# Orexin-A Levels in Non-Alcoholic Fatty Liver Disease: A Case-Control Study Exploring Metabolic and Hepatic Correlations

**Original Article**

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## ABSTRACT

**Background:** Nonalcoholic fatty liver disease (NAFLD) is a global health concern, closely associated with obesity, type 2 diabetes mellitus (T2DM) and dyslipidemia. Orexin-A, a neuropeptide involved in metabolic regulation, has been implicated in NAFLD pathophysiology.

**Aim of the work:** To assess peripheral Orexin-A levels in NAFLD patients and their correlation with metabolic risk factors.

**Patients and Methods:** This case-control study included 45 participants: 15 NAFLD patients without diabetes (Group 1), 15 NAFLD patients with diabetes (Group 2), and 15 healthy controls (Group 3). Lipid profile, HbA1c, and NAFLD fibrosis score (NFS) were analyzed. A Sandwich ELISA assay was employed to determine serum Orexin-A levels.

**Results:** NAFLD patients exhibited significantly lower Orexin-A levels compared to controls (0.34 vs. 2.12 pg/mL, $P < 0.001$). Orexin-A correlated negatively with serum albumin ($r = -0.361$, $P = 0.05$) and blood urea in diabetic NAFLD patients ($r = -0.612$, $P = 0.015$). ROC analysis demonstrated that Orexin-A ≤0.66 pg/mL effectively differentiated NAFLD patients from controls (AUC = 0.877, sensitivity = 90%, specificity = 86.67%).

**Conclusion:** Orexin-A is significantly reduced in NAFLD patients, suggesting its potential role as a biomarker for NAFLD detection and disease severity assessment.

**Key Words:** Metabolic syndrome, NAFLD, orexin-A, type 2 diabetes mellitus.

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## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a prevalent liver disorder globally, strongly associated with obesity, dyslipidemia, type 2 diabetes mellitus (T2DM), and metabolic syndrome. Approximately 20% of cases progress to cirrhosis, increasing the risk of liver-specific and overall mortality[1]. NAFLD can evolve into more severe hepatic conditions, including fibrosis, cirrhosis, and hepatocellular carcinoma (HCC)[2], with poor diet and physical inactivity being primary contributing factors[3].

The hypothalamus plays a crucial role in regulating peripheral metabolism, including liver function, by releasing the neuropeptide orexin-A, also known as hypocretin-1[4,5]. Hypothalamic orexin-A exerts protective effects against hepatic endoplasmic reticulum stress and chronic inflammation in obesity via autonomic nervous system regulation, thereby influencing the pathogenesis of nonalcoholic steatohepatitis, while also modulating physical activity and autonomic function to prevent obesity[4]. Additionally, orexin-A functions as an anti-inflammatory mediator, with its serum levels showing an inverse correlation to pro-inflammatory markers like C-reactive protein[6].

Orexin-A plays a crucial role in insulin sensitivity, energy expenditure, and metabolic rate[6], while its deficiency may contribute to obesity and physical inactivity[4]. It is also considered a potential biomarker for T2DM and may protect against pancreatic cell failure[7]. Additionally, orexin-A modulates baseline and postprandial insulin secretion, exerts a direct lipogenic effect on adipose tissue, enhances insulin release in response to glucose stimulation, delays gastric emptying, and increases leptin secretion, thereby improving postprandial glycemic control and reducing baseline insulin secretion[8].

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## AIM OF THE WORK

This study aims to assess peripheral Orexin-A levels NAFLD patients, both obese and non-obese, in comparison to healthy controls. Additionally, it seeks to correlate Orexin-A levels with obesity, T2DM, severity of liver disease, and dyslipidemia.

## ETHICAL CONSIDERATION

Between August 2023 and February 2024, this case-control study was conducted at the Outpatient Clinic of the Internal Medicine Department, Ain Shams University Hospital, following approval from the ethical committee (Approval Code: FMASU MS 235/2023). Informed consent was obtained from each participant in accordance with the department's research plan. The study included 45 participants: 30 patients with NAFLD, with or without T2DM, and 15 age- and gender-matched healthy controls.

