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Paper

Biochemical study of the risk of diabetes, prediabetic and insulin resistance in car painters and its association with mercury exposure: a retrospective case–control study

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Purpose: There is controversy about the effect of mercury (Hg) exposure on developing diabetes and insulin resistance. This study aimed to assess the risk of diabetes and insulin resistance in car painters using biochemical markers and serum Hg levels.
Methods: A retrospective case–control study involving 210 male participants aged between 25 and 50 years. The participants were divided into two groups: Car painters for at least one year and healthy people who had not worked as car painters and had no health concerns or chronic diseases.
Results: The serum levels of Hg, MDA (malondialdehyde), interleukin (IL)-1β, visfatin, fasting insulin, and fasting blood glucose (FBG) were evaluated. Serum Hg levels were significantly higher in car painters compared to the control group (19.00 $\pm$ 7.20 vs. 8.339 $\pm$ 3.916 μg/L, *P*-value $<$ 0.001). Serum levels of visfatin, MDA, insulin, FBG, and IL-1β were significantly higher in the car painter compared to the control (*P*-value $<$ 0.001). There was a significantly higher proportion of people with diabetes in car painters compared to control (8.6% vs. 0%) and higher prediabetic (30.5% vs. 13.3%, *P*-value $<$ 0.001). In car painter workers, levels of Hg were significantly higher in DM compared to prediabetic and normoglycemic car painter workers (27.01 $\pm$ 1.59, 23.98 $\pm$ 4.31, and 15.39 $\pm$ 6.41 μg/mL, respectively, *P*-value $<$ 0.001); additionally, levels of Hg were significantly higher car painter with insulin resistance compared to non-insulin resistance workers (21.18 $\pm$ 7.29 vs. 16.79 $\pm$ 16.7 μg/mL, *P*-value $<$ 0.001).
Conclusions: Increased serum Hg in car painters increases the risk of insulin resistance and diabetes/prediabetes status.

Keywords: Mercury; Prediabetic; Insulin resistance; Oxidative stress; Diabetic.

1. Introduction

Diabetes mellitus (DM), a prevalent and increasing condition worldwide, is a major contributor to mortality, progressive vision loss, and long-term kidney dysfunction. Additionally, it is a significant contributing factor to vascular disorders such as myocardial infarction, stroke, and peripheral vascular disease. Efforts to decrease the occurrence of DM have been strengthened due to the rise in social costs caused by illness or mortality connected to DM. The increasing prevalence of DM is believed to be linked to lifestyle changes and other contributing variables, such as exposure to various environmental contaminants and industrial toxins.[1] Due to fast industrial expansion, there has been an increase in exposure to many environmentally hazardous substances, which is thought to be associated with an increase in the incidence of DM. The US Environmental Protection Agency has designated environmental agents that induce endocrine disruption as "endocrine-disrupting chemicals."[2] Trace elements, including some heavy metals, are naturally occurring inorganic elements in trace levels within the body. They are indispensable for crucial physiological activities. Heavy metals are typically characterized as metals that have high densities, atomic weights, or atomic numbers. Heavy metals and metalloids, including lead, mercury (Hg), cadmium, and metalloid arsenic, can potentially disrupt hormonal activity; these substances are widely considered toxicants and endocrine-disrupting chemicals. These toxic metals have detrimental impacts on the body's physiological processes. They could be linked to the occurrence of DM in certain populations.[1,3] There is limited research on whether exposure to heavy metals in the workplace directly or indirectly impacts the body or the development of specific diseases. Several studies on a population heavy metal level have examined the link between exposure to metals and diabetes, but the findings have been conflicting.[4–6]

Mercury is a dense metallic element recognized for its harmful effects on living organisms and can be found in several chemical states. Inorganic mercury consists of elemental mercury (Hg^0) and mercurous (Hg^{+1}) or mercuric (Hg^{+2}) salts. On the other hand, organic mercury refers to compounds where mercury is bound to a structure that contains carbon atoms, such as ethyl, methyl, phenyl, and so on.[7] These forms exhibit significant variations in their biological activity and toxicity.[7] Hg exposure can have a wide range of harmful consequences on several systems in the body, including the cardiovascular, pulmonary, hematological, digestive, renal, immunological, neurological, endocrine, and reproductive systems.[8] Regarding diabetes, Hg can specifically affect β-cells in the pancreas, leading to their malfunction and death.[9] Multiple

