

FIRAT UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
T Ü R K İ Y E



**EVALUATION OF SERUM SELENIUM LEVELS IN TERM OF
GLYCEMIC CONTROL IN PATIENTS WITH TYPE 2 DIABETES
MELLITUS IN DUHOK CITY-IRAQ**

Abdulraheem Taher KHALID

Master's Thesis

DEPARTMENT OF CHEMISTRY

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THESIS APPROVAL

This thesis, which was prepared according to the thesis writing rules of the Graduate School of Natural and Applied Sciences, Firat University, was evaluated by the committee members who have signed the following signatures and was unanimously approved after the defense exam made open to the academic audience.

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DECLARATION

I hereby declare that I wrote this Master's Thesis titled “Evaluation Of Serum Selenium Levels in Term of Glycemic Control in Patients with Type 2 Diabetes Mellitus in Duhok City-Iraq ” in consistent with the thesis writing guide of the Graduate School of Natural and Applied Sciences, Firat University. I also declare that all information in it is correct, that I acted according to scientific ethics in producing and presenting the findings, cited all the references I used, express all institutions or organizations or persons who supported the thesis financially. I have never used the data and information I provide here in order to get a degree in any way.

12 January 2025

Abdulraheem Taher KHALID



PREFACE

This study evaluates the serum selenium (Se) levels in relation to glycemic control in patients with type 2 diabetes mellitus (T2DM) attended Endocrine and Diabetes Unit of Azadi Teaching Hospital in Duhok city, Iraq. Currently, T2DM is a crucial public health problem; our goal was to find a potential relationship between selenium levels and T2MD. Selenium is considered as an important essential element in the human body due to its antioxidant properties and its role in metabolic health. To our best knowledge, there is no prior data available on serum selenium levels in diabetic patients within this region, underscoring the necessity of this research.

Challenges that we faced during this study; the sampling process was one of the key difficulties, since patients with T2MD needed regular treatment, and the prolonged fasting was almost impractical for them. Some patients could not allocate time for participation, while others refused altogether. On the other hand, some patients did not fully understand that they were to be a part of such a study, which added much more delay in the sample collection process.

Additionally, our study was allocated to a specific population in a limited geographical region, restricting the generalization of the findings. In addition, processing and interpreting a large volume of collected clinical and demographic data imposed a significant challenge and it was time-consuming and required many efforts. The limited availability of research techniques and resources further complicated the research process, with the measurements of selenium levels being the most challenging aspect of the research. Despite all the above obstacles, the study was pursued by a commitment to improving diabetes research and public health in the region. This commitment enabled the successful completion of the work.

First of all, I thank God so much who gave me the strength, health, and direction I needed to continue and finish this work.

I am deeply grateful to my thesis supervisor, Prof. Dr. Habibe Özmen, for her guidance and encouragement throughout my work.

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I am profoundly grateful forever to my family and friends for their constant support and encouragement during this work.

Finally, I sincerely appreciate the laboratory staff at Duhok Azadi Teaching Hospital for their invaluable support and cooperation during my research, particularly in facilitating access to laboratory equipment.

The present work was done under the permission of the Ethics Committee of the Duhok Directorate General of Health, and I want to thank them for their cooperation. Without the support and help of these individuals and sections, this work would not have been ended.

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ELAZIG, 2025

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ABSTRACT

Evaluation Of Serum Selenium Levels in Term of Glycemic Control in Patients with Type 2 Diabetes Mellitus in Duhok City-Iraq

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Master's Thesis

FIRAT UNIVERSITY
Graduate School of Natural and Applied Sciences
Department of Chemistry

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Type 2 Diabetes Mellitus (T2DM) is a common metabolic disorder that causes comorbidities such as oxidative stress and inflammation through unmanaged hyperglycemia. Selenium is an active component of glutathione peroxidase, an enzyme that acts as an anti-free radical and reduces oxidative stress. As reported in many studies, the relationship between selenium and T2DM is paradoxical, and further research is needed. This study aimed to evaluate serum selenium levels among glycemic index and different variables in patients with T2DM and controls in Duhok, Iraq. This cross-sectional study was conducted with 99 participants: 59 (59.6%) as the patient group and 40 (40.4%) healthy individuals as the control group. Demographic and clinical characteristics such as body mass index (BMI), waist circumference (WC), and blood pressure were recorded. Biochemical parameters such as fasting blood sugar (FBS), glycated hemoglobin A1c (HbA1c), and lipid profiles were analyzed using Roche analyzers (Cobas), and serum selenium levels were analyzed using an inductively coupled plasma mass spectrometer (ICP-MS). Selenium levels were significantly higher in the patients ($129.42 \pm 33.70 \mu\text{g/L}$) compared to the controls ($115.94 \pm 24.06 \mu\text{g/L}$; $p = 0.032$). A negative correlation was obtained between HDL-C and selenium. No significant difference was found in selenium levels among the subgroups categorized according to HbA1c and other biochemical parameters. Selenium levels are altered by lipid metabolism rather than glycemic status. As a result, the use of Se-containing drugs is not recommended for diabetic patients in the studied population. More research is needed to understand the link between selenium and T2DM.

Keywords: Type 2 diabetes mellitus (T2DM), Glycemic Control, Selenium, Duhok, Iraq.

ÖZET

Irak'ın Duhok şehrinde Tip 2 Diyabetli Hastalarda Glisemik Kontrol Açısından Serum Selenyum Düzeylerinin Değerlendirilmesi

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Tip 2 Diabetes Mellitus (T2DM), yönetilemeyen hiperglisemi yoluyla oksidatif stres ve inflamasyon gibi komorbiditelere neden olan yaygın bir metabolik bozukluktur. Selenyum, anti-serbest radikal olarak görev yapan ve oksidatif stresi azaltan bir enzim olan glutatyon peroksidazın aktif bir bileşenidir. Birçok çalışmada bildirildiği gibi, selenyum ve T2DM arasındaki ilişki paradoksaldır ve daha fazla araştırmaya ihtiyaç vardır. Bu çalışmanın amacı, Irak'ın Duhok kentinde T2DM hastalarında ve kontrol grubunda serum selenyum düzeylerini glisemik indeks ve farklı değişkenler açısından değerlendirmektir. Bu kesitsel çalışma 99 katılımcı ile gerçekleştirilmiştir: Hasta grubu olarak 59 (%59,6) ve kontrol grubu olarak 40 (%40,4) sağlıklı birey. Vücut kitle indeksi (BMI), bel çevresi (WC) ve kan basıncı gibi demografik ve klinik özellikler kaydedilmiştir. Açlık kan şekeri (AKŞ), glikozlu hemoglobin A1c (HbA1c) ve lipid profilleri gibi biyokimyasal parametreler Roche analizörleri (Cobas) kullanılarak, serum selenyum düzeyleri ise indüktif eşleşmiş plazma kütle spektrometresi (ICP-MS) kullanılarak analiz edilmiştir. Selenyum düzeyleri hastalarda ($129,42 \pm 33,70 \mu\text{g/L}$) kontrol grubuna ($115,94 \pm 24,06 \mu\text{g/L}$; $p = 0,032$) kıyasla anlamlı derecede yüksek bulunmuştur. HDL-K ile selenyum arasında negatif bir korelasyon elde edilmiştir. HbA1c ve diğer biyokimyasal parametrelere göre kategorize edilen alt gruplar arasında selenyum düzeylerinde anlamlı bir fark bulunmamıştır. Selenyum seviyeleri glisemik durumdan ziyade lipid metabolizması tarafından değiştirilmektedir. Sonuç olarak, çalışılan popülasyondaki diyabetik hastalar için Se içeren ilaçların kullanımı önerilmemektedir. Selenyum ve T2DM arasındaki bağlantıyı anlamak için daha fazla araştırmaya ihtiyaç vardır.

Anahtar Kelimeler: Tip 2 diabetes mellitus, Glisemik Kontrol, Selenyum, Duhok, Irak.

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ABBREVIATIONS

ADA	: American Diabetes Association
AD	: Anno Domini
ALT	: Alanine Aminotransferase
ANOVA	: Analysis of Variance
BMI	: Body Mass Index
CRP	: C-Reactive Protein
DPP-4	: Dipeptidyl Peptidase-4
DM	: Diabetes Mellitus
EDTA	: Ethylenediaminetetraacetic acid
EM	: Electron Multiplier
ESRD	: End-Stage Renal Disease
GDM	: Gestational Diabetes Mellitus
GLUT4	: Glucose Transporter Type 4
Hb1Ac	: Hemoglobin A1c
HDL-C	: High-Density Lipoprotein-cholesterol
HR	: Hazard Ratio
ICP-MS	: Inductively Coupled Plasma-Mass Spectrometry
IDDM	: Insulin- Dependent Diabetes Mellitus
IDF	: International Diabetes Federation
IQR	: Inter Quartile Ratio
LDL-C	: Low-Density Lipoprotein-cholesterol
NHANES III	: Third National Health and Nutrition Examination Survey
NIDDM	: Non-Insulin Dependent Diabetes Mellitus
S-Cr	: Serum Creatinine
SD	: Standard Division
SGLT2	: Sodium-glucose co-transporter-2
SPSS	: Statistical Package for the Social Sciences
T2DM	: Type 2 Diabetes Mellitus
TSH	: Thyroid Stimulating Hormone
WC	: Waist Circumference
WHO	: World Health Organization

1. INTRODUCTION

1.1. Diabetes Mellitus

Diabetes mellitus, historically, is a disease involving hyperglycemia that, if not managed, results in severe consequences [1]. Some symptoms of this disease, like polyuria and polydipsia, were described more than 3,500 years ago in Egyptian medical papyri, but the illness itself has not been described [2]. Polyuria was described as far back as 1,550 years ago in a medical papyrus originating from Egypt, discovered in the year 1862 AD, and although it was categorized as a group of diseases, it can be considered one of the earliest signs of diabetes mellitus [3].

Early Indian medical literature described two forms of diabetes: One which a person is born with and another they develop at some other time in their life. Even the ancient Indian physicians considered diabetes as related to obesity, diet, and other components of lifestyle and offered early treatment. Furthermore, the 5th–6th century AD Indian physician Sushruta mentions the sweet smell of diabetic urine and flies appearing to be attracted to it, hence the name ‘honey urine’ [2].

The word diabetes, literally meaning to pass through, was first used by Apollonius of the 3rd century B.C., who attributed the condition to the frequent passage of urine due to kidney affection. In the 2nd–3rd century AD, Aretaeus of Cappadocia was the first to introduce diabetes into medical practice. Originally, the word mellitus was a Latin word meaning honey added to the term diabetes by John Rollo, a British Army surgeon, in the late 17th century to differentiate diabetes mellitus, which presents sweet urine, from diabetes insipidus, which does not [3].

Diabetes mellitus is characterized by high blood glucose levels as a result of insulin problems: inadequate insulin hormone production, poor uptake of the hormone by the body’s cells, or a combination of both. This disease has become one of the most widespread and complicated diseases in the field of health care in the world. It has been estimated that it is currently the fifth leading cause of death worldwide. This makes diabetes a special disease, as it comes with many complications. The most common complications are kidney failure and retinopathy, which may result in blindness, neuropathy, hypertension, and an increased risk of amputation. These complications help reduce patient life spans and introduce various costs to the global healthcare system. As a result, diabetes mellitus is considered one of the most epidemic diseases of the twenty-first century [4].

1.2. Classification of Diabetes Mellitus (DM)

The major types of diabetes mellitus (DM) are:

1.2.1. Type 1 Diabetes Mellitus (T1DM)

Type 1 diabetes mellitus, or insulin-dependent diabetes mellitus (IDDM), is an autoimmune-mediated disease in which the insulin-secreting beta cells of the pancreas are destroyed by T-cells, leading to an absolute lack of insulin. T1DM, though constituting 5 to 10% of the total of diabetic patients, happens mostly among children and adolescents [5]. It is thought that it is hereditary and the result of exposure to viruses and toxins [6]. Further, it is indicated that there is a presence of beta cell autoantibodies before clinical diagnosis, reflecting the ongoing autoimmune process of the subjects [7].

1.2.2. Type 2 Diabetes Mellitus (T2DM)

Type 2 Diabetes Mellitus (T2DM) is one of the commonly observed metabolic diseases in which there is a reduced ability of the pancreas to produce insulin, a hormone that regulates blood sugar in the body, as well as insulin resistance by body cells, tissues, and organs. It is a multicausal disease where inflammatory indices, measures of oxidative stress, and special indicators of trace elements are considered pathogenetically significant [8].

T2DM is best described as a pandemic that has been linked to obesity in most world regions. Patients with T2DM are more likely to develop microvascular diseases like retinopathy, nephropathy, and neuropathy as well as macrovascular diseases like cardiovascular disease. These complications are otherwise due to hyperglycemia individually and the separate components of the insulin resistance syndrome. These include obesity, unhealthy diets, physical inactivity, and genetic factors that cause pathophysiological derangement, resulting in different degrees of impaired glucose homeostasis in T2DM [9].

Frequently labeled as adult-onset diabetes, T2DM is characterized by a slowly deteriorating insulin secretion in addition to insulin resistance. People with this type of diabetes often have a measured resistance to insulin in their bodies. Microvascular diseases that involve blood vessels, kidneys, eyes, and nerves are serious in both types of diabetes and constitute a major source of morbidity and mortality. T2DM is caused by age-specific, genetic risk factors, obesity, and physical inactivity, with middle-aged and older persons bearing a disproportionately large burden [10].

About 90% of all diabetes cases are T2DM. One of the characteristic features of T2DM is that the body's response to insulin reduces gradually and vanishes in the end. Therefore, insulin can no longer regulate blood glucose levels, and the body tries to produce even more insulin to

compensate for the insulin reduction. However, this compensatory mechanism at some time ceases to work and results in decompensation, leading to T2DM. Although T2DM used to be found among people aged 45 years and older, nowadays, younger generations are also diagnosed with T2DM. This might be due to increased rates of obesity, physical inactivity, and high-calorie intake [5].

1.2.3. Gestational Diabetes Mellitus (GDM)

According to the current classification, gestational diabetes mellitus (GDM) is defined as glucose intolerance of varying severity that is detected during pregnancy. It develops during pregnancy and frequently improves after delivery. Nevertheless, children of mothers with GDM are exposed to obesity and type 2 diabetes later in life due to the effects of intrauterine hyperglycemia [10]. Pregnancy affected by GDM is known to be free from complications in only 7% of the cases. Mothers with GDM, as well as their children, are at a higher risk of developing diabetes later in life [11]. Furthermore, GDM is also associated with hypertension, preeclampsia, and hydramnios, which add to the chance for a surgical intervention [5].

1.2.4. Other Specific Types of Diabetes Mellitus (Monogenic Types)

Monogenic diabetes, by definition, comes from a mutation in one gene as opposed to more common forms of diabetes, such as type 1 and type 2 diabetes, which are polygenic disorders; they both involve multiple genes and environmental factors. This form of diabetes is relatively rare and is estimated to be the cause in only 1.5–2% of diabetic patients; nonetheless, the accuracy of this statistic is uncertain because monogenic diabetes is often initially diagnosed as type 1 or type 2 diabetes [12].

1.3. Global Prevalence of Diabetes Mellitus (DM)

According to WHO, in 2014, worldwide, 8.5% of the population of all adults with 18 years and above were diagnosed with diabetes. In 2019, it caused 1.5 million deaths, and 48% of these were people who died below the age of 70 years. About 460,000 deaths were due to kidney disease, and approximately 20% of the deaths from cardiovascular-related deaths were attributed to high blood glucose levels. Between 2000 and 2019, the mortality rates for diabetes increased by 3% in total and by 13% in lower-middle-income countries [10].

