Diagnostic Value of Acyl-Ghrelin in Type 2 Diabetic Patients with Non-Alcoholic Fatty Liver Disease

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Abstract

BACKGROUND: Nonalcoholic fatty liver disease (NAFLD) has become the most leading cause of chronic liver disease worldwide. Type 2 diabetes mellitus (T2DM) is described as one of the most significant risk factors for developing NAFLD, non-alcoholic steatohepatitis, and advanced cirrhosis. The high incidence of NAFLD in T2DM patients, as well as its serious clinical consequences, is both reasons for concern. Therefore, it is becoming critically needed to develop simple, low-cost, and noninvasive test for diagnosis and management of NFLD. Accordingly, the objective of this study was to examine the diagnostic value of acyl ghrelin (AG) for detecting NAFLD in T2D patients.

AIM: The aim of this study is to examine the accuracy of AG as a non-invasive biomarker to effectively diagnose diabetic patients with NAFLD.

METHODS: A total of 61 patients with T2D were selected from internal medicine outpatient clinic in National Research Centre, Egypt. 29 diabetic patients were free of NAFLD while the other 32 were diagnosed with NAFLD. Measurements of lipid profile, fasting glucose, liver enzyme activities, and AG levels were collected. Data management and analysis were performed using SPSS version 20.

RESULTS: A comparison between diabetic subjects with and without NAFLD showed some metabolic abnormalities including central obesity, significant increases in waist circumference, body weight, fasting blood sugar, triglycerides, low-density lipoprotein, liver enzymes levels, and a significant decrease in high-density lipoprotein in diabetic with NAFLD patients. Increases in total cholesterol and AG levels were observed; however, none of these differences were significant when compared with control diabetic subjects.

CONCLUSIONS: The association between elevated AG level and NAFLD is clearly supported by the current findings. However, more studies are needed to consider it as diagnostic marker in NAFLD patients with T2D.

Introduction

Nonalcoholic fatty liver disease (NAFLD) can broadly be defined as conditions caused by excessive fat accumulation in hepatocytes not promoted by excessive alcohol consumption. NAFLD is found to be correlated with a cluster of metabolic disturbances such as, insulin resistance (IR), visceral and subcutaneous fat accumulation, atherogenic dyslipidemia, and type 2 diabetes [1], [2], [3]. It is now well established from a variety of studies that imbalances between liver lipid output and input are the most important components in fatty liver development. The main factors influencing this energy disturbance are: (1) Increased systemic free fatty acid (FFA) and IR as a result of increased adipose tissue lipolysis [4], [5]; (2) increased dietary fat consumption as a source of FFAs; (3) increased hepatic de novo lipogenesis [6]; (4) change in lipoprotein production or secretion [6], [7], [8]; (5) Decreasing FA β-oxidation in mitochondria [9], [10], [11]. The cellular shift in metabolism from fatty acid oxidation to de novo lipid synthesis is regulated by the activity of the three known transcription factors PPAR-γ [12], ChREBP, and SREBP-1c [13], [14]; all of them are essential modulators of hepatic triglycerides (TG) contents through controlling target genes involved in lipid synthesis.

Ghrelin is a novel peptide hormone of 28 amino acid residues that has a n-octanoyl group at the serine three residues. It is an endogenous ligand of the growth hormone secretagogue receptor (GHS-R) that mostly produced by the stomach [15]. According to Dezaki et al. in 2004, exogenous ghrelin is thought to stimulate appetite and food consumption through induction of hypothalamic neuropeptide Y/agouti-related peptide neurons expressing GHS-R type 1a [16]. Moreover, other types of ghrelin effects on energy metabolism, lipid and glucose homeostasis, are mediated at the peripheral level by modulating insulin secretion and sensitivity in pancreatic b-cells [17] and increasing glucose output by primary hepatocytes [18].
Among the peptides generated from the preghrelin gene are acylghrelin (AG), des-acyl ghrelin (DAG), and obestatin. Recent research has demonstrated that both AG and DAG may modulate peripheral biological actions antagonistically [19], [20].

Despite the importance of AG role in obesity, IR and diabetes mellitus (DM) [16], [17], [18], [21], to date very little attention has been paid to it in liver disease. Therefore, the purpose of this study is to examine the accuracy of AG as a non-invasive biomarker to effectively diagnose diabetic patients with NAFLD.

