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ORIGINAL ARTICLE

Niemann-Pick Disease, Type C1 Gene Expression in PBMCs is Associated with Interleukin 10 Serum Concentration: a Case-Control Study

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SUMMARY

Background: **Recent studies showed that atherosclerosis is a lysosomal storage disease (LSD) and Niemann-Pick disease type C1 (NPC1) is the most important protein of the lysosomal membrane that is involved in the removal of FC from lysosomes. Whereas several *in vitro* and *in vivo* studies have described the crosstalk between lysosomal cholesterol accumulation and increased inflammation, there is no study addressing the correlation between NPC1 gene expression and an anti-inflammatory cytokine, interleukin 10 (IL-10) serum concentration in atherosclerotic patients.**

Methods: **IL-10 and 25-hydroxyvitamin D serum concentrations were quantified by enzyme-linked immunosorbent assay (ELISA) in atherosclerotic patients (n = 40) and a control group (n = 40). NPC1 gene expression analysis was performed by quantitative real-time PCR, and correlation between the two parameters was assessed.**

Results: **Mean IL-10 serum concentration and peripheral blood mononuclear cells' (PBMCs) gene expression of NPC1, adjusted for drug consumption, age, and BMI, was not significantly different between the patient and control groups (p = 0.6 and 0.67 respectively). However, NPC1 gene expression showed positive significant correlation with IL-10 serum concentration (p = 0.04, r = 0.29). We also observed lower serum concentration of IL-10 in the subjects with lower 25-hydroxyvitamin D serum concentration (p = 0.034).**

Conclusions: **Our findings supported the previous observations showing the contribution of lysosomal lipid homeostasis of PBMCs to inflammation and pathogenesis of atherosclerosis.**

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INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of global morbidity and mortality, and it alone is responsible for half of the deaths resulting from non-communicable diseases [1]. Atherosclerosis, the primary cause of CVD worldwide, is a chronic, progressive, and inflammatory disease [2]. The hallmark feature in the pathogenesis of atherosclerosis is lipid (specially cholesterol ester) laden macrophages [3]. It is believed that the progression and inflammatory response of athero-

sclerosis can be improved if cholesterol homeostasis in plaque macrophages occurs more efficiently [3]. It has been demonstrated that cholesteryl ester (CE) accumulation in cells includes cytoplasmic and lysosomal CE accumulation [4,5]. Most studies were mainly focused on the mechanisms mediating the abnormal intracellular cholesterol trafficking and cytoplasmic accumulation of CE [6]. However, it has been shown that atherosclerotic macrophages display lysosomal dysfunction, including lysosomal enlargement, increased membrane permeability and lysosomal pH, and decreased proteolytic capacity [7,8]. Since lysosomes serve as the main metabolic organelles in hydrolyzing ox-LDL, it is critical to understand the factors that influence lysosomal CE accumulation thereby influencing macrophage lipid accumulation and pathogenesis of atherosclerosis [5]. It has been demonstrated that the initiation phase of atherosclerosis is mainly accompanied by the CE accumulation within the cytoplasmic lipid droplets; however, during the late-stage of atherosclerosis, CE and free cholesterol (FC) significantly accumulated within the lysosomal lipid droplets [4]. Lysosomal FC accumulation can directly cause an increase in lysosomal membrane cholesterol content and inactivation of V-ATPase pump. Inactivation of this acidification pump disturbs the function of lysosomal hydrolase enzymes and, finally, leads to formation of lipid laden lysosomes or foam lysosomes. Thus, abnormal function of lysosomes, especially disturbed lysosomal FC efflux to the cytosol, is a main contributor to the late-stage of atherosclerosis [4, 9-11].

Niemann-Pick type C1 (NPC1) is known as the most important protein involved in lysosomal FC efflux its function is coupled with NPC2. The NPC2 protein is a soluble FC-binding protein in the lysosomal lumen but the NPC1 protein is a transmembrane protein with a FC binding site. NPC1 first receives FC from NPC2 and then delivers the lysosomal FC to ER and plasma membrane [12]. The NPC1 defect is related to Niemann-Pick Disease Type C (NPC) disease. The most noticeable biochemical feature of NPC1-deficient cells is an excessive lysosomal CE accumulation. Also, subjects with NPC1 deficiency display decreased plasma high density lipoprotein-cholesterol (HDL-C) levels and decreased reverse cholesterol transport (RCT) [13].

