Original Research Article

The Egyptian Journal of Immunology

The Egyptian Journal of Immunology
Volume 29 (2), 2022: 76–86.
www.Ejimmunology.org

Role of Thymosin Beta 4 in the diagnosis of Nonalcoholic Fatty Liver and its relation to Metabolic Syndrome in Egyptian patients

Ahmed E. Ahmed, Wesam A. Ibrahim, Zeinab M. Nabil, Khaled A. Mansour, Ahmed M. F. Mansour and Ahmed M. ElGhandour

Gastroenterology and Hepatology Unit, Internal Medicine Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Corresponding author: Zeinab M. Nabil, Gastroenterology & Hepatology Unit, Internal Medicine Department, Faculty of Medicine, Ain Shams University, 11566 Cairo, Egypt.
Email: zeinab.mohamed@med.asu.edu.eg.

Abstract

Nonalcoholic fatty liver disease (NAFLD) is a spectrum of hepatic diseases linked to metabolic and cardiovascular disorders that impair quality of life and increase morbidity and mortality. There has been significant interest in replacing conventional diagnostic tools such as liver biopsy with non-invasive biomarkers for the diagnosis of NAFLD. Thymosin Beta 4 (Tβ4) is a G-actin sequestering peptide involved in many critical biological processes. This study aimed to evaluate the role of Tβ4 in the diagnosis of NAFLD, and its relation to metabolic syndrome. Eighty patients were enrolled in this study, divided into two equal groups of NAFLD cases (n=40) and a control group (n=40). The two groups were subjected to history taking, physical examination, measurement of waist circumference and body mass index (BMI). Laboratory workup included serum Tβ4, insulin resistance (HOMA-IR), fibrosis-4 score (FIB-4), fatty liver index (FLI) and NAFLD fibrosis score (NFL) were calculated for both groups. Serum Tβ4 was significantly lower in NAFLD patients (P <0.001) and there was a significant positive correlation between serum Tβ4 and HDL (P = 0.034). On the other hand, there was a significant negative correlation between serum Tβ4 and waist circumference (P <0.001), total cholesterol level (P <0.001), insulin level (P <0.001), HOMA-IR (P <0.001), serum triglycerides (P= 0.025) and FLI (P = 0.004). Serum Tβ4 at a cut-off value of ≤900 ng/ml had 100 % sensitivity, 100 % specificity, 100% positive predictive value and 100% negative predictive value for the prediction of NAFLD. In conclusion, serum Tβ4 could be used as a biomarker for the diagnosis of NAFLD.

Keywords: Thymosin Beta 4, Nonalcoholic Fatty Liver, Metabolic Syndrome.

Date received: 12 February 2022; **accepted:** 26 March 2022.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is rapidly becoming the leading cause of end-stage liver disease and consequently liver transplantation with significant morbidity and mortality.[1] Metabolic syndrome is not only common in NAFLD patients, but its components also increase the risk of developing NAFLD.[2] Consequently, improving insulin resistance, for

instance, may reduce the prevalence of NAFLD and nonalcoholic steatohepatitis (NASH).[3] There has been a lot of interest in developing clinical prediction rules and non-invasive biomarkers to replace liver biopsy or identify candidates for liver biopsy.[4]

Thymosin beta 4 (Tβ4) is a G-actin sequestering peptide and is mainly related to the regulation of actin polymerization in living cells.[5] It participates in various critical biological processes, including apoptosis, cell migration, angiogenesis, fibrosis, and wound healing.[6]

Inflammation is a key process in NAFLD pathogenesis. The development of NAFLD is accompanied by obesity as well as metabolic disruptions that cause excessive hepatic lipid accumulation. Liver steatosis increases the vulnerability of the liver to oxidative stresses or proinflammatory insult, resulting in NAFLD. Thus, measures that suppress oxidative stress and inflammation could prevent the development of NAFLD.[7] Some studies have observed that Tβ4 has anti-inflammatory effects.[8,9] Other studies suggested that Tb4 is negatively correlated with endotoxemia and could suppress proinflammatory TLR signaling and reduce inflammatory cytokines.[10,11] The main aim of this study was to evaluate the role of serum Tβ4 in the diagnosis of nonalcoholic fatty liver disease and its relation to metabolic syndrome.

Materials and Methods

This case-control study was conducted on 80 subjects recruited from the Internal Medicine and Hepatology department at Ain Shams University Hospital from August 2019 to July 2020. The Research Ethics committee at the Faculty of Medicine, Ain Shams University reviewed and approved the protocol of the study (FMASU/MD/126/2019). Informed consent was taken from all subjects participated in the study.

