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Detection of Some Heavy Metals (Mercury and Lead) Pollutants and their Association with Type 2 Diabetes Mellitus (T2DM) in Baghdad, Iraq

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Abstract:

Due to the increased use of products carrying heavy metals caused by human factors in the streets of the city of Baghdad and their direct impact on the air, water quality and soil pollution, heavy metals pollution is one of the important environmental problems. As heavy metals cannot be degraded, they accumulate biologically, and, therefore, can directly threaten higher organisms including humans. The current study focused on detecting heavy metals (mercury and lead) to determine the adverse effects and risk of developing type 2 diabetes mellitus (T2DM) in humans. One hundred total blood samples were collected from healthy people and the patients of both sexes who were suffering from T2DM, with mean age of 31 to 75 years, after 8 to 12 hours of fasting. The first group - G1 the control group consisted of 20 health people (6 males and 14 females). The second group - G2 included 40 patients (20 males and 20 females) who had T2DM and were not exposed to any pollutants. The third group - G3 included 40 males with T2DM and also who were exposed to smoke from local electricity generators, as these samples were collected from workers or living near generators and were exposed to air pollution for a long time. Blood samples were collected from each individual by drawing blood from a vein to evaluate each of the biochemical tests, as well as detecting of some heavy metals such as mercury and lead. The results showed that there was a highly significant increase ($p \leq 0.01$) when both fasting blood sugar (FBS) and HbA1c were analysed. As for the results of the analysis of the types of lipid profile, there is no significant difference in cholesterol, while there was a significant increase ($p \leq 0.05$) in both low-density lipoprotein (LDL) cholestrol and very low density lipoprotein (VLDL) cholestrol and a highly significant increase ($p \leq 0.01$) for both triglyceride and high density lipoporotein cholesterol (HDL) cholestrol. The results of the liver function analysis showed that there was no significant difference in the results of the enzyme serum glutamic oxaloacetic transaminase (SGOT) as well as total bilirubin, while there was a significant increase ($p \leq 0.05$) in serum glutamate pyruvate transaminase (SGPT). Significant increase ($p \leq 0.01$) was detected in the alkaline phosphatase (ALP) enzyme for each of the studied groups. The results of the detection of the presence of heavy metals using the atomic absorption spectrophotometer showed that there was a significant increase ($p \leq 0.01$) in lead, and as for mercury, no significant difference was noticed.

Keywords: Heavy metals, Type 2 Diabetes Mellitus, Liver, Lead and Mercury.

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الكشف عن بعض ملوثات المعادن الثقيلة (الزئبق والرصاص) وارتباطها بمرض السكري من النوع الثاني في بغداد، العراق

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الخلاصة

يعد التلوث بالمعادن الثقيلة من المشاكل البيئية المهمة ، وذلك بسبب زيادة استخدام منتجاتها بفعل العوامل البشرية في شوارع مدينة بغداد وتأثيرها المباشر على عملية تلوث الهواء والماء والتربة. تتوزع الكائنات الحية على نطاق واسع ، حيث لا يمكن أن تكون المعادن الثقيلة قابلة للتحلل ، ولكنها تتراكم بيولوجيًا ، ويمكن أن تهدد بشكل مباشر الكائنات الحية الأعلى بما في ذلك البشر. ركزت الدراسة على الكشف عن المعادن الثقيلة (الزئبق والرصاص) لتحديد مخاطر الآثار السلبية للتطور لدى الأشخاص المصابين سكري من النوع الثاني. في بداية البحث ، تم جمع ١٠٠ عينة من الأشخاص الأصحاء والمرضى الذين يعانون من مرض السكري من النوع الثاني و من كلا الجنسين بعد ٨ إلى ١٢ ساعة من الصيام. تراوح متوسط اعمار العينات المأخوذة من ٣١ إلى ٧٥ سنة لكل من الذكور والإناث. تم جمع عينات الدم من جميع مجموعات الدراسة الحالية. المجموعة الأولى – G1 كانت مجموعة السيطرة وتتكون من ٢٠ (٦ ذكور و ١٤ إناث)، الذين كانوا يتمتعون بصحة جيدة. المجموعة الثانية – G2 تضم ٤٠ شخص (٢٠ ذكر و ٢٠ أنثى) مصابين بالسكري من النوع الثاني ولا يتعرضون لأي ملوثات. المجموعة الثالثة – G3 تضم ٤٠ ذكرًا مصابًا بسكري من النوع الثاني يتعرضون لدخان من مولدات الطاقة الكهربائية الاهلية، حيث تم جمع هذه العينات من العمال أو الذين يعيشون بالقرب من المولدات وتعرضوا لملوثات الهواء لفترة طويلة. بعد ذلك ، تم جمع عينات الدم من كل فرد عن طريق سحب الدم من الوريد لتقييم كل من الاختبارات الكيموحيوية، وكذلك الكشف عن بعض المعادن الثقيلة مثل الزئبق والرصاص. اظهرت نتائج اختبارسكر الدم الصائم و السكر التراكمي ان هناك هنالك ارتفاعا معنويا عاليا ($p\leq0.01$) بين مجاميع الدراسة لكل من الاختبارين. اما فيما يخص نتائج تحاليل انواع الدهون، وجدت الدراسة انه لا يوجد اختلافا معنويا فيما يخص الكوليسترول، بينما هنالك ارتفاعا معنويا ($p\leq0.05$) لكل من تحليل البروتين الدهني منخفض الكثافة والبروتين منخفض الكثافة جدا، وارتفاعا معنويا عاليا ($p\leq0.01$) لكل من الدهون الثلاثية والبروتين الدهني عالي الكثافة. بينما اوضحت نتائج اختبار وظائف الكبد ان لا يوجد اختلاف معنوي في نتائج إنزيم أسبارتيت أمينوترانسفيريز وايضا البيليروبين الكلي، بينما كان هنالك ارتفاعا معنويا ($p\leq0.05$) فيما يخص إنزيم ناقلة أمين الألانين، اما فيما يخص أنزيم الفوسفاتاز القلوي فقد سجل ارتفاعا معنويا عاليا ($p\leq0.01$) في كل من المجاميع المدروسة. أظهرت نتائج الكشف عن وجود معادن ثقيلة باستخدام مقياس الامتصاص الذري وجود زيادة معنوية ($p\leq0.01$) بالنسبة للرصاص ، أما بالنسبة للزئبق فلم يكن هناك فرق معنوي.

