MJMR, Vol. 35, No. 2, 2024, pages (130-135)

Open Access
ISSN: 2682-4558

Research Article

Pattern of PNPLA3 gene polymorphism among MAFLD patients in Minia Governorate, Egypt.

Mahmoud Abu El-Eneen Khattab[1], Yousef Ismail Moussa[1], lamia Hamdy[2], Omnia Ahmed Hussein[1] and Elham Ahmed[1]

[1] Department of Internal Medicine, Faculty of Medicine, Minia University, Egypt.
[2] Department of Clinical Pathology, Faculty of Medicine, Minia University, Egypt

DOI: 10.21608/mjmr.2024.216304.1414

Abstract

Background: The frequency of MAFLD/NAFLD is surpassing twenty percent and increased in most areas of the world. Egypt is considered among the most 10 countries in predominance of obesity which forms one of the major components of MAFLD. The "multiple hit" concept shows that MAFLD is activated by a combination of several insults acting on susceptible individuals. The PNPLA3 gene is expressed in both adipocytes and hepatocytes and It's an essential factor in controlling the livers lipid metabolism. The PNPLA3 type is communicated in both adipocytes and hepatocytes, and It's fundamental in controlling the liver's lipid metabolism. **Aim:** The objective of this research was to study the pattern of PNPLA3 polymorphism C/G (rs738409) among Egyptian MAFLD. **Patients Methods:** This study included 80 participants with MAFLD and 20 healthy control. Demographic, biochemical, and hematological tests were performed on all participants. The diagnosis of liver steatosis was done by Conventional and Dixon MRI examination of liver. Liver fibrosis was assessed by share wave elastography Detection of PNPLA3 polymorphism C/G (rs738409) in the serum was performed with real-time PCR. **Results:** The objective of our work was to show the form of PNPLA3 polymorphism C/G (rs738409) among Egyptian MAFLD persons. Methods: We included 80 persons who had MAFLD and 20 healthy controls. We performed Demographic, biochemical, and hematological laboratory investigations on all groups of the study. We assessed Liver steatosis using Conventional and Dixon MRI examination of liver. We assessed liver fibrosis using share wave elastography. Detection of PNPLA3 polymorphism C/G (rs738409) at the serum was by real-time PCR. Results: The mutant PNPLA3 polymorphism (GG) was higher in 59 MAFLD members (74%) than the controls around 5 members (25%). This finding was detected regardless of presence or absence of diabetes or obesity. (P value < 0.001). **Conclusion:** The mutation of PNPLA3 plays role in pathogenesis of MAFLD in Egyptian patients.

Keywords: MAFLD, PNPLA3, SNP

Introduction

Non-alcohol fatty liver disease (NAFLD) was slated for replacement in 2020 by the more accurate nomenclature of metabolic associated fatty liver disease (MAFLD). The MAFLD diagnosis is by hepatic steatosis as well as one of the three metabolic diseases overweight/obesity, diabetes mellitus type 2, or signs of metabolic dysregulation (MD) in lean people [1].

Detection of fatty liver is by hepatic steatosis in more than five percent of hepatocytes as decided by histological examination or >5.6% as decided by quantitative fat/water selective magnetic resonance imaging (MRI) [2].

The prevalence of MAFLD in Egypt had changed. It becomes 56.7% instead of 46.7%, with fibrosis influencing 56.7% of people. [3].

The process by which MAFLD is created is still obscure. The "multiple hit" hypothesis is a more exact clarification of pathogenesis since it analyzes how many insults working together on people who are genetically susceptible to developing MAFLD can create the condition. Insulin resistance, hormones delivered by fat tissue, dietary factors, intestinal microbiota, as well as genetic and epigenetic variables are all included in this category [4].

MAFLD is characterized by insulin resistance, which can be caused by many causes involving soluble mediators released by immune cells and/or adipose tissue, for illustration TNF-α & IL-6 [5].