Subjects were divided into two groups; GROUP I (Case group), included 40 Egyptian patients diagnosed with NAFLD using abdominal ultrasonography and abdominal computed tomography (CT) while GROUP-II included 40 apparently healthy Egyptian controls verified via outpatient physical examinations, normal liver ultrasound imaging and routine blood, liver and kidney functions, blood lipids and blood glucose tests.

Patients consuming alcohol >20 g/day (women) and >30 g/day (men), patients with concomitant HBV, HCV infection, hereditary metabolic disease (hemochromatosis and Wilson's disease), liver cirrhosis and malignant tumors were excluded from the study. Serum samples from study participants were collected and immediately aliquoted, frozen, and kept at −80°C until tested.

All study subjects were subjected to the following**:** full history taking and clinical examination, calculation of body mass index (BMI) based on the formula: BMI = kg/m² where kg is a person's weight in kilograms and m² is their height in meters squared.[12], and waist circumference.

Laboratory investigations included complete blood count (CBC) by an automated analyzer (Sysmex XN-1000™ Hematology Analyzer, USA). And, fasting blood glucose (FBG), liver function tests (LFTs) which included serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), total and direct bilirubin, serum albumin, and gamma-glutamyl transferase (GGT), serum creatinine, and lipid profile, all were assessed using an automated analyzer (AU480 chemistry analyzer- Beckman Coulter, USA). In addition, HBsAg, HCVAb, and HIVAb were measured an analyzer (The cobas® e 411 analyzer-Roche, USA). Prothrombin time and INR were evaluated by an automated analyzer (Sysmex CS-1600 Coagulation Analyzer, USA). All were performed according to the manufacturer's instructions.

Serum Thymosin Beta 4 (Tβ4) levels were measured by an enzyme-linked immunosorbent assay (ELISA) kit (Bioassay Technology laboratory, Shanghai, China, Cat. No E4436Hu), according to the manufacturer's instructions. The kit employs an antibody specific for human Tβ4 coated on a 96-well plate. Standards and samples were pipetted into the wells, and Tβ4 present in a sample was bound to the wells by the immobilized antibody. The wells were

washed, and the biotinylated anti-human Tβ4 antibody was added. After washing away the unbound biotinylated antibody, horseradish peroxidase (HRP)-conjugated streptavidin was pipetted to the wells. The wells were again washed, and a tetra- methylbenzidine (TMB) substrate solution was added, and color developed in proportion to the amount of Tβ4 bound. The Stop Solution changed the color from blue to yellow, and the intensity of the color was measured by Multiskan™ FC Microplate Photometer (Thermo Fisher 51119000, USA) at 450 nm. The mean absorbance for standards and samples was calculated. A standard curve was plotted on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. A best-fit straight line through the standard points was drawn, sensitivity: 15.79ng\ml, standard curve:30 ng\ml-9000 ng\ml.

HbA1C was measured by a colorimetric method (Biosystems S.A. Costa Brava, 30.08030 Barcelona, Spain). Fasting Insulin hormone level was measured by an ELISA kit (Precheck Bio, Inc., Anaheim, USA, CA 92801), according to the manufacturer's instructions (Normal values: 0.7-9 µU/ml. (Or mU/L).

Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated to evaluate IR and β-cell function using the following formula: HOMA-IR = ([fasting glucose (mg/dL) × fasting insulin (µU/mL)]/405). Normal values=0.5–1.4. Above 1.9 indicates early insulin resistance, above 2.9 indicates significant insulin resistance.[13]

Imaging

Abdominal ultrasonography.[14]

Non-contrast abdominal CT, in accordance with the diagnosis criteria of NAFLD as follows: hepatic attenuation 10 Hounsfield units (HU) less than splenic attenuation on unenhanced CT, or an absolute liver attenuation of less than 40 HU on unenhanced CT, which are criteria commonly used in the clinical practice for diagnosis.[15]

Fatty liver Index (FLI): this algorithm, special formula, was used to support the diagnosis of fatty liver and the referral of suspected patients to ultrasonography based on patient BMI, waist circumference, and serum triglyceride and gamma-glutamyl-transferase levels.[16]

Fibrosis-4 score (FIB-4): this non-invasive scoring system was used to estimate the amount of scarring in the liver; (Age* x AST) / (Platelets x √(ALT)) (not to be used in patients <35 or >65 years old, as the score has been less reliable in these patients).[17]