Although in the past, the studies on the subject of lysosomal CE accumulation was limited to its association with plaque severity, and recently, a growing body of evidence supports the pivotal link between lysosomal CE accumulation and inflammation [14]. Atherosclerosis has been proposed as an inflammatory disease which involves the pathogenesis many different pro- and anti-inflammatory cytokines [15]. Interleukin 10 (IL-10) is one of the most important anti-inflammatory cytokines that is mainly secreted from activated peripheral blood mononuclear cell (PBMCs) [15,16]. Principal roles of IL-10 include modulating multiple atherogenic macrophage functions, inhibition of matrix metalloproteinases (MMP), proinflammatory cytokines production, and activation of anti-apoptotic pathways [17]. In addition, recent studies have shown that IL-10 also has a powerful influence on cellular lipid metabolism by stimulating both cholesterol influx and cholesterol efflux (RCT) [18]. Moreover, it has been demonstrated that overexpression of IL-10 in macrophages inhibits atherogenesis [17].

On the other hand, increasing evidence shows that vitamin D [25-hydroxyvitamin D] deficiency may promote atherosclerosis [19]. Several mechanisms have been suggested which could explain the possible association of vitamin D deficiency and CVD. One of the most important attributed mechanisms of vitamin D in atherosclerosis is the attenuation of inflammation through increasing expression of anti-inflammatory cytokines [20].

Therefore, due to the important regulatory function of IL-10, NPC1, and 25-hydroxyvitamin D in inflammation and cholesterol homeostasis during atherosclerosis, in this study we investigated the serum concentration of IL-10 and 25-hydroxyvitamin D as well as NPC1 gene expression in PBMCs of atherosclerotic patients and controls.

MATERIALS AND METHODS

Study population (sampling)

Eighty men aged 50 years or more participated in this case-control study. Patients (n = 40) and controls (n = 40) who had undergone coronary angiography examination were recruited from Tehran Heart Center, Tehran, Iran for this study. Subjects with 50% or more stenosis in at least one of the vessels [right coronary artery (RCA), left coronary artery (LCA) and left anterior descending artery (LAD)] were considered as the patient group. All controls had < 10% stenosis. Every angiogram was scored by at least one attending cardiologist and one attending surgeon. All subjects with liver and kidney diseases, diabetes, stroke, cancer, and MI in the last three months were excluded from the study. The study was approved by the ethical committee of TUMS and informed consent was obtained from all the individuals.

Clinical characteristics and laboratory evaluation

Demographic and anthropometric indices of the study population including age, height, weight, blood pressure (BP), and history were taken and body mass index (BMI) was calculated. Blood samples were collected from all the subjects after an overnight fast. Whole blood (10 mL) was collected into EDTA-K3 tubes (5 mL) for PBMC isolation and molecular tests and into coagulation tubes (5 mL) for the evaluation of lipid profile, IL-10, and 25-hydroxyvitamin D serum concentrations. The IL-10 serum concentration was measured by immunoassay (ELISA), according the manufacturer's instructions (Human IL-10 ELISA Kit (Interleukin-10) (Abcam, ab46034). According to manufacturer's claim,

the ELISA intra- and inter- assay coefficients of variation and sensitivity of the IL-10 were 3.2% and 7.3%, < 5 pg/mL, respectively. A standard curve was plotted using the average of optical densities (ODs) and concentrations of standards. The results were within the linear range of the standard curve. The serum concentration of 25-hydroxyvitamin D was assessed using a commercial kit (Immunodiagnostic Systems, UK). The ELISA intra- and inter- assay coefficients of variation were < 8% and < 10%, respectively.

Ficoll-Hypaque (lympholyte, Cedarlane, Canada) density gradient separation was used for isolation of PBMCs, and the cells were washed twice in PBS.

Serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were determined enzymatically (Pars Azmoon kits, Iran) using a fully automated auto analyzer.

