Punjab University Journal of Zoology

OPEN ACCESS

41(1): 75-83 (2026)
https://dx.doi.org/10.17582/journal.pujz/2026/41.1.75.83

Check for updates

Research Article

# Blood-Based Expression of Metabolic (*LEP*, *ADIPOQ*, *INS*) and Inflammatory (IL-10, TNF-α) Biomarkers in Iraqi Individuals with Obesity and Diabetes: A Case-Control Study

**Hadeel Jabar Neama Almuoswi[1*], Israa Allawi Luaibi Al-Hraishawi[1], Jameel K. Alumeri[2] and Ahmed Jasim Neamah[3]**

[1]*Department of Medical Biotechnology, College of Biotechnology, University of Al-Qadisiyah, Iraq.*
[2]*Department of Biology, College of Education, University of Al-Qadisiyah, Iraq*
[3]*Department of Agriculture Biotechnology, College of Biotechnology, University of Al-Qadisiyah, Iraq.*

**Article History**
Received: December 26, 2025
Revised: February 25, 2026
Accepted: March 03, 2026
Published: April 24, 2026

**Authors' Contributions**
HJNA designed the study. IALA performed data collection and laboratory work. JKAconducted data analysis. AJN contributed to manuscript writing and revision. All authors have read and approved the final version of the manuscript.

**Keywords**
*LEP*; *ADIPOQ*; *INS*; Inflammatory Biomarkers; Obesity; Diabetes



**Abstract** | This case-control study aimed to determine the blood-based gene expression of the metabolic biomarkers (*LEP*, *ADIPOQ*, and INS) and the values of the circulating inflammatory cytokines (IL-10 and TNF-α) in Iraqi people with obesity and diabetes. The participants were divided into six categories (n = 6 in each category) comprising of obese and non-obese subjects including type 1 or type 2 diabetes, obese healthy subjects, and normal control subjects. qRT-PCR with the comparative 2-ΔΔCt procedure was applied to quantify the levels of gene expression and ELISA was used to measure the levels of cytokines. *LEP* expression was significantly elevated among all groups of patients in comparison to controls. Only in the case of obese patients with type 1 diabetes, the expression of *ADIPOQ* was lowered significantly, but in the chosen groups of patients, *INS* expression was lowered significantly. *LEP*/*ADIPOQ* ratio was greatly accelerated in obese type 1 diabetic and obese non-diabetic. In terms of inflammatory markers, the values of IL-10 markedly differed greater in-patient groups whereas the levels of TNF-α were lower in most of the groups with controls having a significantly higher level. These findings should be interpreted with caution since the sample size is very small. The relationships that can be observed indicate possible changes in the expression of adipokine genes and cytokine patterns among the Iraqi obese and diabetic population, but the research needs to be represented on a larger scale to confirm these provisional exploratory results and determine their clinical implications.

**Novelty Statement** | The paper analyzes blood-based expression of important metabolic markers (LEP, ADIPOQ and INS) and inflammatory cytokines (IL-10 and TNF- a ) of obese and diabetic people in Iraq. It involves adipokine gene profiling and cytokine evaluation in one cohort. The results give some initial evidence on population-specific immunometabolic patterns.

**To cite this article:** Almuoswi, H.J.N., Al-Hraishawi, I.A.L., Alumeri, J.K. and Neamah, A.J., 2026. Blood-based expression of metabolic (*LEP, ADIPOQ, INS*) and inflammatory (IL-10, TNF-α) biomarkers in Iraqi individuals with obesity and diabetes: A case-control study. *Punjab Univ. J. Zool.*, **41**(1): 75-83. https://dx.doi.org/10.17582/journal.pujz/2026/41.1.75.83

## Introduction

Obesity and diabetes mellitus (DM) are some of the most common chronic metabolic conditions on the global level and they are key causes of disease incidence

**Corresponding Author: Hadeel Jabar Neama Almuoswi**
hadeel.jabbar@qu.edu.iq

and death rate (WHO, 2024, 2025). Insulin resistance, dysfunctional adipose tissue, and sustained subclinical inflammation form an interrelated pathogenic network in which each factor reinforces the other. This shared biological basis explains their concurrent development, a metabolic state termed diabesity (Hossain *et al.*, 2007; Schwartz *et al.*, 2017). The incidence of diabetes is increasing in Middle Eastern populations, and Iraq is no exception where the high rate of overweight, obesity, and type 2 diabetes is attributed to the rapid lifestyle changes.

Adipose tissue is currently being acknowledged as more than an organ of energy preservation but also a metabolically active endocrine organ that produces bioactive molecules called adipokines (Fasshauer and Blüher, 2015; Coelho *et al.*, 2013). Among adipokines, *Leptin* (*LEP*) and adiponectin (*ADIPOQ*) are dramatically researched since they serve a key function in the management of appetite, energy balance, glucose metabolism, and insulin sensitivity. Excess adiposity is commonly characterized by elevated serum leptin levels, a state frequently linked to reduced leptin sensitivity, altered metabolic signaling, and increased cardiovascular vulnerability (Zhang *et al.*, 1994; Frühbeck *et al.*, 2018). Conversely, adiponectin concentrations are typically diminished in obesity and type 2 diabetes, and lower levels correlate negatively with insulin resistance and chronic inflammatory activity (Yamamoto *et al.*, 2014; Wang *et al.*, 2018).

There is mounting evidence that the *Leptin*-to-adiponectin (L/A) ratio could be a more powerful marker of metabolic dysregulation than either of the indicators by itself, and that the ratio of pro- and anti-metabolic pathways (Frühbeck *et al.*, 2018). Besides, variations in the expression levels of the insulin (INS) gene can indicate the predisposition of the body to the violation of glucose homeostasis and the work of β-cells, especially in the presence of metabolic stress related to obesity.

