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Original Article

ASPROSIN AS A PREDICTOR OF INSULIN RESISTANCE IN NEWLY DIAGNOSED PREDIABETICS IN ASSOCIATION WITH GLYCEMIC INDICES.

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ABSTRACT

Prediabetes is a critical and sensitive stage between normal glucose levels and developing type 2 diabetes mellitus (T2DM), in which a good control can delay T2DM progression as it is closely linked to insulin resistance. The recent discovered adipokine secreted by adipose tissues, asprosin, influence glucose production and insulin sensitivity, making it a potential biomarker for insulin resistance and diabetes risk. The aim of the current study is to compare the evaluated serum asprosin levels among prediabetics and apparently healthy individuals and examine their association with glycemic indices. This case-control study was done at Duhok City, Kurdistan Region, Iraq, from October 2024 to January 2025. A total of 150 samples were collected, 75 were newly diagnosed prediabetics and 75 were apparently healthy for the control group. HbA1c%, blood sugar, insulin, and asprosin were all evaluated. Serum asprosin levels were higher in prediabetics (26.19 ± 1.49 ng/mL) compared to the control group (17.63 ± 0.51 ng/mL, $p < 0.001$). Within the prediabetic group, asprosin showed no significant variation across demographic or anthropometric variables, but it was significantly elevated in those with glucose levels between 140–199 mg/dL (21.95±1.25 ng/mL, $p = 0.011$). Moreover, asprosin was significantly higher in individuals with higher glucose levels, and strongly positively correlated with FBS, insulin, and HOMA-IR.

KEYWORDS: Asprosin, Insulin resistance, Prediabetes, Glycemic indices, Adipokines.

1. INTRODUCTION

The transition stage between normal glucose control and the development of type 2 diabetes mellitus (T2DM) is known as prediabetes (Abdullah & Salih, 2023; Ibrahim & Salih, 2022). It is a critical and sensitive stage, in which a good control can delay T2DM progression and its associated complications such as thyroid problems and cardiovascular diseases (CVDs) (Othman *et al*., 2023; Suleiman *et al*., 2023a; Zhang *et al*., 2023). Early identification and treatment are crucial because prediabetes is usually with no symptoms, so an individual may develop diabetes without noticing (Salih, 2024; Summers *et al*., 2023). Insulin resistance is the main mechanism that include prediabetes and T2DM, as it is the reduced ability of insulin to effect on cells uptake and/or utilize glucose mainly cells of muscles, liver, and adipose tissues (Shabir *et al*., 2021; Suleiman *et al*., 2023a; Summers *et al*., 2023; Zhang *et al*., 2023). If the cells are not responding, the pancreas is forced to secret more insulin, leading to hyperinsulinemia, overtime high blood glucose concentration and the onset of prediabetes (Summers *et al*., 2023; Zhang *et al*., 2023).

A recent discovered adipokine called asprosin, secreted by white adipose tissues, has gained attention to be a potential biomarker for insulin resistance (Gozel & Kilinc, 2021; Hekim *et al*., 2021). It plays a significant role in glucose homeostasis by stimulating liver to release glucose via the olfactory receptor 734 (OR734) during the fasting status to regulate energy balance (Gozel & Kilinc, 2021; Hekim *et al*., 2021; Shabir *et al*., 2021; Summers *et al*., 2023). Moreover, some studies have shown that high levels of asprosin are associated with higher waist circumference (WC) and body mass index (BMI) in which both play as a good indicator of metabolic health problems (Suleiman *et al*., 2023b; Summers *et al*., 2023; Zhang *et al*., 2023). Fasting blood sugar (FBS), Postprandial glucose, and HbA1c% are the critical parameters for the assessment of glucose metabolism and the risk of diabetes (Hekim *et al*., 2021; Summers *et al*., 2023). The recent data suggests that asprosin may influence these glycemic indices by modulating glucose production, altering insulin sensitivity, and triggering hyperglycemia, those suggesting it as a potential biomarker to identify individuals that are at risk of insulin resistance and diabetes (Gozel & Kilinc, 2021; Hekim *et al*., 2021; Shabir *et al*., 2021; Zhang *et al*., 2023). Several studies highlighted the potential of asprosin as a diagnostic tool, as they have shown that significantly higher asprosin concentrations are seen in individuals with insulin resistance compared to healthy individuals even after adjusting confounding factors such as age, gender, and BMI, hence suggesting that it can be used in with the known traditional biomarkers to improve the accuracy of detecting insulin resistance (Gozel & Kilinc, 2021; Hekim *et al*., 2021; Zhang *et al*., 2023). A study in Duhok found significantly higher serum asprosin levels in participants with metabolic syndrome compared to healthy controls, and strong positive correlations with FBS, insulin, HOMA-IR, BMI, and lipid parameters (Sulaiman, 2021). Another recent study in Baghdad found

