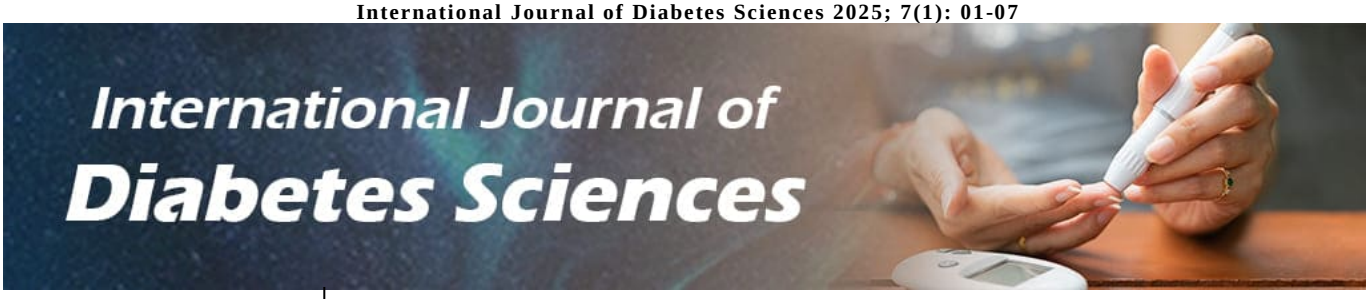

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Study of the relationship between Afamin and insulin resistance with type2 diabetes and fatty liver patients in Thi-Qar governorate

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Abstract
Background & Objective: The Insulin resistance (IR) and compromised with beta cell (β-cell) function are traits of diabetes type 2. It has been discovered that among the risk factors for NAFLD is type 2 diabetes (T2DM) development on its own. Compared to people without diabetes, Individuals who have type 2 diabetes have 80% more fatty livers.
Simple steatosis to the more advanced type known as non-alcoholic steatohepatitis are all included in the spectrum of NAFLD, or non-alcoholic fatty liver disease. Metabolic disorders like obesity, diabetes, insulin resistance, and dyslipidemia are linked to non-alcoholic fatty liver disease (NAFLD). Regarding Afamin is a significant factor in people and a sign of having fatty liver or non-alcoholic fatty liver disease in diabetics.
Methodology: August through October 2024, 80 diabetic patients participated in a case control study. They were split into two groups based on ultrasound diagnoses: T2D free of NAFLD (non-alcoholic fatty liver disease) and T2D with NAFLD. For comparison, 40 healthy controls made up a third group. Determine age the Bio-Rad D-10 HPLC analyzer (Bio-Rad Laboratories, USA) was used to detect the amount of glycated hemoglobin (HbA1C) in serum samples, insulin resistance, and afamin (AFM) levels using ELISA kits. The atomic absorption spectrophotometer was used to measure the lipid profile (TC, TG, VLDL, LDL, and HDL). Descriptive statistics were used to examine the data using the statistical program for social sciences (SPSS).
Results: The study found significantly elevated (AFM) levels in T2D patients in comparison to NAFLD, with significant differences between T2D subgroups. This suggests that type 2 diabetes and fatty liver disease may have an impact on the relationship between age, lipid profile, and afamin levels. Afamin claims that this implies that fatty liver disease and type 2 diabetes may have an impact on the relationship between HbA1c levels and AFAMIN levels. The exhibits a little negative correlation with insulin resistance.
Conclusions: Elevated levels of afamin (AFM), regardless of the study suggests the existence of non-alcoholic fatty liver as a potential biomarker for T2D diagnosis substantial association between insulins and afamin (AFM) in patients with non-alcoholic fatty liver disease (T2D). In people with type 2 diabetes, NAFLD prevalence was surprisingly high. Age, lipid profile, insulin resistance, and high HbA1c have no bearing on the study. These biomarkers may aid in early diagnosis and disease monitoring for the treatment of type 2 diabetes.
Abbreviations: (AFM) afamin enzyme-linked immune sorbent assay for FA fatty acid, or HbA1C glycated hemoglobin PSS Statistical Package for Social Sciences IR insulin resistance picogram Diabetes mellitus type 2 (NAFLD) Fatty liver without alcohol.