NAFLD fibrosis score (NFS): this scoring system was used to independently identify NAFLD patients with and without advanced fibrosis. NAFLD Score = -1.675 + (0.037*age [years]) + (0.094*BMI [kg/m2]) + (1.13*IFG/diabetes [yes = 1, no = 0]) + (0.99*AST/ALT ratio) – (0.013*platelet count [×109/L]) – (0.66*albumin [g/dl]).[18]

Statistical analysis

The collected data were coded, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 20. Quantitative data were expressed as mean ±SD (standard deviation) for normally distributed data. In quantitative data, independent t-test was used to compare two independent groups with normally distributed data and paired t-test in cases of two dependent groups with normally distributed data. Analysis of variance (ANOVA) was used to compare more than two independent groups with normally distributed data, followed by post hoc test to compare between each two groups. In qualitative data, inferential analyses for independent variables were done using Chi Square (X^2) test for differences between proportions. Correlations were done using Pearson correlation coefficient test. Multiple regression analysis was done to assess the strength of the relationship between the dependent variable and several predictor variables as well as the importance of each of the predictors to the relationship, often with the effect of other predictors statistically eliminated. The receiver operating characteristic (ROC) curve was constructed to obtain the most sensitive and specific cutoff values for different parameters. A p value less than 0.05 was considered statistically significant.

Results

Group I included 40 NAFLD patients, comprising 23 (57.5%) males and 17 (42.5%) females, with a mean age of 34.775 ± 6.878 years. Group II included 40 Egyptian volunteers as controls, comprising 26 (65%) males and 14 (35%) females, with a mean age of 32.225 ± 6.573 years. There was no significant difference between the two groups regarding age and sex (P>0.05).

NAFLD patients had statistically significant higher BMI, waist circumference and incidence of hypertension than controls as outlined in Table 1.

Table 1. Comparison between the NAFLD patients and control groups regarding demographic findings

	Group				
	Group I (cases)		Group II (controls)		
Age					[a]*P*-value
Range	25 - 58		21 - 45		NS
Mean ±SD	34.775 ± 6.878		32.225 ± 6.573		
Sex	N	%	N	%	[b]*P*-value
Male	23	57.50	26	65.00	Ns
Female	17	42.50	14	35.00	
BMI (kg/m²)					[a]*P*-value
Range	28.4 - 42.9		19.1 - 25.6		<0.001
Mean ±SD	34.323 ± 3.639		22.743 ± 1.310		
Waist (cm)					[a]*P*-value
Range	101 - 130		74 - 95		<0.001
Mean ±SD	113.750 ± 7.503		84.725 ± 6.202		
Hypertension	N	%	N	%	[b]*P*-value
No	33	82.50	40	100.00	0.006
Yes	7	17.50	0	0.00	

Abbreviations: SD, standard deviation; N, number; BMI, body mass index. [a]T-Test [b]Chi-Square.

P value >0.05 is. Not significant (NS).

Concerning laboratory findings, NAFLD patients had statistically significant higher levels of HDL, Total Cholesterol, Serum Triglycerides, Serum Insulin levels, HBA1C and insulin resistance (HOMA-IR) as compared to controls (P<0.001), Table 2.

Fatty liver index and NAFLD fibrosis scores were significantly higher in NAFLD patients than in the controls (P<0.001). However, there was no significant difference in Fibrosis-4 score between the two groups (Table 3).

Table 2. Comparison of laboratory data between the NAFLD patients' group and the controls.

		Group		*P-value
		Group I (cases)	Group II (controls)	
Triglycerides (mg/dl)	Range	111-204	78-135	<0.001
	Mean ±SD	158.425±24.063	111.525±15.359	
Total Cholesterol(mg/dl)	Range	119-288	119-186	<0.001
	Mean ±SD	198.050±31.902	143.200±16.610	
HDL (mg/dl)	Range	29-60	52-90	<0.001
	Mean ±SD	43.450±8.006	67.625±9.366	
AST (IU/L)	Range	8-40	12-33	NS
	Mean ±SD	20.775±8.813	21.975±5.994	
ALT (IU/L)	Range	9-36	9-43	NS
	Mean ±SD	22.700±7.187	20.500±9.362	
Albumin (g/dl)	Range	3.5-5	3.5-4.8	NS
	Mean ±SD	4.255±0.357	4.105±0.421	
Insulin level (µIU/ml)	Range	10-40	2-9	<0.001
	Mean ±SD	20.900±6.242	5.200±2.053	
HOMA-IR	Range	2-13	0.4-1.9	<0.001
	Mean ±SD	5.533±2.484	1.073±0.449	
HBA1C	Range	4.9-11	4.5-5.5	<0.001
	Mean ±SD	6.805±1.644	4.975±0.326	

Abbreviations: SD, standard deviation; HDL, high density lipoprotein; AST, aspartate transaminase; ALT, alanine transaminase; HOMA-IR, insulin resistance; HbA1c, hemoglobin A1c. *T-Test. *P* value >0.05 is. Not significant (NS).