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significant positive correlations between asprosin with BMI, FBS, and insulin levels, showing how can asprosin work as a metabolic biomarker to detect metabolic dysregulations (Ali *et al*., 2025).

The aim of the current study is to evaluate serum asprosin levels among prediabetics compared to apparently healthy individuals. Moreover, it is the first study conducted to specifically measure asprosin levels in prediabetics and its association with other glycemic indices in Kurdistan Region, filling a regional gap in biomarker research and enhancing the early detection of prediabetes at a time where it is becoming increasingly common.

2. MATERIALS AND METHODS

This case-control study was conducted at Duhok University, College of Health Science, Medical Laboratory Department. The study duration lasted from October 2024 to January 2025. A total of 150 participants from Duhok province were included in this study. Of these, 75 were newly diagnosed prediabetic patients aged 30-69 years who attended the Diabetes Unit at Azadi Teaching Hospital. The remaining 75 were healthy controls, matched for age and gender with the prediabetic group, and selected from medical staff, university employees, and other apparently healthy individuals with normal glycemic indices. Biochemical parameters measurement was performed at Vin Private Medical Laboratory. The diagnosis of prediabetes is based on the criteria of ADA, FBS between 100 mg/dL and 125 mg/dL (indicating IFG), or 2-hour plasma glucose (2-h PG) during a 75-g OGTT between 140 mg/dL and 199 mg/dL (indicating IGT), or HbA1c% levels between 5.7% and 6.4% (ADA, 2022).

Study participants were chosen by convenience, non-random sampling method. All participants completed a questionnaire form, which included personal information and a special code for each participant to ensure their privacy. As for physical activity, it was differentiated into two types, active lifestyle (150 minutes of moderate or 75 minutes of vigorous activity per week) and nonactive lifestyle (below these thresholds) (Bull *et al*., 2020). Anthropometric measurements such as height, weight, and WC were measured. As for BMI, it was calculated by using this formula: weight (kg) / (height (m)^2 (WHO, 2024).

After an overnight (12 hours) fasting, 8ml of blood was collected from the participants, then transferred to serum separation tubes and EDTA tube, labeled by their unique ID code. Serum separation tubes were applied to centrifuge to obtain serum from the blood. Serum glucose and insulin measurements were performed by Roche/Hitachi Cobas 6000. Insulin levels were measured by electrochemiluminescence immunoassay method, whereas colorimetric enzymatic principle were used for glucose estimation (Ibrahim & Salih, 2022; Sathiya & Kumar, 2024). Serum HbA1c% was measured by HPLC, based on separating hemoglobin variants through charge differences (Rathod *et al*., 2024). Asprosin was estimated by using Human Asprosin ELISA kit - BT LAB depending on the sandwich principle that utilizes two antibodies (Mishra & Chopra, 2022). As for HOMA-IR, it was determined by this calculation: HOMA-IR = [FBS (mg/dL) × Fasting Insulin (μU/mL)]/405. A level of 3 or more was identified as insulin resistance (Suleiman *et al*., 2023). Individuals experiencing the following conditions were excluded from the study: liver, kidney, or thyroid diseases, pregnancy, any systemic diseases, or use of medications such as Glucophage or Thiazolidinediones that could alter study biochemical parameters.

