ORIGINAL ARTICLE

Relationship of lipid profile and erythrocyte indices in non-anaemic elderly

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ABSTRACT

Objectives: Several human studies have reported a variety of morphologic alterations in erythrocyte in relation to serum lipid components in different pathological states, but there are few reports about the influence of lipid profiles on heamatologic parameters in healthy individuals. This study evaluated the effect of elevated serum level of the lipid indices on the alteration of the RBC indices in healthy elderly persons.

Methods: 275 non-anaemic elderly people were assessed for the association between dyslipidemia and circulating erythrocyte indices in the southwest of Iran. The student t-test and Chi-square test were used for statistical comparison.

Results: There was a statistically significant difference between elderly women with and without hypercholesterolemia and hypertriglyceridemia, and in age-matched men with regard to the red cell count, haemoglobin, and haematocrit (p<0.001) but not for mean cell volume (p>0.05). Fasting levels of LDL-C were significantly associated with erythrocyte number, hematocrit, and haemoglobin concentration (p<0.001) in healthy elderly men and women while HDL-C levels were only associated with red cell count (p<0.001) and haemoglobin (p<0.05).

Conclusions: We conclude that dyslipidemia appears to have a significant effect on red cell indices in non-anaemic elderly persons.

Key words: Dyslipidemias; erythrocyte indices; elderly, non-anaemic.

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INTRODUCTION

Red blood cell's (RBC) plasma membrane is composed of the phospholipid bilayer, transmembrane (integral) proteins, and a cytoskeletal network of protein fibres that help to maintain the normal structure and function of the erythrocytes (1). The structural elements and biochemical composition of the RBC membrane have been well characterised (2) in that proteins constitutes approximately 52% of lipids (including cholesterol and phospholipids) 40% of carbohydrates and 8% of the mass of the RBC membrane (3). The major class of lipid composition of RBC membranes are either phospholipids or neutral lipids and unesterified cholesterol, found in about equimolar quantity (4).

The reverse cholesterol transport pathway involves the removal of cholesterol from peripheral tissue for excretion in the faeces (5). The RBC cholesterol pool is more readily exchangeable facilitating peripheral cholesterol efflux to the faeces, particularly in subjects with low HDL-c (6). Because RBCs do not contain intracellular membranous structures, a bidirectional *in vivo*, and *in vitro* efflux of free cholesterol between plasma lipoprotein and RBC plasma membranes (7) cause alterations in the fluid properties and function of the erythrocyte membrane with potential pathophysiological consequences (8). The results of one study confirmed that abnormalities in the composition of plasma lipoproteins in patients with hypercholesterolemia is associated with erythrocyte deformability and corresponding modifications in erythrocyte membrane lipid composition (9). Furthermore, another study reflects that the effects of postprandial plasma cholesterol and triglyceride (TG) levels lead to enhanced RBC aggregation accompanied by changes in RBC membrane lipid composition (10). The anaemia of severe burned patients is characterised by a decreased RBC half-life and abnormal RBC morphology (predominantly echinocytes) and is correlated with decrease in plasma lipid concentrations (5). Various studies have consistently reported a variety of morphologic alterations in erythrocyte indices in relation to serum lipid components among patients with various diseases, including sepsis-associated inflammation, (11) and chronic alcoholism (12).

A few reports have determined the influence of lipid profiles on haematologic parameters in apparently healthy individuals (5). More research is needed that provides insight into advancing our understanding of the underlying association between hyperlipidemia and erythrocyte indices in humans. We therefore investigated how elevated serum levels of the lipid panel [total cholesterol (TC), high density cholesterol (HDL-C), low density cholesterol (LDL-C) and triglycerides] alters RBC indices with a negative impact on the distribution of red blood cell mean corpuscular volume (MCV) in non-anaemic elderly individuals.

MATERIALS AND METHODS

The present-based cross-sectional study was conducted on an elderly healthy population in the southwest of Iran from September 2017 to April 2018. The Cochran formula was used for calculating of the sample size.

$n = ((z^2pq) / e^2)/1 + 1/N((z^2pq) / e^2 - 1))$ = 269

n = sample size, N = population size (900 households), z = standard normal deviation set at 95%, confidence level = 1.96, p = percentage picking a choice or response = 0.5, e = margin of error (desired level of precision) = 0.05.

