The Egyptian Journal of Hospital Medicine (July 2021) Vol. 84, Page 1786-1790

Chemerin As A Non-Invasive Serum Marker for Non-Alcoholic Fatty Liver Disease

Mohamed M Salama, Essam Byoumy, Azza Mohamed, Wesam Ibrahim,
Ahmed El-shafie, Mohamed Abdallah, Ghada A Mohamed*
Gastroenterology and Hepatology Unit, Department of Internal Medicine,
Faculty of Medicine, Ain Shams University, Cairo 11591, Egypt
***Corresponding author:** Ghada Abdelrahman Mohamed, Gastroenterology and Hepatology Unit, Department of Internal Medicine, Faculty of Medicine, Ain Shams University, Cairo 11591, Egypt.
E-mail ghadaabdelrahman@med.asu.edu.eg http://orcid.org/0000-0003-0320-1011

ABSTRACT

Background: Attempts have been made to recognize noninvasive markers for the identification of non-alcoholic fatty liver disease (NAFLD). Chemerin is a newly defined adipokine linked to insulin resistance and adipogenesis.

Objective: This study intended to evaluate the diagnostic ability of serum chemerin compared with NAFLD fibrosis score as a noninvasive marker for the diagnosis and grading of NAFLD.

Patients and methods: We enrolled 60 NAFLD patients and categorized them into 3 subgroups based on the fatty liver grade by ultrasound. Thirty healthy participants were recruited as a control group. ELISA method was used for serum chemerin levels measurement. **Results:** Serum chemerin levels were significantly higher in NAFLD cases than controls ($p \leq 0.001$). Furthermore, these levels were positively correlated with the fatty liver grade ($p \leq 0.001$). Serum chemerin was comparable to NAFLD fibrosis score as regards NAFLD diagnosis ($p \leq 0.001$). Patients within the gray zone of NAFLD fibrosis score had a significantly higher serum chemerin levels in comparison to patients under the gray zone ($p \leq 0.001$).

Conclusion: Serum chemerin is a promising marker for diagnosing and grading of NAFLD. More research is required to determine its definitive clinical benefit in patients with NAFLD.

Keywords: Non-alcoholic fatty liver disease, Chemerin, Adipokines, Obesity, NAFLD fibrosis score

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease that affect 30% of the population and 40%-70% of overweight persons [(1)]. The occurrence of NAFLD is growing due to the rising pandemic of obesity [(2)]. NAFLD is described as the excess deposition of lipid in the patients' liver in the absence of alcohol abuse and other reasons of hepatic steatosis. It includes a broad scale of severity extending from simple steatosis to non-alcoholic steatohepatitis (NASH), in which steatosis is combined with inflammation and fibrosis [(3)] with the risk of evolution to hepatic cirrhosis and hepatocellular carcinoma [(4)].

NAFLD is the hepatic component of metabolic syndrome [(5)]. This syndrome is characterized by obesity-linked insulin resistance and inflammation which are known by increased inflammatory cytokines levels and pro-inflammatory pathway stimulation [(6, 7)]. Currently, liver biopsy is still the gold method for the precise NAFLD diagnosis. However, it is invasive, expensive, and containing a risk of sampling errors and significant complications [(8)]. Hence, there is a serious need to recognize and validate a simple, noninvasive test that precisely diagnose and determines the stage of NAFLD.

The liver interacts with adipose tissue [(9)], which is not only an energy-storage organ but also considered as an endocrine organ that secrete polypeptides named adipokines [(10)]. An increasing evidence from reports shows that adipokines are linked to many physiological processes, such as inflammation, immunity, insulin resistance, and NAFLD [(11)].

Chemerin is known as retinoic acid receptor response protein 2 (RARRES2). It is produced as prochemerin (inactive form) then stimulated through C-terminal cleavage by coagulation and inflammatory serine proteases [(12)]. Chemerin can activate the immune inflammatory reaction by binding to chemerin-like receptor 1 (CMKLR1), chemerin receptor (ChemR) 23, and chemokine (CCmotif) receptor-like (CCRL) 2 [(13)]. The visceral adipose tissue and the hepatocytes are the main sources of chemerin construction [(14)]. Studies found that obesity and insulin-resistance cause a stimulation of the chemerin/ CMKLR1 signalling path, which subsequently trigger the inflammatory response [(15, 16, 17)].