The IDF Diabetes Atlas 10th edition (2021) revealed that more than 10% of the adult population was living with diabetes, and these figures are expected to further increase to 12-13 %, i.e., over 780 million in 2045. This prevalence is almost equal in both genders but rises more steeply at 70 years and older. The rates differ with the living area and lifestyle and are expected to be higher

in middle-income countries. In 2021, the costs of diabetes in the world amounted to more than 950 billion USD, which are expected to exceed 1,050 billion USD by 2045 [12].

The high rates of T2DM are due to growing urbanization, aging populations, obesity, and sedentary lifestyles. In regions like North America and European Union countries, obesity accounts for 70-90% of T2DM cases in adults. However, the relationship between obesity and insulin resistance or T2DM pathogenesis is not well understood and is hypothesized to involve increased plasma-free fatty acids, adipokines, and leptin [13]. In the last two to three decades, the world has woken up to the fact that complications of diabetes are enormous. Newly, type 2 DM establishes itself as an exacerbated worldwide health issue, which manifests hand in hand with accelerated cultural and social transformation, aging populations, increased urbanization, dietary shifts, decreased physical activity, and other pathological and unsustainable lifestyle and behavioral trends. Several complications also occur with Type 2 DM globally that lead to increased morbidity and mortality; they include cardiovascular disease, neuropathy, retinopathy, and amputations [12].

Lifestyle and behavioral therapies, weight management approaches, and pharmacological treatments are critical components of stated management procedures for persons with Type 2 diabetes [14].

1.4. Pathophysiology of Type 2 Diabetes Mellitus

Diabetes mellitus type 2, also known as non-insulin-dependent diabetes mellitus (NIDDM), is conventionally polygenic or polyetiological in nature. The alterations that define T2DM include impaired β -cell function, insulin resistance, inflammation, and oxidative stress, all of which contribute to compromised glycemic control and the development of microvascular and macrovascular complications [9].

T2DM is primarily defined by three metabolic defects that act synergistically: an enhanced rate of hepatic glycogenesis, a decreased rate of peripheral glucose utilization, particularly by the skeletal muscles and other tissues, and less insulin available to be released into circulation. They contribute to β -cell dysfunction with time. Available evidence on the heritability of T2DM is overwhelming; for example, if one twin in a given pair has T2DM, the other is almost certain to have it or impaired glucose intolerance. Nevertheless, no increase is associated with a certain T2DM risk, which is why the disease is thought to be polygenic [15].

Insulin resistance can be congenital but most often occurs when the body does not respond appropriately to the insulin that is produced, the latter being attributed to genetic factors dominant in T2DM. Another essential cause is what can only be termed as acquired causes, which include but are not limited to inactivity, an increase in age, and obesity, among other things. Such individuals may at first observe hyperinsulinemia and elevated levels of insulin resistance with normal blood glucose levels. However, severe ARI progresses to impaired glucose tolerance, highly

contributing to diabetes. Chronic hyperinsulinemia following excessive insulin secretion may eventually lead to the loss of β -cell function due to stress and apoptosis and worsening of metabolic control [8].

These mechanisms are crucial for making intervention and treatment approaches that would address T2DM, as elevated blood glucose levels for a long time create a significant burden on T2DM individuals' health.

1.5. Complications of Type 2 Diabetes Mellitus

T2DM is accompanied by a wide range of complications that can impact an individual's health status and overall quality of life. Hyperglycemia is a chronic condition in T2DM that can cause both microvascular and macrovascular complications by oxidative stress and inflammation. Diabetes, when persistent, results in complications, some of which are fatal [16].

Microvascular complications: Diabetic retinopathy, nephropathy, and neuropathy are the chief manifestations of microvascular complications of diabetes. Diabetic retinopathy is caused by injury to the retinal blood vessels, which then gets worse from non-proliferative changes to pupillary changes to proliferative changes in the retina, and if not treated, it leads to blindness. Nephropathy is a chronic pathology in diabetic patients associated with the development of glomerular proteinuria and progressive renal dysfunction that can cause end-stage renal disease (ESRD) requiring dialysis or transplantation. Peripheral neuropathy is the most common form of neuropathy, causing pain, numbness, or decreased sensitivity in the hands or feet, which results in an increased risk of formation of foot ulcers and infections leading to amputation [17].

Macrovascular complications involve diseases of the large blood vessels, including coronary artery diseases, stroke, and peripheral vascular diseases. These conditions arise from atherosclerosis because diabetes exposes the body to high levels of blood glucose for long, hypertension, and dyslipidemia. Cardiovascular disease is a major concern in T2DM since people with this illness are twice to three times more likely to develop cardiovascular disease than those without diabetes, thus becoming a major determinant of morbidity and mortality among this population [17].

Also noteworthy is that patients with T2DM are likely to develop other complications, including increased immunocompromising due to suboptimal immune function and slower levels of wound healing. These complications not only raise the costs of health care but also equally put emotional and psychological burdens on the patients and their families. These findings signify the need for strategies to address all four vectors of impact, given that the management of diabetes involves controlling blood glucose levels and encouraging personal behavior changes, as well as screening for complications to prevent adverse effects [18]. Fortunately, all these dangerous complications can be avoided with suitable management of diabetes.

1.6. Risk Factors of Type 2 Diabetes Mellitus

Two category groups can be developed with type 2 diabetes mellitus as a risk factor: modifiable and non-modifiable factors. Among the risk variables that are uncontrollable or unavoidable are age, genes, family history, and environment. The factors that cannot be controlled have a big role in raising the risk of developing type 2 diabetes.

Conversely, controllable risk factors include excessive caloric intake, a sedentary lifestyle, being overweight or obese, hypertension, and physical inactivity, which are the major causes of T2DM and can be modified. For instance, metabolic risks, including obesity as represented by the body mass index, and behavioral risks, including smoking, lack of physical activity, and poor diets, are strong precursors of the disease.

A prospective investigation revealed that a meal with a low fiber content can cause a high glycemic index, which is linked to an increased risk of Type 2 Diabetes Mellitus. Furthermore, its association with T2DM has been researched to be specifically occasioned by the consumption of red meat [19]. It was shown that these modifiable attributes can be influenced through diet and sustained levels of exercise to greatly reduce the propensity of developing new T2DM cases. Diabetes mellitus poses a great impact on the patient's quality of life and health worldwide as well.

Diabetic patients develop complications as a result of continued interaction between the disease and their bodies and hence are physically challenged, emotionally stricken, and less healthy. Most of these complications are chronic and demand constant medical intervention and further treatment, which strains the health systems through increased hospitalization rates, expenditure, and resource utilization. Thus, regular, proper management and prevention of such impacts are necessary [20].

1.7. Diagnostic Criteria for Diabetes Mellitus

Diabetes mellitus (DM) can be diagnosed based on specific glucose tests or HbA1c levels, as outlined by established guidelines using the standard diagnostic criteria suggested by the International Diabetic Federation (IDF) and the World Health Organization (WHO) in 2021. Diabetes should be diagnosed if one or more of the criteria described in Table 1.1 are met [12].

Table 1.1. Diagnostic Criteria for Diabetes Mellitus

Tests	Diagnostic Criteria
Fasting Plasma Glucose (FPG)	≥ 7.0 mmol/L (126 mg/dL)
Two-hour Plasma Glucose After a 75g oral glucose tolerance test (OGTT).	≥ 11.1 mmol/L (200 mg/dL)
HbA1c (%)	≥ 48 mmol/mol (6.5%)
Random Plasma Glucose (RPG) with Symptoms of Hyperglycemia.	≥ 11.1 mmol/L (200 mg/dL)

1.8. Current strategies for managing type 2 diabetes mellitus

The treatment and management of T2DM involve lifestyle changes, medical management, and the monitoring of blood glucose. Blood glucose control measures include diet, exercise, and weight control, and these form the basis of any diabetic management program [16]. Oral antidiabetic drugs such as metformin are usually the first-line treatment options available. Others are sulfonylureas, dipeptidyl peptidase-4 (DPP-4) inhibitors, sodium-glucose co-transporter-2 (SGLT2) inhibitors, or injectables such as insulin may also be used [21]. Since glycemic control must be continuously assessed and adjusted to reflect the immediate modifications to the treatment, the blood glucose level has to be checked frequently and, therefore, usually checked via self-monitoring and repeating HbA1c testing. Issues are minimized, and overall health status is improved by the addition of patient education and preventative care measures such as managing cardiovascular risks, foot care, and eye checks [14]. The goals of managing type 2 diabetes mellitus include the maintenance of a good quality of life and the reduction of acute and chronic complications. Due to the chronic and non-curable nature of type 2 diabetes, the management plan must be extensive, intricate, and designed to deal with this fact [22].

1.9. HbA1c as a Biomarker for Glycemic Control

1.9.1. Introduction to HbA1c

HbA1c is an independent prognostic marker of glycemic control in diabetic individuals for the previous 2 to 3 months. It estimates how ‘glycated’ the hemoglobin is, a protein in red blood cells that latches on to glucose in the blood. HbA1c level characterizes the average blood glucose level during the previous 2–3 months—the life cycle of red blood cells (about 120 days). When blood glucose concentrations rise, a higher percentage of glucose goes on to bind to hemoglobin, producing high levels of HbA1c [23].

1.9.2. Role of HbA1c in the Diagnosis and Management of Diabetes

HbA1c is typically used in diabetes diagnosis as well as in the checkout of diabetes management. A cut-off point of 6.5% or above for the diagnosis of diabetes was recommended by the American Diabetes Association (ADA). Since it provides a single glimpse of how effective the diabetes treatment has been in achieving glycemic control over a long time, clinical practitioners often use it to monitor the diabetes treatment progress. Ideally, HbA1c levels should be kept at an optimum in every person suffering from diabetes because high levels of HbA1c increase the risks of neuropathy, retinopathy, and cardiovascular diseases. The ADA proposed specific HbA1c cut points for individuals with diabetes to be less than 7% for most adults [23]. However, the setting of these targets may be modified by other options, including age, duration of DM, presence of complications, and other comorbidities.

1.9.3. Classification of Glycemic Control Based on HbA1c Levels

Based on the American Diabetes Association (ADA) 2020, to evaluate the degree of glycemic control, glycated hemoglobin A1c levels are mostly divided into three sub-groups: poor control ($\text{HbA1c} \geq 8\%$), moderate control (HbA1c between 7-8%), and good control ($\text{HbA1c} < 7\%$). Diabetic patients with a glycated hemoglobin A1c level of less than 7% are considered to have good control of the disease management; patient groups with a glycated hemoglobin A1c level of 7-8% are considered to have moderate control of diabetes management, while patients with a glycated hemoglobin A1c level of more than 8% are more likely to develop diabetes complications, which underlines the need for control and treatment so that the best control of blood glucose levels is maintained. Complication rates for diabetes are directly proportional to decreased glycemic control and underline the importance of timely screening and treatment to achieve optimal diabetes mellitus control [23].

1.9.4. Factors Affecting HbA1c Levels

Compared to the several indicators that can be measured pointwise and are expressed in mmol/L or mg/dL (e.g., FBG), HbA1c is an effective marker of long-term blood glucose control; however, several factors affect the measurement accuracy and interpretation of its results. Other diseases associated with red blood cells' survival rate include anemia and hemoglobinopathy, for example, sickle cell disease and renal diseases, which in turn impact the glycated hemoglobin A1c level [24]. Also, differences in HbA1c levels have been presented depending on age, race, and gender. For instance, researchers have opined that racial and ethnic differences might play a role in differences in HbA1c levels; African Americans, for instance, are reported to have higher HbA1c even at the same glycemic levels [25]. Moreover, obesity and certain medicines also affect the

HbA1c value [24]. That is why the mentioned variables should be taken into account when evaluating HbA1c levels because they can mislead the clinicians in the given patient's real glycemic status.

1.9.5. HbA1c and Selenium in Type 2 Diabetes

Previous research has also investigated the relationship between selenium and glycemic status. Selenium is an antioxidant trace element; it plays a role in insulin metabolism and glucose metabolism in the body. It was reported that selenium can affect the level of insulin or glucose metabolism and thereby the HbA1c of patients with type 2 diabetes [26].

1.9.6. Limitations of HbA1c

The frequent assessment of glycated hemoglobin A1c values carries some risks. Unlike other types of monitoring parameters, it will not be able to give information about recent variations in blood glucose level and can only be used to give the average blood glucose level of the last three months, making it so inefficient in measuring the short-term changes in glucose level due to exercise, diet, or stress, as reported by Suastika et al., 2021 [27]. Moreover, several studies have shown that HbA1c does not give information about short-term changes, which are fundamental to assessing urgent glycemic control, including hypoglycemia or sharp fluctuations in blood glucose levels [28].

Health issues can interfere with blood sugar levels that are used to estimate HbA1c. Factors that affect the rate of red blood cell production, including anemia, hemoglobinopathies (sickle cell anemia, thalassemia), and kidney disease, can lead to an artefactual increase or decrease in HbA1c levels. For instance, premature red blood cells breaking down, a condition known as hemolysis, causes low HbA1c levels, while kidney diseases produce carbamylate hemoglobin, which produces high HbA1c levels [27]. In addition, technical factors such as differences in the mean red blood cell lifespan, as in pregnancy or with common hemoglobinopathies, introduce variability in the estimation of HbA1c, as reported by Davidson et al., 2010 [29].

HbA1c as a diagnostic tool for diabetes mellitus has been made more challenging by ethnic and racial differences. Such human groups, for example, non-Hispanic Blacks and Mexican Americans, may have elevated HbA1c levels even if corrected for glucose levels. This results in poor diagnostic accuracy or underdiagnosis of diabetes, as evident in studies done among the NHANES III population, where non-Hispanic Blacks have been found to register higher HbA1c levels across categories of glucose levels known to affect the test [29].

Other limitations are involved in the methods used for the determination of HbA1c. The significance of the outcome may also, in some ways, differ contingent on the nature of the analytical

technique that is used, which can be either immunoassays or enzymatic assays, because these make a difference in recognizing the hemoglobin variants. For instance, ion-exchange chromatography could be affected by carbamylated hemoglobin, giving a wrong reading [27]. These variations make it difficult to compare results in two or more laboratories, hence making interpretation of the HbA1c value undesirable. Therefore, HbA1c as a technique for long-term control of blood glucose must be accepted with certain drawbacks, especially when diagnosing diabetes or assessing glycemic control in patients with a pathological condition that influences their red blood cell lifetime. This is compounded by ethnicity and racial differences [29].

Altogether, HbA1c is a useful diagnostic marker, effective for evaluating diabetes and the risk of complications based on the level of glycemic regulation for the previous 2-3 months. Classification of HbA1c into good, moderate, and poor glycemic control is useful in developing care plans for patients with diabetes. The purpose of this study is to understand, where possible, a relationship between serum selenium levels and HbA1c in type 2 diabetes, which may once again help in resolving the diabetes pathophysiology and identifying therapeutic targets.

1.10. Selenium

The chemical element selenium has an atomic mass of 34 with the chemical symbol Se [30]. Because the human body requires it in limited quantities to support the multiple functions in the body, it belongs to the group of micronutrients. Since it is a part of the selenium-containing proteins, or selenoproteins, as well as having a very crucial role to play in several vital biochemical processes, that makes it essential.

These selenoproteins thus regulate the immune system and thyroid hormone metabolism and also act as antioxidants. Yet, the failure or the deficiency of this element may lead to severe health complications [31].

Selenium, as an active component of the enzyme glutathione peroxidase that counteracts free radicals and the resulting cellular damage, performs several indispensable functions, with protection against oxidation being the most important [32]. Further, it has a role in the conversion of thyroid hormone T4 to the metabolically active form, T3, needed in the regulation of metabolic rates and maintenance of energy levels [33]. Selenium also has benefits for the reproductive system, perhaps primarily with an enhancement of sperm mobility, and for the immune system through stimulation of the production and activity of white blood cells [34].

