Short Research Article

Levels of oxidized low-density lipoprotein and high sensitive C-reactive protein in retinal vascular occlusions: a pilot study

ABSTRACT

BACKGROUND

Oxidized low-density lipoprotein (OX-LDL) is believed to be a key trigger factor in the genesis of atherosclerosis. It has been postulated that it can activate inflammatory mediators such as high sensitive C-reactive protein (hs-CRP), interleukin 6 (IL6), and tumour necrosis factor (TNF). These mediators have been demonstrated to have an atherogenic effect.

AIMS

This pilot study examined levels of OX-LDL and hs-CRP in patients with retinal vascular occlusive disorders.

METHODS

We enroled 7 patients with retinal vascular occlusions during a two-month period. A high sensitive CRP of greater than 3mg/l was considered significantly elevated and a median oxidised LDL value greater than 59 U/L was considered significantly elevated.

RESULTS

There were 4 cases of retinal vein occlusion, 1 case of amaurosis fugax, 1 case of cilioretinal artery occlusion and a case of cholesterol embolus in the retinal vasculature. Mean OX-LDL (55.6 ± 21.1 U/L) was borderline, but within the normal range (< 59 U/L) but hs-CRP (3.9 ± 3.1 mg/l) were elevated compared to the normal range (0 -3 mg/l). Oxidized LDL was elevated in three patients (43%), and bordeline (between 55 and 59 U/L) in two patients (28%). No significant correlation was found between hs-CRP and OX-LDL values (Pearson's correlation: r = 0.256; p = 0.456).

CONCLUSIONS

This study is the first to suggest that OX-LDL and hs-CRP may be elevated in retinal vascular disease. Further investigations are required to determine the significance of these findings

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Introduction

Retinal vascular occlusive diseases include a spectrum of clinical conditions such as, retinal venous occlusion (branch, hemiretinal or central), retinal arterial occlusion (branch or central) and transient occlusive events (amaurosis fugax). There are several different mechanisms by which these different clinical scenarios may occur and thus many varied risk factors have been implicated in the aetiology of retinal vascular occlusions. These risk factors include factors in the wall of the vessels, factors affecting flow through the vessels and variations of the constituents of the blood flowing through the vessel, collectively known as Virchov's triad.[1] The risk factor common to all forms of retinal vascular disease is atherosclerosis, which may lead to thrombotic and/or embolic events that may transiently or permanently occlude a retinal artery, causing amaurosis fugax or retinal artery occlusion, respectively. Atherosclerosis is also the most common underlying cause of retinal vascular occlusions.[2]

Recently, research has focused upon subcategories of low-density lipoprotein (LDL) in the context of atherosclerosis. It has been shown that a subtype of LDL termed oxidized (Ox-LDL) has a strong predictive role for the presence of vascular atherosclerosis.[3]

Inflammation has been implicated to play a pivotal role in all phases of atherosclerosis, from the fatty streak lesion formation to the acute coronary event due to vulnerable plaque rupture. There is evidence that vascular inflammation contributes to the pathogenesis of atherosclerosis, and later in the disease process, it is a major determinant for the acute coronary syndromes. High-sensitive C-reactive protein (hs-CRP), a simple downstream marker of inflammation, is a plasma protein synthesised by the liver. In the absence of large inflammatory spikes, the year-to-year within person variations in hs-CRP concentration are similar to those in total cholesterol concentration and systolic blood pressure. In a literature-based meta-analysis of 22 prospective studies, the relative risk for coronary heart disease was 1·6 (95% CI 1·5–1·7) in a comparison of people in the top third (mean 2·4 mg/L) and bottom third (1·0 mg/L) of the hs-CRP distribution.[4]

Elevated baseline concentrations of hs-CRP are associated with the risk of atherosclerotic events in general populations and show a predictive value even in terms of secondary prevention, both in patients with chronic stable angina and acute coronary syndromes.[5]

To date there has been no published data regarding a possible link between either OX-LDL or hs-CRP and retinal vascular occlusive disorders. The purpose of our study is to evaluate if such a possible link exists.