Patients and Methods

This cross-sectional study was conducted on 61 type 2 DM (T2DM) patients in association with or without NAFLD. The participants aged over 18 years and were selected from the internal medicine outpatient clinic of National Research Centre, Dokki, Egypt. All subjects underwent routine anthropometric measurements, physical and biochemical examinations after receiving informed consent and approval from research ethics committee (number 1451022021).

Thirty two diabetic patients had NAFLD which was diagnosed by abdominal ultrasonography (US). Ultrasonographic measurements were performed by experienced radiologists based on 4 known criteria (hepatorenal echo contrast, liver brightness, deep attenuation, and vascular blurring). The participants were required to have hepatorenal contrast and liver brightness to be given a diagnosis of NAFLD. All patients were asked about their medical history and alcohol consumption. Patients with hepatitis B virus antigen or hepatitis C virus antibody, and the patients with chronic liver disease were excluded from the study.

Anthropometric measurements and laboratory tests were carried out included weight (kg), height (cm), and waist circumference (cm). Body mass index (BMI) was calculated by dividing the weight in kilograms by the height in meters squared. Blood pressure (BP) was measured twice, with the subjects in a sitting position, using an automated device; the mean of two measurements was calculated.

Blood samples were collected after a fasting period of at least 12 h, plasma were separated by centrifugation for the following laboratory investigations:

1. Liver function tests including the activity levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) using the calorimetric assay from Bio diagnostic and Research Reagents company, Dokki, Egypt

2. Fasting blood sugar using enzymatic colorimetric assay from Bio diagnostic and Research Reagents company, Dokki, Egypt

3. Lipid profile, the concentrations of cholesterol, high-density lipoprotein (HDL), and TG were measured using colorimetric enzymatic assays. Low-density lipoprotein (LDL) concentration was calculated using the Friedewald formula. Some of Plasma samples were immediately frozen at –80°C for acylated ghrelin test using ELISA technique.

For statistical analysis

Data were coded, entered, and analyzed using statistical package for social sciences version 20. The quantitative variables were compared using paired t-test or one-way analysis of variance. The comparison of qualitative variables were performed using chi-square test or Fisher’s exact test.

p-value level of significance.

- p > 0.05: Non-significant
- p < 0.05: Significant
- p < 0.01: Highly significant.

Results

A total number of T2DM patients were enrolled in this study. 32 diabetic patients with US-proven NAFLD have mean age 48 ± 10 years, female/male 26/6 while those without NAFLD, control group, have mean age 45 ± 7 years, female/male 13/16 (Table 1).

Table 1: Age and sex distribution in study population

| Variable | Control (T2D) n (29) | NAFLD and T2D n (32) |
|----------|----------------------|----------------------|
| Age      | 45 ± 7.8             | 48 ± 10.9            |
| Sex      | 16                   | 6                    |
|          | Male                 | 16                   |
|          | Female               | 13                   |

NAFLD: Nonalcoholic fatty liver disease.

Results of demographic parameters in the Table 2 and Figure 1 show, the mean of pulse, BP (Systolic and Diastolic) 79 ± 4.5, (125 and 77), respectively for group control, while in case of group with T2D and NAFLD 80 ± 3.9, (127 and 80), the distribution of groups does not show any statistically significant of data.

Table 2: Demographic parameters in examined patients (M ± SD)

| Parameter                   | Control (T2D) n (29) | NAFLD and T2D n (32) | p-value | Statistically significant |
|-----------------------------|----------------------|----------------------|---------|--------------------------|
| Age, years                  | 45 ± 7.8             | 48 ± 10.9            | 0.252   | N. S                     |
| Puls/Min                    | 79 ± 4.5             | 80 ± 3.9             | 0.214   | N. S                     |
| BP                          | 125 ± 12.5           | 127 ± 15.6           | 0.434   | N. S                     |
| Systolic mm Hg              | 77 ± 8.4             | 80 ± 10.2            | 0.298   | N. S                     |
| Diastolic mm Hg             | 80 ± 10.2            | 127 ± 15.6           | 0.434   | N. S                     |

Statistical test used: Low sample t-test. P<0.05 considered statistically significant (95% confidence interval).
NAFLD: Nonalcoholic fatty liver disease. BP: Blood pressure.
A significant increase (p < 0.05) in body weight and waist circumference in T2D and NAFLD group was recorded compared with control (Table 3). BMI was elevated also in T2D and NAFLD patients but no significant difference between the two groups was evident. The mean value of body weight, length, waist circumference, and BMI are 87.7 ± 7.3, 158 ± 6.3, 95 ± 3.8, and 34 ± 4.2, respectively, for control group, and in T2D and NAFLD group are 93.2 ± 14.5, 165.4 ± 7.9, 115.4 ± 7.6, and 37 ± 5.1.

