http://doi.org/10.36582/j.Alkuno.2023.06.06
Al-Kunooze University College
Journal homepage: http://journals.kunoozu.edu.iq/1/archive &
http:// www.iasj.net

Evaluation of some hematological parameters abnormalities in patient with non-alcoholic fatty liver disease in basrah city

Dalal F. Al-Akabi *

Department of medical laboratory techniques, Al-Kunooze University College, Basrah, Iraq

Email: dalal.f.m@kunoozu.edu.iq

Abstract

Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver diseases worldwide, it is a set of graded cases that not associated with drinking alcohol, which is characterized by accumulation of fat in the liver, especially in people who are overweight and smokers. The aim of the current study is to evaluate the serum erythropoietin (EPO) and complete blood count (CBC) levels in NAFLD patients. 45 NAFLD blood samples and 45 control samples were collected, the whole blood used to perform CBC and the serum for EPO test which it was measured by ELIZA technique, all results statistically analyzed by SPSS based on the questionnaire information. The results showed a significant difference in EPO and some of CBC parameters in NAFLD patients compared with healthy people, where disease severity, smoking and BMI factors increase the abnormalities in the study parameters. In conclusion EPO and other hematological parameters may effect by NAFLD pathophysiology and worsen by different factors and this calls for more research to study the full dimensions of such changes in these patients.

Keywords

NAFLD, Erythropoietin, EPO, CBC

* *Corresponding author*. Tel.: 07746320087.
E-mail address: dalal.f.m@kunoozu.edu.iq

Introduction

NAFLD is the most common liver disease around the world, especially in developing countries with prevalence of 25% of the adults [1, 2]. The life style and genetic factors involve in the development of NAFLD and most of the infected people are asymptomatic for a long period of time, so that they are difficult to diagnose before the disease progresses to more severe conditions like liver cancer [3]. NAFLD includes several levels of severity, where it starts with fatty liver steatohepatitis (NASH) and cirrhosis [4]. NAFLD increases among patients with type 2 diabetes, metabolic syndrome and obese people also it is a major cause of liver cirrhosis, which requires transplantation and a special methods are used to diagnose liver fibrosis, such as transient elastography, ELF test and fibrosis Score [5]. NAFLD prevalence among obese and non-obese people also non-obese patients suffer from fibrosis and NASH, but at a lower rate than what occurs with obese patients also in non-obese patients, the metabolic syndrome is associated with the NAFLD progresses and some parameters can be used to diagnosing the progression of NAFLD in non-obese patients [6]. Smoking increases the risk of NAFLD occurrence, and this risk increases with the strictness of smoking, but no clear association was shown in the newly smoked [7]. Hypoxia contributes to the development of hepatosteatosis in NAFLD patients through its role in liver lipid accumulation via regulation of Hypoxia-inducible factor 1α and 2α in the liver which induces steatosis by induction of lipogenesis, hepatic free fatty acid uptake and oxidation [8]. EPO is a hormone and a cytokine known to play a vital role in erythropoiesis, the production of red blood cells, and its deficiency in blood is a major cause of anemia in kidney disease, where in the laboratory mice, it was observed that EPO receptors when restricted in hematopoietic tissues suffering from insulin resistance and obesity, where fat cells and the amount of white adipose tissue were elevated, and EPO lost its important role in energy regulation [9,10]. As well as EPO role in erythropoietsis, it plays an important role in healing wounds, protecting cells from apoptosis and inhibiting proinflammatory cytokines [11]. EPO is produced in kidney and its receptors are expressed in different places such as bone marrow, heart, brain and skeletal muscle which plays an important role in regulating sugar and fat levels by protecting fatty tissue from inflammations and when its rise above the normal value a defect in the formation of fats and bone tissue will occure [12]. When environmental oxygen decreases, the body tries to compensate for this loss by stimulating the production of RBCs by several factors such as EPO, leading to anemia or erythrocytosis, and any defect in EPO trascription leads to mutations in HIF which is a major cause of erythrocytosis [13]. EPO is known for its role in regulating lipid metabolism as it inhibits hepatic endoplasmic reticulum stress and stimulates the

secretion of fibroblast growth factor -21 in mice [14]. The aim of the presesnt study is to study the possible changes in the serum EPO level and the hematological parameters in NAFLD patients under different conditions to record any relationship between disease physiology and
Study criteria.