Patients and Methods

We present a pilot study of patients with retinal vascular occlusions (retinal venous occlusion, retinal arterial occlusion or amaurosis fugax), enrolled during a two-month period. Patients gave informed consent and this study adhered to the tenents of the declaration of Helsinki. Local ethics committee approval was obtained.

All patients underwent a dilated ophthalmic biomicroscopic examination. In addition to routine serological investigations we performed analysis of fasting LDL, Ox-LDL and hs-CRP. Ten eyes in 10 patients had retinal vascular pathology during the study period. Patients who were already

taking acetylsalicylic acid and statins (HMG-CoA reductase inhibitors, n = 3) were excluded as these medications have been shown to reduce the levels of circulating oxidized LDL and hs-CRP.[6,7] Patients with any evidence of intercurrent infection (n = 0) were also excluded, as this can alter hs-CRP levels.[8] The seven patients who remained were included in the analysis.

Analysis of hs-CRP was performed with a Beckman Immage immunochemistry system (Beckmann Coulter, Brea, CA, USA), using Beckman hs-CRP reagent for the quantitaive determination of hs-CRP in serum or plasma by rate turbidimetry. A value for hs-CRP of greater than 3mg/l was considered significantly elevated.

OX-LDL analysis was carried out using an enzyme linked immunosorbent assay kit (ELISA; Mercodia AB, Uppsalla, Sweden). In this assay oxidized LDL levels were measured by competitive ELISA utilising a specfic murine monoclonal antibody mAB -4E6. The Mercodia kit is a capture ELISA in which wells of the microplate are coated with a capture antibody. A median value greater than 59 U/L was considered significantly elevated. OX-LDL is a very complicated particle, a number of epitopes exist, the method described in this study detects the epitope particular to the apolipoprotein B-100 moiety of LDL. Mercodia OX-LDL ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the oxidized apolipoprotein B molecule.

Mean values were compared using a students t-test, correlations were performed utilizing Pearson's Coefficient, a p value of < 0.05 was taken a statistically significant.

Results

We present the analysis of 7 patients with retinal vascular occlusions. There were four male patients (57%) and three female patients (43%). There were 3 (43%) cases of central retinal vein occlusion (CRVO), 2 (29%) cases of amaurosis fugax, 1 (14%) case of cilioretinal artery occlusion and a case (14%) of branch retinal artery occlsion (BRAO) secondary to a cholesterol embolus in the retinal vasculature. Only three of the patients (43%) had underlying systemic risk factors for atherosclerotic disease namely: hypertension, diabetes and hypercholesterolemia.

The mean age (± standard deviation, SD) of the patients was 61.0 (±16.0) years. The mean LDL, OX-LDL and hs-CRP (± SD) for all patients, along with those patients with and without comorbidity are displayed in table 1.

Four patients (57%) had hs-CRP levels above the upper limit of normal (3mg/l; Table 2). Two of the patients had elevated LDL levels (normal reference range: 2.5 – 3.5 mmol/L). Four patients (57%) had an LDL level ($\geq$ 3.0 mmol/L) above the advised level for moderate risk patients.[9] With regard to OX-LDL levels, three patients (43%) had elevated levels.

LDL was not significantly correlated with OX-LDL (Pearson's correlation: $r = 0.67$; $p = 0.565$), nor did OX-LDL levels not show a significant correlation with hs-CRP (Pearson's correlation: $r = 0.256$; $p = 0.456$). Age was not significantly correlated with LDL, OX-LDL or hs-CRP (Pearson's correlation: $r = -0.237$; $p = 0.538$, $r = 0.121$; $p = 0.756$, and $r = 0.294$; $p = 0.442$, respectively).

Discussion

Holovet et al. found significantly elevated mean levels of OX-LDL and LDL in patients with atherosclerotic coronary artery disease. Patients had 139% higher mean OX-LDL and 10% higher mean LDL, when compared to controls.[10] A study of patients with atherosclerotic peripheral vascular disease, found 20% higher mean OX-LDL levels and 26% higher mean LDL levels, compared to controls.[11] Our study found 54% higher mean OX-LDL levels and 3% higher mean LDL in patients with retinal vascular occlusions, ~~compared to controls~~. While there is considerable variation regarding the overall figures between these studies, they do reflect a general trend towards increased OX-LDL and LDL levels in the context of atherosclerotic disorders.

