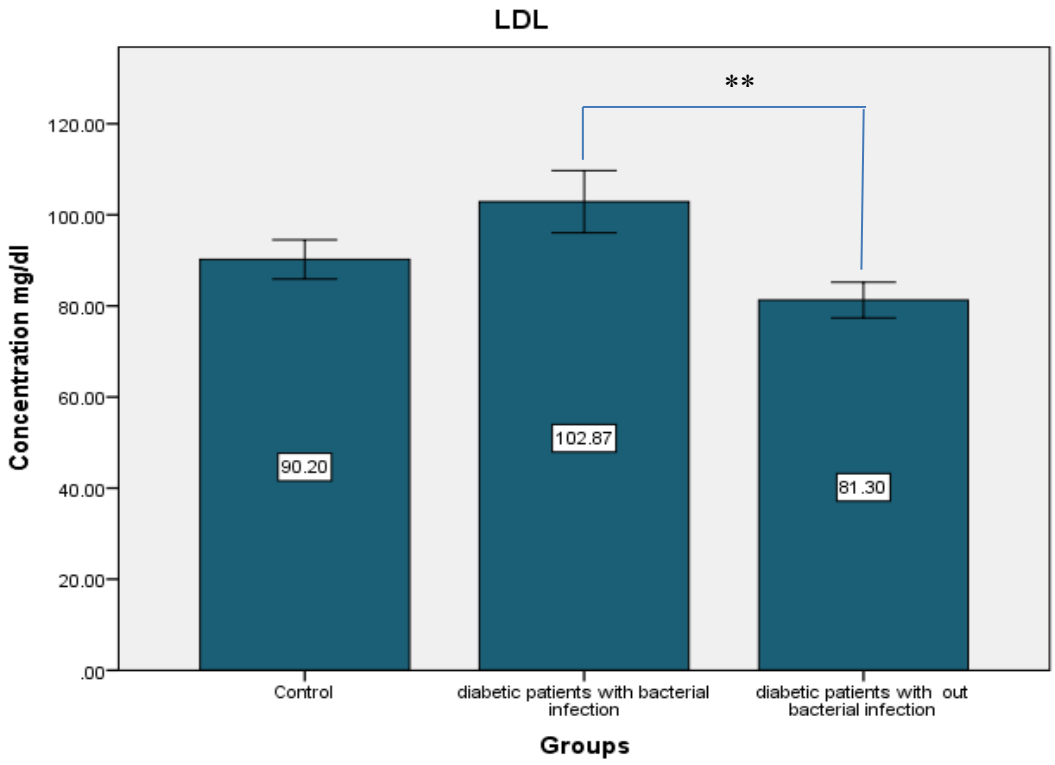

Figure **4.18 : Concentration of LDL in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups . The level of probability was indicated as **$P \leq 0.01$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.**

4.5.5. High-density lipoprotein cholesterol(HDL-C) concentration

Figure 4.19 shows the average serum HDL-C level in control group and two groups of diabetic patients. There was high significant increase in the mean level of HDL-C for diabetic patients with bacterial infection group compared to control group 54.53 ± 1.95 mg/dl and 49.40 ±1.10 mg/dl, respectively in ($P \leq 0.05$).

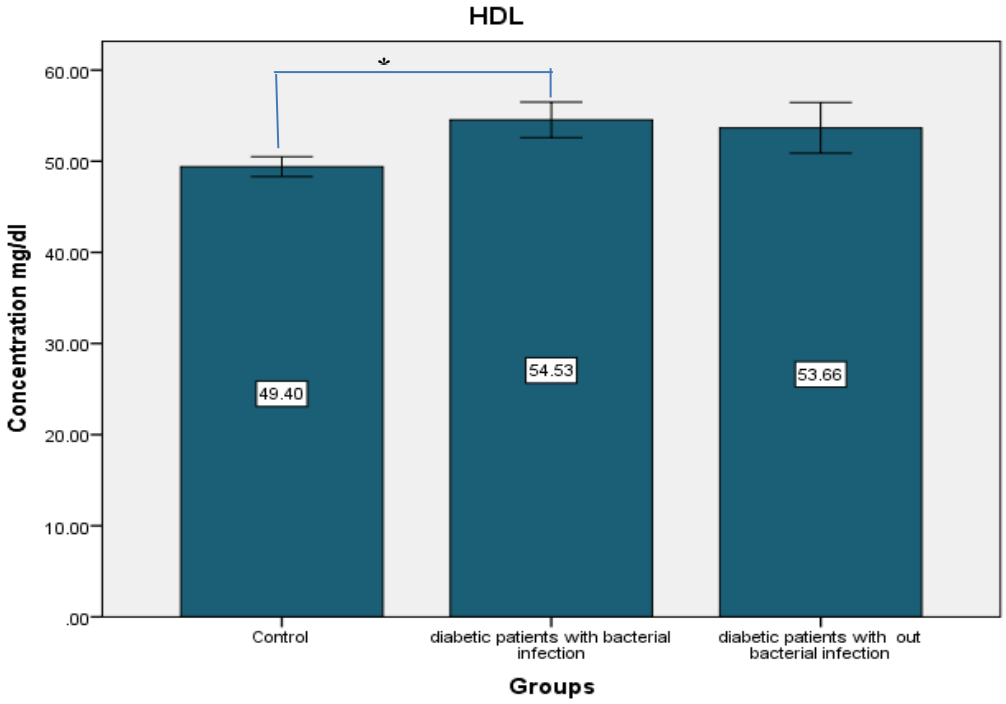

Figure 4.19 : Concentration of HDL in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups . The level of probability was indicated as *$P \leq 0.05$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.5.6. Non-high-density lipoprotein cholesterol (Non-HDL-C) concentration

Showed in Figure 4.20 that serum Non-HDL-C levels were significantly elevated for diabetic patients with bacterial infections group 122.73 ±7.76 mg/dl compared to the patients without bacterial infection group 104.27 ±3.93 mg/dl ($P \leq 0.05$).

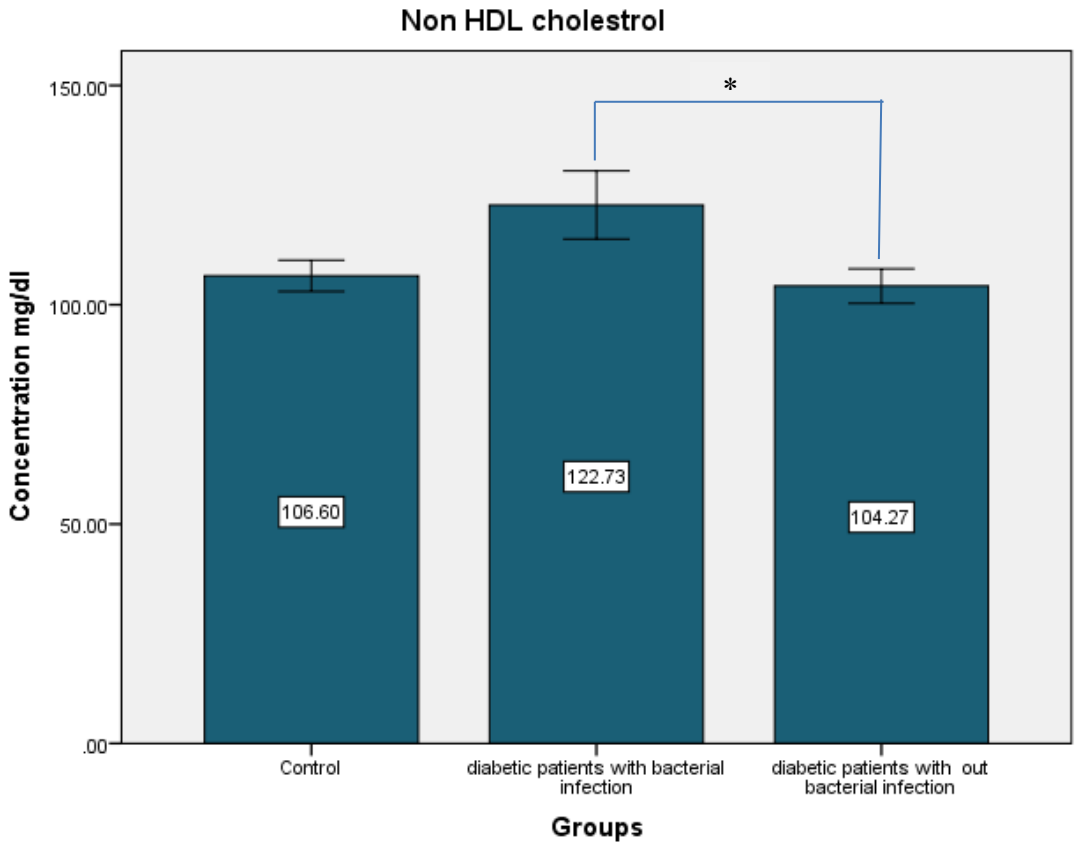

Figure 4.20 : Concentration of Non HDL cholesterol in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups . The level of probability was indicated as *$P \leq 0.05$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.5.7. Alanine transaminase (ALT) concentration

Recorded results showed a decreasing in ALT concentration in serum of patients in compassion to the control group. The results also showed a significant decrease in ALT levels for T1DM with bacterial infection the mean was (15.60 ± 1.36) IU/L and control the mean was (19.70 ± 0.76) IU/L on level ($P \leq 0.01$) (Figure 4.21).

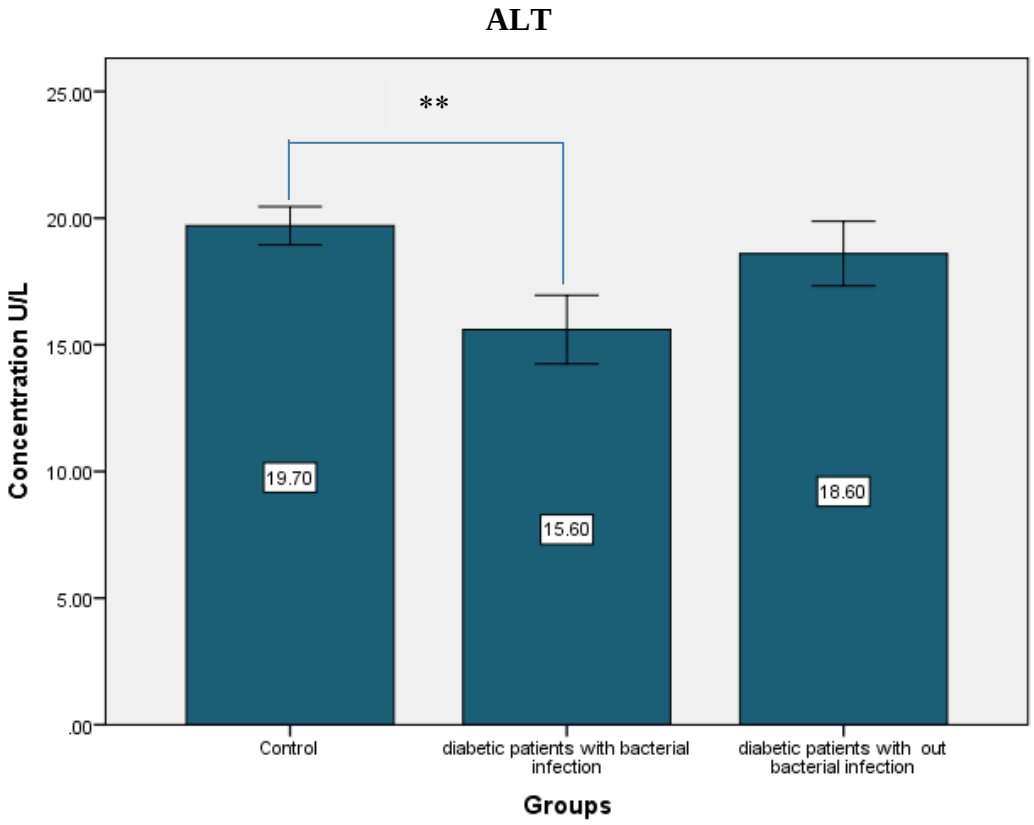

Figure 4.21 : Concentration of GPT in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups . The level of probability was indicated as **$P \leq 0.01$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.5.8. Aspartate transaminase (AST) concentration

The results also showed a significant decrease in AST levels for T1DM with bacterial infection the mean was 22.47 ± 1.21 U/L while without bacterial infection the mean was 23.83 ± 1.32 U/L on level $P \leq 0.001$ and $P \leq 0.01$ respectively, compared to the control 27.80 ± 0.87 U/L (Figure 4.22) .

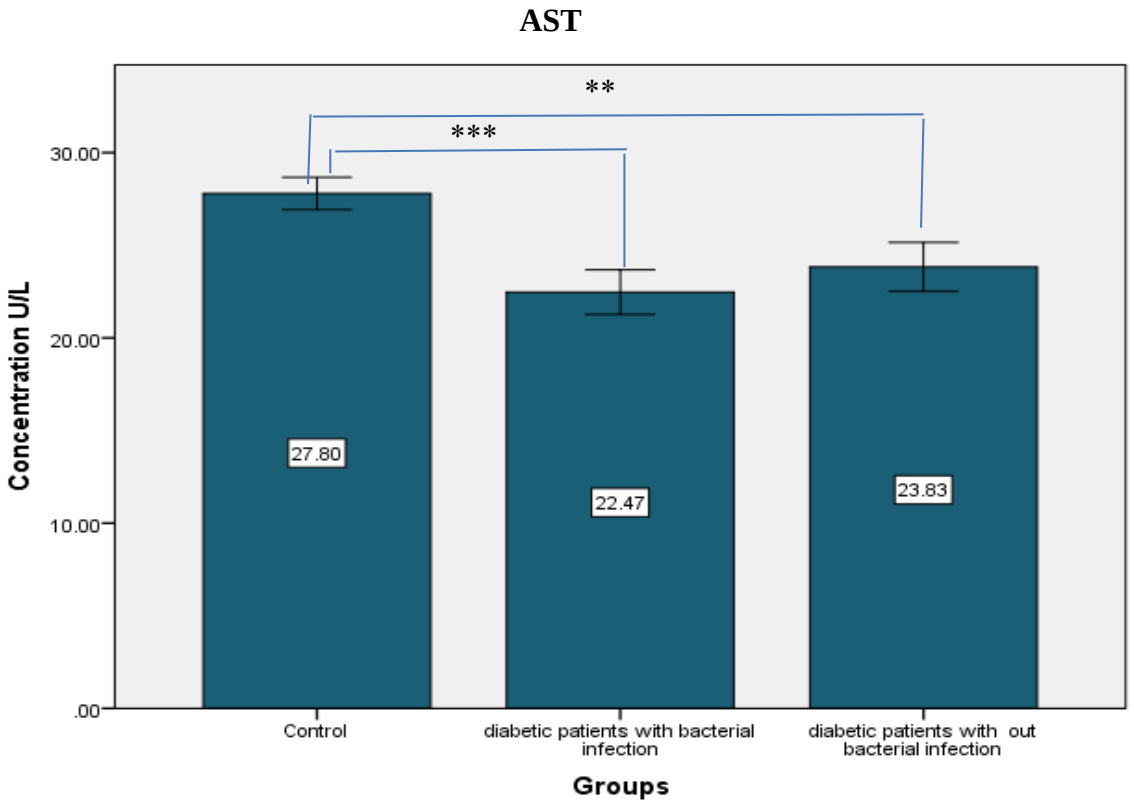

Figure 4.22: Concentration of AST in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups. The level of probability was indicated as **$P \leq 0.01$ and *** $P \leq 0.001$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.5.9. Alkaline phosphatase (ALP) concentration

The results demonstrated show in Figure 4.23, that there was an increasing in ALP level in serum of diabetic patients without bacterial infection compared to diabetic patients with bacterial infection and controls (269.80 ± 19.31 U/L, 223.83 ±15.80 U/L and 217.40±12.16 U/L) respectively. The ALP concentration was significantly elevated for diabetic patients without bacterial infections compared to the control in $P \leq 0.05$.

