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Non-Alcoholic Fatty Liver Disease and Metabolic Syndrome: Diagnostic and Laboratory Approach

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ABSTRACT

Both Non-Alcoholic Fatty Liver Disease (NAFLD) and metabolic syndrome are health problems worldwide. Various studies suggest that NAFLD and metabolic syndrome have a two-way relationship. Metabolic syndrome can be preceded by NAFLD and NAFLD can be a manifestation of the metabolic syndrome. Because of the relationship between the two, the diagnosis and management of NAFLD and metabolic syndrome are important to prevent complications such as cardiovascular disease, liver cirrhosis, and malignancy. The diagnosis of metabolic syndrome can be made based on various diagnostic criteria determined by several health organizations, such as WHO, IDF, and NCEP-ATP. Since NAFLD is asymptomatic until advanced disease, many patients are only identified at advanced stages. Liver biopsy is currently the gold standard for diagnosing NASH, which is a type of NAFLD. This procedure is invasive, and many studies are currently looking for and assessing non-invasive markers for NAFLD and metabolic syndrome. Laboratory as diagnostic support plays an important role in the diagnosis of NAFLD and metabolic syndrome. Non-invasive laboratory tests with high sensitivity and specificity are expected to contribute to the early diagnosis of NAFLD and metabolic syndrome. Various laboratory parameters have been developed to support the diagnosis of NAFLD and metabolic syndrome.

Keywords: NAFLD, metabolic syndrome, early diagnostic

INTRODUCTION

Non-Alcoholic Fatty Liver Disease (NAFLD) is one of the most common causes of impaired liver function and liver disease worldwide. The prevalence of NAFLD is currently increasing, making it a public health problem. Disease progression is associated with some risk factors and varies between individuals. NAFLD is associated with other conditions such as metabolic syndrome, obesity, cardiovascular disease, diabetes, and cancer.[1,2]

Various cross-sectional studies have shown that NAFLD is associated with metabolic syndrome. Early diagnosis of NAFLD and metabolic syndrome is necessary to prevent complications that result in increased morbidity and mortality.[1,3] This literature review was compiled to find out more about the diagnostic and laboratory approach of NAFLD and metabolic syndrome.

NAFLD and Metabolic Syndrome

NAFLD is a fatty liver disease caused by excess nutrition as in most cases of central obesity. The limitation of non-alcoholic is alcohol consumption no more than 40 g/day. An increase in liver fat >5% is reported in NAFLD. The histological spectrum of NAFLD includes simple steatosis (simple steatosis) and steatohepatitis Non-Alcoholic Steatohepatitis (NASH).[4,5]

Metabolic syndrome is defined as a group of metabolic disorders that coexist. These metabolic disorders include central obesity, hyperglycaemia, hypertriglyceridemia, low HDL cholesterol, and hypertension. The presence of three of the five metabolic disorders can be defined as metabolic syndrome.[6] There are several diagnostic criteria for the metabolic syndrome according to the World Health Organization (WHO), International Diabetes Federation (IDF), and the National Cholesterol Education Program Adult Treatment Panel (NCEP-ATP III) (Table 1).[7]

Several studies have found a bidirectional relationship between NAFLD and metabolic syndrome. NAFLD has been associated with a predisposition to metabolic syndrome. Recent studies have shown that NAFLD in its entire spectrum from pure fatty liver to NASH represents a hallmark of the metabolic syndrome. Similarly, the metabolic syndrome is associated with a 4 to 11-fold risk of fatty liver in NAFLD and may precede the onset of fatty liver by several years. NAFLD is often considered a hepatic manifestation of metabolic syndrome.[8]

