Hematological Inflammatory Markers in patients with clinically confirmed familial hypercholesterolemia

Golnaz Vaseghi
Isfahan University of Medical Sciences
Kiyan Heshmat-Ghahdarijani
Isfahan Cardiovascular Research Center
Zahra Shafiee
Isfahan Cardiovascular Research Center
Roghayeh Aliyari
Tarbiat Modares University Faculty of Medical Sciences
Shaghayegh Haghjooy-Javanmard
Isfahan University of Medical Sciences
Shabnam Hajian
Isfahan Cardiovascular Research Center
Nizal Sarrafzadegan (nsarrafzadegan@gmail.com)
Isfahan Cardiovascular Research Center

Research

Keywords: Familial hypercholesterolemia; Cell blood inflammatory factor; Cholesterol

Posted Date: February 20th, 2020

DOI: https://doi.org/10.21203/rs.2.24107/v1

Version of Record: A version of this preprint was published at BioMed Research International on January 17th, 2022. See the published version at https://doi.org/10.1155/2022/5051434.

Abstract

Background and aims:

Familial hypercholesterolemia (FH) is an autosomal dominant genetic disorder of lipid metabolism which leads to premature cardiovascular diseases. In patients with FH, blood inflammatory markers may be disrupted; however, its extent is unclear. In this study we aimed to evaluate, the NLR: Neutrophil to lymphocyte ratio, PLR: Platelet count to lymphocyte count ratio, MPV: Mean platelet volume, RPR: Red blood cell distribution width to platelet count ratio, WBC: White blood cell, PDW: Platelet distribution width and platelets count.

Methods:

The study group consisted of 331 patients with FH and 260 control patients. Controls had a history of hyperlipidemia and both groups could be on pharmacotherapy or not.

Results:

None of the CBC inflammatory markers were not significantly difference in patients with FH than non-FH. Platelet count was significantly higher among patients with FH when compared to control patients (240.43±54.15 vs 228.52±47.37 p= 0.049*). When we used linear regression analysis, only RLR was independently associated with total cholesterol (b=0.000, p=0.13). Here we showed that CBC markers are nor different between FH and non-FH patients, however PLR is associated with cholesterol level not FH.

Conclusions:

Our results may show the important of high cholesterol on platelets activity and highlight the use of lipid lowering drugs in patients with hyperlipidemia.

Background

Familial hypercholesterolemia (FH) is a monogenic disorder which inherited in an autosomal dominant trait [1]. lipoprotein metabolism is impaired in FH and results in severe elevation of low-density lipoprotein cholesterol (LDL-C) concentration. Patients with FH have the greatest risk of premature cardiovascular disease (CVD) [1]. A mutation in LDL receptor gene, Apo lipoprotein (Apo) B100 gene or proportion convertase subtilisin/kexin type 9 (PCSK9) can be identified in 30% to 80% of patients with clinically-diagnosed FH. Alternatively, about 20% of clinical FH is thought to have a polygenic cause [2].

Molecular diagnosis is recommended for FH patients but still not easily available and also recent reclassification of genetic variants associated with FH limits its routine use. Therefore, FH remains sub diagnosed and inadequately treated till now. New FH clinical diagnostic criteria like FAMCAT are being tested and seems to be more accurate than the classical ones [3]. It has been shown that FH is associated with inflammation, endothelial activation and oxidative stress [4–5].

The complete blood cell (CBC) count, an easily available test, is used wildly in clinical practice. Recently, the indices derived from CBC are recognized as novel inflammatory markers and predictors of outcome in chronic inflammatory diseases [6] and risk predictor for coronary heart disease [7]. Mean platelet volume (MPV), Platelet distribution width (PDW) and platelet-to-lymphocyte ratio (PLR) are new inflammatory markers which have been recognized for the assessment of inflammation and endothelial dysfunction in many inflammatory and cardiovascular diseases [8]. The neutrophil–lymphocyte ratio (NLR), another CBC derived marker, is considered to evaluate systemic inflammation and endothelial function. It has been shown that NLR is associated with the severity of coronary artery disease [9].

Animal studies show the link between dyslipidemia and increased leukocytosis, however, this association in human has not been well understood [10]. It has been shown that increased triglyceride is associated with increased total white blood cell, lymphocyte, neutrophil, and monocyte counts [11]. It also has been reported that increased LDL cholesterol levels are related to increased lymphocyte numbers [12–13] but lower total white blood cell and monocyte and neutrophil counts [14]. A positive correlation between non-HDL cholesterol levels and platelet counts has also been reported [15].