الكلمات المفتاحية: المعادن الثقيلة، مرض السكري من النوع 2،الكبد ،الرصاص،الزئبق.

1- Introduction

Any moderately dense metal that one can mention has the potential to be harmful, especially when used in environmental settings [1]. Lead and mercury are specifically mentioned in this regard. These substances are of utmost importance to the general public according to the World Health Organization's (WHO) list of the ten most dangerous substances. Other substances of concern included are manganese, chromium, cobalt, nickel, copper, zinc, selenium, antimony and thallium [2]. Heavy metals can be found naturally in the ground where human activity has concentrated them. It can enter plants, animals, or human tissues through inhalation, food consumption and physical contact. Once connected, they

disrupt the functions of essential cellular components. The ancients were aware of the toxicity of mercury and lead, however systematic research on the toxicity for both mercury and lead didn't start until 1868. Although both mercury and lead are harmful to human health even in small quantities, poisoning in humans is often treated by taking chelating medicines that attach to metal atoms inside cells and prevent poisoning in reducing its effects on body tissues [3].

According to conventional definitions, heavy metals including mercury and lead, have relatively large densities, atomic weights, or atomic numbers (with an A density that is more than 5 g/cm3 times more than that of water and are highly toxic even at low doses). Depending on the author and context, different standards are employed, and metalloids are considered. For instance, in metallurgy, a heavy metal may be classified according to density, but in physics, the defining characteristic may be an atomic number, and a chemist would likely be more interested in chemical behavior [4]. Numerous metals, such as copper, iron, manganese, nickel, and zinc play crucial roles in biology as cofactors in enzyme activities or as components of the secondary and tertiary structures of proteins. Heavy metals such as mercury (Hg) and lead (Pb), on the other hand, are not known to play any biological roles. Instead, these metals are categorized as hazardous heavy metals because they are very dangerous when taken by humans [5]. The World Health Organization includes the metals Pb and Hg in its top ten list of chemicals that pose the greatest threat to human health. Exposure to these metals has been related to several human disorders. The likelihood that humans will come into contact with these metals through the air, water, contaminated soil, and food has increased even though they are naturally occurring. These metals are typically found in the subsurface and are linked to human activities, particularly through mining and industrial processes [6].

Both natural and manmade causes contribute to the release of heavy metals especially Pb into the environment. According to previous studies, they may penetrate soils and groundwater, are highly reactive and frequently dangerous at low doses. They bio-accumulate in food webs [7]. Toxicology of heavy metals that behave as pseudo-elements, and some of them, like aluminum, may be eliminated by bodily elimination mechanisms. At specific moments in the body, they may interfere with metabolic processes, summarizes a heavy metal attack on a live cell, the equilibrium between reactive oxygen species (ROS), the subsequent defense provided by antioxidants, and how others get accumulated in the human body and food chain leading to chronic effects [8, 9]. As a result of the effects of any heavy metal, such as Pb, the amount of ROS increased while the antioxidant level (such as glutathione) reduced in the cell which shielded it from free radicals. One of the most significant health and social-economic issues in developing nations is diabetes [10].