Table 5 and Figure 4)

In addition, significant increase of fasting blood sugar (FBS), TG, LDL, ALT, and AST levels was found in T2D and NAFLD group compared with control group, however, no significant differences in Tc level between groups. The mean of FBS, Tc, TG, HDL, LDL, ALT, and AST are 123.7 ± 20.7, 185.7 ± 28.6, 128.4 ± 14.4, 59.3 ± 9.3, 119 ± 22.9, 18.6 ± 5, and 20 ± 4 respectively for control group, and in T2D and NAFLD group are 144.2 ± 25.9, 196.7 ± 26.8,164.5 ± 39.4, 40.9 ± 8.5,125.8 ± 34.6, 21.1 ± 5.7, and 32.3 ± 2.1 (Table 4 and Figure 2).

Figure 1: Demographic parameters in examined patients

Blood pressure

![Blood pressure graph](image1)

Figure 2: Biochemical parameters in examined patients (M ± SD)

![Biochemical parameters graph](image2)

Clear increment was observed in fasting AG level of T2D and NAFLD group; however, it was not significant compared with control group (Table 5 and Figure 3).

In Table 5, only ALT did not show significant correlation while all other parameters show significant correlation with serum AG as shown in (Table 5 and Figure 4).

Table 3: Physical examination parameters in examined patients (M ± SD)

| Parameter                  | Control (T2D) | NAFLD and T2D | p-value  | Statistically significant |
|----------------------------|---------------|---------------|----------|--------------------------|
| Weight (kg)                | 87.7 ± 7.3    | 93.2 ± 14.5   | 0.087    | Sig.                     |
| Length (cm)                | 158 ± 6.3     | 165.4 ± 7.9   | <0.0001  | Sig.                     |
| waist circumference (cm)   | 95 ± 3.8      | 115.4 ± 7.6   | 0.0034   | Sig.                     |
| BMI                        | 34 ± 4.2      | 37 ± 5.1      | 0.1      | N.S                      |

Statistical test used: T-test. P≤0.05 considered statistically significant (95% confidence interval). NAFLD: Nonalcoholic fatty liver disease.

Discussion

NAFLD, the common cause of liver related morbidity and mortality, is a growing public health concern worldwide [22], [23]. Several studies have reported that T2DM patients are expected to be at a higher risk of developing liver disease including fibrosis, cirrhosis, and hepatocellular carcinoma compared with healthy populations [24], [25]. Moreover, a large proportion of them are diagnosed long after the onset of their diabetes which means that it is difficult to determine the duration and the risk of developing NAFLD [26]. Until recently, there has been little interest of safe accurate estimate of NAFLD in T2DM patients especially in populations with a high prevalence of NAFLD. In this regard, our study set out with the aim of assessing the possibility of using serum AG hormone as reliable noninvasive marker of NAFLD in diabetic patients.

The effects of ghrelin on hepatic fatty acid metabolism have been observed in previous experimental models, and has been recently proposed as a link of NAFLD development in patients with T2D [27], [28]. In line with these observations, our data showed elevation of AG level in T2D and NAFLD group. Although this difference was not significant compared with control, it was positively correlated with FBS, Tc, TG, HDLc, LDLc, and AST. These findings are in agreement with those observed in recent studies of Mykhalchyshyn et al., 2015, Lui et al. 2020, and others [2], [16], [19], [29], [30], [31]. Manuela et al. in 2014 partly explained these relationships through a well proved mechanism of AG action in different tissues [32]. It stimulates both lipogenesis and gluconeogenesis, raises TG levels, and reduces fatty acid oxidation-stimulating activity. Its impact on TG deposition is greater in liver than skeletal muscle.

