Original
ISSN 2521-8492

Role of Mesencephalic Astrocyte-Derived Neurotrophic Factor Levels in Pathogenesis of Non-Alcoholic Fatty Liver

Ibtihal Hameed Gazi[1], Fadhil Jawad Al-Tu'ma[1*], Atheer Hameid Odda[1], Jawad Fadhil Al-Tu'ma[2]

[1]Department of Chemistry and Biochemistry, College of Medicine, University of Kerbala/Kerbala – Iraq.
[2]Department of Internal Medicine, Al-Zahraa Teaching Hospital, Al-Hussein Medical City, Kerbala Health Directorates/Kerbala – Iraq.
[*]Correspondence to: Fadhil Jawad Al-Tu'ma (E-mail: fadhil.jawad.altuma@gmail.com)
(*Submitted: 20 March 2023 – Revised version received: 06 April 2023 – Accepted: 23 April 2023 – Published online: 29 September 2023*)

Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) has a global prevalence of 25% and is one of the main causes of cirrhosis and hepatocellular carcinoma. Recently, the stress response protein mesencephalon-astrocyte-derived neurotrophic factor (MANF) has been shown to regulate hepatic and systemic metabolic homeostasis.

Objectives: The main purpose of this study is to investigate the relationship between mesencephalon-astrocyte-derived neurotrophic factor levels with other anthropometric indicators, and its function in the pathogenesis of non-alcoholic fatty liver disease.

Materials and Methods: A total of 120 patients with ages ranging between 40 to 73 years were included in this study and their serum samples were collected and kept at –2°C. The liver function test, lipid profile, and albumin were determined using the automated biochemistry analyzer, while the mesencephalic astrocyte-derived neurotrophic factor biomarker was determined by the ELIZA technique.

Results: Our study showed that MANF levels decrease with age, and decreased MANF levels are associated with inflammatory phenotypes. The mean levels of ALT, ALP, AST, TSB, and the ALT/AST ratio in the non-alcoholic fatty liver patients were significantly higher than that for the non-fatty liver patients. As well, the mean level of MANF in the non-fatty liver patients was 305.25 ± 110.49 mg/dl which was significantly higher in the non-alcoholic fatty liver group (157.52). ($P \leq 0.001$).

Conclusion: A novel finding of our study is that the reduction of serum MANF levels is strongly associated with the pathogenesis of non-alcoholic fatty liver disorders and could be used as a potential therapeutic target in the treatment of hepatic disorders.

Keywords: Non-alcoholic fatty liver disease, mesencephalon-astrocyte-derived neurotrophic factor, liver disorders

Introduction

Non-alcoholic fatty liver disease (NAFLD) stands out as a major contributor to the occurrence of chronic liver disease worldwide. The incidence of NAFLD and the number of people with advanced liver disease are anticipated to continue to climb in light of the ongoing pediatric obesity pandemic, the rise in diabetes, and other variables.[1] The term "non-alcoholic fatty liver disease" (NAFLD) has been redefined as "metabolic dysfunction-associated fatty liver disease".[2,3] In recent years, there have been advancements in understanding the development of NAFLD. The currently accepted theory is the "multiple-hit model," which suggests that genetic, dietary, and environmental factors interact to contribute to the development of this condition. These factors lead to insulin resistance in adipose tissue, resulting in the breakdown of fat and dysfunction of adipocytes. As a result, there is an increase in the influx of free fatty acids into the liver.[4] This leads to the accumulation of triglycerides and other lipid metabolites in the liver, such as diacyl-glycerols, long-chain acylcarnitines, and ceramides. These harmful intermediates cause mitochondrial dysfunction, oxidative stress, and chronic inflammation in the liver, which contribute to the progression of the disease. Obesity, diabetes, and hyperlipidemia are often associated with NAFLD and are present in approximately 80% of individuals with metabolic syndrome.[2–4] Non-alcoholic fatty liver disease (NAFLD) is a range of liver conditions ranging from benign steatosis to cirrhosis that necessitates liver transplantation. Several factors such as metabolic syndrome, pregnancy, nutrition, drugs, pollutants, etc. are involved in developing NAFLD. Patients with diabetes and obesity are more likely to experience it. It may also appear in asymptomatic patients. Common clinical symptoms in NAFLD patients are pain and/or discomfort in the right upper quadrant, increased liver enzyme levels, and usually an increased ALT:AST ratio, which can be treated in the early stages by modifying the diet, increasing physical activity, and weight loss. Medications that target insulin resistance, such as metformin, thiazolidinediones, lipid modifiers, and the antioxidant vitamin E, can be used to enhance the treatment effectiveness.[5]

