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ORIGINAL RESEARCH PERIOPERATIVE MEDICINE

Clinical significances of circulating serum fetuin-A, netrin-1, and α-hydroxybutyrate levels in type 2 diabetes mellitus patients with and without hypertension

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ABSTRACT

Background & Objective: Type 2 diabetes (T2D) is a rising global health issue, with biomarkers such as fetuin-A (Fet-A), netrin-1 (NTN-1), and alpha-hydroxybutyrate (α-HB) showing potential for early diagnosis and management. These biomarkers can help predict T2D risk and understand insulin resistance (IR), emphasizing the need for further research. The current investigation evaluated the effectiveness of Fet-A, NTN-1, and, α-HB as novel biomarkers to diagnose T2D with hypertension.

Methodology: A cross-sectional study was conducted from August to December 2023, involved 60 diabetic participants, which were divided into two groups: T2D without hypertension and T2D with hypertension. A third group consisted of 30 healthy controls (HC) for comparison. Serum samples were analyzed for fasting blood glucose (FBG) using the Roche/Cobas c111 system, as well as insulin, Fet-A, NTN-1, and α-HB levels using kits for the enzyme-linked immune-sorbent assay (ELISA). Descriptive statistics were used in the statistical package for social sciences (SPSS) for data analysis.

Results: The study found significantly elevated Fet-A, NTN-1, and α-HB levels in T2D patients compared to HC, with no significant differences between T2D subgroups. Fetuin-A and α-HB showed non-significant correlations with FBG and homeostatic model assessment of IR (HOMA-IR) across all groups. NTN-1 positively correlated with FBG and HOMA-IR in T2D patients with hypertension.

Conclusions: Elevated levels of fetuin-A and netrin-1, regardless of the presence of hypertension, are suggested by the study as possible biomarkers for the diagnosis of T2D. Netrin-1's significant correlation with HOMA-IR in hypertensive T2D patients underscores its utility in assessing insulin resistance severity. Although alpha-hydroxybutyrate levels were higher in T2D patients, their non-significant correlation with FBG and HOMA-IR requires further research. These biomarkers could aid in early diagnosis and disease monitoring for T2D management.

Abbreviations: α-KB - α-ketobutyrate; ELISA - enzyme linked immune-sorbent assay; FA - fatty acids; FBG - fasting blood glucose; Fet-A - fetuin-A; HOMA-IR - homeostatic model assessment of IR; IR - insulin resistance; NAD - nicotinamide adenine dinucleotide; NADH - nicotinamide adenine dinucleotide hydrogen; ng - nanograms; NTN-1 - netrin-1; PB - peripheral blood; pg - picogram; SPSS - Statistical Package for Social Sciences; T2D - Type-2 diabetes mellitus; α-HB - alpha-hydroxybutyrate

Keywords: Diabetes, Fetuin-A, Netrin-1, α-HB, HOMA-IR.

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1. INTRODUCTION

One of the biggest health issues of our day is type-2 diabetes mellitus (T2D), whose incidence will rise by more than 50% worldwide by 2045.[1] It is linked to disturbances in protein, fat, and carbohydrate metabolism.[2] T2D has unclear risk factors, although there is a clear correlation with aging, ethnicity, overweight, obesity, and familial history of diabetes mellitus (DM).[3] Insulin resistance and malfunctioning beta (β) cells are two hallmarks of T2D. Insulin secretion first rises, maintaining normal blood glucose levels. Because of β-cell alterations brought on by the disease's development, insulin secretions cannot maintain glucose homeostasis, which results in hyperglycemia.[4]

Reactive oxygen species are produced in diabetes due to impairment of mitochondria and elevated blood sugar levels. Diabetes-related vascular issues, such as retinopathy, nephropathy, and cardiovascular diseases (CVD), are accelerated by this oxidative stress, which also harms endothelial cells and blood vessels.[5] The risk of CVD increases when diabetes and hypertension coexist, which are hallmarks of metabolic syndrome. Fifty to eighty percent of T2D patients suffer consistently high blood pressure (BP).[6] The ever increasing incidence of DM and its complications is getting worse every year, highlighting the need for an effective biomarker to aid in early identification and appropriate therapeutic management in the fight against additional comorbidities. However, biomarkers are crucial for evaluating the most effective treatment regimens. Hence, finding possible new T2D diagnostic biomarkers was the main objective of the present study.

Using secreted proteins to communicate with peripheral tissues and carry out autocrine, paracrine, and endocrine functions is a crucial method of monitoring metabolic balance. Disruption of target-tissue activity and protein synthesis is the primary cause of metabolic dysfunction, which includes insulin resistance (IR).[7] Identification, as well as understanding of the etiology of IR can be achieved with relative ease and minimal invasiveness by the quantitative detection of circulating protein biomarkers. Several parameters of metabolic homeostasis disruption, including insulin susceptibility, tolerance for glucose, circulating levels of lipids, and circulatory levels of pro- and anti-inflammatory agents, have been significantly linked to fetuin-A (Fet-A).[8] It has been noted that hypertriglyceridemia is linked to higher Fet-A levels. Thus, it is evident that Fet-A has the potential to be a new biological marker for the identification of dyslipidemia and associated metabolic diseases such as T2D,[9] strengthening the theory that high levels of circulating Fet-A are linked to IR in humans and that Fet-A is implicated in the pathophysiology of IR in T2D,[10] indicating that it might be a new mechanism in the pathogenesis of T2D.