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mechanisms are implemented, including modifying Ca^{2+} balance, activating the phosphatidylinositol 3-kinase (PI3K) Akt signaling pathway, and generating reactive oxygen species (ROS).[10] Several research has examined the occurrence of this metal in the scalp hair,[11] urine,[12] and blood[13–16] of diabetic patients to investigate potential connections between its levels and type 2 DM (T2DM); however, the findings are inconsistent. Several studies have shown a correlation between T2DM and elevated levels of Hg in the bloodstream. Some research has shown a correlation between T2DM and levels of Hg in blood,[17–22] urine,[23] hair,[24] and toenail;[25] other studies have not shown any such association.[26,27]

Prolonged and significant contact with heavy metals can lead to elevated concentrations of heavy metals in the bloodstream or urine. In contrast, minimal exposure results in extremely low concentrations, prolonged and moderate exposure to heavy metals may result in only minimal quantities in the bloodstream or urine, and accumulating heavy metals in organs might have detrimental effects. Accumulation of heavy metals in the liver and pancreas can disrupt the process of gluconeogenesis in the liver and impact the production of insulin, ultimately affecting the occurrence of diabetes mellitus. The purpose of this study was to examine the potential association between occupational exposure to Hg and its biochemical pathways and the risk of developing DM. The objective of this study was to examine the potential correlation between blood mercury levels and other biological indicators, such as malondialdehyde (MDA), interleukin-1beta (IL-1beta), visfatin, insulin, and fasting blood glucose (FBG), and the potential risk of DM and prediabetic among a group of car painters' workers in Iraq.

2. Materials and methods

2.1. Study design

This was a retrospective case–control study involving 210 male participants aged between 25 and 50 years. The participants were divided into two groups: 105 who had worked as car painters for at least one year and 105 healthy people who had not worked as car painters and had no health concerns or chronic diseases.

2.2. Participants selection and sample size calculation

The inclusion criteria include healthy participants without any history of chronic disease or chronic medication, aged 18 years or older, male, non-obese, and worked at least one year as car painters. The control group was chosen based on living in residence similar to the car painters' group, but their work does not involve working as car painters; in addition to choosing similar socioeconomic backgrounds, this criterion ensures reducing the risk of bias originating from sociodemographic and economic characteristics. Regarding the exclusion criteria, participants in the control group had no local automobile painting facilities near their residency and no history of mercury exposure. Additionally, they had a body mass index (BMI) below 30 kg/m^2. Regarding the car painters' group, all participants had no chronic illness before entering the study.

All participants were surveyed to evaluate their likelihood of exposure to Hg, and no one disclosed any exposure other than that associated with their current occupation.

The sample size was determined using G*Power version (3.1.9.7).[28,29] The effect size was 0.5, the significance threshold (α) was set at 0.05, the type II error rate (β) was set at 0.05 with 95% detection power, and a two-tailed t-test was used. The total sample size was 210, with 105 participants in each group.

2.3. Study settings

The study was conducted in the Baghdad governorate, Iraq. The study was done in an industrial location, where we employed a random sampling technique to pick 105 personnel who specialize in automobile painting. The participants were selected using a non-random quota sampling technique, with their distribution decided by their place of residence, as shown by the statistics provided by the agriculture organization in Baghdad. The mean weekly work hours were 87.2 ± 8.2 h, and most workers have been employed for a minimum of 1 year. The study was conducted between 2022 March 1, and 2022 October 1.

2.4. Laboratory analysis

The authors were accountable for the acquisition of the blood samples. Approximately 10 mL of venous blood was collected using disposable syringes from the peripheral forearm vein. The blood sample was centrifuged at 5000 rpm for 10 min to separate serum. The serum was stored at −20 °C until the analysis day.

2.4.1. *Measurement of Hg ($\mu g/mL$)*

The samples were processed in a controlled environment with a clean room that met Class 10,000 cleanroom standards[30] All glass and plastic ware was cleaned by soaking in 10% HNO3 for 24 h and rinsing several times with deionized water. Blood samples (2 mL) were mixed with 250 mg of potassium persulphate, 2 mL of HNO_3 solution, 0.5 mL of H_2SO_4, and 5 mL of deionized water. The mixture was heated for half an h at 80 °C.

