https://doi.org/10.31689/rmm.2024.31.3.243

ORIGINAL PAPERS

Differences Platelets Count, IL-6, Aldosterone Level and Fatty Liver Occurrence Between Obese and Non-Obese Adult Young Women

Farah Hendara NINGRUM[1], Ignatius RIWANTO[2], Hermina SUKMANINGTYAS[3], Kusworo ADI[4], Tjokorda Gde Dalem PEMAYUN[5], Antonius Gunawan SANTOSO[6], Rr. Lydia Purna Widyastuti Setjadiningrat KUNTJORO[6], Miftahul JANNAH[7], Meita HENDRIANINGTYAS[8]

Abstract

Background: Non-Alcoholic Fatty Liver Disease (NAFLD) is common liver disease often found and occurs in community. Prevalence of liver disease (NAFLD) has risen rapidly along with an increase obesity incidence in women. Inflammation condition in obese condition causing an increase in IL-6, Platelets Count (PLTC), and Aldosterone level increased risk of developing NAFLD.
Aim: To analyze differences IL-6, PLTC, and Aldosterone level between Obese and Non-Obese Women.
Methods: This study was an observational cross-sectional analytical study that conduct in November-Januari 2021 at Diponegoro National Hospital (RSND) Semarang. Inclusion criteria for respondent in this study were being middle-aged women (30-49 years), healthy, and willing to take part in this study. Total respondent in this study was 60 people then divided into two groups that is Obese and Non-Obese Group. The degree of fatty liver was examined using the ultrasound method. Platelete count (PLTC) were measured by Indico Blood Analyzer. Meanwhile IL-6 and Aldosterone level analyzed by the enzyme-linked immunosorbent assay (ELISA) method. Fatty liver was diagnosed by abdominal ultrasound. Data were analyze using SPSS 18.0 that including normality, different test, and regression test.
Result: Incidence of fatty liver in the obese group was significantly different from the non-obese group ($p<0.005$). No significant different average aldosterone serum, and AST between two group. Platelete Count (PLTC), IL-6 between two groups was showed significantly difference between two group. Conclusion: Obesity will trigger an increase in IL-6 and PLT which can increase the risk of fatty liver.

Keywords: Obesity, NAFLD, IL-6, Platelete Count, Aldosterone, Inflammation

[1]Doctoral Study Program of Medical and Health Science, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
[2]Department of Surgeon, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
[3]Department of Radiology, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
[4]Department of Physics, Faculty of Science and Mathematics, Universitas Diponegoro, Semarang, Indonesia
[5]Department of Internal medicine, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
[6]Department of Radiology, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
[7]Department of Radiology, Dr. Kariadi General Hospital, Semarang, Indonesia
[8]Department of Clinical Pathology, Universitas Diponegoro, Semarang, Indonesia

***Corresponding author:**
Farah Hendara NINGRUM, Doctoral Study Program of Medical and Health Science, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
E-mail: farahhendara@fk.undip.ac.id

BACKROUND

Non-Alcoholic Fatty Liver Disease (NAFLD) is common liver disease that is often found and occurs in society. NAFLD was characterized by hepatic steatosis when no other causes for secondary hepatic fat accumulation (e.g., excessive alcohol consumption) can be identifed[1]. Highest level of severity of NAFLD is non-alcoholic steatohepatitis (NASH) that can caused fibrosis and cirrhosis that irreversible[2]. Prevalence of liver disease (NAFLD) has risen rapidly with a worldwide prevalence of 25% [3]. NAFLD case found in almost 45,2% in Diabetes Mellitus Type 2 patient and 51% in general population[4]. Central obesity, Type 2 Diabetes Mellitus, Dyslipidemia, and Metabolic syndrome are becoming major risk factor for NAFLD. People with NAFLD usually have one or more components of the metabolic syndrome (MS) like systemic including hypertension, dyslipidemia, insulin resistance, or overt diabetes[4]. There is increasing evidence that visceral obesity is a risk factor for NAFLD and it also has to be taken into account that MS is a known risk factor in cardiovascular disease development[5].

The most common technique used to asses NAFLD in population studies is Conventional B-mode ultrasonography. NAFLD diagnosed based on the following ultrasound parameters: parenchymal brightness, liver-to-kidney contrast, deep beam attenuation, bright vessel walls, and gallbladder wall definition. Qualitative grade of fatty liver categorized with Grade 0 to 3. Grade 0 is defined as non-fatty liver or liver with normal condition. Grade 1 is Mild, Grade 2 is Moderate, and Grade 3 is Severe[6].

