Relation between hypercholesterolaemia and vascular endothelial microinflammation

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العلاقة بين فرط كوليستيرول الدم وبين الالتهابات الدقيقة في الخلايا البطانية للأوعية الدموية

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الخلاصة: تم في إطار هذه الدراسة بحث علاقة الترابط بين فرط كوليستيرول الدم وزيادة الأكسدة من جانب، وبين مستوى ب — سيليكتين والانترلوكين — 6، من جانب آخر بوصفها من العلامات التي تميز الوضع الراهن للخلايا البطانية. وأُجريت الدراسة على 40 من المصريين البالغين الذين لا تظهر عليهم أعراض فرط كوليستيرول الدم، و20 من الشواهد المماثلين لهم في العمر والجنس. ولوحظ ارتفاع يُعتدّ به إحصائياً في مستوى أكسدة الشحوم (P<0.001) في مجموعة الدراسة، كما لوحظ ترابط إيجابي بينه وبين مستوى الكوليستيرول (P<0.001) والبروتين الشحمي المنخفض الكثافة LDL (P<0.002). وكان مستوى نشاط إنزيم بيروكسيداز الغلوتاثيون مرتفعاً أيضاً بشكل يُعتدّ به إحصائياً (P<0.001)، وترابط نشاطه ترابطاً إيجابياً مع مستوى الكوليستيرول (P<0.001) ومستوى البروتين الشحمي المنخفض الكثافة (P<0.001). ولوحظ أيضاً ارتفاع واسمات وظائف الخلايا البطانية بشكل يُعتدّ به إحصائياً في المجموعة الخاضعة للدراسة (P<0.001) مع ترابط إيجابي بمستوى الكوليستيرول (P<0.001) ومستوى البروتين الشحمي المنخفض الكثافة (P<0.001). وخلصت الدراسة إلى أن فرط كوليستيرول الدم يسبِّب الالتهابات الدقيقة في الخلايا البطانية للأوعية الدموية، وأن ب — سيليكتين والإنترلوكين — 6 قد يكونان من عوامل اختطار الإصابة بمرض القلب الوعائي.

ABSTRACT We investigated the correlation between hypercholesterolaemia and oxidative stress and P-selectin and interleukin-6 (IL-6) as markers for endothelial status. We studied 40 Egyptian adults with asymptomatic hypercholesterolaemia and 20 age- and sex-matched controls. Lipid peroxidation was significantly higher ($P < 0.001$) in the study group and positively correlated with cholesterol ($P < 0.001$) and low-density lipoprotein (LDL) ($P < 0.002$). Glutathione peroxidase activity was also significantly higher ($P < 0.001$) with positive correlation with cholesterol ($P < 0.001$) and LDL ($P < 0.001$). Markers for endothelial cell function were significantly higher in the study group ($P < 0.001$) with a positive correlation with cholesterol ($P < 0.001$) and LDL ($P < 0.001$). Hypercholesterolaemia causes endothelial microinflammation, and P-selectin and IL-6 may also be risk factors for cardiovascular disease.

Relation entre l'hypercholestérolémie et la micro-inflammation de l'endothélium vasculaire

RÉSUMÉ Nous avons exploré la corrélation entre l'hypercholestérolémie, le stress oxydatif et les marqueurs de la fonction endothéliale que sont la P sélectine et l'interleukine 6 (IL-6). Nous avons étudié 40 Égyptiens adultes présentant une hypercholestérolémie asymptomatique et 20 témoins appariés selon l'âge et le sexe. La peroxydation lipidique est apparue significativement plus intense ($p < 0,001$) dans le groupe expérimental et corrélée positivement au cholestérol ($p < 0,001$) et aux lipoprotéines de basse densité (LDL) ($p < 0,002$). De même, l'activité glutathione peroxidase était significativement supérieure ($p < 0,001$), avec une corrélation positive avec le cholestérol ($p < 0,001$) et les LDL ($p < 0,001$). Les marqueurs de la fonction des cellules endothéliales se sont avérés significativement plus élevés dans le groupe expérimental ($p < 0,001$), en corrélation positive avec le cholestérol ($p < 0,001$) et les LDL ($p < 0,001$). L'hypercholestérolémie génère une micro-inflammation de l'endothélium vasculaire, tandis que la P sélectine et l'IL-6 peuvent également être des facteurs de risque de maladie cardio-vasculaire.

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Introduction

The vascular endothelium is a dynamic endocrine organ that regulates vascular tone, local homeostasis, and the fibro-inflammatory-proliferative process. Many studies have demonstrate that endothelial dysfunction and activation is one of the earliest markers in patients with atherogenic risk factors (e.g. male sex, ageing, hypertension, diabetes mellitus, smoking, family history) in the absence of angiographic evidence of atherosclerosis [*1*].

Oxidative stress to the vascular endothelium is a serious causative factor of vascular endothelial dysfunction, and plays an important role in the pathophysiology of several vascular diseases, including atherosclerosis, diabetes, neuronal disorders, and ischaemia-reperfusion injury [*2*]. It was noted that some of the patients attending 6th October national insurance clinic for annual routine laboratory and clinical examinations were diagnosed as hypercholesterolaemic without any clinical symptoms. A few years later, vascular injury was noted during the annual medical examination. Thus, we carried out this study to confirm the relationship between hypercholesterolaemia and vascular injury.