Our findings in terms of a relation between hyperglycemia and elevated AG in NFLD and T2D reflect those of Broglio et al., 2001, and Dezaki et al., 2004 [16], [19]. They referred it to the direct action of ghrelin on pancreatic α- and β-cells which stimulate glucagon secretion and inhibit glucose-induced insulin release, respectively. This increases hepatic glucose production and decreases glucose uptake and insulin sensitivity in skeletal muscle and adipose tissue, causes of elevated blood glucose.

In addition, the observed positive correlation between body weight, waist circumference and elevated
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AG in NAFLD and T2DM patients are in line with those of previous studies obtained by Neuman et al., 2014.

Table 4: Biochemical parameters in examined patients (M ± SD)

| Parameter          | Control (T2D) | NAFLD and T2D | p-value | Statistically significant |
|--------------------|---------------|---------------|---------|--------------------------|
| FBS (mg/dl)        | 123.7 ± 20.7  | 144.2 ± 25.9  | 0.035   | Sig.                     |
| Tc (mg/dl)         | 185.7 ± 28.6  | 196.7 ± 26.8  | 0.299   | N.S                      |
| TG (mg/dl)         | 128.4 ± 14.4  | 164.5 ± 39.4  | 0.004   | Sig.                     |
| HDL (mg/dl)        | 59.3 ± 9.3    | 40.9 ± 8.5    | 0.288   | Sig.                     |
| LDL (mg/dl)        | 119 ± 22.9    | 125.8 ± 34.6  | <0.0001 | Sig.                     |
| ALT (u/ml)         | 18.6 ± 5      | 21 ± 5.7      | 0.016   | Sig.                     |
| AST (u/ml)         | 20 ± 4        | 32.3 ± 2.1    | 0.01    | Sig.                     |

Statistical test used: Two sample t-test p≤0.05 considered statistically significant (95% confidence interval).

Liu et al., 2020. They suggested that AG could cause IR and promote liver fat deposition through a core hypothalamic mechanism. PPAR- and SREBP1, as well as other fat storage-related proteins including acetyl-CoA carboxylase, lipoprotein lipase, and fatty acid synthase are the most important driving factors [33]. Moreover, the elevated FFA in obese subjects can result in IR and subsequent increase in ghrelin level in NAFLD patients [34].

Table 5: Fasting AG in examined patients (M ± SD)

| Parameter          | Control (T2D) | NAFLD and T2D | p-value | Statistically significant |
|--------------------|---------------|---------------|---------|--------------------------|
| Fasting AG         | 37.8 ± 9.3    | 56.1 ± 10.7   | 0.306   | NS                       |

Statistical test used: Two sample t-test. p-value≤0.05 considered statistically significant (95% confidence interval).

From the other hand, acyl transferase, an enzyme involved in the n-octanoylation of ghrelin, namely Ghelin O-Acyltransferase is another factor that may explain such correlation. Obviously, obesity
influences the expression and/or activity of acyl transferase which causes elevation of AG plasma concentrations [33]. This outcome is contrary to that of Xiaojun et al, in 2004 who found negatively associated plasma ghrelin with fasting glucose in newly diagnosed T2D patients [34] and Olavi et al., 2005, Tschöp et al., 2001who found that low total ghrelin concentration has been associated with some features of metabolic syndrome, including elevated BP, hypertriglyceridemia and obesity [21], [35].

The low level of HDL-C concentration in our study and the high level of TG, the key features of IR dyslipidemia, showed significant differences between patients with and without NAFLD. These findings broadly support the work of other studies in this area linking NAFLD with metabolic syndrome [36], [37], [38].

Table 6: Stepwise multiple linear regression analysis using as dependent variable serum AG level

| Parameter     | NAFLD versus Control         |
|---------------|------------------------------|
| Cut-off value | <0.26                        |
| Sensitivity, % | 48.2                         |
| Specificity, % | 60                           |
| PPV, %         | 9.4                          |
| NPV, %         | 93.1                         |
| AUROC          | 0.569                        |
| 95% confidence interval | 0.4226-0.7154 |
| p-value        | 0.3533                       |

Table 7: Diagnostic accuracy of AG for NAFLD diagnosis and differentiation between patients with elevated and normal values

| Parameter     | NAFLD versus Control         |
|---------------|------------------------------|
| Cut-off value | <0.26                        |
| Sensitivity, % | 48.2                         |
| Specificity, % | 60                           |
| PPV, %         | 9.4                          |
| NPV, %         | 93.1                         |
| AUROC          | 0.569                        |
| 95% confidence interval | 0.4226-0.7154 |
| p-value        | 0.3533                       |

Conclusions

The findings of this study provide insights for AG role, however the generalizability of it as marker of NAFLD condition in T2D patients, needs further studies with large number of patients.