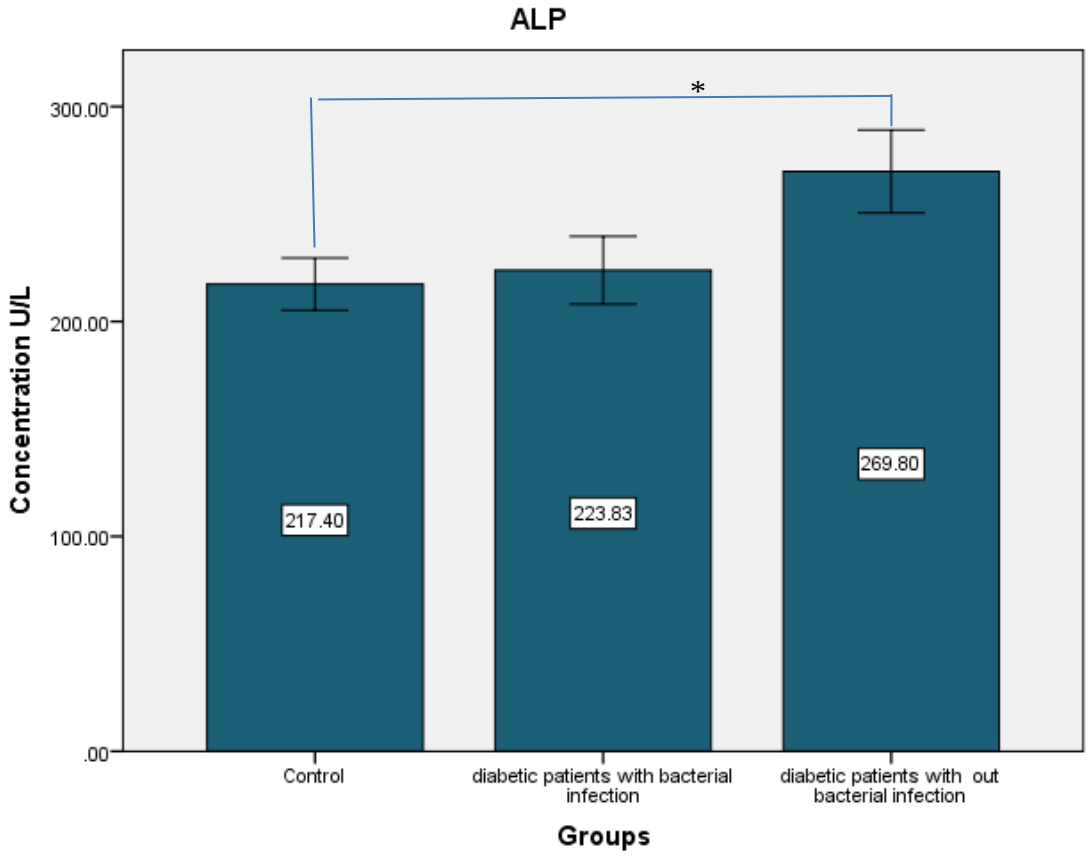

Figure 4.23: Concentration of ALP in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups . The level of probability was indicated as *$P \leq 0.05$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.5.10. Total serum bilirubin (TSB) concentration

TSB levels were significantly elevated in diabetic patients with bacterial infections 0.707 ±0.04 mg/dl compared with patients without bacterial infection 0.560 ±0.02 mg/dl in $P \leq 0.01$ and decreasing in TSB levels concentration of patients without bacterial infections patients in comparable two other groups (Figure 4.24).

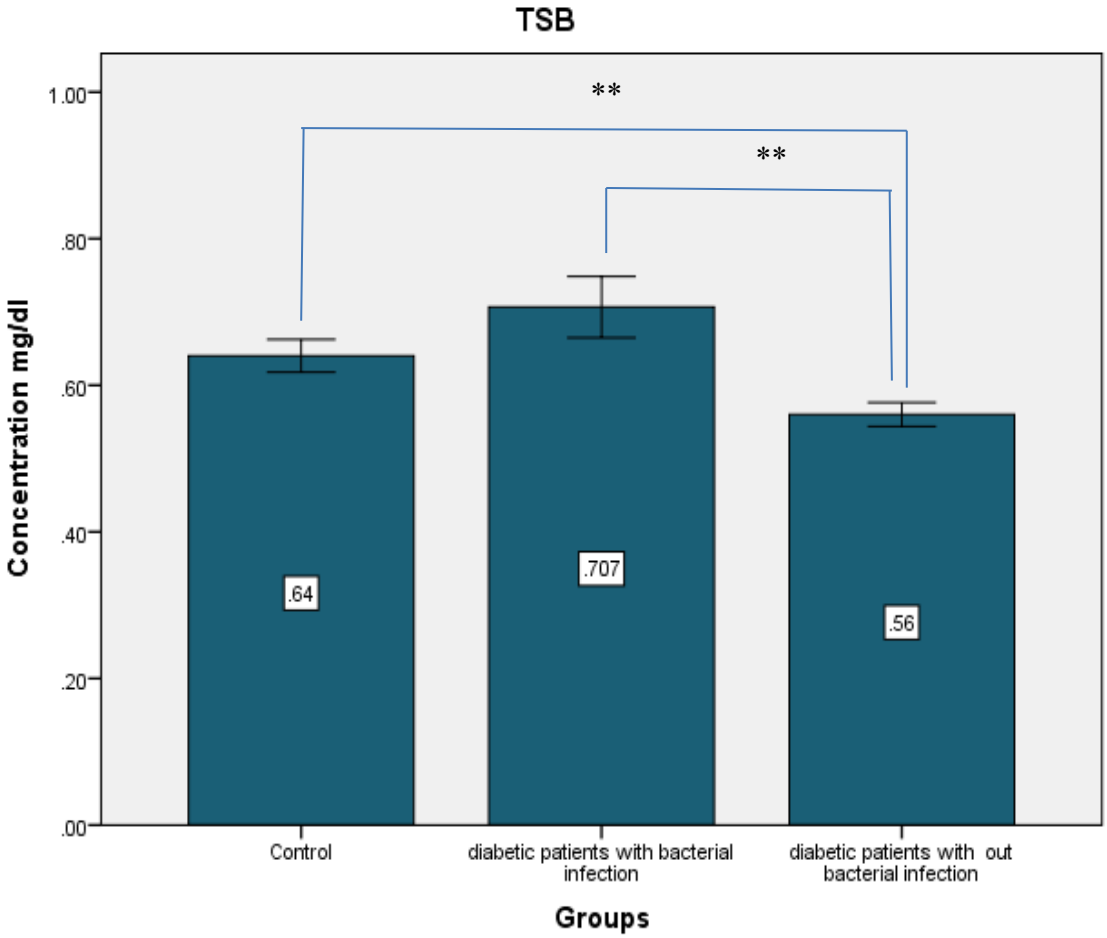

Figure 4.24 : Concentration of TSB in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups. The level of probability was indicated as ** $P \leq 0.01$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.5.11. Blood urea concentration

The results showed a significant increase in the mean level of blood urea in diabetic patients without bacterial infection compared to diabetic patients with bacterial infection and control 19.13 ± 1.21 mg/dl , 16.33 ±0.57 mg/dl and 16.70 ±0.70 mg/dl ($P \leq 0.05$) respectively (Figure4.25).

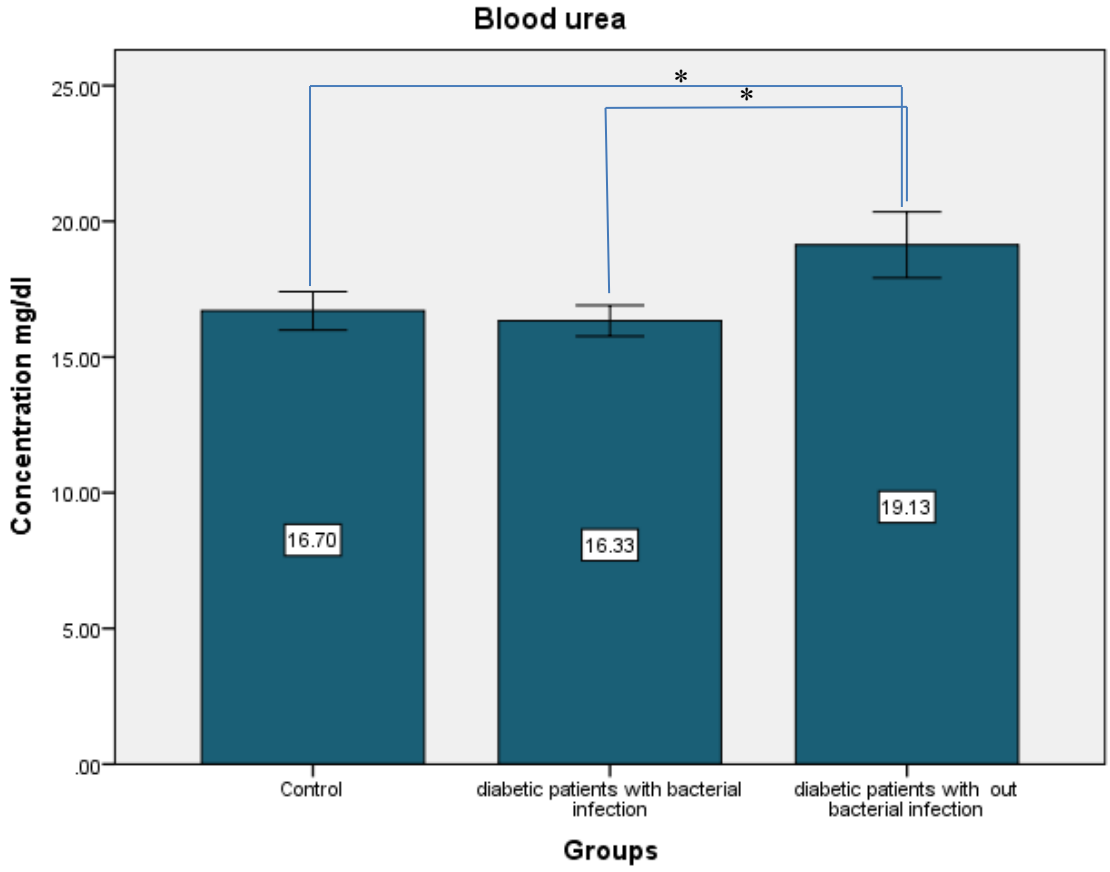

Figure 4.25: Concentration of blood urea in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups . The level of probability was indicated as *$P \leq 0.05$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.5.12. Serum creatinine concentration

As shown in Figure 4.26, the serum creatinine concentration was higher for T1DM with bacterial infections compared to T1DM without bacterial infections and control. The creatinine levels were significantly elevated in diabetic patients with bacterial infections 0.530 ±0.04 mg/dl compared with patients without bacterial infection 0.443 ±0.02 mg/dl ($P \leq 0.05$) .

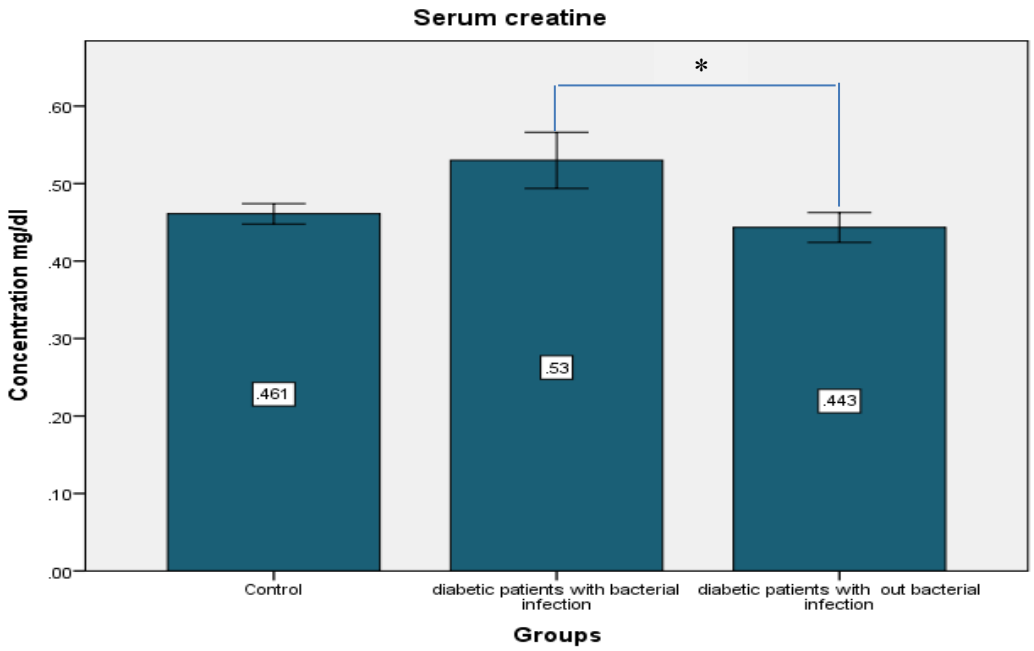

Figure 4.26 : Concentration of serum creatine in control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups. The level of probability was indicated as *$P \leq 0.05$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.6. Distribution of the study population by the age and comparative some immunological parameters

Table 3.4, demonstrates the distribution of three groups for this study by age periods divided to two categories: (5-10 year) and (11-16 year). For the control groups,the percentage of children in the age group (5-10) was 43 %, and for the age group (11-16) it was estimated at 57 % .The D1TM patients with bacterial infections the percentage of children for the age group (5-10) was 27 %, and for the age group (11-16) it was estimated at 73 %. The percentage of children for D1TM patients without bacterial infections the age group (5-10) was 27 %, and for the age group (11-16) it was estimated at 73 %.

4.6.1. The percentage of CD19+IL10 CD24+CD27+ B-reg cellsdepending on the age

Result of current study shown in Figure 4.27 the mean value of CD19+IL10 CD24+CD27+ cellspercentage was highest for age of (11-16 year) and lowest mean value was for period (5-10 year) for all groups study as shown in figure 4.29. Data analysis indicated that there was revealed that a significant rising for period (5-10 year) control compared to diabetic patients with bacterial infections and diabetic patients without bacterial infections for probability level ($P \leq 0.001$ and $P \leq 0.01$) respectively, but was revealed that a significant rising for period (11-16 year) control compared to diabetic patients with bacterial infections and diabetic patients without bacterial infections for probability level ($P \leq 0.0001$) for two comparisons.

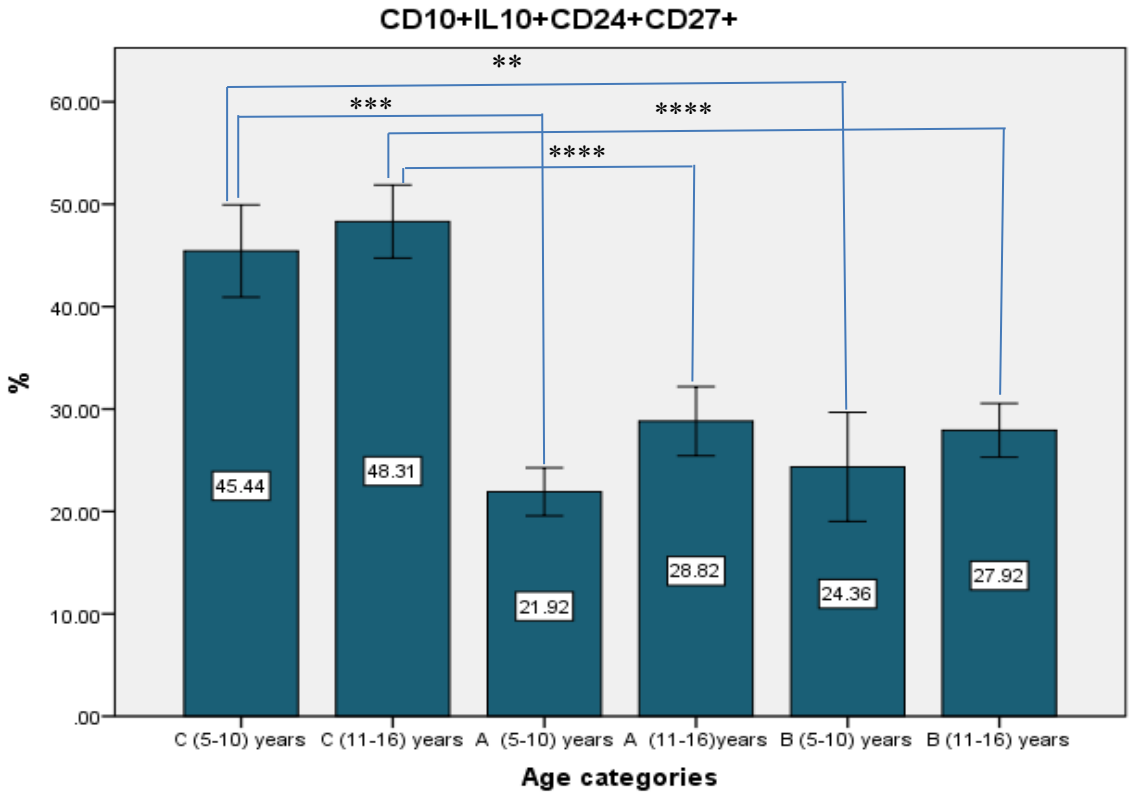

Figure 4.27 : The percentage of CD19+IL10+CD24+CD27+ cells in age categories of control (C), diabetic patients with bacterial infections (A) and diabetic patients without bacterial infections(B) ,the significance value was indicated as * between the groups . The level of probability was indicated as *****P* $\leq$ **0.01,***** ***P*** $\leq$ **0.001 and ****** ***P*** $\leq$ **0.0001. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.**

4.6.2. Expression of co-stimulatory molecules CD80 on B-reg depending on the age

Figure 4.28, showed the mean value of of CD80+B-reg was highest for period (5-10 year) ages for diabetic patients with bacterial infections and diabetic patients without bacterial infections compared to the control. Asignificant increase in CD80-expressing B cells in subjects infected with T1DM with bacterial infections compared to the controls for period (5-10 year) at the probabilistic level $P \leq 0.05$.