In the current study, we intended to evaluate the diagnostic ability of serum chemerin compared with NAFLD fibrosis score as a noninvasive marker for the diagnosis and grading of NAFLD.

PATIENTS AND METHODS

This case-control study included 60 NAFLD cases in addition to 30 healthy controls. All participants were enrolled from Ain Shams University Hospitals. NAFLD patients were further equally categorized into three subgroups according to the grade of the disease.

Cases with other aetiologies of hepatic disease (e.g. alcohol consumption > 20 g/day, viral hepatitis, primary biliary cholangitis, autoimmune hepatitis, primary sclerosing cholangitis, Wilson's disease, hemochromatosis, α1-antitrypsin deficiency, drug-induced liver disease), and those on total parenteral nutrition or taking medications affecting carbohydrate or lipid metabolism were excluded.

NAFLD Evaluation:

Ultrasonography was done to evaluate the grades of steatosis. Grading of NAFLD was done in line with the

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY-SA) license (http://creativecommons.org/licenses/by/4.0/**)**

Received: 5/4/2021
Accepted: 1/6/2021

macrovesicular steatosis and described as grade 0: no steatosis; grade I: up to 33% steatosis; grade II: 33-66% steatosis; grade III: > 66% steatosis [18].

NAFLD Fibrosis Score:

According to **Angulo *et al.* [19],** NAFLD fibrosis score < -1.455 = F0 – F2, NAFLD fibrosis score = -1.455 – 0.675 = undetermined score, and NAFLD fibrosis score > 0.675 = F3 – F4.

Serum chemerin level measurement:

Serum chemerin level was measured using enzyme immunoassay kit (Chongqing Biospes Co. Ltd., China). The lower recognition limit for the measurement of serum chemerin levels was 0.044 ng/mL. Mean inter- and intra-assay coefficients of variation (CV%) were 5.2 and 6.7%, respectively.

Ethical approval:

This study followed the ethics regulations of the 1975 Declaration of Helsinki and its appendices and was accepted by the Ethical Board of the Faculty of Medicine, Ain Shams University (FWA 000017585). Written informed approval was gained from each participant enrolled in this work.

Statistical analysis:

The Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) software version 20 was used for analysis. The following tests were utilized to assess differences for significance: difference of qualitative variables by Chi square test (X^2), differences between parametric numerical data by *t*-test, differences between non-parametric numerical data by Mann-Whitney test, and multiple parametric differences by One-way ANOVA test, or Kruskal-Wallis test in non-parametric variables. Correlations were performed by Pearson's or Spearman's ρ-correlation test, as appropriate. ROC curve was applied to evaluate the diagnostic performance of serum chemerin for the diagnosis of NAFLD. P value of ≤ 0.05 was considered statistically significant.

RESULTS

The current study was performed on 60 cases with NAFLD and 30 controls, they were 46 females (51.1%) and 44 males (48.9%). Cases' characteristics are presented in Table (1). Serum chemerin levels were significantly higher in NAFLD cases than controls ($p \leq 0.001$) (Table 1). Furthermore, these levels were positively correlated with the NAFLD grade ($p \leq 0.001$) (Table 2 and Fig. 1). Other laboratory differences according to the grade of NAFLD are presented in Table (2).