Hypercholesterolaemia is frequently associated with enhanced lipid peroxidation [*3*]. We therefore aimed to investigate the correlation between hypercholesterolaemia and oxidative stress on the one hand and P-selectin and interleukin-6 (IL-6) (pro-inflammatory cytokine) as markers of endothelial functional status on the other.

Methods

We contacted asymptomatic Egyptian adult employees of the National Research Centre (males and females) who had been examined at the health insurance outpatient clinic between the beginning of October 2004 and the end of December 2004 and who had been identified as having hypercholesterolaemia (total cholesterol > 200 mg/dL). We excluded any patient with cardiovascular disease. Of those we invited to take part in our study 65% refused to participate and 35% agreed (40 patients). During the same period, 20 healthy age- and sex-matched personnel whose medical records showed total cholesterol and triglycerides were within normal levels and who had no cardiovascular disease were selected from the medical staff of the National Research Centre to participate in the study as controls. This group was retested to confirm their blood lipid status before participating in the study.

After taking verbal agreement, all participants (patients and controls) underwent the following investigations.

Blood samples (10 mL) were collected from each participant at the health insurance clinic or the clinic in the National Research Centre, as convenient for the participant. Each sample was divided into 2 × 5 mL portions: in the first portion, serum was separated, divided into aliquots and preserved at –20 °C until used; the blood in the second portion was collected over EDTA and assayed on the same day for the determination of glutathione peroxidase activity and lipid peroxidation, measured as thiobarbituric acid-reactive substance (TBARS).

Lipid peroxidation was determined as TBARS value according to the method of Mitsura and Midori. The TBARS value was measured as the difference in optical density read at 535 nm and 520 nm [*4*].

Glutathione-peroxidase activity in whole blood was determined using Ransel kit (Randox Laboratories, Crumlin, UK) according to the manufacturer's instructions.

Total serum cholesterol was determined using a commercial kit (catalogue number

07986B-07/98BioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions].

Serum high-density lipoprotein (HDL) cholesterol was determined using a BioMérieux kit (catalogue number 00232B-04/96) according to the manufacturer's instructions.

Serum low-density lipoprotein (LDL) cholesterol was measured directly using a BioMérieux kit (catalogue number 00238 B-04/96) according to the manufacturer's instructions. It was read at wavelength 500 nm and calculated using the formula: $A_{sample}/A_{standard} \times n$ mg/dL (dilution coefficient n = 387).

Human P-selectin was measured by enzyme-linked immunosorbent assay kit for quantitative detection of soluble human P-selectin (Bender MedSystems, Vienna, Austria) according to the manufacturer's instructions.

IL-6 was measured using an immunoenzymometric assay kit (IL-6 EASIA, Biosource, Nivelles, Belgium) for the quantitative measurement of human IL-6 in serum according to the manufacturer's instructions.

Results were expressed as mean and standard deviation (SD). Data were analysed using *SPSS*, version 10. Data were compared using a paired *t*-test for independent variables. Values of $P < 0.05$ were considered significant.

Pearson correlations between different variables were done. Values of $P < 0.01$ were considered significant (2-tailed).

Results

Levels of lipid peroxidation (TBARS) ($P < 0.001$) and glutathione peroxidase activity as markers for oxidative stress were statistically significantly higher in the hypercholesterolaemic (patient) group ($P < 0.001$). P-selectin (marker for endothelial cell function) and IL-6 (pro-inflammatory cytokine) were also markedly higher in the patient group ($P < 0.001$) (Table 1).

Table 2 shows the correlation (Pearson coefficient, *r*) between total cholesterol

Table 1 **Comparison between hypercholesterolaemic patients (total cholesterol > 200 mg/dL) and controls (total cholesterol ≤ 200 mg/dL) for total cholesterol and markers of oxidative stress and endothelial function**

Variable	Patients Mean (SD)	Controls Mean (SD)
Cholesterol (mg/dL)	291.85 (73.35)	115.3 (20.58)
Oxidative stress marker		
TBARS difference between OD (535–520 nm)	0.8705 (8.488E-02)	0.3111 (4.977E-02)
Glutathione peroxidase activity (units/L whole blood)	132.37 (24.38)	41.25 (12.319)
Endothelial function marker		
P-selectin (ng/mL)	791.5 (322.57)	184.88 (114.98)
Interleukin-6 (pg/mL)	66.35 (39.69)	4.1 (2.3722)

TBARS = thiobarbituric acid-reactive substance, indicator of lipid peroxidation, indicated as difference in optical density read at 535 nm and 520 nm.
P < 0.001 for all variables.
SD = standard deviation.

level and LDL-cholesterol level and oxidative stress, expressed by lipid peroxidation (TBARS) and glutathione peroxidase activity. Also shown is the correlation with endothelial function, expressed by P-selectin and the pro-inflammatory cytokine IL-6.