Chronic inflammation represents a fundamental contributor to the pathogenesis of diabesity. As adipose tissue mass increases, it facilitates immune cell infiltration and stimulates the secretion of pro-inflammatory cytokines, including TNF-α, a mediator closely associated with impaired insulin sensitivity (Akash *et al.*, 2018; Alzamil, 2020). Conversely, IL-10 exerts anti-inflammatory effects and participates in the regulation of immune and metabolic homeostasis, although its impact on obesity and type 2 diabetes varies according to physiological and pathological conditions (Hong *et al.*, 2009; Haamid *et al.*, 2022). The interaction of adipokines and cytokines is dynamic, which highlights the complicated nature of immunometabolism of diabesity.

Although many studies have assessed the level of circulating adipokines and cytokines, less research has been conducted to determine the blood-based gene expression of *LEP*, *ADIPOQ* and *INS* especially among the Middle East population. Additionally, there is limited data that combines the expression of adipokine genes and inflammatory cytokine profiling in the same cohort of Iraqis. Since ethnicity and genetic background may have an impact on adipokine regulation (Rasmussen-Torvik *et al.*, 2012; Mente *et al.*, 2010), adipokine regulation in particular population should be investigated.

Thus, the current case-control study sought to examine the blood-based expression of *LEP*, *ADIPOQ*, and *INS* genes and serum IL-10 and TNF-α levels in obese and diabetic Iraqi participants. Our hypothesis was that metabolic status would be related to changes in adipokine gene expression and inflammatory cytokine profiles in this cohort.

## Materials and Methods

*Study cohorts*

A total of 36 subjects were recruited for this case–control study. Participants were subsequently allocated into six groups as illustrated in Figure 1: Group I comprised obese patients diagnosed with T2DM (n = 6); Group II included non-obese patients with T2DM (n = 6); and Group III consisted of obese patients with T1DM (n = 6), non- obese with T1DM group IV (n=6), obese non-diabetic group V (n=6), and control healthy (non-diabetic and non-obese) group VI (n=6). The 18 obese subjects, with BMI more than or equal to 25 kg/m$^2$ per WHO, were recruited from obesity and internal medicine outpatient clinics of the in El-Koufa Governorate City, Iraq. This study was conducted during the year 2024 and all study participants did give their written informed consent before they were subjected to the study. Prior to initiation, the study protocol was evaluated and authorized by the Institutional Research Ethics Committee, University of Al-Qadisiyah (Approval No. 122/2024). All experimental and clinical procedures adhered strictly to the ethical guidelines set forth in the Declaration of Helsinki.

Since this research is exploratory and the number of participants is limited, 36 participants were enrolled (n = 6 in each group). The formal calculation of a priori power was not made, so the findings can be regarded as preliminary and hypothesis-generating. These observations should be validated by future studies that involve larger sample sizes.

*Study design*

The above-mentioned six groups enrolled in this study were subjected to blood samplings. Each group included 6 study participants. Each study participant underwent a blood sampling as mentioned below. Each blood sample belonging to each study participant was subjected to laboratory analytic procedures to estimate

the levels of the metabolic biomarkers *LEP*, *ADIPOQ*, and insulin using qRt-PCR and quantifying the values of the inflammatory biomarkers IL-10 and TNF-α using indirect ELISA approach (Figure 1). Lastly, all laboratory data was subjected to strict statistical analysis using Prism GraphPad 8.0.

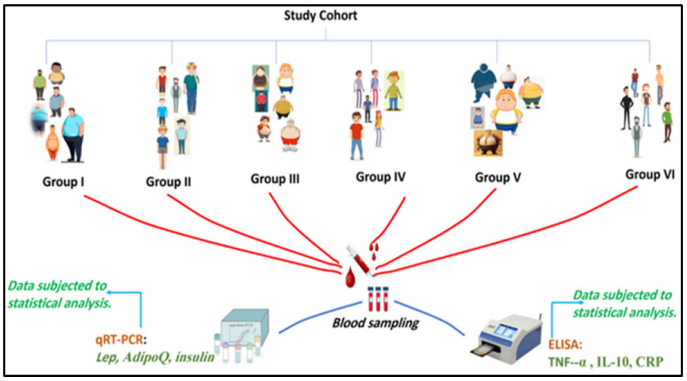


**Figure 1: A chart illustrating case-control diabesity study design. Group 1: obese participants with T2DM, Group II: non-obese study participants with T2DM, Group III: obese study participants with T1DM, Group IV: non-obese study participants with T1DM, Group V: obese study participants without DM, and Group VI: healthy control study participants; non-obese without DM. qRT-PCR: quantitative reverse transcriptase-polymerase chain reaction. ELISA: Enzyme linked immunosorbent assay. *Lep*: *leptin* gene, *AdipoQ*: *Adiponectin* gene, *insulin*: *insulin* gene, TNF-α: tumor necrosis factor alpha, IL-10: interleukin-10.**

*Blood sampling*

The sampling in blood was done in the period between November 2024 and April 2025. Each of the participants had 2 mL of venous blood collected under sterile conditions in EDTA tubes. Whole blood samples were frozen temporarily at -20 °C briefly before the RNA isolation. Total RNA was then isolated to be analyzed on gene expression and isolated RNA samples were stored at −80 °C until analysis.

To determine the cytokines, the blood that was treated with EDTA was centrifugated to isolate plasma, which was then utilized in the determination of the concentration of IL-10 and TNF-α by ELISA.

*Total RNA isolation*

Total RNA isolation from whole blood human samples collected from the 36 study participants enrolled in this study employing QIAamp RNA Blood Mini Kit (Qiagen Co., Germany) following the manufacturer's guidelines as elucidated in Figure 2. The eluted RNA was subjected to quality and quantity assessment study. The quality and quantity of the extracted total RNA was determined using the Thermo Scientific™ NanoDrop™ Ultra Microvolume UV-Vis Spectrophotometer (ThermoFisher Scientific™, USA). An RNA sample was considered pure if its Ab260/Ab280 and Ab262/Ab230 were 2.0 and >2.0, respectively. A concentration of an RNA sample of 500 ng/µL was considered a fit concentration appropriate for the subsequent reverse transcription of RNA. All RNA specimens were preserved at -80 °C until being further processed.

