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Inverse Association Between Serum Interleukin-40 and Glycemic Status in Postmenopausal Women: A Cross-Sectional Study

Chiman Kanaan Shekho[1] and Mohammad Ahmad Hamza[2,*]

[1] *Department of Biology, College of Science, University of Zakho, Duhok, Iraq.*
[2] *Department of Chemistry, College of Science, University of Zakho, Duhok, Iraq.*

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ABSTRACT

Background: The link between interleukin-40, a newly discovered cytokine, and the increased risk of type 2 diabetes after menopause has not been previously explored.
Objectives: To assess the correlation between blood levels of IL-40 and glycemic status in post-menopausal women.
Materials and methods: In a cross-sectional study, HbA1c was employed to categorize 80 menopausal women into three groups: normoglycemic (n = 22), prediabetic (n = 22), and type 2 diabetes (n = 36). Serum IL-40 levels were measured by an enzyme-linked immunosorbent assay (ELISA) kit. Besides, hematologic tests, liver and kidney indicators, and the fundamental indices (glycemic, inflammatory, and atherogenic) were assessed. Statistical studies performed included receiver operating characteristic (ROC) analysis, group comparisons, correlation, and logistic regression.
Results: IL-40 concentrations were significantly lower in prediabetic women (P-value = 0.031) than in normoglycemic individuals. Additionally, they demonstrated a negative connection with HbA1c (r = -0.264, P-value = 0.018) and hemoglobin (r = -0.227, P-value = 0.043). For every 1 ng/mL increase in IL-40, the risks of prediabetes reduce by 16% (OR = 0.84, 95% CI: 0.73–0.97, P-value = 0.015). Although C-reactive protein (CRP) had a higher overall (AUC) area under the curve (0.841), IL-40 demonstrated acceptable discrimination (AUC = 0.726) with higher specificity (86.4%) after recoding prediabetes as the positive state. When controls were classified as positive, ROC analysis showed an inverse effect (AUC = 0.274).
Conclusion: IL-40 differentiates between normoglycemic and prediabetic women in post-menopausal women with higher specificity. It also has a negative correlation with glycemic status and hemoglobin levels. In this high-risk group, these results suggest that it may have clinical significance and diagnostic utility as a supplementary biomarker alongside CRP and fasting glucose, warranting confirmation in longer-term research.
Keywords: Biomarker; Interleukin-40; Insulin Resistance; Post-menopause; Type 2 Diabetes Mellitus.

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INTRODUCTION

A woman's aging process is significantly impacted by menopause, which also causes hormonal changes, especially a significant decrease in estrogen levels. These changes have been biologically linked to a state of systemic, low-grade inflammation and raise the risk of metabolic diseases, such as type 2 diabetes mellitus (T2DM) [1, 2]. Insulin resistance, the main pathophysiological hallmark of T2DM, is more likely to occur during this change in hormones [3]. Glycated hemoglobin (HbA1c) values can be used to periodically evaluate sustained glycemic control, which should be the goal of T2DM treatment in this population. Reducing long-term diabetic complications is closely linked to keeping its levels within the normal range [4, 5]. Higher β-cell activity, as estimated by the Homeostatic Model Assessment 2 of β-cell Function (HOMA2-B%), is linked to higher levels of inflammatory markers such as

* Corresponding author: E-mail: mohammad.hamza@uoz.edu.krd

white blood cells, lymphocytes, granulocytes, and Mixed Cell Count (MID) even in healthy women of reproductive age. This suggests that compensatory insulin secretion occurs concurrently with mild inflammation prior to the development of severe diabetes [6]. Although lifestyle and socioeconomic variables are known to increase this risk [7], research is still ongoing to determine the molecular and cellular mechanisms that lead to post-menopausal women's changes from normal glucose tolerance to detected diabetes.

One of the main risk factors for T2DM, insulin resistance, is mostly caused by chronic low-grade inflammation [8]. Pro-inflammatory cytokines, including tumor necrosis factor (TNF), interlukin-1 (IL-1), and IL-6, which can directly prevent insulin from sending signals, are mostly found in adipose tissue [9]. Because of this data, researchers are searching for new biomarkers that can help them better understand the complex immunopathology of diabetes and assess their risk of developing the condition [10]. This complex cytokine network includes the recently identified IL-1F10, commonly referred to as IL-40. Although IL-40 was first connected to two autoimmune diseases, ankylosing spondylitis and systemic lupus erythematosus [11, 12]. Its function is quickly spreading to other inflammatory diseases.