According to the WHO estimates around 347 million people globally have diabetes [11]. Due to the ongoing and fast growth of the condition, type 2 diabetes mellitus (T2DM) is expected to overtake heart disease as the seventh leading cause of death worldwide by 2030 [12]. Diabetes causes glucose not to be converted into energy. This results in the presence of excess energy in the blood which the cells still need. Over the years, hyperglycemia (hyperglycemia: the origin of the word is Latin) develops, causing severe damage to nerves and blood vessels. This can lead to complications such as heart disease, stroke, kidney disease, blindness, diabetic neuropathy, gingivitis, diabetic foot and possibly even amputation [13]. The main hormone that controls the movement of glucose from the blood to the majority of the body's cells, particularly muscle cells and fat cells, is insulin which is generated by the pancreas. Any sort of diabetes pattern is brought on by a shortage of insulin or a body that does not respond to it [14, 15]. Glucose is the main source of energy in the body that can mainly be obtained from the carbohydrates such as sugar and grains. It is then transported

through the blood to the cells of the body that use it as a source of energy. The body regulates blood glucose levels using insulin, a hormone that pulls glucose from the bloodstream into cells to be used for energy [16]. After the process of digesting carbohydrates, glucose molecules are released, the intestine absorbs glucose and releases it through the blood and goes to the rest of the body's cells. Excess glucose is removed from the bloodstream and gets converted into its stored form of glycogen. The liver which stores glucose in the form of glycogen, changes glycogen into glucose. This process is called glycogenolysis [17].

The hormone glucagon which functions differently from insulin, is primarily responsible for controlling this process causes the glucose to be produced from glycogen. Chronic environmental cadmium exposure has been linked to T2DM in the "beginning before age 50" in Black residents of a lead smelter in Dallas, Texas [18]. According to a study of Korean inhabitants of cadmium-contaminated mining sites, men's diabetes and cadmium have been linked together [19]. Another American study discovered that teenage females' methyl mercury levels were linked to increased overall cholesterol levels [20]. Persons with diabetes had greater cadmium and lead levels in their hair than people without diabetes did in adults from metropolitan regions of Pakistan and Ireland, while essential mineral levels were lower [21]. A research was conducted in Iraq to find out if there is a link between iron and lead levels and diabetes. This study included 30 diabetic patients, and the age groups were confined between 30-70 years with the duration of the disease between 1-22 years, compared to 20 healthy subjects. No significant differences in the level of lead and iron in the serum of diabetic patients were noticed compared to healthy subjects [22]. According to several human epidemiological research, those who are exposed to some heavy metals more often have a greater chance of developing T2DM. Long-term, longitudinal studies that track individuals over time are among the supporting evidence. Studies in the lab using cells or animals (mice) have shown that exposure to particular heavy metals can have biological consequences connected to diabetes and obesity. Studies have also discovered some connections between exposure to heavy metals and the likelihood of developing problems from T2DM [23].

Heavy metals have been linked to obesity, hyperglycemia and possibly diabetes, and have also been linked to chronic inflammation and oxidative stress. Arsenic, iron, mercury, lead, cadmium, and nickel have all been investigated as potential risk factors for diabetes and hyperglycemia. Another set of metals that includes vanadium, chromium, zinc, and magnesium, can induce hypoglycemia through various methods. Lack of zinc, magnesium and chromium is linked to a higher risk of developing diabetes [24]. The aim of the current study was to support future studies that justified the validity of the association and investigate whether there was an association of heavy metals with the risk of developing T2DM.

2- Materials and Methods

This study was approved by the research ethics at the University of Baghdad, College of Science, Department of Biology.

2.1- Experimental Design

In the current study, the sampling process included two parts, the first part samples were collected from the Department of Health Baghdad, Rusafa primary health care sector in the first municipalities/Al-Moallemin Dispensary Typical, from December 2020 to June 2021. One hundred persons with ages between 31-75 years were selected for the study. The second part was collected from patients who were working or lived near local Electricity generators and were exposed for long periods to smoke-polluted air from local electricity generators. The groups were divided into:

1- Group one (G1): Included 20 healthy persons, 6 males and 14 females (Control group).

2- Group two (G2): Included 40 patients, 20 males and 20 females with T2DM but were not exposed to any pollution.
3- Group three (G3): Included 40 male patients with T2DM who were exposed to the smoke polluted air emitting from local electricity generators.

2.2 - Blood Samples Collection

After eight to twelve hours of fasting (between 8:30 - 12 AM), one hundred blood samples from healthy people and patients with T2DM of both sexes, were recruited for this study. . Using disposable syringes 10 ml of blood specimens were collected from each person through venipuncture from veins in the arm or elbow area in the three study groups mentioned earlier. The samples drawn were then preserved in a deep freezer (-20º C) to be used for the biochemical tests such as lipid profile, liver function, and detections of some heavy metals for all aforementioned groups.

2.3 - Investigation of Heavy Metals Concentrations

After samples collection concentration of heavy metals was measured in two ways: Cold Vapor Atomic Absorption Spectrometry (CVAAS) was used to measure mercury while flame or flameless atomic absorption Spectrophotometry (NOVAA -400) model was used to detect other heavy metals like lead. The samples were digested in several steps including:
1- All glass and plastic utensils were cleaned by immersing nitric acid solution, and then rinsed with DDW, and finally dried with air before use.
2 - The acids were diluted u through the application of the dilution formula in accordance with the principles of dilution to obtain acid with a concentration of 10% for each concentrated nitric acid 65% and perchloric acid 70%. DDW was employed as the diluent in this process.
3 - 0.75 ml of 10% diluted nitric acid HNO_3 and 0.75 ml of 10% diluted perchloric acid $HClO_4$ were added to each sample serum to make 1.5 ml of $HNO_3/HCLO_4$ (1:1 v/v) which was then added to 0.5 ml serum sample [25 , 26].
4 - The mixture was shaken (serum and diluted acids $HNO_3/HCLO_4$) after placing it in heat resistant fuzzy plane tube. Each tube was numbered according to the sample number to distinguish between samples and groups that were to be measured by a device.
5 - The samples were then heated according to the following temperature/time: 175°C/60 minute [26].
6 - The samples were then left to cool down to room temperature. After that the samples were filtered using either filter paper made of cellulose with a porosity of less than 0.4µm or a Millipore filter (sterile syringe filter) with a porosity of 0.45µm before finally reading on the device [26].