Table 3. Comparison of serum biomarkers between the NAFLD patients' group and the controls.

		Group		*P-value
		Group I (cases)	Group II (controls)	
Fatty liver Index	Range	68-100	10-29	<0.001
	Mean ±SD	87.375±7.999	18.425±5.505	
Fibrosis-4 Score	Range	0.23-1.03	0.26-1.54	NS
	Mean ±SD	0.544±0.207	0.625±0.324	
NAFLD fibrosis score	Range	-3.38--0.84	-5.37--1.35	<0.001
	Mean ±SD	-2.161±0.504	-3.450±1.023	

Abbreviations: SD, standard deviation; n, NAFLD, nonalcoholic fatty liver disease .*T-Test.
P value >0.05 is. Not significant (NS).

Serum Tβ4 levels were significantly lower in the NAFLD patients than in controls (*P*<0.001) (Table 4). For NAFLD patients' group, there was a statistically significant positive correlation between serum Tβ4 levels and serum HDL (r = 0.337, *P* = 0.034). However, there was a statistically significant negative correlation between serum Tβ4 level and waist circumference (r = -0.571, p <0.001), serum total cholesterol (r = -0.531, *P* <0.001), serum

insulin levels (r = -0.584, *P*<0.001), HOMA-IR (r = -0.538, *P* <0.001 (as shown in Figure 1) and serum triglycerides (r = -0.354, *P*= 0.025) (Table 5).

Furthermore, serum Tβ4 levels were significantly negatively correlated with fatty liver index (r= -0.445, *P* = 0.004) (as shown in Figure 2) while there was no significant difference between serum Tβ4 levels and Fibrosis-4 score (r = 0.123, *P* = 0.448) or NAFLD fibrosis score (r = -0.008, *P* = 0.962) (Table 5).

Regression analysis showed that waist circumference, serum total cholesterol, HDL and insulin level had the highest impact on serum Tβ4 (Table 6).

Table 4. Comparison between serum concentrations of Thymosin Beta 4 in the NAFLD patient group and the control group.

Thymosin Beta 4 (ng/ml)	Group		*P-value
	Group I (cases)	Group II (controls)	
Range	300-900	1000-9600	<0.001
Mean ±SD	663.750±175.041	3316.250±2363.841	

*T-Test Abbreviations: SD, standard deviation, *P* value <0.05 is. Significant.

Table 5. Correlation between serum Thymosin Beta 4 levels and other study parameters.

Study parameters	Thymosin Beta 4	
	R	*P*-value
Age	0.086	NS
BMI	-0.061	NS
Waist	-0.571	<0.001
Triglycerides	-0.354	0.025
Total Cholesterol	-0.531	<0.001
HDL	0.337	0.034
AST	-0.036	NS
ALT	-0.081	NS
Albumin	0.195	NS
Insulin level	-0.58	<0.001
HOMA-IR	-0.538	<0.001
HbA1c	-0.017	NS
Fatty liver index	-0.445	0.004
Fibrosis-4 score	0.123	NS
NAFLD fibrosis score	-0.008	NS

Abbreviations: BMI, body mass index; HDL, high density lipoprotein; AST, aspartate transaminase; ALT, alanine transaminase; HOMA-IR, insulin resistance; HbA1c, hemoglobin A1c; NAFLD, nonalcoholic fatty liver disease.

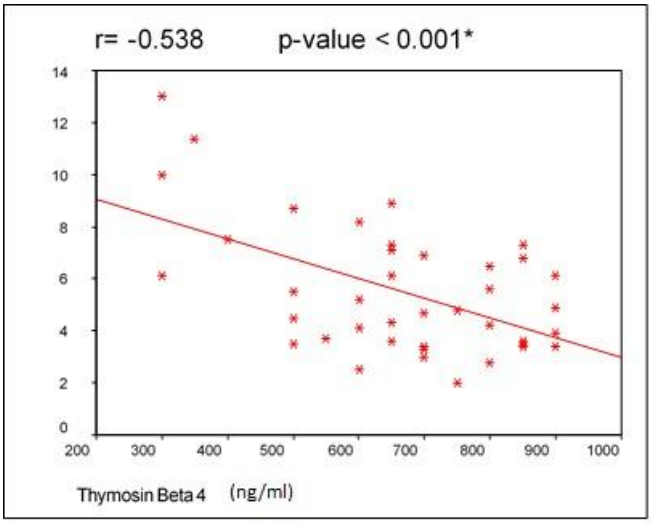

Figure 1. Scattered blot to measure the correlation between serum Tβ4 and HOMA-IR.