Obesity more common in women than men caused by sedentary lifestyle[7,8]. Sedentary lifestyle like unbalanced nutrition intake, lack of physical activity that occur in society, increasing incidence of obesity every year[9]. Obese condition that characterized by high lipid accumulation can cause dysregulation of adipokines such as IL-6, CRP, Adiponectin, lipotoxicity, oxidative stress, dysbiosis of the gut microbiota and endocrine disruptors. Combined effect of some of these factors, are initially accumulated in the hepatocytes leading to NAFLD[5].

Obesity is a condition that can trigger inflammation caused by an increase in body fat[10]. Previous studies show that platelet profile plays an important role in a number of diseases whose pathogenesis is strongly influenced by the inflammatory process[11]. Platelet profile can be an alternative inflammatory parameter with the advantage of easier and cheaper examination compared to pro-inflammatory cytokines[12].

Previous studies describe that aldosterone levels are higher in overweight/obese individuals, especially those with excessive visceral fat[13]. Correlation between obesity and aldosterone levels caused by adipose tissue produces factors that can independently stimulate aldosterone secretion from the adrenal glands[14]. Aldosterone in obesity also correlated with hypertension that become one of the criteria for metabolic syndrome.

NAFLD is an reversible condition that can which can be controlled so that the clinical manifestations do not become more severe to the point of causing irreversible conditions such as cirrhosis. Research related to early detection of NAFLD is very necessary as early prevention for women with obesity. Conducting an analysis regarding the correlation of IL-6, PLTC, and Aldosterone as an alternative parameter for initial screening for NAFLD.

METHOD

This study was using cross-sectional observational research method and approved by Health Research Ethics Committee, Faculty of Medicine, Diponegoro University Semarang No.195/EC/KEPK/FK-UNDIP/VI.2021. The research was conducted during November-Januari 2021 at the Diponegoro National Hospital (RSND) Semarang.

Respondent of this study were young women aged 30-49 years who lived in Semarang, Indonesia. Inclusion criteria respondent was having normal body temperature (36.5–37.5°C), aged 30-49 years and willing to take part in the study. Exclusion criteria were pregnant women, hemolysis specimens, frozen specimens, heart disease, diabetes mellitus, and blood disorders.

Sample divided into two groups that is obese group and non-obese group based in BMI score. Women with BMI > 25 kg/m^2 classified as obese group and BMI ≤ 25 kg/m^2 classified as Non-Obese Group. MI was calculated using this formula:

$$\text{BMI} = \frac{Weight\ (cm)}{Height\ (m)^2}$$

Respondent had 3 cc of blood taken in the EDTA tube, then an examination was carried out at the RSND outpatient laboratory. Blood biochemistry was analyzed

using an automatic hematology analyzer Sysmex XS-500i. In addition, anthropometric measurements were also carried out on research respondents. IL-6 and Aldosterone serum was analyze by ELISA kit in GAKY Laboratory, Faculty of Medicine, Diponegoro University.

Degree of fatty liver in respondent was determined using Ultrasonography (USG) Infinity type by Philip. Ultrasound is widely recommended as the first-line imaging test for individuals with suspected NAFLD[15]. Identification degree of fatty liver using ultrasonography characterized by slightly enlarged liver size, echoparenchyma increases homogeneously, and there is deep attenuation in the section posterior[16]. Degree I (Mild) is indicated by an increase ecogeneity is just increased. Grade II (Moderate) is indicated when liver echogenic obscuration echogenic walls of the portal vein branch. Degree III (Severe) is indicated when liver echogenicity obscures the outline of the diaphragm[17].

Collected data in this study was analyzed using software SPSS 21 ver. Normality test using Shapiro-Wilk. Different test between two group using independent t test if data was normally distributed and mann whitney if data was not distributed normally. Fisher test exact was used to analyze differences fatty liver occurrence between two group in this study.

RESULT

There was no significant average age between obese group and non-obese group respondent in this study. Not only age, but also aldosterone serum between two group was not significantly different. BMI, Platelete Count (PLTC), IL-6 between two groups was significantly different with obese group showing higher values (Table 1).