References

1. Wong RJ, Aguilar M, Cheung R, Perumpail RB, Harrison SA, Younossi ZM, et al. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. Gastroenterology. 2015;148(3):547-55. http://doi.org/10.1053/j.gastro.2014.11.039 PMid:25461851
2. Tomah S, Alkhouri N, Hamdy O. Nonalcoholic fatty liver disease and Type 2 diabetes: Where do diabetologists stand? Clin Diabetes Endocrinol. 2020;6:9. http://doi.org/10.1186/s40842-020-00097-1 PMid:32518675
3. Godoy-Matos AF, Júnior WS, Valerio CM. NAFLD as a continuum: From obesity to metabolic syndrome and diabetes. Diabetol Metab Syndr. 2020;12(1):60. http://doi.org/10.1186/s13098-020-00570-y PMid:32684985
4. Polyzos SA, Koutrouzas J, Zavos C. Nonalcoholic fatty liver disease: The pathogenic roles of insulin resistance and adipocytokines. Curr Mol Med. 2010;9(3):299-314. http://doi.org/10.2174/156652409787847191 PMid:19355912
5. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, et al. Nonalcoholic steatohepatitis: Association of insulin resistance and mitochondrial abnormalities. Gastroenterology. 2001;120(5):1183-92. http://doi.org/10.1053/gast.2001.23256 PMid:11266382
6. Fujita K, Nozaki Y, Wada K, Yoneda M, Fujimoto Y, Fujitake M, et al. Dysfunctional very-low-density lipoprotein synthesis and release is a key factor in nonalcoholic steatohepatitis pathogenesis. Hepatology. 2009;50(3):772-80. http://doi.org/10.1002/hep.23094 PMid:19650159
7. Musso G, Gambino R, Cassader M. Recent insights into hepatic lipid metabolism in non-alcoholic fatty liver disease (NAFLD). Prog Lipid Res. 2009;48(1):1-26. http://doi.org/10.1016/j.plipres.2008.08.001 PMid:18824034
8. Qureshi K, Abrams GA. Metabolic liver disease of obesity and role of adipose tissue in the pathogenesis of nonalcoholic fatty liver disease. World J Gastroenterol. 2007;13(26):3540-53. http://doi.org/10.3748/wjg.v13.i26.3540 PMid:17659704
9. Schwarcz JM, Linfoot P, Dare D, Aghajanian K. Hepatic de novo lipogenesis in normoinsulinemic and hyperinsulinemic subjects consuming high-fat, low-carbohydrate and low-fat, high-carbohydrate isocaloric diets. Am J Clin Nutr. 2003;77(1):43-50. http://doi.org/10.1093/ajcn/77.1.43 PMid:12499321
10. Gárcia-Ruíz C, Baulies A, Mari M, García-Rovés PM, Fernandez-Checa JC. Mitochondrial dysfunction in non-alcoholic fatty liver disease and insulin resistance: Cause or consequence? Free Radic Res. 2013;47(11):854-68. http://doi.org/10.3109/10715762.2013.830717 PMid:23915028
11. Schadinger SE, Bucher NL, Schreiber BM, Farmer SR. PPARγ2 regulates lipogenesis and lipid accumulation in steatotic hepatocytes. Am J Physiol Endocrinol Metab. 2005;289(6):E1195-205. http://doi.org/10.1152/ajpendo.00513.2004 PMid:15644454
12. Shimomura I, Bashmakov Y, Horton JD. Increased levels of nuclear SREBP-1c associated with fatty livers in two mouse models of diabetes mellitus. J Biol Chem. 1999;274(42):30028-32. http://dx.doi.org/10.1074/jbc.274.42.30028
PMid:10514498

13. Kojima M, Kosoda H, Kangawa K. Ghrelin, a novel growth-hormone-releasing and appetite-stimulating peptide from stomach. Best Pract Res Clin Endocrinol Metab. 2004;18(4):517-30. http://dx.doi.org/10.1016/j.beem.2004.07.001
PMid:15337737