According to recent studies, IL-40 is an effective regulator of inflammatory responses and has important effects on metabolic health. Its inhibition has been shown to decrease the formation of neutrophil extracellular traps (NETosis), hence reducing multi-organ damage in severe inflammatory illnesses such as sepsis [13]. More precisely, in connection with metabolic diseases, IL-40 has been suggested as a potential biomarker that may increase the prevalence of T2DM and help in predicting the effectiveness of insulin resistance treatment [14, 15]. Its potential for diagnostic use in relation to the consequences of diabetes, including diabetic kidney disease, is also being investigated [16]. However, the precise function of IL-40 in the distinct physiological environment of post-menopause and how its expression relates to different glycemic control levels have not been previously explored.

By classifying post-menopausal women according to the American Diabetes Association's (ADA) HbA1c diagnostic criteria, the study aimed to explore the relationship between IL-40 levels and glycemic status. Due to conflicting information about the role of IL-40 in diabetes and the lack of data in post-menopausal women, individuals who are more susceptible to glucose dysregulation and inflammation, a critical knowledge gap exists. In this high-risk susceptible population, we aim to determine whether IL-40 is a protective signal, a pathogenic marker, or a clinically relevant biomarker by exploring the link between this unique cytokine and a gold-standard metabolic marker.

MATERIALS AND METHODS

Study Design

The current cross-sectional study was performed at Zakho General Hospital in Zakho City, Kurdistan Region, Iraq, from October 2024 to March 2025. The Ethics Committee of the University of Zakho approved the project research (Reference: SEP2024/UOZ14). The work aligned with the ethical standards of the 1964 Helsinki Declaration and its later amendments. Informed consent was obtained from the women before enrollment.

Inclusion criteria

A total of 80 post-menopausal women aged between 50 and 75 years were recruited for this study. Menopause was confirmed for each woman who experienced at least 12 consecutive months of amenorrhea with no other obvious pathological or physiological cause and exhibited signs and symptoms consistent with post-menopausal syndrome, such as hot flashes and general weakness. Every woman had previously received a postmenopausal diagnosis from a gynecologist.

A history of T2DM and HbA1c levels were used to categorize postmenopausal women in compliance with ADA recommendations. In addition to ensuring that classification reflects long-term glycemic control, this combination method reduces the possibility of confusion caused by sudden changes in fasting glucose.

The following is how the groupings were defined:

Twenty-two normoglycemia cases: HbA1c less than 5.7% and no history of T2DM.

Twenty-two prediabetes cases: HbA1c 5.7%–6.4%, no history of T2DM.

Thirty-six T2DM cases: HbA1c $\geq$ 6.5% or history of T2DM.

Exclusion criteria

Post-menopausal women who had a history of malignancy, acute infection, thyroid disease, recent major surgery, renal impairment, or severe depression were not allowed to take part. Women who had taken drugs recreationally and women who underwent hormone replacement therapy were also eliminated from the study.

Power Analysis

A prior power analysis was calculated by G*Power (version 3.1.9.4), depending on the previous study that reported IL-40 across glycemic groups as 30, 23, and 16 pg/mL with a standard deviation (SD) of 8.4; the effect size was about 0.67 [17]. The three group comparison by one-way ANOVA yielded a statistical power of 0.99 at an alpha level of 0.05 with a total sample size of 80, which was adequate to detect the significant difference between groups.

Data Collection and Anthropometric Measurements

Participants were selected during their regular visit, and their information was recorded in a questionnaire that included: Age, height, weight, current medication, medical history, and demographic information. All women reported neither smoking nor regular exercise. The body mass index (BMI) was calculated by dividing weight (kg) by height squared (m^2). Systolic and diastolic blood pressures were recorded after ten minutes of rest using the automatic Omron blood pressure monitor.