Hyperinsulinemia, which is activated by peripheral insulin resistance, can increase hepatic denovo lipogenesis by keeping up parts of the insulin signaling pathway which is known as specific selective insulin resistance. A dysregulation of fat tissue lipolysis can happen when there is insulin resistance in fat tissue. This may raise the stream of fatty acids from adipocytes to the liver [6].

The I148M variation of the human patatin-like phospholipase domain-containing 3 gene (PNPLA3) has been examined broadly in a number of clinical settings due to its relationship with a higher probability of hepatic steatosis and more serious shapes of the condition, including nonalcoholic steatohepatitis, progressed fibrosis & cirrhosis [7].

The rs738409 C>G single nucleotide polymorphism (SNP) encodes the isoleucine to methionine substitution at position 148 (I148M) inside the PNPLA3 gene. Triglyceride and retinyl palmitate esterase actions are due to PNPLA3. The replacement of isoleucine instead of methionine causes a loss of function in these enzyme activities resulting in an impairment of lipid catabolism, lipid droplet remodeling and VLDL secretion this would promote hepatocellular triglyceride accumulation during insulin resistance [8].

People who carry the PNPLA3 I148M variation have a substantially greater likelihood of having cirrhosis and hepatocellular carcinoma and this hazard is independent of their susceptibility to develop steatosis this demonstrates that PNPLA3 is directly implicated in both the process of fibrogenesis as well as the process of carcinogenesis [9].

Patients & Methods

Our retrospective Case control study was conducted at an outpatient clinic of hepatology and gastroenterology at Minia university hospital. It included 80 cases with MAFLD, and 20 people as control matched for age and sex. The research protocol was performed in agreement with the Declaration of Helsinki and with the approval of the Institutional Ethics Committee.

Patients

The study included 100 persons who were divided according to ultrasound findings into 5 groups: first group included 25 over weight patients who are diagnose according their BMI ≥25 with type 2 DM patients ,second group included 15 lean patients with BMI ≤ 25 with type 2DM patients ,the third group included 20 MAFLD ,non-diabetic obese patients, the fourth group included 20 MAFLD ,non-diabetic lean patients and The fifth group consisted of 20 healthy individuals serving as a case control population.

Methods

All subjects were exposed to: Complete history with specific attention to age, sex, residence, smoking history, alcohol intake, drug history, family history of liver disease, history of diabetes mellitus with full clinical examination was done by measuring systemic blood pressure, BMI, waist and waist hip ratio. The height weight measuring scale was employed to determine weight and height. Calculation of BMI was done through the equation: [BMI= Weight (kg) / Height2 (m2) [10].

Whole blood samples were collected by venipuncture, taken care to avoid hemolysis, under complete aseptic

conditions in sterile tubes and containers. 15 ml of blood was withdrawn from all study subjects fasting 10 hours. This sample was divided as follow: Four ml blood were collected in 2 sterile tube containing EDTA solution, 2ml used for CBC that It was influenced by automated cell counter, Sysmex KX-21N (TAO Medical Incorporation, Japan).

Morever 2 ml Blood stored at -80 OC for DNA extraction, Prothrmbin time, concentration and international normalized ratio (INR) were done using thrombrel-s (human throplastin containing calcium) from Behring diagnostic Inc. USA[11]. By STAGO ANALYSER.

Three ml blood on the plane tube was left to clot in the incubator then centrifuged. Expressed serum is utilized for determination of LFTS, renal function tests, fasting BG and fasting insulin level were determined by fully automated chemistry auto-analyzer system SELECTRA PRO XL (ELI TECH GROUP clinical system) (France).

Three ml blood on the plane tube was left to coagulate in the incubator then centrifuged. Expressed serum is utilized for estimation of thyroid function by ELISA, ferritin, ceruloplasmin, anti-nuclear antibody (ANA) was estimated by ELISA, HBs Ag (ELISA), HCV Abs (ELISA) and lipid profile which include Triglycerides (TG): Serum sample stored at -20°C by spectrophotometer or colormeter. Total cholesterol (TC): serum sample stored at -20°C by spectrophotometer or colormeter, High density lipoprotein cholesterol (HDL): From serum was precipitated by phosphotungstate in the presence of Mg ions, after centrifugation the supernatant containing HDL, The HDLc determined using the total cholesterol enzymatic reagent (Kaplan, 1984). Low density lipoprotein cholesterol (LDL) = Total cholesterol –HDL- (Triglycerides/5) [12].