Table (1): Comparison between cases and controls as regards patients' characteristics

		Group		P
		Control (n = 30)	Case (n = 60)	
Age (year)		39.80 ± 7.17	41.20 ± 7.25	0.122
BMI		24.32 ± 2.84	37.58 ± (5.52	0.00
Sex	Female	14 (46.7%)	32 (53.3%)	0.55
	Male	16 (53.3%)	28 (46.7%)	
Smoking	No	12 (40%)	48 (80%)	0.00
	Yes	18 (60%)	12 (20%)	
HTN	No	30 (100%)	51 (85%)	0.025
	Yes	0 (0.0%)	9 (15%)	
DM_IGT	No	30 (100%)	36 (60%)	0.00
	Yes	0 (0.0%)	24 (40%)	
NAFLD fibrosis score		-4.12 (-5.5 - -2.9)	0.045 (-2.8 -1.76)	0.00
Serum chemerin (ng/mL)		87.58 ± 8.47	227.38 ± 44.72	0.00
Serum cholesterol (mg/dL)		165.13 ± 12.62	212.36 ± 19.45	0.00
Serum triglycerides (mg/dL)		124.63 ± 9.99	183.05 ± 18.98	0.00
INR		1.04 ± 0.05	1.12 ± 0.07	0.00
RBS mg/dL		104.16 ± 14.26	191.96 ± 8.40	0.00
AST (IU/L)		20.10 ± 5.49	49.95 ± 4.44	0.00
ALT (IU/L)		19.86 ± 3.87	49.80 ± 14.35	0.00
Serum Albumin (g/dL)		4.44 ± 1.28	3.76 ± 0.28	0.077
Platelets (per mcL)		319833 ± 141	200000 ± 656	0.00
Total Bilirubin (mg/dL)		0.58 ± 0.16	0.88 ± 0.17	0.00
Total Protein (g/dL)		6.95 ± 0.84	6.57 ± 0.65	0.022
Creatinine (mg/dL)		0.73 ± 0.14	0.73 ± 0.12	0.999
Urea (mg/dL)		29.40 ± 4.43	30.23 ± 4.64	0.418
Hemoglobin (g/dL)		13.88 ± 0.74	13.51 ± 1.28	0.150

BMI: body mass index, HTN: Hypertension, DM_IGT: diabetes and impaired glucose tolerance, INR: international normalized ratio, RBS: random blood sugar, AST: aspartate transaminase, ALT: alanine transaminase.

Table (2): Comparison between NAFLD patients' characteristics according to the grade of NAFLD

	Grade I (n = 20)	Grade II (n = 20)	Grade III (n = 20)	P
Serum chemerin (ng/mL)	175.36 ± 16.41	228.76 ±14.07	278.02 ± 13.95	0.00
NAFLD fibrosis score	-2.03- (-2.87 – -1.28)	0.10 (-1.07 - 1.19)	1.46 (1.22 - 1.76)	0.00
BMI	30.76 ± 1.42	36.11 ± 1.87	45.87 ± 3.37	0.00
Cholesterol (mg/dL)	191.20 ± 6.35	210.80 ± 5.97	235.10 ± 9.00	0.00
Triglycerides (mg/dL)	163.50 ± 5.28	180.25 ± 5.82	205.40 ± 11.01	0.00
INR	1.09 ± 0.05	1.14 ± 0.07	1.14 ± 0.08	0.065
RBS (mg/dL)	171.25 ± 5.38	185.35 ± 8.83	219.30 ± 8.12	0.159
AST (IU/L)	35 ± 2.63	47.40 ± 5.13	67.45 ± 7.10	0.00
ALT (IU/L)	35.80 ± 3.34	46.40 ± 3.89	67.20 ± 8.72	0.00
Albumin (g/dL)	3.90 ± 0.32	3.65 ± 0.23	3.75 ± 0.24	0.021
Platelets per (mcL)	268250 ± 408	174600 ± 660	157150 ± 219	0.00
Total Bilirubin (mg/dL)	0.67 ± 0.13	0.84 ± 0.21	1.13 ± 0.09	0.00
Total Protein (g/dL)	6.82 ± 0.60	6.39 ± 0.63	6.51 ± 0.68	0.097
Creatinine (mg/dL)	0.75 ± 0.14	0.68 ± 0.12	0.75 ± 0.11	0.162
Urea (mg/dL)	29.10 ± 4.55	30.85 ± 5.21	30.75 ± 4.14	0.416
Hemoglobin (g/dL)	13.64 ± 1.16	13.57 ± 1.60	13.34 ± 1.07	0.746