3 - Statistical Analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effects of difference factors in study parameters. Least significant difference (LSD) test (analysis of variance-ANOVA) was performed to compare between means in this study [27].

4 - Results and Discussion

4.1 - Fasting Blood Glucose (FBG) and Hemoglobin A1C (HbA1C)

The FBG findings revealed a highly significant ($p \leq 0.01$) increment in diabetic group G2 (222.74 ±15.37 mg/dl) and patients with T2DM who were exposed to local electricity generators in group G3 (195.47 ±12.93 mg/dl) compared with healthy controls group G1 (100.08 ±1.96 mg/dl) (Table 1). Also, highly significant ($p \leq 0.01$) increment in HbA1c in diabetic G2 (8.64 ±0.32 %) and G3 (8.17 ±0.32 %) was noticed as compared with the healthy group G1 (5.22 ±0.09 %) (Table 1).

Table 1: Comparison between different groups from FBG test and HbA1c test

Group	Mean ± SE FBG (mg/dl)	Mean ± SE HbA1c %
G1	100.08 ±1.96 b	5.22 ±0.09 b
G2	222.74 ±15.37 a	8.64 ±0.32 a
G3	195.47 ±12.93 a	8.17 ±0.32 a
LSD value	41.336 **	0.954 **
P-value	0.0001	0.0001
Means having different letters in same column differed significantly. ** ($P \leq 0.01$). NS: Non-Significant		

FBG level in groups G2 and G3 had higher levels than those in control group G1. FBG levels in diabetic patients raised regarding the second group G2 of diabetic patients. The current study agrees with most of previous research that found plasma levels of 20 trace elements as well as those of heavy metals including lead, mercury, and cadmium in a Han Chinese population to investigate the relationships between the prevalent risk of T2DM [28, 29, 30]. Numerous metals, such as manganese, copper, zinc, arsenic, selenium, and cadmium have been linked to diabetes morbidity and have been found to increase the chances of developing the disease in plasma [31]. There are studies that that have shown that exposure to environmental pollutants that carry heavy metals continuously and for a long period of time, can cause the risk of developing diabetes, especially mercury and cadmium [32].

In China aluminum, titanium, cobalt, nickel, copper, zinc, lead, selenium, rubidium, strontium, molybdenum, cadmium, antimony, barium, and tungsten have all been linked to diabetes, high fasting glucose, or impaired fasting glucose [33]. Lead has also been linked to T2DM, higher fasting glucose, higher HbA1c average glucose, higher insulin levels, and greater insulin resistance in Chinese individuals [34]. A collection of metabolic conditions known as hyperglycemia includes chronic diabetes. The increase in FBG level may have been brought on by problems with insulin production and insulin action. The results of the FBG test are closely correlated with the degree of diabetes mellitus. Therefore, the rise in FBG levels found in this investigation was consistent with what has been shown by [35].

For HbA1c the results indicated that G2 and G3 had a significantly higher HbA1c than the control group G1 which is consistent with the results of a patient population examined by another researcher [36]. HbA1c below 7% for most patients to indicates excellent glycemic control, whereas the International Diabetes Federation (IDF) recommends HbA1c levels below 6.5% [37]. Testing HbA1c is attractive because it monitors chronic glycemia in DM patients. The elevations in HbA1c have been linked to the rise in FBG in diabetic groups. It has been used to monitor diabetic patients as an objective metric of average glucose control [38]. Increased non-enzymatic glycosylation of different bodily proteins, including hemoglobin and albumin, is one of the main side effects of hyperglycemia. Therefore, the increased HbAlc levels in this study suggests that the blood glucose levels are not as well controlled [39].

In humans with DM, the serum glucose is unstable, and testing HbA1c, which provides the average blood glucose level over the previous two to three months, is an efficient technique to monitor it. In epidemiological research, the HbA1c can be a useful complement to blood glucose measurements [29]. According to the findings, T2DM patients' insulin levels significantly increased when compared to the control group [40]. Another study with 500 T2D patients discovered a substantial correlation between having diabetes for a longer period and having an HbA1c level of over 8% [41].

Hyperglycemia and hyperinsulinemia in humans can affect insulin secretion and insulin sensitivity which is a confusing issue. With longer time spent living with diabetes, the body becomes less sensitive to insulin, resulting in a situation where the body's insulin levels are high or normal yet there isn't enough insulin to go around. Due to the level of insulin resistance present in the majority of T2D individuals, developing diabetes is almost impossible unless the ability to release extra quantities of insulin to counteract the insulin resistance is compromised [42].