Keywords: Diabetes, afamin, insulin resistance

Introduction
The inability of the body to produce or react to endocrine signals and so maintain appropriate blood sugar (glucose) levels is a hallmark of diabetes mellitus, a macromolecule metabolism disease (Ahmad *et al.*, 2021). A group of metabolic diseases known as diabetes mellitus are characterized caused by consistently elevated blood sugar levels and varied degrees of protein, fat, and and carbohydrates. Although there are several causes of diabetes mellitus, issues with the generation of insulin by the pancreas are always one of them (Al-Musawi, Al-Lami, and Al-Saadi 2021). More than type 2 diabetes, or T2DM, affects 400 million individuals globally. By 2040,

more than 640 million people will have diabetes globally. The prevalence of type 2 diabetes is expected to quadruple over the next 20 years due to factors including obesity, aging, and the number of high-risk ethnic groups in the population (Marín-Peñalver *et al.* 2016) [13]. Medical treatment's shortcomings and restrictions (ADA, 2019). Blood purification from insulin is dependent on insulin receptors and degradation enzymes. Pathophysiology of Diabetes mellitus type 2 the pathophysiology of the disease states that a malfunction in the feedback loops between insulin action and secretion causes abnormally high blood glucose level. When β-cells malfunction, insulin production decreases, restricting the body's ability to keep glucose levels within normal ranges. On the other hand, IR increases the liver's synthesis of glucose while decreasing its absorption in muscle, liver, and adipose tissue. β-cell dysfunction is frequently more severe than IR, even though it happens early in the pathophysiology and contributes to the course of the disease. IR and β-cell dysfunction can exacerbate hyperglycemia and hasten the progression of type 2 diabetes (Galicia-Garcia *et al.* 2020). Fatty liver was originally described by Addison in 1836. For decades afterward, pathologists identified the parallels between the alterations in liver histology shown in diabetics and people with severe obesity and those observed in alcoholics. The pathologist Rokitansky noted hepatic fat accumulation in autopsy specimens in 1838, which may have been the cause of cirrhosis (Lonardo *et al.* 2020) [9]. Fat-infiltrating hepatocytes in people without an alcohol-related past consumption or competing etiologies for hepatic steatosis. Anon-alcoholic fatty liver disease (NAFLD) is the spectrum of illnesses characterized by steatosis of the liver (triglycerides). The illness's symptoms can include fatty steatosis, what is known as "silent liver disease," (Milić *et al.*, 2014) [17]. Insulin resistance, also known as impaired insulin sensitivity, is commonly characterized by decreased skeletal muscle glucose clearance, impaired hepatic regulation of glucose synthesis, and decreased adipose tissue lipolysis rates (Roden *et al.*, 2024). Because it arises when insufficient insulin production by the pancreas makes up for the deficit in insulin action, type 2 diabetes was the first clinical syndrome to be linked to insulin resistance (IR) (Zhou and Xu 2024) [37]. Insulin signaling that stimulates the process of making fatty acids and suppresses the generation of glucose in a healthy liver (Bo *et al.*, 2024). Afamin (AFM) is a new human serum protein with unique vitamin E binding characteristics that is found on chromosome 4 and belongs to the albumin family. (Li *et al.* 2022) [8]. AFM is mostly produced by the liver, although it can also be found in human cerebrospinal fluid and ovarian follicles. Age, gender, menstrual cycle, circadian rhythms, and prandial state had little effect on the levels of afamin in the blood (Abed, Farhan, and Salman 2023). The glycoprotein known as human afamin (AFM) has an apparent molecular weight of 87 kDa and 34% identity and 55% amino acid sequence similarity. In 1994, it was discovered through cloning and sequence analysis to be the fourth member of the human albumin gene family, which also contains vitamin D-binding protein (DBP), α-fetoprotein (AFP), and albumin (ALB). (Dieplinger and Dieplinger 2015) [1]. Metabolic syndrome is linked to elevated afamin levels components and insulin resistance (IR). It could be employed as a biomarker to identify aberrant glucose levels.) (Abed *et al.*, 2023). Vitamin E is a poten anti-inflammatory and antioxidant qualities t, and human plasma afamin is a particular binding protein for it. Afamin transports vitamin E across the blood-brain barrier in an appropriate cell culture mode, according to a study (Sami and Hamodat 2023)., elevated afamin levels are linked to type 2 diabetes mellitus and all of the main a metabolic syndrome characteristics, including high blood pressure, dyslipidemia, obesity, and high blood glucose. According to (Naschberger *et al.* 2017). The main source of circulating afamin is the liver. An increased risk of type 2 diabetes, non-alcoholic fatty liver disease, and hepatic insulin resistance are all linked to excessive hepatic lipid buildup. However, a decrease in serum afamin is linked to severe alcoholic liver cirrhosis, possibly as a result of compromised hepatic synthesis. The gene for afamin (Nuñez-Calonge *et al.*, 2021).