Statistical analysis:

Recording and data analysis was done by using Statistical Package for Social Sciences (SPSS), version 26, and normality was assessed using Shapiro-Wilk test. General data and lab results were recorded as number, percentage, means ± standard error (SE) and p-values. One way ANOVA and independent t-test were used for the comparison between the groups, and the Pearson chi-square test to determine significant association between categorical variables. Pearson Correlation was used to determine the relationship between serum asprosin levels and study biochemical parameters in prediabetics. Statistical significance was defined as a p-value (< 0.05).

3. RESULTS

The demographic and anthropometric characteristics of overall study participants are shown in Table 1. Both characteristics, age and gender, had no statistically significant difference. The Mean ± SE was (47.51 ± 1.23) in prediabetic group and (48.13 ± 1.22) in control group, prediabetic group included 38 males (50.7%) and 37 females (49.3%), while the control group included 40 males (53.3%) and 35 females (46.7%). As for anthropometric parameters, WC and BMI were significantly higher in the prediabetic group (103.12 ± 1.59) and (31.17 ± 0.56) compared to the apparently healthy group (96.44 ± 1.89) and (28.32 ± 0.52), with a p value = 0.001 and < 0.001. Additionally, physical activity levels were notably lower in the prediabetic group, where only 20% were physically active (p = 0.008).

Table 1: Demographic and anthropometric characteristics between studied groups (150 participants).

Characteristics	P rediabetics N=75 Mean ± SE, N (%)	Controls N=75 Mean ± SE, N (%)	*p* value
Gender			
Male	38 (50.7%)	40 (53.3%)	0.870
Female	37 (49.3%)	35 (46.7%)	
Age (years)	47.51 ± 1.23	48.13 ± 1.22	0.718
30-39	21 (28%)	20 (26.7%)	
40-49	21 (28%)	21 (28%)	
50-59	20 (26.7%)	21 (28%)	0.997
60-69	13 (17.3%)	13 (17.3%)	
WC (cm)	103.12 ± 1.59	96.44 ± 1.89	**0.001**
Male			
<102	16 (21.3%)	24 (32%)	0.088
≥102	22 (29.3%)	16 (21.3%)	
Female			
<88	4 (5.3%)	8 (10.6%)	0.214
≥88	33 (44%)	27 (36%)	

BMI (kg/m2)	31.17 ± 0.56	28.32 ± 0.52	**<0.001**
Normal 18.5 - 24.9	5 (6.7%)	21 (28%)	
Overweight 25 - 29.9	28 (37.3%)	25 (33.3%)	**0.002**
Obese ≥30	42 (56%)	29 (38.7%)	
Physical Activity			
Active	15 (20%)	31 (41.3%)	**0.008**
Non-active	60 (80%)	44 (58.7%)	

Statistical analysis was conducted using Pearson chi-square and independent t-test. Values in bold are considered statistically significant.

Biochemical parameters, HbA1c%, Serum insulin, HOMA-IR, and asprosin were all greater in prediabetics compared to apparently healthy individuals as shown in Table 2**.** The Mean ± SE values for prediabetics versus apparently healthy individuals were HbA1c% (5.81 ± 0.04 vs. 5.05 ± 0.05), serum insulin (18.07 ± 0.97 vs. 11.75 ± 0.68 µIU/mL), HOMA-IR (5.02 ± 0.32 vs. 2.63 ± 0.16), and asprosin (26.19 ± 1.49 vs. 17.63 ± 0.51 ng/mL), all with a p value of <0.001.

Table 2: Biochemical parameters between prediabetics and apparently healthy individuals (150 participants)

Biochemical parameters	**Prediabetics N=75 Mean ± SE N (%)**	**Controls N=75 Mean ± SE N (%)**	***p* value**
HbA1c (%)	5.81 ± 0.04	5.05 ± 0.05	**<0.001**
<5.7	23 (30.7%)	75 (100%)	
57-6.4	52 (69.3%)	0 (0%)	**<0.001**
Insulin (µu/L)	18.07 ± 0.97	11.75 ± 0.68	**<0.001**
2.6-24.9	64 (85.3%)	71 (94.7%)	
≥25	11 (14.7%)	4 (5.3%)	**0.100**
HOMA-IR	5.02 ± 0.32	2.63 ± 0.16	**<0.001**
<3.0	12 (16%)	53 (70.7%)	
≥3.0	63 (84%)	22 (29.3%)	**<0.001**
Asprosin (ng/mL)	26.19 ± 1.49	17.63 ± 0.51	**<0.001**

Statistical analysis was conducted using Pearson chi-square and independent t-test. Values in bold are considered statistically significant.

