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Resistin: A Novel Diagnostic and Prognostic Biomarker for Metabolic Dysfunction-Associated Steatotic Liver Disease

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Abstract:

Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a prevalent health problem affecting more than 1/3 of the world population. It has similar cardiovascular risk factors to those of the metabolic syndrome and represents an independent risk factor of atherosclerotic ischemic heart disease and chronic renal impairment with significant morbidity & mortality implications. Its long-term outcomes are liver fibrosis, cirrhosis, and hepatic carcinoma. More extensive research is still required to improve the diagnostic and therapeutic options for MASLD and its severe form, metabolic dysfunction-associated steatohepatitis (MASH. Resistin is an adipokine with pro-inflammatory properties, produced by adipose tissues & some inflammatory cells such as macrophages, monocytes as well as hepatic stellate cells. It contributes to the pathogenesis of MASLD inflammatory process & MASLD-induced fibrogenesis.

Methods: This case-control study was performed in Zagazig University hospitals over a period extending from July 2023 till July 2024. It enrolled 69 Subjects, who were divided into 3 equal groups: MASLD, MASH & control subjects. The patients were evaluated by history taking, thorough clinical examination, abdominal ultrasonography, blood workup, and serum resistin level.

Results: The study included 39.1% males and 60.9%females with a mean age of 43 years old. There was a statistically significant difference between the studied groups regarding body mass index (BMI), hemoglobin, platelet count, liver enzymes, total & LDL-cholesterol, triglycerides, blood glucose level & serum resistin level. The best cutoff values of serum resistin were ≥28.25 & ≥903.5 pg/ml in MASLD & MASH respectively.

Conclusion: Serum Resistin level is a proper & valid serum biomarker for MASLD & MASH

Keywords: Metabolic dysfunction-associated steatotic liver disease (MASLD), metabolic dysfunction-associated steatohepatitis (MASH), liver steatosis, liver cirrhosis, hepatocellular carcinoma, Resistin.

Introduction:

Steatotic liver disease (SLD) is considered when there is excess fat (>5%) deposited inside the hepatocytes. MASLD is the most novel term of metabolic liver steatosis defining the causal relationship between excess fat deposition in the liver and the cardiovascular risks and components of metabolic syndrome. It is emerging as the leading aetiology of chronic liver disease globally. This new term emphasizes that MASLD is an independent risk factor for cardiovascular & renal derangement**.[1]**

MASLD affects more than 35% of the population of the world. This increasingly high prevalence is attributed to the worldwide spread of obesity, which has been considered a global epidemic by the World Health Organization (WHO) since 1997, and its accompanying metabolic abnormalities.**[2]**

Inflammatory ballooning of liver cells with excess deposition of inflammatory cells in the hepatic lobules is the hallmark in the pathogenesis of MASH which is the severe clinical form of MASLD. MASH is considered a significant risk for irreversible fibrosis with subsequent loss of the normal liver architecture.**[3]**

MASLD and its complications, particularly end-stage liver cirrhosis and hepatocellular carcinoma (HCC), are the principal causes of hepatic mortality globally in the recent decades. HCC may develop in MASLD patients without being preceded by liver cirrhosis. The health burden of MASLD is projected to continue to increase in the next few decades secondary to the absence of early screening programs and lack of effective therapeutic options. The major challenge that the research community of liver diseases should address is the discovery of novel modalities for early recognition of MASLD patients with the highest risk for hepatic, cardiovascular & renal complications.**[4]**

Excess fat accumulation in the adipose stores and ectopically is the widely accepted mechanism that initiates SLD. This expansion of fat stores induces excess macrophages infiltration with subsequent release and accumulation of pro-inflammatory cytokines that produce a state of insulin resistance in the visceral adipose tissues (2-hit hypothesis). Excess lipolysis in adipose tissues stores in association with insulin resistance inappropriately deliver & deposit excess amounts of fatty acids into the hepatocytes.**[5]**

Most MASLD patients have no symptoms and are discovered accidentally when a routine blood workup reveals abnormal liver functions tests or abdominal ultrasound shows hepatomegaly and liver steatosis. The invasive liver biopsy remains the gold standard for confirmation of MASLD diagnosis. However, liver biopsy has multiple complications & is rarely done in the routine clinical evaluation of MASLD patients**.[6, 7]**