BMI: body mass index, INR: international normalized ratio, RBS: random blood sugar, AST: aspartate transaminase, ALT: alanine transaminase

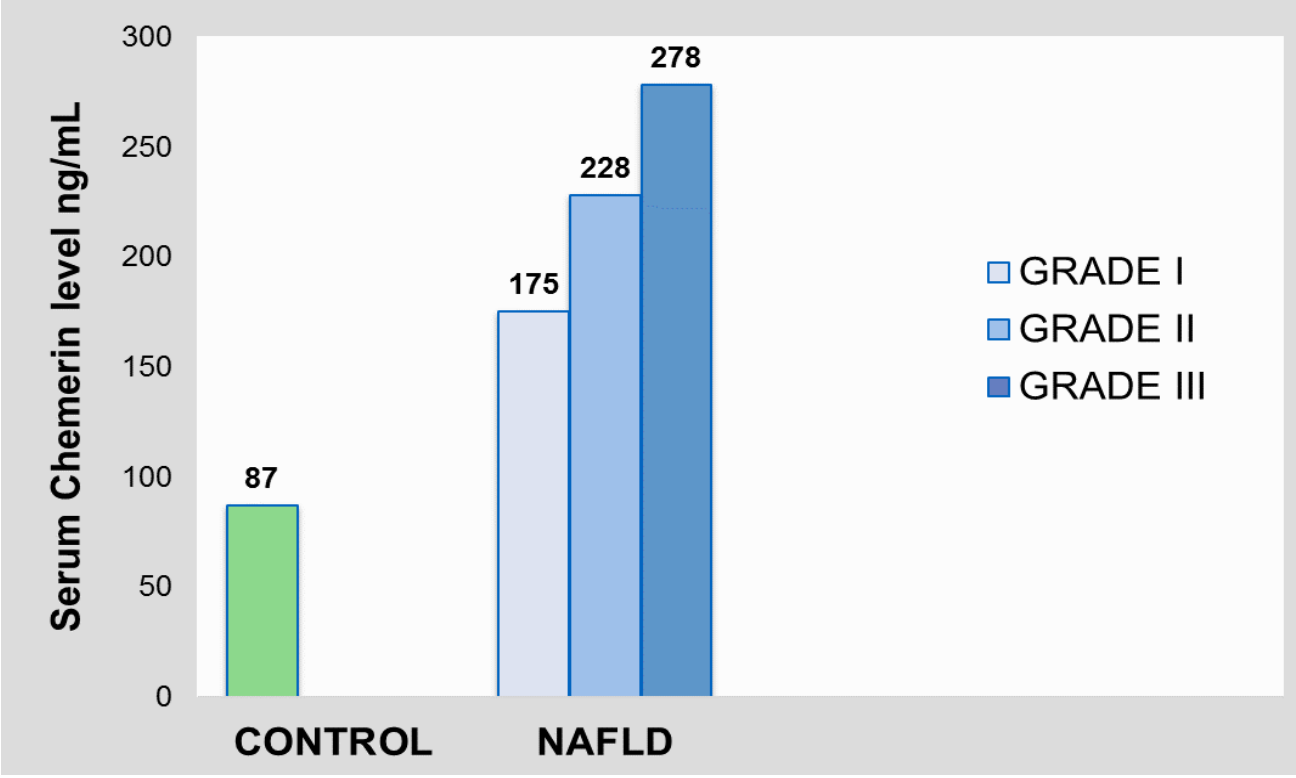

Figure (1): Serum chemerin levels in controls and NAFLD patients according to the grade of the fatty liver.

Patients within the gray zone of NAFLD fibrosis score (n = 18) had a significantly elevated serum chemerin levels in comparison to patients under the gray zone (n = 15) with a mean serum level of 213.83 ± 13.74 vs. 114.48 ± 35.41 ng/mL, respectively, ($p \leq 0.001$).