It is important to understand that the selenium levels in the general population differ by dietary intake, geographical location, and certain demographic variables concerning age and gender. Regions having low selenium levels in the soil are normally likely to produce food with low selenium levels, while high-level soil selenium areas will yield foods containing high levels, thus increasing levels in the population.

Since both excess and shortage of selenium may have detrimental consequences for human health, such fluctuation is critical [35].

1.10.1. Sources of selenium in the diet

The main sources of selenium are nuts, especially Brazil nuts, sea products like fish and other kinds of seafood, organ meats, cereals, and eggs. The type of soil that people or animals are fed on—plants or animals—influences the level of selenium in food. Some diseases that are caused by a lack of selenium include impaired cognitive function, immune system compromise, and increased susceptibility to certain forms of cancer. On the other hand, excessive intake of selenium, essentially due to over-supplementation, can result in selenium toxicity, the symptoms of which include brain damage, hair loss, and digestion complications [36]. Also, selenium at high and low levels can cause many health problems [37].

The reactions of selenium on thyroid function and as an antioxidant are part of the associated metabolism and may present ameliorative effects. Selenium is important for a healthy metabolism and warding off oxidative stress, which is detrimental to metabolism. Selenium could affect insulin receptor sensitivity and glucose homeostasis and thereby could affect disease management in metabolic disorders, including diabetes mellitus type II [38].

1.10.2. Selenium and Diabetes

The published data provide contradicting information on selenium and its relation to diabetes mellitus. Some works are showing the protective properties of selenium regarding diabetes mellitus. It has been discovered that selenium supplements can benefit human health in numerous ways; while some researchers indicate that selenium could help reduce diabetes mainly due to antioxidant activity, at the same time, other studies showed that more selenium intake leads to the development of type 2 diabetes. These conflicting results might be due to differences in the study method, dose of selenium, or perhaps differences in populations, which need larger studies to resolve these discrepancies [39].

Many papers have been published evaluating the effect of selenium levels and diabetes mellitus, especially T2DM. The studies on selenium and type 2 diabetes mellitus indicated in the recent meta-analyses described here reveal inconsistencies.

Four of the seven meta-analyses identified a positive association between selenium levels and the risk of developing type 2 diabetes, suggesting that higher selenium concentrations might increase the likelihood of diabetes [40] [41] [42] [43]. Interestingly, the two meta-analysis studies pointed out that selenium does not affect the development of diabetes; therefore, it may not be responsible for the development of diabetes [44].

Also, one meta-analysis reported a possible U-shaped dose-response, like both low and high selenium concentrations increased type 2 diabetes risk; this means that both low and high selenium intakes could be dangerous [37].

These findings highlight the unclear relationship between selenium and diabetes and confirm the need for more research to establish clear recommendations for diabetes management.

1.10.3. Impact of diabetes on selenium levels

Oxidative stress is one of the complications of diabetes, which might lower selenium levels in the body. Selenium is a crucial part involved in the synthesis of selenoproteins, which aid in protecting the body from oxidative stress as a result of free radicals. Diabetes mellitus has been shown to cause increased oxidative stress within body cells, and this may cause demand for selenium [45].

Inflammation, as one of the most related complications of diabetes, can also impact selenium levels. Inflammatory processes might also change the distribution and use of selenium and thus result in decreased selenium concentrations. It may also lead to increased oxidative stress [46].

Selenium status under type 2 diabetes is influenced by insulin resistance because the condition affects how the body utilizes essential trace elements. The disrupted path in which selenium concentrations change can result from the alteration of cellular signaling mechanisms that manage selenium's activities inside cells [47].

Dietary Intake and Absorption: Many changes in selenium take place because of the diabetic influence on the functioning of the digestive system and food consumption. For example, diabetic neuropathy and gastroparesis may interfere with selenium. Pathologic nutrition patterns characteristic of diabetic diets can also result in low selenium consumption [48]. Diabetes can cause nephropathy because of kidney dysfunction, while kidney failure may result from either the accumulation of selenium or its deficiency based on the severity of disease, leading to changing selenium levels in the body [49].

1.10.4. Interaction between selenium and diabetes

The relationship between selenium and diabetes is more complex and may be thought of as involving several mechanisms. **Antioxidant activities:** Selenium is an integral part of the active centers of selenoproteins, enzymes that have antioxidant activity. These proteins play an important role in diminishing the levels of oxidative stress through the elimination of free radicals. Since oxidative stress is recognized to occur in diabetes by causing insulin resistance and beta-cell dysfunction, having enough selenium may lessen such effects, thus reducing the risk of the disorder [45], [50]. Selenium may affect diabetes-related molecules and insulin signaling. A number

of the works present selenium as able to increase the sensitivity of insulin receptors and the rate of glucose assimilation. This could affect insulin resistance positively and keep blood sugar in a normal range [51], [52]. Selenium might prevent inflammation, which is known as the cause of insulin resistance and diabetes [53]. Hormones and Metabolism: Selenium plays a vital role in the thyroid gland, which functions as a metabolic rate regulatory gland. Diabetes: Thyroid hormones influence carbohydrate metabolism, while selenium deficiency leads to thyroid disorder and fluctuation in blood glucose levels [54]. Researchers suggest a U-shaped link between selenium and diabetes, meaning that both low and high serum selenium concentrations are related to diabetes risk, but moderately high serum selenium levels appear to be more favorable. This might be due to selenium's role in overall health, while too much selenium can be dangerous and disable metabolic processes [55]. These mechanisms prove that selenium can influence diabetes in a bidirectional manner.

1.11. Problem Statement

1.11.1. Identification of the Problem

This research aims to understand the correlation between selenium levels and type 2 diabetes mellitus (T2DM) in the earlier stages. While several studies suggest that diabetes alters the metabolism of a few trace elements, among them being selenium, there is limited understanding of how type 2 diabetes impacts selenium levels. Based on present research, there is no clear evidence of the connection between selenium and glycemic control in diabetes mellitus patients. Additionally, much is still unknown about the impact of diabetes-caused metabolic changes on both the excretion and absorption of selenium [50].

This discrepancy is the biggest area of knowledge gap in the relationship between diabetes and selenium level with health effects on patients [55].

1.11.2. Significance of the Problem

Both normal and abnormal selenium metabolisms are important to understand, particularly in cases like diabetes mellitus because selenium is involved in many metabolic processes, including antioxidant protection and regulation of thyroid hormone concentration. A better understanding of this relationship could eventually create better diet advice and supplement dosing advice for patients with diabetes. If a link is found, it could modify clinical interventions related to selenium testing and treatment in diabetic people, thus improving such diabetic patients' outcomes by correcting imbalances in selenium levels [41].

This work is relevant to the modern state of health care since diseases such as type 2 diabetes mellitus are becoming chronic in many regions of the world. Further research and studies on the effect of diabetes on micronutrients, including the above-stated selenium, can lead to new nutrition intervention goals that will help to prevent or alleviate the effects of the disease, like poor blood sugar regulation. Further, these results may help in designing public health measures involving the proper diet for diabetic people [36].

1.11.3. Consequences of Not Addressing the Issue

Failure to fill this gap may mean that practice uncertainty surrounding dietary advice and supplementation in diabetes persists alongside uncertainty about selenium intake, potentially promoting suboptimal healthy intakes in those with diabetes [56]. Thus, T2DM patients' oxidative stress and metabolic dysfunction may not significantly improve if diabetes management strategies do not receive clear guidelines [57]. In that regard, the investigators could be correct in presuming that, while overlooking the fundamental ignorance of the relationship of selenium to diabetes, the implications might become dangerous in the long run. Given such an approach, it is possible to make the mistake of not recognizing selenium as a potentially modifiable factor in diabetes control. It is almost possible to end up with inadequate management efforts leading to a higher rate of complications and an additional burden on the healthcare system. Similar to the diet for diabetic patients, the public health initiatives informing dietary guidelines in regard to selenium may do so without adequate evidence as well [58].

1.12. Objectives

1.12.1. Main Objective

To evaluate the serum selenium levels in terms of glycemic control (HbA1c), good, moderate, and poor control in patients with type 2 diabetes mellitus.

1.12.2. Specific Objectives

To compare serum selenium concentration between T2DM patients and healthy controls across subgroups based on participant characteristics like age, gender, BMI, biochemicals, and glycemic control, and determine whether selenium levels significantly differ among these subgroups.

To investigate whether parameters such as FBS, cholesterol, triglycerides, HDL-C, LDL-C, and family history of T2DM influence serum selenium concentration.

1.13. Primary and Secondary Outcomes

The primary outcome of this research will give us more understanding of the link between serum selenium concentration and glycemic control (HbA1c) in patients with type 2 diabetes mellitus. Secondary endpoints will also look at the correlation with selenium levels and other metabolic markers, including lipid profile. These measurements will provide information about the role of selenium in diabetes mellitus [55].

1.14. Contribution to the Field

The work aimed to find out how blood selenium levels are affected by diabetes. By comparing the level of this mineral in the body with diabetes and showing whether the disease inhibits selenium metabolism in the body. The results may also impact clinical recommendations by encouraging the reconsideration of dietary selenium intake for diabetic patients in the various nations with selenium-poor soils, depending on the availability of selenium in the soils, to improve nutrient-targeted ways. By describing how selenium influences glycemic regulation in diabetic patients, this work may develop bases for subsequent works that examine selenium as a supplementary treatment to diabetes [59].

The results from this study may give new ideas into whether changes in selenium levels are associated with diabetic control and, as a result, lead to better management and dietary recommendations for diabetes patients [60].

1.15. Hypothesis and Research Questions

1.15.1. Hypothesis

The present study postulates that T2DM affects the serum selenium levels in diabetic patients. More precisely, the null hypothesis (H_0) assumes that there are no differences in serum selenium concentrations in type 2 diabetes mellitus patients with good, moderate, and poor glycemic control. On the other hand, the alternative (H_1) hypotheses propose that there is a significant impact of T2DM on serum selenium levels; hence, there should be an observable difference among these categories.

1.15.2. Research Questions

Primary Question:

- What is the relationship between serum selenium levels and glycemic control (good, moderate, or poor glycemic control) in patients with type 2 diabetes mellitus (T2DM)?

Secondary Questions:

- What is the relationship between serum selenium levels and parameters like age, gender, BMI, and duration of diabetes?
- How can metabolic parameters like, HDL, LDL, cholesterol, and triglycerides affect serum selenium concerning T2DM?
- How do serum selenium levels in patients with type 2 diabetes mellitus compare to serum selenium levels in healthy individuals in terms of correlation?

1.16. Significance of the Study

The main objective of this study is to produce new knowledge about selenium and diabetes mellitus. The research aims to establish the relationship between serum selenium levels and glycemic control in T2DM patients that will help in detecting the contribution of selenium in diabetes. If a connection between selenium levels and glycemic control is observed, it might mean that selenium level assessment and possibly selenium levels modulation may become one of the emergent sides of diabetes management. Such a situation may create grounds for the identification of dietary guidelines or the use of supplementation protocols that would help enhance glycemic efficacy and the general state of diabetics.

This research could benefit several groups working in this field: Doctors: The study could provide useful information on how to control the level of selenium concerning diabetes and, hence, help come up with proper patient treatment programs and personalized treatment plans. Patients: People with type 2 diabetes might better understand the role of trace elements such as selenium for their condition, enabling them to optimize their health outcome by finding a suitable strategy to adopt. Type 2 diabetes is a chronic ailment that affects millions of people all over the world. The present study would be useful in expanding the current knowledge base concerning diabetes and micronutrients, thereby providing a starting point and springboard for other studies in the field. The study will be useful to researchers who are looking at the metabolic component of diabetes and the micronutrient components of the condition in planning out future research.

Overall, this work tries to fill gaps in the existing knowledge as well as offer implementable findings that might improve the care and management of diabetes.

1.17. Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Inductively coupled plasma mass spectrometry (ICP-MS), depicted in figure (1.1), is one of the most widely used analytical techniques to determine the trace elements in clinical and environmental studies. The device uses inductively coupled plasma to ionize materials into the

individual components and a mass spectrometer to separate and detect the ions. The device is multipurpose, it is used in environmental science, the food industry, and pharmaceutical studies. It has high precision and accuracy in detecting toxic heavy metals, trace elements, nutrients, and metal impurities [61].

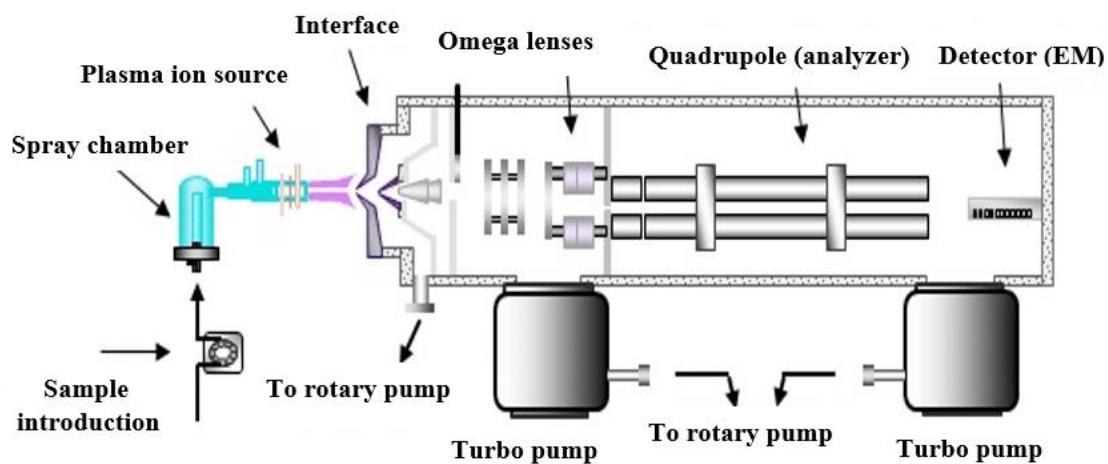


Figure 1.1. Schematic diagram of the ICP-MS

For a biological sample, before the analysis, several steps need to be done, such as digestion, dilution, and filtration; those steps are crucial for accurate quantification of the trace elements. A strong oxidizer such as nitric acid (HNO_3) is used in digestion in line with hydrogen peroxide to ensure protein decomposition. The digestion process is performed under typical conditions in a microwave oven or on a hot plate. Consequently, the sample is serially diluted with a weak acid or ultrapure water. During the dilution, the elemental concentration should match the optimum ICP-MS range. Moreover, elements such as germanium or tellurium are added for the instrumental correction for drift and matrix effects. Then, the sample is centrifuged and filtered to eliminate particulates and unwanted residuals.

The ultra-pure sample is then introduced into the nebulizer, which evaporates it into aerosols or liquid drops in air or other gaseous environments. An inert gas such as argon or nitrogen carries the aerosols to the spray chamber in which larger particles are removed, allowing only fine ones to reach the plasma chamber where an argon plasma generated by radio frequencies ionizes the samples completely to positive ions. Those ions pass through a series of sampler and skimmer cones, reducing the atmospheric pressure and directing ions into the vacuum system. Then, they are focused and accelerated by an ion lens toward the mass spectrometer. The mass analyzer, the quadrupole consisting of four parallel rods vibrating in an electric field, allows ions of specific mass to charge ratio to pass through while filtering other ions, ensuring high specificity. An electron multiplier detector is used to catch the ions reached where the signal is amplified and processed, giving quantitative information about the element of the sample under study.

The ICP-MS is an advanced analytical instrument with extremely low detection limits (sub-ng/L to low $\mu\text{g/L}$ levels). It has a high throughput with minimal sample volume and requires comparatively easy sample preparation. It is an essential instrument for determining the trace and ultra-trace elements with its advanced configurations, such as high-resolution and tandem mass spectrometry (triple-quadrupole), further improving accuracy and specificity. Despite these advantages, the device is not cost-effective, including initial cost, operational, and maintenance costs. Its operation requires high-purity inert gases, and the device should be operated in a controlled environment free from particulates and other dust [62].