14. Ren L, Sun D, Zhou X, Yang Y, Huang X, Li Y, et al. Chronic treatment with the modified Longdan Xiegan Tang attenuates olanzapine-induced fatty liver in rats by regulating hepatic de novo lipogenesis and fatty acid beta-oxidation-associated gene expression mediated by SREBP-1c, PPAR-alpha and AMPK-alpha. J Ethnopharmacol. 2019;232:176-87. http://doi.org/10.1016/j.jep.2018.12.034
PMid:30590197

15. Chen HY, Trumbauer ME, Chen AS, Weingarth DT, Adams JR, Frazier EG, et al. Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. Endocrinology. 2004;145(6):2607-12. http://dx.doi.org/10.1210/en.2003-1598
PMid:14962995

16. Dezaki K, Kosoda H, Kakei M, Hashiguchi S, Watanabe M, Kangawa K, et al. Endogenous ghrelin in pancreatic islets restricts insulin release by attenuating Ca2+ signaling in β-cells: Implication in the glycemic control in rodents. Diabetes. 2004;53(12):3142-51. http://dx.doi.org/10.2337/diabetes.53.12.3142
PMid:15561944

17. Gauna C, Delhanty PJ, Hofland LJ, Janssen JA, Broglio F, Ross RJ, et al. Ghrelin stimulates, whereas des-octanoyl ghrelin inhibits, glucose output by primary hepatocytes. J Clin Endocrinol Metab. 2005;90(2):1055-60. http://dx.doi.org/10.1210/jc.2004-1069
PMid:15536157

18. Ikezaki A, Kosoda H, Ito K, Iwama S, Miura N, Matsuoka H, et al. Fasting plasma ghrelin levels are negatively correlated with insulin resistance and PAI-1, but not with leptin, in obese children and adolescents. Diabetes. 2002;51(12):3408-11. http://dx.doi.org/10.2337/diabetes.51.12.3408
PMid:12453893

19. Broglia F, Gottero C, Prodam F, Gauna C, Muccioli G, Papotti M, et al. Non-acylated ghrelin counteracts the metabolic but not the neuroendocrine response to acylated ghrelin in humans. J Clin Endocrinol Metab. 2004;89(6):3062-5. http://dx.doi.org/10.1210/jc.2003-031964
PMid:15181099

20. Gauna C, Meyler FM, Janssen JA, Delhanty PJ, Abribat T, van Koetsveld P, et al. Administration of acylated ghrelin reduces insulin sensitivity, whereas the combination of acylated plus unacylated ghrelin strongly improves insulin sensitivity. J Clin Endocrinol Metab 2004;89(10):5035-42. http://dx.doi.org/10.1210/jc.2004-0363
PMid:15472202

21. Tschöp M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. Diabetes. 2001;50(4):707-9. http://dx.doi.org/10.2337/diabetes.50.4.707
PMid:11289302

22. Dixon JB, Bhatthal PS, O’Brien PE. Nonalcoholic fatty liver disease: Predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology. 2001;121(1):91-100. http://dx.doi.org/10.1053/gast.2001.25540
PMid:11438497

23. Bellentani S. The epidemiology of non-alcoholic fatty liver disease. Liver Int. 2017;37(Suppl 1):81-4. http://dx.doi.org/10.1111/liv.13299
PMid:28052624

24. Drescher HK, Weiskirchen S, Weiskirchen R. Current status in testing for nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH). Cells. 2019;8(8):845. http://dx.doi.org/10.3390/cells8080845
PMid:31394730

25. Constantino MI, Molyneaux L, Limacher-Gisler F, Al-Saeed A, Luo C, Wu T, et al. Long-term complications and mortality in young-onset diabetes: Type 2 diabetes is more hazardous and lethal than Type 1 diabetes. Diabetes Care. 2013;36(12):3863-9. http://dx.doi.org/10.2337/dc12-2455
PMid:23846814

26. Kobyliaik N, Mykhalchyshyn G, Bodnar P. Relationships between acylated ghrelin and parameters of metabolic profile in patients with non-alcoholic fatty liver disease depending on transaminases activity. Res J Pharm Biol Chem Sci. 2015;6(1):1097-105.