Methods

The present study was achieved in Iraq / Basrah in 2021(December to March), which included 90 sample (45 people with NAFLD, and 45-matched healthy people as control group). NAFLD patients were diagnosed by ultrasound and blood test under the supervision of an internist. The type of samples was classified according to the severity of the disease severity, smoking and BMI factors according to the questionnaire form. Whole blood were used to perform CBC by Mindray device and serum of EPO concentration was measured by ELISA method (Bioassay technology laboratory kit / china) , exclusion criteria for all samples are other liver diseases, anemia, pulmonary diseases, kidney diseases, hormonal diseases , immune diseases and prolonged medication intake,. The statistical analysis is carried out using SPSS ($P<0.05$ is considered statistically significant) and the difference was evaluated by T-Test and one way Anova test.

Results

The current study results showed that there was a significant increase in EPO, WBC, LYM, RBC, HGB and HCT level, a significant decrease in PLT level in NAFLD patients when compared with the control groups, while there were no significant difference in the other parameters, table (1).

Table 1- Comparison of serum EPO and CBC parameters between NAFLD patients and control groups, values were expressed as (mean ± standard deviation).

Study parameters	NAFLD(N=45)	Control(n=45)
EPO(mlIU/ml)	354.35±221.38	116.96±74.40*
WBC (*10^9/L)	9.033±3.1855	7.02±2.09*
LYM (*10^9/L)	5.68±3.39	0.92±0.34*
RBC(*10^{12}/L)	5.91±1.77	4.52±0.88*
HGB(g/Dl)	16.80±.51	12.89±0.90*
HCT%	48.92±8.57	43.72±5. 87*
MCV(fL)	92.07±6.53	89.56±6.88
MCH(pg)	31.34±2.51	31.002±2.3169
MCHC (g/dL)	34.07±1.80	32.69±2.18

RDW-CV(fL)	14.13±1.48	14.002±1.2607
PLT(*10^9/L)	127.17±33.99	318.97±72.90*
MPV(fL)	9.67±1.32	9.47±1.14
PDW%	13.38±2.32	12.91±2.55
PCT%	0.22±0.07	0.20±0.06
*** Significant at P≤0.05.**		

Serum EPO showed a significant positive correlation with RBC, HGB,HCT and a significant adverse correlation with PLT, while serum EPO showed non- significant correlation with WBC and LYM in patients with NAFLD, table (3).

Table 2: Correlation of EPO with CBC parameters.

Variables	WBC	LYM	RBC	HGB	HCT	PLT
EPO	0.190	0.106	0.252*	0.326*	0.166*	-0.184*
***Correlation is Significant at P≤0.05.**						

Depending on the level of disease severity, one way anova test showed a significant increase in EPO, WBC, LYM, RBC level between steatosis, NASH and fibrosis patients groups, where post hoc test showed a significant increase in EPO, WBC, LYM and RBC between steatosis and cirrhosis, also a significant increase in LYM between NASH and cirrhosis was observed, table (2).

Table- 3 Comparison of serum EPO and CBC parameters in NAFLD patients according to disease severity, values were expressed as (mean ± standard deviation).