Resistin, which is also called adipocyte secreted factor (ADSF) and found in inflammatory zone (FIZZ3), is a 108-amino acid peptide hormone with a molecular weight of 12.5 kDa secreted by adipocytes of both white & brown adipose tissues. Its concentration is highest in the ovaries, as well as the subcutaneous, and omental fatty tissues.**[8, 9]**

It is present in the circulation in two forms, the high-molecular mass (HMM), which is more abundant, and the low-molecular mass (LMM) complex, which is more active. Resistin is increased in obesity, its main actions are inhibition of adipocyte differentiation and inhibition of insulin-induced glucose uptake & metabolism by the cells**.** It is expressed in liver cells, and its production is likely to increase with both simple steatosis and in MASH.**[10, 11]**

High-fat diet and obesity increase Resistin-like molecule- β (RELM-β), which is a Resistin homodimer expressed in the colon & not in the adipose tissues. RELM- β induces dysregulation of the gut microbiota, with subsequent excess production & release of endotoxin lipopolysaccharide (LPS). LPS induces hepatic steatosis and cytokines release with further worsening of MASLD & MASH.**[12]**

Objectives of the study

- **Primary**: to assess the serum levels of resistin in MASLD & MASH patients
- **Secondary:** to evaluate the relationship between serum resistin levels and the degree of severity of MASLD

Methods:

This is a case-control study, which was carried out in the outpatient clinic of the Internal Medicine Department, in Zagazig University Hospitals, during the period from July 2023 to July 2024.

The study was approved by the institutional review board & ethical committee of the Faculty of Medicine, Zagazig University (IRB# 10520-2023), and was carried out according to the principles of the Declaration of Helsinki in 1975. All participants provided written informed consent before enrollment**.**

The study enrolled 69 Subjects, who were divided into 3 groups:

Group 1: included 23 subjects apparently healthy & non-obese as control group.

Group 2: included 23 patients with MASLD

Group 3: included 23 patients with MASH

Diagnosis of MASLD: according to the diagnostic criteria of the multi-society Delphi consensus statement for MASLD diagnosis: **[2]**

Hepatic steatosis detected by imaging or biopsy, plus at least 1 of the 5 cardiovascular metabolic risk factors:
(1) BMI ≥ 25 kg/m2 or waist circumference > 94 cm in men, > 80 cm in women, or ethnicity adjusted
(2) Fasting serum glucose ≥ 100 mg/dl, or 2-hour post-prandial glucose level ≥ 140 mg/dl, or HbA1c ≥ 5.7% or on specific drug treatment for type 2 diabetes.
(3) Blood pressure ≥ 130/85 mmHg or specific drug treatment for hypertension
(4) Plasma triglycerides ≥ 150 mg/dl or specific drug treatment
(5) Plasma HDL cholesterol < 40 mg/dl for men and < 50 mg/dl for women or specific drug treatment

Criteria of inclusion:

1. Age:18 - 65 years old
2. Free of chronic disease: ischemic heart disease, chronic kidney disease, and thyroid disorders.
3. MASLD patients: meet the multi-society Delphi consensus criteria of MASLD diagnosis **[2]**
4. MASH patients: positive liver biopsy &/or bright liver in abdominal ultrasonography with elevated liver enzymes.**[6]**

Criteria of exclusion:

1. Age: less than 18 years or more than 65 years old
2. Patients with a history of excessive chronic alcohol consumption (> 20 gm/day for women, & > 30gm/day for men)
3. Positive serum viral markers for hepatitis B or C virus
4. History of other metabolic or autoimmune liver diseases.
5. Patients with clinical, laboratory, or imaging criteria of end-stage liver disease
6. Patients who didn't accept to be enrolled in the study.