Sample Collection and Processing

In the morning, between 9:00 and 11:00, a 12-hour fasting blood sample was drawn, 7 ml was added to the serum-separating tube, the blood was incubated for one hour at room temperature, and then centrifuged at 3000 rpm for 10 minutes (Universal 320, Hettich). Sera were stored at -70°C using Eppendorf tubes for the biochemical measurements. An additional 3 mL of blood was added to EDTA tubes for complete blood count (CBC) and HbA1c analysis.

Laboratory Analyses

Automated apparatuses were used for testing all biochemical parameters. Fasting blood sugar (FBS), total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), alanine transaminase (ALT), and aspartate transaminase (AST) were measured on a Cobas 6000 analyzer (c501 and c311 modules, Roche Diagnostics). C-peptide and C-reactive protein (CRP) were determined using electrochemiluminescence immunoassays on a Cobas e 411 analyzer. Serum IL-40 concentrations were quantified using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Sunlong, Cat. No. SL3535Hu) according to the producer's requirements. Hematological parameters for the CBC were determined using a Medonic M-series analyzer.

Calculated Indices and Formulas

Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula: LDL-C = TC - HDL-C - (TG/5). Non-HDL-C was calculated as TC - HDL-C. The Atherogenic Index of Plasma (AIP) was calculated as log10 (TG/HDL-C). Non-HDL-C was determined as Total Cholesterol -HDL-C. The LDL-C/TG ratio was also measured.

The Systemic Immune-Inflammation Index (SII) was equal to the platelet count multiplied by the neutrophil count divided by the lymphocyte count.

Indicators of Insulin resistance (HOMA2-IR), beta-cell function (HOMA2-B), and insulin sensitivity (HOMA2-S) were calculated from fasting C-peptide and glucose values using the official Oxford University calculator.

The estimated glomerular filtration rate (eGFR) was obtained according to the 2021 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation [18].

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) for Windows (IBM Corp., Armonk, NY, USA), Version 26, was used to carry out statistical analysis. The Shapiro-Wilk test was used to analyze the normality of data distribution. Normally distributed data is presented as mean ± standard deviation (SD), while non-normally distributed data are presented as median [interquartile range (IQR)]. Differences between the three glycemic groups were measured via a one-way analysis of variance (ANOVA) with Tukey's post hoc test for normally distributed variables, or the Kruskal-Wallis test for non-normally distributed variables, followed by pairwise comparisons with Bonferroni correction. Using Spearman's rank correlation for skewed data and Pearson's correlation for normal data, correlations between IL-40 and other parameters were examined. To explore how IL-40 and other factors related to the ordered glycemic categories, a binary logistic regression analysis was performed. Moreover, the discrimination ability of IL-40 was analyzed using receiver operating characteristic (ROC) curve analysis in glycemic states, and the AUC was calculated. The Youden's Index was utilized to determine the optimal cutoff values. All tests were statistically significance when the two-tailed P-value was less than 0.05.

RESULTS

Clinical and anthropometric measurements

There were no significant differences in age, BMI, and diastolic blood pressure (DBP) among the three groups. Systolic blood pressure (SBP) increased significantly in the prediabetic, and diabetic groups compared to the normoglycemic group (P-value = 0.002). Glycemic status indicators, including FBS, HbA1c, HOMA2-R, and HOMA2-B, showed significant differences (P-value < 0.05). In addition, TG, AIP, and LDL-C/TG levels were elevated in the diabetic group compared to both the normoglycemic and prediabetic groups (P-value < 0.01 for all). No differences were found in TC, LDL-C, non-HDL-C, and HDL-C levels across the three groups. The diabetic and prediabetic groups exhibited significantly higher levels of CRP compared to the normoglycemic (P-value = 0.001). Similarly, the median Basic Inflammatory Indices (SII) was significantly higher in the prediabetic group than in the normoglycemic group (P-value = 0.037). The analysis of the primary biomarker of interest, IL-40, revealed significant differences across the three glycemic groups (P-value =0.031). At 8.98 ng/mL, the median IL-40 concentration in the normoglycemic group was notably greater than that of the prediabetic group (3.74 ng/mL). Moreover, correlation analyses across the entire cohort showed a substantially significant negative relationship between serum IL-40 and HbA1c (r = -0.264, P-value = 0.018). Similarly, a noticeable negative association was also observed between IL-40 and Hb levels (r = -0.227, P-value = 0.043). No other significant correlations were found between IL-40 and the other measured parameters. A negative correlation was detected between IL-40 and CRP; however, it did not reach significance (r = -0.212, P-value = 0.059) as shown in Table 1.