Study parameters	Steatosis (n=18)	NASH (n=15)	fibrosis (n=12)
EPO(mlIU/ml)	247.40±160.26 ^{aa}	355.47±212.54	513.39±229.20 ^{ab*}
WBC (*10^9/L)	7.47±2.81 ^{aa}	9.02±2.60	11.38±3.11^{ab*}
LYM (*10^9/L)	3.80±2.69^{aa}	5.30±3.25 ^{bb}	8.97±1.87^{ab*}
RBC(*10^{12}/L)	4.96±1.11^{aa}	6.08±1.59	7.13±2.08 ^{ab*}
HGB(g/Dl)	16.68±0.59	16.82±0.45	16.93±0.45
HCT%	47.40±7.88	50.71±7.18	48.95±11.12
PLT(*10^9/L)	131.94±28.09	126.93±45.70	120.33±25.62

***Significant at P≤0.05.**

The current results showed a significant increase in the level of EPO and HGB only in smoking NAFLD patients when compared to non-smoking NAFLD patients, table (4).

Table- 4 Comparison of serum EPO and CBC parameters in NAFLD patients according to smoking factor, values was expressed as (mean ± standard deviation).

Study parameters	Smoking (n=21)	Nonsmoking (n=24)
EPO(mlIU/ml)	457.93±243.42	263.72±154.48*
WBC (*10^9/L)	10.42±3.08	7.81±2.78
LYM (*10^9/L)	7.24±3.30	4.31±2. 88
RBC(*10^{12}/L)	6.43±2.05	5.46±1.38*
HGB(g/Dl)	16.90±0.41	16.71±0.58*
HCT%	50.01±10.15	47.96±6.98
PLT(*10^9/L)	123.14±30.40	130.70±37.14

***Significant at P≤0.05.**

Depending on BMI , one way anova test showed a significant increase in EPO, WBC, LYM, RBC levels between normal weight, pre-obesity and obesity class I NAFLD patients groups, where post hoc test showed a significant increase in EPO normal weight and obesity class I, also a significant increase in WBC, LYM, RBC between normal weight and pre-obesity , pre-obesity and obesity class I, table (5).

Table- 5 Comparison of serum EPO and CBC parameters in NAFLD patients according to BMI factor, values was expressed as (mean ± standard deviation).

Study parameters	Normal weight (n=20)	Pre-obesity (n=14)	Obesity class I (n=11)
EPO(mlIU/ml)	274.17±157.67 aa	318.14±221.81	470.75±239.07^{ab}*

WBC (*10^9/L)	7.20±2.84^{aa}	8.50±2.35^{bb}	11.40±2.88^{ab}*
LYM (*10^9/L)	3.62±2.60 ^{aa}	5.14±3.13 ^{bb}	8.27±2.74 ^{ab}*
RBC(*10^{12}/L)	5.00±1.20 aa	5.60±1.50 ^{bb}	7.13±1.91 ^{ab}*
HGB(g/Dl)	16.75±0.61	16.75±0.51	16.89±0.42
HCT%	47.360±8.6360	52.093±4.8999	47.30±10.77
PLT(*10^9/L)	131.00±22.50	124.13±44.66	126.40±33.24

*Significant at P≤0.05.

Discussion

[15] Showed a simple effect of NAFLD on hematological parameters, whereas a significant increase in haematocrit (HCT) and a significant decrease in platelet count was recorded in NAFLD compared to control group. Elevated WBCs levels in NAFLD patients have been reported in many studies and it is associated with the occurrence of NAFLD regardless of cardiovascular and metabolic syndrome risk factors also inflammations play an important role in the occurrence of disease, especially hepatic steatosis that occurs as a result of systemic inflammation [16, 17, and 18]. Elevated hemoglobin has been observed in several studies and may be a risk factor for NAFLD, RBCs number and RDW abnormalities in NAFLD reflects the genetic changes that occurs in the liver [19, 20]. Hemoglobin elevation in NAFLD patients with cirrhosis may revert to liver hypoxia which leads to elevated erythropoietin levels [21]. Elevated HCT may be an important marker linking between NAFLD and the cardiovascular disease and It can be used as a clinical marker of disease severity [22, 23]. [24] Recoded a significant increase in the level of WBC, Hb, and LYM. An increase in PDW level also a decrease in MPV and PCT level have been observed in NAFLD patients and MPV can be considered an important marker in the diagnosis of NAFLD [25]. High levels of ferritin, HCT and MCH concentration was observed in lean subjects with NAFLD compared to healthy obese subjects and a significant difference in the lipid metabolism and iron status also observed between the two groups [26]. [27] Showed a clear defect in the fatty acids levels t in the RBCs membranes in patients with NAFLD. NASH children have high values of Hb, RDW, and hematocrit when compared with children with NAFLD [28]. EPO was found to reduce fat accumulation in the liver when used as a treatment for NAFLD as it improves lipid metabolism by activating proteins that synthesis lipid in the liver; EPO/EPOR-induced sterol regulatory element-binding protein [29]. EPO therapy relieves simple steatosis, where EPO receptor