Complete blood cell count (CBC), including red cell indices, were measured in 275 elderly persons, aged 50 to 65 years (121 elderly men, mean: 56.71 years; 154 elderly women, mean: 56.95 years). We excluded those taking medications for anaemia, such as for iron deficiency anaemia, chronic kidney disease, chronic inflammation, immune disorders, and smokers. This study was approved by the Ethical Committee of Abadan Faculty of Medical Sciences (IR.ABADANUMS.REC.1396.243) and informed consent was obtained from all subjects. According to the WHO definition of of anaemia, cut-off value of haemoglobin (Hb) levels of < 120 g/L was used for women and < 130 g/L for men. Serum concentrations of cholesterol, triglyceride, LDL-C, and HDL-C were measured with an automatic chemical analyser (Hitachi 747, Hitachi, Tokyo, Japan). The subjects were assigned into two groups: subjects with hypercholesterolemia (serum cholesterol ≥ 5.17 mmol/L) and with normal serum cholesterol levels of < 5.17 mmol/L. In addition, subjects were assigned

into each six groups: subjects with hypertriglyceridemia (serum triglyceride ≥ 2.26 mmol/L) and with serum triglyceride concentrations < 2.26 mmol/L; subjects with elevated serum level of LDL-C and HDL-C(serum LDL-C ≥ 2.59 mmol/L and HDL-C≥ 1.55 mmol/L and with normal serum level of LDL-C concentration < 2.59 mmol/L and HDL-C concentration < 1.55 mmol/L.

After fasting for > 12h, venous blood was drawn into an evacuated serum separator tubes. Full blood cell and haematological parameters (WBC, RBC, Hb, Hct, MVC, MCH, and MCHC) were measured with EDTA-anticoagulated blood using an electronic cell counter (SE 9000, Sysmex, Kobe, Japan). Morphologic changes in erythrocytes were examined in peripheral blood smears from all subjects. Statistical analysis was done by SPSS version 24 statistical software (SPSS Inc., Chicago, Illinois, USA). We used the student t-test for statistical comparison of groups and Chi-square test for calculating of the qualitative variables. Descriptive statistics were generated (means, or percentages and associated standard errors). P < 0.05 was considered statistically significant.

RESULTS

Descriptive characteristics of the subjects stratified by study design

The average age of participants (n=275) was 56.95± 5.35 and 56.71± 4.41 years for men and women, respectively. 121 (44%) men and 154 (56%) women had elevated serum cholesterol and triglyceride levels. Mean levels of total cholesterol and triglyceride in these men and women were 5.17 ± 1.06 mmol/L and 2.40 ± 0.53 mmol/L, respectively. Across this study, the mean (standard deviation) LDL-C level were 3.11 (0.85) mmol/L in men and 3.31 (1.03) mg/dL in women, respectively. In addition, HDL-C levels were 1.3 (0.35) mmol/L in men and 1.38 (0.30) mmol/L in women, respectively. Descriptive characteristics of the mean ±SD for haematologic parameters and red cell indices (WBC, RBC count, Hb, Hct, MCV, MCH, MCHC) are shown in Table 1.

Associations between serum cholesterol and triglyceride concentrations and erythrocyte indices in elderly men and women with and without hypercholesterolemia and hypertriglyceridemia

In case of hypercholesterolemia (serum cholesterol ≥ 5.17 mmol/L) we examined the individual association between serum cholesterol and triglyceride concentrations and erythrocyte indices. A relevant association between RBC count (p=0.011) in older men and haemoglobin (p=0.002) and haematocrit (p<0.001) in older women with high serum levels of cholesterol was found (Table 2). With regard to hypertriglyceridemia (serum triglyceride ≥ 2.26 mmol/L) significant associations between the two groups in the RBC count (p=0.005), Hb (p=0.004), and Hct (p=0.001) were found (Table4). There was no significant differences for MCV (p>0.05) in the hypercholesterolemia and hypertriglyceridemia groups (Tables 2 and 3).