Because FH patients are at greater risk of developing atherosclerosis-related diseases and in earlier aged, the need for early diagnosis is really important to initiate appropriate aggressive treatment. Therefore, we aimed to investigate some hematologic inflammatory factors in patients with FH to find any association for early diagnosis that might need actions.

Methods

2.1.Study Population

The patients in this study were selected from IFH (Isfahan FH registry) [16]. Briefly, the enrollment framework in these approaches was based on first investigating laboratories for contacting patients with high LDL-C to enroll them in our study (National Clinical Trial No.2865694). All individuals aged above 2 years, irrespective of their sex with LDL-C of more than 150 mg/dl (LDL-C > 190 mg/dl or LDL-C > 150 mg/dl but under pharmacological treatment) were contacted by phone to come to our clinic for further evaluation. We used the Dutch Lipid Clinic Network Score (DLCNS), which was based on the clinical symptoms of FH and family history. Patients who were clinically diagnosed with definite or probable FH were enrolled in the study according to the DLCNS as previously described [17].

Key exclusion criteria were secondary causes of hyperlipidemia. All the eligible individuals signed the constant form. The control group were selected from the patients with a history of hyperlipidemia but not diagnosis with FH. Patients on pharmacotherapy were not excluded from our study.

Hypertension was defined as blood pressure ≥ 140/90 mmHg and diabetes mellitus was defined as two fasting glucose levels > 126 mg/dl [18].

2.2.Biochemical measurement

Venous blood samples were taken from patients after ≥ 8 hours of overnight fasting. High-density lipoprotein (HDL-C), and Serum total cholesterol and triglycerides (TG) were measured by enzymatic assays (Boehringer, Mannheim, Germany). LDL-C was measured by the LDL Cholesterol Assay Kit. The automated machine was used to perform fasting blood sugar (FBS) and white blood cell count (WBC). Other factors were measured with standard methods. All measurements were performed at the hospital's central laboratory.

2.3.Statistical analysis

Quantitative variables were described as mean and standard deviation and qualitative variables as frequency and percentage. Kolmogorov test was used for checking the normality distribution. Independent t-test or Mann–Whitneytest was used for comparing quantitative variables between groups in case of normality or non-normality distribution. Chi-square test was used for comparing qualitative variables between groups. Univariate general linear model was applied and all the test were adjusted b age, sex, CVD history, use of lipid lowering drugs and type 2 diabetes. In addition, correlation analysis was performed to identify the relationship of blood inflammatory markers with lipid parameters. Variables showing correlation with cholesterol and LDL-C at a level of significance of P <.05 were included into the linear regression analysis and were made to determine the independent association between those markers and confounders. Statistical significance was defined as a 2-tailed P <.05. Statistical analysis was done by SPSS Ver.14. In all tests, P-value < 0.05 was considered as statistical significance.

Results

The demographic and clinical characteristics of patients with FH and Non-FH group are summarized in table 1. The mean of age in FH and Non-FH group was 50.54±13.58 and 50.96±13.37 respectively. In FH group 42.6% and in another group 50.4% of patients were male. As it is shown in table 1 and table 2, there were no statistically differences between the two groups in respect of age, gender, smoking, anti-platelet drug use, hypertension, diabetes mellitus (DM) type 1 and the level of TG and HDL. On the other hand, these two groups have significant differences in serum LDL, total cholesterol, FBS, LDL/HDL ratio, DM type 2, History of CVD and use of Lipid-lowering Therapy (LLT).

Table 3 showed the CBC derived inflammatory markers in FH and Non-FH patients in our study. The NLR, PLR, WBC, PDW and platelet count were higher in FH group compared with control but not significant. However, RPR was lower in FH group than control but not significant.

Table 4. shows the correlation between LDL-C, cholesterol and hematological inflammation factors, PLR and PRP were associated with cholesterol level,

They both were included into the linear regression analysis and were made to determine the independent association between PLR and confounders. These variables were diabetes, sex, history of CVD, Platelet therapy, lipid lowering therapy and total cholesterol for PLR and cholesterol, TRG, sex and smoking for RPR. Table 5. Shows that the coloration was significant only for PLR.

Discussion

This study for the first time evaluate the colorations of CBC inflammatory markers and FH. After multiple adjusting for relevant covariates, we showed that there is a significant coloration between PLR and cholesterol in FH patients. However, there was not significant differences between two groups regard of CBC derived inflammatory markers.