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Patients with high hs-CRP concentrations are more likely to develop stroke, MI and significant peripheral vascular disease.[4] Mean levels of hs-CRP have been reported to be significantly higher in patients with acute ischaemic stroke (4.87 mg/l for patients and 0.99 mg/l for controls),[12] acute myocardial infarction (2.7 mg/l for patients and 1.0 for controls),[13] and peripheral vascular disease (3.4 mg/l for patients).[14] Our study suggests a similar level of elevation in hs-CRP is associated with retinal vascular occlusions. It is interesting to note that the three parameters of mean hs-CRP, LDL and OX-LDL were even higher in our study when patients with comorbidities were excluded.

It is interesting to examine the evidence regarding these parameters (hs-CRP and OX-LDL) in the context of other ophthalmology disorders. OX-LDL[15] and CRP[16] have both been reported to be significantly higher in patients with neovascular age related macular degeneration (AMD). Atherosclerosis has previously been implicated in the aetiology of this disease.[15] None of our patients had any evidence of AMD.

The small number of patients, lack of control group, along with the heterogenicity of the pathology are obvious limitations to this study. We acknowlegde that the inidvidual retinal vascular disorders have very different underlying proposed mechanisms and this limits the interpretation of our results. However, this is the first report of a possible trend towards higher mean levels of hs-CRP and Ox-LDL in patients with retinal vascular occlusions. This pilot study supports the case for larger prospective studies to eludidate the exact nature of these potential relationships and investigate their clinical significance.

Conclusion

This pilot study suggests a trend of increase levels of OX-LDL and hs-CRP may potentially occur in the context of retinal vascular occluisive disorders.

Competing interests:

The Authors have no competing interests in this study.

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	All Patients (n = 7)	Patients with comorbidy (n =3)	Patients without comorbidity (n = 4)	P Value
Hs-CRP (mg/l)	3.9 ± 3.1	2.8 ± 2.8	4.7 ± 3.4	0.464
LDL (mmol/l)	3.1 ± 0.7	2.6 ± 0.6	3.6 ± 0.5	0.54
OX-LDL (U/L)	55.6 ± 21.1	52.0 ± 10	58.3 ± 28.3	0.735

Table 1.

Table displaying the mean (± standard deviation) parameters of three defined groups. All patients: all study patients with retinal vascular occlusion. Patients without comorbidity: retinal vascular occlusion patients with no documented cardiovascular comorbidity. Hs-CRP: high sensitive C-reactive protein. LDL: low-density lipoprotein. OX-LDL: oxidized low-density lipoprotein. The P value refers to the students t-test results of comparing the parameters of patients with and without comorbidity.

Patient	Age	Sex	Diagnosis	Hs-CRP (mg/l)	LDL (mmols/l)	Ox LDL (u/l)	Systemic condition
1	33	F	CRVO	4.14	4.0	79	-
2	75	M	Cilioret art occl	1.97	3.1	79	-
3	81	F	Am fugax	6.08	2.9	62	DM, chol
4	51	F	Am fugax	3.27	3.2	56	-
5	65	M	Chol embolus	0.86	2.9	52	DM, HTN
6	65	M	CRVO	9.61	4.0	19	-
7	57	M	CRVO	1.57	1.9	42	HTN

Table showing the individual patients demographics, age, sex, retinal diagnosis, levels of High-sensitive C-reactive protein (HS-CRP), low-density lipoprotein (LDL), oxidized LDL and any systemic illnesses with cardiovascular implications. Column one relates to the patient number. Abbreviations: F- female, M- Male, CRVO- central retinal vein occlusion, cilioret art occl- Cilioretinal artery occlusion, Am fugax- Amaurosis fugax, Chol embolus- cholesterol embolus, DM- diabetes mellitus (type 2 in both cases), Chol- hpercholesterolaemia, HTN- hypertension.