Relevant association between serum LDL-C & HDL-C and erythrocyte indices in elderly men and women with normal and high levels of LDL-C and HDL-C

There was a statistically significant difference between elevated level of LDL-C (≥ 2.59 mmol/L) and RBC count (p=0.049) in older men and RBC count (p=0.002), haemoglobin (p<0.001), and haematocrit (p<0.001) in older women (Table4). Although, MCV edied not show a statistically significant difference with high serum level of LDL-C in either men (p=0.329) or women (p=0.702), respectively (Table4). HDL-C levels of ≥ 1.55 mmol/L was associated with both the RBC count (p=0.009) and Hb (p=0.009) in men and Hb (p=0.017) and MCV (p=0.013) in women, while MCV was not significantly different in men (p=0.884), (Table 5).

Table1. Descriptive parameters of study population**.**

Parameters	N	Mean	SD
TC			
<5.17 mmol/L	122	4.33	0.58
>5.17 mmol/L	153	6.17	0.80
TG			
<2.26 mmol/L	216	1.37	0.43
>2.26 mmol/L	59	2.96	1.03
LDL-C			
<2.59 mmol/L	69	2.04	0.37
>2.59 mmol/L	206	3.62	0.74
HDL-C			
<1.55 mmol/L	159	1.25	0.19
>1.55 mmol/L	59	1.79	0.31
Haematologic parameters			
WBC (10^9/l)	275	6.96	2.01
RBC (10^{12}/L)	275	5.10	0.62
Haemoglobin (g/L)	275	13.74	1.56
Haematocrit (Fraction of RBC)	275	0.40	0.04
Red cell indices			
MCV (fl)	275	78.80	7.69
MCH (pg)	275	27.07	3.17
MCHC (g/L)	275	342.9	9.2

Table 2. Red cell absolute values and erythrocyte indices in elderly with and without hypercholesterolemia. Results are mean±SD.

Characteristic	Men	Men	p-value	Women	Women	p-value
	TC < 5.17mmol/L	TC ≥ 5.17mmol/L		TC < 5.17 mmol/L	TC ≥5.17 mmol/L	
No. of cases (n)	58	63		64	90	
Age (years)	56.58±4.82	56.82±4.03		57.20±6.67	56.77±4.19	
Haematologic parameters						
WBC (10^9/l)	6.64±1.75	7.01±1.78	0.092	7.12±2.65	7.02±1.81	0.722
RBC (10^{12}/L)	5.25±0.77	5.41±0.63	0.011	4.90±0.52	4.94±0.47	0.112
Haemoglobin g/L	14.59±1,78	14.52±1.54	0.114	12.87±1.23	13.26±1.06	0.002
Hematocrit (Fraction of RBC)	0.42±0.04	0.42±0.04	0.52	0.37±0.03	0.39±0.03	<0.001
Red cell indices						
MCV (fl)	80.62±0.79	78.39±7.10	0.181	77.39±8.77	78.91±7.08	0.283
MCH (pg)	27.85±3.25	27.01±2.97	0.167	26.53±3.55	27.01±2.93	0.291
MCHC (g/L)	342±10.5	343.9±0.89	0.589	342±10.3	341.7±9	0.605

Table 3. Red cell absolute values and erythrocyte indices in elderly with and without hypertriglyceridemia. Results are mean±SD.

Characteristic	Men		p-value		Women	p-value
	TG < 2.26 mmol/L	TG ≥2.26 mmol/L		TG < 2.26 mmol/L	TG ≥2.26 mmol/ L	
No. of cases (n)	58	63		64	90	
Age (years)	56.59±4.59	57.21±3.57		57.38±5.56	55.52±4.37	
Haematologic parameters						
WBC (10^9/l)	6.66±1.69	7.56±1.96	0.001	6.89±2.31	7.64±1.64	0.282
RBC (10^{12}/L)	5.32±0.70	5.41±0.72	0.005	4.92±0.50	4.94±0.44	0.573
Haemoglobin (g/L)	14.51±1.67	14.74±1.58	0.004	12.98±1.11	13.48±1.19	0.138
Haematocrit fraction of RBC)	0.42±0.04	0.43±0.04	0.001	0.38±0.03	0.39±0.03	0.178
Red cell indices						
MCV (fl)	79.72±7.29	78.34±8.39	0.541	77.99±8.01	79.46±7.20	0.496
MCH (pg)	27.51±3.08	26.99±3.33	0.526	26.61±3.28	27.46±2.87	0.484
MCHC (g/L)	344.5±10.1	343.8±8	0.780	340.8±9.9	345.1±7.5	0.524

Table 4. Red cell absolute values and erythrocyte indices in elderly with low and high levels of LDL-C. Results are mean±SD.