Tabel 1. The several diagnostic criteria for the metabolic syndrome

WHO, 1998	NCEP-ATPIII, 2001	IDF, 2006
IGT or IFG or diabetes and/ or IR (estimated with the euglycemic hyperinsulinemic clamp method) plus two or more of the following:	Three or more of the following:	Central obesity as defined by ethnic/racial, specific WC, but can be assumed if BMI ≥ 30 kg/m^2 and two or more of the following:
	FPG ≥ 100 mg/dl* or on treatment for DM	FPG ≥ 100 mg/dl or on treatment for DM
BP ≥ 140/90 mmHg	BP ≥ 130/85 mmHg	BP ≥ 130/85 mmHg or on anti-hypertensives
Abdominal obesity: WHR > 0.9 for men and > 0.85 for women and/or BMI > 30 kg/m^2	WC: ≥ 102 cm for men, ≥ 88 cm for women	
Triglycerides ≥ 150 mg/dl or on treatment	Triglycerides ≥ 150 mg/dl or on treatment	Triglycerides ≥ 150 mg/dl or on lipid lowering agent
HDL-C: < 35 mg/dl for men and <39 mg/dl for women	HDL-C: < 40 mg/dl for men, < 50 mg/dl for women	HDL-C: < 40 mg/dl for men, < 50 mg/dl for women or on treatment for dyslipidemia
Urine albumin excretion rate ≥ 20 µg/min or urine albumin to creatinine ratio ≥ 30 mg/g		

FPG ≥ 100 mg/dl* modified in 2004 according to the IDF definition of impaired fasting glucose. The 2001 definition of NCEP-ATPIII identified FPG ≥ 110 mg/dl as elevated.
Body mass index (BMI), blood pressure (BP), fasting plasma glucose (FPG), high-density lipoprotein- cholesterol (HDL-C), impaired fasting glucose (IFG), impaired glucose tolerance (IGT), insulin resistance (IR), International Diabetes Federation (IDF), National Cholesterol Education Program Adult Treatment Panel (NCEP/ATP111), waist circumference (WC), waist to hip ratio (WHR), World Health Organization (WHO)

Pathogenesis

The liver is the key organ of insulin action and the main source of endogenous glucose production, the main site for the synthesis and disposal of lipids, and insulin degradation. The main predispositions for NAFLD and metabolic syndrome are overweight and obesity. Excess liver fat is strongly associated with insulin resistance in the liver, muscle, and adipose tissue, leading to a high risk of metabolic syndrome. Insulin resistance will eventually trigger the mechanism of inflammation and fibrosis. This theory is known as the "two hits hypothesis". Another known hypothesis is the "muti-hits hypothesis". This hypothesis involves environmental factors. The multi-hits hypothesis involves gene expression, weight gain, increased FFA mobilization, ectopic fat deposition, and Insulin Resistance (IR).[5]

Environmental factors influence the expression of genes that promote weight gain. The addition of subcutaneous Adipose Tissue (AT) also increases the mobilization of Free Fatty Acids (FFA) resulting in the deposition of visceral and ectopic fat in the muscles and other organs such as the pancreas and liver. This results in IR and decreased glucose uptake in muscle, adipose tissue, and pancreatic steatosis. Adipose tissue insulin resistance leads to central obesity. Increased FFA mobilization to the liver leads to NAFLD. In NAFLD conditions, atherogenic

dyslipidaemia, type 2 diabetes, increased dipeptidyl peptidase 4 (DPP4), and Glucose Resistance (GR) in the liver can occur. Disorders of the liver and pancreas due to the mobilization of these FFAs interfere with the normal functioning of the liver-pancreas axis. As a result, systemic IR occurs, which produces hyperinsulinemia, which can increase sodium reabsorption and increase sympathetic nervous system activity, which contributes to hypertension. The conditions of central obesity, atherogenic dyslipidaemia, type 2 diabetes and hypertension cause the metabolic syndrome (Figure 1).[5]

Laboratory Tests of NAFLD and Metabolic Syndrome

Oral Glucose Tolerance Test (OGTT)

Oral Glucose Tolerance Test (OGTT) is a provocation test to determine the body's efficiency in glucose metabolism. OGTT can detect impaired glucose tolerance. The OGTT is more sensitive than fasting blood sugar for the diagnosis of diabetes. However, OGTT is not recommended for routine diagnostics or daily monitoring of blood glucose control. OGTT is used for the diagnosis of impaired glucose tolerance, impaired fasting glucose, and epidemiological population studies.[9]