Hematological markers

White blood cell (WBC), granulocyte (GRA), and red blood cell (RBC) counts were markedly higher in the prediabetic and diabetic groups. Hb and hematocrit (HCT%) rose significantly in the diabetic group compared with the normoglycemic group (P-value = 0.01). Individuals in the prediabetic group exhibit a markedly elevated median RDW% when compared with the control group, and a significant decrease in median total number of intermediate white blood cells (MID) percent in the diabetic group, in contrast to the control (P-value < 0.05) as indicated in Table 2.

Predictive value of IL-40 for glycemic status

IL-40 was a significant predictor of prediabetes; each 1 ng/mL increase in IL-40 lowered the odds of being prediabetic by about 16% (OR=0.84, 95%CI 0.73–0.97, P-value = 0.015). The model fit the data well (Hosmer–Lemeshow p=0.685) and accounted for approximately 21-28% of the variance in glycemic status. Overall, it accurately classified 73% of cases (64% of normoglycemic and 82% of prediabetic participants), as demonstrated in Table 3.

The diagnostic capabilities for determining the accuracy of diagnosing IL-40, CRP, and fasting glucose in identifying patients with pre-diabetes were examined using a ROC curve (Table 4, Figures 1 and 2). ROC analysis demonstrated that CRP was the strongest discriminator of prediabetes (AUC = 0.841, 95% CI: 0.724–0.958, P-value = 0.001), with an optimal cutoff of ≥2.32 mg/L (sensitivity 81.8%, specificity 72.7%). Fasting glucose also showed good discriminatory ability (AUC = 0.742, 95% CI: 0.595–0.888, P-value = 0.006), with an optimal cutoff of ≥101.65 mg/dL (sensitivity 90.9%, specificity 54.5%). IL-40 was a statistically significant predictor (P-value = 0.010), but its association was inverse: when controls were coded as the positive state, the ROC curve appeared below

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Table 1. Characteristics and biochemical parameters (Including IL-40) of postmenopausal women grouped according to American Diabetes Association diagnostic criteria*.