The association of Mean ± SE of asprosin with demographic and anthropometric characteristics in prediabetic participants as shown in Table 3. Asprosin levels were compared across different demographic and anthropometric groups in prediabetic participants according to gender, age, WC, BMI, and physical activity. With no statistical significance, female participants under the age of 60, those who were obese and physically inactive exhibited higher Asprosin levels.

Table 3: Association of asprosin with demographic and anthropometric characteristics in prediabetic participants (75 participants).

Characteristics	**Asprosin (ng/mL) in prediabetics N= 75**		
	N (%)	**Mean ± SE**	**p value**
Gender			
Male	38 (50.7%)	24.73 ± 1.95	0.323
Female	37 (49.3%)	27.71 ± 2.27	
Age (years)			
30-39	21 (28%)	26.89 ± 3.18	
40-49	21 (28%)	27.64 ± 3.10	0.675
50-59	20 (26.7%)	26.56 ± 2.91	
60-69	13 (17.3%)	22.20 ± 2.07	
WC (cm)			
Male			
<102	16 (21.3%)	23.98 ± 2.61	0.663
≥102	22 (29.3%)	25.75 ± 3.04	
Female			
<88	4 (5.3%)	27.49 ± 2.31	0.792
≥88	33 (44%)	29.46 ± 10.17	
BMI (kg/m2)			
Normal 18.5 - 24.9	5 (6.7%)	25.32 ± 2.38	
Overweight 25 - 29.9	28 (37.3%)	26.31 ± 8.30	0.903
Obese ≥30	42 (56%)	26.72 ± 1.98	
Physical Activity			
Active	15 (20%)	25.97 ± 1.61	
Non-active	60 (80%)	27.07 ± 3.95	0.774

Statistical analysis was conducted using an independent t-test and one-way ANOVA

Asprosin association with chosen biochemical parameters in prediabetic individuals is shown in Table 4. A statistically significant difference in asprosin levels was shown between participants grouped by glucose levels. Participants with glucose levels within 140–199 mg/dL had a higher serum asprosin concentrations 28.32 ± 2.10 ng/mL compared to those with glucose levels below 140 mg/dL had 21.95 ± 1.25 ng/mL, with a p-value of 0.011. Participants with HbA1c% in the prediabetic range (5.7–6.4%) and those with high insulin levels had a slightly higher asprosin levels.

Table 4: Association of biochemical parameters with asprosin in prediabetics (75 participants).

Characteristics	Asprosin (ng/mL) in prediabetics, N= 75		
	N (%)	Mean ± SE	p value
Glucose (mg/dL)			
<140	25 (33.3%)	21.95 ± 1.25	**0.011**
140-199	50 (66.7%)	28.32 ± 2.10	
HbA1c (%)			
<5.7	23 (30.7%)	25.21 ± 2.45	0.664
57-6.4	52 (69.3%)	26.63 ± 1.88	
Insulin (µu/L)			
2.6-24.9	64 (85.3%)	21.19 ± 1.97	0.167
≥25	11 (14.7%)	27.06 ± 1.72	
HOMA-IR			
<3.0	12 (16%)	21.14 ± 1.90	0.131
≥3.0	63 (84%)	22.16 ± 1.72	

Statistical analysis was conducted using an independent t-test. Values in bold are considered statistically significant.

Asprosin correlation with the study biochemical parameters is shown in Table 5. There is a strong and statistically significant positive correlation between asprosin levels and several metabolic markers such as FBS (r = 0.854, p < 0.001), insulin levels (r = 0.940, p < 0.001) and HOMA-IR (r = 0.947, p < 0.001). A moderate positive correlation was also found between asprosin and 2-hour post-load glucose (2h-OGT) (r = 0.359, p = 0.002).