Mesencephalic astrocyte-derived neurotrophic factor (MANF) is an endoplasmic reticulum (ER) stress-inducible protein originally known as a survival-promoting factor for brain dopaminergic neurons.[6] In many cell types, MANF expression can be induced by several ER stress inducers and can play a protective role against ER stress-induced damage. ER stress in cells results from the accumulation of unfolded or misfolded proteins which activates signaling cascades that attempt to restore physiological cell activity. A study on mice demonstrated that pancreatic-specific MANF deficiency leads to ER stress, apoptosis of pancreatic beta cells, and diabetes, whereas recombinant MANF enhances pancreatic beta cell proliferation. Furthermore, it has been shown MANF protects human pancreatic beta cells against experimentally stress-induced cell death.[7] MANF supplementation improves age-related metabolic dysfunction and reduces signs of liver aging. It also protects against diet-induced hepatosteatosis.[8]

Cloning and sequencing of MANF cDNA showed that the 4.3 kb gene encoding MANF, which has 4 exons, is located on human chromosome 3.[9] The protein encoded by this gene is localized and secreted in the endoplasmic reticulum (ER) and Golgi. MANF promotes tissue repair and regeneration by modulation of anti-inflammatory response. Decreased expression of this gene increases susceptibility to ER stress-induced

death and leads to cell proliferation.[10] Moreover, a study on newly diagnosed diabetes patients demonstrated the correlation of the circulating levels of MANF with the regulation of systemic metabolic homeostasis.[11] Several studies have shown the important role of MANF in the regulation of liver lipid metabolism and its high expression in the liver.[12] Studies using human and animal models have linked MANF to the emergence of NAFLD. Also, non-alcoholic steatohepatitis patients exhibited significantly lower serum MANF levels.[8,13]

Recent studies have shown that the reduction of MANF expression increases liver inflammation and fibrosis, while the use of MANF supplementation reverses age-related inflammatory changes (Figure 1).[14] Also, MANF plays a significant role in the proliferation and survival of normal cells and protects them from the apoptosis that caused by stresses.[15,16]

In the current work, we aimed to examine the relationship between mesencephalon-astrocyte-derived neurotrophic factor levels with other anthropometric indicators and its function in the pathogenesis of non-alcoholic fatty liver disease.

Materials and Methods

This case-control study included 120 samples of serum obtained from Al-Hussein Medical City during Dec., 2022 to June, 2023 as the source of patient sample collection, taking into account the ethics of taking the sample from patients of both sexes and different weights. Their age ranged between 40 and 73 years and the number of patients with NAFLD was 74, and another 46 samples were obtained from non-alcoholics as apparently healthy controls from relatives of both sexes. Via a sterile disposable syringe, fasting patients' five milliliters of venous blood were obtained. Approximately five minutes at room temperature were spent waiting for the blood in a gel tube to clot, then separated into four Eppendorf tubes and stored at –20°C in the freezer. Serum levels of mesencephalic astrocyte-derived neurotrophic factor, liver function tests, lipid profile, and albumins were determined in the College of Medicine laboratories at the University of Kerbala under ideal circumstances using modern devices and tools. We applied the Automated Biochemistry device to measure liver function and examine the albumin level.