## PATIENTS AND METHODS

### Study design and population

Participants were stratified into three groups according to their clinical and laboratory profiles. Group 1 comprised 15 patients diagnosed with NAFLD in the absence of DM, Group 2 comprised 15 patients with both NAFLD and DM, and Group 3 served as the control group, consisting of 15 apparently healthy volunteers with normal transaminase levels, normal hepatic ultrasound findings, and negative serology for HBsAg and HCV-Ab.

### Eligibility criteria

Inclusion criteria were adult participants of both genders with NAFLD diagnosed by ultrasonographic evidence of a bright liver, regardless of obesity status or the presence of T2DM[9]. Exclusion criteria were participants with alcoholic liver disease or other liver diseases (e.g., viral or bilharzial), advanced organ failure, malignancies, chronic or acute conditions affecting other organs or systems, medications that could interfere with the assay, and neurological or psychiatric conditions that might impact the assay's results.

### All patients were subjected to the following

A comprehensive medical history was obtained from all patients and control participants, followed by a thorough clinical examination. Laboratory assessments were conducted for all subjects, encompassing serum transaminases (ALT, AST), complete blood count (CBC), a full lipid profile (including HDL-C, LDL-C, triglycerides, and total cholesterol), coagulation parameters, serum albumin, renal function tests (serum creatinine and blood urea), and urinalysis. Additionally, screening for viral hepatitis was performed through the detection of anti-HCV antibodies and HBsAg. Serum Orexin-A levels were determined using Sandwich ELISA technique.

### Abdominal ultrasound

To minimize inter-observer variability, hepatic steatosis (bright liver) was assessed in all participants by a single operator using a Hitachi ultrasound system with a 3.5 MHz curved probe. Patients were examined after fasting for at least eight hours in supine, right, and left lateral positions. The confirmation of fatty liver diagnosis was based on hepatic echogenicity surpassing that of the spleen and renal cortex, together with ultrasound wave attenuation, diminished diaphragm delineation, and a poorly defined intrahepatic structure.

### NAFLD-Fibrosis score

The NAFLD Fibrosis Score (NFS) is calculated using six variables: age (years), BMI (kg/m²), impaired fasting glucose (IFG) or diabetes, AST/ALT ratio, platelet count, and albumin (g/dL). Two cutoff points were used to determine fibrosis severity: a score >0.676 indicates the presence of significant fibrosis (F3-F4) with a positive predictive value of 90%, while a score < -1.455 predicts the absence of significant fibrosis (F0-F2) with a negative predictive value of 93%. Scores between -1.455 and 0.675 are considered indeterminate[10].

### Statistical methods

SPSS software (version 27, IBM, Armonk, New York, USA) was used for statistical analysis and data management. Data were described as mean ± standard deviation (SD) for quantitative (numerical) variables and as median & interquartile range (IQR) (categorical) variables. Non-parametric data were compared using Kruskal-Wallis test followed by Mann-Whitney post-hoc test for pair-wise analysis. Kolmogorov-Smirnov Z test was used for comparing the test to control groups (pair-wise analysis). Parametric data were compared using one-way ANOVA test followed by LSD post-hoc test for pair-wise analysis. Correlation of non-parametric data was done using the spearman’s correlation test. All statistical tests were two-sided. Statistical significance was defined as a *p-value* below 0.05.

## RESULTS

No substantial difference was identified between the control and cases groups regarding sex distribution ($P = 0.502$) or mean age ($P = 0.073$).

The cases group had significantly elevated HbA1c ($P = 0.001$), cholesterol ($P < 0.001$), triglycerides ($P < 0.001$), LDL ($P < 0.001$), BMI ($P < 0.001$), NFS ($P < 0.001$). Conversely, HDL ($P < 0.001$) and Orexin A levels ($P < 0.001$) were significantly lower in cases. Ultrasound results revealed that 100% of cases exhibited a bright liver, whereas none of the controls displayed this finding ($P < 0.001$). (Table 1).