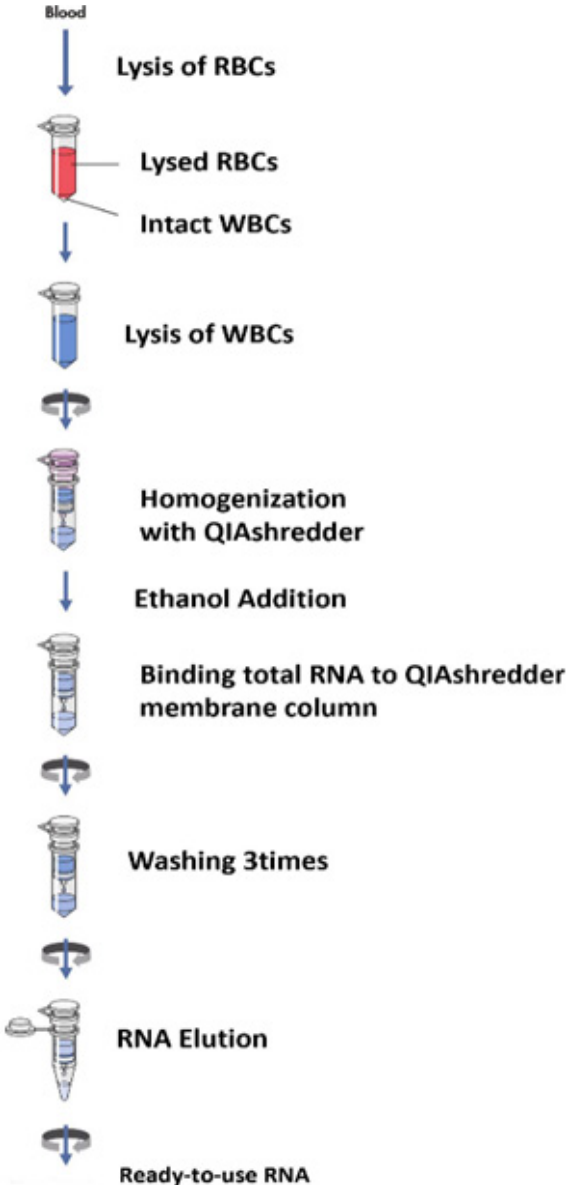


**Figure 2: A flow chart illustrating the steps involved in total RNA isolation from human whole blood samples using QIAamp RNA Blood Mini Kit (Qiagen Co., Germany).**

*RNA retro-transcription step*

The isolated total RNA was retro-transcribed into cDNA using QuantiNova Reverse Transcription Kit (Qiagen Co., Germany) following the manufacturer's guidelines. An equal amount of RNA (1 µg) from all samples was retro-transcribed according to the recipe settled in Table 1. The cDNA synthesis was conducted in PCR thermocycler machine (Biometra Co., Germany). The program for cDNA synthesis was settled to include

three steps: I) 25 °C for 5 min, II) step II (42 °C, 60 min), and step III (70 °C, 5 min). The resulting cDNA was kept at -80°C for subsequent RT-qPCR analysis.

**Table 1: Components and volumes of reactions in the first-strand cDNA synthesis.**

| Component | Volume in µL or amount in µg |
|---|---|
| Template RNA (µg) | 5.0 |
| Oligo (dT)18 primer (µL) | 1.0 |
| Nuclease free water (µL) | *X |
| 5x Reaction Buffer (µL) | 4.0 |
| RiboLock RNAase Inhibitor (20 U/µL) | 1.0 |
| 10 mM dNTP Mix (µL) | 2.0 |
| RevertAid M-MuLV RT (200U/µL) | 1.0 |
| Total reaction volume (µL) | 20.0 |

*Volume of nuclease free water is up to 12 µL.

*qRT-PCR*

Each sample was analyzed in three independent qRT-PCR reactions on a CFX Opus 96 platform operated with CFX Maestro Software v2.3 (Bio-Rad Laboratories, USA). Fluorescent detection of PCR products relied on SYBR Green dye chemistry, consistent with established methodological recommendations (Bustin *et al.*, 2009; Taylor *et al.*, 2019). The primer sequences were designed based on the following NCBI reference sequences (NM002046.3, NM0004797, , NM000230, NM000207 and NM002021.3): *LEP*, *ADIPOQ*, and *INS* focusing on three of them, along with a single reference housekeeping gene (GAPDH). The sequences of the primers were designed using Primer-BLAST online resource.

The reference gene was chosen because it is reportedly stable in peripheral blood samples both in the presence of metabolism and inflammation (GAPDH). The GAPDH Ct values were also compared between study groups, but consistency of the values was checked first before analysis of the gene expression.

A 25 µL of PCR reaction mixture was prepared with 12.5 µL of 2X SYBR 1-Green Universal Master Mix (Applied Biosystems 1, USA), 0.9- µL of gene-specific forward and reverse primers, and 25 ng of cDNA template per reaction. The thermal cycling conditions included the following program: denaturation at 95 °C of 3 min, 45 cycles of denaturation at 95 °Cof 15 sec, and extension/annealing at 60 °C of 1 min. The amplification specificity and the presence of single peaks were verified with the help of a melting curve analysis (55–99 °C).

The comparative 2−ΔΔCt procedure was employed to achieve relative expression levels through the use of healthy group as a calibrator (Livak and Schmittgen, 2001). The efficiency of the primer was confirmed by serial dilution of a representative sample of the RNA and similar efficiency of the primer in amplification of the gene under study and the control gene warranted the application of the 2−ΔΔCt methodology.

*IL-10 estimation*

The Elabscience® Human IL-10 ELISA Kit (Catalog No: E-EL-H6154) was used to measure IL-10 in the plasma samples as per the instructions of the manufacturer. The sensitivity of the assay was 0.94 pg/mL and detection range were 1.56-100pg/mL and intra-assay variation coefficient was less than 10%.