Serum chemerin positively correlated with NAFLD fibrosis score, age, BMI, serum cholesterol, serum triglycerides, random blood sugar, AST, ALT, and total bilirubin, and negatively correlated with platelets and total proteins (Table 3).

Table (3): Correlation of serum chemerin level with other parameters

Chemerin	r	P
NAFLD fibrosis score	0.988	0.001
Age	0.571	0.001
BMI	0.972	0.001
Serum Cholesterol	0.942	0.001
Serum Triglycerides	0.915	0.001
INR	-0.164	0.122
Random blood sugar	0.486	0.001
AST	0.963	0.001
ALT	0.960	0.001
Serum Albumin	-0.154	0.148
Platelets	-0.827	0.001
Total Bilirubin	0.740	0.001
Total Protein	-0.290	0.006

BMI: body mass index, INR: international normalized ratio, AST: aspartate transaminase, ALT: alanine transaminase.

Serum chemerin at a cutoff value > 147.8 ng/ml was comparable to NAFLD fibrosis score for the diagnosis of NAFLD ($p \leq 0.001$) (Table 4). In Addition, serum chemerin level at cutoff value > 245 ng/ml can distinguish grade F3–F4 fibrosis with AUROC = 1.00, CI 95% = 1.00 – 1.00, 100% sensitivity and 100% specificity.

Table (4): ROC curve analysis of serum chemerin and NAFLD fibrosis score for the diagnosis of NAFLD

Variable	Area	Cutoff	P	95% Confidence Interval	
				Lower Bound	Upper Bound
Serum Chemerin	1.000	>147.8 ng/mL	0.001	1.000	1.000
NAFLD fibrosis score	1.000	> -2.8	0.001	1.000	1.000

DISCUSSION

The connection between obesity, metabolic syndrome, and NAFLD was previously proved [2, 3, 5]. As expected according to previous reports [20-25], a significant difference was detected between NAFLD cases and control group in terms of BMI, NAFLD fibrosis score, serum cholesterol, serum triglycerides, international normalized ratio (INR), alanine transaminase (ALT), aspartate transaminase (AST), platelets, total bilirubin, and total protein.

Data on chemerin in human NAFLD are debatable [17]. In the current study, serum chemerin levels were significantly higher in NAFLD cases than controls. Furthermore, these levels were positively correlated with the NAFLD grade. This observation agrees with earlier reports [20, 21, 24, 26, 27, 28].

In contrast, other reports detected no significant difference in chemerin levels between NAFLD and control groups [29, 30, 31]. In agreement with our results, **Zhuang *et al.*** [32] found higher serum chemerin levels in NAFLD cases than controls, which significantly decreased following treatment with metformin, suggesting that NAFLD is closely correlated with serum chemerin level, as well as insulin resistance.

In agreement with previous reports [20, 24, 26, 33], we detected a positive correlation between serum chemerin and NAFLD fibrosis score, age, BMI, serum cholesterol, serum triglycerides, random blood sugar, AST, ALT, and total bilirubin, and a negative correlation with platelets and total proteins. These findings prove the correlation between serum chemerin and the presence of metabolic syndrome, obesity, dyslipidemia, and the grade of NAFLD.

We observed by using ROC curve analysis that serum chemerin at a cutoff value > 147.8 ng/mL was comparable to NAFLD fibrosis score for the diagnosis of NAFLD ($p \leq 0.001$). Similarly in another study [24], serum chemerin at a cutoff value = 410 ng/mL had AUROC = 0.99, sensitivity of 98%, and specificity of 87%. However, the diagnostic performance of chemerin was inferior to our results in another study [26], with a cut-off level of 186.7 ng/mL, serum chemerin had 56.44% sensitivity and 87.72% specificity ($P<0.001$). These discrepancies may be due different kits and various protocols and dilutions advised by the producers.

Given the conflicting data about the pathophysiologic mechanisms linking between adipokines including chemerin and NAFLD, a precise determination of their role in NAFLD is mandatory. Consequently, it may be used as noninvasive biomarkers for the diagnosis of NAFLD and interventions aiming at modulating their levels may result in its use as a novel target in NAFLD therapy [34, 35].