Category	Variable	Normoglycemia (n=22)	Pre-diabetic (n=22)	Diabetic (n=36)	P-value
Demographic, Anthropometric, Glycemic and Insulin Profile	Age (years)	57(9)	60(13)	61.5(14)	0.313^{K}
	Residence (Urban/rural)	(13/9)	(18/4)	(25/11)	0.257^{S}
	BMI (kg/m^2)	27.86(5.07)	28.08(3.15)	29.29(4.39)	0.406^{K}
	Diabetes (yes/no)	(0/22)	(0/22)	(34/2)	0.001^{*S}
	FBS (mg/dl)	$99.8(40.1)^{B}$	$111.9(16.9)^{C}$	$130.7(49.7)^{B,C}$	0.001^{*K}
	HbA1c (%)	$5.5(0.3)^{A,B}$	$5.9(0.4)^{A,C}$	$6.8(1.2)^{B,C}$	0.001^{*K}
	C-peptide (ng/ml)	2.77(1.42)	3.21(1.7)	3.11(2.61)	0.427^{K}
	HOMA2-IR	$2.09(1.14)^{B}$	2.51(1.33)	$2.81(2.10)^{B}$	0.036^{*K}
	HOMA2-B	$121.5(140.8)^{B}$	$120.8(40.7)^{C}$	$82.5(67.6)^{B,C}$	0.001^{*K}
	HOMA2-S	$47.8(24.6)^{B}$	39.8(20.3)	$35.6(27.9)^{B}$	0.036^{*K}
	Diabetic medication (yes/no)	(0/22)	(0/22)	(25/11)	0.001^{*S}
	HTN (yes/no)	(5/17)	(12/10)	(26/10)	0.001^{*S}
	HTN medication (yes/no)	(3/19)	(9/13)	(20/16)	0.007^{*S}
	SBP (mmHg)	$130(20)^{A,B}$	$145(40)^{A}$	$145(20)^{B}$	0.002^{*K}
	DBP (mmHg)	80(13)	80(5)	80(18)	0.119^{K}
Novel Biomarkers	Interleukin 40 (ng/ml)	$8.98(14.87)^{A}$	$3.74(4.63)^{A}$	6(7.88)	0.031^{*K}
Basic Inflammatory Indices	SII	$394.3(237.9)^{A}$	$529.6(166.7)^{A}$	470.4(308.7)	0.037^{*K}
	CRP (mg/l)	$1.99(1.44)^{A,B}$	$3.52(3.93)^{A}$	$5.16(6.76)^{B}$	0.001^{*K}
Lipid Profile and Atherogenic Indice	AIP	$0.43(0.21)^{B}$	$0.40(0.12)^{C}$	$0.62(0.44)^{B,C}$	0.003^{*K}
	Non-HDL-C	146.5±24.5	140.1±42.9	168.08±44.5	0.226^{N}
	TG (mg/dl)	$121.7(44.5)^{B}$	$131(45.3)^{C}$	$178.1(128.9)^{B,C}$	0.002^{*K}
	LDL-C/TG	$0.99(0.46)^{B}$	$0.85(0.54)^{C}$	$0.70(0.42)^{B,C}$	0.004^{*K}
	TC (mg/dl)	196.1±27.6	192.2±45.6	205.1±42.7	0.454^{N}
	HDL-C (mg/dl)	48.7(13)	50.9(16)	45.6(16.5)	0.203^{K}
	LDL-C (mg/dl)	122.2±23.8	113.9±39.7	115.8±35.7	0.694^{N}
Renal and Liver Function Tests	ALT (U/L)	15.7(8.48)	14.7(10.09)	13.2(13.2)	0.874^{K}
	AST (U/L)	19.4(4.35)	16.9(5.18)	18.3(7.9)	0.460^{K}
	Urea (mg/dl)	23.7(8.6)	26.05(7.05)	27(8.9)	0.046^{*K}
	Creatinine (mg/dl)	0.70(0.28)	0.66(0.21)	0.73(0.23)	0.104^{K}
	Uric acid (mg/dl)	4.15(1.41)	4.35(1.85)	4.55(1.23)	0.364^{K}
	eGFR (mL/min/1.73m^2)	95.6(28.5)	103.02(24.2)	91.4(22.2)	0.104^{K}

* HTN: Hypertension, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, BMI: Body mass index, TC: Total cholesterol, HDL-C: High density lipoprotein-cholesterol, LDL-C: Low density lipoprotein-cholesterol, FBS: Fasting blood sugar, HbA1c: Glycated hemoglobin, TG: Triglycerides, HOMA2-IR, HOMA2-B, HOMA2-S homeostasis model assessment for insulin resistance, beta-cell function and insulin sensitivity, SII: Systemic-immune inflammation Index, CRP: C-reactive protein, AIP: Atherogenic index of plasma, Non-HDL: Non-high density lipoprotein-cholesterol, Non HDL: Ratio of non-HDL to HDL cholesterol, ALT: Alanine transaminase, AST: Aspartate transaminase, GFR: Glomerular filtration rate, Data are presented as mean ± SD or as median (interquartile range). N: A one-way ANOVA, K: Kruskal–Wallis test. s: Chi-square test. A: significant between Normal vs. pre-DM, B: Significant between Normal vs. Diabetes, C: Significant between Pre-DM vs DM.*: Significant differences (P-value < 0.05).

the diagonal (AUC = 0.274, 95% CI: 0.116–0.432). After recoding prediabetes as the positive state, the AUC was 0.726 (95% CI: 0.568–0.884), with an optimal cutoff of ≥7.81 ng/mL (sensitivity 63.6%, specificity 86.4%).

DISCUSSION

This study investigated the relation between serum IL-40 levels and glycemic status in post-menopausal women, a population at heightened risk for T2DM due to hormonal and inflammatory changes. The primary and most significant finding is the substantial negative association between serum IL-40 concentrations and worsening glycemic status. We observed that higher levels of this novel cytokine were characteristic of normoglycemia, with a progressive, significant reduction in individuals with prediabetes and a non-significant decrease of approximately 33% in those with overt T2DM. In this high-risk group, this finding suggests that IL-40 may be a possible marker of a healthy metabolic state rather than a diagnostic of illness. A protective or counter-regulatory signal in glucose homeostasis may be the cause of the reduced IL-40 levels observed in prediabetes. Regression analysis also revealed that the probability of developing prediabetes was considerably decreased for every unit increase in IL-40, confirming the strength of this link.