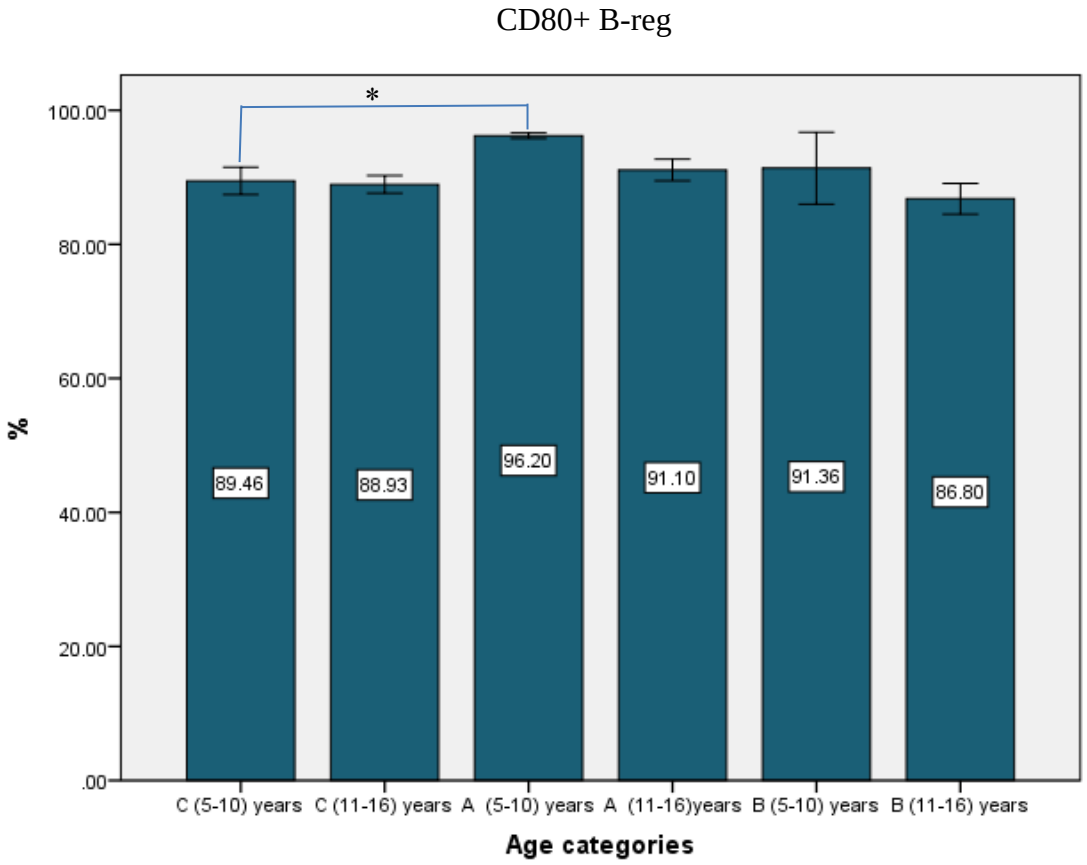

Figure 4.28: The percentage of CD80+ B-reg in age categories of control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups . The level of probability was indicated as *$P \leq 0.05$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.6.3. Mean fluorescence intensity (MFI) of CD80 expression on B-reg depending on the age

Data results show in Figure 4.29, MIF levels were significantly elevated for period (5-10 year) and lowest mean value was for period (11-16year) of all groups**.** In control group the mean value of MIF 136.85± 12.11 and 101.12± 6.75 for period (5-10 year) and (11-16year) respectively**.** The mean value of MIF of DM1T with bacterial infection 153.13 ± 15.95 for period (5-10 year) and 74.64± 14.97 for period (11-16 year) .

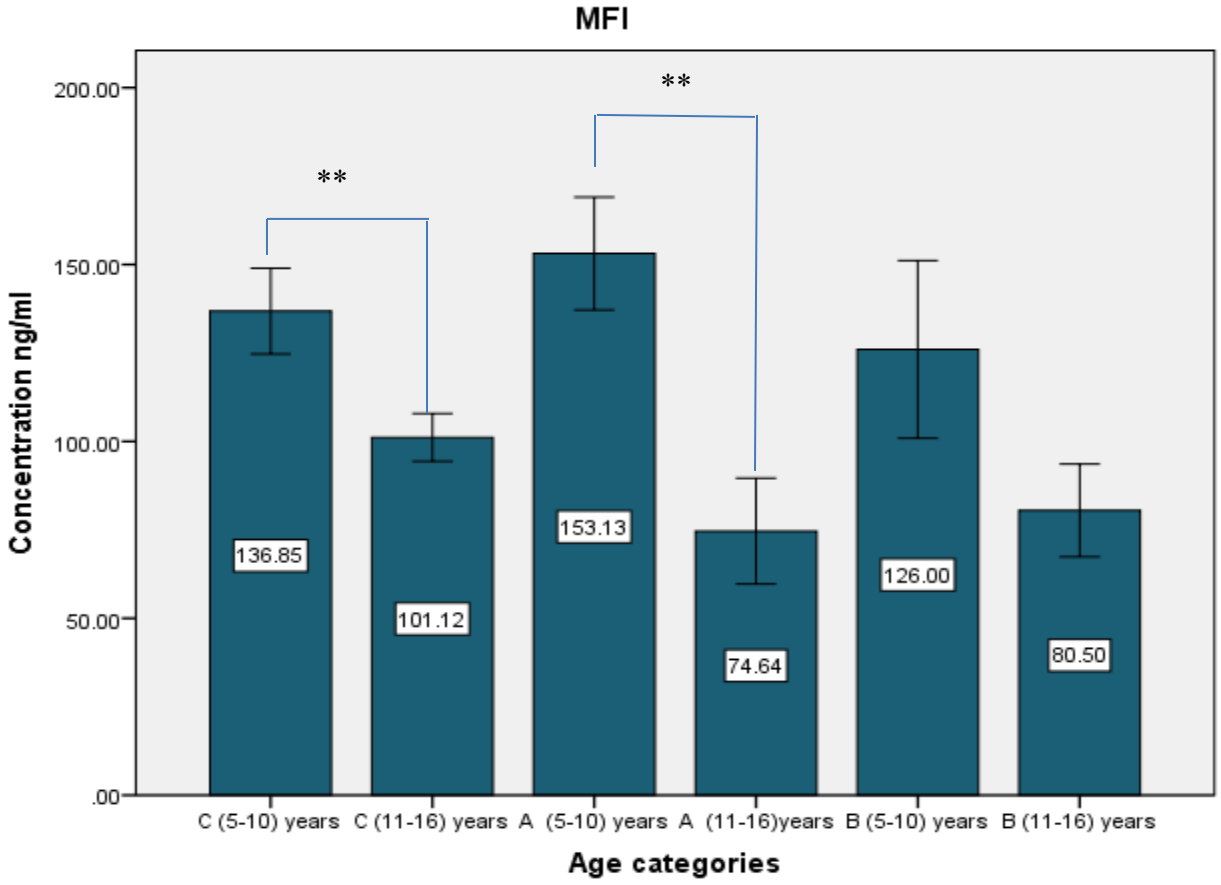

Figure 4.29: Concentration of MFI in age categories of control, diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups . The level of probability was indicated as **$P \leq 0.01$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.7. Distribution of the diabetic patients by the duration of the disease and comparative some immunological parameters

Distribution of T1DM patients by disease duration is demonstrated in Table 3.5 Patients with diabetes are divided to two categories: (1-5 year) and (6-10 year) . The T1DM 57 %, and for the duration of (6-10 year) it was estimated at 43 %. The percentage of children for D1TM patients without bacterial infections the duration of (1-5 year) was 77 %, and for the duration of (6-10 year) it was estimated at 23% .

4.7.1. The percentage of CD19+IL10+CD24+CD27+ B-reg depending on the duration of the disease

Result of current study shows the mean value of CD19+IL10 CD24+CD27+ B-reg was highest for period (6-10 year) disease duration for diabetic patients with bacterial infections and diabetic patients without bacterial infections compared to period (1-5 year) disease duration for the same groups as shown in Figure 4.30 , also show non significant differences between all groups study.

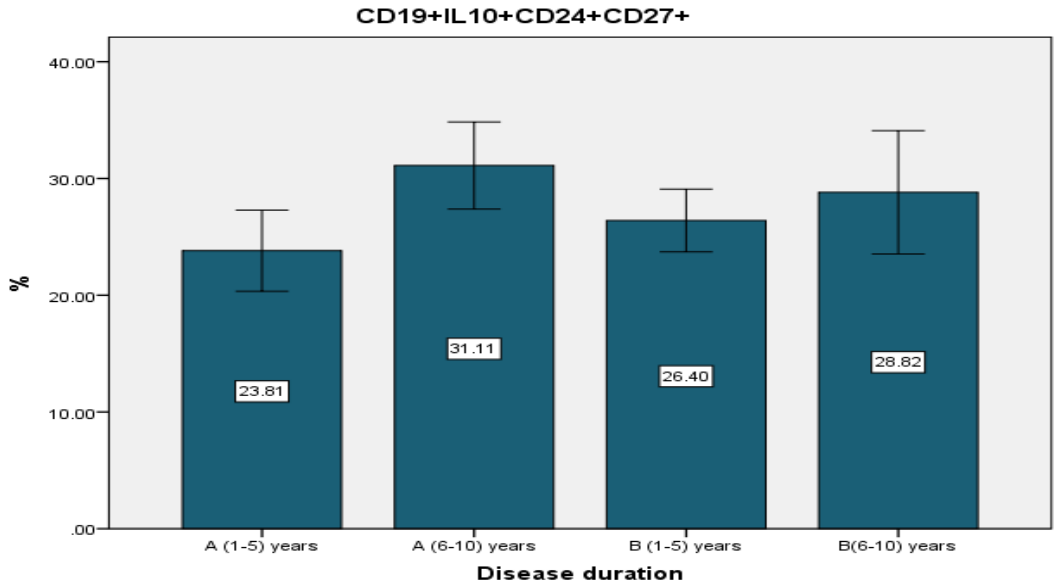

Figure 4.30: The percentage of CD19+IL10 CD24+CD27+ cells in disease duration categories of diabetic patients with bacterial infections (A) and diabetic patients without bacterial (B) . Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.7.2. Expression of co-stimulatory molecules CD80 on B-reg depending on the duration of the disease

As shown in Figure 4.31, The percentage of CD80+ B-reg was significantly elevated in (1-5 years) compared with patients (6-10 years) in diabetic patients with bacterial infections group, also a significant result shown in T1DM with bacterial infections in (1-5 years) compared to diabetic patients without bacterial infections group for period (1-5 years) at the probabilistic level $P \leq 0.05$.

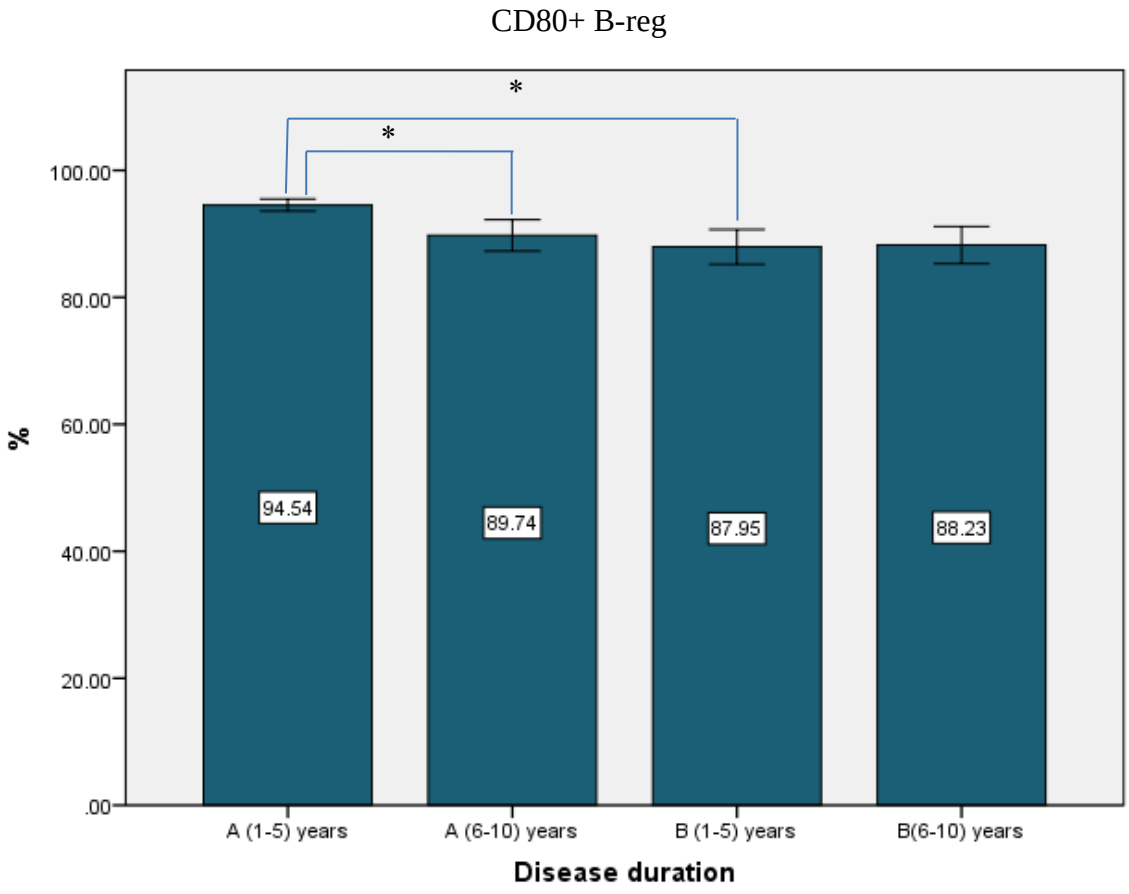

Figure 4.31 :The percentage of CD80+ B-reg in disease duration categories of diabetic patients with bacterial infections and diabetic patients without bacterial infections ,the significance value was indicated as * between the groups . The level of probability was indicated as *$P \leq 0.05$. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.7.3. Mean fluorescence intensity (MFI) of CD80 expression on B-reg depending on the duration of the disease

Result of current study shown that the mean value of MFI of CD80+B -reg was highest for period (1-5 year) disease duration for diabetic patients with bacterial infections and diabetic patients without bacterial infections compared to period (6-10 year) disease duration for the same groups as shown in Figure 4.32 .Data results also show non significant differences among all groups study.

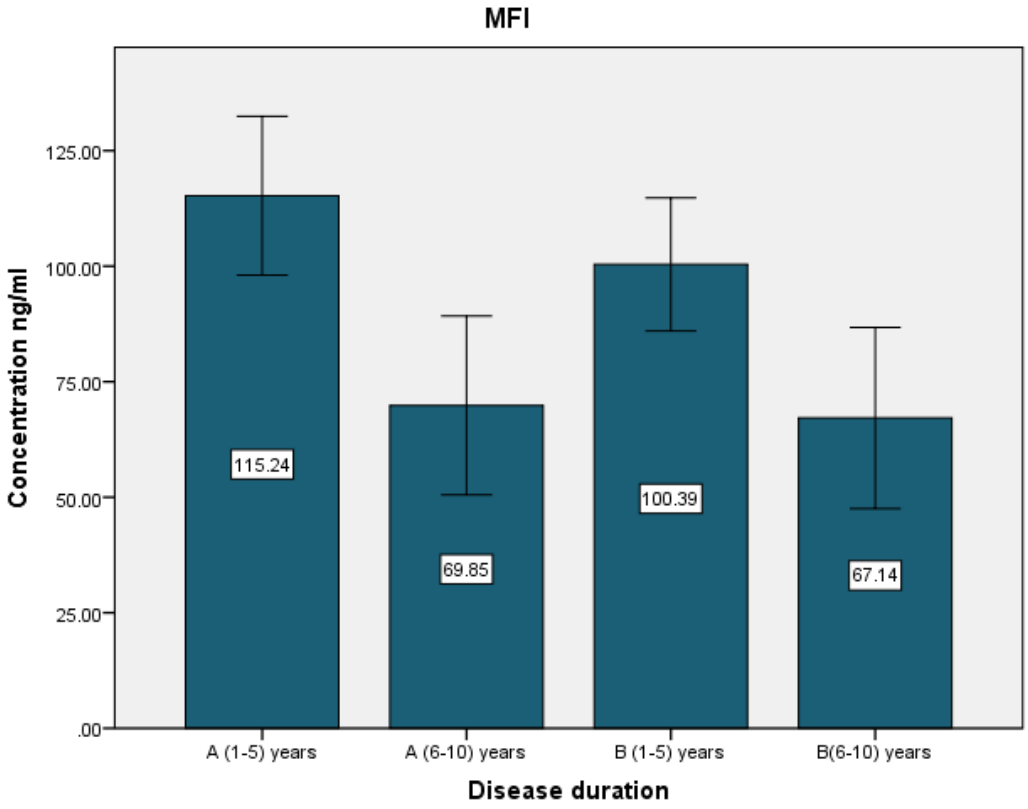

Figure 4.32 : Concentration of MFI in disease duration categories of diabetic patients with bacterial infections and diabetic patients without bacterial infections. Data was presented as mean ± SE,T-test one-way ANOVA, n =30.

4.8. The bacterial etiology of urinary colonization in diabetes

Data results show in Table 4.1 ,The bacterial pathogens that cause UTI belongs to gram negative 54% and gram-positive bacteria 46%. Among Gram negative pathogens, *Escherichia coli* li is the principal etiology of UTI in diabetic individuals If estimated at 35% followed by *Pseudomonas stutzeria* while among Gram positives, *Kocuria rosea* is the major uropathogen estimated at 18.5 % followed by *Streptococcus thoraltensis* , *Streptococcus gallolyticus* , *Staphylococcus haemolyticus* , *Kocuria kristinae*. The results show Vitek 2 automated report on identification and susceptibility of bacteria in appendix 1.