2. LITERATURE REVIEW

The presented review aimed to highlight the current knowledge regarding the relationship between selenium and type 2 diabetes mellitus (T2DM); recent investigations provide multiple connections. Positive associations demonstrate those cases where selenium concentration or supplementation could have a valuable effect on T2DM risk or control. Other studies have revealed that alterations in serum selenium levels are not related to type 2 diabetes. These results suggest that in some populations, the impact of selenium does not contribute to diabetes complications. In addition, U-shaped or non-linear dose-response associations have been postulated, implying that both Se concentrations considered at extremes, either too high or too low, are abstracts for T2DM. This proves the significance of selenium, even though for diabetes, its effect may differ depending on the level in the body.

2.1. Positive Associations Between Selenium and T2DM Risk

Several research works suggest that high levels of selenium might be associated with T2DM by the negative effects of the element on glucose balance.

Stranges et al. (2007), in their systematic review and meta-analysis, did a randomized control trial with 1202 American adults as part of the Nutritional Prevention of Cancer trial. Participants taking 200 µg/day of selenium supplements showed a significantly higher T2DM risk (HR = 1.55, 95% CI: 1.03–2.33) compared to the placebo group, suggesting that high selenium intake may disrupt glucose metabolism [63]. A study by Bleys et al. (2007) and Laclaustra et al. (2009) used data from the third National Health Examination Survey (NHES III), a cross-sectional study with 8,876 U.S. adults, finding a 43% to 50% higher prevalence of diabetes among those with elevated selenium levels (OR = 1.43, 95% CI: 1.07–1.92 for Bleys et al. and OR = 1.50, 95% CI: 1.14–1.96 for Laclaustra et al.). These results indicate that high serum selenium is positively related to T2DM risk [51] [64]. Also, research by Wei et al. (2015) examined 5,423 middle-aged and elderly Chinese adults and found a significant association between high dietary selenium intake and diabetes risk. Subjects in the top quartile had an adjusted OR of 1.52 (95% CI: 1.01–2.28; P = 0.04) compared to the lowest quartile, which means that the intake of selenium-rich foods may also contribute to T2DM risk in non-Western populations [65].

2.2. Non-linear and U-shaped Relationships

According to reports from other studies, there is a non-linear and U-shaped association between elevated risk for type 2 diabetes and both high and low selenium levels;

Vinceti et al. (2021) studied selenium and diabetes in detail as they did a meta-analysis that outlined that there is an inversely proportional relationship between selenium and diabetes when dietary selenium is over 80 µg/day. They also suggest that high or low levels of selenium in the blood could be related to increased diabetes risk [42]. According to research done between 2016 and 2022 by Alharithy and Alafif (2023), elevated serum selenium levels often raise the risk of type 2 diabetes by 25% to 40%. Also, there was unclear information about selenium in diets; some studies reported that there is no relation between selenium and diabetes, while others suggested that selenium has a protective benefit [39]. Further, several studies identified that selenium has significant importance in metabolism, focusing on the antioxidant effect of selenium and the capability of selenium to imitate insulin-like one by Saliha Rizvi et al. (2023) [66]. They found out that selenium consumption for diabetics has a U-shaped effect since it is considered to increase diabetes risk in both high and low intakes.

2.3. Conflicting or No Association Findings

The complexity is increased when some research indicates no meaningful correlation between selenium supplementation or ingestion and the incidence of type 2 diabetes:

Rayman et al. (2012) found that selenium supplementation up to 300µg per day for elderly patients with T2DM in the UK did not elevate T2DM risk. This indicates that moderate selenium supplementation cannot affect the populations having low selenium levels in relation to diabetes risk [67]. A randomized, placebo-controlled trial was carried out by Jacobs et al. (2019) in addition to assessing the impact of selenium on β-cell function and insulin sensitivity. Nearly three years later, they found no meaningful difference between the selenium and placebo groups; they suggested that selenium supplementation at moderate levels might not affect diabetes risk [68]. Also, in a systematic review of several cross-sectional studies that included Chinese patients, Cui et al. (2022) [69] reported no relationship between dietary selenium consumption and the prevalence of T2DM. Nevertheless, they stated that selenium intake played a role in the effects of other nutrients on diabetes. This means that selenium might interfere with other nutrients in some way to impact metabolic health.

Taking into account all these studies, it can be concluded that the relationship between selenium and type 2 diabetes is unclear. Although higher selenium levels are frequently linked to a higher risk of type 2 diabetes, non-linear results suggest that both excess and deficiency may play a role in the development of diabetes. It was also thought that the selenium dosage necessary to maintain the metabolic health of an individual seems to be moderate. The impact of different selenium forms and doses on diabetes should also be the focus of research in different populations. Due to uncertainties with selenium, additional research will be ensuing in the functioning to identify the right dietary level that will be of benefit in reducing the risks associated with type 2 diabetes.

3. MATERIALS AND METHODS

This cross-sectional study was conducted at the Endocrine and Diabetes Unit of Azadi Teaching Hospital in Duhok City, Iraq, from January to June 2024. A convenience sampling method was used. Informed agreement was obtained from each participant before the study, and the research protocol was approved by the Ethics Committee of the Directorate of Health in Duhok Governorate (Appendix 7).

3.1. Subjects and Study Design

Initially, 133 participants were enrolled in this cross-sectional study, including 50 healthy participants (control group) and 73 people with Type 2 diabetes mellitus (patients' group). Both patients and healthy subjects were obtained from the same population.

3.2. Inclusion and Exclusion Criteria

Inclusion Criteria: Patients with type 2 diabetes mellitus without diseases complications. **Exclusion Criteria:** To ensure that the study focuses on patients with only type 2 diabetes without complications, several exclusion criteria were applied: patients with chronic kidney disease (serum creatinine [S-Cr] was measured), thyroid dysfunction (thyroid-stimulating hormone [TSH] was assessed), chronic liver disease (Alanine aminotransferase [ALT] were evaluated), and severe inflammatory conditions (C-reactive protein [CRP] test was performed). Additionally, individuals taking multivitamins (A-Z), selenium-containing supplements, or insulin injections, as well as pregnant women and those diagnosed with type 1 diabetes mellitus (T1DM), were excluded from the study.

3.3. Collection and Processing of Blood Samples

In this study, 73 blood samples were collected from patients diagnosed with T2DM based on WHO protocol, and 50 blood samples were collected from healthy individuals as a control group. Participants attended the Endocrine and Diabetic Unit in Azadi Teaching Hospital, Iraq, Duhok City, after overnight fasting for more than 8 hours. Venous blood samples (8 ml) were collected via venipuncture from the antecubital vein between 8:30 and 11:30 AM. Two ml of the blood was placed in a vacuum tube containing K⁺ EDTA as an anticoagulant for glycated hemoglobin A1c testing, the remaining 6 ml were collected in a separator tube. The serum was separated after clotting for 30 minutes using a HITACHI centrifuge at 6000 r/m for 15 minutes. Serum samples

were divided into two parts; one part was frozen at -30 degree for selenium measurement, the second part was processed immediately to measure other biochemicals.

3.4. Biochemical Analysis

Several biochemical tests were performed on the study subjects to evaluate the metabolic state of the population and search for signs of T2DM abnormalities. The following tests were carried out to measure other critical biochemical values associated with diabetes mellitus and complications using Roche analyzers, Cobas c311 (Appendix 1), Cobas 6000 (Appendix 2), and Cobas e411(Appendix 3).

Most biochemical tests were performed using the Roche, Hitachi, Cobas c311, Cobas 6000, and Cobas e411 analyzers for clinical chemistry. The tests listed below were carried out:

- Glycated Hemoglobin A1c (HbA1c): Measured using the Tina-Quant Hemoglobin A1c Gen.3 assay-150 tests, for Roche Cobas 6000, Reference number 05336163 190. Results are expressed in percentage (%).
- Fasting Blood Sugar (FBS): Measured using the Glucose HK Gen.3 assay-300 tests, for Roche Cobas c311, Reference number 04404483 190. Results are printed in mg/dL.
- Cholesterol: Measured using the Cholesterol Gen.2 assay-400 tests, for Roche Cobas c311 with reference number 03039773 190. Results are expressed in mg/dL.
- Triglycerides (TG): Measured using the Triglyceride assay-250 tests, for Roche Cobas c311, Reference number 20767107 322. Results are printed in mg/dL.
- High-Density Lipoprotein Cholesterol (HDL-C): Measured using the HDL-Cholesterol Gen.4 assay-300 tests, for Roche Cobas c311, Reference number 07528566 190. Results are printed in mg/dL.
- Low-Density Lipoprotein Cholesterol (LDL-C): Measured using the LDL-C Gen.3 assay-200 tests, for Roche Cobas c311, Reference number 07005717 190. Results are expressed in mg/dL.
- Serum Creatinine (S-Cr): Measured using the Creatinine Jaffe-700 tests kit, Reagent for Roche Cobas c311, Reference number 04810716 190. Results are expressed in mg/dL.
- C-Reactive Protein (CRP): Measured using the CRP4-250 tests kit, for Roche Cobas c311, Reference number 07876033 190. Results are expressed in mg/dL.
- Alanine Aminotransferase (ALT): Measured using GPT/ALT-500 test kit, Reagent for Roche Cobas c311, Reference number 20764957 322. Results are expressed in U/L.
- Thyroid-Stimulating Hormone (TSH): Measured using TSH Elecsys-200 tests, Reagent kit, for Roche Cobas e411, Reference number 08429324 190. Results are expressed in μ IU/mL.

3.5. Excluded subjects before selenium determination

During the sampling process, 34 people were excluded from the study population after chemical tests were conducted on the population to find abnormalities that met the criteria of exclusion. Specifically, 24 patients from the T2DM group and 10 subjects from the control group were excluded due to abnormalities detected in one or more biochemical parameters, including thyroid-stimulating hormone (TSH), C-reactive protein (CRP), creatinine levels, and alanine aminotransferase (ALT), to keep the research population's integrity. Due to these exclusions, the final study population consists of 99 participants: 59 patients with T2DM and 40 healthy individuals in the control group. Such strict criteria were utilized in the admission of participants into the study to increase the validity of the results being presented.

3.6. Selenium Determination Procedure

Elemental analysis of selenium was carried out by using Thermo Scientific ICP-MS, an advanced inductively coupled plasma mass spectrometer specifically intended for quantifying many trace elements at significantly sensitive rates (Appendix 4). The ICP-MS operates by ionizing the sample using a plasma torch and detects ions according to their mass-to-charge ratio (m/z) using a quadrupole mass filter. This system can perform multi-elemental analysis with high sample throughput and lower detection limits, making it useful for clinical diagnostics such as the determination of selenium concentrations in serum samples.

3.6.1. Calibration and Quality Control of The Instrument

Multi-element calibration standards containing selenium were prepared by diluting a 1000 mg/L stock solution in deionized water and nitric acid (3% v/v). The calibration standards were obtained by further dilution of stock solution ranging from 1-100 $\mu\text{g/L}$. The calibration curve exhibited a straight-line equation with an R^2 value greater than 0.999, meaning that the analysis process has high accuracy. To justify quality control, both standard reference and spiked samples were analyzed under proper clean room conditions for maximum reduction of interfering substances.

3.6.2. Sample Preparation and Digestion

For the determination of Selenium in serum samples (150 μL aliquots), optima grade nitric acid (HNO_3), trace selective grade hydrogen peroxide (H_2O_2), pre-cleaned polyethylene tubes, ultrapure deionized water, and certified selenium standards (1000 mg/L) were used. Serum samples were thawed at room temperature for 20 min, and 150 μL of the sample was pipetted into pre-cleaned polyethylene tubes. For the decomposition of serum samples, 150 μL of nitric acid and 100

μL of hydrogen peroxide were added to the samples and digested using a microwave digester at 95 °C for 35 min. Finally, the samples were diluted to a final volume of 10 mL for analysis.

3.6.3. Selenium Sample Analysis

Before digesting the samples for analysis by ICP-MS, a calibration curve was established using selenium standards, and each sample was measured in triplicate. Internal standards were employed to correct for potential matrix effects, and the relative standard deviation (%RSD) for triplicate measurements was below 5%. Selenium concentrations in each sample were reported in micrograms per liter (μg/L).

3.7. Statistical Analysis

The general characteristics of patients with type 2 diabetes mellitus and healthy controls were presented as mean (SD), median (IQR), or number (%). Comparisons of general characteristics and lifestyle factors among the study groups were conducted using one-way ANOVA, the Mann-Whitney U test, the independent t-test, the Kruskal-Wallis H test, or Pearson's chi-squared test. Serum selenium levels in patients with type 2 diabetes mellitus and healthy controls were analyzed using bivariate regression. Bivariate correlation analysis was performed to assess the relationship between selenium levels and biochemical parameters, as well as the general characteristics of T2DM. Pairwise comparisons were conducted using Tukey's post hoc test. A p-value of less than 0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 26.

3.8. Variable-Based Subgroups Design for Comparing Selenium Levels Across Study Participants (patient and control group)

Age-based subgroups

The participants were divided into two age groups

- ≤50 years
- >50 years

Family history of diabetes mellitus

Patients were divided into two subgroups

- Have a family history of DM (Male group and Female group).
- No family history of DM (Male group and Female group).

Duration of type 2 diabetes mellitus

Patients were divided into two subgroups

- ≤5 years.

- > 5 years.

Physical activity

According to physical activity, patients were classified into two subgroups

- Physically non-active, where the patient did not exercise at least 30 minutes per day.
- Physically active, where the patient exercises at least 30 minutes or more per day.

Body Mass Index (BMI)

The subjects were divided into three subgroups.

- BMI less than 24.9 kg/m² were estimated normal weight.
- BMI between 25.0 and 29.9 kg/m² were estimated overweight.
- BMI equal to, or more than 30 kg/m² were estimated obese.

$$*BMI = \text{Weight (Kg)} / (\text{Height (m)})^2$$

Waist circumferences (WC)

- For male; < 102 cm, ≥ 102 cm.
- For female; < 88 cm, ≥ 88 cm.

Smoking habit

Based on smoking habits, the subjects were divided into two subgroups.

- Smokers.
- Non-smokers.

Glycemic control (HbA1c)

The patient's readings of HbA1c were categorized into three subgroups.

- Good controlled (HbA1c < 7%).
- Moderate controlled (HbA1c 7–8%).
- Poor controlled (HbA1c > 8%).

Fasting blood sugar

- FBS < 110 mg/dL.
- FBS ≥ 110 mg/dL.

Lipid profile status

lipid cut-off points were done based on National Cholesterol Education Program (NCEP) guidelines.

Total serum cholesterol

- Total serum cholesterol less than 200 mg/dL were considered as normal.
- Total serum cholesterol between 200 and 239 mg/dL was considered borderline.
- Total serum cholesterol ≥ 240 mg/dL was considered as hypercholesterolemia.

Serum triglyceride (S.TG)

- S.TG less than 150 mg/dL was considered as normal.
- S.TG between 150 and 199 mg/dL was considered as high.
- S.TG more than 200 mg/dL was considered as hypertriglyceridemia.

Serum high-density lipoprotein-cholesterol (S.HDL-C)

S.HDL-C was measured as follows:

- For Male: > 40 mg/dL, ≥ 40 mg/dL.
- For Female: > 50 mg/dL, ≥ 50 mg/dL.

Serum low-density lipoprotein-cholesterol (S.LDL-C)

- S.LDL-C less than 100 mg/dL was considered as optimal.
- S.LDL-C between 100 and 129 mg/dL was regarded as near-optimal.
- S.LDL-C between 130 and 159 mg/dL was considered as a borderline high-risk factor.
- S.LDL-C equal to or more than 160 mg/dL was considered as a high-risk factor.