The serum level of the mesencephalic astrocyte-derived neurotrophic factor was determined using the enzyme-linked immune sorbent assay (ELISA) on the other hand, lipid profile and liver function tests were determined using the colorimetric method. The following formula was used to obtain body mass index (BMI) and categorize patients into normal (BMI 18.5–24.9), overweight (25–29.9), and obese (30–34.9).[17]

$$\text{BMI (kg/m}^2\text{)} = \text{Weight/(Height)}^2$$

Results

A total of 120 participants were included in this study, 74 of them with fatty liver, and the remaining 46 samples were taken from normal cases acting as non-alcoholic fatty liver. Patients groups were divided into subgroups based on age, gender, metabolic syndrome, lifestyle, T2DM, hypertension, and smoking. The clinical demographics and laboratory test results of the study groups are compiled in Table 1. The percentage of gender obtained was 56 males of 74 patients used in this study (75.6%), whereas the remaining 18 of 74 patients (24.3%) were females, and their mean ± SD of the age of them (50.78 ± 8.21 years) and (55.21 ± 6.82 years), respectively. The overall age range of participants was (45.94%) within (40–50) years, (32.44%) within (51–60) years, and the remaining (21.62%) more than 60 years (Table 1).

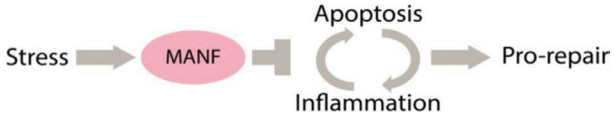

Fig. 1 **Association between MANF and liver dysfunctions.**

The difference between the level of biomarkers in the fatty liver and non-fatty liver groups indicated that patients with alcoholic fatty liver disease showed an increasing level in serum level of ALP when compared with the non-fatty liver groups, while the level of MANF was decreased compared to non-fatty liver.

As shown in Figure 2, the obtained results indicate a statistically significant difference in all biomarkers between the two groups. The mean ± SD level of ALP activity was elevated (264.57 ± 49.61 U/L), while the level of MANF decreased (157.51 ± 57.23 pg/ml) in the non-alcoholic fatty liver group, while in the non-fatty liver group, the mean ± SD level of ALP was 202.00 ± 30.50 U/l and the serum level of the MANF was 305.25 ± 110.49 pg/ml.

Furthermore, the analysis of the serum level of biomarkers in the non-fatty liver and non-alcoholic fatty liver groups showed that patients with non-alcoholic fatty liver disease had higher serum levels of ALT and AST compared to the non-fatty liver groups. The mean ± SD level of ALT and AST activity levels were (34.76 ± 10.7 U/L) and (34.62 ± 6.33 U/L) in

Table 1. **Descriptive of the demographic characteristics of the study population**

Variables	Groups	Alcoholic fatty liver *N* = 74	Non-alcoholic fatty liver *N* = 46
Age, year	40–50 years	34/74 (45.94%)	16/46 (34.8%)
	51–60 years	24/74 (32.44%)	26/46 (56.5%)
	More than 60 years	16/74 (21.62%)	4/46 (8.7%)
BMI, kg/m²	Normal weight	4	18
	Over weight	18	14
	Obese	52	14
Gender%	Male	56/74 (75.6%)	34/26 (73.9%)
	Female	18/74 (24.3%)	12/46 (26.1%)
T2DM	No	54	40
	Yes	20	6
Hypertension	No	66	36
	Yes	8	10
Metabolic syndrome	No	58	21
	Yes	16	2
Lifestyle	Sedentary lifestyle	46	24
	Active lifestyle	28	22

alcoholic fatty liver (264.57 ± 49.61 U/L), while in the non-fatty liver were (29.26 ± 11.26 U/L) and (26.96 ± 5.53 U/L) respectively (Figure 3).

In non-alcoholic fatty liver, there were significantly lower serum levels of TSB (1.03 ± 0.46 mg/dl) and ALT/AST activity (0.68 ± 0.26) compared to non-fatty liver, where the serum levels of TSB were 1.23 ± 0.33 mg/dl and ALT/AST activity was 0.99 ± 0.33 u/l. However, there was no significant difference in the serum level of albumin between the two groups (*P* value > 0.05) (Figure 4).

As shown in Figure 5, in the age groups of 40–50 years, the mean level of MANF was 408.75 ± 152.79 pg/ml, while in the age group 51–60 years it was 66.42 ± 05. 332 pg/ml and in more than 60 years was lower than other groups (Post HOC test).

Also, the results of the effect of BMI on the serum level of the MANF demonstrated that in the group 1 (normal weight) the mean ± SD level of MANF was 576.47 ± 123.94 pg/ml, while, in the group 2 (overweight) was 421.65 ± 124.97 pg/ml, and in the group 3 (obesity) was 389.85 ± 139.73 pg/ml (Figure 6).