This study is restricted by the relatively small sample size and not performing a liver biopsy due to its invasiveness.

CONCLUSION

Serum chemerin is a promising marker for the diagnosis and grading of NAFLD. More research is required to determine its definitive clinical benefit in patients with NAFLD.

ACKNOWLEDGMENT: Not applicable.

REFERENCES

1. **Reccia I, Kumar J, Akladios C *et al.* (2017):** Non-alcoholic fatty liver disease: a sign of systemic disease. Metabolism, 72: 94-108.
2. **Bekaert M, Verhelst X, Geerts A *et al*. (2016x):** Association of recently described adipokines with liver histology in biopsy proven non-alcoholic fatty liver disease: a systematic review. Obesity Reviews, 17 (1): 68–80.
3. **Buzzetti E, Pinzani M, Tsochatzis E (2016):** The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism, 65: 1038-48.
4. **Anty R, Gual P (2019):** Pathogenesis of non-alcoholic fatty liver disease. Presse Med., 48: 1468–1483.
5. **Polyzos SA, Bugianesi E, Kountouras J *et al.* (2017):** Nonalcoholic fatty liver disease: updates on associations with the metabolic syndrome and lipid profile and effects of treatment with PPAR-γ agonists. Metabolism, 66: 64-8.
6. **Salama M, Kabiel W, Hana S *et al.* (2020):** Correlation of serum betatrophin levels with disease severity and the emergence of insulin resistance in cirrhotic patients. Egypt Liver Journal, 10: 29.
7. **Käräjämäki A, Bloigu R, Kauma H *et al.* (2017):** Non-alcoholic fatty liver disease with and without metabolic syndrome: Different long-term outcomes. Metabolism, 66: 55-63.
8. **Sumida Y, Nakajima A, Itoh Y (2014):** Limitations of liver biopsy and noninvasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World J Gastroenterol., 20: 475-485.
9. **Scheja L, Heeren J (2016):** Metabolic interplay between white, beige, brown adipocytes and the liver. J Hepatol., 64: 1176-86.
10. **Polyzos S, Mantzoros C (2015):** Leptin in health and disease: facts and expectations at its twentieth anniversary. Metabolism, 64: 5-12.
11. **Boutari C, Perakakis N, Mantzoros C (2018):** Association of Adipokines with Development and Progression of Nonalcoholic Fatty Liver Disease. Endocrinol Metab., 33: 33-43.
12. **Mattern A, Zellmann T, Beck-Sickinger A (2014):** Processing, signaling, and physiological function of chemerin. IUBMB Life, 66: 19-26.
13. **Ferland D, Watts S (2015):** Chemerin: a comprehensive review elucidating the need for cardiovascular research. Pharmacol Res., 9: 351–61.
14. **Krautbauer S, Wanninger J, Eisinger K *et al.* (2013):** Chemerin is highly expressed in hepatocytes and is induced in non-alcoholic steatohepatitis liver. Exp Mol Pathol., 95: 199–205.
15. **Buechler C, Feder S, Haberl E *et al.* (2019):** Chemerin Isoforms and Activity in Obesity. Int J Mol Sci., 20 (5): 1128.
16. **Mirmajidi S, Izadi A, Saghafi-Asl M *et al.* (2019):** Inflammatory Potential of Diet: Association With Chemerin, Omentin, Lipopolysaccharide-Binding Protein, and Insulin Resistance in the Apparently Healthy Obese. J Am Coll Nutr., 38 (4): 302-310.
17. **Polyzos S, Kountouras J, Mantzoros C (2016):** Adipokines in nonalcoholic fatty liver disease. Metabolism, 65 (8): 1062-79.
18. **Sanyal A (2002):** AGA technical review on nonalcoholic fatty liver disease. Gastroenterology, 123: 1705-1725.