Blood Hg levels were determined using cold Hg vapor atomic absorption spectrophotometry (Shimadzu atomic absorption spectrophotometry model (AA 630–12)) and Hg hollow cathode lamp at 253 nm. A quartz cell of 12 cm in length and 8 mm in diameter with quartz windows and outlet ends has been used for measurements.[20,31]

Eight mL of digested blood sample was introduced in reducing vessels, 2 mL of $SnCl_2$ solution was added and mixed using a magnetic stirrer for 2 min, then Hg vapor forced by nitrogen gas with a rate of 0.25 L/min,[32] a calibration curve prepared from the aqueous standard of Hg^{+2} solution to which $SnCl_2$ was added and forced by the same previous procedure.

2.4.2. *Measurement of biomarkers*

The serum samples were used to determine the MDA, visfatin, IL-1β, and insulin levels by ELISA technique, which was done following the manufacturer's procedure (Sunlong biotech®, China). Frozen serum samples were thawed to room temperature (25 °C), then 50 μl of serum samples were placed in the wells of ELISA plates for 2 h at room temperature. Subsequently, 50 μL of detection antibody was introduced and allowed to incubate for 90 min.[33] Subsequently, three washes are performed using a pre-prepared washing buffer. The spectrophotometer quantified the samples' optical density (450 nm), while a standard curve determined their concentration using an ELISA reader from Diagnostic Automation (Cortez Diagnostics®, California, USA).

2.4.3. *Measurement of fasting blood glucose*

FBG was determined immediately by the Accu-Chek® Performa glucometer (Roche Diagnostics, Switzerland) following the manufacturer's instructions. All patients fasted for at least 12 h before taking the test, and all measurements were performed by experienced, trained laboratory staff.

Table 1. Assessment of demographic parameters.

Parameters	Control	Car painter	p-value
Number	105	105	-
Age (year), mean ± SD	39.10 ± 7.38	38.48 ± 6.94	0.532
BMI (kg/m^2), mean ± SD	25.20 ± 2.53	25.34 ± 2.52	0.690
Smoking, no. (%)	19 (18.1%)	25 (23.8%)	0.309
Married	80 (76.2%)	75 (71.4%)	0.433
Fish intake (≥1 serving per day)	16 (15.2%)	26 (24.8%)	0.084
Physical activity (<21.5 min/day)	75 (71.4%)	82 (78.1%)	0.266
Education level			0.138
Illiterate	17 (16.2%)	10 (9.5%)	
Primary or secondary	77 (73.3%)	76 (72.4%)	
College	11 (10.5%)	19 (18.2%)	
Monthly income			0.094
Poor	16 (15.2%)	13 (12.4%)	
Low	30 (28.6%)	43 (41.0%)	
Middle	51 (48.6%)	36 (34.3%)	
High	8 (7.6%)	13 (12.4%)	

BMI: body mass index, SD: standard deviation

2.5. Homeostatic model assessment for insulin resistance (HOMA-IR)

The HOMA-IR is a method that utilizes fasting glucose and plasma insulin levels to define insulin resistance for clinical and research applications across various populations:

$$HOMA - IR = \frac{Fasting\ insulin\ \left(\frac{mU}{L}\right)\ x\ FBG\ \left(\frac{mg}{dL}\right)}{405}$$

In the current study, insulin resistance was defined as a cut-off of less than 2.9 for HOMA-IR.[34]

2.6. Ethics approval

The study received approval from the Research Ethics Committee at Al-Mustafa University College (Approval number: AP012, research no.: 12, date: 2022 January 20). All participants provided written informed consent.

2.7. Statistical analysis

The current study's statistical analysis was conducted using GraphPad Prism version 10.0.1. normality test was undertaken using the Anderson-Darling test, and variables followed a normal distribution. The descriptive data were presented as the mean ± standard deviation (SD). The independent t-test was used to determine the statistical significance of the difference between the analyzed groups. One-way ANOVA with post hoc Tukey test assessed the difference between normoglycemic, diabetic, and prediabetic. Chi-square analysis to assess the difference in categorical variables. Pearson correlation is used to assess the relationship between different variables. The groups were deemed statistically significant when the P-value was less than 0.05.

3. Results

The study included 210 male non-obese participants, and as illustrated in Table 1, there was no significant difference in their age, BMI, smoking habit, education level, monthly income, and other parameters between both groups.