The liver is where this glycoprotein is primarily produced and released into the blood. Afamin may play a role in controlling the uptake and α-tocopherol's passage through the blood-brain barrier. Insulin resistance and other metabolic syndromes are associated with this protein characteristics, and it also participates in antiapoptotic cellular processes linked to oxidative stress. (Polkowska *et al.*, 2019).

Aim of the Study

1. Assessment of serum levels of Afamin (AFM) as new biomarkers for Compared to healthy individuals, type 2 diabetes and nonalcoholic fatty liver disease
2. Evaluation of HOMA-IR, HbA1C, in individuals with type 2 diabetes and NFLD and comparison with healthy individuals.
3. Serum lipid profiles of NFLD and type 2 diabetes patients are estimated and compared to those of healthy people.
4. Know the correlations relationship between Afamin (AFM) and each one of parameters in patients of NAFLD.

Methodology

Study design/Subjects

The trial included 120 participants, 80 of whom had type 2 diabetes and 40 of whom were purportedly healthy controls. They ranged in age from 35 to 65. This study is a case-control research carried out at the Thi-Qar Specialized Diabetes Endocrine and Metabolism Center (TDEMC), Iraq, between August and October of 2024. Three groups participated in the study: 40 people without T2D or evenly split between the sexes made up the control group. 42 T2D patients without nonalcoholic fatty liver, equally divided between men and women, made up the second group. The third group consisted of 38 T2D Men and women make up the same proportion of patients with nonalcoholic fatty liver disease.. The nonalcoholic fatty liver was assessed using ultrasonography, a widely used and inexpensive clinical technology that can help detect the exact amount of the fat accumulation inside the liver. Individuals with cancer, those who had received the blood transfusion during the previous six months, and those who did not smoke or consume alcohol were excluded from the study. Those who do not have specific liver disease etiologies, such as viral or metabolic, include those who have other forms of diabetes mellitus, chronic inflammatory or autoimmune diseases, are taking medications that may alter biochemical parameters within 24 hours of blood collection, are pregnant or nursing,

are receiving corticosteroid therapy for four weeks or longer, or are undergoing biological agent therapy. Participants in this trial required to be T2D patients who had not eaten or drunk anything within the last 12 hours and who did not fit any of the following requirements. People in the HC group had to be free of infections, have never had blood transfusions or surgery, be nonsmokers, avoid getting pregnant or nursing (if a woman provided the sample), not have a family history of diabetes of any kind, and not have taken any biological agents.

Sample collection

Five milliliters of blood from the veins were extracted from type 2 diabetic patients and controls after they had fasted for about eight hours the previous night. The blood was then transferred to a plain tube, allowed Seven milliliters were allowed to coagulate at room temperature before being centrifuged for ten minutes at 3000 rpm to extract the serum.. After that, unless they were needed immediately, the serum samples were kept at -50˚C for use in further biochemical parameter assays.

Biochemical Assays

Fasting blood glucose serum levels (lipid profile) were measured using an atomic absorption spectrophotometer. Following the manufacturer's instructions, the test procedure was conducted. Assess serum samples for glycated hemoglobin (HbA1C) utilizing Bio-Rad Laboratories' D-10 HPLC analyzer (USA).Using an ELISA kit (Elabscience), afamin was detected and titrated in micro-international units (µIU)/mL., United States of America (USA)) that was based on sandwich-ELISA technique. The results were given in milligrams (mg) per deciliter (dL). Following the guidelines in the handbook, The Thi-Qar governorate's Specialized Diabetes Endocrine and Metabolism Center served as the site for the assay procedure.