The glucose load given to adults is 75 g glucose and children 1.75 g/kg BW to 75 g glucose. The patient is required to fast for 12 hours prior to test. Plasma glucose samples are taken 10 minutes before the glucose load and 120 minutes after the glucose load. Table 2 shows the classification of glucose tolerance from the results of the OGTT based on WHO. OGTT is affected by metabolic stress from several clinical conditions and drug use, such as steroids, thiazides, phenytoin, estrogen, and thyroxine.[9,10]

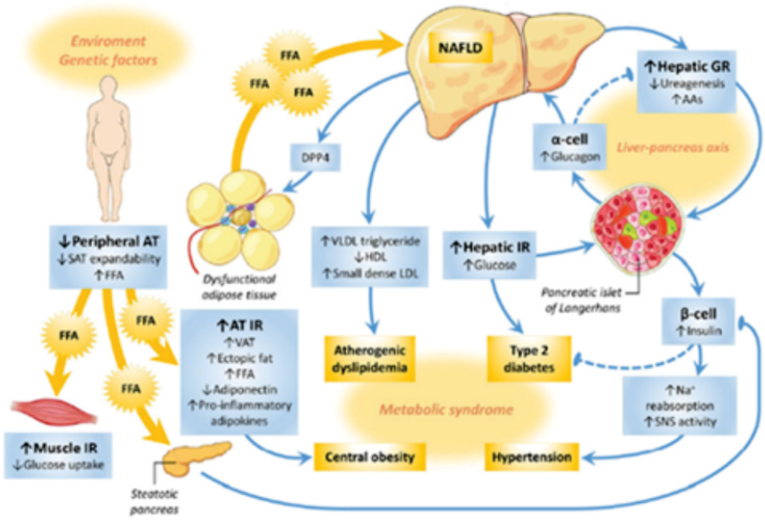

Figure 1. Pathogenesis of NAFLD and Metabolic syndrome. Environmental genetic factors can cause obesity, which can increase the mobilization of Free Fatty Acids (FFA) to visceral and ectopic tissues such as muscle. FFA deposition in muscle results in increased Insulin Resistance (IR) and reduced glucose uptake. The FFA mobilization also induces Adipose Tissue Insulin Resistance (ATIR) resulting in dysfunctional adipose tissue and central obesity. Dysfunctional adipose tissue facilitates lipolysis and increases FFA flux to the liver, resulting in NAFLD. In NAFLD there is lipotoxicity, mitochondrial dysfunction, and endoplasmic reticulum stress, which causes hepatocyte damage, apoptosis, and fibrosis. These dysfunctional hepatocytes synthesize and secrete dipeptidyl peptidase 4 (DPP4), which triggers inflammation in AT macrophages and more IR. FFA flux to the liver induces hepatic IR and increases glucose production, de novo hepatic lipogenesis, and VLDL release, resulting in type 2 diabetes and atherogenic dyslipidaemia. FFA flux to the liver also induces hepatic Glucagon Resistance (GR) reducing ureagenesis and resulting in amino acid hyperactivity. As compensation for hepatic GR, an increase in AA stimulates glucagon production. The circle of this process is called the liver-pancreatic axis. Increased FFA in the pancreas causes lipotoxicity resulting in the dysfunction of pancreatic cells. Pancreatic-cell dysfunction results in hyperglycaemia, diabetes, and hypertension. The presence of a state of central obesity, hypertension, type 2 diabetes, and atherogenic dyslipidaemia can be called as metabolic syndrome

Table 2. Classification of glucose tolerance based on WHO[10]

Classification	Fasting Plasma Glucose		2-h Plasma Glucose*
Impaired Fasting Glucose	6.1 to 6.9 mmol/L (110 mg/dL to 125 mg/dL)	and	<7.8mmol/l (<140 mg/dL) (if measured)
Impaired Glucose Tolerance	<7.0 mmol/L (<126 mg/dL)	and	≥7.8 and <11.1 mmol/L (≥140 mg/dL and < 200 mg/dL)
Diabetes	≥7.0 mmol/L (≥126 mg/dL)	or	≥11.1 mmol/L (≥200 mg/dL)

* Venous plasma glucose 2–h after ingestion of 75g oral glucose load
* If 2–h plasma glucose is not measured, status is uncertain as diabetes or IGT cannot be excluded