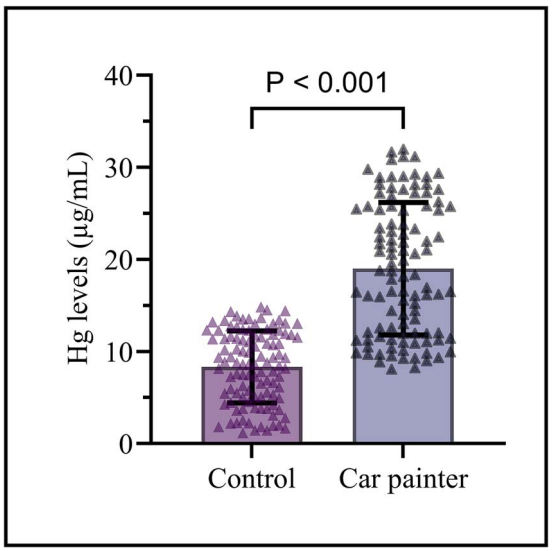

Fig. 1. Assessment of mercury levels in the study groups.

Serum Hg levels were significantly higher in car painters compared to the control group (19.00 ± 7.200 vs. 8.339 ± 3.916, P-value < 0.001), as illustrated by Fig. 1.

Serum levels of visfatin, MDA, insulin, FBG, and IL-1β were significantly higher in the car painter than in the control (P-value < 0.001), as illustrated by Table 2.

The HOMA-IR value was significantly higher in car painters compared to the control group (3.441 ± 1.706 vs. 2.186 ± 0.722, P-value < 0.001). As illustrated by Fig. 2, 50.5% of the car painters had insulin resistance (IR), compared to 15.2% of the control group.

According to "Standards of Care in Diabetes" in the American Diabetes Association guidelines in 2023, levels of FBG < 100 mg/dL are considered normal, between 100–125 mg/dL are considered prediabetic, and levels ≥126 mg/dL are considered diabetic (type II),[35] according to this classification, there was a significantly higher proportion of diabetics in car painters compared to control (8.6% vs. 0%) and higher prediabetic (30.5% vs. 13.3%, P-value < 0.001), as illustrated by Table 3.

Table 2. Assessment of investigated biomarkers.

Parameters	Control	Car painter	P-value
Number	105	105	-
MDA (nmol/mL), mean ± SD	0.80 ± 0.33	2.02 ± 0.69	< 0.001
Visfatin (ng/ml), mean ± SD	1.06 ± 0.23	2.45 ± 0.71	< 0.001
Insulin (mU/L), mean ± SD	10.09 ± 3.10	14.18 ± 5.89	< 0.001
FBG (mg/dL), mean ± SD	87.77 ± 10.29	97.62 ± 19.24	< 0.001
IL-1B (pg/mL), mean ± SD	6.71 ± 2.51	16.93 ± 8.59	< 0.001