Netrin-1 (NTN-1) has been shown in previous investigations to have pro-angiogenic, anti-apoptotic, and anti-inflammatory qualities. In addition, it has been linked to the migration of leukocytes in peripheral organs, tissue regeneration, and the management of inflammatory-based diseases.[11] The specific signaling mechanism responsible for mediating the beneficial outcomes of NTN-1, as well as the impact of NTN-1 on macrophage in cases of acute pancreatitis, has not yet been established.[12] An increased risk for glycemic disorders is associated with NTN-1. Diminished NTN-1 concentrations have been linked to metabolic traits as a risk factor and could be a T2D prediction biomarker.[13] However, the research that has been published has not fully examined NTN-1's potential as a biomarker of diabetes or its long-term effects.

Alpha-hydroxybutyrate (α-HB) is emerging as a potential biomarker for various metabolic conditions, including T2D.[14] Research suggests that levels of α-HB are inversely related to insulin sensitivity. In individuals with IR, α-HB levels tend to be higher.[15] Elevated levels of α-HB have been observed in individuals with T2D. This could be due to increased lipolysis and ketogenesis seen in diabetes, which results in higher circulating levels of ketone bodies including α-HB.[16] Studies have explored α-HB as a potential biomarker for predicting the risk of developing T2D. Elevated levels may precede the clinical diagnosis of diabetes, offering a window for early intervention and preventive measures. Alpha-hydroxybutyrate measurements can provide insights into metabolic health beyond traditional glucose and lipid biomarkers. They may help in monitoring response to interventions such as diet and exercise, and in assessing the effectiveness of treatments aimed at improving insulin sensitivity.[17] According to this, α-HB shows promise as a biomarker for T2D due to its association with IR and metabolic dysregulation. To completely understand its function and clinical relevance in diabetes diagnosis and prediction, more research is required. Thus, the purpose of the current investigation was to determine if Fet-A, NTN-1, and α-HB, as novel biomarkers, are useful in the diagnosis of T2D (with and

without hypertension), and whether the severity of the disease affects the levels of these biomarkers.

2. METHODOLOGY

2.1. Study design/Subjects

A cross-sectional investigation was carried out at Al-Nasiriya Teaching Hospital and Al-Rifae Teaching Hospital in Thi-Qar province, Iraq, from August 2023 to December 2023. The study involved three groups: the first group consisted of 30 individuals with T2D who did not have hypertension, with an equal number of men and women. The second group included 30 individuals with T2D who also had hypertension, again with an equal distribution of males and females. The blood pressure was measured using a computerized patient monitor made by UTAS Technologies in Slovakia.[18] The third group was the HC group, comprising 30 individuals without T2D or hypertension, equally divided between the sexes. All subjects in the study were aged between 34 and 56 years.

The patients with the following criteria were excluded; people who had had a blood transfusion in the last six months, cancer patients, people with chronic inflammation or auto-immune diseases, people with other types of DM, individuals who had medications that could change the biochemical parameters within 24 h of the blood collection, women who were pregnant or breastfeeding, people who had been on corticosteroid therapy for four weeks or more, and people who were on biological agent therapy. Participants in this study were those who satisfied the following criteria; patients with T2D who had not eaten or drank anything in the last 12 h and did not meet any of the above excluded criteria.

For the HC group, people without a family history of any type of diabetes, non-smokers, non-pregnant or breastfeeding (if the sample is from a woman), with no infection, not had a blood transfusion or surgery, or taken any biological agent.

2.2. Sample collection

From each participant 3-5 mL of peripheral blood was collected by venipuncture. The blood was allowed to clot at room temperature in a gel vacuum tube, and the serum was separated after 10 min of centrifugation at 3600 xg. If not utilized immediately, the gathered sera were cryopreserved at −80 °C for later examination.

2.3. Biochemical Assays

The Roche/cobas c111 system was utilized to measure the serum levels of fasting blood glucose (FBG). The test procedure was carried out in accordance with the manufacturer's instructions. The results were reported in milligrams (mg) per deciliters (dL).

Human insulin was detected and titrated in the micro-international unit (μIU)/mL using an insulin ELISA kit (Elabscience, United State of Americans (USA)) which was based on sandwich-ELISA technology as a method. The formula listed below was used to determine the HOMA-IR:[19]

$$\text{HOMA-IR} = \text{fasting insulin (μIU)} \times \text{FBG (mg/dl)}/405$$

Serum Fet-A, NTN-1, and α-HB levels were measured in nanograms (ng)/mL, picogram (pg)/mL, and micromoles (μmol)/L, respectively. Enzyme linked immunosorbent assay kit from Elabscience (USA) was used to measure Fet-A and NTN-1, and α-HB was measured using a kit from Mybiosource (San Diego, CA, USA). In this case, the Sandwich-ELISA method was used, and the assay procedure was carried out at Al-Rifae Teaching Hospital in Thi-Qar governorate, according to the instructions in the manual.