The results of the ultrasound examination showed that there were 30 respondents in non-obese group did not have fatty liver. In obese group, 5 respondents did not have fatty liver, 16 respondents have fatty liver degree I, 6 respondents having fatty liver degree II, and 2 respondents having fatty liver degree III.

Result from differences test in this study showed that there was significant differences Platelets count (PLTC) and IL-6 between two group (p=0.025; p=0.000) and there was no significant difference aldosterone between two group (p=0.077). (Table 1.).

Table 1. Comparison Inflammatory Parameters and Fatty Liver Incident in Obese and Non-Obese Group

Characteristic	Obese (Mean±SD) N=30	Non-Obese (Mean±SD) N=30	p
Age	31.33 ± 10.07	31.13 ± 4.48	0.324
IMT	31.86 ± 5.16	20.16 ± 3.75	0.002*
PLTC (Platelete Count)	327.61 ±57.11	291.63 ±57.66	0.025*
IL-6	33.10 ± 30.59	5.94 ± 5.28	0.000*
Aldosteron	34.61 ± 16.99	35.60 ± 35.98	0.077
Fatty Liver Incident			
- Non-Fatty Liver	7 (23.3%)	30 (100 %)	
- Mild	16 (53.3%)	0 (0 %)	
- Moderate	5 (16,7%)	0 (0 %)	
- Sever	2 (6.7%)	0 (0%)	0.000**

*Note: *Significant* in Mann Whitney Test (p<0.05)

** *Significant in Fisher Test (p<0.05)*

DISCUSSION

Non-Alcoholic Fatty Liver Disease (NAFLD) is common chronic hepatic diseases in many developed countries. This disease has broad range of hepatic disorders from simple fat accumulation in hepatic cells (simple steatosis) to hepatic tissue[18]. Diagnosis of NAFLD is of special importance because of the odds of its progressing to more critical stages. Furthermore, in cases of diagnosis at early stages. At present, liver biopsy is the gold standard for diagnosing nonalcoholic fatty liver, but this method is not only invasive and expensive but

also has the important limitations of pain, reluctance of patients, risk of severe complications and being subject to sampling error[19]. The primary imaging modality in suspected NAFLD is sonography. It is inexpensive, widely available, free of side effects, can be repeated as often as required, and has a high level of patient acceptance due to the direct examination by the doctor.

In this study, degree of fatty liver in respondent was determined using Ultrasonography (USG). B-mode ultrasound is an excellent method to detect moderate and severe steatosis in patients with NAFLD as an alternative screening method beside liver biopsy as gold standard[20]. Fisher test analysis for fatty liver occurrence in this study describe that there are significantly difference between two group in this study ($p<0.005$). More respondents in the obese group experienced fatty liver than other groups. This result in line with previous theory that describe obesity is one condition that elevated risk for fatty liver.

Obesity is one of the major risk factors of Non-Alcoholic Fatty Liver Disease (NAFLD). Excessive body fat in obese condition led to increase IL-6 secretion. IL-6 is one of the cytokines pro inflammations produce by adipose tissue[20]. Obesity can start with the development of leptin resistance. Reducing peripheral leptin resistance will restore leptin function to break down fat tissue so that IL-6 levels in serum will decrease. Result in this study in line with previous study that describe IL-6 serum respondent in Obese group was higher than Non-Obese Group. IL-6 serum obese group was significantly different than Non-Obese Group. Obese group have average IL-6 serum (33.10 ± 30.59 μg/ml) and Non-Obese Group was (5.94 ± 5.28). It has been shown from the previous study that the inflammatory cytokines played an important role in the development of NAFLD by activating various inflammatory pathways that interfered with insulin signaling[21]. Previous research also found that macrophages secreted IL-1b and IL-6, which led to hepatocyte injury and liver fibrosis[22].

Platelete Count (PLTC) becoming one of the low inflammation biomarkers. Analytic statistic from two groups shows that there are significant different between PLTC from respondent in Obese Group and Non-Obese Group. Respondent in Obese group have higher PLTC with (327.61 ±57.11) than Non-Obese Group (291.63 ±57.66). Spearman rank test between NAFLD and PLTC also indicate a significant correlation between that two variable.

NAFLD caused by a chronic inflammatory state in liver. The inflammatory state is mostly due to metabolic syndrome characterized by obesity, insulin resistance and diabetes (type 2). Platelets are involved in pathological processes such as chronic inflammation and atherothrombosis and possibly fibrogenesis[23].