MDA: malonaldehyde, IL-1: interleukin – 1

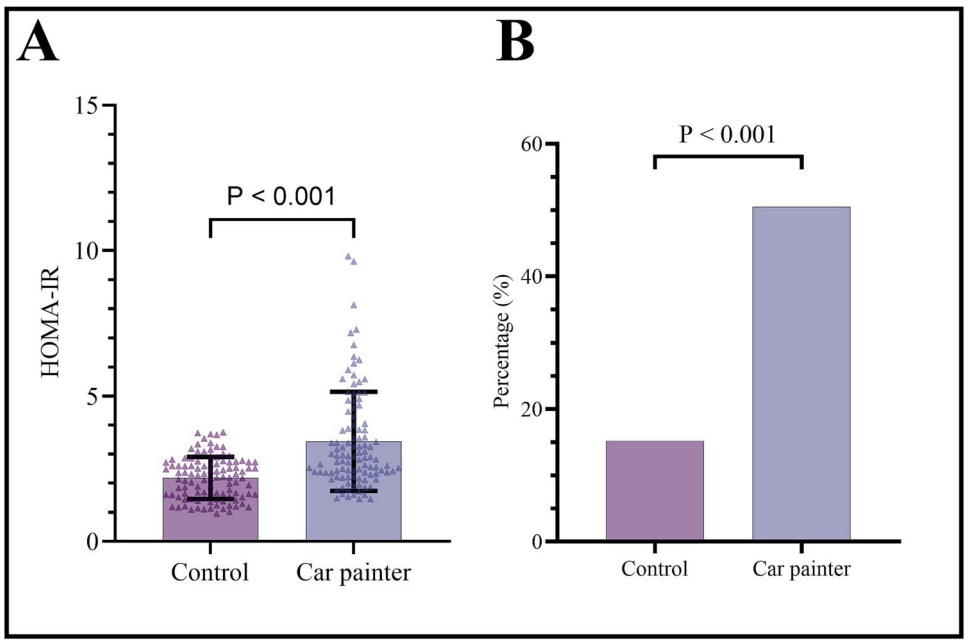

Fig. 2. Assessment of insulin resistance status in the studied groups. (A) Value of HOMA-IR, (B) overall insulin resistance status (defined as HOMA-IR > 2.9).

Table 3. Assessment of diabetic and prediabetic according to study groups.

Parameters	Control	Car painter	P-value
Number	105	105	-
Normal	91 (86.7%)	64 (61.0%)	< 0.001
Prediabetic	14 (13.3%)	32 (30.5%)	
Diabetic	0 (0%)	9 (8.6%)	

In car painter workers, levels of Hg were significantly higher in DM compared to prediabetic and normal FBG workers (27.01 ± 1.59, 23.98 ± 4.31 μg/L, and 15.39 ± 6.41 μg/mL, respectively, *P*-value < 0.001), as seen in Fig. 3A; additionally, levels of Hg was significantly higher car painter with insulin resistance compared to non-IR workers (21.18 ± 7.29 vs. 16.79 ± 16.7 μg/mL, *P*-value < 0.001), as seen in Fig. 3B.

Mercury showed a significant direct correlation with MDA (moderate correlation), visfatin (moderate correlation), FBG (moderate correlation), and HOMA-IR (moderate correlation). In contrast, its correlation with IL-1β was weak, as illustrated by Fig. 4.

4. Discussion

Diabetes mellitus is a persistent metabolic condition induced by various triggers. Certain environmental elements are considered risk factors for diabetes.[36,37] Mercury is a redox-passivating metal, and recent studies indicate that it may provoke oxidative stress by diminishing the enzymatic activity of superoxide dismutase, reducing antioxidants, or binding to protein-sulfhydryl (-SH) groups.[38,39] The harmful effects of mercury are associated with mitochondrial malfunction, which correlates with oxidative damage and inflammation of the β-cell apoptotic signaling system, resulting in diminished β-cell activity and heightened vulnerability to diabetes. Oxidative stress, apoptosis, and inflammation contribute to mercury-induced diabetes.[36] Nonetheless, their specific roles and mechanisms in islet beta-cell activity and glucose regulation remain ambiguous.[26]

In the present study, we examined Hg levels in car painters and compared them to healthy matched control; Hg levels were significantly higher in car painters compared to the control group (*P*-value < 0.001). Furthermore, in the car painter group, Hg levels were significantly higher in diabetics than in normoglycemic participants (*P*-value < 0.001); additionally, prediabetic car painters showed significantly higher Hg levels than in normoglycemic car painters (*P*-value < 0.001). In the car painter group, Hg levels were significantly higher in participants with insulin resistance than those without insulin resistance (*P*-value = 0.001). These findings suggest that Hg is associated with the pathogenesis of diabetes and insulin resistance in car painters.

Our findings were in agreement with a previous study in which Hg was moderately correlated with metabolic syndrome and its