Table 4.1. Bacterial etiology of UTI in diabetic individuals

No.	Types of bacteria	Number of strains	%
1	***Escherichia coli***	19	35
2	***Pseudomonas stutzeria***	10	18.5
3	***Kocuria rosea***	10	18.5
4	***Staphylococcus haemolyticus***	2	4
5	***Streptococcus gallolyticus***	4	7
6	***Kocuria kristinae***	1	2
7	***Streptococcus thoraltensis***	8	15
		∑= **54**	

4.9. Summary of B-cells phenotypes and some immune parameters data

The data in the Tables 4.2 and 4.3 was showed the number of sample size, mean values of the data, standard error and *p* value (when compared between diabetic patients groups and controls) of B-cells phenotypes and some immune parameters.

Table 4.2. Summary of cluster of differentiation expressed on the B-cells population

Cell types	Groups	N	Mean	± SE	*P* value Δ
CD19+ B-cell	Control	30	6.55	.36	
	T1DM with UTIs	30	3.08	.26	0.000
	T1DM without UTIs	30	3.43	.46	0.000
CD19+IL10+ B-reg	Control	30	33.03	2.84	
	T1DM with UTIs	30	24.51	3.17	0.050
	T1DM without UTIs	30	33.46	5.26	0.945
CD19+IL10+CD24+CD27+ B-reg	Control	30	47.06	2.78	
	T1DM with UTIs	30	26.98	2.59	0.000
	T1DM without UTIs	30	26.97	2.36	0.000
CD19+IL10+CD24+CD27+CD80+B-reg	Control	30	89.16	1.14	
	T1DM with UTIs	30	92.46	1.25	0.057
	T1DM without UTIs	30	88.01	2.19	0.642
Expression of co-stimulatory molecules CD80 on B-reg (MFI)	Control	30	116.60	7.17	
	T1DM with UTIs	30	95.57	13.30	0.169
	T1DM without UTIs	30	92.63	12.08	0.093

T1DM: Type 1 diabetes mellitus , Standard error (SE) , UTIs: Urinary tract infection , N. : number of sample size , Δ: compared diabetic patients groups with controls .

Table 4.3. Summary of some immune parameters in study groups

Parameters	Groups	N	Mean	± SE	P value Δ
Interleukin -35 (IL35)	Control	30	5.51	0.20	
	T1DM with UTIs	30	10.42	1.56	0.003
	T1DM without UTIs	30	7.09	0.34	0.000
Transforming growth factor-β (TGF-β)	Control	30	278.91	15.00	
	T1DM with UTIs	30	8576.91	8276.67	0.320
	T1DM without UTIs	30	230.01	11.50	0.012
Macrophage migration inhibitory factor (MIF)	Control	30	4.69	0.52	
	T1DM with UTIs	30	29.68	7.18	0.001
	T1DM without UTIs	30	9.05	1.47	0.007
Lipopolysaccharide Binding Protein(LBP)	Control	30	540.29	54.89	
	T1DM with UTIs	30	3620.80	456.24	0.000
	T1DM without UTIs	30	1230.89	138.96	0.000
Toll-Like Receptors TLR4	Control	30	2.52	0.09	
	T1DM with UTIs	30	4.58	0.50	0.000
	T1DM without UTIs	30	2.85	0.12	0.039

T1DM: Type 1 diabetes mellitus , Standard error (SE) , UTIs: Urinary tract infection

N. : number of sample size , Δ: compared diabetic patients groups with controls .

Chapter Five
Discussions

Chapter Five : Discussions

5.1. Diagnosing type 1 diabetes in children

T1DM is a common chronic autoimmune diseases characterized by the destruction of insulin-producing pancreatic β-cells by their own immune system, resulting in lifelong insulin deficiency. In this exploratory, case - control study was examined the levels of fasting blood glucose level and Hemoglobin A1C (HbA1c) to confirm that patients have diabetes but anti-GAD and C-peptide to prove that they had type 1 diabetes. All routine testing used in this study is based on current American Diabetes Association standards of medical care for diabetes (Goyal *et al*., 2020).

In comparison to fasting blood glucose levels, HbA1c Figure (4.1 and 4.2) provides a number of benefits, including higher convenience (fasting is not necessary), greater preanalytical stability, and fewer daily fluctuations due to stress, dietary changes, or sickness. However, these advantages may be offset by the lower sensitivity of HbA1c at the designated cut point, greater cost, limited availability of HbA1c testing in certain regions of the developing world, and the imperfect correlation between HbA1c and average glucose in certain individuals. It is crucial to understand that HbA1c is an indirect indicator of average blood glucose levels when using it to diagnose diabetes and to take other factors into account that may have an impact on hemoglobin glycation independently of glycemia, such as pregnancy, hemodialysis, HIV treatment, age, genetic background, race/ethnicity, and hemoglobinopathies. The diagnosis is made on the basis of the confirmatory screening test , if a patient meets the diabetes criterion of the HbA1c (results $\geq$ 6.5% [48 mmol/mol]) and FBG ($\leq$126 mg/ dL [7.0 mmol/L]), that person should be considered to have diabetes (Burrack *et al*., 2017).

After confirming that the children had diabetes, GAD autoantibody and and C-peptide tests were conducted to prove that they had type 1 diabetes Figure (4.3 and 4.4) . The utoantibodies multiple because of beta cells failure and undergo programmed death or degradation by immune cells and may serve as an indication for type 1 diabetes but the undetectable levels of plasma C-peptide can indicate the presence or absence of insulin secretion (Caso *et al*., 2018).

This finding may interpret reduced insulin production by autoantibody mediated attacking of beta cells in double positive antibodies patients (Pipi & Tsirogianni, 2014). The opposed correlation between anti-GAD and C-peptide concentration that was agreed with study Mendivil *et al*.,(2017) that found anti-GAD positivity is frequent and likely related to predict complete beta-cellsfailure. Anti-GAD tend to remain high even when C-peptide secretion becomes undetectable.

5.2.Variation in the concentration of cytokines in T1DM and the effect of UT bacterial infection

Cytokines are low molecular weight extracellular proteins that mediate immune responses. They function in incredibly intricate circuits that control the inflammatory process, and they are crucial for coordinating the body's reaction to the lesion site (Gouda *et al*., 2018). Cytokines play crucial roles in orchestrating complex multicellular interactions between pancreatic β-cells and immune cells in the development of type 1 diabetes (Lu *et al*., 2020). Cytokines that can induce anti-inflammatory roles (regulatory functions), such as IL-10 , IL-35 and TGF-β are thought to restore immune tolerance and prevent β-cells damage (Zhang *et al*., 2023). By contrast, cytokines such as IL-1β, IL-6, IL-17, and MIF which promote the differentiation and function of diabetogenic immune cells, are thought to lead to T1DM onset and progression (Günther *et al*., 2019). However, targeting these dysregulated cytokine networks does not always result in consistent effects because anti-inflammatory or proinflammatory functions of cytokines, responsible for β-cellsdestruction, are context dependent (Gouda *et al*., 2018).

5.2.1 MIF pro-inflammatory cytokine

Pro-inflammatory cytokines, MIF is major inflammatory inducers that could directly relate to the pathogenesis of T1DM (Gouda *et al*., 2018). MIF is a protein that acts as a cytokine-, it binds to the cell-surface receptor CD74 in association with CD44, which activates the downstream signal transduction pathway (Günther,2019).

MIF showed in the present study higher serum level in T1DM patients compared to the control (Figure 4.12). This finding is consistent with Ismail *et al*., (2016), who noted that this cytokine contributes to the destruction of pancreatic beta-cells by the immune system, favoring the inflammatory response and autoimmune that is typical of T1DM. Furthermore, our findings are consistent with those of do Nascimento de Oliveira *et al*., (2018), who found elevated levels of inflammatory markers in patients with newly diagnosed T1DM, indicating that systemic inflammation may have contributed to the disease's genesis. However, the increased peripheral MIF levels in childhood indicate a potent role during the first years of the disease, which can contribute to the cytokine storm associated with the first stage of T1DM. Primary prevention strategies targeting inflammatory-mediated comorbidity may prevent secondary complications in the future for these patients.

During insulitis, high levels of MIF are secreted by effector T-cells to trigger the β-cellsdestruction process. Furthermore, MIF has been recognized as important molecule to the development of T1DM complications such as diabetic foot disease and is known to promote inflammatory cytokine and palmitic acid-induced pancreatic islet apoptosis (Sánchez-Zamora and Rodriguez-Sosa,2014). After successful antibody and pharmacological inhibitor-mediated MIF neutralization, MIF was proposed as a new target strategy for the treatment of T1DM (Wołoszyn-Durkiewicz and Myśliwiec, 2019).

5.2.2 Anti-inflammatory cytokines IL-35 and TGF-β1

The body produces the immunosuppressive and anti-inflammatory compounds IL-35 and TGF-1,which control immune response (Li *et al*., 2017). Our results demonstrated that diabetic patients' serum levels of IL-35 and TGF-1 were significantly higher than those of controls in (Figure 4.10 and Figure 4.11). These results are consistent with a studies performed by Espes *et al*., (2017) and Zhang *et al*., (2023) demonstrated that the elevated levels of anti-inflammatory cytokines in T1DM patients may be due to the production from a compensatory mechanism to the rise of pro-inflammatory cytokines. Accordingly, the cytokines linked to the pathogenesis of T1DM are crucial for the development of the illness, acting both in the positive control (pro-inflammatory

cytokines) and in the negative control (anti-inflammatory cytokines) of the inflammation, and they can act singly or in a cascade.

It also shows the results for the present study, the significantly elevated for IL-35 and TGF-β1 levels in T1DM with UTIs compared to T1DM without UTIs. Both innate and adaptive immune systems collaboratively orchestrate robust effector immune responses against invading pathogens, such as bacterial, viral, fungal, and other parasitic pathogens. However, the resolution of inflammation mediated by regulatory mechanisms is crucial to prevent unwarranted immunopathology (Ye *et al*., 2021).T-reg and B-reg have critical roles as a negative regulator of immunity for bacterial infections , mainly due to the fact that they secret a high level of TGF-β and IL-35. From immune evasion mechanisms, the induction of immunosuppression by T-reg and B-reg through TGF-β and IL-35 generates the most effective roles, which optimize the conditions for the survival of pathogens (Yu *et al*., 2022).This mechanism is also used by widespread bacteria and viruses that are capable to chronically persisting in the human body, such as hepatitis viruses and human immunodeficiency virus (Garib and Rizopulu, 2015).

5.3. The concentration of LBP and TLR4 in serum

UTI is the second most common bacterial infection, after otitis media, in T1DM children. The mechanisms of disease susceptibility and the role of immunity in the pathogenesis of UTI in children have been evaluated. In recent years, TLRs have been recognized as specific components of the innate immune system constituting important mediators in host immune recognition (Karananou *et al*., 2016).

Present study investigated the soluble form of LBP and TLR4 in serum for T1DM without and with UTIs compared with control. The major findings of the study are: 1. T1DM subjects with/without UTIs had significantly higher levels of LBP and TLR4 compared with control. 2. A significant increasing of LBP and TLR4 in T1DM with UTIs patients compared to T1DM without UTIs and control (Figure 4.13 and Figure 4.14).

High levels of LBP inhibit the LPS responses in monocytes and can protect both humans and mice from septic shock caused by LPS or gram-negative bacterial infection. Several immune and non-immune cells express LPS receptor (TLR4) and respond to LPS stimulation by secreting pro-inflammatory cytokines (Ciesielska *et al*., 2021). The end result is a systemic increase in the levels of TGF-β1, MIF, and IL-35, in the T1DM subjects as seen in this study.

5.4. Flow cytometry analysis of regulatory B-cell

5.4.1 CD19+B cells in human peripheral blood

B-cells have been generally considered to be positive regulators of immune responses because of their ability to produce antigen-specific antibodies and to activate T- cells through antigen presentation. Impairment of B-cellsdevelopment and function may cause autoimmune diseases (Miyagaki, 2015). Moreover, study by Kendall *et al*., (2007) has suggested that B-cells are among the earliest cells to infiltrate the pancreatic islets inT1DM mice, which is where they organize with T-cells into lymphoid structures within germinal centers that promotes the selection of autoreactive B-cells , begin to generate at the early stage of peri-insulitis. There is an increasing evidence that B-cells play a pathogenic role in the initiation of T1DM, targeting B cells may be a potential approach to treating B-cells mediated autoimmune diabetes.

In the present study, was employed a flow cytometry approach to characterize the CD19+ B-in the peripheral blood of T1DM male children with and without UTIs compared with healthy individuals to assess whether these cells are implicated in the regulation of this immune-mediated disease.

The results showed in (Figure 4.5) that a significant decreased in the percentage of CD19+ lymphocyte cells(B-cell) on level ($P \leq 0.0001$) in both patients groups of T1DM , 3.428 ± 0.46 % in T1DM without bacterial infection and 3.077 ± 0.26 % in T1DM with bacterial infections compared with control 6.547 ± 0.36 % . The results also did not observe a distinct alteration in the overall frequency of total peripheral

CD19+ B-cells in T1DM children with UTIs compared patients without UTIs. Consequently, this significant result was agreed with Thompson, *et al*., (2014).

Hanley and his colleagues used fresh peripheral blood and larger patient's numbers to study circulating B lymphocyte by flow cytometric analysis. They found that T1D patients had decreased percentages of B10 B cells compared to healthy controls the mean percentage of CD19+B cells was 10.09 ± 5.77 for T1D patients and 11.97 ± 5.93 for controls (Hanley *et al*., 2017) .

The result is inconsistent with what was found El-Mokhtar *et al*., (2020) they did not observe any significant difference in the frequency of total CD19+B-cells between T1DM patients and controls , the mean of CD19+ B-cells percentage was 14 ± 6.6 and 9.7 ± 2.1 in T1DM patients and controls , respectively.

These findings suggest that peripheral B cells maturation is disrupted in up to 50% of patients with T1DM. This decline may be involved in loss of self-tolerance and β-cellsdestruction and could be used as a biomarker and potential target for immunological intervention. Overall, the pathogenesis of type 1 diabetes is intricate and multi-staged, and it necessitates a variety of disease-causing cellstypes. It is also obvious that regulatory cells, which comprise both T and B cells subsets, act as a counterbalance to these pathogenic cells. Understanding the functions of these less well-studied B-reg subgroups will be crucial for future research in humans with the goal of expanding treatment options.

5.4.2 The frequency of B-reg cells in B-lymphocyte

T1DM may be impacted by a range of regulatory actions that B10 cells have that are IL-10 dependent. Multiple pathways involving the immune system's innate and adaptive components are involved in how IL-10 reduces inflammation, include the reduction of co-stimulatory molecules such MHC-II, which lowers T-cellsactivation, and the downregulation of proinflammatory cytokine production. B10 cells restrict the proliferation of Ag-specific CD4+CD25+T-cells and control the activation of monocytes,

all of which are negatively regulated by dendritic cells' capacity to present antigen (Kalampokis *et al*., 2013).

Although evidence has been provided in mouse models that IL-10+B-cells can control autoimmune diabetes Hussain and Delovitch, (2007), the proportion of IL-10-producing B-cells that have manifested illness has rarely been studied. Therefore was limited grasp of how these B-reg subgroups contribute to the pathophysiology of this illness. In this study was focused on B-reg frequency for T1DM human disease in the current investigation. The data showed that a significant decreased of the percentage of B-reg in T1DM with UTIs compared to T1DM without UTIs and control. The frequency of B-reg increased in the group of T1DM without bacterial infection , the mean of percentage was (33.46 ± 5.26) % compared to infected patients, suggesting that the bacterial infections led to a decrease in the frequency of regulated B cells (Figure 4.6).

The heterogeneity of numerically Breg, both in T1DM with bacterial infection and T1DM without bacterial infection, led to the hypothesis that any B-cells can differentiate into a B-reg depending on their prevailing environment, rather than a subset derived from a distinct lineage. Indeed, signals required for the induction or the promotion of regulatory B-cells are the result of an activated inflammatory environment, including pro-inflammatory cytokines, engagement of Toll-like receptors (TLRs) and costimulatory signals.

In autoimmune diseases, high B-reg frequency is linked to preventing the development of more severe phases of inflammation. Although the roles played by these cells subsets are very similar to those of T-reg, it is thought that these roles alternate during inflammation, with B-reg being more active at the beginning of inflammation and T-reg acting at the conclusion. Numerous studies that show that B-reg are isolated more frequently in healthy people but eventually get depleted as inflammatory responses intensify support this idea . Studies concentrating on various inflammatory responses utilizing murine and human models have noted the similar "depletion-reappearing" characteristic of these cells (Motaung and Loxton, 2019).