4.2 - Parameters of Lipid Profile

Total cholesterol level was not significantly different for G2 (183.06 ±5.92 mg/dl) and G3 (181.21 ±6.96 mg/dl) groups compared to the healthy control G1 (169.23 ±5.57 mg/dl). On the contrary, triglycerides (T.G) level showed a highly significant ($p \leq 0.01$) increase in diabetic G2 (214.88 ±17.37 mg/dl) and G3 (234.30 ±20.40 mg/dl) groups in comparison with healthy G1 (100.97 ±6.91 mg/dl) group.High density lipoprotein (HDL) cholestrol level decreased highly significantly ($p \leq 0.01$) in diabetics for both G2 (40.84 ±1.92 mg/dl) and group G3 (37.48 ±2.04 mg/dl) groups in comparison with healthy control G1 (62.91 ±4.10 mg/dl) group.

While low density lipoprotein (LDL) cholestrol showed a significant ($p \leq 0.05$) difference in G2 (105.73 ±5.54 mg/dl) and G3 (99.38 ±5.77 mg/dl) in comparison with the healthy control G1 (125.97 ±4.13 mg/dl). Also, the very low density lipoprotein (VLDL) cholestrol level showed significant ($P \leq 0.05$) increment in diabetic G2 (46.12 ±3.26 mg/dl) and G3 (48.16 ±3.95 mg/dl) groups compared with the healthy control G1 (33.02 ±3.93 mg/dl) (Table 2).

Table 2: Comparison between difference groups for lipid profile.

Group	Mean ± SE (mg/dl)				
	Cholesterol (mg/dl)	**Triglyceride (T.G)** (mg/dl)	**HDL** (mg/dl)	**LDL** (mg/dl)	**VLDL** (mg/dl)
G1	169.23 ±5.57	100.97 ±6.91 b	62.91 ±4.10 a	125.97 ±4.13 a	33.02 ±3.93 b
G2	183.06 ±5.92	214.88 ±17.37 a	40.84 ±1.92 b	105.73 ±5.54 b	46.12 ±3.26 a
G3	181.21 ±6.96	234.30 ±20.40 a	37.48 ±2.04 b	99.38 ±5.77 b	48.16 ±3.95 a
LSD value	19.623 NS	55.53 **	7.121 **	16.977 *	11.27 *
p-value	0.379	0.0001	0.0001	0.0153	0.0381
Means having with the different letters in same column differed significantly. *($p \leq 0.05$), **($p \leq 0.01$). NS: Non-Significant					

A changed lipid profile is referred to as dyslipidemia which includes elevated TG levels, lower HDL levels, higher LDL levels, and higher VLDL levels [43]. It has been discovered that dyslipidemia is linked to both insulin resistance and coronary heart disease. High triglyceride and low HDL cholesterol levels are the most typical dyslipidemia profiles in T2DM. LDL cholesterol levels in people with T2DM were found to be considerably high ($p<0.01$) by [44].

In T2DM patients, however, there was a bad connection between LDL and HDL, proving that those with T2DM may have inadequate fibroblast LDL absorption [45, 46] which could cause HDL cholesterol levels to drop. The same outcomes have been reported by [47] where they noted a decrease in HDL cholesterol in T2DM patients as a result of elevated hepatic lipase activity which is crucial for HDL metabolism. Contradictory studies on the other hand claim that HDL cholesterol levels in diabetes individuals did not decrease significantly [48]. Patients with diabetes who have dyslipidemia often have high triglyceride levels and poor HDL cholesterol [49]. Patients who have high triglycerides are more likely to develop life-threatening consequences such as diabetic ketoacidosis and coronary artery disease [50]

which, as a result of a rise in triglycerides, occurs more frequently in diabetics than in non-diabetics [51, 52].

Previous studies have reported that lead and nickel both have harmful effects on the immune system [53, 54]. Studies have shown the most important damages that can be caused by exposure to lead and cadmium (Pb-Cd) together which can induce lipid metabolism issues by altering the metabolism of linoleic acid, sphingolipids, and glycolipids. It can also enhance oxidative stress by raising lactate dehydrogenase (LDH) activity that will be beneficial in assessing tissue damage, the test checks the amount of LDH in blood or other bodily fluids, superoxide dismutase (SOD) which is an enzyme that alternatively catalyzes the dismutation of the superoxide radical (O^{-}_{2}) into regular molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). As a by-product of oxygen metabolism, superoxide is created and, if unchecked, can result in a variety of cell damages [55, 56, 57].

Increased TG deposition in non-adipose tissue, including the heart, liver, pancreas, and skeletal muscle, is linked to obesity and T2DM. The strongest correlation between the risk of metabolic and cardiovascular problems and obesity is the accumulation of fat in the subcutaneous abdominal and visceral depots [58]. Lipid levels in T2DM compared to healthy control, with the bulk of studies demonstrated higher TG levels in diabetic patients. The human metabolism is catabolic, thus increasing levels of insulin resistance, decreases adipose tissue lipoprotein lipase (LPL) activity, and increasing levels of lipolysis all work together to ensure the availability of useful substances like glucose, fatty acids, and ketone bodies [59].