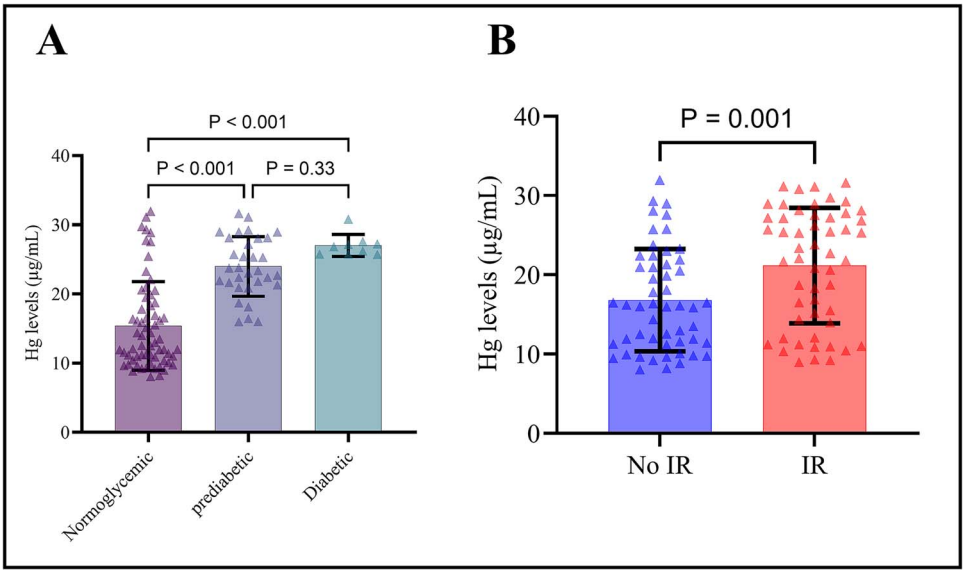

Fig. 3. Assessment of mercury levels according to (A) diabetic status and (B) insulin resistance status.

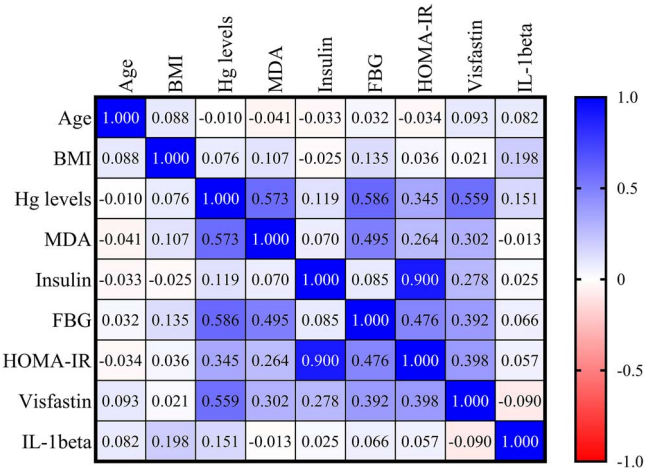

Fig. 4. Correlation matrix between Hg and various parameters in the car painter group.

components (waist circumference, systolic and diastolic blood pressure, triglyceride, blood glucose, insulin, and insulin resistance).[22] However, unlike this study, the authors did not examine the relationship Hg and diabetic.[22] In another study that examined the Hg levels in the blood of the adult Inuit population in Greenland (A community characterized by abnormally elevated levels of mercury consumption resulting from the consumption of marine mammals and fish as part of their diet), the authors reported a direct association between blood Hg and FBG; furthermore, they reported an association between Hg blood levels with increased risk of DM (with each 5 μg/L there is 2%–3% increased odds of DM).[19]

Mercury has been recognized for its ability to harm organ functions and disturb physiological balance. Mercury poisoning has been linked to the development of cardiovascular disease[40] and beta-cell apoptosis[41] in Faroese men. A prospective investigation discovered a notable linear correlation between reduced beta-cell function and the presence of methyl mercury in toenails.[25] The scientists identified this connection as the fundamental reason that accounted for the positive correlation they observed between mercury and the occurrence of T2DM. A study conducted in Korea found no significant correlations between blood mercury levels and both insulin resistance (measured as HOMA-IR) and beta-cell function (measured as HOMA-beta) in a sample of individuals from the general community who do not have diabetes.[42]

In contrast to our findings, earlier research has not shown any evidence of a connection between methyl mercury and the development of diabetes in two US prospective cohorts.[43] Moreover, a comprehensive analysis determined inadequate evidence to support a connection between type 2 diabetes and exposure to mercury.[44] The conclusions were derived from analyses of both toenail and blood mercury readings. The mean blood mercury level was 4.37 ± 1.81 μg/L for people without diabetes and 4.42 ± 1.96 μg/L for participants with diabetes.[42] The conclusions reported in both studies were derived from research conducted exclusively on Western or Korean populations. This study was conducted on a community in Iraq that was at a higher risk of exposure to mercury due to their work as car painters.