Real time PCR analysis

Gene All kit was utilized for total RNA extraction from PBMCs according to the protocol. Reverse transcription of total RNA was performed using the Revert Aid First Strand cDNA synthesis kit (Thermo Scientific, #K1622), according to the protocol. Gene expression analysis was assessed by Quantitive real-time PCR, using SYBR Premix Ex TaqTMII (Takara, Japan) in a final volume of 20 µL on a Rotor Gene real-time thermocycler (Qiagen, Germany). The following reaction conditions were applied: 2 minutes at 95°C, 40 cycles of 15 seconds at 95°C, and 30 seconds at 60°C, and a melting curve protocol from 65°C to 95°C. Beta-actin (as the endogenous control) and NPC1 primers are listed in Table 1. Amplification efficiency of primers was calculated by real time PCR of 5X serial dilutions of pooled cDNA.

Statistical analysis

The data were analyzed using SPSS 20 software. Independent *t*-test was used to compare the parametric parameters between groups. Comparison of qualitative variables between groups was performed using the Chi-square test. The quantitative parameters were reported as mean ± SEM. A p-value < 0.05 was taken as statistically significance. IL-10 serum concentration and NPC1 gene expression correlation was performed using univariate multiple linear regression. This correlation analysis was adjusted for age, BMI, statin, anticoagulant, and nitrate drugs consumption. The standardized regression coefficients were indeed the same as correlation coefficients after controlling for the above-mentioned confounding factors.

Also, univariate analysis was performed to correct the data of IL-10 and 25-hydroxyvitamin D serum concentration and NPC1 gene expression between patient and control groups for the mentioned confounding factors.

RESULTS

Clinical characteristics of study population

Demographic and anthropometric characteristics, laboratory findings, and medical history of the subjects are presented in Table 2. Atherosclerotic patients were older than the control group (p = 0.001). In addition, they were more likely to have a familial history of cardiovascular disease (CAD) and hyperlipidemia history (p = 0.002, 0.023 respectively). HDL-C was lower in the atherosclerotic group compared to the control group (p = 0.023). TG, TC, and LDL-C indicated no significant difference between the patients and controls (p = 0.702, 0.156, 0.821, respectively).

IL-10 serum concentration analysis

IL-10 serum concentration was significantly higher in the control group compared to the atherosclerotic group (p < 0.001). However, after a full adjustment for age, BMI, statin, anticoagulant, and nitrate drugs consumption, no significant difference was observed between the atherosclerotic and control groups (p = 0.6) (Table 2).

NPC1 gene expression analysis in PBMCs

To evaluate the expression of NPC1 in PBMCs, relative quantitative real-time PCR was performed. mRNA expression of NPC1 in the PBMCs of the control group was significantly higher compared to that of patients (p < 0.001). However, after a full adjustment for age, BMI, statin, anticoagulant, and nitrate drugs consumption, no significant difference was observed between the atherosclerotic and control groups (p = 0.67) (Figure 1).

Association between NPC1 gene expression and IL-10 serum concentration

Because of available evidence that linked lysosomal lipid homeostasis and inflammation, we evaluated the NPC1 gene expression in the subjects divided by IL-10 serum concentration (group 1: IL-10 < 12 pg/mL, group 2 ≥ 12 pg/mL), we observed higher expression of NPC1 in group 2 compared to group 1 (p = 0.006), after a full adjustment for the mentioned factors (Figure 2).

We then evaluated the correlation between NPC1 gene expression and IL-10 serum concentrations in the subjects. NPC1 gene expression was positively associated with the IL-10 serum concentration, after a full adjustment for age, BMI, statin, anticoagulant and nitrate drugs (p = 0.04, r = 0.29) (Table 3).

25-hydroxyvitamin D serum concentration analysis

After a full adjustment, 25-hydroxyvitamin D serum concentration was higher in the control group compared to the atherosclerotic group, but not significant (p = 0.086) (Table 2).

We further divided the subjects into two groups, group 1; 25-hydroxyvitamin D < 30 ng/mL and group 2; 25-hydroxyvitamin D > 30 ng/mL, and we observed a higher level of IL-10 serum concentration in group 2 compared to group1 (p = 0.034) (Figure 3).

Table 1. Primers for real time PCR.