5.4.3 Frequency of CD19+IL10 +CD24+CD27+ B-reg cells

IL-10 competence remains the best phenotypic marker for defining human B10-cells, which were readily identified by their ability to express cytoplasmic IL-10 by intracellular staining flow cytometry. However, freshly isolated blood B10 and B10pro cells have express important surface markers that contribute to and from its organizational work as $CD24^{hi}CD27+$. Demonstrate the results of this study reduction in the number of CD19+IL10+CD24+CD27+ B-reg could be responsible for breaking immune tolerance and for T1DM development in children (Figure 4.6). This CD19+IL10+CD24+CD27+ B-reg has a significant immunoregulatory potential for both innate and acquired immunity. It is involved in the resolution of inflammation during acute infections at the local and systemic level. It can inhibit the activity of Th1, natural killer cells and macrophages, so it may have equal capability to detract pathogen clearance as well as to prevent tissue damage.

5.4.4.Expression of co-stimulatory molecules CD80 on B-reg

The functions and mechanisms of co-stimulatory molecules in the etiology of diabetes have up until now remains poorly understood, despite extensive basic and clinical research of these molecules . Co-stimulatory dyads frequently play a paradoxical role in the development of diabetes because of the disease's complex and specific microenvironment. Following are some potential explanations for the conflicting functions of co-stimulatory dyads in diabetes mellitus:1) The maintenance of T-reg populations requires the basal expression of co-stimulatory molecules like B7-1 (CD80) and B7-2 (CD86) ; 2) Different co-stimulatory molecules may be differentially regulated by a variety of inflammatory cytokines during the course of diabetes; 3) CD80 can lead to induction of hypoproliferative T cells that produce both IL-10 and TGF-β1 and act as adaptive Treg (Perez et al 2008) and 4) Different co-stimulatory molecules have distinct effects on different cells populations, which results in disparate outcomes (Zhong *et al*., 2018).

Although, in this study a slightly elevated or equal expression of CD80 B-reg for T1DM compared with control, but the concentration of CD80+ molecule on B-reg decreased on the surface of B-reg in T1DM patient for both groups compared to control (Figure 4.8 and Figure 4.9).

In a mouse model of colitis, groundbreaking investigations revealed that CD80 is important in B cells inhibition of pathogenic T cells and in avoiding disease development (Mann *et al*., 2007). These findings were further supported for human IL-10+ Breg, where IL-10, CD80, and CD86 cooperate to inhibit Th1 responses (Hu *et al*., 2019). Studies in both people and mice have shown that CD80/CD86 and CD28 contact is necessary for peripheral Treg homeostasis, whereas CTLA-4 engagement is crucial for Treg-mediated suppression (Salomon *et al*., 2000). Later, it was discovered that CD80/CD86-deficient B cells could not activate T-reg (Mann *et al*., 2007).

It has been established that B-cells antigen presentation is necessary for the best effector immune reactions. However, antigen presentation by B-cells lacking CD80 can result in anergy or T-reg in T-cells (Mauri and Blair, 2010).

5.5. Measurement of some physiological parameter levels

5.5.1 Lipid profile

One of the primary findings was that a higher mean of CHOL, TG, and HDL-C levels increased for both T1DM groups compared with controls (Figure 4.15, Figure 4.16 and Figure 4.19), respectively . Lipid profile is influenced by T1DM, because of a lower intake of carbohydrates and fiber accompanied by a higher intake of saturated fats, compared with an average diet. A recent systematic review published in 2019 highlighted on the association between increased prevalence of weight gain, high blood glucose levels, and a worse lipid profile in T1DM (Ewers *et al*., 2019). A recent large population study showed improved lipid profiles in children and adolescents with T1DM treated with continuous subcutaneous insulin infusion (CSII) therapy as compared with injection therapy (IT) (Kosteria *et al*., 2019). Individuals with T1DM have a high risk of endothelial dysfunction, cardiovascular morbidity and mortality (Tell *et al*., 2020).

When related to UTIs, CHOL, TG, TC/HDL-C, LDL-C, HDL-C, and Non-HDL-C levels were significantly higher in positive than in negative cases. Similar results were previously reported (Kayar *et al*., 2015). The fact that UTIs cause high levels of interleukin-6 and interleukin-8 may help to explain the link between lipid profile and UTI infection (Al Rushood et al 2020). An increase in these cytokines may 1) cause endothelial dysfunction and insulin resistance (Rehman andAkash, 2016) , 2) Pro-inflammatory cytokines play a crucial role in the connection between metabolic and liver problems in the fat buildup by increasing the generation of hepatic gluconeogenesis and triglycerides and altering the lipid levels by blocking lipoprotein lipase activity and activating hepatic lipogenesis (Das and Balakrishnan, 2011).

5.5.2. Liver functions

The metabolism, digestion, detoxification, and removal of chemicals from the body are all significantly influenced by the liver. Alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) are frequently included in liver function tests. Depending on the pattern of elevation, these tests can assist in organizing a differential diagnosis and in identifying a potential site of liver injury (Lala *et al*., 2021). Hepatocellular disease is indicated by increases in ALT and AST that are not proportional to increase in alkaline phosphatase and bilirubin. A cholestatic pattern would be characterized by an increase in alkaline phosphatase and bilirubin that is out of proportion to ALT and AST (Kwo *et al*., 2017).

In the present results, the mean concentration of ALT and AST significantly decreased in T1DM serum compared to control group,with urinary tract infection it was more lower (Figure 4. 21 and Figure 4.22), respectively. The mean activates of ALP was significantly elevated in T1DM without UTIs compared to controls (Figure 4.23). On the other hand, the decrease was in ALP levels for T1DM with UTIs compared to T1DM without UTIs and controls. That is, the bacterial infection led to a significant decrease in the concentration.

The factors which can affect liver enzyme include BMI, activity, and race of African Americans (20% lower in exercisers). Metronidazole's relatively high concentration and absorbance near 340 nm could lead it to interfere with ALT procedures (Knorr *et al*., 2012),

5.5.3. Kidney functions

In the present results there were significant increases in the levels of total serum bilirubin and creatinine in T1DM with UTIs when compared to controls and T1DM without UTIs (Figure 4.24 and Figure 4.26), respectively. On the other hand, a general decrease was observed in T1DM without UTIs compared to the other two groups. But with regard to the blood urea concentration, the opposite was seen, it increased in T1DM without UTIs and decreased in T1DM with UTIs compared to healthy individuals (Figure 4.25).

Creatinine is a waste product that is typically filtered from the blood and expelled with the urine. Urea is created by the liver as a byproduct of protein breakdown. Changes in the levels of creatinine and urea, indices of kidney function, can signal renal disorders (Debra Manzella, 2008). As a result, the findings suggested that many of the T1DM study participants still have early-stage illness. The fact that more than half of the patients had diabetes for less than five years was clear evidence of this.

Bacterial infections led to an increase in the concentration of this titrant, which is an indication of the onset of kidney damage caused by it . Nevertheless, it was challenging to pinpoint the beginning of such changes in TSB and creatinine concentrations, which may have produced contentious findings (Varghese *et al*., 2001; El-Meligi *et al*., 2003). To ascertain how much function the kidneys have, the creatinine levels must be carefully monitored, and this does fluctuate slightly.

5.6. Distribution of the study population by the age and comparative some immunological parameters

Age at diagnosis might be important in type 1 diabetes. It could carry information about—and thus act as a proxy for—several important factors, such as total glycaemic load, varying autoimmune mechanisms, age-related variations in clinical care, differences in ability to cope with the disease, and so on (Du *et al.,* 2022 ; Scherm *et al.*, 2022).

Accordingly, current study showed that the mean value of CD19+IL10+ CD24+CD27+ cells percentage was higher for period (11-16 year) and lower mean value was for period (5-10 year) for all groups (Figure 4.27). The concentration of co-stimulatory molecules CD80 on B-reg was no significant differences between the diabetic patients compared with control groups but the concentration of CD80 molecule was significantly elevated for period (5-10 year) and lowest mean value was for period (11-16year) for all groups**.**

In a study by Coppieters *et al.,* (2012) it was found that people with a younger age of onset and with short disease duration generally have a more varied amount and character of immune cells entering the islets. Consecutive sections from HLA-A2–expressing individuals were probed for CD8 T-cells reactivity against six defined islet autoantigens associated with T1DM by in situ tetramer staining. Both single and multiple CD8 T cells autoreactivities were detected within individual islets in a subset of T1D patients with disease duration ranging from 1 wk to 8 yr after clinical diagnosis.

The results of the study carried out by Leete *et al.,* (2016) showed, that the extent of B cells infiltration of islets is directly associated with age at diabetes onset. Immune infiltration of islets is more aggressive in individuals diagnosed < 7 y and includes significant numbers of CD20+ B cells ($CD20^{hi}$), compared with patients diagnosed 13 y, in whom a more pauci-immune response is observed, with very few CD20+ B cells ($CD20_{Lo}$) .

5.7. Distribution of the diabetic patients by the duration of the disease and comparative some immunological parameters

Result of current study showed that the mean value of CD19+IL10 CD24+CD27+ B-reg was higher for period (6-10 year) disease duration for T1DM with UTIs and T1DM without UTIs compared to period (1-5 year) disease duration for the same groups as shown in Figure 4.30. The percentage of CD80+ B-reg was significantly elevated in (1-5 years) compared with patients (6-10 years) in diabetic patients with bacterial infections group (Figure 4.31). The mean value of concentration of CD80 molecule of CD80+B -reg was higher for period (1-5 year) disease duration for diabetic patients compared to period (6-10 year) disease duration as shown in Figure 4.32.

The innate and adaptive immune systems are both involved in the three stages of T1DM development, which typically spans several years. Stages one and two, which are referred to as "prediabetes" because they occur prior to the clinical diagnosis, are identified by the emergence of two or more autoantibodies, which are followed by dysglycemia (when blood glucose levels are higher than normal after a tolerance test but do not meet the diagnostic criteria for diabetes) (Insel *et al*., 2015). The third stage, Hyperglycemia, a condition with high blood glucose levels, and the emergence of symptoms are what lead to the diagnosis. The prevailing consensus is that -cells destruction occurs before symptoms manifest, and with time, autoantibody levels should decrease (Katsarou *et al*., 2017). The idea that T1DM is highly heterogeneous and that disease progression can depend on a variety of factors, including age at diagnosis, family history, immunogenetic profile, and disease duration, have been challenged by studies that found a positive correlation between T cells autoimmunity to islet antigens and disease duration.

5.8. Isolation of bacteria from T1DM with urinary tract infections

The most prevalent bacterial infection in humans is the urinary infection, and the urinary system is the second most common site for bacterial infection after the respiratory tract. The prevalence of DM is dramatically rising and is emerging as a severe public health issue, particularly in developing nations. It has a long-term impact on the prevalence of UTI, which has been found to be around four times greater in diabetic individuals compared to non-diabetic ones (Patil *et al*., 2012). The most common bacteria isolated in the current study were Escherichia coli 35% ,*Pseudomonas stutzeria* 19% and *Kocuria* rosea19%. This was shown by a previous study conducted by Alekseenko *et al*. (2018) who reported that *E.coli* and *Klebsiella* spp. were commonly associated with UTIs for T1DM (Table 4.1).

E coli is the most frequent cause of UTI in both men and women with DM. However multiple series show that this organism only accounts for a small part of both nosocomial and community-acquired UTI in diabetic patients when compared to age-matched nondiabetic individuals. It is challenging to generalize these results because most series consist of unselected participants, comprising both type 1 and type 2 diabetes, both sexes, and large age ranges, and many studies were not controlled for concomitant illnesses(Al-Ofairi *et al*., 2018).

The exact reasons are still unknown for UTIs greater in diabetic individuals compared to non-diabetic ones. A few studies have shown that the reason could be the presence of static pools of urine due to dysfunctional bladders contracting poorly, immunodeficiency, a modified urothelium (resulting in a higher bacterial adhesion), which serves a favorable media for bacterial growth while others suggest that hyperglycemic urine promotes rapid bacterial growth and colonization (Patil *et al*., 2012).

A systemic inflammatory response brought on by bacterial infections can result in serious organ damage or even death. Acute and chronic inflammations are greatly influenced by bacterial endotoxins and LPS. LPS causes the innate immune response, which is marked by the release of cytokines and the activation of the immune system. A

distinctive glycolipid called LPS is found at the surface of Gram-negative bacteria. Different areas of the body, such as the oral mucosa and the gastrointestinal, genitourinary, and respiratory tracts, are susceptible to colonization by these potentially dangerous bacteria (Wendel *et al*., 2007; Simonsen *et al*., 2021).

Upon bacterial stimulation, tissue macrophages produce a variety of cytokines that orchestrate the immune response that clears the infection, peripheral blood mononuclear cells (PBMCs) from human infected with Gram-negative bacteria are more likely to produce IL-6, IL-8, and IL-10 than Gram-positive bacteria, which produce higher quantities of interleukin-12 (IL-12), interferon- (IFN-), and tumor necrosis factor (TNF) (Jönsson, 2009).

Conclusions

1- The percentages of all populations of CD19+B cells and CD19+IL10 +CD24+CD27+ B-reg were significantly decreased in both T1DM patients groups compared to control.

2- The percentage of CD19+IL-10+ B cells was significantly decreased in T1DM with bacterial infection group compared to control group but increased in the group of T1DM without bacterial infection, This result indicated that bacterial infections led to minimize the frequency of regulatory B-cells.

3- Bacterial infection caused a significantly increased of co-stimulatory molecules CD80 expression on B-reg, but it had no effect on its mean fluorescence intensity on B-reg cells surface.

4- The difference in the cytokines concentration for T1DM children with bacterial infection, may involve in the loss of auto-tolerance ensuing death of pancreatic cells.

Recommendations

1- Further research is highly recommended on regulatory B cell, as well as costimulation by Breg and pathways involved in causing and developing for type1diabetes patients and other autoimmune diseases.

2-Estimation of immune cells phenotypes changes is needed for infected with bacterial infections to determine the reason behind the immune imbalance that occurs when infected.

3- Further research is highly recommended on urinary tract infection among other autoimmune diseases.

4- Molecular study on B-reg change in different autoimmune disease or different severity in the one autoimmune disease.

References

References

Abraham, S. N., & Miao, Y. (2015). The nature of immune responses to urinary tract infections. *Nature reviews. Immunology*, *15*(10), 655–663. https://doi.org/10.1038/nri3887

Ahmad, S. F., Zoheir, K. M., Ansari, M. A., Nadeem, A., Bakheet, S. A., Al-Hoshani, A. R., ... & Attia, S. M. (2015). Histamine 4 receptor promotes expression of costimulatory B7. 1/B7. 2 molecules, CD28 signaling and cytokine production in stress-induced immune responses. *Journal of Neuroimmunology*, *289*, 30-42.

Akil, A. A. S., Yassin, E., Al-Maraghi, A., Aliyev, E., Al-Malki, K., & Fakhro, K. A. (2021). Diagnosis and treatment of type 1 diabetes at the dawn of the personalized medicine era. *Journal of Translational Medicine*, *19*(1), 1-19.

Akkaya, M., Kwak, K., & Pierce, S. K. (2020). B cell memory: building two walls of protection against pathogens. *Nature Reviews Immunology*, *20*(4), 229-238.

Al Rushood, M., Al-Eisa, A., & Al-Attiyah, R. (2020). Serum and Urine Interleukin-6 and Interleukin-8 Levels Do Not Differentiate Acute Pyelonephritis from Lower Urinary Tract Infections in Children. *Journal of inflammation research*, *13*, 789–797. https://doi.org/10.2147/JIR.S275570

Althuwaiqeb, S. A., & Bordoni, B. (2022). Histology, B Cell Lymphocyte. In *StatPearls [Internet]*. StatPearls Publishing.

American Diabetes Association. (2021). Classification and diagnosis of diabetes: standards of medical care in diabetes—2021. Diabetes care, 44(Supplement 1), S15-S33.

Atkinson, M. A. (2012). The pathogenesis and natural history of type 1 diabetes. *Cold Spring Harbor perspectives in medicine*, *2*(11), a007641.

Baugh, J. A., & Bucala, R. (2002). Macrophage migration inhibitory factor. Critical care medicine, 30(1), S27-S35.

References

Blair, P. A., Noreña, L. Y., Flores-Borja, F., Rawlings, D. J., Isenberg, D. A., Ehrenstein, M. R., & Mauri, C. (2010). CD19+ CD24hiCD38hi B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic lupus erythematosus patients. *Immunity*, *32*(1), 129-140.

Bloem, S. J., & Roep, B. O. (2017). The elusive role of B lymphocytes and islet autoantibodies in (human) type 1 diabetes. *Diabetologia*, *60*(7), 1185-1189.

Bouaziz, J. D., Calbo, S., Maho-Vaillant, M., Saussine, A., Bagot, M., Bensussan, A., & Musette, P. (2010). IL-10 produced by activated human B cells regulates CD4+ T-cell activation in vitro. *European journal of immunology*, *40*(10), 2686-2691.

Burrack, A. L., Martinov, T., & Fife, B. T. (2017). T Cell-Mediated Beta Cell Destruction: Autoimmunity and Alloimmunity in the Context of Type 1 Diabetes. Frontiers in endocrinology, 8, 343. https://doi.org/10.3389/fendo.2017.00343.

Calandra, T., & Roger, T. (2003). Macrophage migration inhibitory factor: a regulator of innate immunity. *Nature reviews immunology*, *3*(10), 791-800.

Cardoso, A. P., Goncalves, R. M., Antunes, J. C., Pinto, M. L., Pinto, A. T., Castro, F., ... & Oliveira, M. J. (2015). An interferon-γ-delivery system based on chitosan/poly (γ-glutamic acid) polyelectrolyte complexes modulates macrophage-derived stimulation of cancer cell invasion in vitro. *Acta biomaterialia*, *23*, 157-171.