Genomic DNA was extracted from frozen human whole blood by PREP-NA DNA/RNA extraction minikit from DNA-TECHNOLOGY Biosystems, Thermo Fisher Scientific, Waltham, Massachusetts United States. Single nucleotide polymorphism (SNP) for PNPLA3 I148M (rs738409) was genotyped by TaqMan allelic discrimination, using predesigned TaqMan SNP genotyping assays. Kits supplied by Applied (Biosystems, Thermo Fisher Scientific, Waltham, Massachusetts United States) which used the 5′ nuclease assay for amplifying and detecting specific SNP alleles in purified genomic DNA samples. MAFLD was diagnosed according to recent criteria [2] and liver steatosis was estimated by Conventional and Dixon MRI and liver fibrosis was estimated by Elastography shear wave for determining the degree of fibrosis.

Biostatistical analysis:

Unless otherwise specified, data is provided as medians and ranges. IBM SPSS version 23 (IBM, New York, USA) was utilized to conduct statistical analyses. Parametric and nonparametric data were distinguished using the Kolmogorov-Smirnov test for normality. As applicable, independent sample t-tests were utilized for the analysis of quantitative data. Chi-squared (X2) measurement. For all tests, the probability (p) was deemed insignificant if≥ 0.05 and significant if < 0.05

Results

Table (1) shows the demographic and descriptive characteristics of the study groups. The group's study showed highly significant differences in gender, DM, and hypertension in MAFLD patients compared to healthy controls (P=0.015, <0.001 & 0.002) correspondingly. This denotes that female sex is a risk factor of MAFLD especially in presence of obesity and diabetes. And hypertension presents more frequently in MAFLD patients who are obese and diabetic than in the other groups. DM is a risk factor in diabetic MAFLD patients. And hypertension presents more frequently in MAFLD patients who are obese and diabetic than in the other groups. DM is a risk factor in diabetic MAFLD patients.

The table (2,3,4) showed that the mutant type of PNPLA3 polymorphism gene was frequently higher in MAFLD patients than control group regardless of presence or absence of diabetes or obesity. (P value < 0.001), and the mutant type of gene was significantly associated with higher degree of steatosis. Although our data show higher frequency of this mutant type of PNPLA-3 gene (GG genotype) with higher grades of fibrosis, yet the p value was not statistically significant (p=0.527).

Table (1): Demographic and clinical characteristics of groups study:

		Obese diabetics (I)	**Lean diabetics (II)**	**Obese non-diabetics (III)**	**Lean non-diabetics (IV)**	**Control (V)**	**P. value**
		N=25	N=15	N=20	N=20	N=20	
Age	*Range* *Mean ± SD*	(21-63) 44±11.5	(20-52) 40±9.1	(27-59) 44.3±10.2	(27-65) 40.1±10.6	(22-65) 41±11.5	*0.539*
Sex	*Male* *Female*	3(12%) 22(88%)	8(53.3%) 7(46.7%)	3(15%) 17(85%)	9(45%) 11(55%)	7(35%) 13(65%)	*0.015**
DM2	*No* *Yes*	0(0%) 25(100%)	0(0%) 15(100%)	20(100%) 0(0%)	20(100%) 0(0%)	20(100%) 0(0%)	*<0.001**
HTN	*No* *Yes*	11(44%) 14(56%)	10(66.7%) 5(33.3%)	15(75%) 5(25%)	17(85%) 3(15%)	19(95%) 1(5%)	*0.002**

Table (2): Comparison of pattern of PNPLA3 gene polymorphism among study groups:

		Groups					**P. value**
		Obese diabetics (I)	**Lean diabetics (II)**	**Obese non-diabetics (III)**	**Lean non-diabetics (IV)**	**Control (V)**	
		N=25	**N=15**	**N=20**	**N=20**	**N=20**	
PNPLA3	**Wild** **Hetero** **Mutant**	0 (0%) 4 (16%) 21 (84%)	3 (20%) 4(26.7%) 8(53.3%)	2 (10%) 2 (10%) 16 (80%)	2 (10%) 4 (20%) 14 (70%)	9 (45%) 6 (30%) 5 (25%)	**0.001***

Table (3): Showed relation between pattern of PNPLA3Cand degree of fibrosis by share wave

		PNPLA3			**P. value**
SW		**wild**	**hetero**	**Mutant**	0.527
	F0-1	0	3	10	
	F1-2	0	0	3	
	F2	2	3	7	
	F2-3	1	4	7	
	F3	4	4	27	
	F4	0	0	5	

Table (4): Showed relation between pattern of PNPLA3 and degree of steatosis by MRI:

MRI		PNPLA3			P. value
		wild	hetro	Mutant	0.042
	1	6 (85.7%)	10(71.4%)	22(38.6%)	
	2	1(14.3%)	3(2.4%)	27(47.4%)	
	3	0(0%)	1(7.2%)	10(17.5%)	

Discussion

Our research concluded that the mutation of PNPLA3 polymorphism gene was higher in MAFLD patients than control group regardless of presence or absence of diabetes or obesity. (P value < 0.001). The mutation of gene increased between patients with increased degree of steatosis. Additionally The mutation of gene increased between patients with increased degree of fibrosis (f4), These findings coordinate with the study of Guangrong Dai,et al, 2019 which reported strong relation between PNPLA3 rs738409 polymorphism and NAFLD. Additionally, the study detected strong relation between PNPLA3 rs738409 polymorphism and NAFLD aggressive[13]. The gene polymerphism was shown to be increased in children susceptible to NAFLD especially the sever form [14].

An Italian PNPLA3 mutation had no relation with BMI but the variant was so important in lean persons with NASH or F2 [15].

A research was done in Japan on adults who had NAFLD detected increased G allele in normal weight (18.5 kg/m2 −22.9 kg/m2) (OR 3.52; 95%-CI: 1.42–8.71; P = 0.0063) and overweight patients (23 kg/m2–24.9 kg/m2) (OR 2.60; 95%-CI: 1.14–5.91; P = 0.0225), but not in obese patients (BMI ≥ 25 kg/m2) [16].

Another study from of Japan also found a higher incidence of the GG allele (47.8% vs 36.5% P = 0.02) in non-obese NAFLD (BMI < 25 kg/m2) vs obese (BMI ≥ 25 kg/m2 [17].

Huapeng Lin, et al,2021 study was on 904 persons. Amongst those with NAFLD, lean subjects (30.3%) had higher PNPLA3 rs738409 GG genotype than overweight (17.9%) and obese subjects (17.4%) (P = .003). The GG genotype was higher in lean subjects than the cc genotype [18].

The exact role of PNPLA3 rs738409 in pathogensis of NAFLD is not clear but some studies had explained that PNPLA3 mutation has tricylglycerol (TG) hydrolase and lysophosphatidyl acyltransferase (LPAAT) and calcium independent phospholipase A2 activities. Additionally, PNPLA3 is important for regulation of lipid metabolism. PNPLA3 mutant protein results in hydrolysis of hepatic glycerol-lipid in the liver and inhibits fat release into peripheral fatty tissue which causes fatty liver and its complications [19].

References

1. Eslam M, Newsome P, Sarin S, et al., (2020). A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. J Hepatol.; 73: 202–209.
2. Zheng, K. I., Fan, J. G., Shi, J. P., et al., (2020). From NAFLD to MAFLD: a “redefining” moment for fatty liver disease. Chinese medical journal, 133(19), 2271.
3. Tomah S, EID EM, Abouelmagd MM, Hassan AH, Eldib AH, et al., (2019). 214‐LB: Vibration controlled transient elastography reveals alarming prevalence of nonalcoholic fatty liver disease and fibrosis among young adults in Egypt. Am Diabetes Assoc.;68(Supl 1).
4. Buzzetti, E., Pinzani, M., & Tsochatzis, E. A. (2016). The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism, 65(8), 1038-1048.