Primer	Forward	Reverse
Beta-actin	5′-GGACTTCGAGCAAGAGATGG-3′	5′-AGCACTGTGTTGGCGTACAG-3′
NPC1	5′-GGTCCGCCTGTGTACTTTGT-3′	5′-GGCTTCACCCAGTCGAAATA-3′

Table 2. Clinical characteristics and laboratory findings.

Variable	Controls (40)	Patients (40)	p-value
Age (year) *	58.90 ± 1.24	64.92 ± 1.27	0.001
BMI (kg/m2) *	28.2 ± 0.86	25.9 ± 0.81	0.06
TG (mg/dL) *	138.8 ± 16.25	124.7 ± 12.19	0.702
TC (mg/dL) *	159.45 ± 6.68	145.41 ± 7.15	0.156
LDL-C (mg/dL) *	95.86 ± 5.38	94.29 ± 5.06	0.821
HDL-C (mg/dL) *	43.21 ± 1.52	37.68 ± 1.77	0.023
SBP *	114.81 ± 1.95	121.12 ± 2.90	0.076
DBP *	72.43 ± 1.45	74.20 ± 1.65	0.423
Family history of CAD **	6 (15)	19 (46.3)	0.002
Hyperlipidemia **	9 (22.5)	19 (46.3)	0.023
Hypertension **	14 (35)	22 (53.7)	0.09
Smoking **	9 (22.5)	17 (41.5)	0.06
Physical activity **	13 (67.5)	18 (56.1)	0.29
IL-10 serum concentration (pg/mL) *	17.57 ± 0.78	18.66 ± 0.91	0.6
25-hydroxyvitamin D serum concentration (ng/mL) *	27.57 ± 5.09	8.15 ± 6.84	0.086

Data are expressed as mean ± SEM (*), and patient number (percentage) (**) as appropriate. [Body Mass Index (BMI), TG (Triglyceride), TC (total cholesterol), LDL-C (low density lipoprotein cholesterol), HDL-C (high density lipoprotein cholesterol). SBP (systole blood pressure), DBP (diastole blood pressure)].

Table 3. Association between NPC1 gene expression and IL-10 serum concentration in study population.

	p-value	Standardized regression coefficients
NPC1 gene expression and IL-10 serum concentration correlation	0.04	0.29

DISCUSSION

According to the literature review, this is the first study to examine the correlation of IL-10 serum concentration with NPC1 gene expression in the context of CAD. Our results indicated that after full adjustment, NPC1 gene expression in PBMCs was lower, but not significant, in patients compared to controls. Although we did not find a significant difference in IL-10 serum concentration between the atherosclerotic and control groups, the subjects with lower NPC1 gene expression were more likely to have lower IL-10 serum concentration. Our linear regression analysis also showed a significant positive correlation between NPC1 gene expression and IL-10 serum concentration. Furthermore, when divided into two groups according to the serum 25-hydroxyvitamin D level (group 1 < 30 ng/mL and group 2 > 30 ng/mL), the subjects with 25-hydroxyvitamin D deficiency demonstrated lower IL-10.

Since atherosclerosis is an inflammatory disease, many

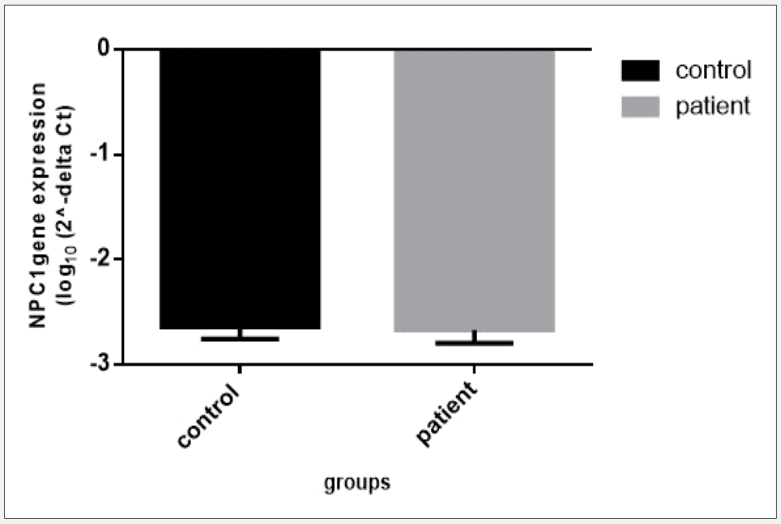

Figure 1. NPC1 gene expression in study population (p = 0.67 adjusted for age, BMI, statin, anticoagulant and nitrate drugs).