Characteristic	Men		p-value		Women	p-value
	LDL-C < 2.59 mmol/L	LDL-C ≥2.59 mmol/L		LDL-C < 2.59 mmol/L	LDL-C ≥2.59 mmol/L	
No. of cases (n)	32	89		37	111	
Age (years)	55.87±4.48	57.01±4.37		57.67±7.81	56.72±4.29	
Haematologic parameters						
WBC (10^9/l)	6.55±1.89	6.93±1.72	0.879	7.07±1.94	7.06±2.26	0.764
RBC (10^{12}/L)	5.23±0.92	5.37±0.61	0.049	4.85±0.53	4.95±0.47	0.002
Haemoglobin (g/L)	14.77±2.17	14.47±1.43	0.190	12.89±1.28	13.16±1.09	<0.001
Haematocrit (fraction of RBC)	0.42±0.06	0.42±0.04	0.120	0.37±0.03	0.38±0.03	<0.001
Red cell indices						
MCV (fl)	81.46±8.56	78.74±6.99	0.329	78.24±8.46	78.34±7.65	0.702
MCH (pg)	28.18±3.58	27.14±2.92	0.285	26.81±3.40	26.83±3.14	0.586
MCHC (g/L)	345.3±12.4	344±8.6	0.940	342±10	341.8±9.4	0.372

Table 5. Red cell absolute values and erythrocyte indices in elderly with low and high levels of HDL-C. Results are mean±SD.

Characteristic	Men		p-value		Women	p-value
	HDL-C< 1.55 mmol/L	HDL-C ≥1.55 mmol/L		HDL-C < 1.55 mmol/L	HDL-C ≥1.55 mmol/L	
No. of cases (n)	32	89		37	111	
Age (years)	57.25±4.30	55.94±4.39		56.81±4.23	56.63±4.56	
Haematologic parameters						
WBC (10^9/l)	6.89±1.68	6.80±1.86	0.377	7.36±2.43	6.48±1.79	0.094
RBC (10^{12}/L)	5.35±0.62	5.46±0.48	0.069	4.95±0.52	4.90±0.35	0.454
Haemoglobin (g/L)	14.50±1.43	14.33±1.40	0.009	13.16±1.17	13.20±0.95	0.052
Haematocrit fraction of RBC)	0.42±0.04	0.42±0.03	0.009	0.38±0.03	0.39±0.02	0.017
Red cell indices						
MCV (fl)	79.02±6.83	77.47±7.42	0.884	78.24±8.30	79.26±5.98	0.013
MCH (pg)	27.27±2.86	26.43±3.22	0.685	26.85±3.35	27.03±2.60	0.035
MCHC (g/L)	344.5±8.5	340.3±12.9	0.358	342.4±9.5	340.7±8.6	0.561

DISCUSSION

Our study showed that non-anaemic elderly with hypercholesterolemia and hypertriglyceridemia had significantly higher RBC count, Hb, and Hct compared to non-anaemic elderly with normal cholesterol and triglyceride levels. Those with a high serum fasting level of LDL-C had an increase in erythrocyte number, Hct, and Hb concentration, while high HDL-C level (serum HDL cholesterol ≥ 1.55 mmol/L), was associated with a decrease in RBC count and Hb.

Conditions, such as dyslipidemia/hyperlipidemia along with plasma membrane cholesterol accumulation in erythrocytes are all modifiable risk factors for hypercholesterolemic patients (13). Clinical case studies with small number of patients have provided strong evidence that there is a probable reciprocal interaction between cholesterol loading and human population erythrokinetics and the platelet count. Experimental *in vitro* studies also demonstrated the efflux of cholesterol from RBCs for generation of serum lipoproteins because of their remarkable capacity for high cholesterol absorption (14). More human subject research is needed to provides insight into advancing our understanding of the underlying association between hyperlipidemia and erythrocyte indices, which in this study was studied. In our study, in case of hypercholesterolemia, we fully agree with Choi *et al.* (14) that there is no statistically significant variations among the mean values of red cell indices between subjects with and without hypercholesterolemia and hypertriglyceridemia. We also have shown that hyperlipidemia (hypercholesterolemia and hypertriglyceridemia) is related to RBC count, Hb, and Hct, consistent with another study (14). However, recently no differences were reported between those with and without hyperlipidemia which is different to our results regarding erythrocyte number, Hct, and Hb (14).