4. RESULT AND DISCUSSION

In this study, the whole study population consisted of 99 participants, 59 (59.6 %) individuals with type 2 diabetes mellitus (T2DM) in the patients' group, and 40 (40.4 %) healthy subjects in the control' group. These participants were examined for several key variables according to the general characteristics of the study population.

Results:

4.1. Study Population's General Characteristics According to Patients and Control Groups

Concerning the age as a continuous variable, participants of the diabetic group were older, with a median of 55 (IQR = 15) years, while the control group was the youngest, with a median age of 48 (IQR = 8) years. The population data analysis showed a significant difference in age between type 2 diabetes and the control group at $p < 0.001$. This result is in line with the fact that aging is a risk factor for type 2 diabetes. There was a statistically significant variation in WC when comparing the diabetic group to the control group, with the mean waist circumference (WC) for the diabetic group being 104.92 ± 9.39 , and the control group's mean waist circumference (WC) being 93.40 ± 14.07 . The results of the independent t-test were significant at $p < 0.05$, indicating that the proportion of abdominal obesity, which is defined by the WC, is higher in those with type 2 diabetes. This aligns with the previous observations that relate the distribution of abdominal adiposity to insulin resistance caused by type 2 diabetes development. The mean body mass index (BMI) was 29.28 ± 5.36 in the diabetic group, and thereby most of the diabetic participants were either overweight or obese; 47.4% of the participants were obese, and 44.1% were overweight. In the control group, the average body mass index (BMI) was 25.77 ± 5.73 , while 17.5% were considered obese, and 55.1% were considered normal weight. In terms of BMI, Pearson's Chi-square test shows a highly significant difference at $p < 0.001$, highlighting the role of BMI in the development of type 2 diabetes. In particular, central obesity is proven to be a negative determinant of insulin sensitivity and a factor promoting metabolic disorder. We did not find substantial differences in terms of gender between the two groups, with Fisher's expected chi-square equal to 238. With regards to categorical data, Table 2 shows that a majority of the participants in both groups were male, with 61 (64.9%) in the diabetic group and 72.5 (72.5%) in the control group. This result suggests that the gender of this population may not be a highly influencing factor for the development of type 2 diabetes. Specifically, regarding smokers, the relative percent of the diabetic group was 30.5%, and the relative percent of the control group was 42.5%. When comparing the two groups, no significant difference was observed ($p = 0.202$), indicating that smoking status was not a strong determinant of the prevalence of type 2 diabetes among this group.

of patients. This may be because the risk factors for developing the disease are numerous and often overlap with one another. There was a strong relationship between having type 2 diabetes and having one or more first-degree relatives with the disease. A significantly higher proportion of the diabetics provided a positive response regarding family history when compared to the control, 71.2% and 32.5%, respectively ($p < 0.001$). This underscores the genetic contribution of type 2 diabetes, which is an important element of the disease's causation. People in the diabetic group had a significantly higher prevalence of high blood pressure, with 64.4% of them confessing to having high blood pressure as against none in the control group ($p < 0.001$). This high prevalence of high blood pressure in our patient with type 2 diabetes supports previous researches, which describe hypertension as both a complication and complication risk factor for type 2 diabetes, commonly presenting as metabolic syndrome. The diabetic group was less of a physically active group; only 16.9% were physically active, while 60% of the control group were active ($p < 0.001$). Lack of exercise is an established risk factor for type 2 diabetes, implying that people who fail to exercise may be at a higher risk of developing diabetes health complications. Among diabetic participants, 43 (72.9%) had the disease for less than five years, while 16 (27.1%) had the disease for five years or more. This distribution may be attributed to the study's inclusion criteria, which required participants with T2DM to have no complications. As a result, the majority of participants were in the earlier stages of the disease, with a relatively short duration of diabetes. The findings from all the evaluated studies mentioned in this paragraph are systematically summarized in Table 4.1.

Table 4.1. Study Population's General Characteristics According to Patients and Control Groups

Subject's Characteristics (n=99)	Frequency Distribution		p-value	Comparison test
	Mean ± Sd or Median IQR			
	Diabetic (n=59)	Control (n=40)		
Continues variables				
Age	55(50-65)	48(44-52)	<0.001	Mann-Whitney U-test
≤ 50 years	16 (27.1%)	27 (67.5%)	<0.001	Pearson Chi-Square
> 50 years	43 (72.9%)	13 (32.5%)		
WC	104.92±9.39	93.40±14.07	<0.001	independent T-test
Male				
< 102 cm	15 (25.4%)	22(55%)	0.004	Pearson Chi-Square
≥ 102 cm	22 (37.3%)	7(17.5)		
Female				
< 88 cm	0	4 (10%)	0.008	Pearson Chi-Square
≥ 88 cm	22 (37.3%)	7 (17.5%)		
BMI	29.28±5.36	25.77±5.73	0.002	independent T-test
Normal	5 (8.5%)	22(55.1%)	<0.001	Pearson Chi-Square
Overweight	26 (44.1%)	7 (17.5%)		
Obese	28 (47.4%)	11 (27.5%)		
Categorical variables				
Gender				
Male	36 (61.0%)	29 (72.5%)	0.238	Pearson Chi-Square
Female	23 (39.0%)	11 (27.5%)		
Smoking Habits				
Smoker	18 (30.5%)	17 (42.5%)	0.202	Pearson Chi-Square
Non-smoker	41 (69.5%)	23 (57.5%)		
F-history of T2DM				
Positive	42 (71.2%)	13 (32.5%)	<0.001	Pearson Chi-Square
Negative	17 (28.8%)	27 (67.5%)		
Blood Pressure				
Positive	38 (64.4%)	0	<0.001	Pearson Chi-Square
Negative	21 (35.6%)	40 (100%)		
physically				
Active	10 (16.9%)	24 (60%)	<0.001	Pearson Chi-Square
Non-active	49 (83.1%)	16 (40%)		
Duration of Diabetes				
<5 years	43 (72.9%)	0	-	-
≥5 years	16 (27.1%)	0		

4.2. Comparisons of Biochemical Measurements Among Study Groups

Table 4.2, also gives diabetics and control group comparison of different biochemical parameters. The levels of fasting blood glucose were significantly higher in the diabetic patients than in the control group; 176.0 mg/dL, IQR = 102 for the diabetic group compared to 99.0 mg/dL, IQR = 17 for the control group; $p < 0.001$. Likewise, there was evidence of poor glycemic control in the diabetic group as found out by the higher mean HbA1c level of 8.35% than the control group's mean HbA1c level of 5.31%, with $p < 0.001$. In the diabetic group, cholesterol concentrations were marginally higher at 177.0 mg/dL, IQR = 42 than the control group at 167.5 mg/dL, IQR = 62, $p =$

0.043 there is evidence of dyslipidemia in type 2 diabetes. The result from this study shows that the diabetic group had a higher triglyceride level: 171.0 mg/dL (IQR = 150) compared to 92.5 mg/dL of the control group (IQR = 52), $p < 0.001$. Nevertheless, the present study could not record any substantial difference for the group means in the HDL-C ($F = 1.101$, $p = 0.315$) as well as the LDL-C ($F = 0.832$, $p = 0.459$) suggesting that these lipid phenotypes may not be highly tender in type 2 diabetes in this population. The mean level of C-reactive protein was significantly raised in the diabetic group (mean 2.5mg/dL; IQR = 3.8) compared to the control group (mean 1.45 mg/dL; IQR = 2.37) $p = 0.053$ a trend toward significant difference. These alterations were not significant in terms of liver functions between case and control groups as there was no difference in the GPT levels ($p = 0.745$). A slightly higher mean of serum creatinine concentrations of 0.595 ± 0.181 mg/dL were recorded in the diabetic population than the control one where the mean was 0.64 ± 0.16 mg/dL, and thus there was no significant difference in the renal function between these two groups ($p = 0.214$). TSH values differed between diabetic and control groups: mean $2.49 \pm SD 1.04$ in the diabetic group versus mean $1.50 \pm SD 0.67$ in the control group. The p -value = 0.004 thus additional thyroid function testing could be important in Type 2 diabetes. Last, selenium was slightly higher in the diabetic group (mean = 129.42 μ g/L; SD = 33.70) compared to the control group (mean = 115.94 μ g/L; SD = 24.06) $p = 0.032$ but still within the normal range. This indicates that although the selenium level may be somewhat higher in type 2 diabetic patients, the differential is not sufficient to point to the severity of deficiency or excess to warrant further investigation of selenium in diabetes. These results are discussed in Table 4.2 below.

Table 4.2. Comparisons of Biochemical Measurements Among Study Groups

Biochemical Parameters (n=99)	Mean $\pm$ SD or Median IQR		P-value	Comparison test
	T2DM (n=59)	Controls (n=40)		
FBS (mg/dL)	176.0 (102)	99.0 (17)	<0.001	Mann-Whitney U Test
HbA1c (%)	8.35 $\pm$ 2.03	5.31 $\pm$ 0.45	<0.001	Independent t-test
Cholesterol (mg/dL)	177.0 (42)	167.5 (62)	0.043	Mann-Whitney U Test
Triglyceride (mg/dL)	171.0(150)	92.5(52)	<0.001	Mann-Whitney U Test
HDL-C (mg/dL)	45.0 (13)	43.5 (13)	0.630	Mann-Whitney U Test
LDL-C (mg/dL)	101 (54)	97 (48)	0.459	Independent t-test
CRP (mg/dL)	2.5 (3.8)	1.45 (2.37)	0.053	Mann-Whitney U Test
GPT (U/L)	18 (11)	18 (8.75)	0.745	Mann-Whitney U Test
S-Cr (mg/dL)	0.595 $\pm$ 0.181	0.64 $\pm$ 0.16	0.214	Independent t-test
TSH (mIU/L)	2.49 $\pm$ 1.04	1.50 $\pm$ 0.67	0.004	Independent t-test
Selenium (μ g/L)	129.42 $\pm$ 33.70	115.94 $\pm$ 24.06	0.032	Independent t-test

4.3. Selenium Levels in T2DM and Healthy Subjects in Relation to Age

Table 4.3 presents the cross-tabulation analysis of age and serum selenium in both diabetic and non-diabetic individuals. Overall, 27.1% of diabetic participants were 50 years old or younger; their selenium level was 126.76 µg/L (IQR = 63.9); 72.9% of the participants were more than 50 years old, and their selenium level was 130.31 µg/L (IQR = 49.3). Because of the above observation, it was observed that the p-value for inter-group comparison was 0.682 indicating no statistical significance of age influencing selenium level in diabetic subjects and hence concluded that age is not an influential factor affecting the selenium level in patients with type 2 diabetes. In the control group therefore, only 67.5% of the participants were less than or equal to 50 years, with a mean selenium concentration of 119.41µg/L (IQR=39.3) while 32.5% were above 50 years of age, mean selenium concentration at 111.36 µg/L (IQR=18.7). The p-value for this comparison was 0.231, which means there were no significant differences in selenium levels between the age groups in the control group. Taken collectively, these results also point to the fact that age per se has negligible impact on selenium levels in the studied diabetic and otherwise healthy subjects so it is tentative to assume that metabolic and/or clinical factors might play a more critical role in regulating selenium levels.

Table 4.3. Selenium Levels in T2DM and Healthy Subjects in Relation to Age

Subject's Characters (n=99)	Selenium in T2DM (n=59)			Selenium in Control (n=40)		
	N (%)	Median IQR	P- value	N (%)	Median IQR	P- value
		(µg/L)			(µg/L)	
Age						
≤50 years	16 (27.1%)	126.76 (63.9)	0.682	27(67.5%)	119.41(39.3)	0.231
< 50 years	43 (72.9%)	130.31 (49.3)		13(32.5%)	111.36(18.7)	
Mann-Whitney U-test was performed for statistical analysis.						

4.4. Selenium Levels in T2DM and Healthy Subjects in Relation to BMI

Analyzing whether BMI impacts selenium concentrations by comparing diabetic and control groups according to BMI categories. In the diabetic group, 8.5% of participants had normal body mass index, 44.1% were overweight while the rest were obese and they had mean selenium levels of 128.32 µg/L (SD = 18.71), 125.84 µg/L (SD = 37.77) and 132.93 µg/L (SD = 32.28) respectively. The p-value for this comparison is 0.747 which means that there was no statistically significance difference in the mean selenium levels between the different BMI groups in the diabetic group,

therefore, BMI does not have an impact on selenium levels in this population. In the control group, 55.1% had normal BMI and the mean selenium level was 117.57 $\mu\text{g/L}$ (SD = 27.25); 17.5% of overweight people with mean selenium level of 106.82 $\mu\text{g/L}$ (SD = 15.12) and 27.5% obese individuals with mean selenium level of 118.47 $\mu\text{g/L}$ (SD = 22.12). The p-value for comparison in the control group was found 0.553, thus specifying that there was no statistical difference observed in between the BMI categories for the control group subjects also toward selenium level. Taken together, these findings imply that BMI does not significantly influence the selenium levels in the diabetic as well as control populations. More studies may be required to find possible relationships of BMI with selenium which can be affected by other exposures; insulin resistance, inflammation, or dietary intake. Details of these findings are presented in Table 4.4.

Table 4.4. Selenium Levels in T2DM and Healthy Subjects in Relation to BMI

Subject's Characters (n=99)	Selenium in T2DM (n=59)			Selenium in Control (n=40)		
	N (%)	Mean $\pm$ SD ($\mu\text{g/L}$)	P- value	N (%)	Mean $\pm$ SD ($\mu\text{g/L}$)	P- value
BMI						
< 24.9 kg/m^2	5 (8.5%)	128.32 $\pm$ 18.71	0.747	22 (55.1%)	117.57 $\pm$ 27.25	0.553
25-24.9 kg/m^2	26 (44.1%)	125.84 $\pm$ 37.77		7 (17.5%)	106.82 $\pm$ 15.12	
$\geq 30 \text{ kg/m}^2$	28 (47.4%)	132.93 $\pm$ 32.28		11 (27.5%)	118.47 $\pm$ 22.12	
ANOVA one-way was performed for statistical analysis						

4.5. Selenium Levels in T2DM and Healthy Subjects in Relation to WC

Table 4.5 below aims to determine the correlation between waist circumference (WC) and selenium among the diabetic and a control group. Data from the male participants with T2DM revealed that the majority 38.9% had a WC less than 102 cm with a mean selenium level of 121.49 $\mu\text{g/L}$ (SD = 37.24); while a minority of 61.1% had a WC of greater or equal to 102 cm with a higher average selenium level of 143.66 $\mu\text{g/L}$ (SD = 34.98). These results summarized that the p-value equal to 0.079 implicates that there is no significant difference in selenium levels between these two WC categories in male T2DM participants. In the control group, 75.9% of male participants had a WC less than 102 cm with a mean selenium level of 114.92 μg selenium per liter of blood (SD = 18.55), and only 24.1% of the participants with WC more than or equal to 102 cm the mean selenium level was 107.37 μg selenium per liter of blood (SD = 19.04). The observed F-test value of .329 indicates no significant difference in selenium levels between the two WC categories in male participants in the control group because the p-value is 0.360. Because all the female participants in the T2DM group had WC >88 cm, selenium levels could not be compared in smaller

WC >88 cm groups. Among the female participants in the control group, 36.4 percent had a WC less than 88 cm with a mean selenium level of 129.54 µg/L (SD = 52.58), 63.6 percent of the participants had WC greater than or equal to 88 cm with a mean selenium level of 119.95 µg/L (SD = 24.10). The calculated p-value of 0.683 indicates evidence of no differences in selenium levels between the two WC categories in female participants of the control group. In our study, no correlations were obtained between gross WC and selenium concentrations both in diabetic subjects and in the control group evidenced by p-values equal to 0.079, 0.360, and 0.683. The results of this study imply that at least body fat mass, as indexed by waist circumference, does not modulate selenium levels in this sample of participants.