Care, D. (2022). Care in Diabetes—2022. *Diabetes Care*, *45*, S17.

Caso, F., Costa, L., Nucera, V., Barilaro, G., Masala, I. F., Talotta, R., ... & Atzeni, F. (2018). From autoinflammation to autoimmunity: old and recent findings. *Clinical Rheumatology*, *37*(9), 2305-2321.

Castañeda-Sánchez, J. I., Duarte, A. R. M., Domínguez-López, M. L., Cruz-López, J. J. de la, & Luna-Herrera, J. (2017). B Lymphocyte as a Target of Bacterial Infections. InTech. doi: 10.5772/intechopen.69346

References

Catalán, D., Mansilla, M. A., Ferrier, A., Soto, L., Oleinika, K., Aguillón, J. C., & Aravena, O. (2021). Immunosuppressive mechanisms of regulatory B cells. Frontiers in Immunology, 12, 611795.

Cederbom, L., Hall, H., & Ivars, F. (2000). CD4+ CD25+ regulatory T cells down-regulate co-stimulatory molecules on antigen-presenting cells. *European journal of immunology*, *30*(6), 1538-1543.

Cerutti, A., Cols, M., & Puga, I. (2013). Marginal zone B cells: virtues of innate-like antibody-producing lymphocytes. *Nature Reviews Immunology*, *13*(2), 118-132.

Chaurasia, R., & Jain, A. (2019). Self Assessment & Review of Microbiology & Immunology. JP Medical Ltd.

Chen, C., Xu, H., Peng, Y., Luo, H., Huang, G. X., Wu, X. J., ... & Xu, J. F. (2020). Elevation in the counts of IL-35-producing B cells infiltrating into lung tissue in mycobacterial infection is associated with the downregulation of Th1/Th17 and upregulation of Foxp3+ Treg. *Scientific Reports*, *10*(1), 1-14.

Chen, J. H., Lin, X., Bu, C., & Zhang, X. (2018). Role of advanced glycation end products in mobility and considerations in possible dietary and nutritional intervention strategies. Nutrition & metabolism, 15(1), 1-18.

Ciesielska, A., Matyjek, M., & Kwiatkowska, K. (2021). TLR4 and CD14 trafficking and its influence on LPS-induced pro-inflammatory signaling. *Cellular and molecular life sciences CMLS*, *78*(4), 1233–1261. https://doi.org/10.1007/s00018-020-03656-y

Cognasse, F., Hamzeh-Cognasse, H., Lafarge, S., Chavarin, P., Pozzetto, B., Richard, Y., & Garraud, O. (2008). Identification of two subpopulations of purified human blood B cells, CD27- CD23+ and CD27high CD80+, that strongly express cell surface Toll-like receptor 9 and secrete high levels of interleukin-6. *Immunology*, *125*(3),430–437.https://doi.org/10.1111/j.13652567.2008.02844.x

References

Collison, L. W., Workman, C. J., Kuo, T. T., Boyd, K., Wang, Y., Vignali, K. M., ... & Vignali, D. A. (2007). The inhibitory cytokine IL-35 contributes to regulatory T-cell function. Nature, 450(7169), 566-569.

Coppieters, K. T., Dotta, F., Amirian, N., Campbell, P. D., Kay, T. W., Atkinson, M. A., ... & von Herrath, M. G. (2012). Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. *Journal of Experimental Medicine*, *209*(1), 51-60.

Couper, K. N., Blount, D. G., & Riley, E. M. (2008). IL-10: the master regulator of immunity to infection. *The Journal of Immunology*, *180*(9), 5771-5777.

Dahman, L. S. B., Sumaily, K. M., Sabi, E. M., Hassan, M. A., Thalab, A. M. B., Sayad, A. S., ... & Alhadhrmi, F. M. (2022). A Comparative Study for Measuring Serum Ferritin Levels with Three Different Laboratory Methods: Enzyme-Linked Immunosorbent Assay versus Cobas e411 and Cobas Integra 400 Methods. *Diagnostics*, *12*(2), 320.

Das, S. K., & Balakrishnan, V. (2011). Role of cytokines in the pathogenesis of non-alcoholic fatty liver disease. *Indian Journal of Clinical Biochemistry*, *26*, 202-209.

De Giacomo, A., Gargano, C. D., Simone, M., Petruzzelli, M. G., Pedaci, C., Giambersio, D., ... & Ruggieri, M. (2021). B and T immunoregulation: a new insight of B regulatory lymphocytes in autism spectrum disorder. *Frontiers in Neuroscience*, 1381.

Dekaboruah, E., Suryavanshi, M. V., Chettri, D., & Verma, A. K. (2020). Human microbiome: an academic update on human body site specific surveillance and its possible role. *Archives of microbiology*, *202*, 2147-2167.

Dema, B., & Charles, N. (2016). Autoantibodies in SLE: specificities, isotypes and receptors. *Antibodies*, *5*(1), 2.

References

Dhande, S., Khan, M., Muglikar, S., Chaudhari, S., Jangale, S. A., & Jangale, A. G. (2022). Diabetes and Periodontal Disease: The Reciprocal Relationship. Journal of General Dentistry, Vol.3, Issue 2, 001-008.

do Nascimento de Oliveira, V., Lima-Neto, A. B. M., Van Tilburg, M. F., de Oliveira Monteiro-Moreira, A. C., Duarte Pinto Lobo, M., Rondina, D., ... & Guedes, M. I. F. (2018). Proteomic analysis to identify candidate biomarkers associated with type 1 diabetes. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, 289-301.

Du, C., Whiddett, R. O., Buckle, I., Chen, C., Forbes, J. M., & Fotheringham, A. K. (2022). Advanced Glycation End Products and Inflammation in Type 1 Diabetes Development. *Cells*, *11*(21), 3503. https://doi.org/10.3390/cells11213503

El-Mokhtar, M. A., Elsherbiny, N. M., Sayed, D., Raafat, D. M., Askar, E., Hussein, A., ... & Shalaby, A. M. (2020). Altered regulatory B cell subsets in children with type 1 diabetes mellitus. *Journal of Immunology Research*, *2020*.

Espes, D., Singh, K., Sandler, S., & Carlsson, P. O. (2017). Increased interleukin-35 levels in patients with type 1 diabetes with remaining C-peptide. *Diabetes Care*, *40*(8), 1090-1095.

Ewers, B., Trolle, E., Jacobsen, S. S., Vististen, D., Almdal, T. P., Vilsbøll, T., & Bruun, J. M. (2019). Dietary habits and adherence to dietary recommendations in patients with type 1 and type 2 diabetes compared with the general population in Denmark. *Nutrition*, *61*, 49-55.

Ferreira, L. M., Muller, Y. D., Bluestone, J. A., & Tang, Q. (2019). Next-generation regulatory T cell therapy. *Nature reviews Drug discovery*, *18*(10), 749-769.

Garib, F. Y., & Rizopulu, A. P. (2015). T-regulatory cells as part of strategy of immune evasion by pathogens. *Biochemistry (Moscow)*, *80*, 957-971.

References

Gouda, W., Mageed, L., Abd El Dayem, S. M., Ashour, E., & Afify, M. (2018). Evaluation of pro-inflammatory and anti-inflammatory cytokines in type 1 diabetes mellitus. *Bulletin of the National Research Centre*, *42*(1), 1-6.

Goyal, A., Gupta, Y., Singla, R., Kalra, S., & Tandon, N. (2020). American diabetes association "standards of medical care—2020 for gestational diabetes mellitus": a critical Appraisal. *Diabetes Therapy*, *11*, 1639-1644.

Günther, S., Fagone, P., Jalce, G., Atanasov, A. G., Guignabert, C., & Nicoletti, F. (2019). Role of MIF and D-DT in immune-inflammatory, autoimmune, and chronic respiratory diseases: From pathogenic factors to therapeutic targets. *Drug discovery today*, *24*(2), 428-439.

Ha, E. K., Kim, J. H., Yon, D. K., Lee, S. W., Kim, M., Lee, K. S., ... & Han, M. Y. (2021). Association of serum lipopolysaccharide-binding protein level with sensitization to food allergens in children. *Scientific reports*, *11*(1), 1-8.

Halliday, N. A. (2019). *CD4 regulatory T cells: their requirement for CD80 and CD86 costimulation and their role in autoimmune hepatitis* (Doctoral dissertation, UCL (University College London)).

Hanley, P., Sutter, J. A., Goodman, N. G., Du, Y., Sekiguchi, D. R., Meng, W., ... & Prak, E. T. L. (2017). Circulating B cells in type 1 diabetics exhibit fewer maturation-associated phenotypes. *Clinical Immunology*, *183*, 336-343.

Herzenberg, L. A. (2000). B-1 cells: the lineage question revisited. *Immunological reviews*, *175*(1), 9-22.

Hillier, T. A., Pedula, K. L., Ogasawara, K. K., Vesco, K. K., Oshiro, C. E., Lubarsky, S. L., & Van Marter, J. (2021). A pragmatic, randomized clinical trial of gestational diabetes screening. *New England Journal of Medicine*, *384*(10), 895-904.

References

Hlela, C., Shepperd, S., Khumalo, N. P., & Taylor, G. P. (2009). The prevalence of human T-cell lymphotropic virus type 1 in the general population is unknown. *Aids Rev*, *11*(4), 205-214. https://doi.org/10.2337/dc22-S002.

Hu, H. T., Ai, X., Lu, M., Song, Z., & Li, H. (2019). Characterization of intratumoral and circulating IL-10-producing B cells in gastric cancer. *Experimental cell research*, *384*(2), 111652.

Huai, G., Markmann, J. F., Deng, S., & Rickert, C. G. (2021). TGF-β-secreting regulatory B cells: unsung players in immune regulation. *Clinical & translational immunology*, *10*(4), e1270. https://doi.org/10.1002/cti2.1270

Hussain, S., & Delovitch, T. L. (2007). Intravenous transfusion of BCR-activated B cells protects NOD mice from type 1 diabetes in an IL-10-dependent manner. *The Journal of Immunology*, *179*(11), 7225-7232.

Insel, R. A., Dunne, J. L., Atkinson, M. A., Chiang, J. L., Dabelea, D., Gottlieb, P. A., ... & Ziegler, A. G. (2015). Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. *Diabetes care*, *38*(10), 1964-1974.

Ismail, N. A., Abd El Baky, A. N., Ragab, S., Hamed, M., Hashish, M. A., & Shehata, A. (2016). Monocyte chemoattractant protein 1 and macrophage migration inhibitory factor in children with type 1 diabetes. *Journal of Pediatric Endocrinology and Metabolism*, *29*(6), 641-645.

Iwata, Y., Matsushita, T., Horikawa, M., DiLillo, D. J., Yanaba, K., Venturi, G. M., ... & Tedder, T. F. (2011). Characterization of a rare IL-10–competent B-cell subset in humans that parallels mouse regulatory B10 cells. *Blood, The Journal of the American Society of Hematology*, *117*(2), 530-541.

Kakleas, K., Soldatou, A., Karachaliou, F., & Karavanaki, K. (2015). Associated autoimmune diseases in children and adolescents with type 1 diabetes mellitus (T1DM). *Autoimmunity reviews*, *14*(9), 781-797.

References

Kalampokis, I., Yoshizaki, A., & Tedder, T. F. (2013). IL-10-producing regulatory B cells (B10 cells) in autoimmune disease. *Arthritis research & therapy*, *15 Suppl 1*(Suppl 1), S1. https://doi.org/10.1186/ar3907.

Karananou, P., Fleva, A., Tramma, D., Alataki, A., Pavlitou-Tsiontsi, A., Emporiadou-Peticopoulou, M., & Papadopoulou-Alataki, E. (2016). Altered expression of TLR2 and TLR4 on peripheral CD14+ blood monocytes in children with urinary tract infection. *BioMed research international*, *2016*.

Katsarou, A., Gudbjörnsdottir, S., Rawshani, A., Dabelea, D., Bonifacio, E., Anderson, B. J., ... & Lernmark, Å. (2017). Type 1 diabetes mellitus. *Nature reviews Disease primers*, *3*(1), 1-17.

Kendall, P. L., Yu, G., Woodward, E. J., & Thomas, J. W. (2007). Tertiary lymphoid structures in the pancreas promote selection of B lymphocytes in autoimmune diabetes. *The Journal of Immunology*, *178*(9), 5643-5651.

Kennedy, A., Waters, E., Rowshanravan, B., Hinze, C., Williams, C., Janman, D., ... & Sansom, D. M. (2022). Differences in CD80 and CD86 transendocytosis reveal CD86 as a key target for CTLA-4 immune regulation. *Nature Immunology*, *23*(9), 1365-1378.

Knorr, J. P., Javed, I., Sahni, N., Cankurtaran, C. Z., & Ortiz, J. A. (2012). Metronidazole-induced encephalopathy in a patient with end-stage liver disease . *Case Reports in Hepatology*, Volume 2012 | Article ID 209258 | https://doi.org/10.1155/2012/209258.

Kosteria, I., Schwandt, A., Davis, E., Jali, S., Prieto, M., Rottembourg, D., & SWEET study group. (2019). Lipid profile is associated with treatment regimen in a large cohort of children and adolescents with Type 1 diabetes mellitus: a study from the international SWEET database. *Diabetic Medicine*, *36*(10), 1294-1303.

Kucheria, R., Dasgupta, P., Sacks, S., Khan, M., & Sheerin, N. (2005). Urinary tract infections: new insights into a common problem. *Postgraduate medical journal*, *81*(952), 83.

References

Kwo, P. Y., Cohen, S. M., & Lim, J. K. (2017). ACG clinical guideline: evaluation of abnormal liver chemistries. *Official journal of the American College of Gastroenterology| ACG*, *112*(1), 18-35.

Lala, V., Goyal, A., & Minter, D. A. (2021). Liver function tests. In *StatPearls [internet]*. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK482489/

Leete, P., Willcox, A., Krogvold, L., Dahl-Jørgensen, K., Foulis, A. K., Richardson, S. J., & Morgan, N. G. (2016). Differential insulitic profiles determine the extent of β-cell destruction and the age at onset of type 1 diabetes. *Diabetes*, *65*(5), 1362-1369.

Li, C., Jiang, P., Wei, S., Xu, X., & Wang, J. (2020). Regulatory T cells in tumor microenvironment: new mechanisms, potential therapeutic strategies and future prospects. Molecular Cancer, 19(1), 1-23.

Li, M. O., & Flavell, R. A. (2008). TGF-beta: a master of all T cell trades. Cell, 134(3), 392–404. https://doi.org/10.1016/j.cell.2008.07.025

Li, M., Eckl, J., Geiger, C., Schendel, D. J., & Pohla, H. (2017). A novel and effective method to generate human porcine-specific regulatory T cells with high expression of IL-10, TGF-β1 and IL-35. *Scientific Reports*, *7*(1), 1-13.

Li, X., Zhong, H., Bao, W., Boulad, N., Bussel, J. B., & Yazdanbakhsh, K. (2010). Regulatory B cell defects in patients with immune thrombocytopenia (ITP). *Blood*, *116*(21), 379.

Lu, J., Liu, J., Li, L., Lan, Y., & Liang, Y. (2020). Cytokines in type 1 diabetes: mechanisms of action and immunotherapeutic targets. *Clinical & translational immunology*, *9*(3), e1122.

Lue, H., Kleemann, R., Calandra, T., Roger, T., & Bernhagen, J. (2002). Macrophage migration inhibitory factor (MIF): mechanisms of action and role in disease. Microbes and infection, 4(4), 449-460.

References

Lugovaya, A. V., Mitreikin, V. P., & Kalinina, N. M. (2022). The Role of Apoptosis in Autoimmune Destruction of Pancreatic b-Cells. In *Type 1 Diabetes Mellitus*. IntechOpen. DOI: 10.5772/intechopen.108290

Mann, M. K., Maresz, K., Shriver, L. P., Tan, Y., & Dittel, B. N. (2007). B cell regulation of CD4+ CD25+ T regulatory cells and IL-10 via B7 is essential for recovery from experimental autoimmune encephalomyelitis. *The Journal of Immunology*, *178*(6), 3447-3456.

Marcucci, F., & Rumio, C. (2021). Depleting tumor cells expressing immune checkpoint ligands—A new approach to combat cancer. *Cells*, *10*(4), 872.

Mauri, C., & Blair, P. A. (2010). Regulatory B cells in autoimmunity: developments and controversies. *Nature Reviews Rheumatology*, *6*(11), 636-643.

Mayes, P. A., Hance, K. W., & Hoos, A. (2018). The promise and challenges of immune agonist antibody development in cancer. *Nature Reviews Drug Discovery*, *17*(7), 509-527.

Mendivil, C. O., Toloza, F. J., Ricardo-Silgado, M. L., Morales-Álvarez, M. C., Mantilla-Rivas, J. O., Pinzón-Cortés, J. A., & Lemus, H. N. (2017). Antibodies against glutamic acid decarboxylase and indices of insulin resistance and insulin secretion in nondiabetic adults: a cross-sectional study. *Diabetes, metabolic syndrome and obesity : targets and therapy*, *10*, 179–185. https://doi.org/10.2147/DMSO.S137216

Meng, L., Song, Z., Liu, A., Dahmen, U., Yang, X., & Fang, H. (2021). Effects of lipopolysaccharide-binding protein (LBP) single nucleotide polymorphism (SNP) in infections, inflammatory diseases, metabolic disorders and cancers. *Frontiers in Immunology*, *12*, 681810.