*TNF-α estimation*

The TNF-α concentration in plasma samples was determined using the Elabscience® Human TNF-α ELISA Kit (Catalog No: E-EL-H0109) as per the protocols set by the manufacturer. The sensitivity of the assay was 4.69 pg/mL, the assay range was 7.81 to 500 pg/mL, and the variation coefficient was less than 10%.

*Statistical analysis*

Data analysis was performed with GraphPad Prism software, version 8.0 (GraphPad Software, USA). Intergroup comparisons across the six study groups were assessed using one-way ANOVA, and significant effects were further examined through Tukey's post hoc test. Statistical significance was defined at $p < 0.05$. The information is represented in the form of mean + SD as it is shown in the figures. Taking into consideration that the sample size (n = 6 per group) is small, the findings can be viewed with caution and are exploratory.

## Results

*Adipocytokines genes expression profile among study cohorts*

The gene expression profile of the three adipocytokines *LEP, ADIPOQ,* and *Insulin* genes were monitored among the six groups of the study cohort as portrayed in Figure 3 and Table 3. The level of *LEP* gene expression varied considerably between study groups (Figure 3 and Table 3). Group I through Group V had a significant high level of *LEP* expression as relative to the healthy control group (Group VI) ($p<0.05$). Group III (obese people with T1DM) showed the highest relative expression. All values are expressed as mean ± SD, based on six participants in each group.

The expression profile of *ADIPOQ* was not comparable to that of *LEP* (Figure 3 and Table 3). The only case in which a significant decrease was observed in the *ADIPOQ* gene expression was the Group III (obese with T1DM) when relative to the healthy control group (Group VI) ($p < 0.05$). There was no significant difference was observed in Groups I, II, IV, or V as compared to controls. The data is represented as the mean + SD (n= 6/group).

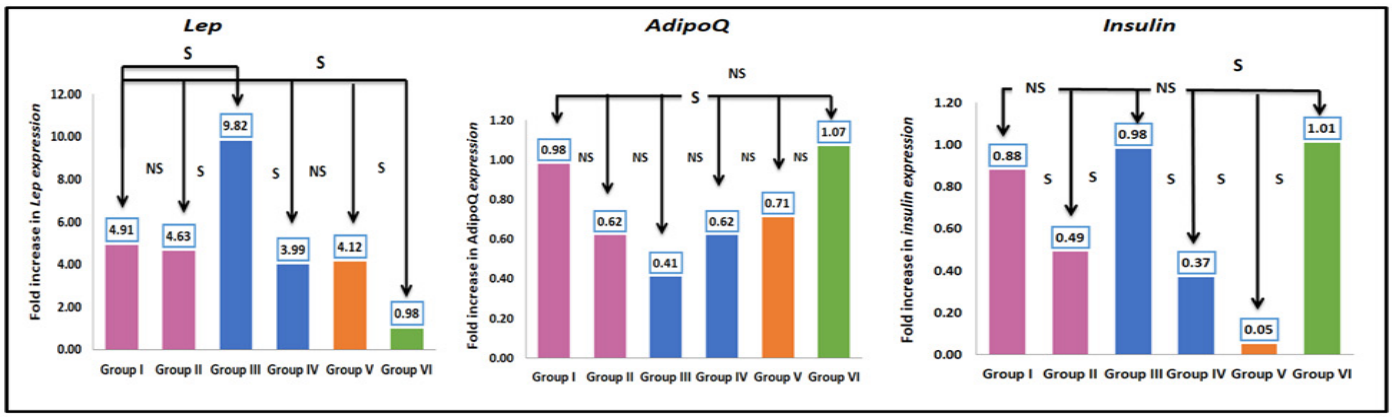


**Figure 3: Fold of gene expression for three adipokines: adipocyte-derived cytokines genes (*Lep* and *AdipoQ*) and adipogenic hormone (*insulin*) among the six groups of study cohort. Values represent mean ± SE (n = 6 per group).**

**Table 2: Primer sequences to be used in qRT-PCR analysis and sizes of the anticipated amplicons.**

| Gene name | Accession number * | Primer sequence (5'-3') | Product length (bp) |
|---|---|---|---|
| *Lep* | NM_000230 | Fw: GCTGTGCCCATCCAAAAAGTCC<br>Rv: CCCAGGAATGAAGTCCAAACCG | 135 |
| *AdipoQ* | NM_004797.4 | Fw: AGGCCGTGATGGCAGAGATG<br>Rv: GGTTTCACCGATGTCTCCCTTAG | 91 |
| *Insulin* | NM_000207 | Fw: CAGGACAGGCTGCATCAGAA<br>Rv: TGTTGGTTCACAAAGGCTGC | 138 |
| *GAPDH* | NM_002046.3 | Fw: ACCCACTCCTCCACCTTTG<br>Rv: CTCTTGTGCTCTTGCTGGG | 178 |

*Accession numbers of the gene sequence as retrieved from GenBank database.

**Table 3: Fold of gene expression for three adipokines: adipocyte-derived cytokines genes (*Lep* and *AdipoQ*) and adipogenic hormone (insulin) among the six groups of study cohort.**

| Groups | *Lep* gene | *AdipoQ* gene | *Insulin* gene |
|---|---|---|---|
| Group I | $4.91 \pm 0.36^{A}$ | $0.98 \pm 0.12^{A}$ | $0.88 \pm 0.23^{A}$ |
| Group II | $4.63 \pm 0.28^{A}$ | $0.62 \pm 0.11^{AB}$ | $0.49 \pm 0.10^{B}$ |
| Group III | $9.82 \pm 0.78^{B}$ | $0.41 \pm 0.03^{B}$ | $0.98 \pm 0.18^{A}$ |
| Group IV | $3.99 \pm 0.52^{A}$ | $0.62 \pm 0.12^{AB}$ | $0.37 \pm 0.09^{B}$ |
| Group V | $4.12 \pm 0.34^{A}$ | $0.71 \pm 0.14^{AB}$ | $0.05 \pm 0.003^{C}$ |
| Group VI | $0.98 \pm 0.16^{C}$ | $1.07 \pm 0.21^{A}$ | $1.01 \pm 0.27^{A}$ |
| p-value | 0.001*† | 0.001*† | 0.001*† |

Means followed by different letters are significantly different according to Tukey, Means followed by the same letter are not significantly different. SD: standard deviation; †: one way ANOVA; **: significant at $P > 0.05$

Expression of the *INS* genes showed a fluctuating pattern in the groups of the studies (Figure 3 and Table 3). The *INS* expression was significantly reduced in Groups II (non-obese with T2DM), IV (non-obese with T1DM), and V with (obese non-diabetic) as relative to the healthy control group (Group VI) ($p < 0.05$). No significant differences were noted between Groups I and III compared to controls. The data is shown in mean + SD (n = 6/group).