Aldosterone is the only variable in this study that was not significantly correlate with fatty liver. Different test between Obese Group and Non-Obese Group also describes that there was no significantly different between Aldosterone Serum. Non-significant correlation between these two variables in this study maybe caused by not considering the amount of visceral fat from respondent. In brief, a related mechanism is that an increase in the amount of visceral fat in the body triggered an increase in the hormone leptin which then caused an increase in the activation of the hormone aldosterone[24]. Related mechanism is that an increase in the amount of visceral fat in the body triggered an increase in the hormone leptin which then caused an increase in the activation of the hormone aldosterone[25].

Aldosterone secretion also strongly correlated with decrease insulin function that did not found in this study. Respondent in this study was not in pre diabetic or diabetic condition that caused hyperaldosteronism condition. Metabolic syndrome that consists of not only obesity but also hyperglycemia, hypertension, and hipertriglyceride that not fulfilled from respondent in this study because of limitation in this study might be the cause of this unsignificant correlation.

Respondent in this study was limited only 60 people and it became our limitation for this study. Another limitation for this study was there are many confounding variables that we cannot analyze in this study. Further research related to this topic on a larger scale needs to be carried out

CONCLUSION

Inflammation parameter such as PLTC and IL-6 significantly correlated with risk of NAFLD in obese women that caused by increase low inflammation in body. Aldosterone that part of RAA mechanism was not significantly correlated with NAFLD in women with obesity because hyper aldosteronism usually find in metabolic syndrome condition that describe as more complex condition that not only caused by obesity. ILC-6 and PLTC can be used as alternative parameters for early detection of NAFLD.