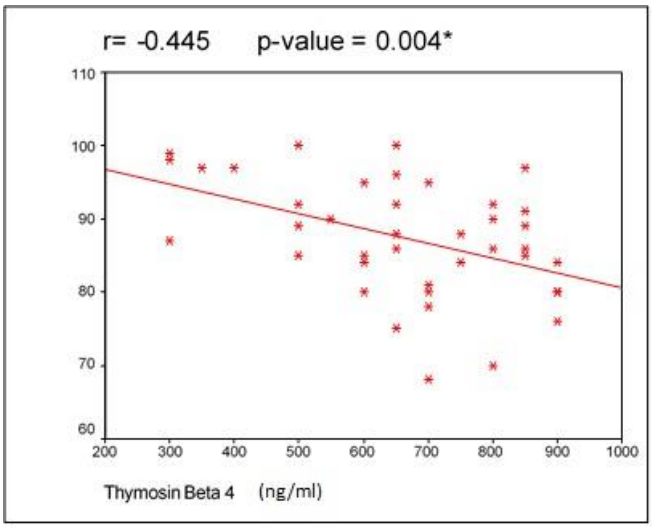

Figure 2. Scattered blot to measure the correlation between serum Tβ4 and Fatty liver index.

Table 6. Regression analysis of serum Thymosin Beta 4 and all other variables in the study.

	Unstandardized Coefficients		Standardized Coefficients	*P*-value
	B	Std. Error	Beta	
Waist	-9.235	2.778	-0.396	0.002
Triglycerides	1.086	0.979	0.149	NS
Total Cholesterol	-1.605	0.755	-0.293	0.041
HDL	6.520	2.407	0.298	0.011
Insulin level	-18.740	8.618	-0.668	0.037
HOMA-IR	22.369	21.617	0.217	NS

Dependent Variable: Thymosin Beta 4. Abbreviations: HDL, high density lipoprotein; HOMA-IR, insulin resistance

P value >0.05 is. Not significant (NS).

The ROC curve analysis of serum Tβ4 for diagnosis of NAFLD showed the following: serum Tβ4 level at a cutoff value ≤900 ng/ml had a 100% sensitivity, a 100% specificity, 100% positive predictive value (PPV) and 100% negative predictive value (NPV) for the prediction of NAFLD patients (as shown in Figure 3).

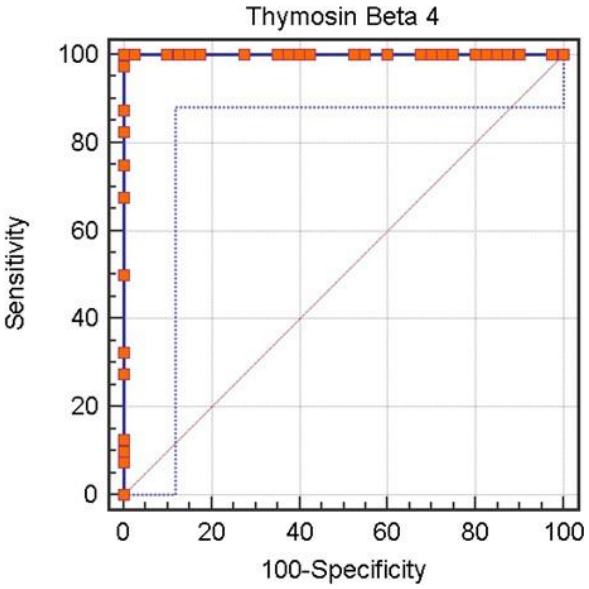

Figure 3. ROC curve of the best cut-off value of serum Tβ4 in discriminating the NAFLD patients (Tβ4 ≤900 ng/ml) from the control subjects (Tβ4 >900 ng/ml).

Discussion

Thymosin beta 4, a major actin-sequestering protein, may serve as a non-invasive biomarker for diagnosing NAFLD and determining its severity.[19] In this study, NAFLD was found to be more prevalent in males than females, where the total studied NAFLD patients comprised 23 males (57%) and 17 females (43%); this conformed with the findings of a study conducted in 2017.[20] This may be attributed to the protective role of the estrogen in females against the development of NAFLD.[21] Furthermore, waist circumference and body mass index were significantly higher in NAFLD patients than in the study controls. Also, hypertension was more prevalent in NAFLD patients than in the study controls.