2.4. Statistical analysis

The data analysis and visualization were done using SPSS (version 22). Descriptive statistics were used for means, frequencies, and standard deviation. The Chi-Square statistical test was used to compare categorical data. The study used a one-way analysis of variance to determine the statistical significance of differences in the means of continuously dispersed, normally distributed data. Non-normally distributed variables were tested using the Kruskal-Wallis test. The correlation coefficients (r) for simple linear regression were also calculated to examine the relationships between the variables. A $P < 0.05$ was considered to be statistically significant.

3. RESULTS

From August 2023 to December 2023, three study groups participated in the present research, ranging in age from 34 to 56 years.

The T2D patients with and without hypertension exhibited significantly ($P < 0.05$) elevated Fet-A levels, 60% and 73.3%, respectively, compared to the HC group (6.7%). There was no statistically significant difference ($P > 0.05$) between T2D patients with and without hypertension. The highest mean Fet-A level was observed in T2D without hypertension (9.66 ± 7.74 ng/mL) and T2D with hypertension (5.52 ± 3.28 ng/mL) with significant differences ($P < 0.05$) compared to the control group (2.02 ± 1.44 ng/mL). In addition, the difference in the mean Fet-A value between T2D patients with and without hypertension was not statistically significant ($P > 0.05$) (Table 1).

Table 1: The result of fetuin-A levels in the three study groups

Biomarker		G1 (n = 30)	G2 (n = 30)	G3 (n = 30)	Total (n = 90)
⸸Fet-A (ng/mL) (FR%)	Below normal (< 2)	3 (10)	3 (10)	14 (46.7)	20 (22.2)
	Normal (2-4)	5 (16.7)	9 (30)	14 (46.7)	28 (31.1)
	Above normal (> 4)	22 (73.3)	18 (60)	2 (6.7)	42 (46.7)
Mean ± SD		9.66 ± 7.74	5.52 ± 3.28	2.02 ± 1.44	4.83 ± 3.77
P-values: G1 × G2 > 0.05; G1 × G3 < 0.05*; G2 × G3 < 0.05*					

Abbreviations: *G1: T2D without hypertension, G2: T2D with hypertension, G3: control group, Fet-A - Fetuin-A; FR - frequency; *significant differences; ng - nanogram; × - indicates comparison.*

⸸The Fet-A scales were calculated according to the findings of the HC group (G3).

Table 2: The result of netrin-1 in the three study groups

Biomarker		G1 (n = 30)	G2 (n = 30)	G3 (n = 30)	Total (n = 90)
⸸ NTN-1 (pg/mL) (FR%)	Below normal (< 5)	0 (0)	0 (0)	3 (10)	3 (3.3)
	Normal (5-15)	12 (40)	15 (50)	20 (66.7)	47 (52.2)
	Above normal (> 15)	18 (60)	15 (50)	7 (23.3)	40 (44.4)
Mean ± SD		30.01 ± 25.88	28.49 ± 20.38	11.14 ± 6.99	18.11 ± 14.25
P-values: G1 x G2 > 0.05; G1 x G3 < 0.05*; G2 x G3 < 0.05*					

Abbreviations: *G1: T2D without hypertension, G2: T2D with hypertension, G3: control group, NTN-1 - netrin-1; FR - frequency; *significant differences; pg - picogram; × - indicates comparison.*

⸸The NTN-1 scales were calculated according to the findings of the HC group.

Table 3: The results of alpha-hydroxybutyrate in the three study groups

Biomarker		G1 (n = 30)	G2 (n = 30)	G3 (n = 30)	Total (n = 90)
⸸α-HB (µmol/L) (FR%)	Below normal (< 300)	0 (0)	4 (13.3)	29 (96.7)	33 (36.7)
	Normal (300-400)	23 (76.7)	19 (63.3)	1 (3.3)	43 (47.8)
	Above normal (> 400)	7 (23.3)	7 (23.3)	0 (0)	14 (15.6)
Mean ± SD		377.8 ± 32.34	397.35 ± 51.8	206 ± 30.21	320.49 ± 87.35
P-values: G1 x G2 > 0.05; G1 x G3 < 0.05*; G2 x G3 < 0.05*					

*Abbreviations: G1: T2D without hypertension, G2: T2D with hypertension, G3: control group, α-HB - alpha-hydroxybutyrate; FR - frequency; *significant differences; µmol - micromole; × - indicates comparison.*

⸸The α-HB scales were calculated according to the findings of the HC group.

NTN-1 levels were significantly higher among T2D patients without and with hypertension groups compared to the HC group (P < 0.05). There was no significant variation between the T2D groups (P > 0.05) (Table 2).

The frequency of lower than normal α-HB levels was significantly (P < 0.05) lower in the T2D groups without and with hypertension (0% and 13.3%, respectively) compared to the control group (96.7%). There was no significant difference (P > 0.05) in between the two T2D groups. The mean titer of α-HB was significantly higher in T2D with hypertension and T2D without hypertension compared to HC (P < 0.05). There was no significant disparity between the T2D patients with or without hypertension (P > 0.05) (Table 3).