To determine the ratios of adipokine signals, *LEP*/*ADIPOQ* (L/A) expression ratio was determined (Figure 4 and Table 4). Group III (obese with T1DM) and Group V (obese non-diabetic) had a much greater L/A ratio than the healthy control group (Group VI) ($p < 0.05$). No significant difference was observed in Groups I, II, or IV with respect to controls. The data are provided in the form of mean + SD (6 per group).

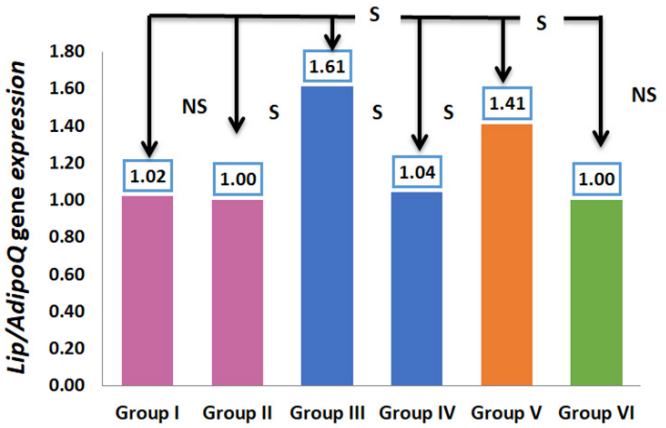


**Figure 4: The L/A ratio was calculated using fold-change values derived from the 2^-ΔΔCt method. The ratio was obtained by dividing the relative expression of *LEP* by that of *ADIPOQ* for each group.**

*Cytokines profile among study cohorts*

The levels of the IL-10 and TNF-α were displayed in Figure 5 and Table 5. The levels of IL-10 in the plasma of the study groups varied significantly (Figure

5 and Table 5). The IL-10 levels were also found to be much higher in Groups I-V ($p < 0.05$) as relative to the healthy control group (Group VI). Group I (obese with T2DM) registered the highest mean IL-10 and Group V (obese non-diabetic) registered the lowest level among the patient groups. Results are given in terms of mean±SD (n=6/group). Study groups significantly differed in TNF-α level plasma (Figure 5 and Table 5). A highly elevated level of TNF-α was found in Group I (obese and T2DM) versus in the healthy control group (Group VI) ($p < 0.05$). Groups II-V, on the contrary, showed much lower levels of TNF-α compared to controls ($p < 0.05$). Data is reported in mean+SD (n= 6/group).

**Table 4: Lep/ AdipoQ (L/A) ratio among the six groups enrolled in the cohort study.**

| Groups | *Lep/AdipoQ* (L/A) gene |
|---|---|
| Group I | $1.02 \pm 0.18^{A}$ |
| Group II | $1.00 \pm 0.14^{A}$ |
| Group III | $1.61 \pm 0.28^{B}$ |
| Group IV | $1.04 \pm 0.21^{A}$ |
| Group V | $1.41 \pm 0.22^{B}$ |
| Group VI | $1.00 \pm 0.12^{A}$ |
| p-value | 0.001*† |

Means followed by different letters are significantly different according to Tukey, Means followed by the same letter are not significantly different. SD: standard deviation; †: one way ANOVA; **: significant at $P > 0.05$

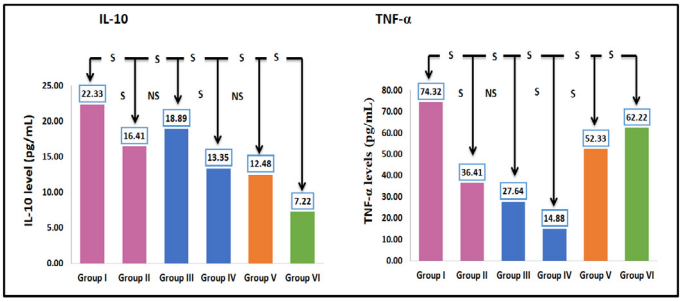


**Figure 5: Levels of inflammatory cytokines among the six groups of study cohort. Values are the mean of six readings (n=6) ±SE bars.**

**Table 5: Levels of inflammatory cytokines among the six groups of study cohort.**

| Groups | IL-10 level (pg/Ml) | TNF-α level (pg/Ml) |
|---|---|---|
| Group I | $22.33 \pm 3.18^{A}$ | $74.32 \pm 14.32^{A}$ |
| Group II | $16.41 \pm 3.11^{BC}$ | $36.41 \pm 8.12^{B}$ |
| Group III | $18.89 \pm 4.21^{AB}$ | $27.64 \pm 6.64^{B}$ |
| Group IV | $13.35 \pm 2.98^{C}$ | $14.88 \pm 3.48^{C}$ |
| Group V | $12.48 \pm 2.22^{C}$ | $52.33 \pm 12.51^{D}$ |
| Group VI | $7.22 \pm 1.14^{D}$ | $62.22 \pm 11.41^{E}$ |
| p-value | 0.001*† | 0.001*† |

Means followed by different letters are significantly different according to Tukey, Means followed by the same letter are not significantly different. SD: standard deviation; †: one way ANOVA; **: significant at $P > 0.05$