Serum Insulin Level

Insulin is an anabolic hormone that is important in the regulation of plasma glucose homeostasis. Measurement of insulin and blood glucose levels is useful for estimating insulin resistance in patients with metabolic syndrome. However, there has been no a universal cut-off value of insulin levels for the diagnostic of insulin resistance. A fasting insulin level of 12 U/L with normoglycemia indicates insulin resistance. Fasting blood insulin levels also depend on pancreatic beta cell reserves.[8,9]

Homeostatic Model Assessment (HOMA)

Homeostatic model assessment is another method for assessing pancreatic beta cell function and insulin resistance using fasting glucose and insulin levels or C-peptide levels. The HOMA test has the terms of Homeostatic Model Assessment-Insulin Resistance (HOMA-IR) and Homeostatic Model Assessment-Cell Function (HOMA-). HOMA-IR assesses insulin resistance while HOMA- assesses pancreatic beta cell function. A high HOMA-IR score indicates low insulin sensitivity (insulin resistance) and a higher HOMA- score indicates a higher number of beta cells, which must secrete insulin. Salgado et al. found that the normal value of HOMA-IR for healthy individuals ranged from 1.7 to 2.0. A HOMA-IR value greater than or equal to 2.0 or 2.5 indicates a diagnostic value for differentiating NAFLD and control group individuals. According to Esteghamati *et al.* the optimal cut-off value of HOMA-IR for the diagnosis of metabolic syndrome was 1.775 in non-diabetics and about 4 in diabetic individuals.[11]

The following is the formula for calculating HOMA-IR and HOMA-[11]

$$\text{HOMA-IR} = \frac{\text{Glucose (mmol/L)} \times \text{Insulin (μU/L)}}{22.5}$$

$$\text{HOMA-IR} = \frac{\text{Glucose (mg/dL)} \times \text{Insulin (μU/L)}}{405}$$

$$\text{HOMA-} = \frac{20 \times \text{Insulin (μU/L) \%}}{\text{Glucose (mmol/L)} - 3.5}$$

$$\text{HOMA-} = \frac{360 \times \text{Insulin (μU/L) \%}}{\text{Glucose (mg/dL)} - 63}$$

Under physiological conditions, fasting plasma glucose and insulin concentrations represent a balance between hepatic glucose output and insulin secretion. The assessment of the homoeostatic model is unable to distinguish between hepatic insulin resistance and peripheral insulin resistance.[12]

Hyperinsulinemic-Euglycemic Clamp

Hyperinsulinemic-euglycemic clamp technique is widely accepted as the reference standard for determining metabolic insulin sensitivity. This test involves the infusion of insulin with simultaneous glucose infusions at variable rates to maintain blood glucose concentrations between 5 and 6 mmol/L. Assuming that the state of hyperinsulinemia is sufficient to suppress hepatic glucose production, the Glucose Infusion Rate (GIR) must equal to the glucose disposal rate (M). Alternatively, the insulin sensitivity index derived from the clamp data can be defined as SI Clamp = M/(GxI), where M is normalized for G (steady-state blood glucose concentration) and I (difference between fasting and steady-state plasma insulin concentrations). Figure 2 describes the dynamics of the "steady state" of glucose and insulin during the hyperinsulinemic-euglycemic clamp.[13]

The rate of glucose infusion is the same as the rate of glucose removal under normal conditions. Insulin-sensitive subjects have a glucose removal rate (or M-value) greater than 7.5 mg/kg/min. In contrast, people with insulin resistance have a low M-value, less than 4 mg/kg/minute. This test is invasive, technically demanding and expensive.[13,14]