Statistical Analysis

The SPSS (version 22) was used for data analysis and visualization. Standard deviation and means are used in descriptive statistics. To ascertain the statistical significance of variations using regularly distributed, continuously scattered data, A study employed an examination of variance in one direction. Utilizing the Kruskal-Walli test to investigate variables that were not regularly distributed. To investigate the correlations between the variables, the basic linear regression's correlation coefficients (r) were also computed. The relevance of the data was defined with a p-value below 0.05.

Results

August through October of 2024, Three research groups took part in the current investigation, between the ages of 35 and 65 years.

Table (3-1) and Figure (3-1) below show the ages of patients and control. Shows as Although No statistically significant difference exists.

(P = 0.1931), the mean age off The T2D groups appears to be slightly older than that of the Control group.

Table (3-1): Age with control and patients group.

Variables	Control Group (N=40)	T2D without NAFLD (N=38)	T2D with NAFLD (N=42)	P-value
Age (years)	46.75±9.023	50.18±9.458	49.43±7.878	0.1931

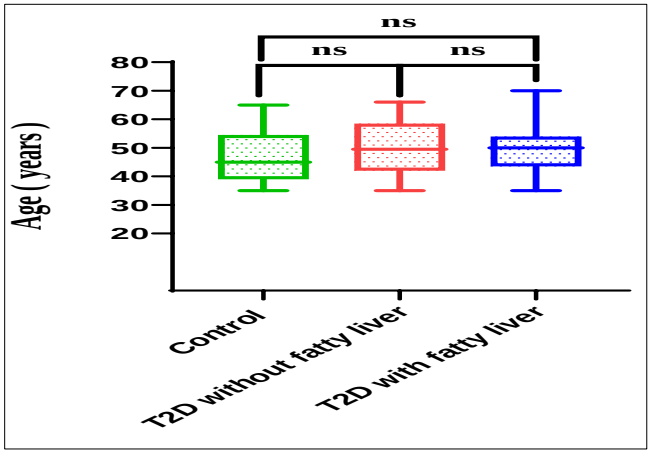

Fig (3-1): Age with control and patients group.

Control and Type 2 Diabetes Glycemic Characteristics of Patients with NAFLD and Normal Liver

Table (3-2) and figure (3- 2)) demonstrates a notable rise in the concentration of HbA1c, HOMAIR) in fatty Liver and normal liver Group in In contrast with the Control group (P ≤ 0.0001). Also, It is observing a substantial increase in the concentration of serum HOMAIR in the fatty liver groups in comparison With the normal liver group. Also, The HbA1c ratio does not differ significantly between normal and categories of fatty livers.

Table (3-2): Control and Type 2 Diabetes Glycemic Characteristics of Patients with NAFLD and Normal Liver

Parameter	Control Group No=40	T2D without NAFLD No=38	T2D with NAFLD No=42	P-value
Insulin (µIU\ml)	4.755±2.237	10.64±6.327	23.57±18.07	<0.0001
HOMA-IR	1.047±0.5608	6.013±5.774	13.84±11.52	<0.0001

A New Biomarker for Type 2 Diabetes and Control in Patients with Normal Liver and NAFLD

Table (3-3) there is a huge increase in the concentration of AFM in fatty Liver and normal liver Groups compared to the healthy group under control ($P \leq 0.0001$). Also, it is observing a noteworthy increase in the concentration of serum AFM in the fatty liver group in comparison with the normal liver group.

Table (3-3): The Serum Afamin concentrations of control and patients groups

A novel biomarker	Control Group No=40	T2D without NAFLD No=38	T2D with NAFLD No=42	p.value
Afamin (AFM) (ng\ml)	7.205±6.592	15.49±5.132	18.83±7.634	<0.0001