**Table 1:** Comparison of laboratory parameters, ultrasound findings, and Orexin-A levels in study groups.

| Parameter | Controls ($n = 15$) | Cases ($n = 30$) | *P-value* |
|---|---|---|---|
| ALT (U/L) | 16.27 ± 4.48 | 18.53 ± 7.34 | 0.28 |
| AST (U/L) | 18 (16 – 23) | 18 (14 – 21) | 0.79 |
| S. albumin (g/dL) | 3.51 ± 0.25 | 3.37 ± 0.20 | 0.054 |
| HB (g/dL) | 11.63 ± 1.50 | 11.94 ± 1.13 | 0.44 |
| PLT (×10⁹/L) | 311.67 ± 50.53 | 266.13 ± 83.62 | 0.06 |
| TLC (×10⁹/L) | 7.34 ± 2.09 | 7.78 ± 1.92 | 0.489 |
| Negative HBsAg | 15 (100.0%) | 30 (100.0%) | – |
| Negative HCV Ab | 15 (100.0%) | 30 (100.0%) | – |
| HbA1c (%) | 4.88 ± 0.49 | 6.74 ± 1.97 | 0.001* |
| Bl. Urea (mg/dL) | 33.33 ± 6.82 | 38.90 ± 10.06 | 0.06* |
| S. Creat (mg/dL) | 0.86 ± 0.18 | 0.93 ± 0.22 | 0.232 |
| Cholesterol (mg/dL) | 165.00 ± 22.43 | 257.90 ± 48.55 | <0.001* |
| Triglyceride (mg/dL) | 124.13 ± 15.43 | 279.17 ± 63.89 | <0.001* |
| LDL (mg/dL) | 48.93 ± 9.14 | 130.09 ± 35.80 | <0.001* |
| HDL (mg/dL) | 55.20 ± 6.41 | 42.37 ± 8.72 | <0.001* |
| Weight (kg) | 64.07 ± 10.48 | 102.43 ± 14.49 | <0.001* |
| Height (m) | 1.57 ± 0.07 | 1.57 ± 0.10 | 0.962 |
| BMI (kg/m²) | 25.88 ± 2.95 | 41.65 ± 4.54 | <0.001* |
| NAFLD Fib Score | -2.99 (-3.3 – -1.99) | -0.04 (-1.04 – 0.69) | <0.001* |
| Ultrasound | Free: 15 (100.0%) | Bright Liver: 30 (100.0%) | <0.001* |
| Orexin A (pg/mL) | 2.12 (1.58 – 3.34) | 0.34 (0.21 – 0.6) | <0.001* |

Data are presented as Mean ± SD, Median (IQR), ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, TLC: Total Leukocyte Count, S. albumin: Serum Albumin, HB: Hemoglobin, PLT: Platelets, HbA1c: Glycated Hemoglobin, HBsAg: Hepatitis B Surface Antigen, HCV Ab: Hepatitis C Virus Antibody, Bl. Urea: Blood Urea, S. Creat: Serum Creatinine, LDL: Low-Density Lipoprotein, HDL: High-Density Lipoprotein, NAFLD: Non-Alcoholic Fatty Liver Disease, BMI: Body Mass Index, Fib Score: Fibrosis Score, DM: Diabetes Mellitus, *: Statistically significant *p-value*.

Group 2 and Group 1 exhibited significantly higher BMI ($p<0.001$), NAFLD fibrosis score ($p<0.001$), cholesterol ($p<0.001$), triglycerides ($p<0.001$), and LDL ($p<0.001$) compared to the control group. HbA1c was significantly higher in diabetics compared to both non-diabetics and controls ($p<0.001$). Blood urea was also significantly elevated in diabetics compared to both groups ($p=0.024$). No substantial variations were observed in ALT ($p=0.303$), AST ($p=0.565$), hemoglobin ($p=0.666$), platelet count ($p=0.071$), or total leukocyte count ($p=0.736$) among the groups. (Table 2).