Table 5: The correlation of Asprosin with biochemical markers in Prediabetic participants (75 participants)

Biochemical parameters and characteristics	Asprosin (ng/mL) in prediabetics N= 75	
	Pearson correlation (r)	Sig.(2-tailed)
FBS (mg/dL)	0.854	**<0.001**
2h-OGT (mg/dL)	0.359	**0.002**
HbA1c (%)	0.042	0.721
Insulin (µu/L)	0.940	**<0.001**
HOMA-IR	0.947	**<0.001**

Statistical analysis was conducted using Pearson correlation. Values in bold are considered statistically significant.

A significant very strong positive correlation was seen between Asprosin levels and FBS in prediabetic subjects, as shown in the simple scatter plot in Figure 1. The Y-axis represents Asprosin while X-axis stands for FBS. The moderate correlation of Asprosin with 2h post-load glucose levels is shown in Figure 2, where Y-axis represent Asprosin and X-axis represent 2h-OGTT, showing a moderate increase in Asprosin levels as 2h-OGTT increases.

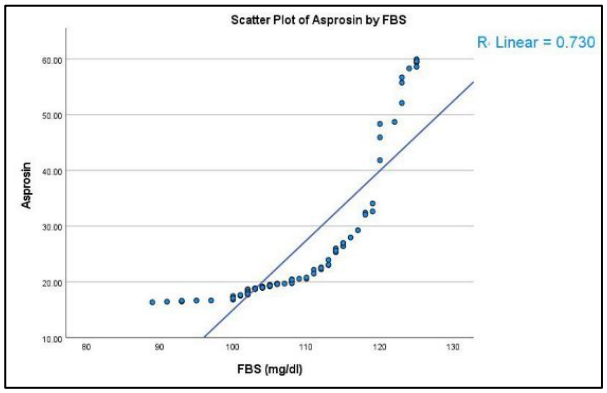

Figure 1: Serum asprosin and FBS correlation in prediabetics.

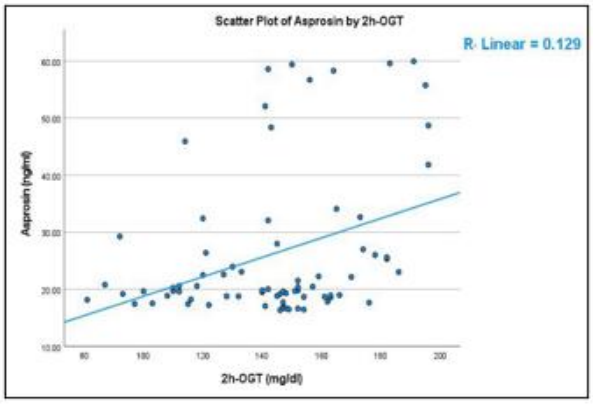

Figure 2: Serum asprosin and 2h-OGT correlation in prediabetics.

Serum asprosin had a very strong positive correlation with fasting insulin concentrations and HOMA-IR in prediabetic participants as shown in figures 3 and 4. Y-axes represent Asprosin while X-axis means Insulin levels or HOMA-IR, suggesting a close and semi constant linear relationship (r = 0.940, p < 0.001) and (r = 0.947, p < 0.001).