Moreover, the result of the correlation of lipid profiles including TC, TG, LDL-C, VLDL-C, and HDL-C with MANF showed a significantly negative relationship with MANF serum level, only in the case of HDL-C this relationship was not significant (Table 2).

Binary logistic regression was performed to analyse the association between MANF, albumin, ALT, ALP, AST and TSB levels in the non-alcoholic fatty liver patients. It was found that all the levels of the investigated biomarkers act as a risk factor, only TSB is a protective factor, but it was not statistically significant (Table 3) (*P* value < 0.05).

The true positive rate (TPR), also known as sensitivity or recall, represents the proportion of actual positive instances correctly classified as positive by the model. On the other hand, the false positive rate (FPR) indicates the proportion of actual negative instances incorrectly classified as positive.

By plotting TPR against FPR at various classification thresholds, the ROC curve provides a comprehensive evaluation of a classification model's performance across different operating points. Each point on the curve corresponds to a specific threshold setting, and moving along the curve reflects adjusting this threshold. The results of the AUC analysis and ROC curve for the MANF are strongly potential diagnostic parameters. The biomarkers performed well in terms of diagnosis of the non-alcoholic fatty liver compared to the non-fatty liver group; data are presented in Figure 7 and Table 4.

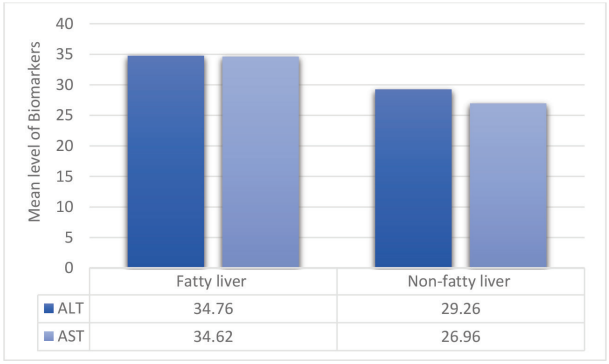

Fig. 3 **Results of analysis of ALT and AST serum levels in non-alcoholic fatty liver and non-fatty liver patients.**

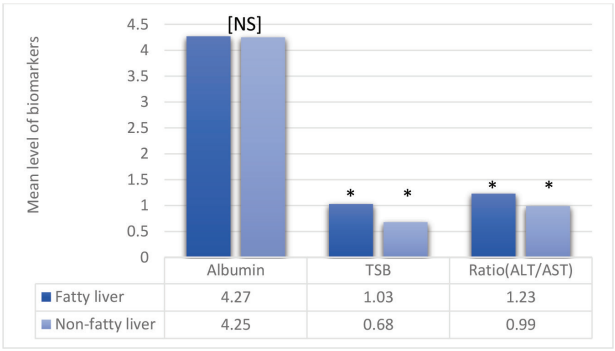

Fig. 4 **Results of analysis of Albumin, TSB, and ALT/AST serum levels in non-alcoholic fatty liver and non-fatty liver patients.**

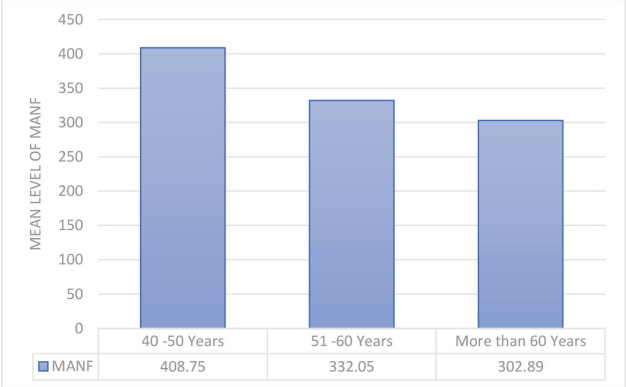

Fig. 5 **The effect of age groups 40–50, 51–60, and more than 60 years on the serum level of MANF.**