Our findings align with a recent study by Abdulraheem et al. [17], which also showed that individuals with T2DM had decreased serum levels of IL-40. Our findings, however, contrast with those of Abed and Muttaleb [15] and Nussrat and

Table 2. Comparing complete blood count parameters among groups based on to the American Diabetes Association diagnostic criteria*.

Variable	Normoglycemia(n=22)	Pre-diabetic(n=22)	Diabetic (n=36)	P-value
Hb (g/dl)	$12.9(1.3)^{B}$	$13.65(2.3)^{C}$	$13.9(1.5)^{B,C}$	0.002^{*K}
RBC (10^{12}/I)	$4.43(0.60)^{A,B}$	$4.75(0.65)^{A}$	$4.98(0.58)^{B}$	0.001^{*K}
WBC (10^{9}/I)	$6.02\pm1.32^{A,B}$	7.97 ± 2.22^{A}	8.13 ± 2.14^{B}	0.001^{*N}
PLT (10^{9}/I)	240(78)	257(90)	262.5(119)	0.275^{K}
HCT (%)	$37.5(3.8)^{B}$	$38.3(7.9)^{C}$	$40(4.8)^{B,C}$	0.002^{*K}
MID (10^{9}/I)	0.4(0.3)	0.5(0.2)	0.48(0.3)	0.672^{K}
MID (%)	$7.7(2.5)^{B}$	6.8(1.3)	$6.16(3.1)^{B}$	0.003^{*K}
GRA (10^{9}/L)	$3.7(1.5)^{A,B}$	$4.9(1.5)^{A}$	$4.6(2.2)^{B}$	0.001^{*K}
GRA (%)	56.8 ± 8.08	60.5 ± 8.31	61.06 ± 7.66	0.133^{N}
LYM (10^{9}/L)	2.13 ± 0.55	2.46 ± 0.81	2.56 ± 0.71	0.079^{N}
LYM (%)	35.5 ± 7.04	32.5 ± 7.38	32.9 ± 7.44	0.330^{N}
MCV (fl)	83.5(5.4)	81.1(9.5)	82.8(6.3)	0.301^{K}
MCH (pg)	29.2(1.8)	28.9(4.02)	28.7(1.65)	0.307^{K}
MPV (fl)	9.5 ± 0.93	9.5 ± 1.01	9.5 ± 0.90	0.982^{N}
MCHC (g/dl)	34.8(1.3)	34.9(1.9)	34.6(2.4)	0.432^{K}
RDW (%)	$11(1.2)^{A}$	$12.05(1.8)^{A}$	11.4(1.9)	0.032^{*K}
PCT (%)	0.23(0.06)	0.24(0.08)	0.24(0.08)	0.261^{K}

* Hb: Hemoglobin, RBC: Red blood cell, WBC: White blood cell, PLT: Platelet count, HCT: Hematocrit, MID: Total number of intermediate white blood cells, which include lymphocytes and monocytes, GRA: Total number of granulocytes, LYM: Total number of lymphocytes, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MPV: Mean platelet volume, MCHC: Mean corpuscular hemoglobin concentration, RDW: Red cell distribution width, PCT: Plateletcrit, data are presented as mean ± SD or as median (interquartile range). N: One-way ANOVA, K: Kruskal–Wallis test. A: Significant between Normal vs. pre-DM, B: Significant between Normal vs. DM, C*: Significant between Pre-DM vs. DM *: Significant differences (P-value < 0.05).

Table 3. Binary logistic regression of IL-40 predicting prediabetes in postmenopausal women*.

Predictor	B	SE	Wald (df=1)	P value	OR (95% CI)
Constant	1.282	0.566	5.13	0.024	3.60
IL 40	-0.172	0.071	5.87	0.015	0.84 (0.73-0.97)

* B = Regression Coefficient; S.E. = Standard Error; Wald: Wald Chi-Square statistics, df: Degrees of freedom, OR = Odds Ratio; CI = Confidence Interval. Significant P-value < 0.05.