## Discussion

The current study investigated blood-based gene expression of key adipokines (*LEP*, *ADIPOQ*, and *INS*) alongside circulating inflammatory cytokines (IL-10 and TNF-α) in Iraqi individuals with obesity and diabetes. The findings demonstrate differential patterns of metabolic and inflammatory markers across the study groups, suggesting potential associations between adipokine signaling and metabolic status within this cohort. However, given the restricted sample size and observational cross-sectional approach, these findings should be interpreted cautiously and considered exploratory rather than definitive. A plethora of literature have addressed the interplay between the three adipokines namely *LEP*, *ADIPOQ*, and insulin to incite serious complications and bad predictive progression of the dual chronic disease (i.e., diabesity). The previous results have focused on two distinctive parameters, mRNAs and serum, to help track the levels of adipokines in a given population of certain ethnic group. There is a scarcity in the number of studies targeting these adipokines from blood samplings. The majority of studies did target the se adipokines either from their main synthesizing factor or from circulating quantities in plasma and serum.

The changed patterns of expression of *LEP* and *ADIPOQ* in this study are widely in line with the already reported results in the cases of obesity and type 2 diabetes. A high level of *Leptin* and a decline in adiponectin have been noted repeatedly in obese and insulin-resistant population (Mir *et al.*, 2022; Zuo *et al.*, 2013; Wang *et al.*, 2018; Yamamoto *et al.*, 2014).

Several Iraqi studies have reported altered leptin and adiponectin values in patients with type 2 diabetes mellitus; one showed a markedly greater leptin/adiponectin ratio in T2DM patients compared with controls (Tahir *et al.*, 2017), while another reported significantly different levels of both hormones among diabetic smokers and non-smokers (Al-Kadium *et al.*, 2013). However, disparities in the level of adiponectin have been reported among the ethnicities, and some studies noted population-specific differences and uneven correlations with metabolic status (Mente *et al.*, 2010).

The strongest decline in the *ADIPOQ* expression was found in the obese study cohort with T1DM, which is partly consistent with the previous Iraqi results that indicated a change in the regulation of adiponectin in diabetic individuals (Akram *et al.*, 2024). Such disparities can be due to population-specific genetic, metabolic or environmental effects given that there are known to be differences in adipokine regulation among different ethnic origins (Rasmussen-Torvik *et al.*, 2012). However, as a result of the relatively small cohort, one should be wary of directly comparing the results.

From another side, some studies addressed the potential of L/A ratio in prognosis of bad complications likely to occur in obese T2DM patients and other obesity co-morbidities rather than tracing *LEP* and *ADIPOQ* levels each alone. These previous studies outlined a conclusive remark stating that high L/A ratio was reported in obese T2DM with IR and other obese co-morbidities as well compared to healthy group with high insulin sensitivity (Frühbeck *et al*., 2018; Sweis *et al*., 2025). A previous study addressed an increase of Adpn/*LEP* ratio among obese with T2DM patients after conducting Roux-en-Y Gastric Bypass (RYGB) compared to that ratio obtained in the same patients prior to RYGB (Unamuno *et al*., 2019).

On top and above, the discrepancy among results addressing the levels of the three biomarkers *LEP*, adiponectin, and insulin in relation to obesity and T2DM is well addressed among various populations of discrete ethnic groups (Jung *et al*., 2010; Mente *et al*., 2010). Ethnic group imposes an effect on the levels of the adipokines biomarkers both alone or as a ratio due to the substantial difference in the genetic makeup among various populations that would impose differences.

In the current study, the IL-10 concentration was considerably higher in the entire patient population in contrast with the healthy population, whereas TNF-α was markedly greater in the obese patients with T2DM and lower in the rest. Such results are in contrast to multiple reports of increased TNF-α and decreased IL-10 in obesity and type 2 diabetes (Alzamil, 2020; Rai *et al*., 2025). The elevated TNF-α and reduced IL-10 have been typically understood as an indicator of unrelenting low-grade inflammation linked to metabolic dysregulation (Tiantian and Chengqi, 2018; Gul and Yilmaz, 2024).

Nevertheless, cytokine patterns have been known to be dynamic, not static and can change according to the length of illness, control of metabolism, treatment, and demographic features (Ghazaryan *et al*., 2023). The anti-inflammatory cytokine IL-10 can rise in compensation with the inflammatory stimuli in some metabolic diseases (Belkina *et al*., 2020; Rangel *et al*., 2021; Andriankaja *et al*., 2023; Novianti *et al*., 2024). Additionally, inter-study variability can be caused by variations in assay procedure, sample (plasma and serum) and storage setting, and the sensitivity of commercial ELISA kits (Valaperti *et al*., 2020).

Population specific reasons could also be the cause of variations in cytokine expression patterns. Past literature has indicated differences in the values of TNF-α and IL-10 in individuals with diabetes or obesity who have various ethnicities (Gul and Yilmaz, 2024; Rai *et al*., 2025). Thus, the patterns of cytokines in this Iraqi cohort can be the immunometabolic reactions to the situation, rather than the uniform inflammatory patterns.

However, the current study has cross-sectional pattern and small sample size, so one should take these results with caution. The invigorating longitudinal studies, bigger in size and strength, are needed to conclude whether the recorded cytokine changes are specific to the population or are caused by sampling error.

## Conclusion

This research examined the gene expression of *LEP*, *ADIPOQ*, and *INS* in blood and the values of circulating IL-10 and TNF-α in obese and diabetic Iraqis. The results show that metabolic and inflammatory markers vary differently in study groups, and there is a possibility of correlating the adipokine signaling and metabolic status in this population. Despite the fact that there were alternative results of cytokine findings, the variability can be attributed to population peculiarities, differences in methods, or sampling. These results can be discussed as initial and hypothesis-generating due to a small sample size and cross-sectional design. These observations need to be confirmed by further large-scale, longitudinal studies that can further elucidate the clinical significance of adipokine and cytokine changes in diabesity.