In T2D without hypertension and HC groups, there was a minor negative correlation between Fet-A level and

FBG level with no significant differences (r = −0.03, P = 0.76 and r = −0.007, P = 0.65). For T2D with hypertension group, there was a positive association between Fet-A and FBG levels with no significant differences (r = 0.06, P = 0.68) (Figure 1-A). The results of the present study identified a non-significant positive correlation (r = 0.06, P = 0.16 and r = 0.09, P = 0.08) between serum Fet-A and HOMA-IR levels in T2D with hypertension and HC groups, respectively. However, the results of T2D without hypertension group showed the opposite without a significant difference (r = -0.08, P = 0.63) (Figure 1-B).

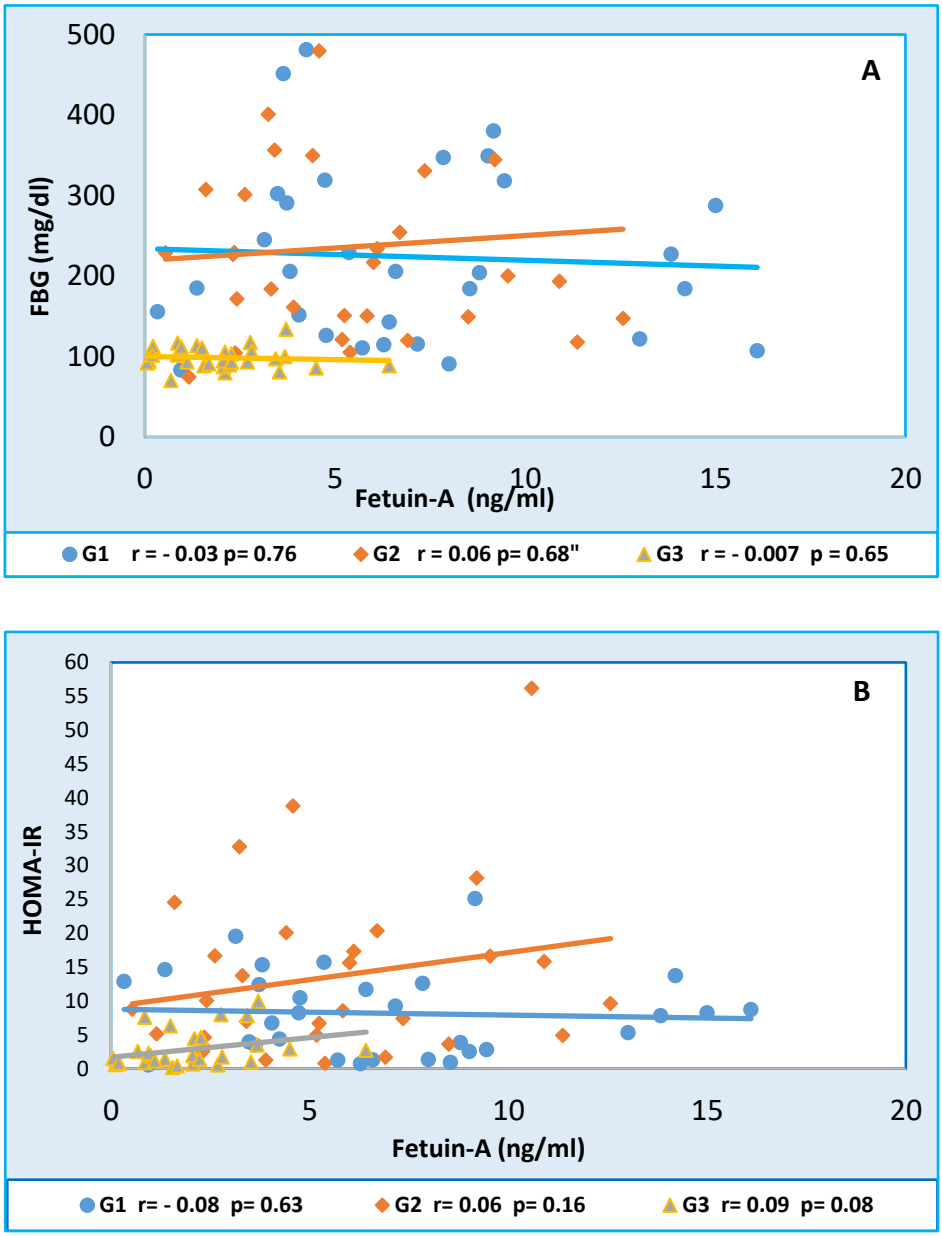

Figure 1: The correlation between fetuin-A and fasting blood glucose (A) and homeostatic model assessment of insulin resistance (B) G1: T2D without hypertension, G2: T2D with hypertension, G3: control group).

For T2D with hypertension group, significantly increased FBG levels were associated with elevated NTN-1 levels (r = 0.038, P = 0.0003) (Figure 2-A). Regarding T2D without hypertension group, the same results profile was documented in the same figure, but without significant differences (r = 0.1, P = 0.07). However, the HC group revealed an inverse non-significant (r = −0.01, P = 0.52) association between serum NTN-1 and FBG levels (Figure 2-A). The results of regression analysis (Figure 2-B) showed that there was a positive significant (r = 0.56, P ≤ 0.00001) relationship between NTN-1 level and HOMA-IR level in T2D with hypertension group. The same was true for T2D without hypertension and HC groups, but without significant difference (r = 0.03, P = 0.3 and r = 0.01, P = 0.58, respectively).