The current study also showed that the levels of Triglycerides, HDL, HBA1C and fasting blood glucose were significantly higher in NAFLD patients than in the controls. Numerous cross-sectional studies illustrated that NAFLD is strongly related to metabolic Syndrome.[22, 23, 24] Almeda et al., (2009)[25] also reported that the development of NAFLD is strongly associated with the metabolic syndrome, as reflected by the observation that approximately 90% of the NAFLD patients have more than one feature of metabolic syndrome and about 33% have three or more criteria.

Other studies indicated that NAFLD can predict a higher incidence of metabolic Syndrome.[26, 27, 28] Ballestri et al., (2016)[29] reported that NAFLD is related to almost two-fold increase in the incidence of type 2 diabetes and metabolic syndrome.

In the present study, HOMA-IR was significantly higher in NAFLD patients than in the study controls. Multiple studies demonstrated

that NAFLD is strongly associated with both hepatic and adipose tissue insulin resistance.[30, 31, 32]

In this study, there was no statistical difference in serum ALT and AST levels between the NAFLD patients and the controls. These findings are consistent with the finding that up to 50% of NAFLD patients can have normal serum ALT and AST levels.[33] Also, there was no statistical difference in serum albumin, GGT levels and Fibrosis-4 score. On the other hand, fatty liver index and NAFLD fibrosis score were significantly higher in patients with NAFLD than in the controls.

This study showed that serum Tβ4 was significantly lower in NAFLD patients than in the controls. Jiang et al., (2019)[34] studied 76 patients with NAFLD and 130 controls and reported that serum Tβ4 levels in NAFLD patients were significantly lower than in the controls. Also, Dong et al., (2013)[35] compared the concentration of serum Tβ4 between NAFLD patients and normal controls and reported that serum Tβ4 levels in patients with NAFLD were significantly lower. After treatment and subsequent improvement in liver function, the concentration of Tβ4 was increased. Tian et al., (2014)[36] studied 83 cases of NAFLD and 80 healthy controls. They reported that Tβ4 level could effectively be used as a biomarker of liver function, as increased Tβ4 level indicated improved liver function and decreased Tβ4 level revealed severe liver damage. Liang et al., (2016)[37] concluded that serum Tβ4 levels in patients with chronic hepatitis B (CHB) combined with NAFLD were not different from those with simple CHB, and Tβ4 expression in serum and tissues was lower in the severe inflammation and fibrosis group. They suggested that Tβ4 is also involved in the regulation of chronic inflammation and fibrosis, playing a defensive role in the disease progression of CHB combined NAFLD patients. The decreased Tβ4 concentration suggested a more significant inflammation and fibrosis progression.

Jiang et al., (2018) studied 24 patients with NAFL and 21 patients with NASH diagnosed with liver biopsy. Serum Tβ4 was significantly lower in patients with NASH than NAFL and negatively correlated with histological activity score and the overall NAFLD activity score. Furthermore, it was also related to the condition of oxidative stress. Therefore, they concluded that serum concentrations of Tβ4 could be used to differentiate between NASH and NAFL.[38]

In the present study, regression analysis of serum Tβ4 showed that the waist circumference, total serum cholesterol, HDL and fasting insulin levels have the highest impact on serum Tβ4 with strong statistical significance between them and serum Tβ4.

Jiang et al., (2019)[34] calculated the correlation coefficients between serum Tβ4 levels and other study parameters. They found that serum Tβ4 was positively correlated with serum adiponectin levels, which can decrease insulin resistance. While, Tβ4 was negatively correlated with total cholesterol, triglyceride, total bilirubin, gamma GT, HOMA-IR, and IL-6, which are closely related to insulin resistance in NAFLD.

Zhu et al., (2012)[39] studied the effects of Tβ4 on hyperglycemia and insulin sensitivity in a type 2 diabetes mellitus mouse model and reported that Tβ4 decreased glucose intolerance and improved insulin resistance. Another study demonstrated that Tβ4 could reduce mean fasting and 2-hour blood glucose levels during oral glucose tolerance testing.[40] These studies suggested that Tβ4 treatment may improve insulin sensitivity and glucose tolerance in NAFLD.

Finally, ROC curve analysis, in the present study, demonstrated the best cut-off of serum Tβ4 that can discriminate the NAFLD patients from the healthy controls. A cut off value of ≤900 IU/L for serum Tβ4 is noted with a 100 % sensitivity and 100% specificity. This finding suggests that Tβ4 can be used as biomarker for diagnosis of NAFLD. In conclusion, data of our study indicated that serum Tβ4 could be used as a biomarker for the diagnosis of NAFLD and determination of its severity.