stimulates autophagy in the liver cells via Sirtuin 1 dependent deacetylation of LC3 protein and increases autophagosomes and hepatocytes lipid [30]. The most important complication of NAFLD is hepatitis, which leads to a disturbance in the level of body lipids and increased oxidative stress, where EPO can be used in the treatment of NAFLD in high quantities, and the reason for this weak response is due to liver inflammation [31]. An increase in EPO levels and inflammation markers was recorded in NAFLD patients compared to control group, and many research showed that high EPO level is associated with high interlukine-6, while with the high level of transaminases, EPO will decreases [32]. In laboratory mice, EPO was shown to treat liver damage in sepsis patients, as it reduces mitochondrial damage and this action stimulated by lipopolysaccharide through inhibiting the signaling of NLRP3 [33]. EPO is a hormone consist of 165 amino with four glycan that is produced in the kidneys under the control of hypoxia-inducible transcription factors (HIF), in NAFLD the accumulation of fat in the liver cells affects tissue oxygen levels, leading to hypoxia , so the body resorts mechanisms to maintain tissue oxygenation and genetic studies confirm hypoxia involved in the dysfunction of the adipose tissue, leading to fibrosis and inflammation, where hypoxia affects the liver leading to fat accumulation [34]. Several studies have shown the high levels of blood parameters such as RBC, HGB, HCT, neutrophil, PLT and WBC also the low levels of erythropoietin and interleukine-7 in cigarette smoking, which indicates that smoking causes inflammatory issues and polycythemia in smoker people [35,36] Obesity and smoking are factors affecting hematological parameters, for example, obesity affects HCT, smoking affects MCV, HCT and MCH, obesity and smoking raise the level of HGB, while obesity raises the RBC count [37]. In Conclusion EPO and CBC parameters may effect by NAFLD and worsen disease severity, smoking and obesity ,where requires studying other aspects of the study criteria in these patients.

Acknowledgments

I extend my thanks to the specialized medical staff who helped me collecting sample sing and analyzing them, and the[first] international Scientific Conference for Medical Sciences that was held at Al-Kunooze University College, in which I participated in my research

References

1. .Byrne CD and Targher G. Non-alcoholic fatty liver disease-related risk of cardiovascular disease and other cardiac complications. Diabetes Obes Metab 2022; 24: 28-43.