Table 4.5. Selenium Levels in T2DM and Healthy Subjects in Relation to Waist Circumference (WC)

Subject's Characters (n=99)	Selenium in T2DM (n=59)			Selenium in Control (n=40)		
	N (%)	Mean ± SD	P- value	N (%)	Mean ± SD	P- value
		(µg/L)			(µg/L)	
WC						
Male						
< 102 cm	14 (38.9%)	121.49±37.24	0.079	22 (75.9%)	114.92±18.55	0.360
≥ 102 cm	22 (61.1%)	143.66±34.98		7 (24.1%)	107.37±19.04	
Female						
< 88 cm	-	-	NA	4 (36.4%)	129.54±52.58	0.683
≥ 88 cm	23(100%)	120.62±26.15		7 (63.6%)	119.95±24.10	
NA: no association. Independent t-test was performed for statistical analysis						

4.6. Selenium Levels in T2DM and Healthy Subjects in Relation to Gender

A correlation was also made between gender and selenium among the diabetic and control groups to establish whether selenium levels are affected by gender. Within the diabetic group, more than half (61.0%) of the participants were males with a selenium level of 135.05 µg/L (SD = 37.00) as compared to female participants (39.0 %) with a selenium level of 120.62 µg/L (SD = 26.15). The p-value obtained for gender – 0.085 between diabetic patients, meant no significant gender difference regarding selenium plasma level, although the mean value of selenium level was higher in males. In the control group, male participants 72.5% had a selenium level of 113.1 µg/L (SD = 18.61) while female participants 27.5% had a selenium level of 123.0 µg/L (SD = 34.66). For the control group, the p-value for comparison of the selenium level between genders was 0.365, which indicated that there was no meaningful variation of the selenium level in different genders. These results are shown in Table 4.6.

Table 4.6. Selenium Levels in T2DM and Healthy Subjects in Relation to Gender

Subject's Characters (n=99)	Selenium in T2DM (n=59)			Selenium in Control (n=40)		
	N (%)	Mean $\pm$ SD ($\mu\text{g/L}$)	P- value	N (%)	Mean $\pm$ SD ($\mu\text{g/L}$)	P- value
Gender						
Male	36 (61.0%)	135.05 $\pm$ 37.00	0.085	29 (72.5%)	113.1 $\pm$ 18.61	0.365
Female	23 (39.0%)	120.62 $\pm$ 26.15		11 (27.5%)	123.0 $\pm$ 34.66	
Independent t-test was performed for statistical analysis						

4.7. Selenium Levels in T2DM and Healthy Subjects in Relation to Smoking Habits

Smoking habits were also analyzed concerning selenium status in both the diabetic and the control subjects. Diabetic participants in the current study included 30.5% smokers, with a mean selenium of 141.45 $\mu\text{g/L}$ (SD = 34.66), and 69.5% non-smokers, with a mean selenium of 124.13 $\mu\text{g/L}$ (SD = 32.29). The p-value for this comparison was 0.069 indicating that there is no statistical difference between smokers and non-smokers in the diabetic group although selenium level in smokers is slightly high. While 42.5% of the control group are smokers and 57.5% non-smokers the mean (SD) selenium in smokers was 118.49 (17.26) $\mu\text{g/L}$ and that of non-smokers, 114.05 (28.30) $\mu\text{g/L}$. However, for the smokers against the control group, the distributions of selenium levels were not significantly different from that observed in the control group, with a p = 0.570. The findings outlined in this study are presented in Table 4.7.

Table 4.7. Selenium Levels in T2DM and Healthy Subjects in Relation to Smoking Habits

Subject's Characters (n=99)	Selenium in T2DM (n=59)			Selenium in Control (n=40)		
	N (%)	Mean $\pm$ SD ($\mu\text{g/L}$)	P- value	N (%)	Mean $\pm$ SD ($\mu\text{g/L}$)	P- value
Smoking Habits						
Smokers	18 (30.5%)	141.45 $\pm$ 34.66	0.069	17 (42.5%)	118.49 $\pm$ 17.26	0.570
Non-smokers	41 (69.5%)	124.13 $\pm$ 32.29		23 (57.5%)	114.05 $\pm$ 28.30	
Independent t-test was performed for statistical analysis						

4.8. Selenium Levels in T2DM and Healthy Subjects in Relation to Family History of T2DM

The interaction of the family history of T2DM with selenium was compared in both groups. Hence 71.2% of diabetic patients reported a positive family history of T2DM while only 28.8% of

patients reported a negative history of T2DM. The patients with positive family history in the diabetic group had lower selenium levels of $121.35 \mu\text{g/L} \pm \text{SD } 32.49$, while those with negative family history had selenium levels of $149.34 \mu\text{g/L} \pm \text{SD } 28.60$, the p-value obtained was 0.003, which means there was a statistically significant difference in selenium levels based on family history of T2DM. In the control group, 32.5% participants had positive family history of T2DM, they had a mean selenium level of $109.75 \mu\text{g/L}$, (SD 19.16), while 67.5% had negative family history, with mean selenium level of $118.91 \mu\text{g/L}$, (SD 25.89), with a p-value of 0.265, indicating that there is no statistically significant difference for selenium between the subgroups of the control group. Based on these observations, negative family history of T2DM may be related to elevated selenium levels in patients with T2DM. Still, it was not confirmed in the control group. These observations are presented in Table 4.8.

Table 4.8. Selenium Levels in T2DM and Healthy Subjects in Relation to Family History of T2DM

Subject's Characters (n=99)	Selenium in T2DM (n=59)			Selenium in Control (n=40)		
	N (%)	Mean $\pm$ SD ($\mu\text{g/L}$)	P-value	N (%)	Mean $\pm$ SD ($\mu\text{g/L}$)	P-value
F-history						
Positive	42 (71.2%)	121.35 ± 32.49	0.003	13 (32.5%)	109.75 ± 19.16	0.265
Negative	17 (28.8%)	149.34 ± 28.60		27 (67.5%)	118.91 ± 25.89	
Independent t-test was performed for statistical analysis						

4.9. Selenium Levels in T2DM and Healthy Subjects in Relation to Physical Activity

Again, the correlation between physical activity and selenium levels was tested for the diabetic and the control group. Non-active physical participants were 16.9% and 83.1% in the diabetic group, with median selenium level of $131.73 \mu\text{g/L}$, IQR = 47.39 and $127.47 \mu\text{g/L}$, IQR = 51.42 respectively, with p-value of 0.824. Indicating no significant differences between the two groups. The control group included 60.0% physically active participants with a selenium median of $115.15 \mu\text{g/L}$, IQR= 26.66, while 40.0% non-active participants with a selenium median of $114.67 \mu\text{g/L}$, IQR= 33.06. With a p-value of 0.689 for this comparison, the difference in selenium levels between the active and non-active study participants was not significant. Based on these observations it may be concluded that regular physical activity does not have any influence on selenium concentration in either examined diabetic or control group in this study. These findings are presented in Table 4.9 below.

Table 4.9. Selenium Levels in T2DM and Healthy Subjects in Relation to Physical Activity

Subject's Characters (n=99)	Selenium in T2DM (n=59)			Selenium in Control (n=40)		
	N (%)	Median IQR ($\mu\text{g/L}$)	P-value	N (%)	Median IQR ($\mu\text{g/L}$)	P-value
physically						
Active	10 (16.9%)	131.73(47.39)	0.824	24 (60%)	115.15(26.66)	0.689
Non-active	49 (83.1%)	127.47(51.42)		16 (40%)	114.67(33.06)	
Mann-Whitney U-test was performed for statistical analysis						

4.10. Selenium Levels in T2DM and Healthy Subjects in Relation to Duration of T2DM

Comparisons between selenium levels based on the T2DM duration were conducted in the patient group. Among patients who had diabetes for less than 5 years with a median selenium of 133.16 $\mu\text{g/L}$ (IQR 40.36) compared with 83 (27.1%) of the diabetic participants with diabetes for 5 years or more with a median selenium of 127.23 $\mu\text{g/L}$ (IQR 50.35). The P-value for this comparison was 0.465 showing that there was no significant difference in selenium levels among participants with diabetic duration < 5 years and participants with diabetic duration $\geq$ 5 years. The results have been summarized in Table 4.10 below.

Table 4.10. Selenium Levels in T2DM and Healthy Subjects in Relation to Duration of T2DM

Subject's Characters (n=99)	Selenium in T2DM (n=59)			Selenium in Control (n=40)		
	N (%)	Median IQR ($\mu\text{g/L}$)	P-value	N (%)	Median IQR ($\mu\text{g/L}$)	P-value
Having T2DM						
<5 years	43 (72.9%)	133.16(40.36)	0.465	-	-	NA
$\geq$ 5 years	16 (27.1%)	127.23(50.35)		Healthy	Group	
NA: no association. Mann-Whitney U-test was performed for statistical analysis						

4.11. Selenium Levels in T2DM and Healthy Subjects in Relation to FBG

To explore the link between fasting blood glucose and selenium, selenium concentrations were compared between subgroups in both diabetic patients and healthy individuals. Among diabetic participants, 8.5% had FSG less than 110mg/dL, with a selenium level of 125.1 $\mu\text{g/L}$ (IQR: 55.7) of the participants, while 91.5% of the participants had FSG of $\geq$ 110 mg/dL, with a selenium level of 128.9 $\mu\text{g/L}$ (IQR: 51.8). The p-value for this comparison was 0.211, indicating there was no difference in the medians of the two subgroups in this regard and selenium was therefore

comparable in the two subgroups. In the control participants, 92.5% had FSG < 110, and the median of their selenium level was 114.7 µg/L (IQR = 30.33), while 7.5% of participants had FSG ≥ 110, and the median of their selenium level was 128.9 µg/L (IQR = 36.0). The p-value for this comparison was equal to 0.090, indicating that the difference in selenium concentration is not statistically significant. These findings reveal that FBG levels have no significant influence on selenium levels in both diabetic and control groups of this work. These findings are presented below, in Table 4.11.

Table 4.11. Selenium Levels in T2DM and Healthy Subjects in Relation to FBG

Subject's parameters (n=99)	Selenium in diabetic (n=59)			Selenium in Control (n=40)		
	N (%)	Median IQR (µg/L)	P- value	N (%)	Median IQR (µg/L)	P- value
FSG						
<110 mg/dL	5 (8.5%)	125.1 (55.7)	0.211	37 (92.5%)	114.7 (30.33)	0.090
≥110 mg/dL	54 (91.5%)	128.9 (51.8)		3 (7.50%)	128.9 (36.0)	
Mann-Whitney U-test was performed for statistical analysis						

4.12. Selenium Levels in T2DM and Healthy Subjects in Relation to Cholesterol

Hence the correlation between cholesterol and serum selenium present was made with the diabetics as well as the control group. In the diabetic group, 69.5% had cholesterol levels below 200 mg/dL, 20.3%, had cholesterol levels between 200-239 mg/dL, and 10.2%, had cholesterol levels above 240 mg/dL, the median selenium level was 13.31 (IQR = 40.91), 120.36 (IQR = 87.3), and 119.89 (IQR = 67.6), respectively. The p-value of 0.970 for this comparison shows that there is no statistically significant difference in the distribution of selenium in these subgroups of cholesterol in the diabetic group. About 77.5% of the control group participants had cholesterol levels of less than 200 mg/dL, median selenium levels of 114.67 µg/L (IQR = 33.2), and 22.5% had a cholesterol level between 200–239 mg/dL, with a median selenium level of 120.36 µg/L (IQR = 24.9). The p-value for this comparison was 0.331 showing that there was no significant difference in selenium levels among subgroups. These results indicate that serum cholesterol does not have a strong relationship with serum selenium levels in the study population. The following findings are summarized in Table 4.13 further.

Table 4.12. Selenium Levels in T2DM and Healthy Subjects in Relation to Cholesterol

Subject's parameters (n=99)	Selenium in diabetic (n=59)			Selenium in Control (n=40)		
	N (%)	Median IQR (µg/L)	P- value	N (%)	Median IQR (µg/L)	P- value
Cholesterol						
< 200 mg/dL	41 (69.5%)	13.31 (40.91)	0.970	31 (77.5%)	114.67 (33.2)	0.331
200 – 239 mg/dL	12 (20.3%)	120.36 (87.3)		9 (22.5%)	120.36 (24.9)	
≥240 mg/dL	6 (10.2%)	119.89 (67.6)		-	-	
Kruskal-Wallis H-test was performed for statistical analysis.						

4.13. Selenium Levels in T2DM and Healthy Subjects in Relation to Triglyceride

In both the diabetic and control groups, the relation between triglyceride and serum selenium concentrations was compared. 40.7% of participants in the diabetic group had triglyceride levels above 200 mg/dL, with a median selenium level of 113.96 µg/L (IQR = 55.96); 13.6% had triglyceride levels between 150 and 199 mg/dL, with a median selenium level of 135.05 µg/L (IQR = 57.66); and 45.7% had triglyceride levels below 150 mg/dL, with a median selenium level of 130.31 µg/L (IQR = 41.23). There was no statistically significant difference in selenium levels among the triglyceride subgroups in the diabetic group, according to the comparison's p-value of 0.698. In the control group, 82.5% of participants had triglyceride levels below 150 mg/dL, with a median selenium level of 117.99 µg/L (IQR = 32.23), and 17.2% had triglyceride levels between 150 and 199 mg/dL, with a median selenium level of 114.67 µg/L (IQR = 25.59). The p-value for this comparison was 0.859, suggesting no significant difference in selenium levels across these subgroups in the control group. These findings suggest that triglyceride levels do not significantly affect selenium concentrations in this study population. These findings are outlined in Table 4.13.

Table 4.13. Selenium Levels in T2DM and Healthy Subjects in Relation to Triglyceride

Subject's parameters (n=99)	Selenium in diabetic (n=59)			Selenium in Control (n=40)		
	N (%)	Median IQR (µg/L)	P- value	N (%)	Median IQR (µg/L)	P- value
Triglyceride						
<150 mg/dL	27 (45.7%)	130.31(41.23)	0.698	33 (82.5%)	117.99(32.23)	0.859
150 – 199 mg/dL	8 (13.6%)	135.05(67.66)		7(17.2%)	114.67(25.59)	
≥200 mg/dL	24 (40.7%)	113.96(55.96)		-	-	
Kruskal-Wallis H Test was performed for statistical analysis						

4.14. Selenium Levels in T2DM and Healthy Subjects in Relation to HDL-C

Table 4.14 examines the relation of high-density lipoprotein with serum selenium levels of the subjects in the diabetic and control groups. The male participants in the diabetic group, with an HDL-C concentration less than 40 mg/dL, represented 36.1% (mean selenium level 136.53 µg/L, SD 40.96); those with HDL-C ≥ 40 mg/dL represented 63.9% (mean selenium level 134.20 µg/L; SD 35.51). With a p-value of 0.859 indicating that the difference in selenium levels is statistically not significant. Males in the control group, 40.1% had HDL-C < 40 mg/dL with a mean selenium level of 119.46 µg/L (SD = 20.22), whereas 59.9% had HDL-C ≥ 40 mg/dL with a mean selenium level of 109.20 µg/L (SD = 16.97). For this comparison, the p-value obtained was 0.153, hence implying that there was no significant difference in the selenium levels in the subgroups of males in the control group. While 60.9% of female participants in the diabetic group had affixed HDL-C less than 50 mg/dL, with a selenium level of 129.25 µg/L (SD = 22.80), 39.1% had affixed HDL-C of 50 mg/dL and above and had a selenium level of 107.19 µg/L (SD = 26). The p-value for comparison between the two groups of females was 0.045, indicating a statistically significant difference. Among the females of the control group, 45.5% had HDL-C less than 50 mg/dL, and the mean selenium level was 125.48 with SD = 19.22, while 54.5% had high HDL-C of 50 mg/dL, and the mean selenium was 121.47 with SD = 45.82. The p-value for this comparison was 0.869, meaning that there was no statistically significant difference in selenium levels between the female subgroups within the control group.