The levels of FBG and α-HB exhibited a minor positive correlation in the T2D with and without hypertension, but the differences were not statistically significant (r = 0.06, P = 0.16 and r = 0.008, P = 0.63, respectively). For the HC group, the results showed a poor negative correlation between the two parameters without significant differences (r = −0.02., P = 0.44) (Figure 3-A). Regression analysis results revealed a negative non-significant relationship between serum α-HB and HOMA-IR in T2D groups with and without hypertension (r = −0.001, P = 0.85 and r = −0.017, P = 0.48, respectively). For the HC group, the result revealed a positive non-significant (r = 0.12, P = 0.057) relationship between both biomarkers (Figure 3-B).

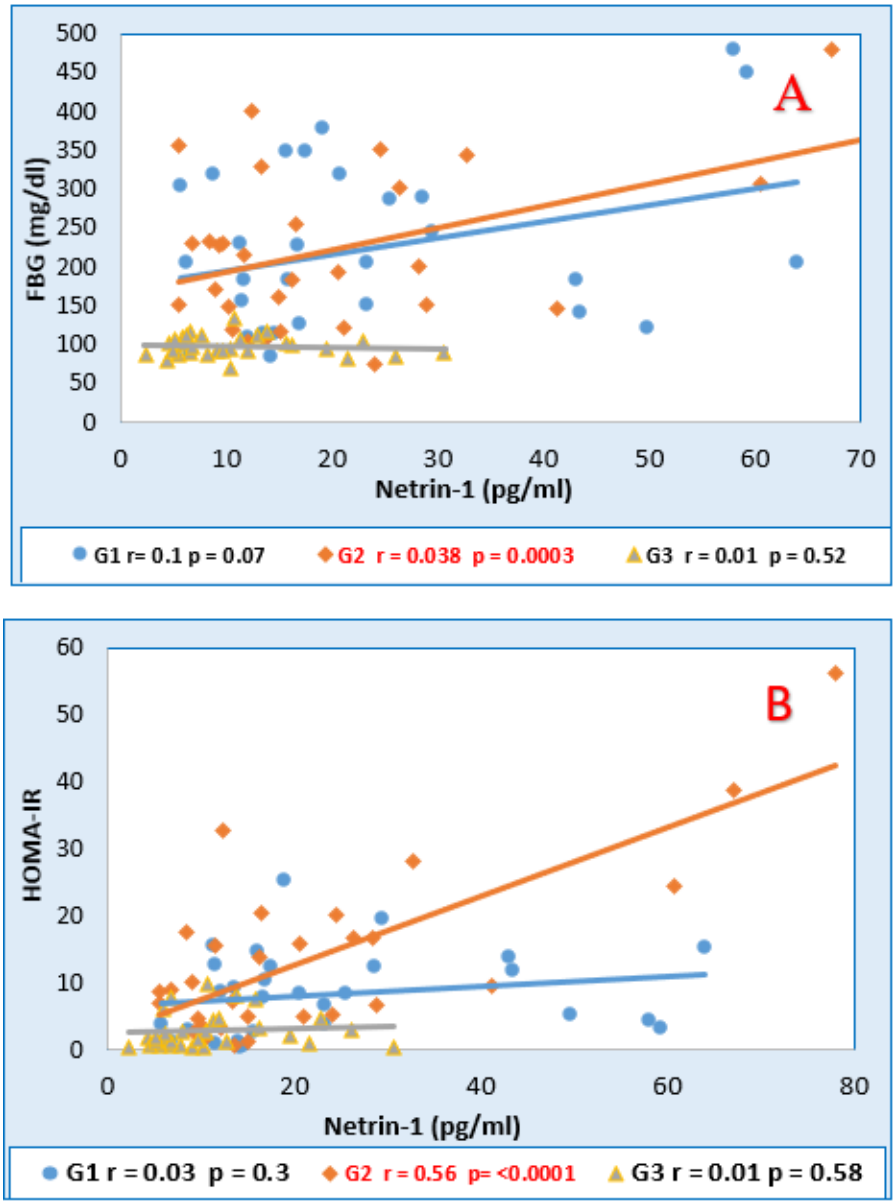

Figure 2: The correlation between netrin-1 and fasting blood glucose (A) and homeostatic model assessment of insulin resistance (B) (G1: T2D without hypertension, G2: T2D with hypertension, G3: control group).