**Table 2:** Comparison of demographics, anthropometrics, lab tests, ultrasound, and Orexin-A levels among study groups.

| Parameter | Controls (*n*=15) | Non-Diabetics (*n*=15) | Diabetics (*n*=15) | *P-Value* |
|---|---|---|---|---|
| Females | 14 (93.3%) | 14 (93.3%) | 12 (80.0%) | 0.407 |
| Males | 1 (6.7%) | 1 (6.7%) | 3 (20.0%) | |
| Age (Years) | 42.93 ± 10.93 2,3 | 53.20 ± 6.07 1 | 52.47 ± 7.33 1 | 0.002* |
| Weight (kg) | 64.07 ± 10.48 2,3 | 101.80 ± 15.05 1 | 103.07 ± 14.41 1 | <0.001* |
| Height (m) | 1.57 ± 0.07 | 1.57 ± 0.11 | 1.57 ± 0.08 | 0.999 |
| BMI (kg/m²) | 25.88 ± 2.95 2,3 | 41.36 ± 4.11 1 | 41.95 ± 5.06 1 | <0.001* |
| NAFLD Fib Score | -2.99 (-3.3 – -1.99) 2,3 | -0.8 (-1.05 – 0.31) 1,3 | 0.61 (-0.48 – 1.15) 1,2 | <0.001* |
| ALT (U/L) | 16.27 ± 4.48 | 17.20 ± 5.37 | 19.87 ± 8.89 | 0.303 |
| AST (U/L) | 18 (16 – 23) | 18 (13 – 21) | 19 (14 – 24) | 0.565 |
| S. Albumin (g/dL) | 3.51 ± 0.25 | 3.42 ± 0.18 | 3.32 ± 0.21 | 0.073 |
| HB (g/dL) | 11.63 ± 1.50 | 11.83 ± 0.97 | 12.05 ± 1.29 | 0.666 |
| PLT (×10⁹/L) | 311.67 ± 50.53 | 247.86 ± 90.74 | 284.40 ± 74.41 | 0.071 |
| TLC (×10⁹/L) | 7.34 ± 2.09 | 7.64 ± 1.39 | 7.91 ± 2.38 | 0.736 |
| HbA1c (%) | 4.88 ± 0.49 3 | 4.99 ± 0.54 3 | 8.49 ± 1.08 1,2 | <0.001* |
| Bl. Urea (mg/dL) | 33.33 ± 6.82 3 | 35.60 ± 8.58 3 | 42.20 ± 10.61 1,2 | 0.024* |
| S. Creat (mg/dL) | 0.86 ± 0.18 | 0.90 ± 0.18 | 0.97 ± 0.25 | 0.340 |
| Cholesterol (mg/dL) | 165.00 ± 22.43 2,3 | 261.73 ± 32.93 1 | 254.07 ± 61.38 1 | <0.001* |
| Triglyceride (mg/dL) | 124.13 ± 15.43 2,3 | 275.73 ± 55.86 1 | 282.60 ± 72.87 1 | <0.001* |
| LDL (mg/dL) | 48.93 ± 9.14 2,3 2,3 | 142.73 ± 33.49 1,3 | 117.44 ± 34.51 1,2 | <0.001* |
| HDL (mg/dL) | 55.20 ± 6.41 2,3 | 39.93 ± 9.05 1 | 44.80 ± 7.94 1 | <0.001 |
| Ultrasound | Free: 15 (100.0%) | Bright Liver: 15 (100.0%) | Bright Liver: 15 (100.0%) | <0.001 |

1: Significantly different from Control Group, 2: Significantly different from Non-Diabetics Group, 3: Significantly different from Diabetics Group, Data are presented as Mean ± SD, Median (IQR), BMI: Body Mass Index, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, HB: Hemoglobin, PLT: Platelets, S. albumin: Serum Albumin, HCV Ab: Hepatitis C Virus Antibody, TLC: Total Leukocyte Count, LDL: Low-Density Lipoprotein, HBsAg: Hepatitis B Surface Antigen, HbA1c: Glycated Hemoglobin, Bl. Urea: Blood Urea, S. Creat: Serum Creatinine, NAFLD: Non-Alcoholic Fatty Liver Disease, HDL: High-Density Lipoprotein, Fib Score: Fibrosis Score, DM: Diabetes Mellitus, *: Statistically significant *p-value*.