Acknowledgements

The authors express their gratitude to staff members of Internal Medicine Department, Hepatology and Gastroenterology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Author Contributions

AMF, KAM, WAH proposed the subject for research and conducted final revision of gathered data. ZMN, AEM collected the relevant data. ZMN, AMF prepared the manuscript. AME, WAI outlined the study design and revised the manuscript. All authors read and approved the final manuscript.

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.

Funding

The author(s) denies receipt of any financial support for the research, authorship, and/or publication of this article.

Ethical approval

Research Ethics committee at the Faculty of Medicine, Ain Shams University reviewed and approved the protocol of the study (FMASU/MD/126/2019).

Informed consent

Informed consent was taken from all subjects participated in the study.

References

1. Carr RM, Oranu A, Khungar V (2016). Nonalcoholic Fatty Liver Disease: Pathophysiology and Management. *Gastroenterol Clin North Am.* Dec; 45(4): 639-652.

2. Fujioka K (2015). Current and emerging medications for overweight or obesity in people with comorbidities. *Diabetes Obes Metab*; 17: 1021-1032.

3. Ratziu V, Bellentani S, Cortez-Pinto H, et al. (2010): A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol*; 53: 372–384.

4. Chalasani N, Younossi Z, Joel E, et al. (2018). The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology*; 67(1): 328-357.

5. Ballweber E, Hannappel E, Huff T, et al. (2002). Polymerization of chemically crosslinked actin: thymosin beta (4) complex to filamentous actin: Alteration in helical parameters and visualization of thymosin beta (4) binding on F-actin. *J Mol Biol*; 315: 613-25.

6. Sosne G, Qiu P, Goldstein AL, et al. (2010). Biological activities of thymosin beta 4 defined by active sites in short peptide sequences. *Faseb J*; 24: 2144-51.

7. Videla LA, Tapia G, Rodrigo R, et al. (2009). Liver NF-kappaB and AP1 DNA binding in obese patients. *Obesit*y (Silver Spring); 17:973e979.

8. Gupta S, Kumar S, Sopko N, et al. (2012). Thymosin b4 and cardiac protection: implication in inflammation and fibrosis. *Ann N Y Acad Sci*; 1269: 84e91.

9. Qiu P, Wheater MK, Qiu Y, et al. (2011). Thymosin beta4 inhibits TNF-alpha-induced NF-kappaB activation, IL-8 expression, and the sensitizing effects by its partners PINCH-1 and ILK. *FASEB J*; 25: 1815e1826.

10. Santra, M, Chopp, M, Zhang, Z, et al. (2012). Thymosin beta 4 mediates oligodendrocyte differentiation by upregulating p38 *MAPK. Glia*, 60(12), 1826–1838.

11. Santra M, Zhang ZG, Yang J, et al. (2014). Thymosin b4 up-regulation of microRNA-146a promotes oligodendrocyte differentiation and suppression of the Toll-like proinflammatory 438 pathway. *J Biol Chem*; 289: 19508-19518.

12. Gadzik J. (2006). "How much should I weigh?" Quetelet's equation, upper weight limits, and BMI prime. *Conn Med.* Feb ;70(2).

13. Matthews DR, Hosker JP, Rudenski AS, et al. (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia;* 28: 412–419.

14. Paul J (2020). Recent advances in non-invasive diagnosis and medical management of non-alcoholic fatty liver disease in adult. *Egypt Liver Journal;* 10: 37.446.

15. Qian Li, Manish Dhyani, Joseph R Grajo, et al (2018). Current status of imaging in nonalcoholic fatty liver disease. *World J Hepatol;* 10(8): 530-542.

16. Bedogni G, Bellentani S, Miglioli L, et al. (2006): The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol*; 6: 33.452.

17. Sterling RK, Lissen E, Clumeck N, et al. (2006). Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology*; 43:4551317-1325.

18. Angulo P, Hui JM, Marchesini G, et al. (2007). The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology*; 45: 846-854.458.