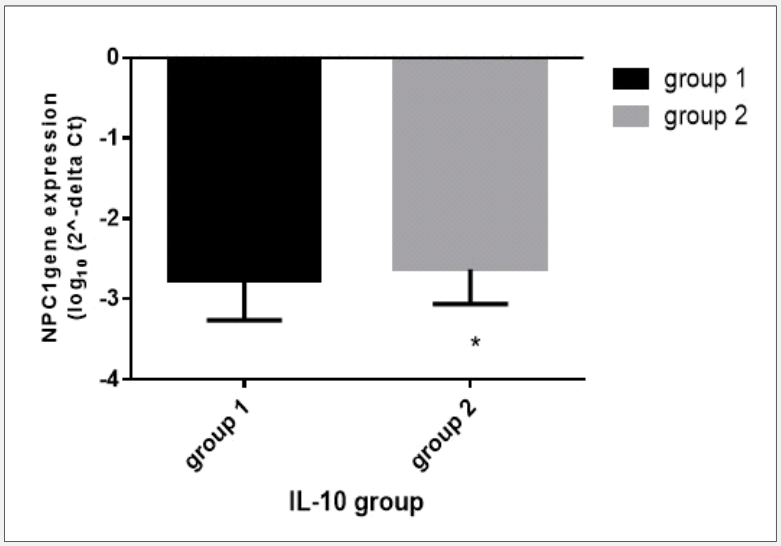

Figure 2. NPC1 Gene expression in the groups divided by IL-10 concentration (group 1: IL-10 < 12 pg/mL, group 2: ≥ 12 pg/mL) (p = 0.006 adjusted for age, BMI, statin, anticoagulant and nitrate drugs).

different anti-inflammatory cytokines are involved in the protection against progression of this disease [21, 22]. It is well-established that IL-10 is one of the most important anti-inflammatory and counter-regulatory cytokines which limits the inflammatory responses and effects [18,23]. A previous study demonstrated lower levels of IL-10 in coronary heart disease patients compared to controls [24]. Seyrek et al. and also Fichtlscherer et

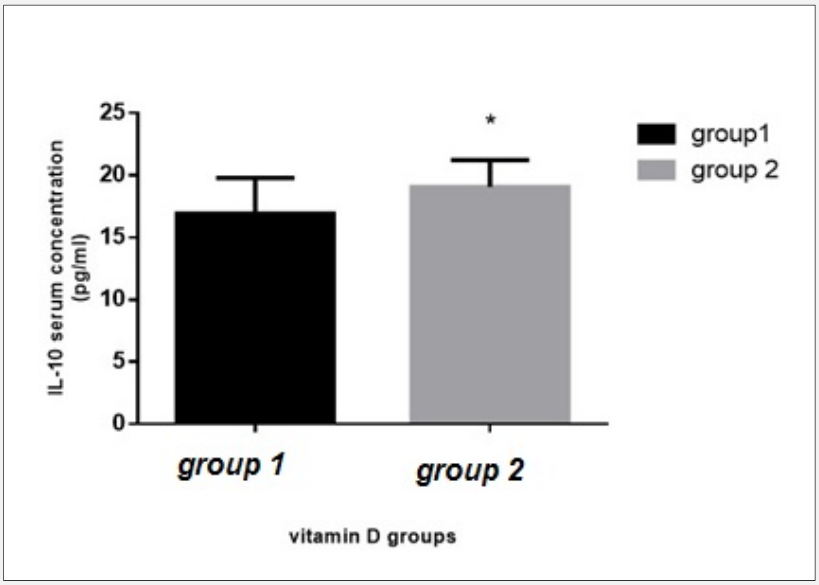

Figure 3. IL-10 serum concentration in vitamin D groups (group 1: < 30 ng/mL and group 2: ≥ 30 ng/mL) (p = 0.034 adjusted for age, BMI, statin, anticoagulant, and nitrate drugs).

al. demonstrated that IL-10 serum concentration decreases in atherosclerotic patients [25,26]. Although we also observed lower levels of IL-10 in the patient group compared to the control group, after full adjustment for drug consumption, age, and BMI, we could not see a significant difference between the two groups.