Table 4. Receiver operating characteristic (ROC) curve analysis of interleukin-40 compared to CRP and fasting blood sugar*.

Test Variable	AUC (95% CI)	SE	P-value	Optimal Cutoff	Sensitivity (%)	Specificity (%)
IL-40 (ng/mL)	0.274 (0.116–0.432)* 0.726 (0.568–0.884)¥	0.081	0.010	≥ 7.81	63.6	86.4
CRP (mg/L)	0.841 (0.724–0.958)	0.060	0.001	≥ 2.32	81.8	72.7
FBS (mg/dL)	0.742 (0.595–0.888)	0.075	0.006	≥ 101.65	90.9	54.5

* a. Under the nonparametric assumption.
b. Null hypothesis: True area = 0.5.
* Raw AUC when controls were coded as the positive state (inverse association).
¥ Corrected AUC After recoding prediabetes as the positive state, the AUC was 0.726, reflecting its true discriminatory ability. IL-40: Interleukin-40, CRP: C-reactive protein, FBS: Fasting blood sugar.

Ad'hiah [14], who reported upregulation and notably higher levels of IL-40 in T2DM patients. While not strictly a defect, this visible symmetry provides important information on the situation-specific biology of IL-40. Our sample consisted entirely of postmenopausal women, whereas the populations in previous studies were more heterogeneous. The decline in estrogen levels and low-grade inflammation, characteristics of this group [1], could imply changes in the IL-40 expression and functions.

Detecting the negative correlation between IL-40 and the HbA1C enhances the understanding of the performance of this interleukin beyond simply group-level differences, because the HbA1C is the most accurate parameter for glycemic profile and long-term consequences associated with diabetes [4, 19]. Furthermore, our findings revealed a new negative correlation between IL-40 and Hb levels. This counts as a new observa-

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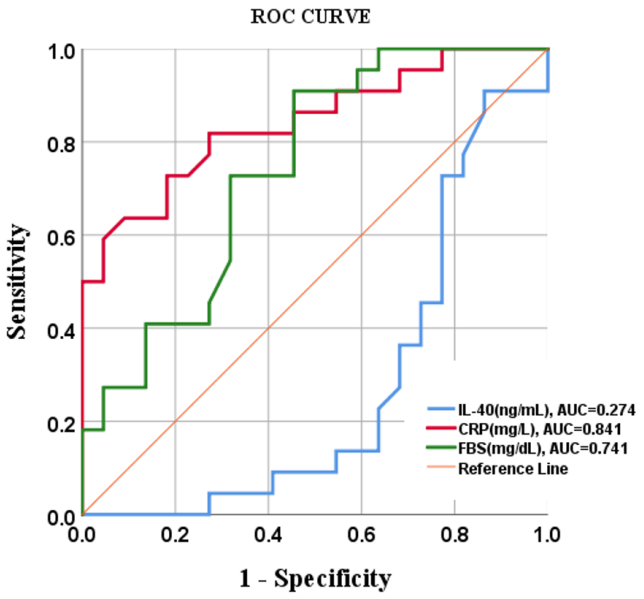

Figure 1. Receiver operating characteristic (ROC) curves for IL-40, CRP, and fasting glucose with controls coded as the positive state. Interleukin-40 (IL-40) appears below the diagonal (AUC = 0.274), reflecting its inverse association with prediabetes.