2. Petroni ML, Brodosi L, Bugianesi E, Marchesini G. Management of non-alcoholic fatty liver disease. bmj 2021;372-391.
3. Sharma P and Arora A. Clinical presentation of alcoholic liver disease and non-alcoholic fatty liver disease: spectrum and diagnosis. Clin Transl Gastroenterol 2020; 5:19-28.
4. Maurice J and Manousou P. Non-alcoholic fatty liver disease. Clin Med 2018; 18; 245–250.
5. Tan EXX, Lee JWJ, Jumat NH, Chan WK, Treeprasertsuk S, Goh GBB, et al. Non-obese non-alcoholic fatty liver disease (NAFLD) in Asia: an international registry study. Metab 2022; 126: 154911-154918.
6. Akhavan Rezayat A, Dadgar Moghadam M, Ghasemi Nour M, Shirazinia M, Ghodsi H, Rouhbakhsh Zahmatkesh MR, et al. Association between smoking and non-alcoholic fatty liver disease: a systematic review and meta-analysis. SAGE open medicine 2018; 6: 1-12.
7. Isaza SC, del Pozo-Maroto E, Domínguez-Alcón L, Elbouayadi L, González-Rodríguez Á, García-Monzón C. Hypoxia and non-alcoholic fatty liver disease. Front Med 2020; 7: 578001-578006.
8. Teng R, Gavrilova O, Suzuki N, Chanturiya T, Schimel D, Hugendubler L, et al. Disrupted erythropoietin signalling promotes obesity and alters hypothalamus proopiomelanocortin production. Nat commun 2011; 2: 1-12.
9. Jelkmann W. Physiology and pharmacology of erythropoietin. Transfus Med Hemotherapy 2013;40(5), 302-309
10. Peng B, Kong G, Yang C, Ming Y. Erythropoietin and its derivatives: from tissue protection to immune regulation. Cell death & dis, 2020;11:1-12
11. Suresh S, Rajvanshi PK, Noguchi CT. The many facets of erythropoietin physiologic and metabolic response. Front Physiol 2020; 10:1534-1553.
12. Franke K, Gassmann M, Wielockx, B. Erythrocytosis: the HIF pathway in control. Blood, Am J Hematol 2013; 122: 1122-1128.
13. Hong T, Ge Z, Zhang B, Meng R, Zhu D, Bi Y. Erythropoietin suppresses hepatic steatosis and obesity by inhibiting endoplasmic reticulum stress and upregulating fibroblast growth factor 21. Int J Mol Med 2019; 44: 469-478.
14. Das SK, Mukherjee S, Vasudevan DM, Balakrishnan V. Comparison of haematological parameters in patients with non-alcoholic fatty liver disease and alcoholic liver. Singapore Med J 2011; 52:175-181.
15. Lee YJ, Lee HR, Shim JY, Moon BS, Lee JH Kim, JK. Relationship between white blood cell count and nonalcoholic fatty liver disease. Dig Liver Dis 2010; 42: 888-894.
16. Wang S, Zhang C, Zhang G, Yuan Z, Liu Y, Ding L, et al. . Association between white blood cell count and non-alcoholic fatty liver disease in urban Han Chinese: a prospective cohort study. BMJ open, 2016; 6:1-7.