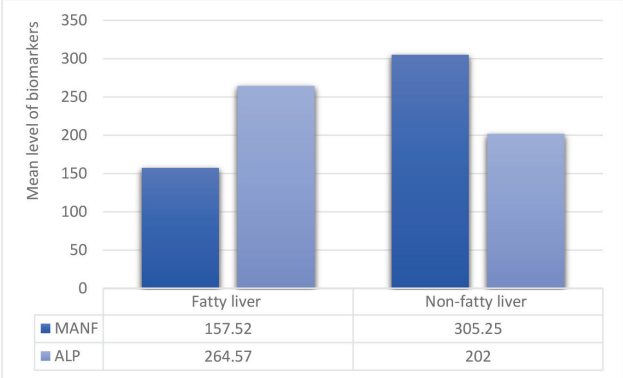

Fig. 2 **Results of analysis of ALP and MANF serum levels in non-alcoholic fatty liver and non-fatty liver patients.**

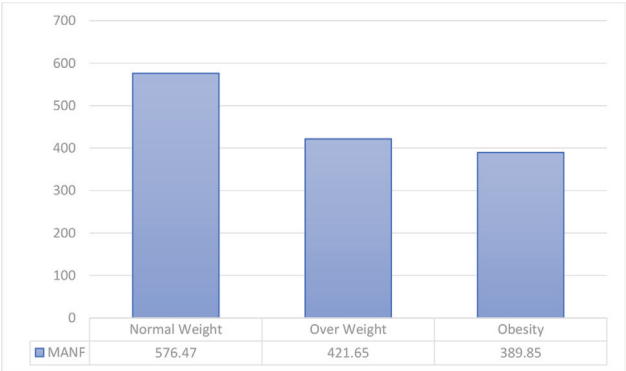

Fig. 6 **Effect of BMI groups (Normal weight), (Over weight), and (Obesity) on MANF level.**

Table 2. **Correlations between MANF with lipid profile**

Biomarkers		Correlation coefficient (*r*)	*P* (2-tailed)
	TC (mg/dl)	–0.5	**<0.001 [S]**
	TG (mg/dl)	–0.4	**<0.001 [S]**
MANF, (pg/ml)	**HDL-C (mg/dl)**	–0.3	0.09 [NS]
	LDL-C (mg/dl)	–0.4	**0.036 [S]**
	VLDL-C (mg/dl)	–0.4	**0.038 [S]**

P < 0.05 considered significantly different, [S] = Significant, [NS] = Non-significant, TC: Total cholesterol; TG: triglyceride; HDL-C: High density lipoprotein-cholesterol; LDL-C: Low density lipoprotein-cholesterol; VLDL-C: Very low-density lipoprotein-cholesterol.

Table 3. **Association between the investigated biomarkers in sera of alcoholic fatty liver as compared with that in non-alcoholic fatty liver group with respect to BMI groups (overweight and obesity)**

Variables	Groups	OR (Lower – upper)	*P* value
	Normal weight	1[a]	–
MANF (pg/ml)	**Overweight**	1.107 (0.997–1.230)	0.058 [NS]
	Obesity	1.109 (0.998–1.232v	**0.05 [S]**
	Normal weight	1[a]	–
Albumin (mg/dl)	**Overweight**	2. 5 (1.65–4.481)	0.059 [NS]
	Obesity	6.11 (1.45–8.080)	0.079 [NS]
	Normal weight	1[a]	–
ALT (U/l)	**Overweight**	1.049 (0.777–1.417)	0.755 [NS]
	Obesity	1.098 (0.816–1.477)	0.539 [NS]
	Normal weight	1[a]	–
ALP (U/l)	**Overweight**	1.001 (0.998–1.020)	0.761 [NS]
	Obesity	1.003 (0.954–1.044)	0.942 [NS]
	Normal weight	1[a]	–
AST (U/l)	**Overweight**	0.606 (0.336–1.093)	0.096 [NS]
	Obesity	0.578 (0.321–1.041)	0.068 [NS]
	Normal weight	1[a]	–
TSB (mg/dl)	**Overweight**	21.063 (0.12–24.182)	0.191 [NS]
	Obesity	1.006 (0.995–1.017)	**0.008 [S]**

P < 0.05 significantly different, [S] = Significant, [NS] = Non-significant, 1[a]: reference category is control.