Orexin-A levels significantly differed among the studied groups ($P < 0.001$). Post hoc (pairwise) analysis demonstrated that it was markedly elevated in the controls group (2.12 [1.58 – 3.34] pg/mL) compared to both Non-Diabetics Group (0.42 [0.17 – 0.62] pg/mL, $P < 0.001$) and Diabetics Group (0.33 [0.21 – 0.51] pg/mL, $P < 0.001$). The comparison of Orexin-A levels between the Non-Diabetic and Diabetic groups did not yield a statistically notable variation ($P > 0.05$). (Figure 1)

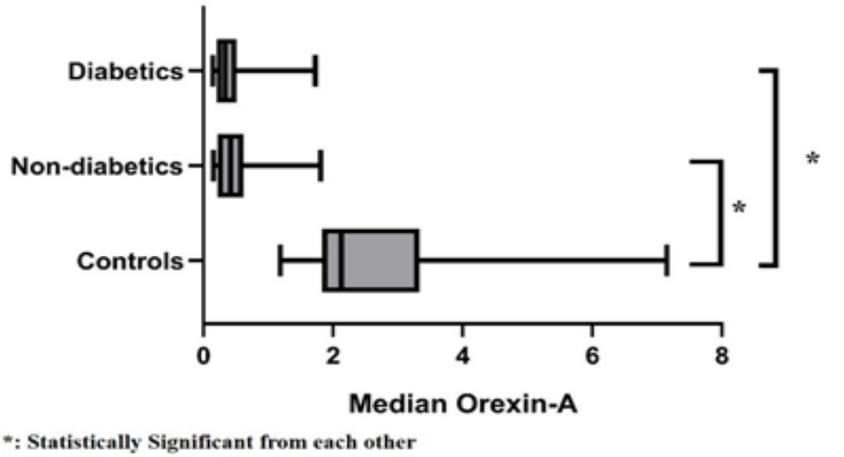


**Fig. 1:** Median Orexin-A among the study groups.

Orexin-A levels showed a substantial negative correlation with serum albumin among all cases (r = -0.361, $p$ = 0.05) and diabetic patients (r = -0.583, $p$ = 0.022). In diabetic patients, Orexin-A levels also exhibited a notable negative correlation with blood urea (r = -0.612, $p$ = 0.015) and a positive correlation with weight (r = 0.585, $p$ = 0.022) and height (r = 0.652, $p$ = 0.008). Among non-diabetic patients, Orexin-A levels correlated negatively with height (r = -0.606, $p$ = 0.017) and positively with hemoglobin (r = 0.521, $p$ = 0.047). Additionally, a significant positive correlation was observed between Orexin-A and the NAFLD fibrosis score across all cases (r = 0.440, $p$ = 0.015). (Table 3).

**Table 3:** Correlation of Orexin-A levels with clinical parameters in all, non-diabetic, and diabetic patients.

| | All cases | | Non-Diabetics | | Diabetics | |
|---|---|---|---|---|---|---|
| | r | *P-value* | r | *P-value* | r | *P-value* |
| Age (Years) | 0.215 | 0.253 | 0.224 | 0.423 | 0.156 | 0.58 |
| ALT (U/L) | 0.078 | 0.683 | 0.073 | 0.797 | 0.074 | 0.792 |
| AST (U/L) | -0.124 | 0.515 | -0.151 | 0.592 | 0.022 | 0.939 |
| S.albumin (g/dL) | -0.361 | 0.05* | -0.2 | 0.475 | -0.583 | 0.022* |
| HB (g/dL) | 0.273 | 0.145 | 0.521 | 0.047* | 0.119 | 0.673 |
| PLT (×10⁹/L) | -0.279 | 0.135 | -0.111 | 0.693 | -0.427 | 0.112 |
| TLC (×10⁹/L) | -0.068 | 0.72 | -0.207 | 0.46 | 0.07 | 0.805 |
| HbA1c (%) | 0.027 | 0.887 | 0.228 | 0.414 | 0.234 | 0.402 |
| Bl.Urea (mg/dL) | -0.214 | 0.255 | 0.041 | 0.884 | -0.612 | 0.015* |
| S.Creat (mg/dL) | -0.104 | 0.583 | 0.063 | 0.823 | -0.273 | 0.324 |
| Cholesterol (mg/dL) | 0.098 | 0.606 | 0.348 | 0.204 | -0.247 | 0.376 |
| Triglyceride (mg/dL) | 0.112 | 0.556 | -0.183 | 0.513 | 0.258 | 0.354 |
| LDL (mg/dL) | 0.228 | 0.227 | 0.369 | 0.176 | -0.115 | 0.682 |
| HDL (mg/dL) | -0.197 | 0.298 | -0.235 | 0.399 | -0.096 | 0.734 |
| Weight (kg) | 0.065 | 0.734 | -0.305 | 0.269 | 0.585 | 0.022* |
| Height (m) | -0.096 | 0.615 | -0.606 | 0.017* | 0.652 | 0.008* |
| BMI (kg/m²) | 0.267 | 0.153 | 0.382 | 0.16 | 0.07 | 0.805 |
| NAFLD Fib score | 0.440 | 0.015* | 0.416 | 0.123 | 0.483 | 0.068 |

ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, S. albumin: Serum Albumin, HB: Hemoglobin, PLT: Platelets, TLC: Total Leukocyte Count, HbA1c: Glycated Hemoglobin, Bl. Urea: Blood Urea, S. Creat: Serum Creatinine, LDL: Low-Density Lipoprotein, HDL: High-Density Lipoprotein, BMI: Body Mass Index, NAFLD: Non-Alcoholic Fatty Liver Disease, Fib Score: Fibrosis Score, DM: Diabetes Mellitus, *: Statistically significant *p-value*.

Orexin A demonstrated strong diagnostic accuracy for NAFLD, with an optimal cutoff of ≤0.66 distinguishing NAFLD patients from normal individuals (AUC = 87.7%, sensitivity = 90%, specificity = 86.67%). In non-diabetic NAFLD patients, the same cutoff (≤0.66) yielded AUC = 87.6%, sensitivity = 86.67%, specificity = 86.67%. Among diabetic NAFLD patients, a slightly lower cutoff (≤0.64) achieved AUC = 87.8%, sensitivity = 93.33%, specificity = 86.67%. (Figure 2 (A-C)).

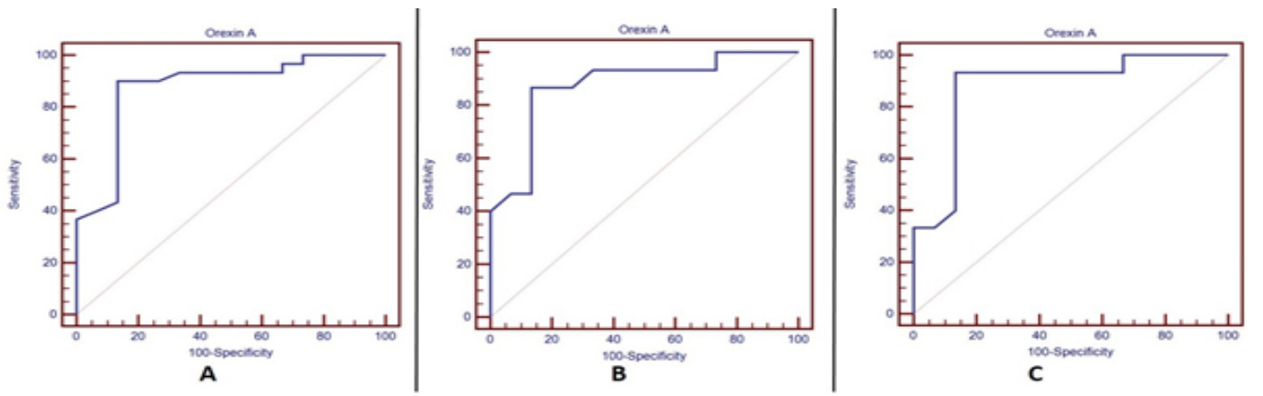


**Fig. 2:** ROC curve to assess Orexin-A to differentiate between (A) control and patient groups, (B) control and non-diabetic patients, (C) control and Diabetic patients

## DISCUSSION

NAFLD, a significant etiological factor in cirrhosis and HCC, evolves from simple hepatic steatosis to non-alcoholic steatohepatitis (NASH), which is characterized by necroinflammation and an accelerated fibrotic process[11].

NAFLD is strongly linked to metabolic syndrome components, including obesity, insulin resistance, hyperlipidemia, and hypertension. Orexins, polypeptides derived from the lateral hypothalamus, particularly orexin-A, regulate feeding behavior, glucose homeostasis, and energy expenditure[8, 12]. Central orexin activity has been implicated in preventing NASH, HCC, and obesity by promoting physical activity[4].