References

1. Pouwels S, Sakran N, Graham Y, Leal A, Pintar T, Yang W, Kassir R, et al. Non-alcoholic fatty liver disease (NAFLD): a review of pathophysiology, clinical management and efects of weight loss. BMC Endocrine Disorders. (2022);22:63.
2. Machado MV, Diehl AM. Pathogenesis of nonalcoholic Steatohepatitis. Gastroenterology. 2016;150(8):1769–77.
3. Maurice J, Manousou P. Non-alcoholic fatty liver disease. Clinical Medicine 2018 Vol 18, No 3: 245–50.
4. Sulaiman, Andri Sanityoso; Hasan, Irsan; Lesmana, Cosmas Rinaldi A.; Kurniawan, Juferdy; Jasirwan, Chyntia Olivia Maurine; Nababan, Saut Horas H.; Kalista, Kemal Fariz; Aprilicia, Gita; and Gani, Rino Alvani (2023) "Perlemakan Hati Non-Alkoholik dan Risiko Fibrosis Hati pada Pasien Hepatitis B Kronik," Jurnal Penyakit Dalam Indonesia: Vol. 10: Iss. 3, Article 2. DOI: 10.7454/jpdi.v10i3.1456 Available at: https://scholarhub.ui.ac.id/jpdi/vol10/iss3/2
5. Polyzos SA, Kountouras J, Mantzoros CS. Obesity and non-alcoholic fatty liver disease: From pathophysiology to therapeutics. Metabolism. 2019 Mar;92:82-97. doi: 10.1016/j.metabol.2018.11.014. Epub 2018 Nov 29. PMID: 30502373.
6. Mahale AR, Prabhu SD, Nachiappan M, Fernandes M, Ulla S. Clinical Relevance Of Reporting Fatty Liver On Ultrasound In Asymptomatic Patients During Routine Health Checkups. Journal of International Medical Research. 2018;Vol. 46(11):4447–4454.
7. Harbuwono DS, Pramono LA, Yunir E, Subekti I. Obesity and Central Obesity in Indonesia: Evidence From A National Health Survey. Med J Indonesia.June 2018;Vol. 27, No. 2:114-120.
8. Hariyadi D, Puspita WL , Anwar T, Wardoyo S. A Path Model of Factors Associated with Hypertension and Disease: Analysis of Indonesian Basic Health Survey Year 2018. Modern Medicine. 2024; 31(2): 135-142. https://doi.org/10.31689/rmm.2024.31.2.135.
9. Saliha MHARCHI, Abdellatif MAAMRI. Diet, Physical Activity, and Their Impact on Chronic Diseases (Hypertension and T2DM) among North-Eastern Morocco's Population. Modern Medicine. 2024; 31(1): 37-47. https://doi.org/10.31689/rmm.2024.31.1.37.
10. Soesilo N, Hendrianingtyas M, Rachma DE, Limijadi EKS. Monocyte to HDL Cholesterol Ratio (MHR) and Monocyte to Lymphocyte Ratio (MLR) in Overweight and Obese Women. Modern Medicine. 2023; 30(4): 335-339. https://doi.org/10.31689/rmm.2023.30.4.335.
11. Cecen S. Platelet Activation is a Risk Factor for Obesity. The Turkish Journal of Endocrinology and Metabolism. 2020; 24:132–7. DOI: 10.25179/tjem.2019-72995.
12. Cohen E, Margalit I, Shochat T, Goldberg E, Krause I. Markers of Chronic Inflammation in Overweight and Obese Individuals and the Role of Gender: A Cross-Sectional Study of a Large Cohort. J Inflamm Res. 2021 Feb 25; 14:567-573. DOI: 10.2147/JIR.S294368.
13. Cooper JN, Fried L, Tepper P, Mitchell EB, Conroy MB, Evas RW, et al. Changes in serum Aldosterone are Associated with Changes in Obesity-related Factors in Normotensive Overweight and Obese Young Adults. Hypertension Research. 2013: 36, 895–901; doi:10.1038/hr.2013.45.
14. Ding X, Wendy BB. Obesity, Hipertension and Aldosteron is Leptin the Link. Int Journal Endocrinelology. 2016: 230, F7–F11.
15. Gabriel Leivas, Obesity Research & Clinical Practice, https://doi.org/10.1016/j.orcp.2021.09.002
16. Kasim S, Arief M, Sulaeman A, Widodo J. Relationship between Obesity and Hypertriglyceridemia on Fatty Liver in Patients at Makassar. J Farm Klin Indones. 2012;1(4):136–46
17. Singh D, Das CJ, Baruah MP. Imaging of nonalcoholic fatty liver disease: A road less travelled. Indian J Endicronolgy Metab. 2013;17(6):990–5.
18. Hadizadeh F, Faghihimani E, Adibi P. Nonalcoholic fatty liver disease: Diagnostic biomarkers. World J Gastrointest Pathophysiol. 2017 May 15;8(2):11-26. doi: 10.4291/wjgp. v8.i2.11. PMID: 28573064; PMCID: PMC5437499.
19. Petzold G. Role of Ultrasound Methods for the Assessment of NAFLD. J Clin Med. 2022 Aug 5;11(15):4581. doi: 10.3390/jcm11154581. PMID: 35956196; PMCID: PMC9369745.
20. Chen Y, Yu CY, Deng WM. The role of pro-inflammatory cytokines in lipid metabolism of metabolic diseases. Int Rev Immunol. 2019;38(6):249-266. doi: 10.1080/08830185.2019.1645138. Epub 2019 Jul 28. PMID: 31353985.
21. Duan Y, Pan X, Luo J, Xiao X, Li J, Bestman PL and Luo M. Association of Inflammatory Cytokines With Non-Alcoholic Fatty Liver Disease Front. Immunol. 2022;13:880298. doi: 10.3389/fimmu.2022.880298.
22. Gao B, Tsukamoto H. Inflammation in Alcoholic and Nonalcoholic Fatty Liver Disease: Friend or Foe? Gastroenterology.2016;150(8):1704–9.doi: 10.1053/j.gastro.2016.01.025.
23. Dalbeni A, Castelli M, Zoncapè M, Minuz P and Sacerdoti D.Platelets in Non-alcoholic Fatty Liver Disease. Front. Pharmacol. 2022;13:842636. doi: 10.3389/fphar.2022.842636.
24. Shibayama Y, Wada N, Baba S, Obara S, Sakai H, Usubuchi H, Terae S, Nakamura A, Atsumi T. The risk factors for hepatic steatosis in patients with primary aldosteronism. Endocr J. 2020 Jun 29;67(6):623-629. doi: 10.1507/endocrj. EJ19-0600. Epub 2020 Mar 24. PMID: 32213734.
25. Ceccoli, L., Ronconi, V., Giovannini, L., Marcheggiani, M., Turchi, F., Boscaro, M., & Giacchetti, G. (2013). Bone health and aldosterone excess. Osteoporosis International, 24(11), 2801–2807. doi:10.1007/s00198-013- 2399-1.