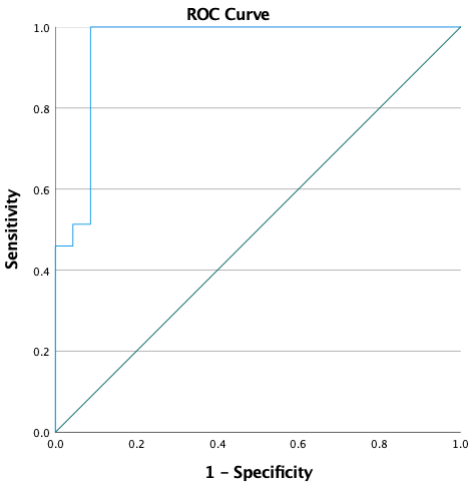

Fig. 7 **Receiver operating characteristics (ROC) curve analysis of MANF levels in patients.**

For MANF levels: (sensitivity = 98%, specificity = 91.3%) at a level = 194.527. Accordingly, the distribution of patients using MANF cut-off values is presented in Table 4.

The *P*-values of the AUC were <0.05 and statistically significant. Youden's J statistics of the parameters in Table 4 confirm these results.

It was estimated that when the cut-off value of MANF was 194.527, giving a sensitivity = 98%, specificity = 91.3% negative predictive value (NPV) was 97.6%, and positive predictive value PPV was 94.8 (Table 5).

Discussion

As indicated in this study, we focused on non-alcoholic fatty liver disease because it is an important topic and has been widely spread recently.[1] In general, high levels of liver enzymes including ALT, AST, ALP, and TSB levels were observed compared to the control group. Since the activity levels of liver enzymes are affected in all liver diseases, they may be less

Table 4a. **AUC, the ideal threshold, the proposed marker's sensitivity, and its specificity were determined by the ROC curves for MANF**

Test	AUC	Specificity %	Sensitivity %	*P* value	Cut-off points	Youden index	CI (95%)
MANF (pg/ml)	9%	91.3%	98%	**<0.001**	194.527	0.913	0.894–1.000

Table 4b. **Statistical significance and performance metrics: *P* value, accuracy, PPV, NPV**

Variables	*P* value	Accuracy	PPV	NPV
MANF (pg/ml)	**<0.001**	96.6%	94.8%	97.6%

Table 5. **Distribution of patients according to the MANF cut-off values in the main group**

Cut-off	Alcoholic fatty liver	Non-alcoholic fatty liver
<194.527	72	4
>194.527	2	42
Total	74	46

affected in fatty liver. The first step and the first to arise from these enzymes is the ALT alanine aminotransferase enzyme in non-alcoholic fatty liver disease, and this has been confirmed by previous studies.[18,19] Albumin testing was not affected in this study because the samples were from individuals with grade I nonalcoholic fatty liver disease, whereas another study showed that albumin is decreased in liver disease and liver failure.[20] The level of mesencephalic astrocyte-derived neurotrophic factor (MANF), which is a new biomarker, according to the results observed in this study, MANF was lower in the patient's serum compared to the healthy group, because the lack of MANF level leads to accumulation. Previous studies also confirmed that reduced MANF levels contribute to age-related inflammation. Liver damage and fibrosis are clinical manifestations in patients with non-alcoholic steatohepatitis. The fact that non-alcoholic hepatitis and low levels of MANF in the blood were confirmed by this study.[8] Through the results obtained in this study, we found that the level of MANF decreases with age, which is consistent with other studies that have also confirmed that the low level of MANF is associated with age, as its level decreases in old age.[8,21] Regarding obesity, the observed results showed that the level of MANF is affected by obesity, as its level decreases in patients with obesity. Our data was in disagreement with another study that showed an inverse relationship with obesity.[12] While other studies have shown that the higher the level of MANF, the greater the obesity constitutes a noticeable.[22] The lower level of MANF in other studies did not show any effect between the obesity of patients and the control group.[23] Through our results in this study, we conclude that MANF is a protective factor against non-alcoholic fatty liver. Other studies also have confirmed that MANF is a protective factor against fatty liver, and there was agreement with the results obtained in this work that MANF prevents the deposition of fat in hepatocytes, and this was confirmed by another study performed recently.[13–16] The statistical results indicated a statistically significant difference between non-alcoholic fatty liver and these markers such as ALT, AST, TSB, ALP, and MANF. The ALT/AST ratio also acts as a good marker for the identification of fatty liver disease.[19,24] This ratio indicates that the rate of the disease increases in older people and in obese people as the level of MANF decreases, and obesity acts as one potential contributor to the buildup of fatty liver in addition to diabetes, lifestyle, and other reasons, which was statistically significant in this study. This has been confirmed by many other studies in nonalcoholic fatty liver disease, which indicate the relationship between the level of MANF and lipid profiles including TC, TG, LDL-C, and VLDL-C. The data obtained revealed that there is a negative relationship between the lower level of MANF and the higher levels of these markers, and the higher the level of MANF, the lower the levels of these markers, as in a study in 2022, the MANF pathway might serve as the connecting bridge through which hypolipidemia.[25] The correlation between the results of all these markers and MANF was statistically significant. These findings were consistent with a previous study that showed MANF levels were negatively associated with total cholesterol and LDL cholesterol.[2–4]