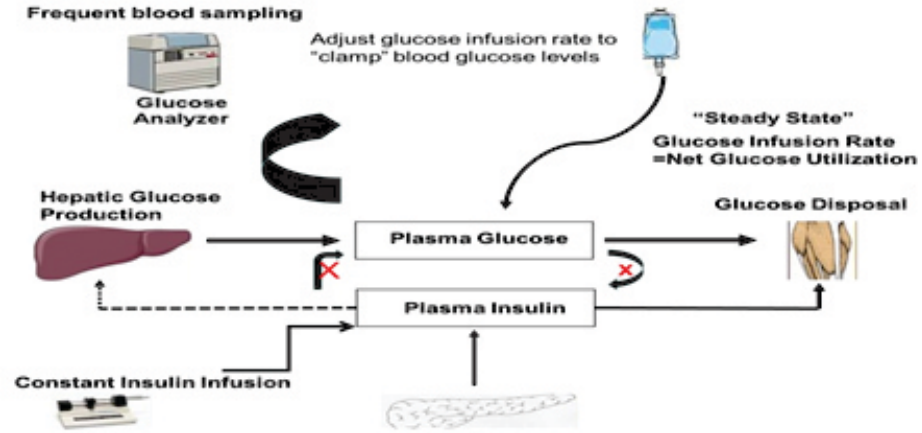

Figure 2. Schematic drawing of the steady state dynamics of glucose and insulin during the hyperinsulinemic-euglycemic clamp. Simultaneous insulin infusion and glucose infusion at variable rates, and blood glucose levels are monitored periodically. The GIR is assumed to be the same as the steady-state glucose disposal rate[14]

Quantitative Insulin Sensitivity Check Index (QUICKI)

Katz *et al*. introduced this method in 2000. This method uses insulin and fasting glucose levels. QUICKI value less than or equal to 0.33 indicates insulin resistance.[15]

The formula for this method is as follows:

$$QUICKI = 1/ (\log Io + \log Go)$$

Abbreviation: fasting insulin level in µU/mL (Io), fasting glucose level in mg/dL (Go)

Triglycerides

Triglycerides are the main form of storage and transport of fatty acids in cells and plasma. High triglycerides are a component of metabolic syndrome and a risk factor for NAFLD. Research shows that the longitudinal association between triglycerides and other components of metabolic syndrome increases over time. Table 3 shows the classification of triglyceride levels according to NCEP-ATP III.[16]

Table 3. Classification of triglyceride levels

Triglyceride Level		Classification
(mg/dL)	(mmol/L)	
< 150	< 1.70	Normal
150–199	1.70–2.25	Mild
200–499	2.26–5.65	High
500	5.65	Very high

HDL-Cholesterol

HDL-cholesterol is an important component of metabolic syndrome. Low levels of HDL-cholesterol are found in metabolic syndrome, associated with insulin resistance, hypertriglyceridemia, and NAFLD. Rahmayani *et al.* found a relationship between HOMA-IR and cholesterol total/HDL ratio, which means that there is a correlation between insulin resistance with cholesterol total/HDL ratio. The prevalence of low HDL-cholesterol is 33-34% in males and 39-40% in females.[17,18]

Retinol-binding protein 4

Measurement of Retinol-Binding Protein 4 (RBP4) is useful as a marker of insulin sensitivity. RBP4 is produced by adipocytes and the serum concentration is directly proportional to fat mass. There was a positive correlation between serum RBP4 concentrations and several components of the metabolic syndrome including fasting triglyceride concentrations, fasting insulin concentrations, and systolic blood pressure. Serum RBP4 concentration can be potentially used as a simple marker of metabolic syndrome. RBP4 levels were measured using ELISA and Western blotting. Although an upper limit for RBP4 levels in normal subjects has not been established, minimum RBP4 concentrations have been reported to be 15 to 30 g/mL among healthy subjects without vitamin A deficiency.[19]

Aspartate transaminase (AST) and Alanine transaminase (ALT)

The main laboratory abnormality in NAFDL is the

elevation of serum aspartate transaminase (AST) and alanine transaminase (ALT) levels. Damage to hepatocytes will increase AST and ALT levels. Changes in aminotransferases are not in line with changes in the fibrosis stage. The Framingham study showed that the ALT/AST ratio can identify hepatic steatosis more accurately than using ALT or AST alone.[2,20]

Gamma Glutamyl Transferase (GGT)

Serum GGT is frequently elevated in patients with NAFLD and several components of metabolic syndrome including type 2 DM, IR, and obesity. The mechanism of increased GGT in NAFLD may be due to abnormal fat deposition in the liver with a secondary inflammatory response and oxidative stress. GGT serum level is higher in NASH than in simple steatosis. Gamma glutamyl transferase normal value range 5-30 IU/L.[21,22]