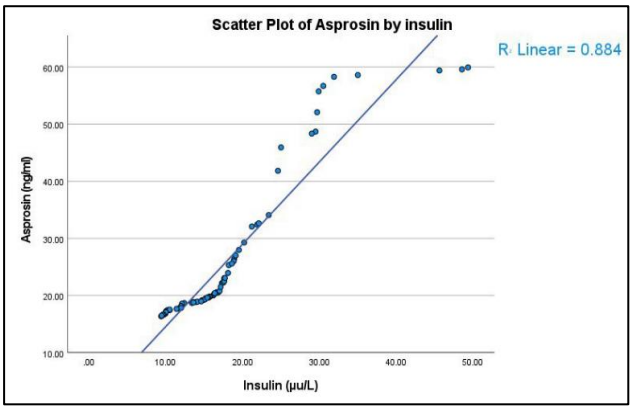

Figure 3: Serum asprosin and insulin correlation in prediabetics

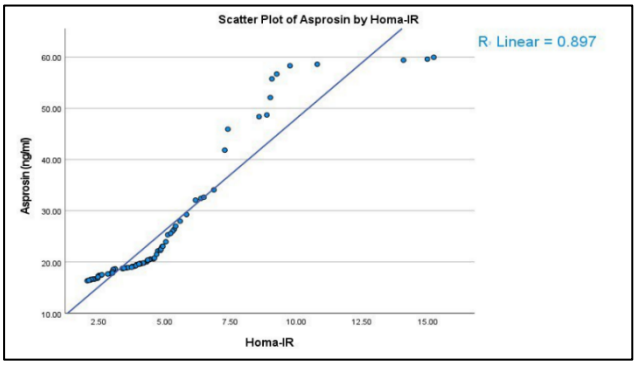

Figure 4: Serum asprosin and HOMA-IR correlation in prediabetics.

4. DISCUSSION

In this study, the comparison of demographic and anthropometric characteristics between prediabetes and apparently healthy individuals was consistent with the glycemic profile dysregulations those referring to the abnormalities in blood sugar levels. The lack of significant differences within age and gender groups minimizes the bias and enhances the reliability for comparisons between other variables. Nevertheless, the current study showed a higher prevalence of prediabetes among participants that are under the age of 60, particularly among individuals that are obese and physically non-active. BMI and WC both were significantly higher in prediabetics, as the larger proportion was classified as obese, and only a few had a normal weight compared to apparently healthy individuals. This observation was supported by another study that used data from two large cohorts in US and UK, demonstrating that both overall and abdominal obesity contributes to insulin resistance and prediabetes development (Li *et al*., 2024). Excess fat interferes with insulin signalling by triggering inflammatory cytokines, TNF-α, IL-6, and reducing adiponectin levels, which together impair insulin receptor activity and glucose uptake by cells (Garg *et al*., 2023; Tylutka *et al*, 2024). Furthermore, physical activity showed a significant difference between the groups. Lower levels of physical activity were more common in individuals with prediabetes, indicating a sedentary lifestyle. This finding is mentioned in couple clinical and health researches as regular physical activity in improves insulin sensitivity and preventing the progression to diabetes, as the reduced energy demand by muscles leads to reduced glucose uptake and utilization (Huang *et al*., 2021; Małkowska, 2024).

In terms of biochemical parameters, the distribution of HbA1c% indicated that the majority of prediabetics fell within the prediabetic range. According to ADA (2024), it reveals a chronic dysregulation in sugar levels over past 3 months and enhances the reliability of the diagnosis. Additionally, other biochemical parameters in the current study, insulin and calculated HOMA-IR means, were significantly higher in prediabetics compared to apparently healthy individuals, indicating insulin resistance. The significantly high insulin and HOMA-IR levels reflect impaired insulin sensitivity, as it has been stated in an earlier study, even after adjusting for age and BMI, that links insulin resistance to prediabetic states (Magkos *et al*., 2022). Insulin sensitivity refers to how the body response to insulin, impaired sensitivity means that a larger amount of insulin is required for glucose uptake by cells (Choi *et al*., 2022).

The novel finding in the present study is the significantly elevated serum asprosin in prediabetics compared to apparently healthy individuals. Recent studies identified asprosin as a biomarker for checking metabolic dysfunction in conditions related to insulin resistance, those elevated asprosin levels in prediabetics support its role in the pathophysiology of early glucose dysregulations (Ertuna *et al*., 2023; Farrag *et al*., 2023). Asprosin normally drops after eating carbohydrates because glucose levels would already increase and there will be no need to produce more glucose by the liver. However, in conditions such as insulin resistance, insulin signals are ineffective so the feedback to suppress asprosin levels is impaired hence asprosin levels remain elevated (Farrag *et al*., 2023; Wang *et al*., 2018). Other common factors in individuals with insulin resistance and prediabetes such as obesity is positively correlated with higher asprosin secretion and inflammatory molecules further worsen the situation (Farrag *et al*., 2023; Wang *et al*., 2018). Hence, couple of recent studies suggest that asprosin can be used as a predictor tool for prediabetics who are at risk to develop T2DM (Elnagar *et al*., 2018; Ertuna *et al*., 2023). In contrast, couple studies on children found no correlation or a negative correlation between asprosin and insulin resistance. However, this might be resulted from the difference in children metabolism compared to adults and also the complexity of applying models derived from adults on children (Corica *et al*., 2021; Long *et al*., 2019).