- **All the study subjects underwent:**

1. Complete history taking: including long-standing unexplained fatigue, right upper abdominal pain, intolerance to heavy or fatty meals, history of alcohol intake, history of using Statins, anti-diabetic or anti-hypertensive drugs, and family history of metabolic, autoimmune, or hereditary liver diseases

2. Thorough general and local abdominal examination, including anthropometric measurements of weight, height, and body mass index (BMI).
3. Imaging study: pelvi-abdominal ultrasonography, for detection of hepatic criteria in the study subjects (liver size, echogenicity, brightness, etc) and to exclude features of end-stage liver disease (portal hypertension, ascites, splenomegaly).
4. Laboratory investigations:
   - ❑ Routine laboratory investigations as complete blood count (CBC), fasting blood sugar, 2-hour postprandial blood sugar, glycated hemoglobin (HbA1c), liver and kidney function tests, HCV Ab, HBsAg, and fasting lipid profile.
   - ❑ Special investigation: Serum level of resistin, measured by the enzyme-linked immunosorbent assay (ELISA)

Specimen collection and storage:

- Ten-ml venous blood sample was collected by vein puncture under complete aseptic condition from every subject after overnight fasting for 12 hours and the samples were put in sterile tubes.
- The tubes used for Resistin measurement were left for 45 minutes at room temperature to clot, followed by centrifugation for 20 minutes at the speed of 2000 -3000 RPM.
- Serum samples were removed and kept in the refrigerator at (- 4^{o}C) till analysis for Resistin level, while other parameters were measured fresh.

Measurement of serum Resistin level:

- Human Resistin ELISA Kits, provided by DL-Develop (China) Catalogue No. DL-RETN-Hu, were used to measure serum Resistin levels for our study subjects.
- Kits linearity limit was 2000 pg/mL, with a lower detection limit of 13.6 pg/mL

Test principle:

- The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to measure the level of human Resistin in serum samples.
- Resistin was added to the monoclonal antibody Enzyme well which is pre-coated with human Resistin monoclonal antibody, then was incubated.
- Tenascin-C antibodies labeled with biotin, and combined with Streptavidin-HRP, were added to form an immune complex. The uncombined enzyme was removed by incubating and then washing the immune complex.
- When Chromogen Solutions A and B were added, the color of the liquid changed into blue, and at the effect of acid, the color finally became yellow.
- The chroma of color and the concentration of the human substance (Resistin) of the sample were positively correlated.

Statistical analysis:

Data were represented in tables & figures and were analyzed using the software SPSS (Statistical Package for the Social Sciences) version 26. Categorical variables were described as frequencies and were compared using the chi-square test, and Monte Carlo tests when appropriate. **Kolmogorov**–**Smirnov test** was used to verify assumptions for use in parametric tests. Quantitative variables were described as means and standard deviations or median and interquartile range and were compared using the independent t-test (for normally distributed data) or Mann-Whitney test (for not normally distributed data). To compare quantitative data between two groups, the Kruskal-Wallis test (for not normally distributed data) and the one-way ANOVA test (for normally distributed data) were used. when the difference is significant, pairwise comparison and Tukey HSD were used

to detect the difference between each two individual groups. The ROC curve was used to determine the cutoff value of Resistin level. Spearman rank correlation coefficient was used to measure the strength and association of correlation between two continuous not normally distributed variables. Linear regression analysis was performed to measure associated independent factors for dependent factors. The level of statistical significance was set at $P<0.05$. A highly significant difference was present if $p\leq0.001$.

Results:

This case-control study enrolled 69 patients in three equal groups. As shown in **table (1)**, the patients were 27 (39.1%) males and 42 (60.9%) females with a mean age of 43 years old. There was a statistically non-significant difference between the studied groups regarding age, sex, level of education, height, and blood levels of urea & creatinine.

There was a statistically significant difference between the studied groups regarding weight and BMI. Hemoglobin, platelet count, ALP, ALT & AST, total & LDL-cholesterol, triglycerides, FBS, and PPS (**Table 2**). Post-hoc test showed a significant difference in comparing the control group with each other group and a significant difference between MASH & MASLD groups in most of these aforementioned parameters.

Table (3) showed a statistically significant difference between the studied groups regarding serum Resistin levels. Pairwise comparison showed significant differences between each two individual groups as shown in **table (4) & figure (1)**

Table (5) revealed that when comparing MASLD patients & healthy control subjects, there was a high statistically significant difference and a positive correlation between serum Resistin level and all of the weight, BMI, TLC, ALP, AST, ALT, two-hour postprandial sugar, total cholesterol, LDL cholesterol, and triglycerides. However, there was a statistically significant negative correlation between serum Resistin level and age & fasting blood sugar. However, when comparing MASH & MASLD, there was a statistically significant difference and a positive correlation between serum Resistin and all of height, hemoglobin, AST, total cholesterol, and LDL cholesterol.