This study has been conducted in patients with various level of LDL-C some of them were FH patients with reduced LDL-C and some of them were patients suffering from hyperlipidemia but not FH. We also show that Platelet count is different between two groups.

Severe elevations of total cholesterol and LDL-C levels lead to premature atherosclerosis disease at an early age [19]. High serum cholesterol levels also increase the production of cellular adhesion molecules and pro inflammatory cytokines. Increased in plasma level of this molecules leads to inflammatory status. FH patients also have endothelial dysfunction [20], which can be explained with inflammatory nature of disease.

NLR, PLR and RPR are known as the hematological markers of systemic inflammation. NLR is used to determine the severity of inflammation [21]. Till now, it has been shown that some diseases such as diabetes mellitus, thyroid functional abnormalities, and malignancies may affect the NLR. Here we showed that after multiple adjustments NLR is not different between patients with or without patients. PLR was found to be an independent prognostic risk factor in patients with malignancies such as pancreatic or colorectal cancer [22]. RPR is another valuable laboratory test to predict mortality in some diseases such as hepatic fibrosis and cirrhosis [23–24]. In our study; we assessed PLR and RPR as the predictors of hyperlipidemia. After multiple adjustment we showed that PLR is associated with higher cholesterol.

Previous study showed that MPV is increased in patients with FH and that is independently associated with total cholesterol level [25]. platelet cholesterol (PC) can be correlated with serum LDL-C cholesterol. Increased in PC content may affect platelet membrane fluidity, thereby resulting in platelet hyperactivity [26].

Here we could not find any differences between MPV in FH and control, the different control groups can explain our different results as in the previous study the control was the norm lipid population, however in both studies the platelet count was significantly difference between groups.

Conclusions

It has been suggested that higher total cholesterol is associated with lower total white blood cell count and also lower monocyte count and neutrophil count. The regression analysis proposed that both the associations may be more important at lower total cholesterol levels, and flatter at higher total cholesterol levels, with a threshold at approximately 155 and 204 mg/ld., respectively [27]. In our study all patients

had history of hypercholesterolemia and we did not observed differences between their WBC count however we observed significant differences when compare inflammation markers consists of WBC component and platelet count based on LDL-C and Cholesterol level.

Limitations of the Study

First, study data were collected from one center and also limited population. Second, we did not conduct a genetic test to confirm FH. Third, we included the patients who received any lipid-lowering agent which may affect the sample size after adjustment.

In conclusion, our findings show that PLR is significantly associated with higher cholesterol in patients with or without FH, which emerge the treatment of hyperlipidemia for any reason.

Abbreviations

FH: Familial hypercholesterolemia

PLR: Platelet count to lymphocyte count ratio

MPV: Mean platelet volume

RPR: Red blood cell distribution width to platelet count ratio

WBC: White blood cell

PDW: Platelet distribution width and platelets count

CBC: Complete blood count

LDL-C: low-density lipoprotein cholesterol

HDL-C: High-density lipoprotein

TG: Triglyceride

DLCNS: Dutch Lipid Clinic Network Score

FBS: Fasting blood sugar

IFH: Isfahan FH registry

PC: Platelet cholesterol

PCSK9: Proportion convertase subtilisin/kexin type 9

LLT: Lipid-lowering Therapy

CVD: Cardiovascular disease

BMI: Body mass index

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed in this study are included in this published article and its supplementary information files.

Competing interests:

The authors declare that they do not have the conflict of interest.

Funding

This work was partly supported by National Institute for Medical Research Development (NIMAD) (research grant: No.943785).

Authors' contributions

G.V data gathering, manuscript writhing, data designing and data analysis. K.H manuscript writhing and data design. Sh.H-J manuscript revision. Sh.H and Z.Sh data gathering and manuscript draft. R.A data analysis. N.S idea and manuscript revision. All authors contributed to data interpretation, critically commented on the manuscript for intellectual content, and approved the final manuscript.

Acknowledgements

We want to thank Dr. Azizi (Erythron Lab) for help us to collect patients.

Author information

Affiliations

Applied physiology research center, cardiovascular research institute, Isfahan university of medical sciences, Isfahan, Iran

Golnaz Vaseghi & Shaghayegh Haghjooy-Javanmard

Department of heart failure, school of medicine, Isfahan university of medical sciences, Isfahan, Iran

Kiyan Heshmat-Ghahdarijani

Isfahan cardiovascular research center, cardiovascular research institute, Isfahan university of medical sciences, Isfahan, Iran

Shabnam Hajian &Zahra Shafiee & Nizal Sarrafzadegan

Department of Biostatistics, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran.