4.3 - Liver Enzyme Functions

The serum glutamic oxaloacetic transaminase (SGOT) and bilirubin level was observed to be non-significantly elevated in 31-75 years old T2DM patients, with mean values of GOT: G2 (19.64 ±1.40 U/L); G3 (19.41 ±0.89 U/L) and bilirubin level: G2 (0.391 ±0.04 mg/dl); G3 (0.381 ±0.02 mg/dl) respectively. Their levels were compared to those of an age-matched control G1 (GOT: 22.63 ±1.39 U/L and bilirubin: 0.442 ±0.04 mg/dl respectively).

The mean level of serum glutamate pyruvate transaminase (SGPT) was significantly elevated ($p \leq 0.05$) in diabetic patients G2 (23.03 ±2.21 U/L) and G3 (27.97 ±2.20 U/L) in comparison with the control group G1 (20.03 ±1.52 U/L). Furthermore, there was a highly significant increase ($p \leq 0.01$) in mean serum levels of alkaline phosphatase (ALP) in diabetic patients G2 (104.44 ±4.59 U/L) and G3 (95.04 ±6.51 U/L) in comparison with their levels in the healthy group G1 (73.79 ±4.20 U/L). The previously reported results are detailed in Table

Table 3: Comparison between difference groups in Liver enzyme.

Group	Mean ± SE			
	GOT (U/L)	**GPT (U/L)**	**Bilirubin (mg/dl)**	**ALP (U/L)**
G1	22.63 ±1.39	20.03 ±1.52 b	0.442 ±0.04	73.79 ±4.20 b
G2	19.64 ±1.40	23.03 ±2.21 ab	0.391 ±0.04	104.44 ±4.59 a
G3	19.41 ±0.89	27.97 ±2.20 a	0.381 ±0.02	95.04 ±6.51 a
LSD value	3.701 NS	6.599 *	0.119 NS	16.931 **
P-value	0.232	0.049	0.619	0.0044
Means having with the different letters in same column differed significantly. * ($P \leq 0.05$), ** ($P \leq 0.01$). NS: Non-significant				

Enzymatic activities such as GPT, GOT, and ALP, as well as levels of non-enzymatic liver markers such as total bilirubin and total protein, are all affected by the insulin resistance status in T2DM patients. According to the results of the current study, diabetes individuals had considerably higher blood levels of liver enzymes like GPT and GOT than the control group**.**

Pancreatic islets result in the formation of T2DM. Several studies have revealed that instability in the amount of these metals produces major negative consequences on health [60, 61]. However, some heavy metals such as cadmium, nickel/Ni, mercury, arsenic, and lead, have a high level of toxicity and are harmful to human health. Patients with T2DM have been found to contain several hazardous metals that interfere with glucose absorption and control [62, 63]. Some other studies have demonstrated how various heavy metal concentrations influence and how diabetes mellitus develops. Disruption of glucose absorption and other issues are caused by exposure to hazardous metals including Pb, Ni, Cd, and Hg brought on by unchecked pollution and industrialization. Several channels lead to human exposure [64]. Diabetes patients' liver enzyme activity rises as compared to healthy people. In many different types of illnesses, GPT and ALP are sensitive indications of liver abnormalities [65, 66].

Insufficient insulin signaling may be the cause of elevated GPT levels rather than only liver cell injury. The pathophysiological process causing these increases in transaminase activity may be an indication of systemic inflammation that compromises insulin signaling in the liver. According to research, GPT can lead to insulin resistance regardless of age, gender, or body mass index [67, 68]. GPT is thought to be a helpful diagnostic for identifying patients at high risk for T2DM. The effects of both glycogen and insulin on liver tissues can be used to explain why diabetic individuals in the current research had elevated blood levels of GPT [69].

GOT is regarded as a measure of healthy liver function, however, it is less focused than GPT. As a result, GOT may be seen as a less precise marker of liver damage associated with the emergence of T2DM [70]. Moreover, the higher blood ALP level can be a sign of increased hepatic fat deposition. The major cause of hepatic insulin resistance is the buildup of fatty substances in hepatic tissues which is also connected to the emergence of systemic insulin resistance and insulin hyperactivity. ALP may therefore be a useful indicator of insulin resistance. There is a lot of data to support the idea that ALP is used as a marker for fatty liver and cardiovascular illnesses (CVDs). The release of ALP into the bloodstream is influenced by cellular stress and regular cell turnover [71].

Prolonged oxidative stress impairs insulin production and gene expression which worsens diabetes management. It has been proposed that T2DM and rising levels of oxidative stress are connected. Another study found a strong relationship between oxidative stress and the microvascular and macrovascular consequences of diabetes. A natural antioxidant called bilirubin lowers the quantities of free oxygen radicals and lessens oxidative stress. Hence, by reducing bilirubin levels, free oxygen radicals may rise and worsen diabetes complications [72].