Conclusion

A novel finding of our study is that the reduction of serum MANF levels is strongly associated with the pathogenesis of non-alcoholic fatty liver disorders and could be used as a potential therapeutic target in the treatment of hepatic disorders, this indicate that MANF prevents the deposition of fat in hepatocytes.

Ethical Approval and Consent to Participate

This research protocol was evaluated and approved on 30.05.2022 by research committee of Kerbala University.

Informed Consent

Consent to participate in the study was received from each individual person who took part in the research.

References

1. Cotter, T. G. and Rinella, M. (2020). Nonalcoholic fatty liver disease 2020: the state of the disease. Gastroenterology, 158(7):1851–1864.
2. Nasiri-Ansari, N., Androutsakos, T., Flessa, C. M., Kyrou, I., Siasos, G., Randeva, H. S. and Papavassiliou, A. G. (2022). Endothelial Cell Dysfunction and Nonalcoholic Fatty Liver Disease (NAFLD): A Concise Review. Cells, 11(16), 2511.
3. Day, C. P., and James, O. F. (1998). Steatohepatitis: a tale of two "hits"?. Gastroenterology, 114(4):842–845.
4. Buzzetti, E., Pinzani, M. and Tsochatzis, E. A. (2016). The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism, 65(8): 1038–1048.