4. DISCUSSION

Hepatokines, which are proteins released by the liver that may have effects in other parts of the body, are becoming recognized as important factors in the development of IR in individuals with T2D.[20] Research has linked this hepatokine to various metabolic issues such as diabetes and obesity.[21] A previous study has found that individual levels of Fet-A are more likely to develop diabetes.[22] Guo et al., showed a link between an increased blood level of Fet-A and a higher risk of T2D.[23] In line with these observations, the current study revealed a significantly higher level of Fet-A among both groups of T2D than the HC group (Table 1). A case-cohort research found a strong correlation between elevated levels of Fet-A and an increased risk of incident T2D.[24] Although the exact mechanism by which higher Fet-A levels occur in T2D is unknown, human Fet-A inhibits insulin receptor activation. Fetuin-A was found to prevent insulin-induced protein kinase B activation and glucose transporter 4 translocation in mouse muscle cells. This hepatokine disrupts the insulin signaling pathway's downstream phosphorylation but does not affect insulin binding to the alpha receptor subunit.[25] These findings suggest that T2D occurrence could be predicted from Fet-A. Additional research is required to fully evaluate the correlation between Fet-A and various vascular problems in individuals with T2D.

Netrin-1, a protein, has shown attributes that inhibit inflammation, induce tissue regeneration, and regulate the immune system. Despite its significant role in the inflammation, and in the progression of IR and T2D,[26] there is less knowledge on the potential link between serum NTN-1 and T2D. Serum NTN-1 levels considerably varied between study groups, and there were significant changes between prediabetic and recently diagnosed T2D, according to a cross-sectional study published in an Egyptian research report.[27] According to a study by Yimer et al. in persons with T2D or impaired fasting glucose, compared to the HC group, plasma NTN-1 levels were considerably higher.[28] In line with these findings, the current investigation revealed that both T2D groups had noticeably higher levels of NTN-1 than the HC group (Table 2). The exact explanation for the rise in NTN-1 levels in individuals with T2D remains uncertain, however, some research potentially corroborated our findings, that NTN-1 contributes to

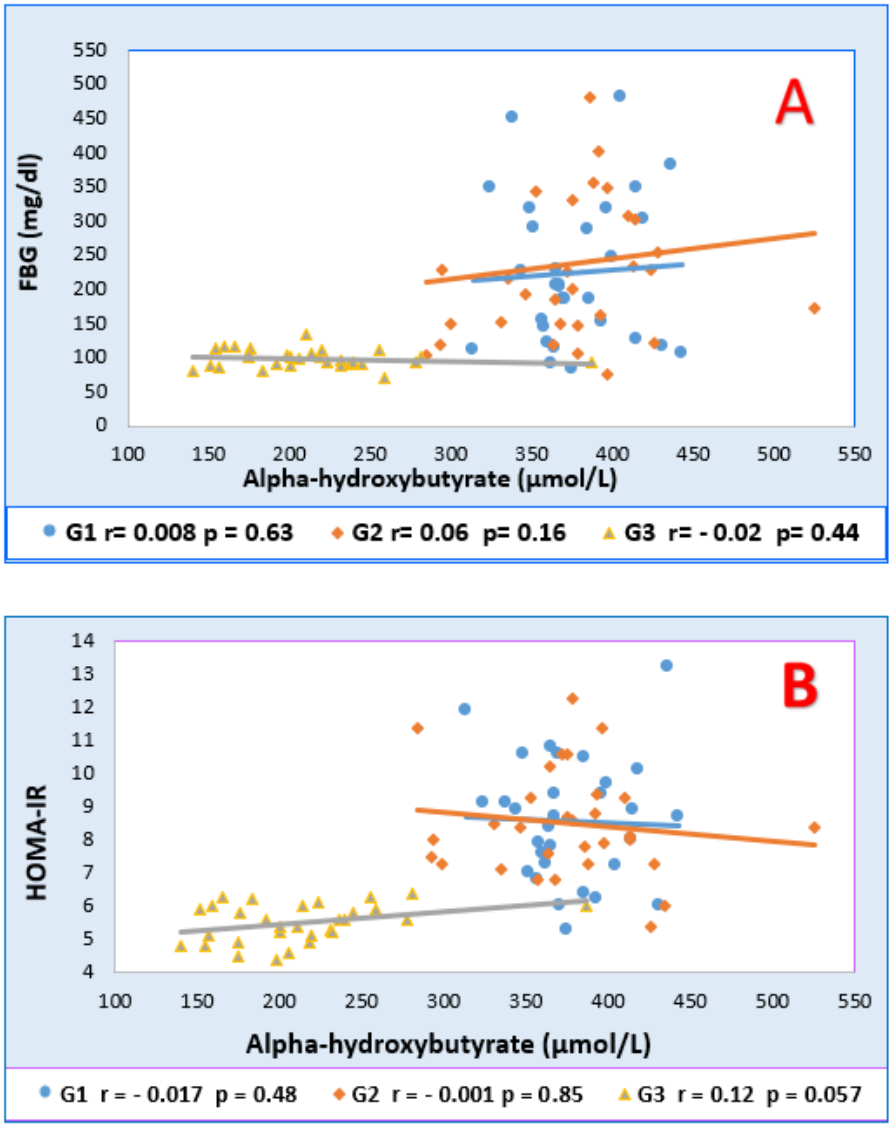

Figure 3: The correlation between alpha-hydroxybutyrate and fasting blood glucose (A) and homeostatic model assessment of insulin resistance (B) (G1: T2D without hypertension, G2: T2D with hypertension, G3: control group).

the development of the pathophysiology of T2D. Natura et al. have reported that NTN-1 is involved in an inflammatory process that may have negatively impacted insulin secretion and exacerbated β-cell dysfunction. [29] Another possible reason is that studies indicate that elevated levels of NTN-1 promote impaired storage of adipose tissue and contribute to metabolic dysfunction, characterized by enhanced chronic inflammation and IR.[30] These observations indicated that NTN-1 has the potential to serve as a biomarker and has therapeutic implications in T2D.