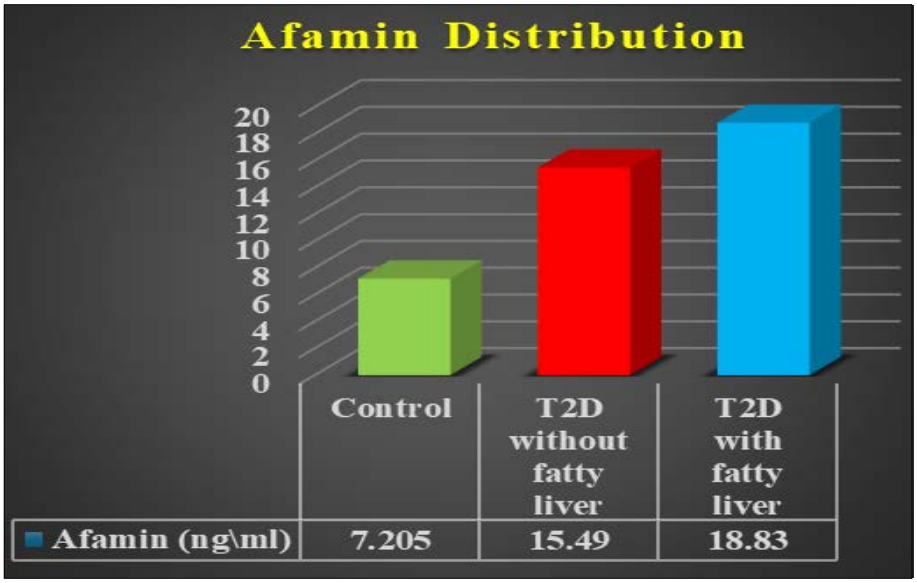

Fig (3-3): Serum Afamin concentrations of the controls and patients Groups

The Lipid profile

The Table (3-4) and Figure (3-4) shows Serum concentration has significantly increased.

TG, TC,, LDL,VLDL in fatty liver and normal liver the groups in Comparisons with the control group ($P \leq 0.0001$). There is a noticeable rise in the concentration of (TG, TC, LDL, and VLDL in group the fatty liver in comparison with the healthy liver group ($P \leq 0.0001$).

We can notes, there is a significant decrease HDL in the fatty liver and liver groups normal when compared with The Control groups ($P \leq 0.0001$). Also, additionally, there is a noteworthy decrease HDL in fatty liver patients when Compared with a Normal liver groups ($P \leq 0.0001$).

Table (3-4): The serum levels of Lipid profile acase study group

Biomarkers	Control Group (N=40)	T2D without NAFLD (N=38)	T2D with NAFLD (N=42)	P-value
TC (mg/dl)	127.9±16.93	176.1±41.64	190.5±3912	<0.0001
TG (mg/dl)	88.55±26.01	173.4±68.21	368.6±131.6	<0.0001
VLDL (mg/dl)	18.30±5.992	34.29±13.68	74.02±26.14	<0.0001
LDL (mg/dl)	59.60±20.19	104.4±43.91	108.52±5.27	<0.0001
HDL (mg/dl)	70.60±11.78	44.37±13.33	40.45±10.28	<0.0001

The Correlation between afamin and HbA1C

The association between HbA1c and AFAMIN levels in three different groups-Control, T2D without fatty liver, and T2D with fatty liver-is displayed in the Figure. In all three groups, the figure shows a weakly negative connection between AFAMIN levels and HbA1c levels. The T2D with fatty liver group seems to have the largest negative association (R = -0.09), followed by positive correlation in the T2D without fatty liver group (R = 0.018), and negative association in the control group (R = -0.01) appears to have the least correlation. This indicates that Fatty liver disease and type 2 diabetes may have an impact on the relationship between HbA1c levels and AFAMIN levels.