Population studies are generally consistent with our study in that LDL-C serum levels are positively correlated with erythrocyte membrane stability and haematocrit (8). Indeed, recent studies indicate that the management of LDL-cholesterol-lowering in hypercholesterolemic multiple sclerosis patients with statin therapy leads to improvement of the erythrocyte stability. Our data can explain that LDL-C, RBC count, and Hct are all associated. And it has already reported of reduced erythrocyte numbers in *in vivo* and in hypercholesterolemia subjects (15). Sebaaly *et al.* have also shown the clinical relevance of a strong direct relationship between increased Hb and hyperlipidemia (16).

Our results provide support that targeting triglyceride and cholesterol levels can influence RBC indexes in hypercholesterolemia and hypertriglyceridemia in the elderly The results of our study are in parallel with a previous study in which a significant relationship between abnormalities in serum triglyceride and cholesterol levels and Hb were observed in patients with moderate iron deficiency anemia (17).

To date there have been a few studies demonstrating a relationship between serum total cholesterol and triglycerides and red cell membrane lipids versus MCV in certain pathological conditions. For example, Shrivani *et al.* demonstrated plasma and red cell lipids alterations in chronic alcoholism with macrocytosis pertaining to increased MCV (17). It has also been reported that plasma levels of triglycerides and RBC cholesterol accessibility were inversely correlated with the MCV in diabetic patients with hypercholesterolemia and /or hypertriglyceridemia (18). In the study of Kim *et al.* no change in red cell MCV in relation to high plasma triglyceride levels was found while mean levels of MCV were inversely correlated with high plasma cholesterol levels (19) However, similar to that study we found no evidence of significant correlations between serum cholesterol and triglyceride concentrations with MCV in healthy elderly in men versus women. It is generally accepted that HDL particles mediate cellular cholesterol efflux through the HDL-mediated pathway of RCT(14). A previous study demonstrated that high HDL concentrations have a highly significant positive correlation related to erythrocytes and platelets count but apparently unrelated to MPV (14). It has also been observed that baseline HDL-C levels were consistently and inversely associated with erythrocyte counts. In that setting, increased erythrocyte membrane cholesterol, as an important source of free cholesterol deriving from HDL-C, can possibly impede the maturation of erythrocyte and increase haemolysis through direct effects on osmotic fragility and deformability (14).

We found no significant increases in Hct, Hb, erythrocyte count, or MCV within both males and females. Table 2 presents mean hematocrit, hemoglobin, erythrocyte count, and MCV by quartiles of the mean serum HDL-C levels. In the unadjusted model, mean erythrocyte count, hematocrit was reduced as HDL-C increased between healthy elderly men and women.

In the unadjusted model, mean erythrocyte count, haematocrit was reduced as HDL-C increased between healthy elderly men and women. In our adjusted analyses there was also a consistent relationship between HDL-C and Hb, unlike other studies which have been disparate. Furthermore, in another study an inverse relationship was found between HDL-C levels and erythrocyte numbers, similar to our finding, whereas no significant differences were observed between themes. Our data suggest that serum HDL-C is related to an abundance of measures of both erythrocytes (erythrocyte number, Hct, Hb, and MCV. Importantly, this controversy observed may be due to the type of studied populations. Our study was in the elderly while other studies have been in the general populations to have an adverse effect on red cell indices.

In conclusion, dyslipidemia appears to have an adverse effect on red cell indices, particularly for RBC count, haemoglobin, and haematocrit, but not for MCV in non-anaemic elderly persons. Furthermore, it is possible that the serum lipid profile and erythrocyte indexes causally influence each other as biomarkers with a cooperative underlying cause.

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