Correlation Between Hyperlipemia and Erythrocytes Indexes

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ABSTRACT

There are few reports that show hyperlipedimia can be cause of erroneous results in the automated cell counters with spurious increased or decreased red blood cell indexes. In this study, we investigated whether increased serum triglyceride or cholesterol concentration can alter red blood cell indices or not. Laboratory results of 1050 outpatient, which included complete blood cell count (erythrocyte indexes), cholesterol, triglyceride and fasting blood sugar, were collected. Results of diabetic and anemic patients omitted due to their possible influence on erythrocyte indexes. Laboratory results were analyzed by nonparametric Spearman's method.

Serum triglyceride level had a positive correlation with MCHC along with cholesterol level. However, there was no correlation with MCV and MCH. Furthermore we did not find any correlation between cholesterol and erythrocyte indexes.

This study showed that hypertriglyceridemia significantly increased MCHC. We did not detect any correlation between hypercholesterolemia and erythrocytes indices.

Key Words: Erythrocyte indexes, Cholesterol, Triglyceride

ÖZET

Hiperlipemi ve Eritrosit İndekslerinin Korelasyonu

Literatürde bir kaç çalışmada hiperlipideminin otomatik hücre sayıcılarında eritrosit indekslerini, yanlışlıkla yüksek ya da düşük gösterebilecekleri belirtilmiştir. Bu çalışmamızda, artmış serum trigliserit veya kolesterol konsantrasyonunun, eritrosit indekslerini değiştirip değiştirmediğini araştırdık. 1050 hastanın laboratuar sonuçları, tam kan sayımı (eritrosit indeksleri), kolesterol, trigliserit ve açlık kan şekerleri toplandı. Diyabetik ve anemik hasta değerleri, eritrosit indeksleri etkilenmiş olabileceği için, çalışma dışında bırakıldı. Laboratuar sonuçları non-parametrik Spearman's yöntemi ile değerlendirildi.

Serum trigliserit düzeyi, MCHC ve kolesterol düzeyleri arasında pozitif korelasyon bulundu. MCV ve MCH arasında korelasyon gösterilemedi. Kolesterol ve eritrosit indeksleri arasında da herhangi bir korelasyon gösterilemedi.

Bu çalışma, hipertrigliserideminin MCHC'yi önemli ölçüde artırdığını göstermiştir.. Hiperkolesterolemi ile eritrosit indeksleri arasında herhangi bir bağlantı gösterilememiştir.

Anahtar Kelimeler: Eritrosit indeksleri, Kolesterol, Trigliserit

INTRODUCTION

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were first introduced by Dr Maxwell Myer Wintrobe in 1929 to define the size (MCV) and hemoglobin content (MCH, MCHC) of red blood cells. Termed red cell indices, these values are useful in elucidating the etiology of anemias. Erythrocyte indices can be calculated if the values of hemoglobin, hematocrit (HCT), and red blood cell count are known. With the general availability of electronic cell counters, erythrocyte indices are now automatically measured in all blood count determinations. Most automated blood cell counters measure the number of red blood cells (RBC), MCV and hemoglobin concentration (HGB). The other red cell parameters, including the HCT, MCH and MCHC, are derived from these primary measurements. The classic method to count and determine the volume of a particle or a cell is electrical impedance. In the electrical impedance instruments the MCV is derived from the mean height of the voltage pulses formed during the red cell count, and the hemoglobin is measured by optical density (1,2).

The sizes of the erythrocytes that are determined by the MCV and hemoglobin content of erythrocytes have traditionally been used to assist in the differential diagnosis of anemia. Anemia may be classified by red cell morphology as macrocytic, normocytic, or microcytic. The erythrocytes indexes have been used to guide the diagnostic workup in patients with anemia, for example testing patients with microcytic anemia for iron deficiency or thalassemia (3,4). Anemia is common, not only in Iranian population but also in other countries (5,6); therefore it is important to us to know about more common errors that influence on the RBC indexes.