The analysis of serum asprosin levels with different demographic and anthropometric characteristics among prediabetics reveals a key finding. No statistically significant differences were observed across the groups examined. Similarly, other studies suggest that asprosin can be used to reflect an early metabolic dysregulation in prediabetics, but the variability in individuals asprosin levels is not significantly related to demographic and anthropometric characteristics. Rather, it is related to different factors such as genetic variation and biochemical signals to stimulate glucose synthesis (Farrag *et al*., 2023; Maylem *et al*., 2021; Wang *et al*., 2018). However, the present study revealed that asprosin levels were non-significantly higher in females compared to males and positively increases with BMI. A study found that even with matched BMI of the participants, women had significantly higher asprosin levels compared to men which usually caused by the differences of body fat distribution such as higher subcutaneous fat and hormonal changes such as estrogen levels in females (Mirr *et al*., 2023).

According to the association of biochemical parameters with asprosin in prediabetics, this study found that prediabetics with elevated glucose levels had significantly higher serum asprosin levels, Although not significantly higher asprosin levels in participants with increased HbA1c% and insulin levels. These findings are supported by previous studies that reported elevated asprosin levels in individuals with impaired glucose metabolism and significant correlations with fasting and postprandial glucose, HbA1c%, and HOMA-IR, suggesting the role of asprosin as a biomarker to detect overall glycemic changes and early metabolic dysregulation in prediabetes (Farrag *et al*., 2023; Wang *et al*., 2020).

Correlation analysis of Asprosin with biochemical markers in prediabetic participants showed a significant positive correlation especially with FBS, insulin, and the strongest link to HOMA-IR. Other studies also found significant positive correlations between serum asprosin and traditional glycemic indices, as asprosin stimulates hepatic glucose production by

activating the cAMP-PKA pathway, leading to increased gluconeogenesis and elevated FBS, additionally, asprosin impair insulin signaling pathways, which explains its strong association with HOMA-IR (Farrag *et al*., 2023; Mirr *et al*., 2023). In contrast, another study included both, adult and pediatric, found no significant correlation between circulating asprosin and FBS, HOMA-IR, or insulin (Zhang *et al*., 2024). A strong association with FBS and a moderate one with 2h post-load glucose in the current study and it suggests that asprosin reflects both fasting and postprandial glycemic status as mentioned in a previous finding (Wang *et al*., 2018).

CONCLUSION

To sum up, in the current study, significant higher levels of asprosin was seen in the prediabetic group compared to apparently healthy participants. A statistically significant association between asprosin and serum glucose concentrations was found, as individuals with a glucose level of (140-199 mg/dL) had statistically higher asprosin level than those with a glucose level of less than 140. Furthermore, asprosin had a strong positive correlation with FBS, insulin, and HOMA-IR and a moderate correlation with 2h post-load glucose levels. It is recommended that further research be conducted to determine how useful asprosin is for standard metabolic dysfunction screening.

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Ethical approval:

The ethical approval was gained from the scientific committee of College of Health Science, University of Duhok, and the Directorate of Health in Duhok governorate with the reference number (25092024-8-22) and the date (25 Sep. 2024). Each participant has voluntarily signed a written consent before participating. The study followed Helsinki and CIOMS guidelines to ensure confidentiality and voluntary participation.

Author Contributions:

The authors collectively participated in the study design and data interpretation. All authors have examined and approved the final manuscript. S.F.S. conceptualized the study and designed the methodology. H.N.S. collected and analyzed the data.

Declaration:

The authors declare no conflicts of interest related to this study.

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