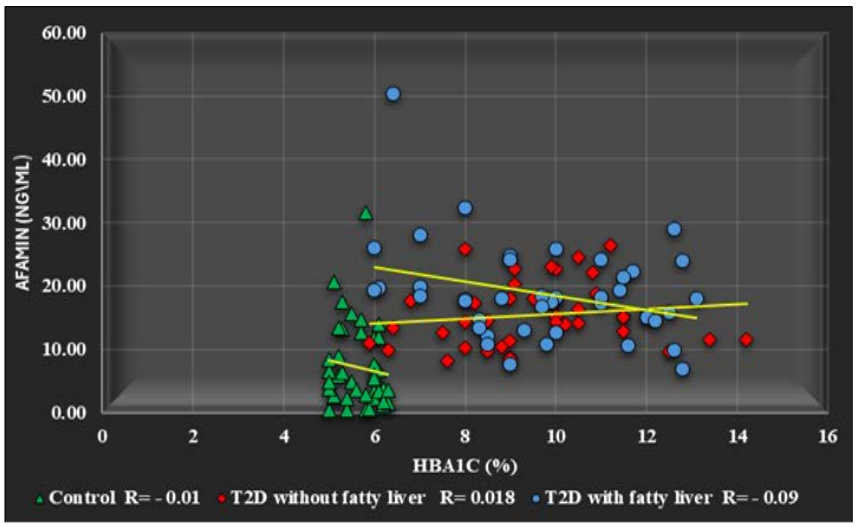

Fig (3-5): Correlation between serum Afamin and HbA1c

The Correlation between afamin with HOMA-IR

In all three groups, HOMA-IR and Afamin levels show a weakly connection. With a R = 0.0002, the control group showed the smallest connection, indicating a minimum relationship between HOMA-IR and AFAMIN levels in those without diabetes. The T2D without fatty liver group shows a significantly larger positive connection (R = 0.029), thereby showing a somewhat better relationship between HOMA-IR and AFAMIN levels within those with the type 2 diabetes but without fatty liver. Suggesting a modest relationship between HOMA-IR and AFAMIN levels in individuals with fatty liver and type 2 diabetes, the T2D with fatty liver group exhibits a little negative correlation (R = 0.016).