There are some physiologic(e.g. age) and pathologic items (e.g. hyperglycemia, monoclonal gammopathy and cold agglutination) that cause of erroneous results in the automated cell counters with spurious increased or decreased red blood cell indexes (7-10). It was implicated hyperlipidemia may cause erroneous PLT and WBC counts increased and also induce serum turbidity, which falsely elevates the MCH and MCHC (2,11). Furthermore there are some paradoxical reports about effect of hyperlipidemia on RBC indexes (12-14).

Relationships between changes in erythrocyte morphology and serum lipid concentrations in the patients with various diseases, such as liver disease and chronic alcoholism have been studied (15-16); however, few studies have closely examined associations between hyperlipidemia and hematologic parameters in apparently healthy subjects.

Hyperlipidemia is common in Iranian population. In one study reported 37.5% of Iranian population had total cholesterol (CH) values over 200 mg/dl with mean values 196.2 + 32.5 and 209.9 + 102.0 for CH and triglyceride (TG) respectively and also TG greater than 200 mg/dl reported in 16% of population in other study (17-19).

We should know about the effect of hyperlipidemia on laboratory results because high prevalence of Hyperlipidemia in general population. In this study, we investigated whether increased serum triglyceride or cholesterol concentration can be altering red blood cell indices or not.

MATERIAL and METHODS

Laboratory results of 1806 outpatient are collected that contained complete blood cell count (CBC) , cholesterol, triglyceride and fasting blood sugar (FBS) from April to October 2007. CBC and red cell indices were measured with K3-EDTA anticoagulated blood using a calibrated electronic counter of Sysmex (K-1000) company. This hematology analyzer works based on electrical impedance technology (2). For all the subjects, peripheral blood smear with Geimsa staining prepared from these drawing blood then morphologic changes in erythrocytes were examined in smears. Also for serum cholesterol, triglyceride and FBS measurement in subjects, after 12 hours fasting ,venous blood was collected, serum separated by centrifugation then these parameters quantitated by enzymatic methods, Mann's company diagnostic Kits and Technicon-RA 1000 instrument.

Samples with abnormal RBC morphology in peripheral blood smear and what belonged to diabetic and anemic patients have obvious influences on erythrocyte indexes (11). Therefore we omitted the results of patients with FBS over 126 mg/dL and also results of patients with hemoglobin below 13.5 g/dL in men and 12.5 g/dL in women. Furthermore

Table 1. Descriptive statistics of laboratory results of patients

	Minimum	Maximum	Mean	SD
TG (mg/dl)	33.00	762.00	155.46	95.48
CHO (mg/dl)	85.00	421.00	203.99	45.38
MCV (fL)	78.00	100.00	83.22	3.89
MCH (pg)	23.10	37.40	28.04	1.65
MCHC (mg/dl)	25.70	39.10	33.68	1.55

we excluded results of patients younger than 20 years due to age related physiologic changes of these indexes.

Finally laboratory results of 1050 patients analyzed by SPSS software (version 11.5) and suitable statistical tests. At first the dispersion of data were determined by Kolmogorov- Smirnov test, then correlation of parameters analyzed by nonparametric Spearman's method and results with p-value under 0.05 ($P \leq 0.05$) was considered statistically significant.

RESULTS

Erythrocyte indices were included from 1050 subjects consist of 633(60.3%) male and 417(39.7%) female. The age range was from 20 to 86 years with 48.27+16.82 for mean + standard deviation (SD). Increasing the subjects' age had positive concordance with triglyceride (P=0.001, r=0.176) and cholesterol (P=0.001, r=0.230). The descriptive data for erythrocyte indexes and also serum TG, CH, FBS are summarized in Table 1.

At first step we evaluated correlation of Hyperlipidemia with RBC indexes. Spearmann test showed that triglyceride had positive correlation with MCHC (P=0.01 r=0.107) and cholesterol (P=0.001, r=0.417) but any correlation with MCV (P=0.12, r=-0.047) and MCH (P=0.87, r=0.053). This statistical test performed again for cholesterol and erythrocyte indices and there were not correlation between cholesterol and MCV (P=0.74 r=0.010), MCH (P=0.34 r=0.029), and also MCHC (P=0.23 r=0.036). These correlations also evaluated in two different genders but the results did not change (Table 2).