Roghayeh Aliyari

Corresponding author

Correspondence to Nizal Sarrafzadegan

References

[1] R. B. Repas, J. R. Tanner. (2014) Preventing early cardiovascular death in patients with familial hypercholesterolemia. J. Am. Osteopath. Assoc. 2014;114 (2) 99–108.

Pubmed. https://www.ncbi.nlm.nih.gov/pubmed/24481802

[2] Trinder M, Li X, DeCastro ML, Cermakova L, Sadananda S, et al. Risk of Premature Atherosclerotic Disease in Patients with Monogenic Versus Polygenic Familial Hypercholesterolemia. J Am Coll Cardiol. 2019;30;74(4):512–522.

Pubmed. https://www.ncbi.nlm.nih.gov/pubmed/31345425

[3] Santos RD. Screening and management of familial hypercholesterolemia. Curr Opin Cardiol. 2019;34(5):526–530.

Pubmed. https://www.ncbi.nlm.nih.gov/pubmed/31393421

[4] Rahman T, Hamzan NS, Mokhsin A, Rahmat R, Ibrahim ZO, et al. Enhanced status of inflammation and endothelial activation in subjects with familial hypercholesterolemia and their related unaffected family members: a case control study. Lipids Health Dis. 2017; 24;16(1):81.

Pubmed. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5404314/

[5] Upadhyay RK. Emerging risk biomarkers in cardiovascular diseases and disorders. J Lipids. 2015;971453.

Pubmed. https://www.ncbi.nlm.nih.gov/pubmed/25949827

[6] Bilgin S, Aktas G, Zahid Kocak M, Atak BM, Kurtkulagi O, et al. Association between novel inflammatory markers derived from hemogram indices and metabolic parameters in type 2 diabetic men. Aging Male. 2019; 28:1–5.

Taylor&Francis online. https://www.tandfonline.com/doi/abs/10.1080/13685538.2019.1632283?src = recsys&journalCode = itam20

[7] Madjid M, Fatemi O. Components of the complete blood count as risk predictor for coronary heart disease: a review. Tex Heart I J. 2013; 40(1):17–29.

Pubmed. https://www.ncbi.nlm.nih.gov/pubmed/23467296

[8] Leader A, Pereg D, Lishner M. Are platelet volume indices of clinical use? A multidisciplinary review. Ann Med. 2012;44(8):80516.

Pubmed. https://www.ncbi.nlm.nih.gov/pubmed/22413913.

[9] Arbel Y, Finkelstein A, Halkin A, Birati EY, Revivo M, et al. Neutrophil/lymphocyte ratio is related to the severity of coronary artery disease and clinical outcome in patients undergoing angiography. Atherosclerosis. 2012; 225(2):456–460.

Pubmed. https://www.ncbi.nlm.nih.gov/pubmed/23040448

[10] Murphy AJ, Akhtari M, Tolani S, Pagler T, Bijl N, et al. Apoe regulates hematopoietic stem cell proliferation, monocytosis, and monocyte accumulation in atherosclerotic lesions in mice. J Clin Invest. 2011;121:4138–4149.

Pubmed. https://www.ncbi.nlm.nih.gov/pubmed/21968112

[11] Bernelot Moens SJ, Verweij SL, Schnitzler JG, Stiekema LCA, Bos M, et al. Remnant cholesterol elicits arterial wall inflammation and a multilevel cellular immune response in humans. Arterioscler Thromb Vasc Biol. 2017;37:969–975.

Pubmed. *https://www.ncbi.nlm.nih.gov/pubmed/28336558[*

12] Oda E. Longitudinal associations between lymphocyte count and ldl cholesterol in a health screening population. J Clin Transl Endocrinol. 2014;1:49–53

Pubmed. *https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5685014/*

[13] Oda E, Kawai R, Aizawa Y. Lymphocyte count was significantly associated with hyper-ldl cholesterolemia independently of high sensitivity c-reactive protein in apparently healthy Japanese. Heart Vessels. 2012;27:377–383.