5. Marchesini, G., Brizi, M., Morselli-Labate, A. M., Bianchi, G., Bugianesi, E., McCullough, A. J., and Melchionda, N. (1999). Association of nonalcoholic fatty liver disease with insulin resistance. The American Journal of Medicine, 107(5): 450–455.
6. Wu, H., Li, H., Wen, W., Wang, Y., Xu, H., Xu, M., ... & Luo, J. (2021). MANF protects pancreatic acinar cells against alcohol-induced endoplasmic reticulum stress and cellular injury. Journal of Hepato-Biliary-Pancreatic Sciences, 28(10), 883–892.
7. Tseng, K. Y., Danilova, T., Domanskyi, A., Saarma, M., Lindahl, M., & Airavaara, M. (2017). MANF is essential for neurite extension and neuronal migration in the developing cortex. eNeuro, 4(5).
8. Sousa-Victor, P., Neves, J., Cedron-Craft, W., Ventura, P. B., Liao, C. Y., Riley, R. R. and Jasper, H. (2019). MANF regulates metabolic and immune homeostasis in aging and protects against liver damage. Nature Metabolism, 1(2):276–290.
9. Mizobuchi, N., Hoseki, J., Kubota, H., Toyokuni, S., Nozaki, J. I., Naitoh, M., ... and Nagata, K. (2007). ARMET is a soluble ER protein induced by the unfolded protein response via ERSE-II element. Cell Structure and Function, 32(1):41–50.
10. Hellman, M., Arumäe, U., Yu, L. Y., Lindholm, P., Peränen, J., Saarma, M. and Permi, P. (2011). Mesencephalic astrocyte-derived neurotrophic factor (MANF) has a unique mechanism to rescue apoptotic neurons. Journal of Biological Chemistry, 286(4):2675–2680.
11. Kim, Y., Park, S. J. and Chen, Y. M. (2017). Mesencephalic astrocyte-derived neurotrophic factor (MANF), a new player in endoplasmic reticulum diseases: structure, biology, and therapeutic roles. Translational Research, 188:1–9.
12. Wu, T., Liu, Q., Li, Y., Li, H., Chen, L., Yang, X. and He, J. (2021). Feeding-induced hepatokine, Manf, ameliorates diet-induced obesity by promoting adipose browning via p38 MAPK pathway. Journal of Experimental Medicine, 218(6):e20201203.
13. He, M., Wang, C., Long, X. H., Peng, J. J., Liu, D. F., Yang, G. Y. and Zhang, L. L. (2020). Mesencephalic astrocyte-derived neurotrophic factor ameliorates steatosis in HepG2 cells by regulating hepatic lipid metabolism. World Journal of Gastroenterology, 26(10):1029.
14. Cordero-Llana, Ó., Houghton, B. C., Rinaldi, F., Taylor, H., Yáñez-Muñoz, R. J., Uney, J. B. and Caldwell, M. A. (2015). Enhanced efficacy of the CDNF/MANF family by combined intranigral overexpression in the 6-OHDA rat model of Parkinson's disease. Molecular Therapy, 23(2):244–254.
15. Ezhilarasan D, Lakshmi T. 2022 A Molecular Insight into the Role of Antioxidants in Nonalcoholic Fatty Liver Diseases. Oxid Med Cell Longev. 2022 May 11: 9233650.
16. Yang, L., Shen, W. W., Shao, W., Zhao, Q., Pang, G. Z., Yang, Y. and Shen, Y. X. (2023). MANF ameliorates DSS-induced mouse colitis via restricting Ly6ChiCX3CR1int macrophage transformation and suppressing CHOP-BATF2 signaling pathway. Acta Pharmacologica Sinica, 44(6):1175–1190.
17. Donini, L. M., Pinto, A., Giusti, A. M., Lenzi, A. and Poggiogalle, E. (2020). Obesity or BMI paradox? Beneath the tip of the iceberg. Frontiers in Nutrition, 7:53.
18. Schindhelm, R. K., Diamant, M., Dekker, J. M., Tushuizen, M. E., Teerlink, T., & Heine, R. J. (2006). Alanine aminotransferase as a marker of non-alcoholic fatty liver disease in relation to type 2 diabetes mellitus and cardiovascular disease. Diabetes/metabolism Research and Reviews, 22(6):437–443.
19. Su, W., Mao, Z., Liu, Y., Zhang, X., Zhang, W., Gustafsson, J. A. and Guan, Y. (2019). Role of HSD17B13 in the liver physiology and pathophysiology. Molecular and Cellular Endocrinology, 489:119–125.
20. Chen, C. B., Hammo, B., Barry, J., & Radhakrishnan, K. (2021). Overview of albumin physiology and its role in pediatric diseases. Current Gastroenterology Reports, 23(8):11.
21. Yang S., Huang S., Gaertig M. A., Li X. J., Li S. (2014). Age-dependent decrease in chaperone activity impairs MANF expression, leading to Purkinje cell degeneration in inducible SCA17 mice. Neuron, 81:349–365.
22. Tang Q, Li Y, He J. MANF: an emerging therapeutic target for metabolic diseases. Trends Endocrinol Metab. 2022 Apr;33(4):236–246. doi: 10.1016/j.tem.2022.01.001. Epub 2022 Feb 5. PMID: 35135706.
23. Galli, E., Rossi, J., Neumann, T., Andressoo, J. O., Drinda, S. and Lindholm, P. (2019). Mesencephalic astrocyte-derived neurotrophic factor is upregulated with therapeutic fasting in humans and diet fat withdrawal in obese mice. Scientific Reports, 9(1):14318.
24. Adams, L. A., Angulo, P. and Lindor, K. D. (2005). Nonalcoholic fatty liver disease. CMAJ, 172(7):899–905.
25. Xu, K., Zheng, P., Zhao, S., Wang, M., Tu, D., Wei, Q. and Xie, P. (2022). MANF/EWSR1/ANXA6 pathway might as the bridge between hypolipidemia and major depressive disorder. Translational Psychiatry, 12(1), 527.