In second step patients were divided based on TG> 400 mg/dL and TG<400 mg/dL to very high level of TG group (31 patients) and not high level of TG

Table 2. The significance of correlation between RBC indexes with TG and CH in two different gender

	RBC indexes	TG	CH
MALE			
	MCV	P=0.33, r=-0.038	P=0.28, r=0.043
	MCH	P=0.87, r=0.007	P=0.10, r=0.065
	MCHC	P=0.24, r=0.046	P=0.28, r=0.043
FEMALE			
	MCV	P=0.29, r=-0.050	P=0.39, r=-0.041
	MCH	P=0.71, r=0.088	P=0.97, r=-0.002
	MCHC	P=0.001, r=0.161	P=0.20, r=0.061

Table 3. The significant differences of RBC indexes between two groups of HTG versus (vs.) Non-HTG and also between two groups of HCH and Non-HCH

RBC indexes	HTG* vs. Non-HTG	HCH** vs. Non- HCH
MCV	P=0.20, t=1.277	P=0.45, t=0.749
MCH	P=0.33, t=-0.965	P=0.23, t=-1.15
MCHC	P=0.004, t=-3.112	P=0.40, t=0.830

* Very high level of Triglyceride ** Very high level of Cholesterol

group respectively then RBC indexes compared in these two groups by T test. This comparison was done for two groups of CH> 350 mg/dL and CH <350 mg/dL or very high level of CH group (7 patients) and not high level of CH group respectively but results approximately was similar first step (Table 3).

DISCUSSION

The widespread use of hematology analyzers has led to a major improvement of cellular hematology, because of quick and accurate results found in most instances. However, in several situations, spurious results are observed. For example in patients with hyperchylomicronemia may form small droplets and disturb platelet counts. Cantero et al. reported a positive interference from lipemia in platelet only on the Technicon H3 that works based on light scattering not on the Coulter counter that works based on electrical impedance (20).

Hemoglobin concentration is measured using a colorimetric (spectrophotometric) method therefore exogenous turbidity can spuriously increased HGB (2). Sandberg et al. reported a correlation between the increased of hemoglobin concentration with hyperlipidemia and this correlation was greatest with the Coulter Counter and the Technicon H 1 followed by the LK 540 and the Technicon H 6000. There was no effect of lipid on the hemoglobin determination using the Reflotron hematology analyzer (19).

It seems the type of hematology analyzer and its technology are two important factors for unmatched results that obtained in different study. We employed of Sysmex analyzer (K 1000) that works based on electrical impedance technology (2).

Although excess of lipids usually spuriously increases hemoglobin, a spurious fall of hemoglobin was also reported. In the study of Bunyaratvej et al. no significant correlation Plasma triglyceride levels with red cell MCV found and plasma cholesterol levels were inversely correlated with MCV of red cells (13). We did not find correlation between TG and MCV similar Bunyaratvej et al. study but our results was not matched with them for correlation between CH and MCV. We agree with Choi et al. and Lee et al. that there are no significant differences in the mean values of red cell indices between the subjects with and without hypercholesterolemia (12, 22). We studied 1050 subjects, this number obviously was more than previous studies such as 463 patients in Choi et al., 24 patients in Lee et al. or 61 patients in Bunyaratvej et al. (12,13, 22).

CONCLUSION

Hypertriglyceridemia can spuriously increase MCHC but can not alter MCV and MCH and also hypercholesterolemia can not significant affects on red cell indices.