19. Kim J, Jung Y (2015). Potential role of thymosin Beta 4 in liver fibrosis. *Int J Mol Sci.;* 16:10624-10635.
20. Ballestri S, Nascimbeni F, Baldelli E, et al. (2017). NAFLD as a Sexual Dimorphic Disease: Role of Gender and Reproductive Status in the Development and Progression of Nonalcoholic Fatty Liver Disease and Inherent Cardiovascular Risk. *Advances in therapy*; 34(6): 1291-1326.
21. Dietrich P, Hellerbrand C (2014). Non-alcoholic fatty liver disease, obesity and the metabolic syndrome. *Best Pract Res Clin Gastroenterol.*; 28(4): 637-53.
22. Chiloiro M, Caruso MG, Cisternino AM, et al. (2013). Ultrasound evaluation and correlates of fatty liver disease: a population study in a Mediterranean area. *Metab Syndr Relat Disord*.; 11(5): 349-58.
23. Juárez-Rojas JG, Medina-Urrutia AX, Jorge-Galarza E, et al. (2013). Fatty liver increases the association of metabolic syndrome with diabetes and atherosclerosis. *Diabetes Care.*Jun; 36(6): 1726-8.
24. Arulanandan A, Ang B, Bettencourt R. et al. (2015). Association Between Quantity of Liver Fat and Cardiovascular Risk in Patients with Nonalcoholic Fatty Liver Disease Independent of Nonalcoholic Steatohepatitis. Clin Gastroenterol Hepatol 13, 1513–1520.e1511.
25. Almeda-Valdés P, Cuevas-Ramos D, Aguilar-Salinas CA (2009). Metabolic syndrome and non-alcoholic fatty liver disease. Ann Hepatol; 8 Suppl 1: S18-24.
26. Adams LA, Waters OR, Knuiman MW, et al. (2009): NAFLD as a risk factor for the development of diabetes and the metabolic syndrome: an eleven-year follow-up study. *Am J Gastroenterol.;* 104(4): 861-7.
27. Ryoo JH, Choi JM, Moon SY, et al. (2013). The clinical availability of nonalcoholic fatty liver disease as an early predictor of the metabolic syndrome in Korean men: 5-year's prospective cohort study. *Atherosclerosis*; 227(2): 398-403.
28. Zhang T, Zhang Y, Zhang C, et al. (2014) Prediction of metabolic syndrome by non-alcoholic fatty liver disease in northern urban Han Chinese population: a prospective cohort study. *PLoS One.* May 6; 9(5): e96651.
29. Ballestri S, Zona S, Targher G, et al. (2016). Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. *J Gastroenterol Hepatol.* May; 31(5):936-44.
30. Utzschneider KM, Kahn SE (2006): Review: The role of insulin resistance in nonalcoholic fatty liver disease. *J Clin Endocrinol Metab*.; 91(12): 4753-61.
31. Bugianesi E, Moscatiello S, Ciaravella MF, et al. (2010). Insulin resistance in nonalcoholic fatty liver disease. *Curr Pharm,* pp. 1941-19.
32. Khan RS, Bril F, Cusi K, et al. (2019). Modulation of insulin resistance in nonalcoholic fatty liver disease*. Hepatology*; 70: 711- 724.
33. Neuschwander-Tetri BA, Caldwell SH (2003). Nonalcoholic steatohepatitis: summary of an AASLD single topic conference. *Hepatology*; 37: 1202–1219.
34. Jiang Y, Zhang Y, Ma CH, et al. (2019). Association between thymosin beta4 and non-alcoholic fatty liver disease. *Rev Esp* Enferm Dig.; 111(4): 308-313.
35. Dong QY, Han T, Wang LF, et al. (2013). The significance of serum thymosin b4 levels in patients with non-alcoholic fatty liver disease. *Tianjin Med J*; 41: 97e100.
36. Tian Y, Wang YB, Tan LL, et al. (2014). Appraisal value of Tb4 on liver function of non-alcoholic liver disease. *Chin J Gastroenterol Hepatol*; 23: 432e434.
37. Liang J, Cai WJ, Han T, et al. (2016). The expression of thymosin b4 in chronic hepatitis B combined nonalcoholic fatty liver disease. *Medicine* (Baltimore); 95: e5763.
38. Jiang Y, Han T, Zhang ZG, et al. (2018). Serum thymosin beta4 as a noninvasive biomarker in patients with nonalcoholic steatohepatitis. *Rev Esp Enferm Dig.* Jan;110(1):19-24.
39. Zhu J, Su L, Ye L, et al. (2012). Thymosin beta4 ameliorates hyperglycemia and improves insulin resistance of KK CgAy/J mouse. Diabetes *Res Clin Pract*; 96: 53e59.
40. Zhu J, Su LP, Zhou Y, et al. (2015). Thymosin b4 attenuates early diabetic nephropathy in a mouse model of type 2 diabetes mellitus*. Am J Ther;* 22: 141-146.