NPC1 is the most important protein in the lysosomal membrane that contributes to cholesterol transport from the lysosome to the cytosol. Several studies have shown that NPC1 deficiency was associated with lysosomal cholesterol accumulation, also reduced ABCA1 sterol transporter and decreased cholesterol efflux and, consequently, progression of atherosclerosis [13,27]. Therefore, NPC1 has an atheroprotective role in CAD [27]. Mosig et al. showed that mRNA expression of NPC1 was decreased in familial hypercholesterolemia monocytes possibly contributing to the early onset of atherosclerosis in this disease [28]. In the present study, although we have seen significantly decreased NPC1 gene expression in the atherosclerotic group, but after a full adjustment, we did not find a significant difference in NPC1 gene expression between two groups, most probably due to relatively small sample size.

However, we found that NPC1 gene expression has a positive significant correlation with IL-10 serum concentration in our study population. According to previous cellular and animal studies, there is a relationship between lysosomal cholesterol accumulation and inflammation [14]. In fact, a wealth of evidence shows that IL-10 can influence cellular lipid metabolism by facilitating both cholesterol uptake and cholesterol efflux [29]. On the other hand, several studies have demonstrated that lysosomal cholesterol accumulation can lead to increased inflammation in the cells by different mechanisms, including disturbance of autophagy due to changes in lysosomal membrane lipid composition [30-32], lysosomal membrane damage that results in release of cathepsins into the cytosol, mitochondrial dysfunction and apoptosis [33,34], and activation of the inflammasome due to cholesterol crystal formation [35]. So, there is a close linking between inflammation and lysosomal cholesterol homeostasis. According to the previous studies, it seems that reduction of lysosomal cholesterol accumulation has beneficial effects in the attenuation of inflammation and improvement of atherosclerosis progression [14]. Moreover, it has been demonstrated that culturing T cells isolated from PBMCs in two media with and without lipid led to changes in lipid rafts, cellular cholesterol content and, notably, IL-10 secretion [36]. Therefore, our finding regarding association of NPC1 gene expression in PBMCs with IL-10 serum concentration could confirm previous *in vitro* and animal studies in clinical context, suggesting that dysregulated NPC1 gene expression in PBMCs of CAD patients might favor lower production of the anti-inflammatory cytokine, IL-10. However, several basic and molecular studies are needed to determine the exact mechanism of correlation between NPC1 and anti-in-

flammatory cytokine levels in atherosclerotic milieu. Several lines of evidence support the correlation of vitamin D deficiency with inflammation [37]. In the current study, we observed higher levels of IL-10 in the subjects with normal 25-hydroxyvitamin D levels (> 30 ng/mL) after adjustment which is consistent with previous reports indicating increased secretion of IL-10 upon vitamin D treatment in innate immune system cells including PBMCs and T cells [37, 38]. In contrast, another study reported that linear regression analysis in a diabetic population divided into deficient and sufficient groups showed inverse correlation between serum 25-hydroxyvitamin D and IL-10 concentration; however, they found no correlation after adjustment for gender, BMI, HbA1c, and diabetes duration [39].

The present study has some limitations. First, the study sample size is relatively small, thus further large-scaled studies are needed to confirm current findings, and second, our study is limited by the lack of data of NPC1 protein qualification in lysates of PBMCs which could verify and give more strength to the declaration that NPC1 is correlated with IL-10 in context of CAD.

CONCLUSION

Our findings revealed a correlation between NPC1 gene expression and IL-10 serum concentration. Although more longitudinal studies are needed, these findings might further advance our understanding of the role of lysosomal lipid homeostasis components in the pathogenesis of inflammatory diseases especially atherosclerosis.

Declaration of Interest:

No conflict of interest was declared by the authors.