Mir, M. A. (2015). Chapter 3—Costimulation immunotherapy in infectious diseases. Developing costimulatory molecules for immunotherapy of diseases, 83-129.

Mirza, B. A., Syed, A., Izhar, F., & Ali Khan, A. (2010). Bidirectional relationship between diabetes and periodontal disease: review of evidence. *Journal of the Pakistan Medical Association*, *60*(9), 766-8.

Miyagaki, T. (2015). Regulatory B cells in human autoimmune diseases. *Nihon Rinshō Men'Eki Gakkai Kaishi= Japanese Journal of Clinical Immunology*, *38*(5), 390-397.

Mohr, A. E., Crawford, M. S., Jasbi, P., Fessler, S., & Sweazea, K. L. (2022). Lipopolysaccharide and the gut microbiota: considering structural variation. *FEBS letters*, *596*(7), 849-875.

Moore, D. K., & Loxton, A. G. (2019). Regulatory B lymphocytes: development and modulation of the host immune response during disease. *Immunotherapy*, *11*(8), 691-704.

Motaung, B., & Loxton, A. G. (2019). Effect of binding immunoglobulin protein on induction of regulatory B cells with killer phenotype during inflammation and disease. *Future Science OA*, *5*(3), FSO379.

Nagendra, L., Boro, H., & Mannar, V. (2022). Bacterial Infections in Diabetes. *Endotext [Internet]*.

Nandi, D., Pathak, S., Verma, T., Singh, M., Chattopadhyay, A., Thakur, S., ... & Gokhroo, A. (2020). T cell costimulation, checkpoint inhibitors and anti-tumor therapy. *Journal of biosciences*, *45*, 1-36.

Nerup, J., Platz, P., Andersen, O. O., Christy, M., Lyngsøe, J., Poulsen, J. E., ... & Svejgaard, A. (1974). HL-A antigens and diabetes mellitus. *The Lancet*, *304*(7885), 864-866.

Ness, D., & Olsburgh, J. (2020). UTI in kidney transplant. World Journal of Urology, 38(1), 81-88.

Nimer, N. A., Al-Saa'da, R. J., and Abuelaish, O. (2016). Accuracy of the VITEK 2 system for a rapid and direct identification and susceptibility testing of Gram-

negative rods and Gram-positive cocci in blood samples. *EMHJ-Eastern Mediterranean Health Journal*, 22(3), 193-200.

Ovcinnikovs, V., Ross, E. M., Petersone, L., Edner, N. M., Heuts, F., Ntavli, E., ... & Walker, L. S. (2019). CTLA-4–mediated transendocytosis of costimulatory molecules primarily targets migratory dendritic cells. *Science immunology*, *4*(35), eaaw0902.

Pascale, A., Marchesi, N., Govoni, S., Coppola, A., & Gazzaruso, C. (2019). The role of gut microbiota in obesity, diabetes mellitus, and effect of metformin: new insights into old diseases. *Current opinion in pharmacology*, *49*, 1-5.

Paudel, S., John, P. P., Poorbaghi, S. L., Randis, T. M., & Kulkarni, R. (2022). Systematic Review of Literature Examining Bacterial Urinary Tract Infections in Diabetes. *Journal of Diabetes Research*, *2022*.

Paul, A. G. (2019). Lab Manual and Workbook in Microbiology 12th Edition. McGraw-Hill Education.

Perez, N., Karumuthil-Melethil, S., Li, R., Prabhakar, B. S., Holterman, M. J., & Vasu, C. (2008). Preferential costimulation by CD80 results in IL-10-dependent TGF-beta1(+) -adaptive regulatory T cell generation. *Journal of immunology (Baltimore,Md.:1950)*, *180*(10),6566–6576. https://doi.org/10.4049/jimmunol.180.10.6566

Pietropaolo, M., Towns, R., & Eisenbarth, G. S. (2012). Humoral autoimmunity in type 1 diabetes: prediction, significance, and detection of distinct disease subtypes. *Cold Spring Harbor perspectives in medicine*, *2*(10), a012831.

Pipi, E., Marketou, M., & Tsirogianni, A. (2014). Distinct clinical and laboratory characteristics of latent autoimmune diabetes in adults in relation to type 1 and type 2 diabetes mellitus. *World journal of diabetes*, *5*(4), 505–510. https://doi.org/10.4239/wjd.v5.i4.505

References

Podojil, J. R., & Miller, S. D. (2009). Molecular mechanisms of T-cell receptor and costimulatory molecule ligation/blockade in autoimmune disease therapy. *Immunological reviews*, *229*(1), 337-355.

Rao, N., & Lipsky, B. A. (2007). Optimising antimicrobial therapy in diabetic foot infections. *Drugs*, *67*, 195-214.

Rehman, K., & Akash, M. S. H. (2016). Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked?. *Journal of biomedical science*, *23*(1), 1-18.

Rico, L. G., Salvia, R., Ward, M. D., Bradford, J. A., & Petriz, J. (2021). Flow-cytometry-based protocols for human blood/marrow immunophenotyping with minimal sample perturbation. *STAR protocols*, *2*(4), 100883.

Rosser, E. C., & Mauri, C. (2015). Regulatory B cells: origin, phenotype, and function. *Immunity*, *42*(4), 607-612.

Sakura, T., Morioka, T., Shioi, A., Kakutani, Y., Miki, Y., Yamazaki, Y., ... & Inaba, M. (2017). Lipopolysaccharide-binding protein is associated with arterial stiffness in patients with type 2 diabetes: a cross-sectional study. *Cardiovascular diabetology*, *16*(1), 1-8.

Salek-Ardakani, S., Choi, Y. S., Rafii-El-Idrissi Benhnia, M., Flynn, R., Arens, R., Shoenberger, S., ... & Salek-Ardakani, S. (2011). B cell-specific expression of B7-2 is required for follicular Th cell function in response to vaccinia virus. *The Journal of Immunology*, *186*(9), 5294-5303.

Salomon, B., Lenschow, D. J., Rhee, L., Ashourian, N., Singh, B., Sharpe, A., & Bluestone, J. A. (2000). B7/CD28 costimulation is essential for the homeostasis of the CD4+ CD25+ immunoregulatory T cells that control autoimmune diabetes. *Immunity*, *12*(4), 431-440.

References

Sánchez-Zamora, Y. I., & Rodriguez-Sosa, M. (2014). The role of MIF in type 1 and type 2 diabetes mellitus. *Journal of diabetes research*, vol. 2014, Article ID 804519, 6 pages, 2014. https://doi.org/10.1155/2014/804519

Sanz, I., Wei, C., Lee, F. E. H., & Anolik, J. (2008, February). Phenotypic and functional heterogeneity of human memory B cells. In *Seminars in immunology* (Vol. 20, No. 1, pp. 67-82). Academic Press.

Saraiva, M., & O'garra, A. (2010). The regulation of IL-10 production by immune cells. *Nature reviews immunology*, *10*(3), 170-181.

Sasano, M., Kimura, S., Maeda, I., & Hidaka, Y. (2017). Analytical performance evaluation of the Elecsys® Cyclosporine and Elecsys® Tacrolimus assays on the cobas e411 analyzer. *Practical laboratory medicine*, *8*, 10-17.

Scapigliati, G., Fausto, A. M., & Picchietti, S. (2018). Fish lymphocytes: an evolutionary equivalent of mammalian innate-like lymphocytes?. *Frontiers in Immunology*, *9*, 971.

Schalkwijk, C. G., & Stehouwer, C. D. A. (2020). Methylglyoxal, a highly reactive dicarbonyl compound, in diabetes, its vascular complications, and other age-related diseases. Physiological reviews, 100(1), 407-461.

Scherm, M. G., Wyatt, R. C., Serr, I., Anz, D., Richardson, S. J., & Daniel, C. (2022). Beta cell and immune cell interactions in autoimmune type 1 diabetes: How they meet and talk to each other. *Molecular Metabolism*, *64*.

Shah, C., Baral, R., Bartaula, B., & Shrestha, L. B. (2019). Virulence factors of uropathogenic Escherichia coli (UPEC) and correlation with antimicrobial resistance. *BMC microbiology*, *19*(1), 1-6.

Shen, P., Roch, T., Lampropoulou, V., O'Connor, R. A., Stervbo, U., Hilgenberg, E., ... & Fillatreau, S. (2014). IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases.Nature, 507(7492), 366-370.

Sumaiya, K., Langford, D., Natarajaseenivasan, K., & Shanmughapriya, S. (2022). Macrophage migration inhibitory factor (MIF): a multifaceted cytokine regulated by genetic and physiological strategies. *Pharmacology & Therapeutics*, *233*, 108024.

Syme, N. R., Toft, A. D., Stoddart, M., & Beckett, G. J. (2011). Clinical performance of the Roche cobas e411 automated assay system for thyrotropin-receptor antibodies for the diagnosis of Graves' disease. *Annals of Clinical Biochemistry*, *48*(5), 471-473.

Tamayo, E., Alvarez, P., & Merino, R. (2018). TGFβ Superfamily Members as Regulators of B Cell Development and Function-Implications for Autoimmunity. *International journal of molecular sciences*, *19*(12), 3928.

Tell, S., Nadeau, K. J., & Eckel, R. H. (2020). Lipid management for cardiovascular risk reduction in type 1 diabetes. *Current opinion in endocrinology, diabetes, and obesity*, *27*(4), 207.

Tenny, S., Kerndt, C. C., & Hoffman, M. R. (2022). Case Control Studies. In *StatPearls*. StatPearls Publishing.

Thayer, T. C., Delano, M., Liu, C., Chen, J., Padgett, L. E., Tse, H. M., ... & Mathews, C. E. (2011). Superoxide production by macrophages and T cells is critical for the induction of autoreactivity and type 1 diabetes. *Diabetes*, *60*(8), 2144-2151.

Thomas, N. J., Lynam, A. L., Hill, A. V., Weedon, M. N., Shields, B. M., Oram, R. A., ... & Jones, A. G. (2019). Type 1 diabetes defined by severe insulin deficiency occurs after 30 years of age and is commonly treated as type 2 diabetes. Diabetologia, 62(7), 1167-1172.

Thompson, W. S., Pekalski, M. L., Simons, H. Z., Smyth, D. J., Castro-Dopico, X., Guo, H., ... & Ferreira, R. C. (2014). Multi-parametric flow cytometric and genetic investigation of the peripheral B cell compartment in human type 1 diabetes. *Clinical & Experimental Immunology*, *177*(3), 571-585.

References

Tullus, K., & Shaikh, N. (2020). Urinary tract infections in children. *The Lancet*, *395*(10237), 1659-1668.

Unnikrishnan, R., & Misra, A. (2020). Infections and diabetes: Risks and mitigation with reference to India. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, *14*(6), 1889-1894.

Vale, A. M., & Schroeder Jr, H. W. (2010). Clinical consequences of defects in B-cell development. *Journal of Allergy and Clinical Immunology*, *125*(4), 778-787.

Vijay K. (2018). Toll-like receptors in immunity and inflammatory diseases: Past, present, and future. *International immunopharmacology*, *59*, 391–412.

Wen, L., Brill-Dashoff, J., Shinton, S. A., Asano, M., Hardy, R. R., & Hayakawa, K. (2005). Evidence of marginal-zone B cell-positive selection in spleen. *Immunity*, *23*(3), 297-308.

Wołoszyn-Durkiewicz, A., & Myśliwiec, M. (2019). The prognostic value of inflammatory and vascular endothelial dysfunction biomarkers in microvascular and macrovascular complications in type 1 diabetes. *Pediatric Endocrinology Diabetes and Metabolism*, *25*(1), 28-35.

Wu, H., Su, Z., & Barnie, P. A. (2020). The role of B regulatory (B10) cells in inflammatory disorders and their potential as therapeutic targets. *International Immunopharmacology*, *78*, 106111.

Xu, L., Li, Y., Sun, H., Zhen, X., Qiao, C., Tian, S., & Hou, T. (2013). Current developments of macrophage migration inhibitory factor (MIF) inhibitors. *Drug discovery today*, *18*(11-12), 592-600.

Ye, C., Yano, H., Workman, C. J., & Vignali, D. A. (2021). Interleukin-35: Structure, function and its impact on immune-related diseases. *Journal of Interferon & Cytokine Research*, *41*(11), 391-406.

References

Yu, C. R., Choi, J. K., Uche, A. N., & Egwuagu, C. E. (2018). Production of IL-35 by Breg is mediated through binding of BATF-IRF-4-IRF-8 complex to il12a and ebi3 promoter elements. Journal of Leukocyte Biology, 104(6), 1147-1157.

Yu, F., Zhu, X., Li, Q., Xu, W., Gao, Y., Wen, Y., ... & Dou, J. (2022). Elevated IL-35 level and iTr35 subset increase the bacterial burden and lung lesions in Mycobacterium tuberculosis-infected mice. *Open Life Sciences*, *17*(1), 312-320

Zajec, A., Trebušak Podkrajšek, K., Tesovnik, T., Šket, R., Čugalj Kern, B., Jenko Bizjan, B., ... & Kovač, J. (2022). Pathogenesis of Type 1 Diabetes: Established Facts and New Insights. *Genes*, *13*(4), 706.

Zhang, S. M., Liang, J., Xia, J. P., Li, L., Zheng, L., Wang, Y. L., ... & Lu, Y. (2023). Interleukin 35: protective role and mechanism in type 1 diabetes. *Central European Journal of Immunology*, *48*(1).

Zhang, S. M., Liang, J., Xia, J. P., Li, L., Zheng, L., Wang, Y. L., ... & Lu, Y. (2023). Interleukin 35: protective role and mechanism in type 1 diabetes. *C.E.J. of Immunology*, *48*(1).

Zhong, J. X., Chen, J., Rao, X., & Duan, L. (2018). Dichotomous roles of co-stimulatory molecules in diabetes mellitus. *Oncotarget*, *9*(2), 2902.

Appendices

Appendices

Appendix 1

Vitek 2 automated report on identification and susceptibility of bacteria (1)

BioMerieux Customer:
Patient Name : 1
Location:
Lab ID:1227

Microbiology Chart Report

Printed June 20,2022 5:58:42 AM CDT
Patient ID:1227
Physician:

Organism Quantity:
Selected Organism : Kocuria rosea

Source:

Collected:

Comments:	

Identification Information	Analysis Time: 7.78 hours	Status: Fnal
Selected Organism	95% Probability Kocuria rosea Bionumber : 0000100000000000	
ID Analysis Messages		

Biochemical Details																	
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	+	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	Dgal	-
38	Drib	-	39	ILATk	-	42	LAC	-	44	NAG	-	45	Dmal	-	46	BACI	-
47	NOVO	-	50	NC6.5	-	52	dMAN	-	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	-	62	dTRE	-	63	ADH2s	-
64	OPTO	-															

Appendices

Appendix 1

Vitek 2 automated report on identification and susceptibility of bacteria (2)

BioMerieux Customer:
Patient Name : 2
Location:
Lab ID:1251

Microbiology Chart Report

Printed July 3,2022 4:40:17 AM CDT
Patient ID:1251
Physician:

Organism Quantity:
Selected Organism : Staphylococcus haemolyticus

Source: **Collected:**

Comments:	

Identification Information	Analysis Time: 7.78 hours	Status: Fnal
Selected Organism	98% Probability Staphylococcus haemolyticus Bionumber : 010006043720271	
ID Analysis Messages		

Biochemical Details																	
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	(-)
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	+
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	Dgal	+
38	dRIB	+	39	ILATk	+	42	LAC	-	44	NAG	+	45	Dmal	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	-	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	+
64	OPTO	+															

Appendices

Appendix 1

Vitek 2 automated report on identification and susceptibility of bacteria (3)

BioMerieux Customer:
Patient Name : 6
Location:
Lab ID:1255

Microbiology Chart Report

Printed July 3,2022 4:09:00 AM CDT
Patient ID:1255
Physician:

Organism Quantity:
Selected Organism : Streptococcus gallolyticus ssp pasteurianus

Source: Collected:

Comments:	

Identification Information	Analysis Time: 7.78 hours	Status: Fnal
Selected Organism	99% Probability **Streptococcus gallolyticus ssp pasteurianus** **Bionumber :** 161011364713731	
ID Analysis Messages		

Biochemical Details																	
2	AMY	+	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	+	11	AGLU	+
13	APPA	+	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	+	23	ProA	-	24	BGURr	-	25	AGAL	+	26	PyrA	-	27	BGUR	-
28	AlaA	+	29	TyrA	+	30	dSOR	-	31	URE	-	32	POLYB	+	37	Dgal	+
38	dRIB	-	39	ILATk	-	42	LAC	+	44	NAG	+	45	Dmal	+	46	BACI	+
47	NOVO	+	50	NC6.5	-	52	dMAN	-	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	+	58	O129R	+	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Appendices

Appendix 1

Vitek 2 automated report on identification and susceptibility of bacteria (4)

BioMerieux Customer:
Patient Name : 5
Location:
Lab ID:1253

Microbiology Chart Report

Printed July 3,2022 7:05:51 AM CDT
Patient ID:1253
Physician:

Organism Quantity:
Selected Organism : Streptococcus thoraltensis
Source: **Collected:**

Comments:	

Identification Information	Analysis Time: 7.78 hours	Status: Fnal
Selected Organism	85% Probability **Streptococcus thoraltensis** **Bionumber :** 177013765773771	
ID Analysis Messages		

Biochemical Details																	
2	AMY	+	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	+	11	AGLU	+
13	APPA	+	14	CDEX	+	15	AspA	+	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	+	23	ProA	-	24	BGURr	-	25	AGAL	+	26	PyrA	+	27	BGUR	-
28	AlaA	+	29	TyrA	+	30	dSOR	+	31	URE	-	32	POLYB	+	37	Dgal	+
38	dRIB	+	39	ILATk	-	42	LAC	+	44	NAG	+	45	Dmal	+	46	BACI	+
47	NOVO	+	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	+	58	O129R	+	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	+
64	OPTO	+															

Appendices

Appendix 1

Vitek 2 automated report on identification and susceptibility of bacteria (5)

BioMerieux Customer:
Patient Name : 1
Location:
Lab ID:1268

Microbiology Chart Report

Printed July 7,2022 4:14:46 AM CDT
Patient ID:1268
Physician:

Organism Quantity:
Selected Organism : Kocuria kristinae

Source: Collected:

Comments:	

Identification Information	Analysis Time: 7.78 hours	Status: Fnal
Selected Organism	90% Probability **Kocuria kristinae** **Bionumber :** 014012702060000	
ID Analysis Messages		

Biochemical Details																	
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	+	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	+	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	+	29	TyrA	+	30	dSOR	+	31	URE	-	32	POLYB	-	37	Dgal	-
38	dRIB	-	39	ILATk	+	42	LAC	-	44	NAG	-	45	Dmal	-	46	BACI	-
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	-	62	dTRE	-	63	ADH2s	-
64	OPTO	-															

Appendices

Appendix 1

Vitek 2 automated report on identification and susceptibility of bacteria (6)

BioMerieux Customer:
Patient Name : 4
Location:
Lab ID:1252

Microbiology Chart Report

Printed July 3,2022 6:51:41 AM CDT
Patient ID:1252
Physician:

Organism Quantity:
Selected Organism : Escherichia coli

Source: Collected:

Comments:	

Identification Information	Analysis Time: 7.78 hours	Status: Fnal
Selected Organism	85% Probability **Escherichia coli** **Bionumber :** 4401610050140210	
ID Analysis Messages		

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	+	5	1ARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BA1ap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	-
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	1LATk	+	41	AGLU	(-)	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	-	53	1HISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	1MLTa	-	62	ELLM	-	64	1LATa	-			

Appendices

Appendix 1

Vitek 2 automated report on identification and susceptibility of bacteria (7)

BioMerieux Customer:
Patient Name : 8
Location:
Lab ID:1246

Microbiology Chart Report

Printed July 1,2022 5:28:03 AM CDT
Patient ID:1246
Physician:

Organism Quantity:
Selected Organism : Pseudomonas stutzeria

Source: Collected:

Comments:	

Identification Information	Analysis Time: 7.78 hours	Status: Fnal
Selected Organism	85% Probability **Pseudomonas stutzeria** **Bionumber :** 0003003100400040	
ID Analysis Messages		

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	1ARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BA1ap	-
23	ProA	+	26	LIP	+	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	-	37	MNT	-	39	5KG	-
40	1LATk	-	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	1HISa	-	56	CMT	-	57	BGUR	-
58	O129R	-	59	GGAA	-	61	1MLTa	+	62	ELLM	-	64	1LATa	-			

Appendices

Appendix 2

Questionnaire

استمارة جمع العينة اوالأستبانة

رقم العينة : **تاريخ اخذ العينة :**

مصاب بالسكري 1 : **العمر:**

الأسم : **تاريخ الولادة :**

محل الولادة : **الجنس :**

العمل: **مدخن :**

مكان السكن الحالي :

مدة الأصابة بداء السكري : نوع العلاج المستخدم :

هل المريض منتظم بأخذ العلاج : الية اخذ العلاج :

هل يعاني المريض من اي امراض اخرى :

هل يعاني المريض من اي اصابات بكتيرية : تذكر ان وجد

هل اصيب مسبقا بألتهابات بكتيرية او فايروسية : تذكر ان وجد:

هل اجرى المريض عملية في السابق : تذكر ان وجد:

عدد ساعات النوم : وقت الأستيقاظ :

عدد افراد العائلة المصابون بالسكري (تذكر قرابتهم من المريض) :

الطول :

الوزن :

محيط الخصر:

نسبة السكر لحضة اخذ العينة :

ظغط الدم لحظة اخذ العينة :

موافقة ولي امر المريض :

اسم ولي الأمر والتوقيع:

رقم الهاتف :

Appendices

Appendix 3

Ethical roles

وزارة الصحة
دائرة صحة كربلاء
مركز التدريب والتنمية البشرية
لجنة البحوث

استمارة رقم ٢٠٢١/٠٣

رقم القرار ٣٧

تاريخ القرار ١٤/٢/٢٠٢٢

قرار لجنة البحوث

درست لجنة البحوث في دائرة صحة كربلاء مشروع البحث ذي الرقم (٢٠٢٢٠٣٢/كربلاء) المعنون:

دور بعض الواسمات المناعية بخطر الاصابة بداء السكري والامراض البكتيرية المصاحبة

والمقدم من الباحثة نور عبد الامير عوده الى وحدة ادارة البحوث والمعرفة في مركز التدريب والتنمية البشرية في دائرة صحة كربلاء بتاريخ ٢٠٢٢/١٢ وقررت:

قبول مشروع البحث اعلاه كونه مستوفيا للمعايير المعتمدة في وزارة الصحة والخاصة بتنفيذ البحوث ولا مانع من تنفيذه في مؤسسات الدائرة.

الدكتور
نعيم عبيد الشهداني
طبيب اخصائي
مقرر لجنة البحوث

14/02/2022

المرفقات:

Choose an item.

ملاحظات:

- تم تخويل عضو لجنة البحوث (د.تقوى خضر عبد الكريم) او مقرر اللجنة (د.نعيم عبيد طلال) للتوقيع على هذا القرار استنادا الى النظام الداخلي للجنة البحوث.
- الموافقة تعني ان مشروع البحث قد استوفى المعايير الاخلاقية والعلمية لإجراء البحث والمعتمدة في وزارة الصحة، اما التنفيذ فيعتمد على التزام الباحث بتعليمات المؤسسة الصحية التي سينفذ فيها البحث.

Appendices

Appendix4

Comprehensive examination committee

Republic of Iraq
Ministry Of Higher Education and Scientific Research
College of Science – University of Kerbala
Division of Postgraduate studies

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء – كلية العلوم
شعبة الدراسات العليا

العدد/ع/6/ 3735
التاريخ/ 28 / 10 / 2021

أمـــر إداري

استناداً إلى مصادقة السيد رئيس الجامعـــة المحترم على أصـــل محضـــر الجـلسة الثالثة المفتوحة لمجلس كليتنا للعـــــام الدراسي 2021-2022 والتـي عقدت للفترة من 14- 2021/10/20 والمرسل إلينا بكتاب امانة مجلس الجامعة ذي العدد /ج/ 1408 بتاريخ 2021/10/26 والمبلغ الينا بكتاب امانة مجلس الكلية ذي العدد م.ك/247 في 2021/10/27 واستنادا للصلاحيات المخولة لنا تقرر:
تشكيل لجنة من التدريسيين المدرجة أسماؤهم في الجدول أدناه لاجراء الامتحان الشامل لطالبة الدراسات العليا/الدكتوراه/ قسم علوم الحياة (**نور عبد الامير عودة**).

علـى ان يكـون موعـد الامتحـان التحريـري فـي يـوم الثلاثـاء الموافـق 2021/12/7 والامتحـان الشـفهي فـي يـوم الثلاثـاء الموافـق 2021/12/21.

ت	اسم التدريسي	اللقب العلمي	المادة الامتحانية المقترحة	المنصب	مكان العمل
1	د. علي حسين ادحية	أستاذ	immunogenetics	رئيسا	كلية العلوم / جامعة بغداد
2	د. ارشد نوري غني	أستاذ	Advanced animal physiology	عضواً	كلية العلوم / جامعة الكوفة
3	د. سهاد هادي محمد	أستاذ	Immunological techniques	عضوا	كلية العلوم الطبية التطبيقية / جامعة كربلاء
4	د. وفاء صادق محسن	أستاذ	Advanced pathogenic bacteria	عضوا	كلية االعلوم/ جامعة كربلاء
5	د. عايد حميد حسن	أستاذ	Blood diseases	عضوا	كلية الطب البيطري / جامعة كربلاء

أ. م.د. جاسم حنون هاشم العوادي
العميد وكالة
2021 / 10 / 28

نسخة منه إلى
- مكتب السيد العميد / للتفضل بالعلم مع التقدير .
- جامعة بغداد/كلية العلوم/ للتفضل بالموافقة على إشراك الأستاذ الدكتور علي حسين ادحية ... مع التقدير ..
- جامعة الكوفة /كلية العلوم / للتفضل بالموافقة على إشراك الاستاذ الدكتور ارشد نوري غني ... مع التقدير ..
- جامعة كربلاء /كلية العلوم التطبيقية / للتفضل بالموافقة على إشراك الاستاذ الدكتور سهاد هادي محمد ...مع التقدير ..
- جامعة كربلاء/ كلية العلوم/ للتفضل بالموافقة على إشراك الأستاذ الدكتور وفاء صادق محسن ...مع التقدير ..
- جامعة كربلاء/ كلية الطب البيطري/ للتفضل بالموافقة على إشراك الأستاذ الدكتور عايد حميد حسن ...مع التقدير ..
- حسابات الكلية /لاجراء اللازم فيما يخص المتطلقات المالية.
- السيد رئيس قسم علوم الحياة. مع التقدير.
- الدراسات العليا مع الأوليات .
- الصادرة

طباعة: Khalaf 2021/10/28A

أ.م.د. زمان حميد كريم
مسؤول شعبة الدراسات العليا
E-mail :science@uokerbala.edu.iq

العنوان : العراق- محافظة كربلاء المقدسة – المدينة الجامعية – كلية العلوم ص .ب. 1125

الخـــــلاصـــــة

داء السكري من النوع الأول هو مرض مناعي ذاتي مزمن يتميز بنقص الأنسولين وتدمير خلايا بيتا بنكرياس. تعد تفاعلات المناعة الذاتية خطوة أساسية خلال تطور مرض السكري من النوع الأول ، حيث تلعب الخلايا التائية دورًا حيويًا. تلعب الخلايا البائية أيضًا دورًا مهمًا في حدوث وتطور السكري من النوع الأول .تم تصنيف الخلايا البائية المنتجة لـ IL-10 على أنها خلايا تنظيمية . يرتبط مرض السكري والالتهابات البكتيرية في اتجاهين . ترتبط العدوى المزمنة بمستويات عالية من السيتوكينات المنشطة للالتهابات ، والتي يمكن أن تؤدي إلى تفاقم مقاومة الأنسولين وتقويض إدارة نسبة السكر في الدم ، بينما يزيد مرض السكري من التعرض للعدوى البكتيرية وعواقبها . هناك ادلة متزايده على أن التغييرات في تركيب الجراثيم قد يكون لها تأثير كبير على كيفية تطور مرض السكري. تهدف الدراسة الحالية إلى توحيد وتحديث المعرفة الحالية حول الآليات التنظيمية الخلوية والجزيئات التي تعرضها خلايا بي التنظيمية لمرضى السكري من النوع الأول و دور المرض في تطور بعض الالتهابات البكتيرية.

اجريت الدراسة في محافظة كربلاء. تم جمع 90 عينة دم و ادرار من الأطفال الذكور تتراوح أعمارهم بين 5-15 سنة. 60 مريض بالسكري من النوع الاول (30 مريض بالسكري مصاب بعدوى المسالك البولية و 30 مريض بالسكري غير مصاب بعدوى المسالك البولية) و30 أفراد أصحاء .تم قياس تردد مجموعات خلايا بي الفرعية باستخدام قياس التدفق الخلوي وزراعة الادرار على وسط المرق المغذي لنمو البكتريا للكشف عن عدوى المسالك البولية. تم قياس بعض المعايير المناعية والفسلجية في مصل جميع المشاركين في الدراسة بمقياس الامتصاصيَّة المناعيَّة للإنزيم المرتبط.

أظهرت نتائج الدراسة الحالية انخفاض معنوي في النسبة المئوية لخلايا B + CD19 عند مستوى $P \leq 0.0001$لمجموعتي مرضى السكري من النوع الأول مقارنة بمجموعة السيطرة. انخفض-B + CD19 + IL10 cell (B-reg)بشكل كبير في السكري من النوع الأول مع عدوى المسالك البولية مقارنةً بمجموعة الأصحاء ولكنه زاد في مجموعة السكري من النوع الأول بدون عدوى المسالك البولية. زاد السكري من النوع الأول مع عدوى المسالك البولية بشكل ملحوظ من تعبير CD80 على B-reg ، في حين أن السكري من النوع الأول بدون عدوى المسالك البولية انخفض ملاحظ عند مقارنته بمجموعة السيطرة . بالإضافة إلى ذلك ، تبين وجود انخفض في تركيز تعبير CD80 على B-reg في كلا المجموعتين التابعة لمرضى السكري من النوع الاول .

كان IL-35 و MIF و LBP و TLR4 أعلى بالنسبة لمجموعتي المرضى من السكري من النوع الأول مقارنة بمجموعة السيطرة ولكن زيادة أكبر في مستويات المعايير لـ السكري من النوع الأول مع العدوى البكتيرية مقارنة بالمجموعتين الأخريين. كان مستوى TGF-β1 أعلى في السكري من النوع الأول مع عدوى المسالك البولية مقارنة بالمرضى الذين ليس لديهم عدوى بكتيرية ومجموعات السيطرة.

تؤدي الالتهابات البكتيرية إلى زيادة في مستوى البلازما لـ CHOL و TC / HDL-C و LDL و-HDL C و Non-HDL-C مقارنة بمرضى السكري الذين لا يعانون من عدوى المسالك البولية والسيطرة عليها. كان تركيز الدهون الثلاثية أعلى بشكل معنوي في مرضى السكري غير المصابين بعدوى بكتيرية مقارنة بـ السكري من النوع الأول مع عدوى المسالك البولية ومجموعة الأفراد الأصحاء.

كان تركيز AST و ALT في مصل الدم يتناقص بشكل ملحوظ في الأطفال المصابين بالسكري من النوع الأول مقارنة بالأفراد الأصحاء . زاد تركيز ALP بشكل ملحوظ في مرضى السكري الذين لا يعانون من عدوى المسالك البولية مقارنة معمجموعة المصابين بالسكري من النوع الأول مع عدوى المسالك البولية و مجموعة الأفراد الأصحاء.لوحظ زيادة كبيرة في مستويات البيليروبين الكلي والكرياتينين في مصل مجموعة المرضى المصابين بالسكري من النوع الأول مع عدوى المسالك البولية مقارنة بمجموعة الأفراد الأصحاء ومجموعة المصابين بالسكري من النوع الأول بدون عدوى المسالك البولية.

تنتمي مسببات الأمراض البكتيرية التي تسبب التهاب المسالك البولية إلى البكتيريا سالبة الجرام بنسبة 54٪ والبكتيريا موجبة الجرام بنسبة 46٪ . تعد بكتريا الإشريكية القولونية اكثر انواع البكتريا المعزولة التي تسبب التهاب المسالك البولية في مرضى السكري من النوع الأول اذا بلغت نسبتها 35 ٪ وبعدها الزائفة شتوتزرية بنسبة 18.5 ٪ و الكوكوريا الوردية بنسبة 18.5%.

تشير هذه النتائج إلى أن النقص العددي لخلايا CD19 + B-cells و CD19 + IL10 + B-cell و CD19 + IL10 + CD24 + CD27 + B-reg واختلاف تركيز السيتوكينات في الأطفال المصابين بالسكري من النوع الأول و المصابين بعدوى بكتيرية الى احتمالية مشاركتها في فقدان التحمل الذاتي للجهاز المناعي الذي ينتج عنه موت خلايا بيتا في البنكرياس ، مما يجعلها هدفًا فعالًا للعلاج المناعي .

جـــامعة كـــربلاء

كليـــــة العلـــــوم

قسـم علوم الحياة

علاقة خلايا البائية المنظمة وبعض المؤشرات المناعية باصابات الجهاز البولي في مرضى السكري من النوع الاول

اطروحـــــة مقدمة الـــى

مجلس كلية العلوم ــ جـامعة كربـلاء

وهي جـزء من متطلبـات نيـل درجـة الدكتـوراة فلسفـة في علـم الاحـياء

من قبل الطالبة

نورعبد الاميرعوده اسماعيل

بكلوريوس علوم الحياة /جامعة كربلاء, ٢٠١١

ماجستير علوم الحياة /جامعة كربلاء, ٢٠١٤

بأشراف

الأستاذ المساعد الدكتور

كوكب عبد الله حسين السعدي

الأستاذ الدكتور

حيدرهاشم محمد علي

١٤٤٤م

٢٠٢٣م