This study aims to evaluate peripheral Orexin A levels in NAFLD patients, obese or non-obese, compared to controls and to explore correlations with obesity, T2DM, and dyslipidemia.

Within our study population, no substantial discrepancy was detected between the case and control groups concerning sex distribution. Similarly, ***Li et al.***[13] reported that obesity substantially increased the likelihood of NAFLD development, irrespective of gender, with a highly significant association ($P < 0.001$). While in the non-obese individuals, NAFLD was more common in male patients than in females ($P$=0.001). Another study by ***Balakrishnan et al.***[14] showed that women have a lower risk of developing NAFLD than men.

Our study also revealed a statistically significant increase in Cholesterol, Triglyceride, LDL, HDL and BMI in cases groups than control group. This suggests there is a strong link between NAFLD and obesity. Nonetheless, albumin levels, platelet count, and serum transaminases (ALT, AST) did not differ markedly between case and control groups. These observations correspond with the findings of ***Li et al.***[13].

A comparison of the anthropometric parameters revealed statistically significant increase in the BMI, triglyceride values between the two groups (obese group and non-obese group), this suggest that BMI and Triglyceride were associated with risk for developing NAFLD in non-obese individuals.

Also, in our study there was statistically significant increase in HbA1c in cases group than control group. Those results were consistent with Masroor and Haque[15] who revealed that among cases and control groups there are 66 % of cases and 32 % of control had HbA1C levels more than 5.7%. It also showed that HbA1C was significantly associated with NAFLD.

Furthermore, abnormally elevated HbA1C had predictive potential of over 70% for NAFLD and after adjustment for body measurement indices, it is the single risk factor most strongly associated with NAFLD. In comparison with other anthropometric measures in the adult population, HbA1C may be provided as a possible biomarker for NAFLD[15].

A recent study conducted on Chinese patients by ***Chen et al.***[16] found that higher levels of HbA1c may lead to the development of NAFLD. It suggests that this type of altered hemoglobin may contribute to the NAFLD development directly, by increasing RAGE (Receptor for Advanced Glycation End products), or indirectly, by promoting hypoxia and inhibiting NO (Nitric Oxide) release, HbA1c levels and NAFLD development are positively influencing each other. This means that there is a strong positive relation between abnormal levels of HbA1c and incidence of NAFLD.

In the current study, there was substantially significant elevation in NFS in cases group than control group and there was statistically significant increase in group 1 than in control group, and there was statistically significant increase group 2 than in control group, also there was statistically significant increase in group 2 than in group 1.

Our results were consistent with ***Rigor et al.***[17] who revealed that NFS has negative predictive value of 93% which is adequate to rule out advanced fibrosis in NAFLD patients. So, it is appropriate for use in healthcare settings as well as part of referral and follow-up programs.

However, a study by ***Graupera et al.***[18] demonstrated that NFS have a weak correlation with liver stiffness, resulting in a significant number of false positives and false negatives. This limitation reduces their utility as screening tools in primary care settings. Therefore, the development of novel noninvasive approaches with improved accuracy is recommended for fibrosis detection, particularly in low-prevalence fibrosis populations.

Another study by ***Kjaergaard et al.***[19] showed that NAFLD Fib Score has higher false positive (45%) and false negative (3%) results when compared to Enhanced Liver Fibrosis test (ELF) alone or combined with FIB-4 score, also it revealed that ELF test accurately categorized 85% of all subjects, whereas NAFLD Fib Score successfully classified only 54%.

Orexin A levels were significantly higher in both Group 1 (non-diabetic) and Group 2 (diabetic) compared to control group, with no substantial variation between patient groups. The optimal cutoff to distinguish cases (diabetic and non-diabetic) from controls was ≤0.66, yielding 90%

sensitivity, 86.67% specificity, and an AUC of 87.7%. For differentiating NAFLD patients without diabetes (Group 1) from controls, the best cutoff was also ≤0.66, with 86.67% sensitivity, 86.67% specificity, and an AUC of 87.6%. To distinguish NAFLD patients with diabetes (Group 2) from controls, the optimal cutoff was ≤0.64, achieving 93.33% sensitivity, 86.67% specificity, and an AUC of 87.8%.