17. Yip TF, Ma AJ, Wong VS, Tse YK, Chan HY, Yuen PC, Wong G. H. Laboratory parameter-based machine learning model for excluding non-alcoholic fatty liver disease (NAFLD) in the general population. Aliment Pharmacol Ther 2017; 46:447-456.
18. Öztürk K, Kurt Ö, Demirci H, Doğan T, Özen A, Çakır M, et al. Association of complete blood count parameters with liver histology and atherosclerosis in non-alcoholic fatty liver disease. Gülhane Tip Dergisi, 2018; 60: 71-77.
19. Papadopoulos C, Tentes I, Anagnostopoulos K. Red Blood Cell Dysfunction in Non-Alcoholic Fatty Liver Disease: Marker and Mediator of Molecular Mechanisms. Maedica 2020; 15:513-516.
20. Yilmaz Y. Is nonalcoholic fatty liver disease the hepatic expression of the metabolic syndrome?. World J. Hepatol 2012; 4: 332–334.
21. Li G, Hu H, Shi W, Li Y, Liu L, Chen Y, et al. Elevated hematocrit in nonalcoholic fatty liver disease: a potential cause for the increased risk of cardiovascular disease?. Clin Hemorheol Microcirc 2012; 51: 59-68.
22. Li, Y, Liu, L, Wang, B, Wang, J, & Chen, D. Hematocrit is associated with fibrosis in patients with nonalcoholic steatohepatitis. Eur J Gastroenterol Hepatol 2014;26: 332-338
23. Ma, X, Yang C, Liang K, Sun B, Jin W, Chen L, et al. A predictive model for the diagnosis of non-alcoholic fatty liver disease based on an integrated machine learning method. Are J Transl Res 2021; 13: 12704–12713.
24. Michalak A, Cichoż-Lach H Guz M Kozicka J, Cybulski M, Jeleniewicz W. Plateletcrit and mean platelet volume in the evaluation of alcoholic liver cirrhosis and nonalcoholic fatty liver disease patients. Biomed Res Int 2021; 2021:1-9.
25. Bernhardt P, Kratzer W, Schmidberger J, Graeter T, Gruener B. Laboratory parameters in lean NAFLD: comparison of subjects with lean NAFLD with obese subjects without hepatic steatosis. BMC Res Notes, 2018;11:1-8
26. Notarnicola M, Caruso MG, Tutino V, Bonfiglio C, Cozzolongo R, Giannuzzi, V, et al. Significant decrease of saturation index in erythrocytes membrane from subjects with non-alcoholic fatty liver disease (NAFLD). Lipids Health Dis 2017; 16: 1-7.
27. Giorgio V, Mosca A, Alterio A, Alisi A, Grieco A, Nobili V, Miele L. Elevated hemoglobin level is associated with advanced fibrosis in pediatric nonalcoholic fatty liver disease. J Pediatr Gastroenterol Nutr 2017; 65: 150-155.
28. Tsuma Y, Mori J, Ota T, Kawabe Y, Morimoto H, Fukuhara S, et al. . Erythropoietin and long-acting erythropoiesis stimulating agent ameliorate non-alcoholic fatty liver disease by increasing lipolysis and decreasing lipogenesis via EPOR/STAT pathway. Biochem Biophys Res Commun 2019;509: 306-313

29. Hong T, Ge Z, Meng R, Wang H, Zhang P, Tang et al. Erythropoietin alleviates hepatic steatosis by activating SIRT1-mediated autophagy. Biochim Biophys Acta Mol Cell Biol Lipids 2018;1863: 595-603
30. Orlic L, Mikolasevic I, Lukenda V, Racki S, Stimac D, Milic S. Nonalcoholic fatty liver disease (NAFLD)–Is it a new marker of hyporesponsiveness to recombinant human erythropoietin in patients that are on chronic hemodialysis?. Med Hypotheses, 2014; 83: 798-801.
31. Dascălu DN. Markers of systemic inflammation in nonalcoholic fatty liver disease. Acta Medica Transilvanica, 2010; 2: 245-7.
32. Zhang GX, Du YJ, Li XH, Feng ZT, Zhao H, Sun Y, et al. Protective effect of erythropoietin against lipopolysaccharide induced inflammation and mitochondrial damage in liver. J Biol Regul. Homeost 2018; 32: 199-206.
33. Lefere S, Van Steenkiste C, Verhelst X, Van Vlierberghe H, Devisscher L, Geerts A. Hypoxia-regulated mechanisms in the pathogenesis of obesity and non-alcoholic fatty liver disease. Cell Mol Life Sci 2016; 73: 3419-3431.
34. Alkhedaide AQ. Tobacco smoking causes secondary polycythemia and a mild leukocytosis among heavy smokers in Taif City in Saudi Arabia. Saudi J Biol Sci 2020; 27: 407-411.
35. Eisenga MF, Kieneker LM, Touw DJ, Nolte IM, van der Meer P, Huls G, et al. Active smoking and hematocrit and fasting circulating erythropoietin concentrations in the general population. In Mayo Clin Proc, Elsevier 2018; 93: 337-343.
36. Yen Jean MC, Hsu CC, Hung WC, Lu YC, Wang CP, Tsai IT et al. Association between lifestyle and hematological parameters: A study of Chinese male steelworkers. J Clin Lab Anal 2019; 33: 1-9.