5. Gonzalez-Jaramillo, V., Portilla-Fernandez, E., Glisic, M., et al., (2019). Epigenetics and inflammatory markers: a systematic review of the current evidence. International journal of inflammation.
6. Jelenik, T., Kaul, K., Séquaris, G., et al., (2017). Mechanisms of insulin resistance in primary and secondary nonalcoholic fatty liver. Diabetes, 66(8), 2241-2253.
7. Francesca Virginia Bruschi, Matteo Tardelli, Thierry Claudel et al., (2017) PNPLA3 expression and its impact on the liver: current perspectives Hepat Med.Nov 6;9:55-66.
8. Pirazzi, C., Valenti, L., Motta, B. M., et al. (2014). PNPLA3 has retinyl-palmitate lipase activity in human hepatic stellate cells. Human molecular genetics, 23(15), 4077-4085.
9. Valenti, L., Dongiovanni, P., Corradini, S. G., et al., (2013). PNPLA3 I148M variant and hepatocellular carcinoma: a common genetic variant for a rare disease. Digestive and Liver Disease, 45(8), 619-624.
10. Romero-Corral A, Somers VK, Sierra-Johnson J, et al., Accuracy of body mass index in diagnosing obesity in the adult general population. Int J Obes. 2008; 32:959–66.
11. Zaman, B. A., Rasool, S. O., Merza, M. A., & Abdulah, D. M. (2021). Hepcidin-to Ferritin Ratio: A Potential Novel Index to Predict Iron Overload-Liver Fibrosis in ß Thalassemia Major. Transfusion Clinique et Biologique.
12. Young, D.S. Effects of disease on clinical lab. Tests, 4th ed AACC, 2001. Grove TH: Effect of reagent pH on Determination of HDL cholesterol by precipitation with phosphor-tungstate-magnesium Clin Chem 25:560, 1979.
13. Guangrong Dai, Pengfei Liu, Xiaomei Li et al., (2019) Association between PNPLA3 rs738409 polymorphism and nonalcoholic fatty liver disease (NAFLD) susceptibility and severity Medicine Baltimore.98(7): e14324.
14. Shan Tang, Jing Zhang, Ting and Ting Mei. (2020) Association of PNPLA3 rs738409 G/C gene polymerphism with nonalcoholic fatty liver disease in children: a meta-analysis BMC Med Genet. 21: 163.
15. Fracanzani AL, Petta S, Lombardi R, et al., (2017). Liver and cardiovascular damage in patients with lean nonalcoholic fatty liver disease, and association with visceral obesity. Clinical Gastroenterology and Hepatology. ;15(10):1604–1611.e1..
16. Nishioji K, Mochizuki N, Kobayashi M, et al., (2015). The impact of PNPLA3 rs738409 genetic polymorphism and weight gain ≥10 kg after age 20 on non-alcoholic fatty liver disease in non-obese Japanese individuals. PLoS ONE. ;10(10).
17. Honda Y, Yoneda M, Kessoku T, et al., (2016) Characteristics of non-obese non-alcoholic fatty liver disease: effect of genetic and environmental factors. Hepatol Res.;46(10): 1011–8..
18. Huapeng Lin, Xinrong Zhang, Guanlin Li, Grace Lai-Hung Wong and Vincent Wai-Sun Wong (2021), Epidemiology and Clinical Outcomes of Metabolic (Dysfunction)-associated Fatty Liver Disease Journal of Clinical and Translational Hepatology vol. 9(6) | 972–982.
19. Pirazzi, C., Valenti, L., Motta, B. M., Pingitore, P., Hedfalk, K., Mancina, R. M., and Romeo, S. (2014). PNPLA3 has retinyl-palmitate lipase activity in human hepatic stellate cells. Human molecular genetics, 23(15), 4077-4085.