Figure (2) showed that the best cutoff value of serum Resistin in differentiating MASLD from healthy control was ≥28.25 pg/ml with the area under curve 1, sensitivity 100%, specificity 95.7%, positive predictive value 95.8%, negative predictive value 100% and overall accuracy 97.8% ($p<0.001$)

Figure (3) showed that the best cutoff value of serum Resistin in differentiating MASH from MASLD ≥903.5 pg/ml with the area under curve 0.989, sensitivity 95.7%, specificity 91.3%, positive predictive value 91.7%, negative predictive value 95.5% and overall accuracy 93.5% ($p<0.001$)

Discussion

MASLD, the hepatic component of metabolic syndrome, is considered nowadays the most prevalent cause of chronic liver disease, with a significantly increased incidence of liver cirrhosis, hepatocellular carcinoma, liver transplantation, and liver-related mortality worldwide. MASH is the progressive necro-inflammatory subtype of MASLD with type 2 diabetes and obesity are the major risk factors and insulin resistance is the cornerstone in the pathophysiology.**[13]**

MASLD nomenclature, the latest term for steatotic liver disease, is appropriately describing the causal relation between liver steatosis and the components of metabolic syndrome and avoids the drawbacks & limitations of the previous NAFLD nomenclature that had exclusionary and potentially stigmatizing criteria**. [14]**

Although MASLD is a highly prevalent disease with severe hepatic, renal, and cardiovascular complications, there is a significantly reduced awareness regarding this disorder among healthcare providers and the general population. Thus, creating a simple and clear national assessment and referral strategy using non-invasive

serological tests is essential for categorizing MASLD patients, identifying patients with MASH, and referring them for more specialized care. **[1]**

Resistin is a pro-inflammatory adipokine, that is highly expressed in adipose tissues and the fatty liver. It antagonizes insulin actions and contributes to the progressive necro-inflammatory cascade of MASH. **[15]**

Our study included 69 subjects, divided into 3 equal groups with matched age & sex criteria.

Our results showed a high statistically significant difference between MASLD & NASH patients on one hand and the control subjects on the other hand regarding body weight, BMI & increased liver enzymes (AST, ALT & ALP), with a statistically significant difference between MASLD & MASH patients in blood levels of hemoglobin & platelets count. These results are similar to those reported by ***Tuzer et al., Dalbeni et al. & Huang et al.*****[16-18]**

Our results showed a high statistically significant difference between MASLD & MASH patients on one hand and the control subjects on the other hand regarding FBS, PPS, and total cholesterol with a statistically significant difference between MASLD & MASH patients in total & LDL-cholesterol. Our results are in match with those reported by ***Lee et al. & Velenosi et al.*****[19, 20]**

Our study revealed that there was a high statistically significant difference and a positive correlation between serum Resistin level and all of the weight, BMI, TLC, ALP, AST, ALT, two-hour postprandial sugar, total cholesterol, LDL cholesterol, and triglycerides on comparing control subjects & MASLD patients. However, there was a statistically significant negative correlation between serum Resistin level and age & fasting blood sugar on comparing both groups. Similar results were reported by ***Fajkić et al.*** **[21]**

We found that there was a statistically significant difference and a positive correlation between serum Resistin and all of height, hemoglobin, AST, total cholesterol, and LDL cholesterol in comparison between MASH & MASLD patients. Similar results were reported by ***Han et al.*** **[22]**

Our results showed that the best cutoff value of serum Resistin in differentiating MASLD from healthy control was ≥28.25 pg/ml with the area under curve 1, sensitivity 100%, specificity 95.7%, positive predictive value 95.8%, negative predictive value 100% and overall accuracy 97.8% ($p<0.001$)

We reported that the best cutoff value of serum Resistin in differentiating NASH from MASLD ≥ 903.5 pg/ml with the area under curve 0.989, sensitivity 95.7%, specificity 91.3%, positive predictive value 91.7%, negative predictive value 95.5% and overall accuracy 93.5% ($p<0.001$)

Conclusion:

Serum Resistin level is a proper & valid diagnostic biomarker for MASLD & MASH at a cutoff value >28.25 pg/ml & > 903.5 pg/ml respectively.

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