Heavy metals, such as lead, cadmium, and arsenic once in the body, are primarily accumulated in the liver, kidney, and pancreas where they negatively affect the way that glucose is metabolized and how it interacts with other metabolic pathways, particularly glycolysis, glycogenesis, and gluconeogenesis. This is done by altering and impairing the specific enzyme activity. The etiology of diabetes mellitus is greatly influenced by the impairment of hepatic glucose balance. Increased blood glucose levels are also greatly influenced by compromised renal, liver, pancreatic, and muscle processes as well as reduced pancreatic and muscle functions. The structure of these enzymes may alter as a result of heavy metals. By damaging the pancreas and adrenal organs, they also disrupt hormonal equilibrium. These metals frequently encourage the production of ROS and impair antioxidant defense systems, which causes harm to numerous organs including the liver [73].

4.4 - Mercury (Hg)

According to the Table 4 below, Hg concentration values in the blood serum were: G1= 0.00418 ±0.0003 mg/L, G2= 0.0055 ±0.0004 mg/L, and G3= 0.0051 ±0.0003 mg/L. The results showed that there were no significant differences between the groups G2, and G3 compared with G1.

Table 4: Comparison between different groups in Hg concentration.

Group	Mean ± SE
	Hg (mg/ L)
G1	0.00418 ±0.0003
G2	0.0055 ±0.0004
G3	0.0051 ±0.0003
LSD value	0.0015 NS
P-value	0.0952
Means having with the different letters in same column differed significantly. * ($p \leq 0.05$), ** ($p \leq 0.01$). NS: Non-Significant	

Some studies and according to information from two other longitudinal studies of American people that agree with the results of the current study that there is no connection between diabetes and mercury levels [74]. Regarding the concentration of mercury shown in the Table 4, the results do not agree with previous studies conducted in Taiwan. RBC-Hg levels were found to be substantially greater in participants with T2DM than in those without T2D. After taking into account all potential confounders such as age, sex, BMI, hypertension, total cholesterol, saltwater fish intake, geographical stratum, seasonality, and hemoglobin, a strong correlation between the RBC-Hg and the prevalence of T2DM was established [75].

The Occupational Safety and Health Administration (OSHA) has established a 0.1 mg/ L (100 µg/m^3) as upper limit for working exposure that is legally enforceable. The recommended exposure limit (REL) for mercury vapor, determined by the National Institute for Occupational Safety and Health (NIOSH) is 0.05 mg/ L (50 µg/m^3) as a Time-Weighted Average (TWA) for up to an 8-hour workday and a 40-hour workweek. As an average exposure for a typical 8-hour workday, the American Conference of Governmental Industrial Hygienists (ACGIH) recommends a threshold limit value of 0.025 mg/ L (25 µg/m^3) of mercury vapor. Additionally, the Ministry of the Environment in Iraq established the allowed limits of 0.05 mg/ L (50 µg/m^3) for mercury and its compounds [76]. Below this level, exposure is deemed to have no harmful consequences, according to the United States Environmental Protection Agency's reference blood mercury content recommendation of 0.0058 mg/L [77, 78].

4.5 - Lead (Pb)

Based on the results shown in Table 5, there were highly significant differences ($p \leq 0.01$) when comparing the second and third groups with the control group where the results showed that in G2 patients' blood contained a concentration of lead (0.280 ±0.03 mg/L). While G3 patients who were exposed to the smoke of local electricity generators and who had T2DM, had lead concentrations of 0.203 ±0.021 mg/L in the blood samples. Also, there was a clear difference and a clear increase in the two groups that have been previously mentioned compared to the control group G1= (0.023 ±0.002 mg/L).

Table 5: Comparison between different groups in Pb concentration.

Group	Mean ± SE
	Pb (mg/ L)
G1	0.023 ±0.002 c
G2	0.280 ±0.03 a
G3	0.203 ±0.021 b
LSD value	0.073 **
p-value	0.0001
Means having with the different letters in same column differed significantly. * ($p \leq 0.05$), ** ($p \leq 0.01$). NS: Non-significant	

According to the United States Centers for Disease Control, the top limit for blood lead in adults is 0.1 mg/L [79]. Lead exposure can occur via tainted food, water, dust, air, or consumer goods and that the brain is the most vulnerable organ affected by lead exposure [80]. Adults who work in specific environments are at a risk of frequently developing lead poisoning. Typically, a diagnosis is done by determining the blood lead level [81].

According to previous studies blood concentrations of lead, mercury and cadmium were slightly higher, but non-significant in participants with diabetes, compared to those without it. After adjustment for age, sex, region, smoking, alcohol consumption, and regular exercise. Lead pollution poses a serious concern to people who live in older, underdeveloped urban neighborhoods [82]. Other previous studies found a connection between exposure to lead and the onset of diabetes and metabolic states [83].

Environmental pollution exposure over time may have negative health effects on people in general, and not only in occupational or unintentional circumstances [84]. Recently, there has been a lot of interest in organic pollutants and their association with risk factors for environmental pollutants like heavy metals, including lead, for diabetics [85].