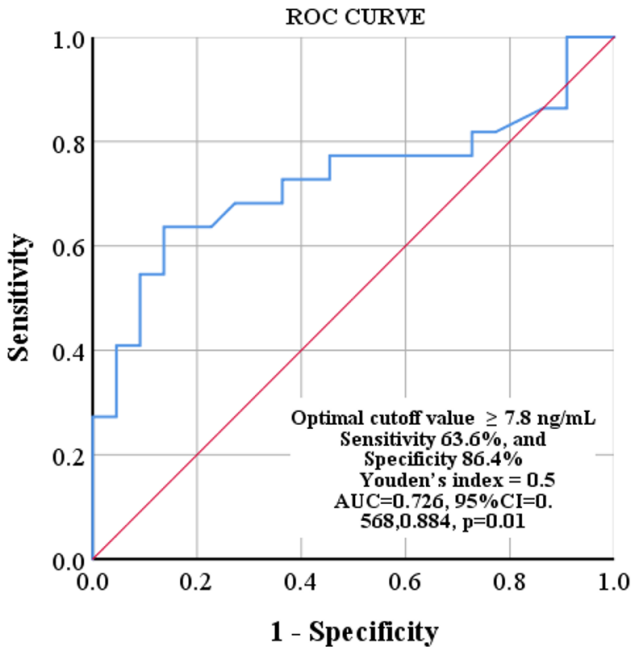

Figure 2. Corrected receiver operating characteristic (ROC) curve for Interleukin-40 (IL 40) with prediabetes coded as the positive state. The curve rises above the diagonal (AUC = 0.726).

tion. Although anemia brought on by chronic inflammation or renal impairment has been associated with reduced Hb levels in diabetic patients [20]. The precise connection between Hb and IL-40 has not yet been documented.

Our findings revealed a significant disparity: whereas the well-known indicators of inflammation, including SII, WBC count, and CRP, all rose as glycemic status worsened, as predicted [21–23], IL-40 showed the reverse pattern. This divergence strongly implies that IL-40 functions as a counter-regulatory signal whose protective effect is diminished or defeated as metabolic disease advances, rather than acting as a typical pro-inflammatory cytokine in the development of T2DM in post-menopausal women. Furthermore, these results show a negative correlation (P-value = 0.059) between CRP levels and IL-40, which may be worth further research, even though it is not statistically significant.

In our ROC curve analysis, though IL-40 by itself had a statistically significant, modest discriminatory ability with a raw AUC = 0.274 in controls that were coded as positive, the corrected AUC was 0.726 after the recoding of prediabetes as the positive state. This suggested that acceptable discrimination existed regarding well-established biomarkers such as CRP (AUC = 0.841). Clinically, IL-40 could serve as a complementary biomarker alongside CRP and FBS, enhancing the ability to discriminate by identifying at-risk individuals before overt diabetes develops. Although CRP achieved a higher AUC and stronger discrimination power, IL-40 showed greater specificity at its optimal cutoff, indicating its value in this specific group of prediabetic post-menopausal women. However, if its counter-regulatory role is confirmed in longitudinal and mechanistic studies, IL-40 could even represent a therapeutic target for modulating inflammation and glucose homeostasis in post-menopausal women.

Because of the study's design, a major limitation of our research is the inability to determine a causal relationship; we could not determine if low IL-40 is a cause or a result of T2DM. Moreover, our results require confirmation in larger, multi-ethnic, longitudinal cohorts because they are unique to postmenopausal women in a single geographic area.

CONCLUSION

The serum IL-40 levels inversely associated with Hb and HbA1c and were considerably lower in women with prediabetes than in those with normoglycemia, according to this study. As glycemic status worsened, IL-40 displayed the opposite pattern to that of standard inflammatory markers like CRP, indicating that it might represent a different regulatory route in glucose metabolism and inflammation. ROC analysis indicated fair discriminatory performance with greater specificity than CRP, highlighting its potential as a complementary biomarker rather than a standalone diagnostic tool. These findings support IL-40 as a physiologically relevant cytokine in post-menopausal women, but longitudinal and mechanistic studies are needed to clarify its prognostic value and therapeutic implications.

ETHICAL DECLARATIONS

Acknowledgments

None.

Ethics Approval and Consent to Participate

The University of Zakho's Ethics Committee granted ethical approval for the research protocol (Reference number: SEP2024/UOZ14). Every method adhered to the ethical criteria of the institutional research committee and the 1964

Helsinki Declaration and its revisions. Before being included in the study, each participant provided informed consent.

Consent for Publication

Not applicable (No personal information was published).

Availability of Data and Material

The data generated and analyzed during this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that there is no conflict of interest.

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Use of Artificial Intelligence

Artificial intelligence has been used in a limited way to correct of spelling, grammar, and punctuation, as well as in specific texts with editing.

Authors' Contributions

Hamza MA handled the conceptualization and methodology for the study, performed statistical analysis, provided supervision, managed project administration, and assisted with the final draft of the manuscript. Shekho CK was responsible for sample collection, conducting laboratory measurements, statistical analysis, and writing the original manuscript draft. Both authors read and approved the final version of the manuscript.

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