## Limitations

The current paper has various shortcomings. To begin with, the sample size (n= 6 per group) has a low statistical power, which limits the extrapolation of the results. Second, cross-sectional design excludes the possibility of causation and it is impossible to examine how levels of adipokines and cytokines change over time. Third, the expression of genes was assessed in peripheral blood as opposed to adipose tissue which is not necessarily reflective of tissue specific adipokine activity. Lastly, the possible confounding variables, including the period of disease, taking of medication, lifestyle and metabolic control, were not effectively controlled. When interpreting the results, one should take these restrictions into account.

## Recommendations

Further research involving bigger and more varied sample groups is justified in order to confirm the current results and enhance the statistical strength. Longitudinal design is especially suggested in order to assess the changes in adipokine gene expression and cytokine profiles over time concerning the disease progression. Also, including the detailed clinical data such as medication history, illness duration, metabolic regulation, and lifestyle characteristics would contribute to explaining the possible confounding effects. Surveys and comparisons of peripheral blood gene

expression and adipose tissue expression could further be used to increase the knowledge on the adipokine regulation of diabesity.

## Declarations

*Acknowledgement*

The authors would like to acknowledge the use of an AI language model for its assistance in improving the English language and readability of this manuscript.

*Funding*

This study received no external funding.

*IRB approval*

The study protocol was reviewed and approved by the Institutional Research Ethics Committee, University of Al-Qadisiyah, Iraq (Approval No. 122/2024).

*Ethical statement*

The informed consent was written and signed by all participants before they were included in the study. Everything was done with regard to the ethical principles of the Institutional Research Ethics Committee and the Declaration of Helsinki.

*Generative AI and AI assisted technology statement*

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

*Statement of conflict of interest*

The authors have declared no conflict of interest.

## References

Akash, M.S.H., Rehman, K., Liaqat, A., Numan, M., Mahmood, Q. and Kamal, S., 2018. Biochemical investigation of gender-specific association between insulin resistance and inflammatory biomarkers in type 2 diabetic patients. *Biomed. Pharmacother.*, **106**: 285–291. https://doi.org/10.1016/j.biopha.2018.06.044

Al-Kadium, T.E., Hammed, I.K. and Rashid, N.F., 2013. Serum level of adiponectin and leptin in type 2 diabetic cigarette smokers. *J. Fac. Med. Baghdad*, **55**: 60–63. https://doi.org/10.32007/jfacmedbagdad.551670

Alzamil, H., 2020. Elevated serum TNF-α is related to obesity in type 2 diabetes mellitus and is associated with glycemic control and insulin resistance. *J. Obes.*, Article ID 5076858. https://doi.org/10.1155/2020/5076858

Andriankaja, O.M., Adatorwovor, R., Kantarci, A., Hasturk, H., Shaddox, L. and Levine, M.A., 2023. Periodontal disease, local and systemic inflammation in Puerto Ricans with type 2 diabetes mellitus. *Biomedicines*, **11**: 1–15. https://doi.org/10.3390/biomedicines11102770

Belkina, A.C., Azer, M., Lee, J.J., Elgaali, H.H., Pihl, R., Cleveland, M. and Nikolajczyk, B.S., 2020. Single-cell analysis of the periodontal immune niche in type 2 diabetes. *J. Dent. Res.*, **99**: 855–862. https://doi.org/10.1177/0022034520912188

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M. and Wittwer, C.T., 2009. The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem.*, **55**: 611–622. https://doi.org/10.1373/clinchem.2008.112797

Coelho, M., Oliveira, T. and Fernandes, R., 2013. Biochemistry of adipose tissue: An endocrine organ. *Arch. Med. Sci.*, **9**: 191–200. https://doi.org/10.5114/aoms.2013.33181

Fasshauer, M. and Blüher, M., 2015. Adipokines in health and disease. *Trends Pharmacol. Sci.*, **36**: 461–470. https://doi.org/10.1016/j.tips.2015.04.014

Frühbeck, G., Catalán, V., Rodríguez, A. and Gómez-Ambrosi, J., 2018. Adiponectin-leptin ratio: A promising index to estimate adipose tissue dysfunction. Adipocyte, 7(1): 57–62. https://doi.org/10.1080/21623945.2017.1402151

Ghazaryan, A., Asoyan, V., Hovhannisyan, A., Kozmoyan, M., Karapetyan, A., Minasyan, A. and Gyulazyan, N., 2023. Comparative study of TNF-α and IL-10 levels at different times of the course of COVID-19. *J. Infect. Dev. Ctries.*, **17**: 1207–1212. https://doi.org/10.3855/jidc.18067

Gul, A. and Yilmaz, R., 2024. Determination of inflammation by TNF-α and IL-10 levels in obese children and adolescents. *Nutr. Hosp.*, **41**: 318–325. https://doi.org/10.20960/nh.05064

Haamid, B., Majid, S., Khan, M.S., Bhat, M.H., Hamid, R., Ashraf, R. and Faiz, S., 2022. Inter-relationship of pro- and anti-inflammatory biomarkers with the development of type 2 diabetes mellitus. *Heliyon*, **8**: e11329. https://doi.org/10.1016/j.heliyon.2022.e11329

Hong, E.G., Ko, H.J., Cho, Y.R., Kim, H.J., Ma, Z., Yu, T.Y. and Kim, J.K., 2009. Interleukin-10 prevents diet-induced insulin resistance by attenuating macrophage and cytokine response in skeletal muscle. *Diabetes*, **58**: 2525–2535. https://doi.org/10.2337/db08-1261

Hossain, P., Kawar, B. and El-Nahas, M., 2007. Obesity and diabetes in the developing world a growing challenge. *N. Engl. J. Med.*, **356**: 213–215. https://doi.org/10.1056/NEJMp068177

Jung, C.H., Rhee, E.J., Choi, J.H., Bae, J.C., Yoo, S.H., Kim, W.J., Park, C.Y., Mok, J.O., Kim, C.H.,

Lee, W.Y. and Oh, K.W., 2010. Relationship of adiponectin/leptin ratio with insulin resistance and metabolic syndrome. *Korean Diabetes J.*, **34**: 237–243. https://doi.org/10.4093/kdj.2010.34.4.237