The organic acid α-HB tends to identify an increased risk of impaired glycemic control and the progression from prediabetic to a clinically apparent diabetic condition.[31] Multiple studies have examined the significance of α-HB, as a predictive biomarker for changes in blood sugar levels in both human and animal models of T2D.[32, 33] Metabolomics analysis revealed that α-HB shows potential as a predictive biomarker for gestational DM.[34] In parallel to these findings, the current study reported a significantly higher concentration of α-HB in both T2D groups compared with the HC group (Table 3). During an oral glucose tolerance test, high levels of fasting serum α-HB were linked to higher 1-hour glucose levels in subjects at increased risk of developing diabetes. This correlation may be attributed to a deceleration in the kinetics of insulin production.[35] The exact mechanism for explaining the elevated level of α-HB in the patient group is still not fully understood, but there are a few pieces of evidence that can be useful to explain these results, one pathologic characteristic commonly observed in IR is increased lipid oxidation in the liver.[36] Elevated nicotinamide adenine dinucleotide hydrogen (NADH)/nicotinamide adenine dinucleotide $(NAD)^+$ levels, which may arise from elevated fatty acid (FA) oxidation in IR, can affect the α-ketobutyrate (α-KB) metabolic pathway, diverting it away from Krebs cycle oxidation and towards the production of α-HB.[37] Increased free FA is associated with α-HB, indicating an elevated NADH/NAD ratio that promoted the conversion of α-KB to α-HB.[38] Elevated levels of α-HB are indicative of metabolic overload, characterized by a higher ratio of NADH to NAD^+ and impaired glucose metabolism. This phenomenon is observed in both IR and the initial stages of dysglycemia.[39]

The current study's findings on Fet-A levels and their relationship with FBG and HOMA-IR in T2D patients, both with and without hypertension, offer intriguing insights. In T2D patients without hypertension and HC, a minor negative correlation between Fet-A and FBG was observed, though this was not statistically significant. On the other hand, a weakly positive relationship between Fet-A and FBG levels was reported in T2D patients with hypertension, also lacking statistical significance (Figure 1-A). Modern research has shown conflicting findings about the relationship between Fet-A and glycemic management. For instance, a study by Ix et al. showed that in older individuals, high Fet-A levels were linked to IR and incident diabetes.[40] Fetuin-A and FBG levels showed a strong positive association in this investigation, indicating that Fet-A may be involved in glucose metabolism and the etiology of DM. Moreover, in T2D patients with hypertension and HC, our investigation found a non-significant positive correlation between serum Fet-A and HOMA-IR, whereas T2D patients without hypertension exhibited a non-significant negative correlation (Figure 1-B). This pattern aligns with findings by Stefan et al.[41] who reported that increased Fet-A levels were associated with higher HOMA-IR values, indicating higher IR. However, their study emphasized a significant relationship, unlike the non-significant trends observed in the present research. Moreover, a recent study by Reinehr et al. investigated how Fet-A affected IR in teenagers and obese children, reporting a positive relationship between HOMA-IR and serum Fet-A, supporting the hypothesis that Fet-A contributes to IR.[42] The minor, non-significant associations in our study could be attributed to different population characteristics, sample sizes, or the presence of hypertension, which might modulate the relationship between Fet-A and IR. In summary, while our findings reveal non-significant correlations between Fet-A and both FBG and HOMA-IR levels across different groups, existing literature generally supports a significant positive association, particularly in populations with varying metabolic conditions. Further research with larger sample sizes and diverse cohorts is essential to elucidate the precise role of Fet-A in glucose metabolism and IR in T2D patients.

For individuals with T2D and hypertension, the study results show a notable positive association between FBG levels and NTN-1 levels. Conversely, the T2D group without hypertension exhibited a similar trend, albeit not statistically significant. In HC, an inverse non-significant relationship was observed between serum NTN-1 and FBG levels (Figure 2-A). These findings are partially consistent with earlier research on NTN-1's function in metabolic diseases. For instance, according to a study by Yimer et al., blood NTN-1 levels are high in T2D patients,[28] which may suggest that NTN-1 is involved in IR and glucose metabolism, which may indicate a role for NTN-1 in IR and glucose metabolism. Additionally, this study showed that T2D patients with complications including hypertension had higher levels of NTN-1, supporting the current findings that NTN-1 is positively correlated with FBG in the hypertensive T2D subgroup. However, the lack of significant association in the non-hypertensive T2D group and the inverse trend in HC warrant further exploration. This could be due to differing pathophysiological mechanisms governing NTN-1 regulation in the presence of hypertension versus its absence. Additionally, the non-significant inverse relationship in HC suggests that NTN-1 might play a distinct role in non-diabetic populations, possibly related to its known functions in inflammation and tissue repair. The regression analysis further underscores the complex interplay between NTN-1 and IR, as measured by HOMA-IR. A considerable positive correlation was seen in the hypertensive T2D group between NTN-1 and HOMA-IR (Figure 2-B). This aligns with findings by Al-Shakour et al., who reported that NTN-1 exacerbates IR through inflammatory pathways, particularly in the presence of comorbidities like hypertension.[43] The non-significant associations in the non-hypertensive T2D and HC groups suggest that NTN-1's impact on IR may be modulated by additional factors present in hypertensive patients. Moreover, these findings resonate with the work of Yimer et al., who found that elevated NTN-1 levels were associated with increased IR and worse metabolic profiles in T2D patients.[28] They proposed that NTN-1 could serve as a biomarker for identifying patients at higher risk of developing complications, particularly in those with coexisting hypertension. The current study's findings are in line with previous literature highlighting the significant association between elevated NTN-1 levels and adverse metabolic outcomes in T2D, particularly in the context of hypertension. The diverse relationship reported across different subgroups highlights the need for more investigation to clarify the fundamental processes and possible therapeutic consequences of aiming at NTN-1 in the management of T2D.