References:

1. Dugani S, Gaziano TA. 25 by 25: Achieving Global Reduction in Cardiovascular Mortality. Curr Cardiol Rep 2016;18:10 (PMID: 26748650).
2. Herrington W, Lacey B, Sherliker P, Armitage J, Lewington S. Epidemiology of Atherosclerosis and the Potential to Reduce the Global Burden of Atherothrombotic Disease. Circ Res 2016;118: 535-46 (PMID: 26892956).
3. Lusis AJ. Atherosclerosis. Nature 2000;407:233-41 (PMID: 1100 1066).
4. Jerome WG. Lysosomes, cholesterol and atherosclerosis. Clin Lipidol 2010;5:853-65 (PMID: 21643524).
5. Xu X, Yuan X, Li N, Dewey WL, Li PL, Zhang F. Lysosomal cholesterol accumulation in macrophages leading to coronary atherosclerosis in CD38(-/-) mice. J Cell Mol Med 2016;20:1001-13 (PMID: 26818887).
6. Yu XH, Fu YC, Zhang DW, Yin K, Tang CK. Foam cells in atherosclerosis. Clin Chim Acta 2013;424:245-252 (PMID: 23782 937).
7. Emanuel R, Sergin I, Bhattacharya S, et al. Induction of lysosomal biogenesis in atherosclerotic macrophages can rescue lipid-induced lysosomal dysfunction and downstream sequelae. Arterioscler Thromb Vasc Biol 2014;34:1942-52 (PMID: 25060788).
8. Moheimani F, Kim CH, Rahmanto AS, van Reyk DM, Davies MJ. Inhibition of lysosomal function in macrophages incubated with elevated glucose concentrations: a potential contributory factor in diabetes-associated atherosclerosis. Atherosclerosis 2012; 223:144-51 (PMID: 22658253).
9. Jerome WG, Cox BE, Griffin EE, Ullery JC. Lysosomal cholesterol accumulation inhibits subsequent hydrolysis of lipoprotein cholesteryl ester. Microsc Microanal 2008;14:138-49 (PMID: 183 12718).
10. Bobryshev YV, Shchelkunova TA, Morozov IA, et al. Changes of lysosomes in the earliest stages of the development of atherosclerosis. J Cell Mol Med 2013;17:626-35 (PMID: 23490339).
11. Karten B, Peake KB, Vance JE. Mechanisms and consequences of impaired lipid trafficking in Niemann-Pick type C1-deficient mammalian cells. Biochim Biophys Acta 2009;1791:659-70 (PMID: 19416638).
12. Maxfield FR. Role of endosomes and lysosomes in human disease. Cold Spring Harb Perspect Bio 2014;6:a016931 (PMID: 24 789821).
13. Yu XH, Jiang N, Yao PB, Zheng XL, Cayabyab FS, Tang CK. NPC1, intracellular cholesterol trafficking and atherosclerosis. Clin Chim Acta 2014;429:69-75 (PMID: 24296264).
14. Hendrikx T, Walenbergh SM, Hofker MH, Shiri-Sverdlov R. Lysosomal cholesterol accumulation: driver on the road to inflammation during atherosclerosis and non-alcoholic steatohepatitis. Obes Rev 2014;15:424-33 (PMID: 24629059).
15. Galkina E, Ley K. Immune and inflammatory mechanisms of atherosclerosis (*). Annu Rev Immunol 2009;27:165-97 (PMID: 193 02038).
16. Pinderski Oslund LJ, Hedrick CC, Olvera T, et al. Interleukin-10 blocks atherosclerotic events *in vitro* and *in vivo*. Arterioscler Thromb Vasc Biol 1999;19:2847-53 (PMID: 10591660).
17. Han X, Kitamoto S, Wang H, Boisvert WA. Interleukin-10 overexpression in macrophages suppresses atherosclerosis in hyperlipidemic mice. FASEB J 2010;24:2869-80 (PMID: 20354139).
18. Han X, Boisvert WA. Interleukin-10 protects against atherosclerosis by modulating multiple atherogenic macrophage function. Thromb Haemost 2015;113:505-12 (PMID: 25373619).
19. Fadaei R, Parvaz E, Emamgholipour S, et al. The mRNA Expression and Circulating Levels of Visfatin and Their Correlation with Coronary Artery Disease Severity and 25-Hydroxyvitamin D. Horm Metab Res 2016;48:269-74 (PMID: 26466019).
20. Akin F, Ayça B, Köse N, et al. Serum vitamin D levels are independently associated with severity of coronary artery disease. J Investig Med 2012;60:869-73 (PMID: 22534630).
21. Tedgui A, Mallat Z. Cytokines in atherosclerosis: pathogenic and regulatory pathways. Physiol Rev 2006;86:515-81 (PMID: 16601 268).
22. Gauss S, Klinghammer L, Steinhoff A, et al. Association of systemic inflammation with epicardial fat and coronary artery calcification. Inflamm Res 2015;64:313-9 (PMID: 25763815).