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EFERENCES

1. Fred HL. Maxwell Myer Wintrobe: new history and a new appreciation. Tex Heart Inst J 34: 328-35, 2007.
2. Michael WM, Frederick RD. Basic examination of blood. In: Clinical Diagnosis and Management by Laboratory Methods. Henry JB. 20th ed. W.B. Saunders. 484-485, 499, 1996.
3. Jain S, Kamat D. Evaluation of Microcytic Anemia. Clin Pediatr (Phila). In press, 2008.
4. Irwin JJ, Kirchner JT. Anemia in children. Am Fam Physician 64: 1379-86, 2001.
5. Fitch CW, Cannon MS, Seidel GE, Krummel DA. Dietary factors affecting iron status of children residing in rural West Virginia. 104:19-22, 2008.
6. Bateni J, Shoghli AR. The Prevalence of Iron Deficiency Anemia (IDA) Based on Hematologic Indices in Non-pregnant Women Aged 15-45 in Zanjan. Journal of Zanjan University of Medical Sciences & Health Services 55: 39-46, 2006.
7. Pappo AS, Fields BW, Buchanan GR. Etiology of red blood cell macrocytosis during childhood: impact of new diseases and therapies. Pediatrics 89:1063-7, 1992.
8. Van Duijnhoven HL, Treskes M. Marked interference of hyperglycemia in measurements of mean (red) cell volume by Technicon H analyzers. Clin Chem 42:76-80,1996.
9. Horstman AL, Serck SL, Go RS. Macrocytosis associated with monoclonal gammopathy. Eur J Haematol 75:146-9, 2005.
10. Zdziarska B, Pa_nicki M. Spurious macrocytosis associated with cold agglutinins: report of two cases. Pol Arch Med Wewn 92:357-60, 1994.
11. Zandecki M, Genevieve F, Gerard J, Godon A. Spurious counts and spurious results on haematology analysers: a review. Part II: white blood cells, red blood cells, haemoglobin, red cell indices and reticulocytes. Int J Lab Hematol 29: 21-41, 2007.
12. Lee CY, Kim KC, Park HW, et al. Rheological properties of erythrocytes from male hypercholesterolemia. Microvasc Res 67:133-8, 2004.
13. Bunyaratvej P, Komindr S, Wisedpanichkij R. Different reticulocyte volume in diabetes mellitus patients with and without hypercholesterolemia and/or hypertriglyceridemia. J Med Assoc Thai 83:790-6, 2000.
14. Cicha I, Suzuki Y, Tateishi N, Maeda N. Enhancement of red blood cell aggregation by plasma triglycerides. Clin Hemorheol Microcirc 24: 247-55, 2001.
15. Owen JS, Brown DJ, Harry DS, et al. Erythrocyte echinocytosis in liver disease. Role of abnormal plasma high density lipoproteins. J Clin Invest 76: 2275-85, 1985.
16. Arienti G, Carlini E, Scionti L, et al . Liver alcoholic cirrhosis and spur-cell (acanthocytic) anaemia. A study of erythrocyte ghost composition and fluidity.Scand J Gastroenterol 30: 1204-9, 1995.
17. Yousefi-nia M, Amani A. A Survey of lipid profile in the population over 30 years old based on Arak healthy heart program. Rahavard Danesh 2: 89-96, 2007.
18. Azizi F, Rahmani M, Salehi P, et al. Serum Lipid levels in Tehranian children and adolescents. Journal of Medical Council of Islamic Republic of Iran 2: 109-117, 2002.
19. Saeidi MR, Ray AR, Rezaei M. Prevalence of hyperlipidemia among adult residents of Kermanshah in 1997-98. Scientific journal of Kurdistan University of Medical Sciences 27: 54-49, 2003.
20. Cantero M, Conejo JR, Jiménez A. Interference from lipemia in cell count by hematology analyzers. Clin Chem 42:987-8, 1996.
21. Sandberg S, Sønstabø K, Christensen NG. Influence of lipid and leukocytes on the hemoglobin determination by Coulter Counter S Plus III, Technicon H 6000, Technicon H 1, LK 540, Reflotron and Hemocap. Scand J Clin Lab Invest 49:145-8, 1989.
22. Choi JW, Pai SH. Influences of hypercholesterolemia on red cell indices and erythrocyte sedimentation rate in elderly persons. Clinica Chimica Acta 341: 117-121, 2004.

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