Springer. http://link-springer-com–443.webvpn.fjmu.edu.cn/article/10.1007%2Fs00380–011–0157-x

[14] Lai YC, Woollard KJ, McClelland RL, Allison MA, Rye KA, et al. The association of plasma lipids with white blood cell counts: Results from theMulti-Ethnic Study of Atherosclerosis. J Clin Lipidol. 2019;15.

Elsevier.https://www.sciencedirect.com/science/article/pii/S1933287419302351?via%3Dihub

[15] Fessler MB, Rose K, Zhang Y, Jaramillo R, Zeldin DC. Relationship between serum cholesterol and indices of erythrocytes and platelets in the us population. J Lipid Res. 2013;54:3177–3188.

Pubmed. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3793622/

[16] Vaseghi G, Arabi S, Haghjooy-Javanmard S, Sabri M, Sadeghi M, et al. CASCADE screening and registry of familial hypercholesterolemia in Iran: Rationale and design. *ARYA Atheroscler.* 2019;15(2):53–58.

Pubmed. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6679656/

[17] Harada-Shiba M, Arai H, Ishigaki Y, Ishibashi S, Okamura T, et al. Guidelines for diagnosis and treatment of familial hypercholesterolemia 2017. J Atheroscler Thromb. 2018; 25(8):751–7.

Pubmed. https://www.ncbi.nlm.nih.gov/pubmed/29877295

[18] Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998;15(7):539–53.

Pubmed. *https://www.ncbi.nlm.nih.gov/pubmed/9686693*

[19] Hovingh GK, Davidson MH, Kastelein JJ, O'Connor AM. (2013) Diagnosis and treatment of familial hypercholesterolaemia. Eur Heart J. 2013;34(13):962–97.

Pubmed. *https://www.ncbi.nlm.nih.gov/pubmed/23416791*

[20] Stokes KY, Cooper D, Tailor A, Granger DN. Hypercholesterolemia promotes inflammation and microvascular dysfunction: role of nitric oxide and superoxide. Free Radic Biol Med. 2002;33(8):1026–

1036.

Pubmed. https://www.ncbi.nlm.nih.gov/pubmed/12374614

[21] Condado JF, Junpaparp P, Binongo JN, Lasanajak Y, Witzke-Sanz CF, et al. Neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) can risk stratify patients in transcatheter aortic-valve replacement (TAVR). Int J Cardiol. 2016;15;223:444–449.

Pubmed. *https://www.ncbi.nlm.nih.gov/pubmed/27544602*

[22] Betterle C, Volpato M, Rees Smith B, Furmaniak J, Chen S, et al. I.Adrenal cortex and steroid 21-hydroxylase autoantibodies in adult patients with organ-specific autoimmune diseases: markers of low progression to clinical Addison's disease. J Clin Endocrinol Metab. 1997; 82(3):932–8.

Pubmed.https://www.ncbi.nlm.nih.gov/pubmed/9062509

[23] Shuster LT, Rhodes DJ, Gostout BS, Grossardt BR, Rocca WA. Premature menopause or early menopause: long-term health consequences. Maturitas. 2010;65: 161–6. 23.

Pubmed. *https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2815011/*

[24] Chen B, Ye B, Zhang J, Ying L, Chen Y. RDW to platelet ratio: a novel noninvasive index for predicting hepatic fibrosis and cirrhosis in chronic hepatitis B. PLoS ONE. 2013; 8:e68780.

Pubmed. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3714293/

[25] Icli A, Aksoy F, Nar G, Kaymaz H, Alpay MF, et al. (2016)Increased Mean Platelet Volume in Familial Hypercholesterolemia. Angiology. 2016;67(2):146–50.

Pubmed. https://www.ncbi.nlm.nih.gov/pubmed/25859052

[26] Jagroop IA, Persaud JW, Mikhailidis DP. A new rapid method to

measure human platelet cholesterol: a pilot study. Clin Appl

Thromb Hemost.2011;17(6):578–584.

Clinical and applied thrombosis/hemostasis.
https://journals.sagepub.com/doi/full/10.1177/1076029611404213

[27] Lai YC, Woollard KJ, McClelland RL, Allison MA, Rye KA.
The association of plasma lipids with white blood cell counts: Results from the Multi-Ethnic Study of Atherosclerosis. J Clin Lipidol. 2019;15.

Sciencedirect.https://www.sciencedirect.com/science/article/pii/S1933287419302351?via%3Dihub#cebib0010

Tables

Due to technical limitations, Tables 1 - 5 are only available for download from the Supplementary Files section.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.docx