This supports the conclusions drawn by ***Tsuneki et al.***[4] whose study on mice models demonstrated that demonstrated that Mice with orexin deficiency developed significant obesity, NASH, and fibrosis. Long-term high fat diet feeding resulted in the HCC. Daily intracerebroventricular supplementation with orexin A mitigated inflammation and hepatic endoplasmic reticulum stress, indicating its potential role in preventing the advancement of NASH, NAFLD, and HCC.

Another study on Kurdish subjects in Iraq by ***Sadiq et al.***[7] showed that Orexin A correlated significantly with fasting blood sugar and HbA1c, and may be a useful biomarker for T2DM. The positive correlation of orexin-A with FBG might be links to the orexin-A role in protecting against pancreatic cell malfunction and stimulating insulin production from pancreatic beta cells in people with T2DM.

## CONCLUSION

Orexin-A is significantly reduced in NAFLD patients, suggesting its potential role as a biomarker for NAFLD detection and disease severity assessment.

## CONFLICT OF INTERESTS

There is no conflicts of interest.

## DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## CONTRIBUTION

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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# مستويات الأوركسين-A في مرض الكبد الدهني غير الكحولي: دراسة الحالات والشواهد لإستكشاف الإرتباطات الأيضية والكبدية

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**المقدمة:** يعتبر مرض الكبد الدهني غير الكحولي مشكلة صحية عالمية ترتبط ارتباطًا وثيقًا بالسمنة، وداء السكري من النوع الثاني ، واضطرابات الدهون. يُعد الأوركسين-A نيوبيبتيدًا مشاركًا في تنظيم عمليات الأيض، وقد تم اقتراح دوره في الفيزيولوجيا المرضية للكبد الدهني غير الكحولي.

هدف الدراسة: تقييم مستويات الأوركسين-A المحيطية في مرضى الكبد الدهني غير الكحولي ومدى ارتباطها بعوامل الخطر الأيضية.

المرضى والطرق: اشتملت هذه الدراسة من نوع الحالة-الشاهد على ٤٥ مشاركًا، مقسمين إلى ثلاث مجموعات: ١٥ مريضًا مصابًا بالكبد الدهني غير الكحولي بدون سكري (المجموعة ١)، و١٥ مريضًا مصابًا بالكبد الدهني غير الكحولي مع السكري (المجموعة ٢)، و١٥ شخصًا من الأصحاء كمجموعة ضابطة (المجموعة ٣). تم تحليل مستويات الدهون، والهيموغلوبين السكري ، ومقياس تليف الكبد الدهني. تم استخدام اختبار الساندويتش ELISA لقياس مستويات الأوركسين-A في المصل.

النتائج: أظهر مرضى الكبد الدهني غير الكحولي مستويات منخفضة بشكل ملحوظ من الأوركسين-A مقارنةً بالمجموعة الضابطة (٠,٣٤ مقابل ٢,١٢ بيكوغرام لكل مل، قيمة الاحتمالية أقل من ٠,٠٠١). كما ارتبطت مستويات الأوركسين-A سلبياً مع الألبومين في الدم (معامل الارتباط يساوي -٠,٣٦١، قيمة الاحتمالية = ٠,٠٥) ومع اليوريا الدموية لدى مرضى الكبد الدهني غير الكحولي المصابين بالسكري (معامل الارتباط يساوي -٠,٦١٢، قيمة الاحتمالية = ٠,٠١٥). أظهرت تحليلات منحنى ROC أن الأوركسين-A بقيمة أقل من أو تساوي ٠,٦٦ بيكوغرام لكل مل كان فعالًا في التمييز بين مرضى الكبد الدهني غير الكحولي والمجموعة الضابطة (المساحة تحت المنحنى =٠,٨٧٧، الحساسية = ٩٠٪، الخصوصية = ٨٦,٦٧٪).

الاستنتاج: يُعد الأوركسين-A منخفضًا بشكل كبير في مرضى الكبد الدهني غير الكحولي، مما يشير إلى إمكانيته كعلامة بيولوجية لتشخيص المرض وتقييم شدته.