The Fatty Liver Index

The fatty liver index is an algorithm to accurately identify NAFLD based on four markers namely BMI, waist circumference, triglycerides, and GGT. This index has been used in population studies and has a sensitivity of 80.3% and specificity of 87.3%, which indicates that the fatty liver index has a high diagnostic value in detecting liver steatosis. The study of Huang *et al.* in a large general Chinese population validated the accuracy of the FLI for predicting NAFLD. The fatty liver index has a score of 0-100. The interpretation of the fatty liver index calculation is as follows: NAFLD can be eliminated if the score is <30 and there is NAFLD if the score is 60. The formula for calculating the fatty liver index is as follows.[2,23]

$$\text{Fatty Liver Index} = [e^{095} \times \log e\ (\text{trigycerides}) + 0.139 \times \text{BMI} + 0718 \times \log e\ (\text{GGT}) + 0.053 \times \text{waist circumference} - 15.745] / [1 + e095 \times \log e\ (\text{triglycerides}) - 0.319 \times \text{BMI} + 0.718 \times \log e\ (\text{GGT}) + 0.053 \times \text{waist circumference} - 15.745] \times 100$$

Abbreviation: Euler's number (e), Body Mass Index (BMI), Gamma-Glutamyl Transferase (GGT)

Adipocytokine

Adipose tissue is currently considered an endocrine organ that secretes some hormones and bioactive substances including adipocytokines. Adipocytokines including leptin, resistin, and adiponectin are associated with metabolic syndrome. Adipocytokine that has been widely studied is adiponectin. The average plasma adiponectin concentration in humans is 5-10 micrograms/mL. There is an inverse relationship between plasma adiponectin concentration and BMI (or visceral adiposity). Plasma adiponectin concentration is negatively correlated with insulin resistance. Shimada *et al.* reported lower adiponectin levels in the early stages of NASH than simple steatosis. Adiponectin with cut-off of 4.0 microgram/mL has a sensitivity of 68% and a specificity of 79% for detection of the early stages of NASH.[4,22,24]

Hyaluronic Acid

Hyaluronic acid levels were reported to be low in normal liver tissue but elevated in fibrotic liver tissue. Measurement of serum hyaluronic acid levels is a fairly specific test for liver fibrosis. Serum hyaluronic acid < 60 mg/L showed no significant fibrosis with a negative predictive value of 93%. The use of this test remains limited because it is only available in large laboratories.[4,22,25]

Cytokeratin-18 Serum (CK-18)

Cytokeratin-18 is one of the most studied markers and one of the most promising non-invasive parameters for the diagnosis of NASH/NAFLD. CK-18 is a caspase-cleaved fragment released by damaged hepatocytes; therefore, serum CK-18 levels can be a marker of liver cell damage. During apoptosis, CK-18 can be cleaved at two sites into three fragments. Serum CK-18 levels can be measured using ELISA.[26] The M30 assay was able to detect caspase-cleared CK-18 fragments using the M30 monoclonal antibody, which indicated a degree of apoptosis. The CK-18 M65 assay uses an M6 capture antibody and M5 detection antibody directed to two different CK-18 epitopes and can recognize the entire CK-18 chain and cleaved CK-18 fragments, regardless of their cleavage by caspases. Serum CK-18 fragments have been evaluated to have a sensitivity of 66% and a specificity of 82% in the diagnosis of NASH.[22,27]

Other Tests

Antinuclear antibodies (ANA), Smooth Muscle Antibodies (SMA), and antimitochondrial antibodies (AMA) are present in 23% to 36% of NAFLD patients. High ANA titers are associated with insulin resistance. Some new markers have been developed for the diagnosis of NASH such as TNF-alpha, IL-6, chemokines MCP-1 and RANTES or Fibroblast Growth Factor 21 (FGF21). However, these new markers have not yet been validated. Diagnostic accuracy for the diagnosis of NAFLD or for differentiating NAFLD from other liver diseases can

be improved by using a panel of multiple markers reflecting different pathophysiological mechanisms. For example, a two-step approach using CK-18 and FGF21 has been shown to further improve accuracy in diagnosing NASH.[22]

CONCLUSION

NAFLD and metabolic syndrome have become important health problems worldwide. There is a strong bidirectional relationship between the metabolic syndrome and NAFLD. NAFLD can be a manifestation of the metabolic syndrome and NAFLD can be a precursor of the metabolic syndrome.