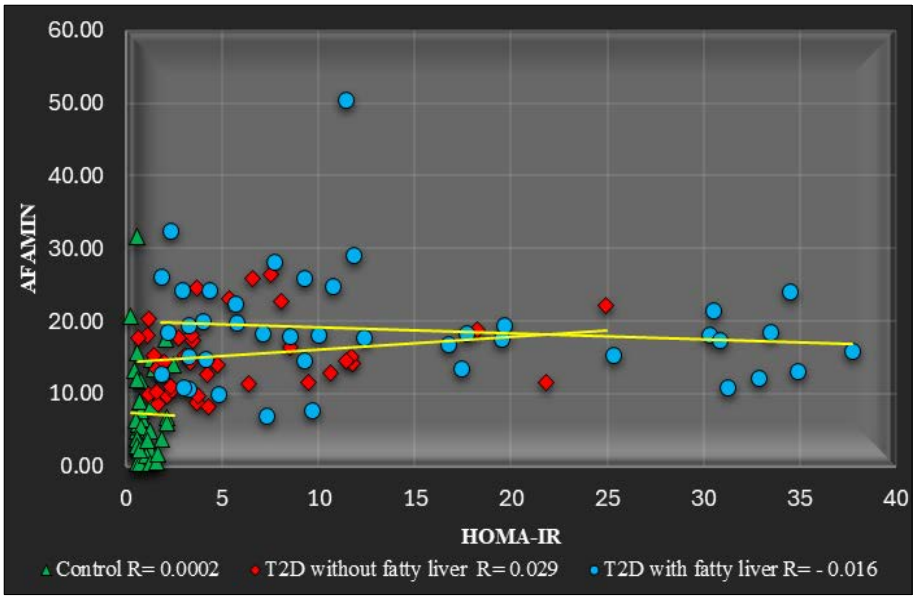

Fig (3-11): Correlation between serum Afamin and HOMA-IR

Discussion

HbA1c this result was in line with the findings of the (Lind *et al.*, 2021) investigation. Due to it indicates amore detrimental Diabetes's glycation aftereffects, include retinopathy and nephropathy., which are thought to be caused by hazardous Advanced Glycation end Products, a high HbA1c rating predicts diabetes problems (Han *et al.*, 2024) [21]. The amount of HbA1C generated is directly correlated with the length of time and frequency of elevated blood glucose levels. Aside from hyperglycemic episodes, biological variations among people can also cause variations in HbA1C (Kanwal *et al.*, 2021) [22]. NAFLD has been found to be strongly predicted by diabetes and obesity. Thus, it is reasonable to expect that people with NAFLD also have elevated HbA1C values (Masroor & Haque, 2021) [25]. The Pancreatic β cells are directly triggered by increased blood glucose levels, which causes a substantial increase in insulin secretion and, ultimately, a reduction in blood sugar levels. The capacity of the body to use pancreatic β cells is impaired under pathological situations. Insulin resistance (IR) develops as a result of this interruption in insulin secretion, which makes it difficult to promote glucose uptake and utilization. (Guo *et al.*, Y. 2024). Insulin signaling is disrupted by excessive hepatic fat accumulation, which results in increased gluconeogenesis and decreased glucose absorption. (Ha *et al.*, 2024) [21]. Higher HOMA-IR, insulin, and glucose levels were linked to increased IR risks. The relevance of pancreatic dysfunction can be seen in this.Catecholamine stress results in ion and decreased β-adrenergic glucose metabolism regulation in hepatocytes (Fujii *et al.*, 2020). Through a number of processes, overnutrition and excessive calorie intake pave the way for IR. According to, (Niranjan *et al.*, 2023). IR and hepatic fat buildup cause oxidative stress and the liver's inflammatory pathways to become active. The correlation between insulin resistance and hepatic fat that is genetically determined was limited to high danger people, indicating that insulin resistance is may be mediated by liver injury rather than being a direct result of hepatic fat accumulation (Dongiovanni *et al.*, 2018) [20]. Afamin protein transports tiny hydrophobic molecules in a manner akin to that of albumin. Although there is a significant amount of this protein in plasma, the majority of circulating plasma afamin comes from the liver (R. J. Ali & Ahmed, 2021). The liver is the primary secretor of AFM, a vitamin E-binding protein that has antioxidant qualities against associated illnesses and damage (Li *et al.*, 2022) [8]. The findings noted that the patient with T2DM had a higher significantly afamin levels. Furthermore, we found a link between afamin levels and hyperglycemia and dyslipidemia (Ardekani *et al.*, 2024). This has been shown that afamin concentration performed a major part in predicting the incidence of future metabolic problems (ALTAIEE *et al.*, 2020). Afamin is a hepatocyte that is secreted into the bloodstream and has a high level of expression in the liver. There is a favorable relationship between serum afamin levels and BMI, or body mass index. NAFLD is linked to serum afamin levels. For the diagnosis of NAFLD, we discovered that serum afamin levels function well as a diagnostic tool (S. Chen *et al.*, 2022). Regardless of established risk factors for NAFLD, Afamin predicted the onset of NAFLD (Pitkänen *et al.*, 2022). The Afamin gene caused a rise in body weight in addition to blood glucose and lipid concentrations (Abdullah *et al.*, 2024). Patients with Diabetes type 2 is more likely to develop hypercholesterolemia than people without the disease because insulin resistance and the resulting increases in Fatty acid transport to the liver induce the increase on the secretion of very low-density Lipoprotein, which in turn

turns into LDL in the blood. The cholesterol (Khil *et al.*, 2023) [24]. Because HDL-C plaies a crucial role in the movement of cholesterol from the body's cells to the living cells, there is a correlation between low HDL-C levels and high cholesterol levels in the body. (Wu *et al.*, 2023). Increased free fatty acid (FFA) stimulates the synthesis of TG, which in turn causes an increase in Apo-lipoprotein B (ApoB) secretion (Sun *et al.*, 2022). Higher susceptibility to diabetes type 2 is connected to higher triglyceride levels within the typical range (R. Guo *et al.*, 2024). These outcomes were in line with the study's findings (Hirano *et al.*, 2022).Hepatic production of apolipoprotein B (apoB) and triglyceride-rich VLDL particles is increased by insulin resistance (Vesa *et al.*, 2020). Particularly in individuals with type 2 diabetes, altered lipoprotein control might result from impaired insulin action and hyperglycemia. Individuals with elevated blood sugar levels have substantial abnormalities in lipoprotein control (Bonilha *et al.*, 2021). The table demonstrating elevated LDL-C in people with fatty liver and elevated blood sugar levels illustrates this. (Cui *et al.*, 2024) [19]. the outcomes aligned with the research findings (Yang & Xu, 2024). The Patients with type 2 diabetes maleties have notable changes in a quantity, makeup, and function of high-density lipoproteins (HDL) (Martagon *et al.*, 2024)., in addition to decreased HDL-C levels. This supports the scientific finding that people with diabetes frequently have dyslipidemia, a condition marked by an aberrant lipoprotein and plasma lipid profile and an elevated risk of cardiovascular atherosclerosis (Sullivan *et al.*, 2024) [29].

Conclusions

Poor control of glucose levels leads to increased levels of Afamin, Regardless matter whether non-alcoholic fatal liver disease or not In people with type 2 diabetes, the prevalence of NAFLD was startlingly high. High levels of HbA1c, HOMA-IR, and Lipid profile. Age has no effect on the study.

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