The accumulation of toxic substances, such as lead and cadmium, in the body causes serious issues in the future. In particular, the human body lacks a mechanism to get rid of toxic heavy metals [86]. Lead and cadmium are among the toxic heavy metals that are known to cause oxidative stress by attaching to protein sulphydryl groups. As a result, numerous enzyme reactions and amino acids are rendered inactive, and antioxidants like glutathione, alpha lipoic acid, and N-acetylcysteine are depleted [87].

Oxidative stress has a critical role in several cellular processes that result in diabetes [88]. The results of current study correspond to this study. When compared to samples from normal people, lead concentrations were greater in the plasma or scalp hair of diabetes patients [89].

In the previous century a direct correlation was found between high lead exposure and high diabetes rates. Even though there isn't much evidence to back this up and the studies on animals were conducted at blood lead concentrations that were higher than what would typically be seen in human exposures (0.14-0.74 mg/L versus 0.05 mg/L for human alertness), the theory has been supported by the few studies which were conducted on animals that at some exposure levels and in combination with other metabolic stresses [90].

Lead promotes the development of diabetes as there is still a large segment of the population exposed to lead from environmental pollution. When one considers that low doses of lead can have a harmful effect, it is clear if lead exposure has effects on the metabolic health of the population, something that is of utmost importance to research [91]. Observations in humans and mice that lead to genome-specific epigenetic changes that last

throughout life and even across generations support the idea that exposure to lead early in life may have physiological repercussions later in life [92].

ROS are known to disrupt several essential insulin signaling pathway components, encouraging the onset of insulin resistance and diabetes [93]. Recent studies have found a strong correlation between blood lead levels and indicators of oxidative stress and have found that even in those with relatively low levels of environmental lead exposure "<0.1 mg/L", oxidative stress should be taken into account in the development of lead-mediated diseases [94]. Pb^{++} cannot change its valence state and cannot produce ROS by the Fenton reaction "The ferrous and/or ferric cations catalytically break down hydrogen peroxide to produce potent oxidizing agents that may break down a variety of organic and inorganic compounds", in contrast to redox-active metals like iron and copper. It is, however, undeniable that Pb^{++} elevates oxidative stress in living things [95, 96].

These four suggested processes, each of which is validated by experimental data, are responsible for:

1. Pb^{++} alters the lipid content of membranes, causing phosphatidyl choline to decline and arachidonic acid to rise [97]. Increased susceptibility to lipid peroxidation is related to the change in lipid composition; for instance, the presence of Pb^{++} is accompanied by a rise in malonyldialdehyde [98, 99].

2. Through binding to oxyhemoglobin, Pb^{++} promotes the production of superoxide [100]. 3. The heme biosynthetic enzyme porphobilinogen synthase {aminolevulinate dehydratase; ALAD} is extremely sensitive to Pb^{++} ions. Through active-site inhibition, lead has a direct impact on ALAD. Particularly, Pb^{++} binding moves a crucial Zn^{++} ion away from the catalytic site which prevents ALAD activation [101]. The blood level of -aminolevulinic acid rises and might perhaps activate an autoxidation pathway to produce superoxide [102]. Lead-exposed humans have elevated amounts of ALA in their blood and urine. 4. Pb^{++} reduces the antioxidant activities of glutathione peroxidase and superoxide dismutase by attaching to sulfhydryl groups or selenocysteine which are necessary for these enzymes' antioxidant activities [103]

Oxidative stress directly affects cellular signaling pathways that affect insulin signaling, hence promoting the diabetes condition. Reactive oxygen species seem to be highly sensitive to pancreatic beta-cells ROS [104, 105]. An extremely low quantity of antioxidant enzymes, including SOD, catalase, and GPx, is thought to be the cause of beta-cells increased sensitivity in comparison to other cell types. Antioxidant medications can be used to make up for the lack of enzymatic antioxidant capability [106]. In T2DM, several antioxidants such as lipoic acid, N-acetyl cysteine, and vitamin C, decrease insulin resistance [107]. By identifying the signaling pathways that may be altered to reduce the level of oxidative stress in pancreatic islets, antioxidant treatment can stop or delay the onset of T2DM [103].

5- Conclusion

The results of this study have important effects on public health, through collecting blood samples from humans for the groups previously mentioned in the current study, where both lead and mercury levels were examined. The findings add to the evidence linking lead and mercury exposure with type 2 diabetes. The results of the study indicate that lead levels were higher in the blood of patients with T2DM compared to the control group and are associated with an increased risk of developing T2DM. The strongest evidence comes about the ability of environmental exposures to contribute to the development of T2DM, especially long-term exposure to environmental pollutants that contain lead. As for mercury, it did not show any

percentage that could pose a risk in the collected blood samples, something that needs further investigation to clarify the role of the developed effect of other several minerals in the T2DM.

Clarification: Predicting toxicity, as well as testing chemicals, all involve the use of animals in experiments. It is considered evidence that supports and complements the identification of the danger of exposure to heavy metals, including lead and mercury, and their association with T2DM. From here, we derive the idea of a human study by taking samples and studying them.

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