The performance of laboratory tests for the diagnosis of NAFLD and metabolic syndrome can be improved when clinical and laboratory parameters are used together for prediction or early detection. Fatty liver index is an algorithm consisting of clinical and laboratory parameters for the diagnosis of NAFLD. Fatty liver index can be an option for early detection of NAFLD because it is non-invasive and has high sensitivity and specificity. Metabolic syndrome can be detected early by periodic examination of the parameters in the diagnostic criteria for metabolic syndrome. The presence of abnormalities in any of the parameters can be an early warning of metabolic syndrome. Further research is needed to increase the diagnostic value of NAFLD and metabolic syndrome from a laboratory approach.

REFERENCES

1. Fattahi MR, Niknam R, Safarpour A, Sepehrimanesh M, Lotfi M. The prevalence of metabolic syndrome in non-alcoholic fatty liver disease; A population-based study. Middle East J Dig Dis, [Internet]. 2016; 8(2): 131-7. Available from: http://dx.doi.org/10.15171/mejdd.2016.18 (accessed July 10, 2022).
2. Neuschwander-Tetri BA. Non-alcoholic fatty liver disease. BMC Med, 2017; 15(1): 1-6.
3. Lonardo A, Ballestri S, Marchesini G, Angulo P, Loria P. Non-alcoholic fatty liver disease: A precursor of the metabolic syndrome. Dig Liver Dis, [Internet]. 2015; 47(3): 181-90. Available from: http://dx.doi.org/10.1016/j.dld.2014.09.020 (accessed July 12, 2022).
4. Dumitrascu DL, Neuman MG. Non-alcoholic fatty liver disease: An update on diagnosis. Clujul Med, 2018; 91(2): 147-50.
5. Godoy?Matos AF, Silva Júnior WS, Valerio CM. NAFLD as a continuum: From obesity to metabolic syndrome and diabetes. Diabetol Metab Syndr, 2020; 12: 60.
6. Sigit FS, Tahapary DL, Trompet S, Sartono E, Willems Van Dijk K, *et al.* The prevalence of metabolic syndrome and its association with body fat distribution in middle-aged individuals from Indonesia and the Netherlands: A cross-sectional analysis of two population-based studies. Diabetol Metab Syndr, [Internet]. 2020; 12(1):1-11. Available from: https://doi.org/10.1186/s13098-019-0503-1 (accessed July 8, 2022).
7. Zafar U, Khaliq S, Ahmad HU, Manzoor S, Lone KP. Metabolic syndrome: An update on diagnostic criteria, pathogenesis, and genetic links. Hormones, 2018; 17(3): 299-313.
8. Wainwright P, Byrne CD. Bidirectional relationships and disconnects between NAFLD and features of the metabolic syndrome. Int J Mol Sci, 2016; 17(3): 367.
9. Jagannathan R, Neves JS, Dorcely B, Chung ST, Tamura K, *et al.* The oral glucose tolerance test: 100 years later. Diabetes, Metab Syndr Obes Targets Ther, 2020; 13: 3787-805.
10. World Health Organization & International Diabetes Federation. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: Report of a WHO/IDF consultation. [Internet]. World Health Organization, 2006; 1-50. Available from: https://apps.who.int/iris/handle/10665/43588 (accessed July 20, 2022).
11. Horáková D, Štěpánek L, Štěpánek L, Pastucha D, Janoutová J, *et al.* What are the real associations of Homeostasis Model Assessment (HOMA) with body mass index and age?. Endokrynol Pol, 2022; 73(4): 736-42.
12. Arellano-Ruiz P, García-Hermoso A, Cavero-Redondo I, Pozuelo-Carrascosa D, Martínez-Vizcaíno V, Solera-Martinez M. Homeostasis model assessment cut-off points related to metabolic syndrome in children and adolescents: a systematic review and meta-analysis. Eur J Pediatr, 2019; 178(12): 1813-22.
13. Fiorentino TV, Marini MA, Succurro E, Andreozzi F, Sesti G. Relationships of surrogate indexes of insulin resistance with insulin sensitivity assessed by euglycemic hyperinsulinemic clamp and subclinical vascular damage. BMJ Open Diabetes Res Care, 2019; 7(1): e000911.
14. Muniyappa R, Madan R. Assessing Insulin sensitivity and resistance in humans [Internet]. Feingold K, Anawalt B, Boyce A, editors. Endotext. South Dartmouth (MA): MDText.com, Inc; 2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK278954/ (accessed July 5, 2022).
15. Mirzaalian Y, Nourian M, Gholamalizadeh M, Doaei S, Hatami M, *et al.* The association of quantitative insulin sensitivity indices (HOMA-IR and QUICKI) with anthropometric and cardiometabolic indicators in adolescents. Arch Med Sci - Atheroscler Dis, 2019; 4(1): 32-7.
16. Karanchi H, K. W. Hypertriglyceridemia - StatPearls - NCBI bookshelf [Internet]. 2017. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459368/?report=reader (accessed July 5, 2022).
17. Nilsson PM, Tuomilehto J, Rydén L. The metabolic syndrome - What is it and how should it be managed?.