23. Yu GI, Cho HC, Cho YK, et al. Association of promoter region single nucleotide polymorphisms at positions- 819C/T and- 592C/A of interleukin 10 gene with ischemic heart disease. Inflamm Res 2012;61:899-905 (PMID: 22592860).

24. Liang K, Dong S, Peng H. Serum levels and clinical significance of IFN-γ and IL-10 in patients with coronary heart disease. Eur Rev Med Pharmacol Sci 2016;20:1339-43 (PMID: 27097956).

25. Fichtlscherer S, Breuer S, Heeschen C, Dimmeler S, Zeiher AM. Interleukin-10 serum levels and systemic endothelial vasoreactivity in patients with coronary artery disease. J Am Coll Cardiol 2004;44:44-9 (PMID: 15234404).

26. Seyrek N, Karayaylali I, Balal M, et al. Is there any relationship between serum levels of interleukin-10 and atherosclerosis in hemodialysis patients? Scand J Urol Nephrol 2005;39:405-9 (PMID: 16257843).

27. Zhang JR, Coleman T, Langmade SJ, et al. Niemann-Pick C1 protects against atherosclerosis in mice via regulation of macrophage intracellular cholesterol trafficking. J Clin Invest 2008;118:2281-90 (PMID: 18483620).

28. Mosig S, Rennert K, Buttner P, et al. Monocytes of patients with familial hypercholesterolemia show alterations in cholesterol metabolism. BMC Medical Genomics 2008;1:60 (PMID: 19040724).

29. Han X, Kitamoto S, Lian Q, Boisvert WA. Interleukin-10 facilitates both cholesterol uptake and efflux in macrophages. J Biol Chem 2009;284:32950-8 (PMID: 19776020).

30. Settembre C, Fraldi A, Jahreiss L, et al. A block of autophagy in lysosomal storage disorders. Hum Mol Genet 2008;17:119-29 (PMID: 17913701).

31. Settembre C, Fraldi A, Rubinsztein DC, Ballabio A. Lysosomal storage diseases as disorders of autophagy. Autophagy 2008;4:113-4 (PMID: 18000397).

32. Koga H, Kaushik S, Cuervo AM. Altered lipid content inhibits autophagic vesicular fusion. FASEB J 2010;24:3052-65 (PMID: 20375270).

33. Li W, Yuan XM. Increased expression and translocation of lysosomal cathepsins contribute to macrophage apoptosis in atherogenesis. Ann N Y Acad Sci 2004;1030:427-33 (PMID: 15659826).

34. Chwieralski CE, Welte T, Buhling F. Cathepsin-regulated apoptosis. Apoptosis 2006;11:143-9 (PMID: 16502253).

35. Duewell P, Kono H, Rayner KJ, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. Nature 2010;464:1357-61 (PMID: 20428172).

36. Chyu KY, Lio WM, Dimayuga PC, et al. Cholesterol lowering modulates T cell function *in vivo* and *in vitro*. PloS One 2014;9:e92095 (PMID: 24647529).

37. Wu E, Cui H. Effect of 1, 25-(OH) 2D3 and lipopolysaccharide on mononuclear cell inflammation in type 2 diabetes mellitus and diabetic nephropathy uremia. Genet Mol Res 2016;15(3):gmr.150385 (PMID: 27706649).

38. Ragab D, Soliman D, Samaha D, Yassin A. Vitamin D status and its modulatory effect on interferon gamma and interleukin-10 production by peripheral blood mononuclear cells in culture. Cytokine 2016;85:5-10 (PMID: 27269178).

39. Talaat I, Nasr A, Alsulaimani A, et al. Association between type 1, type 2 cytokines, diabetic autoantibodies and 25-hydroxyvitamin D in children with type 1 diabetes. J Endocrinol Invest 2016;39:1425-34 (PMID: 27541155).