Livak, K.J. and Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. *Methods*, **25**: 402–408. https://doi.org/10.1006/meth.2001.1262

Mente, A., Razak, F., Blankenberg, S., Vuksan, V., Davis, A.D., Miller, R., Teo, K., Gerstein, H., Sharma, A.M., Yusuf, S. and Anand, S.S., 2010. Ethnic variation in adiponectin and leptin levels and their association with adiposity and insulin resistance. *Diabetes Care*, **33**: 1629–1634. https://doi.org/10.2337/dc09-1392

Mir, M.M., Mir, R., Alghamdi, M.A.A., Wani, J.I., Sabah, Z.U., Jeelani, M., Marakala, V., Sohail, S.K., O'haj, M., Alharthi, M.H. and Alamri, M.M.S., 2022. Differential association of adipocytokines with type 2 diabetes mellitus in Saudi Arabia. *J. Pers. Med.*, **12**: 735. https://doi.org/10.3390/jpm12050735

Novianti, Y. and Nur'aeny, N., 2024. Interleukin-10 levels in diabetes patients with and without oral diseases: A systematic review. *J. Inflamm. Res.*, **17**: 541–552. https://doi.org/10.2147/JIR.S449546

Rai, P.S., Shivarajashankara, Y.M., Prajna, R.H., Anil, R. and Bhandary, Y.P., 2025. Serum adiponectin and tumor necrosis factor-alpha in diabetes: A nutritional perspective. *Biomed. Biotechnol. Res. J.*, **9**: 288–293. https://doi.org/10.4103/bbrj.bbrj_225_25

Rangel, T.P., Reis, A.A., Caponi, L., Pena, L.C., Ruiz, K.G., Santamaria, M.P. and Casarin, R.C., 2021. Subgingival endotoxin and lipoteichoic acid modulate cytokine production in diabetic subjects. *Oral Dis.*, **27**: 1325–1333. https://doi.org/10.1111/odi.13661

Rasmussen-Torvik, L.J., Wassel, C.L., Ding, J., Carr, J., Cushman, M., Jenny, N. and Allison, M.A., 2012. Associations of BMI and insulin resistance with leptin and adiponectin across ethnic groups. *Ann. Epidemiol.*, **22**: 705–709. https://doi.org/10.1016/j.annepidem.2012.07.011

Schwartz, M.W., Seeley, R.J., Zeltser, L.M., Drewnowski, A., Ravussin, E., Redman, L.M. and Leibel, R.L., 2017. Obesity pathogenesis: An endocrine society scientific statement. *Endocr. Rev.*, **38**: 267–296. https://doi.org/10.1210/er.2017-00111

Sweis, N., Jorgensen, J., Zeng, J., Choo-Kang, C., Zapater, J., Bedu-Addo, K., Forrester, T., Bovet, P., Lambert, E.V., Riesen, W. and Korte, W., 2025. Relationship between leptin-to-adiponectin ratio and metabolic syndrome. *Int. J. Obes.*, **49**: 278–285. https://doi.org/10.1038/s41366-024-01655-8

Tahir, N.T., Najim, H.D. and Ashoor, L.S., 2017. Role of leptin/adiponectin ratio in Iraqi type 2 diabetic patients treated with different antidiabetic agents. *Mustansiriya Med. J.*, **16**: 54–62. https://doi.org/10.4103/2070-1128.251019

Taylor, S.C., Nadeau, K., Abbasi, M., Lachance, C., Nguyen, M. and Fenrich, J., 2019. The ultimate qPCR experiment: Producing publication quality reproducible data the first time. *Trends Biotechnol.*, **37**: 761–774. https://doi.org/10.1016/j.tibtech.2018.12.002

Tiantian, W. and He, C., 2018. Pro-inflammatory cytokines: the link between obesity and osteoarthritis. *Cytokine Growth Factor Rev.*, **44**: 38–50. https://doi.org/10.1016/j.cytogfr.2018.10.002

Unamuno, X., Izaguirre, M., Gómez-Ambrosi, J., Rodríguez, A., Ramírez, B., Becerril, S., Valentí, V., Moncada, R., Silva, C. and Salvador, J., 2019. Increase of the adiponectin/leptin ratio in patients with obesity and type 2 diabetes after Roux-en-Y gastric bypass. *Nutrients*, **11**: 2069. https://doi.org/10.3390/nu11092069

Valaperti, A., Li, Z., Vonow-Eisenring, M. and Probst-Müller, E., 2020. Diagnostic methods for the measurement of human TNF-α in clinical laboratory. *J. Pharm. Biomed. Anal.*, **179**: 113010. https://doi.org/10.1016/j.jpba.2019.113010

Wang, Y., Meng, R.W., Kunutsor, S.K., Chowdhury, R., Yuan, J.M., Koh, W.P. and Pan, A., 2018. Plasma adiponectin levels and type 2 diabetes risk: a meta-analysis. *Sci. Rep.*, **8**: 406. https://doi.org/10.1038/s41598-017-18709-9

World Health Organization, 2024. *Diabetes*. Available at: https://www.who.int/news-room/fact-sheets/detail/diabetes (accessed 2025).

World Health Organization, 2025. *Obesity and overweight*. Available at: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (accessed 2025).

Yamamoto, S., Matsushita, Y., Nakagawa, T., Hayashi, T., Noda, M. and Mizoue, T., 2014. Circulating adiponectin levels and risk of type 2 diabetes in the Japanese. *Nutr. Diab.*, **4**: e130. https://doi.org/10.1038/nutd.2014.27

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L. and Friedman, J.M., 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature*, **372**: 425–432. https://doi.org/10.1038/372425a0

Zuo, H., Shi, Z., Yuan, B., Dai, Y., Wu, G. and Hussain, A., 2013. Association between serum leptin concentrations and insulin resistance. *PLoS One*, **8**: e54615. https://doi.org/10.1371/journal.pone.0054615