Eur J Prev Cardiol, 2019; 26(2_suppl): 33-46.
18. Rahmayani R, Aman AK, Safril S. The association of insulin resistance and lipid profile ratio in metabolic syndrome. Indones J Clin Pathol Med Lab [Internet]. 2019; 25(1): 21-5. Available from: https://indonesianjournalofclinicalpathology.org/index.php/patologi/article/view/1484/1055 (accessed August 12, 2022).
19. Majerczyk M, Kocełak P, Choręza P, Arabzada H, Owczarek AJ, *et al.* Components of metabolic syndrome in relation to plasma levels of retinol binding protein 4 (RBP4) in a cohort of people aged 65 years and older. J Endocrinol Invest, [Internet]. 2018; 41(10): 1211-9. Available from: https://doi.org/10.1007/s40618-018-0856-6 (accessed September 10, 2022).
20. Sanyal D, Mukherjee P, Raychaudhuri M, Ghosh S, Mukherjee S, Chowdhury S. Profile of liver enzymes in non-alcoholic fatty liver disease in patients with impaired glucose tolerance and newly detected untreated type 2 diabetes. Indian J Endocrinol Metab, 2015; 19(5): 597-601.
21. Feng G, Feng L, Zhao Y. Association between ratio of γ-glutamyl transpeptidase to high-density lipoprotein cholesterol and prevalence of nonalcoholic fatty liver disease and metabolic syndrome: a cross-sectional study. Ann Transl Med, 2020; 8(10): 634-634.
22. Hadizadeh F, Faghihimani E, Adibi P. Non-alcoholic fatty liver disease: Diagnostic biomarkers. World J Gastrointest Pathophysiol, 2017; 8(2): 11-26.
23. Huang X, Xu M, Chen Y, Peng K, Huang Y, *et al.* Validation of the fatty liver index for nonalcoholic fatty liver disease in middle-aged and elderly Chinese. Med (United States), 2015; 94(40): 1-7.
24. Liu Z, Liang S, Que S, Zhou L, Zheng S, Mardinoglu A. Meta-analysis of adiponectin as a biomarker for the detection of metabolic syndrome. Front Physiol, 2018. Available from: https://www.frontiersin.org/articles/10.3389/fphys.2018.01238/full (accessed September 10, 2022).
25. Gudowska M, Cylwik B, Chrostek L. The role of serum hyaluronic acid determination in the diagnosis of liver fibrosis. Acta Biochim Pol, 2017; 64(3): 451-7.
26. Chang YH, Lin HC, Hwu DW, Chang DM, Lin KC, Lee YJ. Elevated serum cytokeratin-18 concentration in patients with type 2 diabetes mellitus and non-alcoholic fatty liver disease. Ann Clin Biochem, 2019; 56(1): 141-7.
27. Qian L, Zhang L, Wu L, Zhang J, Fang Q, *et al.* Elevated serum level of cytokeratin 18 M65ED is an independent indicator of cardiometabolic disorders. J Diabetes Res, 2020; 2020: 5198359.