19. **Angulo P, Hui J, Marchesini G *et al.* (2007):** The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology, 45 (4): 846-854.
20. **Ismail S, Elgendy N, El Sayed Z *et al.* (2019):** Chemerin and Vaspin as Noninvasive Biomarkers in the Pathogenesis and Diagnosis of Non- Alcoholic Fatty Liver Disease. American Journal of Medicine and Medical Sciences, 9 (1): 7-13.
21. **Zhang Z, Wang J, Wang H (2018):** Correlation of blood glucose, serum chemerin and insulin resistance with NAFLD in patients with type 2 diabetes mellitus. Exp Ther Med., 15 (3): 2936-2940.
22. **Montazerifar F, Bakhshipour A, Karajibani M *et al.* (2017):** Serum omentin-1, vaspin, and apelin levels and central obesity in patients with nonalcoholic fatty liver disease. J Res Med Sci., 22: 70.
23. **Kajor M, Kukla M, Waluga M *et al.* (2017):** Hepatic chemerin mRNA in morbidly obese patients with nonalcoholic fatty liver disease. Pol J Pathol., 68 (2): 117-127.
24. **Hamza R, Elkabbany Z, Shedid A *et al.* (2016):** Serum Chemerin in Obese Children and Adolescents Before and After L-Carnitine Therapy: Relation to Nonalcoholic Fatty Liver Disease and Other Features of Metabolic Syndrome. Arch Med Res., 47 (7): 541-549.
25. **Polyzos SA, Kountouras J, Polymerou V *et al.* (2016):** Vaspin, resistin, retinol-binding protein-4, interleukin-1α and interleukin-6 in patients with nonalcoholic fatty liver disease. Ann Hepatol., 15 (5): 705-714.
26. **Mohamed A, Sabry S, Abdallah A *et al.* (2017):** Circulating adipokines in children with nonalcoholic fatty liver disease: possible noninvasive diagnostic markers. Ann Gastroenterol., 30 (4): 457-463.
27. **Bekaert M, Ouwens D, Hörbelt T *et al*. (2016):** Reduced expression of chemerin in visceral adipose tissue associates with hepatic steatosis in patients with obesity. Obesity (Silver Spring), 24 (12): 2544-2552.
28. **Zwolak A, Szuster-Ciesielska A, Daniluk J *et al.* (2016):** Chemerin, retinol binding protein-4, cytokeratin-18 and transgelin-2 presence in sera of patients with non-alcoholic liver fatty disease. Ann Hepatol., 15 (6): 862-869.
29. **Pohl R, Haberl E, Rein-Fischboeck L *et al.* (2017):** Hepatic chemerin mRNA expression is reduced in human nonalcoholic steatohepatitis. Eur J Clin Invest., 47 (1): 7-18.
30. **Ye Z, Wang S, Yang Z *et al.* (2014):** Serum lipocalin-2, cathepsin S and chemerin levels and nonalcoholic fatty liver disease. Mol Biol Rep., 41 (3): 1317-1323.
31. **Döcke S, Lock J, Birkenfeld A *et al.* (2013):** Elevated hepatic chemerin mRNA expression in human non-alcoholic fatty liver disease. Eur J Endocrinol., 169 (5): 547-557.
32. **Zhuang X, Sun F, Li L *et al.* (2015):** Therapeutic Effect of Metformin on Chemerin in Non-Obese Patients with Non-Alcoholic Fatty Liver Disease (NAFLD). Clin Lab., 61 (10): 1409-1414.
33. **Karczewska-Kupczewska M, Nikołajuk A, Stefanowicz M *et al.* (2020):** Serum and adipose tissue chemerin is differentially related to insulin sensitivity. Endocr Connect, 9 (5): 360-369.
34. **Boutari C, Perakakis N, Mantzoros C (2018):** Association of Adipokines with Development and Progression of Nonalcoholic Fatty Liver Disease. Endocrinol Metab (Seoul), 33 (1): 33-43.
35. **Suliga E, Wawszczak M, Gluszek S (2018):** The roles of vaspin, chemerin, and omentin in the determination of metabolic Syndrome. Medical Studies/Studia Medyczne, 34 (2): 160–177.