Table 4.14. Selenium Levels in T2DM and Healthy Subjects in Relation to HDL-C

Subject's parameters (n=99)	Selenium in diabetic (n=59)			Selenium in Control (n=40)		
	N (%)	Mean ± SD (µg/L)	P- value	N (%)	Mean ± SD (µg/L)	P- value
HDL-C						
Male						
>40 mg/dL	13 (36.1%)	136.53±40.96	0.859	11 (40.1%)	119.46±20.22	0.153
≥ 40 mg/dL	23 (63.9%)	134.20±35.51		18 (59.9%)	109.20±16.97	
Female						
>50 mg/dL	14 (60.9%)	129.25±22.80	0.045	5 (45,5%)	125,48±19,22	0.869
≥ 50 mg/dL	9 (39.1%)	107.19±26.51		6 (54,5%)	121,47±45,82	
Independent t-test was performed for statistical analysis						

4.15. Selenium levels in T2DM and Healthy Subjects in Relation to LDL-C

The link between LDL-C and serum selenium concentrations was examined in both the diabetic and non-diabetic participants. The mean selenium in the diabetic group was 127.91 $\mu\text{g/L}$ (SD = 31.24), for 47.5% of the participants had LDL-C levels under 100 mg/dL, 27.1% had LDL-C levels between 100 and 129 mg/dL (mean selenium level = 129.45 $\mu\text{g/L}$, SD = 32.59), 22% had LDL-C levels between 130 – 159 mg/dL (mean selenium level = 132.38 $\mu\text{g/L}$, SD = 40.3), and 3.4% had LDL-C levels $\geq$ 160 mg/dL (mean selenium level of 131.03 $\mu\text{g/L}$, SD = 62.66). The p-value for this comparison was 0.985, indicating that there was no significant difference in selenium levels among LDL-C subgroups in the diabetic group. In the control group, 55.0% of the participants had an LDL-C level of less than 100 mg/dL with a mean selenium level of 110.55 $\mu\text{g/L}$ (SD = 26.99); 27.5% had an LDL-C level between 100-129 mg/dL, with a mean selenium level of 121.61 $\mu\text{g/L}$ (SD = 23.10); 15% had an LDL-C level between 130-159 mg/dL, with mean selenium levels of 126.36 $\mu\text{g/L}$ (SD = 7.027); and 2.5% had an LDL-C level more than or equal to 160 mg/dL, with a mean selenium level of 109.47. The p-value for this comparison was 0.418, meaning that there is no statistically significant difference in selenium levels according to the LDL-C subgroup within the control group. Based on these results, LDL-C does not have a relationship with selenium levels in the diabetics and controls. These findings are presented in Table 4.15.

Table 4.15. Selenium Levels in T2DM and Healthy Subjects in Relation to LDL-C

Subject's parameters (n=99)	Selenium in diabetic (n=59)			Selenium in Control (n=40)		
	N (%)	Mean $\pm$ SD ($\mu\text{g/L}$)	P-value	N (%)	Mean $\pm$ SD ($\mu\text{g/L}$)	P-value
LDL-C						
<100 mg/dL	28 (47.5%)	127.91 $\pm$ 31.24	0.985	22 (55.0%)	110.55 $\pm$ 26.99	0.418
100 – 129 mg/dL	16 (27.1%)	129.45 $\pm$ 32.59		11 (27.5%)	121.61 $\pm$ 23.10	
130 – 159 mg/dL	13 (22.0%)	132.38 $\pm$ 40.30		6 (15.0%)	126.36 $\pm$ 7.027	
$\geq$ 160 mg/dL	2 (3.40%)	131.03 $\pm$ 62.66		1 (2.5%)	109.47	
ANOVA one-way H Test was performed for statistical analysis.						

4.16. Selenium Levels in T2DM Patients in Relation to Glycemic Control (HbA1c)

The relationship between glycemic control, as measured by HbA1c, and serum selenium concentrations was examined in type 2 diabetes (T2DM) patients, categorized into three subgroups based on HbA1c levels: Classification by control effectiveness can be divided into good control for the values <7%, moderate control for 7-8%, and poor control for >8%. Among the patients' group, 18 (28.1%) of participants with good glycemic control had a selenium level of 124.91 $\mu\text{g/L}$ (SD 36.91), with moderate glycemic control 13 (20.6%) had selenium levels of 126.27 $\mu\text{g/L}$ (SD 32.2), and with poor glycemic control 28 (51.2%) had a selenium level of 133.78 $\mu\text{g/L}$. The p-value for this comparison was 0.644, meaning that there was no statistically significant difference in selenium levels based on the glycemic control subgroups of T2DM patients. These results indicate that the variation of HbA1c does not have an impact on selenium levels in the study population. These findings are presented in Table 4.16.

Table 4.16. Selenium Levels ($\mu\text{g/L}$) in T2DM Patients in Relation to Glycemic Control (HbA1c)

Subject's Characteristics	T2DM patients (n=59)			P-value
	Good (<7%) 18 (28.1%)	Moderate (7 – 8%) 13 (20.6%)	Poor (>8%) 28 (51.2%)	
Se (Mean $\pm$ SD)	124.91 $\pm$ 36.91	126.27 $\pm$ 32.2	133.78 $\pm$ 32.92	0.644
ANOVA one-way-test was performed for statistical analysis.				

4.17. Correlation of Selenium and Continues Variables Among T2DM Patients

According to Pearson and Spearman correlation (r/p), the results of the current study for the patient group showed the correlation between serum selenium levels and various continuous variables; there was a statistically significant poor negative relationship between the HDL-C and serum selenium levels (correlation coefficient = -0.289, p = 0.026). However, the results of the

study do not show significant correlations between selenium levels and other factors, including age (correlation coefficient = 0.128, p-value = 0.336), waist circumference (correlation coefficient = 0.168, p = 0.202), body mass index (BMI) (correlation coefficient = 0.005, p = 0.968), fasting blood sugar (FBS) (correlation coefficient = 0.227, p-value = 0.084), HbA1c (correlation coefficient = 0.189, p = 0.152), cholesterol (correlation coefficient = -0.041, p-value = 0.756), triglycerides (correlation coefficient = 0.000, p-value = 0.998), LDL-C (correlation coefficient = -0.008, p = 0.251), C-reactive protein (CRP) (correlation coefficient = -0.001, p-value = 0.993), alanine aminotransferase (GPT) (correlation coefficient = 0.229, p-value = 0.080), serum creatinine (correlation coefficient = 0.114, p = 0.390), and thyroid-stimulating hormone (TSH) (correlation coefficient = 0.015, p = 0.908) Table 17. Based on the current work observations, it appears that HDL-C may affect selenium concentrations in the blood; however, the other covariates assessed in the present study are not related to selenium levels in this population.

Table 4.17. Correlation of Selenium and Continues Variables Among T2DM Patients

Subject's Characteristics	Correlation coefficient (r/p)	p-value Sig. (2-tailed)	Bivariate Analysis
Age (years)	0.128	0.336	Spearman
Waist Circumference (cm)	0.168	0.202	Pearson
BMI (kg/m ²)	0.005	0.968	Pearson
FBS (mg/dL)	0.227	0.084	Spearman
HbA1c (%)	0.189	0.152	Pearson
Cholesterol (mg/dL)	-0.041	0.756	Spearman
Triglyceride (mg/dL)	0.000	0.998	Spearman
HDL-C (mg/dL)	-0.289*	0.026	Pearson
LDL-C (mg/dL)	-0.008	0.251	Pearson
CRP (mg/dL)	-0.001	0.993	Spearman
GPT (U/L)	0.229	0.080	Spearman
Serum Creatinine (mg/dL)	0.114	0.390	Pearson
TSH (mIU/L)	0.015	0.908	Pearson
*Low correlation (p < 0.05). Pearson or Spearman correlation tests were used based on the data distribution.			

Discussion:

Type 2 diabetes (T2D) is one of the most serious global public health issues, affecting an estimated 400 million people worldwide. Its etiology is complex, with selenium (Se) emerging as a modifiable risk factor for T2D development [54]. Selenium, an essential trace element, plays a crucial role in human health by supporting antioxidant defense mechanisms through its incorporation into selenoproteins, which regulate reproduction, neuroprotection, and thyroid

metabolism. However, Selenium has a narrow range between nutritional sufficiency and toxicity, which varies depending on its chemical form [70]. The impact of selenium on obesity and T2D is linked to disorders in glucose metabolism, insulin signaling, oxidative stress, and inflammation [71]. However, the relationships between dietary selenium intake, serum selenium concentration, plasma glucose levels, glycosylated hemoglobin (HbA1c), and diabetes risk remain under investigation [72]. This cross-sectional study compared the serum selenium levels in terms of glycemic control (HbA1c) and the effect of several biochemical parameters among patients with T2DM and healthy controls.

As a common characteristic of T2DM, fasting blood sugar levels in T2DM patients (176.0 mg/dL) were much higher than those in controls (99.0 mg/dL), Mann-Whitney U Test confirmed statistically significant differences, and the p-value was found to be < 0.001 . These findings agree with the ones reported by Zhang et al. (2019) [73]. These elevated levels in blood glucose are one of the distinctive features of T2MD causing the progress of diabetic complications such as neuropathy, nephropathy, and cardiovascular disease. A study presented by Jalpa Devi et al. (2016) [74], also recorded higher blood sugar levels in diabetic populations, supporting the pathophysiology of hyperglycemia in T2DM.

In a like manner, the T2MD patients had higher levels of Hemoglobin A1c (HbA1c) ($8.35 \pm 2.03\%$) compared to the healthy group of ($5.31 \pm 0.45\%$, $p < 0.001$), which provides a strong relationship between HbA1c high levels and poor glycemic control in diabetes. Similar results were found by Zhang et al. (2019) [73], and Muslim OT et al. (2021) [75]. The HbA1c is one of the critical markers that can be used for monitoring long-term glucose control and its elevated levels are a significant indicator that the T2MD patient might develop complications such as microvascular and macrovascular [76].

Higher Cholesterol levels (177.0 mg/dL) were also recorded in T2MD patients compared to the controls (167.5 mg/dL) ($p = 0.043$). These findings are in line with the ones provided by H. Zhang et al. (2017) [77], While some studies showed no significant relations as reported by Vahid Pouresmaeil et al. (2022) [78]. Cardiovascular disease (CVD) which is the leading cause of morbidity and mortality is strongly linked to elevated levels of cholesterol in diabetes. Therefore, regular monitoring and management of lipid levels in diabetic patients could reduce the risk of diabetes comorbidities [79].

The values of The Triglyceride (TG) in T2MD (171.0 mg/dL) were notably higher than those in controls (92.5 mg/dL) ($p < 0.001$, Mann-Whitney U Test). Similar patterns were observed in studies done by Zhang et al (2019) [73], and Jalpa Devi et al (2016) [74]. While our results are in contradiction to those provided by Alghobashy et al (2018) [80]. However, such high triglyceride levels in T2MD patients are quite common and result from insulin resistance, the key feature of the

disease. The elevated values of triglyceride might increase the risks of atherosclerosis leading to cardiovascular complications [81].

On the other hand, there was no statistical difference ($p = 0.630$) in high-density lipoprotein cholesterol (HDL-C), between T2MD patients and the healthy population. Vahid Pouresmaeil et al. in (2023), presented similar results. Although HDL-C is known to have protective properties against cardiovascular disease, its functionality might be reduced in patients with T2DM. Previously, it has been shown that T2MD patients do not always lower HDL-C levels but its ability to clear cholesterol and exert anti-inflammatory effects might be reduced [78]. Although high-density lipoprotein cholesterol is recognized as having a role in preventing cardiovascular diseases, its functionality may be compromised in T2DM patients. Data from earlier research indicate that T2DM has detrimental effects on the actions of HDL-C, including cholesterol removal and inflammation reduction even if levels of HDL-C are not lowered [81].

In the same manner, there was a significant difference for Low-Density Lipoprotein Cholesterol (LDL-C) ($p = 0.459$, Independent t-test), this agrees with the results reported from studies done by Zixin Cai et al (2021) [79], and one Zhang et al (2019) [73], Other studies such as the ones presented by Vahid Pouresmaeil et al. (2022) [78], and the study by Zhang et al (2019) [73], showed a significantly higher level of LDL-C in T2DM patients. Elevated LDL-C is usually considered a risk factor for atherosclerosis while our results did not find a significant difference, the pattern of lipid dysregulation in diabetes indicates that other factors, such as triglycerides, may have a crucial role in cardiovascular complications in this population [81].

C-reactive protein (CRP), as an indicator of inflammation, had trends toward higher levels (2.5 mg/dL) in the T2MD population compared to (1.45 mg/dL) in controls. However, the comparison was not statistically important ($p = 0.053$, Mann-Whitney U Test) and aligned with the observations given by Rangappa et al. (2023) [82]. High CRP levels are alarming and highlight the inflammatory component of the disease, suggesting that anti-inflammatory therapy is required. Chronic low-grade inflammation is one of the core key factors in the pathogenesis of T2DM that causes insulin resistance, endothelial dysfunction, and increased cardiovascular risks [46].

Similarly, the values of Glutamic Pyruvic Transaminase or Alanine Aminotransferase (GPT, ALT) were close to each other in both T2DM patients and controls showing no statistical significance and suggesting no observable liver dysfunctions in the patients. Regardless of our findings, T2MD patients are at high risk of developing non-alcoholic fatty liver disease (NAFLD) and other liver-related complications over time. Previous observations, including Zhang et al. (2019) [73], also found no remarkable hepatic dysfunction in T2DM patients but alarmed the increased risks of liver dysfunctions in unmanaged long-term diabetes. Higher S-GPT levels might point to early liver dysfunction, chronic hyperglycemia, and insulin resistance can lead to NAFLD and fibrosis [83].

Also, there was no significant difference in Serum Creatinine (S-Cr) levels groups ($p = 0.214$, Independent t-test) such correlation was also supported by Zhang et al. (2019) [73], as no observable renal dysfunction was found in T2MD patients. Although our findings proposed that there is no remarkable renal function impairment in the current cohort, T2DM patients are more likely to have diabetic nephropathy, a condition that might cause chronic kidney disease [84].

Thyroid-stimulating hormone (TSH) was almost doubled in the T2DM group (2.49 ± 1.04 mIU/L) compared to controls (1.50 ± 0.67 mIU/L) ($p = 0.004$), implying that there is a link between thyroid dysfunction and elevated risk factors of diabetes like one by Iwakura et al. (2023) [85]. Subclinical hypothyroidism contributes to insulin resistance, therefore, monitoring thyroid function in diabetic patients is highly important diabetic patients.

As an essential element, selenium has antioxidant properties, and its excess levels in T2DM patients suggest an altered antioxidant response [73]. Although selenium is crucial for maintaining cellular function, some studies have shown that excessive selenium intake may exacerbate oxidative stress and contribute to insulin resistance [77]. The observed results from the current work indicate that T2MD patients had remarkably higher selenium levels (129.42 ± 33.70 $\mu\text{g/L}$) compared to controls (115.94 ± 24.06 $\mu\text{g/L}$, $p = 0.032$). Our results were also confirmed by other studies performed by Sobhy Yakout et al. (2022) [86] and one by Marco Vinceti et al. (2021) [42]. While contrast results are observed by Muslim OT et al. (2021) [75] and Heidari et al. (2017) [87]. Therefore, more research is needed to explore the controversial role of selenium in the metabolic dysregulation observed in T2DM patients.