In the current study, elevated levels of Hg were directly associated with high MDA and visfatin levels but not IL-1B, which suggests that Hg induces oxidative stress through lipid peroxidation pathways. Hg has a lesser effect on inflammatory markers like IL-1B (weak association); oxidative stress plays a role in the advancement of insulin resistance and malfunction of pancreatic beta cells.[45] Shenker et al. discovered that mercury triggers apoptosis in human T lymphocytes. They proposed that the mitochondrion is the specific organelle affected by mercury and that the activation of apoptotic pathways is a result of producing oxidative stress. Their research indicates that methylmercury causes damage to pancreatic beta cells through a process involving oxidative stress. This damage is mediated by a pathway that leads to cell death, namely by an apoptosis mechanism. This pathway is activated by releasing a protein called cytochrome c from the mitochondria and ultimately leads to activating an enzyme called caspase-3.[46]

In a laboratory investigation, exposure to methylmercury at levels equivalent to those found in fish consumed below the acceptable limits set by the US Food and Drug Administration resulted in reduced insulin production and the initiation of apoptosis in HIT-T15 cells and isolated mouse islets. This discovery unequivocally showed that methylmercury's oxidative

stress leads to the death and malfunction of pancreatic beta cells.[9]

During an in vivo experiment, the animals were orally exposed to modest mercury doses for 2 or 4 weeks. This exposure increased lipid peroxidation levels in plasma, a drop in plasma insulin levels, and an elevation in both blood glucose levels and glucose intolerance. N-acetyl-l-cysteine (NAC), a scavenger of ROS, effectively inhibited the reactions caused by mercury.[47]

Both experimental and clinical pathology findings indicate that mercury disrupts the cellular activity of the islets of Langerhans in the pancreas.[48] Nevertheless, there have been few instances of individuals experiencing negative health consequences due to consuming large amounts of fish that contain significant levels of dioxins and mercury. In the current study, we examined fish consumption, and neither group showed a significant difference, indicating that it had a minimal effect on our findings.

These findings, coupled with our increased MDA levels in car painters and its direct association with Hg, suggest that Hg exerts its toxic effects on pancreases via an oxidative stress mechanism with hyperinsulinemia (but it is not clear at this stage whether hyperinsulinemia will remain in these patients or later become hypoinsulinemia at a more advanced stage; this will warrant further study with a prospective cohort).

Hyperinsulinemia is observed in car painters, but we could find a significant association between insulin levels and blood mercury; this raises the question of which came first, insulin resistance or diabetes; further studies are required to determine the exact pathology of diabetes in such patients. Research has demonstrated that peripheral insulin sensitivity is an early impairment in the progression of type 2 diabetes; this was reached by examining studies conducted on genetically predisposed populations and by doing long-term follow-up studies on individuals with normal glucose tolerance who later developed impaired glucose tolerance and type 2 diabetes.[49] In addition, there is a higher proportion of car painters with insulin resistance than that with diabetic/prediabetic, which is highly suggestive that insulin resistance is a cause of the increased risk of diabetic/prediabetic in car painters.

4.1. Study limitations and recommendations

This study investigated the concentrations of Hg in the serum, where Hg becomes attached to protein thiol groups following its absorption into the bloodstream. Within the serum, mercury primarily attaches to proteins such as albumin and certain apolipoproteins. However, in erythrocytes (red blood cells), the hemoglobin molecule possesses more binding sites for mercury. The distribution of mercury in the bloodstream is heavily influenced by its chemical form,[50,51] taking these factors into account, one should carefully interpret serum or blood levels of Hg.

The study's reliance on observation restricts its ability to make broad generalizations. Furthermore, there is a need for nationwide monitoring of mercury exposure among the general population. In order to establish a correlation between Hg exposure and its serum level, the measurement of its concentration in the environment was not carried out, which would provide a more accurate understanding.

5. Conclusion

This study provides evidence that demonstrates a direct correlation between elevated blood mercury levels and heightened susceptibility to insulin resistance and diabetes/prediabetic conditions among individuals working as car painters. The proposed causes for the development of diabetes or prediabetic illness caused by mercury exposure include heightened oxidative stress, elevated levels of visfatin, and insulin resistance.

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Author contribution

Conceptualization, investigation, Manuscript preparation, Numan AT, Jawad NK, and Fawzi HA. Supervision, Jawad NK. Statistical analysis and review of final results, Fawzi HA. Manuscript review and editing, Numan AT, Jawad NK, and Fawzi HA. All authors have read and agreed to the published version of the manuscript.

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Data availability

Zenodo: Fawzi, H. (2024). Mercury and diabetic in car painters [Data set]. Zenodo. https://doi.org/10.5281/zenodo.10866481. Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

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