27. Estep M, Abawi M, Jarrar M, Wang L, Stepanova M, Elariny H, et al. Association of obestatin, ghrelin, and inflammatory cytokines in obese patients with non-alcoholic fatty liver disease. Obes Surg. 2011;21(11):1750-7. http://dx.doi.org/10.1007/s11695-011-0475-1
PMid:21744131

28. Alexander M, Loomis AK, van der Lei J, Duarte-Salles T, Prieto-Alhambra D, Ansell D, et al. Non-alcoholic fatty liver disease and risk of incident acute myocardial infarction and stroke: Findings from matched cohort study of 18 million European adults. BMJ. 2019;367:l5367. http://dx.doi.org/10.1136/bmj.l5367
PMid:31594780

29. Liu X, Guo Y, Li Z, Gong Y. The role of acylated ghrelin and unacylated ghrelin in the blood and hypothalamus and their interaction with nonalcoholic fatty liver disease. Iran J Basic Med Sci. 2020;23(9):1191-6. http://dx.doi.org/10.22038/ijbms.2020.45356.10555
PMid:32963741

30. Mykhalchyshyn G, Kobyliaik N, Bodnar P. Diagnostic accuracy of acyl-ghrelin and it association with non-alcoholic fatty liver disease in Type 2 diabetic patients. J Diabetes Metab Disord. 2015;14(1):44. http://dx.doi.org/10.1186/s40200-015-0170-1
PMid:25995986

31. Neuman MG, Cohen LB, Nanau RM. Biomarkers in nonalcoholic fatty liver disease. Can J Gastroenterol Hepatol. 2014;28(11):607-18. http://dx.doi.org/10.1155/2014/757929
PMid:25575111

32. Yang J, Brown MS, Liang G, Grishin NV, Goldstein JL. Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. Cell. 2008;132(3):387-96. http://dx.doi.org/10.1016/j.cell.2008.01.017
PMid:18267071

33. Ma X, Zhao Y, Wang Q, Wu L, Wang Z, Ma X, et al. Plasma ghrelin concentrations are negatively correlated with urine albumin-to-creatinine ratio in newly diagnosed Type 2 diabetes. Diabetes Care. 2013;36(4):707-9. http://dx.doi.org/10.2337/dc12-2455
PMid:23846814

34. Ukkola O, Pöykö SM, Kesäniemi YA. Low plasma ghrelin concentration is an indicator of the metabolic syndrome. Am J Med. 2006;119(4):274-8. http://dx.doi.org/10.1016/j.amjmed.2006.08.02192
PMid:16754258

35. DeFilippis AP, Blaha MJ, Martin SS, Reed RM, Jones SR, Nasir K, et al. Nonalcoholic fatty liver disease and serum
lipoproteins: The multi-ethnic study of atherosclerosis. Atherosclerosis. 2013;227(2):429-36. http://doi.org/10.1016/j.atherosclerosis.2013.01.022 PMid:23419204

36. Chen SH, He F, Zhou HL, Wu HR, Xia C, Li YM. Relationship between nonalcoholic fatty liver disease and metabolic syndrome. J Dig Dis. 2011;12(2):125-30. http://doi.org/10.1111/j.1751-2980.2011.00487.x PMid:21401898

37. Cheung O, Kapoor A, Puri P, Sistrun S, Luketic VA, Sargeant CC, et al. The impact of fat distribution on the severity of nonalcoholic fatty liver disease and metabolic syndrome. Hepatology. 2007;46(4):1091-100. http://doi.org/10.1002/hep.21803 PMid:17610277

38. Ryysy L, Hakkinen AM, Goto T, Vehkavaara S, Westerbacka J, Halaava J, et al. Hepatic fat content and insulin action on free fatty acids and glucose metabolism rather than insulin absorption are associated with insulin requirements during insulin therapy in Type 2 diabetic patients. Diabetes. 2000;49(5):749-58. http://doi.org/10.2337/diabetes.49.5.749 PMid:10905483

39. Portillo P, Yavuz S, Brl F, Cusi K. Role of insulin resistance and diabetes in the pathogenesis and treatment of nonalcoholic fatty liver disease. Curr Hepatol Reports. 2014;13(2):159-70.

40. Paulo RC, Brundage R, Cosma M, Mielke KL, Bowers CY, Veldhuis JD. Estrogen elevates the peak overnight production rate of acylated ghrelin. J Clin Endocrinol Metab. 2008;93(11):4440-7. http://doi.org/10.1210/jc.2008-0783 PMid:18697865

Author Query???
AQ2: Kindly provide history details