Our results presented in Table 3 suggest that the selenium levels are not affected by age in both patients and control groups. For T2MD patients, there is no significant difference in selenium leaves between the age groups ≤ 50 years and those aged > 50 years, and the p-value is 0.682. Similar results were presented by Bleys et al. (2007) [51], stating that selenium levels might not be age-related but more likely linked to disease-related factors such as insulin resistance and inflammation. Similarly, healthy controls showed no significant difference in selenium levels for age groups ≤ 50 years and those aged > 50 years. This is also in line with a study done by Bleys et al. (2007) [51].

The BMI was categorized as (normal, overweight, and obese) in both patients and healthy groups, and no significant link was found between selenium levels in both groups related to these categories. In patients, the p-value = 0.747, indicating that selenium concentrations were not influenced by the patient weight. Similar findings were recorded by Bleys et al. (2007) [51] where no direct association was found between BMI and selenium levels. For healthy controls ($p = 0.553$), there is also no association between selenium leaves across BMI categories, again confirming that selenium levels in the human body might be linked to weight. Bleys et al. (2007) [51] also reported

stable selenium levels across BMI groups in a general population, further supporting that selenium levels may not be strongly influenced by BMI.

Gender also appeared to not influence selenium levels in both patients and controls. Although female patients had slightly more selenium, the difference was not statistically important ($p = 0.365$), suggesting that the selenium levels are gender irrelevant, which is in agreement with the study by Bleys et al. (2007) [51], which found no remarkable variations in selenium levels when analyzing gender subgroups of U.S. diabetic patients, further supporting that gender might not play a crucial role in determining selenium concentrations.

Smoking habits Table 8 also did not influence the selenium levels in either patients or controls. For T2DM patients, the p-value was 0.069, and for controls p-value of 0.570, despite the increased oxidative stress due to smoking, our findings suggest that smoking might not be affecting the selenium levels. Bleys et al. (2007) [51] also found no significant relationship between smoking and serum selenium concentrations.

Similarly, the selenium levels were not affected by waist circumference (WC) in both patients and controls. Male patients with $WC \geq 102$ cm, had slightly higher selenium but the difference was not statistically important ($p = 0.07$). For the healthy male control group ($p = 0.360$) was also not significant. In females, no differences were found between those with $WC < 88$ cm and ≥ 88 cm in either patients or control ($p = 0.683$) suggesting that waist circumference might not profoundly affect selenium levels, with other factors, such as metabolic disturbances in T2DM, likely playing a more important role, as provided by previous studies by Vahid Pouresmaeil et al. (2022) [78] and Wang et al. (2022) [83].

Diabetic patients with a positive family history Table 8 had much lower selenium levels (121.35 ± 32.49 $\mu\text{g/L}$) compared to those with a negative family history (149.34 ± 28.60 $\mu\text{g/L}$), with a p-value of 0.003 providing a strong inverse relation between selenium levels and patient family history, indicating a genetic or familial predisposition. Contrarily, no such significant difference in selenium levels was observed based on family history in the healthy population ($p = 0.265$). A similar association between patient family history and selenium levels was reported by Jalpa Devi et al. (2016) [74]. Moreover, a study by AL-Auqbi et al. indicated that selenium levels in T2DM patients could be influenced by genetic predisposition, although no significant differences were noted in healthy individuals. These studies also support the idea that a family history of T2DM patients could play a role in selenium concentrations [88].

As shown in Table 9, the selenium levels of 131.73 $\mu\text{g/L}$ were slightly higher in active patients compared to 127.47 $\mu\text{g/L}$ in non-active individuals ($p = 0.824$). The observed values between physically active and non-active individuals in both T2DM patients and healthy controls were statistically not important. Selenium levels were very close to each other in both active (115.15 $\mu\text{g/L}$) and non-active (114.67 $\mu\text{g/L}$) healthy individuals ($p = 0.689$). These results indicate

that other than physical activities, diet, or metabolic control, they may play a more prominent role in regulating selenium concentrations in the human body. This is consistent with results from Pouresmaeil, Vahid, et al. (2023) [78], where no significant association between physical activity and selenium levels was observed.

The duration of the disease also had no significant impact on the selenium levels, as shown in Table 10. ($p = 0.465$) was found for patients having T2DM for less than 5 years compared to those with T2DM for 5 years or more. A similar finding was found by Nashiry, Mohammed, et al. (2019) [89], which also did not observe any significant changes in selenium levels based on the duration of diabetes in Malay patients. In their study, while some trace elements like zinc and copper showed variations in diabetes duration, selenium levels did not significantly change over time.

In terms of fasting blood glucose. The patients were categorized into levels below 110 mg/dL and those with FBG levels above 110 mg/dL, and there was no statistical difference ($p = 0.211$) between the two groups as shown in Table 11. Also, the healthy groups were categorized similarly ($p = 0.090$), showing no significant difference. Based on these findings, the fasting blood glucose in patients or controls does not affect the selenium levels. The results suggest that selenium concentrations are affected by other factors such as insulin resistance or inflammation rather than fasting blood glucose. Previous studies by Strózyk et al. (2019) [90] found no clear association between selenium levels and blood glucose in both diabetic and healthy populations.

As shown in Table 12, the selenium levels were also not significantly affected by cholesterol in T2MD patients or healthy populations. In patients, the median selenium level was 13.31 $\mu\text{g/L}$ for persons with cholesterol < 200 mg/dL, 120.36 $\mu\text{g/L}$ for those who have cholesterol in the range of 200–239 mg/dL, and 119.89 $\mu\text{g/L}$ for those with cholesterol ≥ 240 mg/dL ($p = 0.970$). For, controls, the median selenium level was found to be 114.67 $\mu\text{g/L}$ for people with cholesterol < 200 mg/dL and 120.36 $\mu\text{g/L}$ for those who have cholesterol in the range of 200–239 mg/dL ($p = 0.331$). Our results are in line with those presented by Zhang et al. (2019) [73], and a study by Pouresmaeil, Vahid, et al. (2023), where no significant correlation between selenium and cholesterol was reported [78].

Table 13, provides that there is no association between selenium levels and triglyceride in both patients and controls. There was a marginal difference in selenium levels for patients having triglyceride levels less than 150 mg/dL (130.31 $\mu\text{g/L}$), between 150-199 mg/dL (135.05 $\mu\text{g/L}$), and above 200 mg/dL (113.96 $\mu\text{g/L}$), with a p-value of 0.698. In the same manner, such marginal difference was also observed for controls having triglyceride levels less than 150 mg/dL (117.99 $\mu\text{g/L}$) and between 150-199 mg/dL (114.67 $\mu\text{g/L}$), with a p-value of 0.859. Similar findings were also reported by Pouresmaeil, Vahid, et al. (2023) [78], which reported no clear association between

triglycerides and selenium in general populations, and one by Zhang et al. in (2019) also did not find a strong correlation between lipid profiles and selenium concentrations in T2DM patients [73].

Regarding HDL-C Table 14, the selenium concentration was not significantly different between male patients ($p = 0.859$) or both male and female controls ($p = 0.153$). On the other hand, a significant difference in selenium levels was noticed for female patients with HDL-C < 50 mg/dL and those with HDL-C ≥ 50 mg/dL ($p = 0.045$), with lower selenium levels in the latter group, proposing that HDL-C levels in female patients might have a minor influence on the selenium levels, probably due to the gender-specific metabolism. These findings align with results from a study by Pouresmaeil, Vahid, et al. (2023) [78], which found no clear relationship between HDL-C and selenium in general populations.

For LDL-C, Table 15, in the T2DM patients, selenium levels were almost the same across individuals with LDL-C < 100 mg/dL (127.91 ± 31.24 $\mu\text{g/L}$), LDL-C 100–129 mg/dL (129.45 ± 32.59 $\mu\text{g/L}$), LDL-C 130–159 mg/dL (132.38 ± 40.30 $\mu\text{g/L}$), and LDL-C ≥ 160 mg/dL (131.03 ± 62.66 $\mu\text{g/L}$), with a p-value of 0.985. Likewise, in the control group, selenium levels were comparable across LDL-C sub-groups ($p = 0.418$) and no significant difference in selenium levels was observed across subgroups based on LDL-C in both groups. This is consistent with previous studies by Zhang et al. (2019) [73], and one by Pouresmaeil, Vahid, et al. (2023) which also reported no significant correlation between LDL-C and selenium levels in both diabetic and healthy populations [78].

Regarding glycemic control-based subgroups, in Table 16, the mean selenium level was 124.91 ± 36.91 $\mu\text{g/L}$ in the good control subgroup ($<7\%$), 126.27 ± 32.2 $\mu\text{g/L}$ in the moderate control subgroup ($7\text{--}8\%$), and 133.78 ± 32.92 $\mu\text{g/L}$ in the poor control subgroup ($>8\%$). Despite these marginal changes in the selenium levels, no significant association was observed between selenium levels and the degree of glycemic control ($p\text{-value} = 0.644$). Similar observations were published by Vajdi, Mahdi, et al. (2023) [26].

Using Pearson and Spearman correlation (r/p), our study revealed that selenium level has a statistically significant weak negative correlation with HDL-C ($r = -0.289$, $p = 0.026$). Other factors, like age, waist circumference, BMI, fasting blood sugar, HbA1c, cholesterol, triglycerides, LDL-C, GPT, CRP, TSH, and serum creatinine, showed no statistically significant associations. Suggesting that selenium levels are more likely to be affected by lipid metabolism and oxidative stress. These results are in line with those by Obeid et al. (2008) [91], while in contrast with findings by Devi, Jalpa, et al. (2016) [74].

Conclusion

The results obtained from the current study support the complex relationship between selenium and type 2 diabetes mellitus. Increased serum selenium concentration was observed in T2DM patients compared with the control group, while the results from statistical analyses lacked any relationships between selenium levels and glycemic control (HbA1c), meaning that the relationship between the two is still unclear. Selenium is inversely related to HDL-C. These results bring attention to the precise regulation of selenium and its potential applications in oxidative stress and lipid metabolism in diabetes. Based on the outcomes of this work, T2DM patients in the study population don't need to be treated with selenium-containing supplements or drugs.

Future Directions

Future studies have to expand the study population to include T2DM patients with complications for a better understanding of selenium and diabetes progression, such as neuropathy, nephropathy, and retinopathy which may provide valuable insights into the link between selenium and diabetes.

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APPENDICES

APPENDIX- 1: ROCHE COBAS C311 A BIOCHEMISTRY ANALYZERS



APPENDIX- 2: ROCHE COBAS 6000 A BIOCHEMISTRY ANALYZERS



APPENDIX-3: ROCHE COBAS e411 A BIOCHEMISTRY ANALYZERS:



APPENDIX-4: iCAP RQ ICP-MS ANALYZER



APPENDIX-5: MICROWAVE DIGESTER



APPENDIX-6: RESEARCH QUESTIONNAIRE

Research Questionnaire		
-Subject's Name:		
-Age:	-Gender: (Male) / (Female)	
-Address:	-Tel.:	
Physical examinations		
1. Waist circumferences (WC)..... (cm)		
2. Body weight..... (kg)		
3. Height: (m)		
4. Body mass index (BMI)..... (Weight kg / height m ²)		
Life Style		
Smoker Yes No		
Exercise Yes No		
Family history..... Positive..... Negative		
Duration of disease..... ≤5 Years..... >5 Years		
Diseases History		
-Do you have or take?		
• Kidney diseases:	Yes	No
• cardiovascular disease:	Yes	No
• Liver diseases:	Yes	No
• Multivitamin:	Yes	No
• General inflammation:	Yes	No
• Selenium supplements	Yes	No
• Insulin	Yes	No
• Blood pressure (BP):	Yes	No

APPENDIX-7: FORM (B) APPROVAL OF RESEARCH ETHICS COMMITTEE






Ministry of Health
Duhok Directorate General of Health

University of Duhok

Duhok Polytechnic University

Form B1: Approval of Research Ethics Committee

Date: 27 Mar. 2024

Reference number: 27032024-2-2

Research title: Evaluation of serum selenium level in terms of glycemic control in patients with type 2 diabetes mellitus in Duhok City - Iraq

Principal researcher/investigator name: Abdulraheem Taher Khalid

Supervisors/Co-supervisor(s)/Co-researcher(s)/Co-investigator(s) [if any]: Habibe Özmen

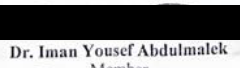
Terms and conditions of approval: The research is approved taking into consideration that the researcher(s) abide by the following terms and conditions:

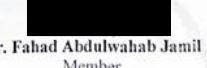
- 1) The researcher(s) shall fill out form C1 (General consent form) for all participants who will be included in the study. The researcher(s) shall store the filled consent forms in a confidential place for at least 5 years from the date of completion of the study for any further reference when deemed needed or asked for by any official authority.
- 2) Participants' identifiable information, including but not limited to their names and telephone numbers, shall not be reported or disclosed.
- 3) Patients'/clients' medical case records, clinical charts, and investigations that show their identifiable information shall not be copied, scanned, or photo-captured.
- 4) The conduct of the research shall not interfere with the daily routine work of any targeted public health facility.
- 5) This approval does not include acceptance to use materials and resources of public health facilities belonging to the Duhok Directorate General of Health.
- 6) Participants shall be given the right to know any potential risks or hazards before participating in this study.


Dr. Aydin Almad Mohammed
 Member


Dr. Alaa Noori Sarkees
 Member


Dr. Mirza Murad Khudeda
 Member


Dr. Iman Yousef Abdulmalek
 Member


Dr. Fahad Abdulwahab Jamil
 Member


Dr. Zeki Ali Mohamed
 Chairman


Dr. Dijwar Ali Haji
 Member


Dr. Dilovan Abdulmajed
 Member

Address:
 Room 8, Floor 2
 Duhok Directorate General of Health – Kurdistan Region of Iraq

Website: www.duhokhealth.org Page 1 of 2

APPENDIX-8: FORM (B) APPROVAL OF RESEARCH ETHICS COMMITTEE



Ministry of Health
Duhok Directorate General of Health



University of Duhok



Duhok Polytechnic
University



Form B1: Approval of Research Ethics Committee

- 7) Participants' inclusion shall be voluntary and they shall have the right to withdraw from the study at any stage of the research.
- 8) Participants shall be fully informed by the researcher(s) about all evidence-based diagnostic protocols and shall be given the full right to choose among these alternatives.
- 9) Neither participants nor Duhok Directorate General of Health shall bear the responsibility for any costs incurred due to this research.
- 10) The researcher(s) shall make it clear to the participants that neither Duhok Directorate General of Health nor members of this research committee is legally and ethically responsible for any adverse event as a result of involvement of participants in this research. The researcher(s) is/are responsible for the legal claims that may arise from the conduct of this research.
- 11) Participants shall be given the right to know the summary of the study results.
- 12) Confidentiality of the data shall be secured.
- 13) The researcher(s) shall not, at any time or circumstance, disclose any information or comment on the studied clients or public health facilities irrelevant to the proposed research to any person, company, institution, or entity. This non-disclosure condition also includes social media, newspapers, blogs, and other verbal and non-verbal media communication means.
- 14) Any change in the study methods needs prior approval from the Research Ethics Committee.
- 15) It is recommended that the researcher(s) share a soft copy of the published results of the research with the Division of Scientific Research – Department of Planning, Duhok Directorate General of Health.
- 16) This approval supposes that the researcher(s) has not yet started the stage of implementation of data collection. According to this letter, the researcher(s) are allowed to begin the process of data collection from the date of this approval.

Dr. Ayad Ahmad Mohammmed
Member

Dr. Alaa Noori Sarkees
Member

Dr. Mirza Mu'rad Khudeda
Member

Dr. Iman Yousef Abdulmalek
Member

Dr. Fahad Abdulwahab Jamil
Member

Dr. Zeki Ali Mohamed
Chairman

Dr. Dijwar Ali Haji
Member

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