The current study's regression analysis reveals intriguing patterns in the relationship between α-HB, FBG, and HOMA-IR across different groups. In the T2D groups, with and without hypertension, the levels of α-HB and FBG exhibited a weakly positive correlation, though these correlations were not statistically significant. This trend contrasts with the HC group, where α-HB and FBG exhibited a poor negative correlation, also non-significant (Figure 3-A). Furthermore, the relationship between serum α-HB and HOMA-IR was negative and non-significant in both T2D groups. Conversely, the HC group showed a

positive but non-significant relationship between these biomarkers (Figure 3-B). These findings align with those of Jebar et al., who also noted the lack of significant correlations between α-HB and markers of glucose metabolism in T2D patients, suggesting that while α-HB is elevated in T2D, its direct relationship with glucose levels and IR might not be strong enough to be significant in statistical analyses.[44] These findings imply that α-HB might not be a reliable standalone marker for assessing glucose control or IR in these patients. The explanation for these findings is that the variability in α-HB levels could be influenced by other metabolic factors not accounted for in these studies, thereby diluting the strength of its association with FBG and HOMA-IR. Interestingly, the T2D groups showed an inverse relationship between α-HB and HOMA-IR, though non-significant, aligns with previous studies suggesting that α-HB might reflect early metabolic changes that precede significant alterations in IR. For instance, Gall et al. indicated that α-HB might be involved in the early dysregulation of lipid metabolism, which could affect insulin sensitivity indirectly over time.[45] The positive, albeit non-significant, correlation between α-HB and HOMA-IR in the HC group could suggest a different metabolic role for α-HB in non-diabetic individuals. Lotta et al. noted that in healthy individuals, α-HB might be linked to physiological variations in energy metabolism, which do not necessarily correspond to pathological IR.[46] The current study's findings highlight some trends, they underscore the complexity of α-HB's role in metabolic regulation. It is possible that α-HB is not a reliable indicator of glucose metabolism or IR on its own, based on the non-significant correlations observed in the T2D and HC groups. Additional investigation is required to clarify the multifaceted interactions between α-HB, glucose regulation, and insulin sensitivity, considering additional metabolic factors and longitudinal data to better understand these relationships.

5. LIMITATIONS

The relatively small sample size (30 participants per group). Measurement techniques for biomarkers may vary in reliability. Confounding variables, not fully accounted for, could influence outcomes. Interpretation of correlations may be limited by small effect sizes. Further studies are needed on large sample sizes to confirm this issue and the prices of these biomarkers should be considered reasonable to include them as routine work in the diagnosis of T2D.

6. CONCLUSIONS

The findings suggest that elevated Fet-A and NTN-1 levels could be potential biomarkers for predicting and diagnosing T2D, given their significant elevation in T2D patients compared to controls. The lack of substantial difference between hypertensive and non-hypertensive T2D patients indicates that these biomarkers are indicative of T2D regardless of hypertension status. A direct association was found between NTN-1 and HOMA-IR, particularly in hypertensive T2D patients, underscores its potential utility in assessing IR severity. Although α-HB levels were higher in T2D patients, their non-significant correlation with FBG and HOMA-IR denotes that more investigation is required to define its function. These biomarkers could aid in early diagnosis, monitoring disease progression, and potentially guiding therapeutic strategies for T2D management.

7. Data availability

The numerical data generated during this research is available with the authors.

8. Conflict of interest

The study utilized the hospital resources only, and no external or industry funding was involved.

9. Ethical Consideration

The Training and Human Development Unit, Thi-Qar Health Department, Ministry of Health, Iraq, granted complete consent for the current study. On July 27, 2023, the research committee decision (number 153/2023) approved this request in compliance with the Helsinki Declaration. The authors obtained each participant's written permission to conduct this study in accordance with international research ethics standards.

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11. Authors contribution

HAAK: worked together on the manuscript's conception, composition, data analysis, assessment, submission, revisions, and final proofreading.

HSS: Data collection, writing, and analysis

AMJ: The originator of the concept and data collection.

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