Table 3 shows the correlation between oxidative stress, expressed by lipid peroxidation and glutathione peroxidase activity, and markers of endothelial function, expressed by P-selectin and pro-inflammatory cytokine IL-6 ($P < 0.001$). Correlation between Il-6 and P-selectin was also statistically significant.

There was a negative, but not statistically significant, correlation between HDL cholesterol and the markers of oxidative stress and endothelial function (Table 3).

Discussion

Hypercholesterolaemia has frequently been associated with enhanced lipid peroxidation [*3*]. In this study we examined the relation between hypercholesterolaemia and increased oxidative stress. We found a very high positive correlation between cholesterol level, LDL cholesterol and raised oxidative stress, expressed as high lipid peroxidation and glutathione peroxidase activity. This is consistent with the result of Davi et al., who stated that they had obtained evidence of enhanced lipid peroxidation (*in vivo*) in hypercholesterolaemic patients [*3*].

Desideri et al. stated that hypercholesterolaemia was associated with endothelial activation and increased lipid peroxidation [*5*]. Lewis et al. showed that oxidative stress stimulates the production and release of platelet-activating factor (PAF) in endothelial cells [*6*]. Other reports from Marathe et al. and Tokumura et al. indicated that PAF as well as PAF-like phospholipids are critical factors in the pathophysiology of vascular endothelial dysfunction under oxidative stress conditions [*7,8*].

According to Lum and Roebuck, oxidative stress to the vascular endothelium is a serious causative factor of vascular endothelial dysfunction and plays an important role in the pathophysiology of several vascular diseases [*2*]. LDL is the cholesterol

Table 2 **Correlation between cholesterol and low-density lipoprotein (LDL) and markers of oxidative stress and endothelial function**

Variable	Cholesterol		LDL	
	r	*P*	*r*	*P*
Oxidative stress marker				
TBARS	0.772	< 0.001	0.665	< 0.002
Glutathione peroxidase activity	0.735	< 0.001	0.759	< 0.001
Endothelial function marker				
P-selectin	0.879	< 0.001	0.876	< 0.001
IL-6	0.782	< 0.001	0.775	< 0.001

Correlation significant at the 0.01 level (2-tailed).
TBARS = thiobarbituric acid-reactive substance, indicator of lipid peroxidation.
IL-6 = interleukin-6.

Table 3 **Correlation between markers for oxidative stress and endothelial function markers and high-density lipoprotein (HDL) cholesterol**

Variable	Oxidative stress marker				Endothelial function marker			
	TBARS		Glutathione peroxidase activity		P-selectin		IL-6	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
P-selectin	0.750	< 0.001	0.812	< 0.001	–		–	
IL-6	0.717	< 0.001	0.805	< 0.001	0.913	< 0.001	–	
HDL cholesterol[a]	–0.217	0.372	–0.097	0.68	–0.113	0.636	–0.086	0.78

Correlation significant at the 0.01 level (2-tailed).
[a]*Not significant.*
TBARS = thiobarbituric acid-reactive substance, indicator of lipid peroxidation.
IL-6 = interleukin-6.

component that is more directly involved in the pathogeneses of vascular dysfunction in hypercholesterolaemic patients.

In our study there was increased level of LDL cholesterol and increased oxidative stress. LDL becomes pathogenic when subjected to oxidation (increase in oxidative stress) and becomes ox-LDL [*9*]. Ox-LDL is, in fact, no longer recognized by the LDL receptors; instead it is taken up by scavenger receptors. These are not subjected to regulation by the intracellular cholesterol level in the subendothelial macrophages which transform to foam cells [*10*]. Egashira showed that LDL, especially oxidized LDL, inhibits endothelial function through inhibition of NO (nitric oxide) activity by down-regulation of endothelial NO synthase expression, decreased receptor-mediated release of NO, and activation of NO via superoxide anion production [*1*].

Aikawa et al. concluded that lipid lowering reduced production of reactive oxygen species, ox-LDL accumulation and plasma level of anti-ox-LDL IgG; VCAM-1 and MCP-1 expression decreased and NO synthase expression increased, and endothelial cells exhibited more normal ultrastructure [*11*].

P-selectin level was much higher in the hypercholesterolaemic group, with a strong positive correlation with cholesterol and LDL-cholesterol levels. This is in agreement with Johnson-Tidey et al., who stated that high P-selectin plasma level in symptomatic hypercholesterolaemic patients may represent an index of the presence of atherosclerotic vascular lesions P-selectin expression is endothelial cells overlying atherosclerotic plaques [*12*].

Davi et al. observed that plasma P-selectin concentration was directly correlated with LDL levels, which suggests that LDL might have an impact on the series of events that lead to P-selectin expression and release *in vivo*. They also found that hypercholesterolaemia was associated with elevated plasma P-selectin [*13*]. P-selectin level may be proposed as a marker of endothelial dysfunction in hypercholesterolaemic patients. This agreed with the results of our study as we found a strong positive correlation between P-selectin and markers of oxidative stress expressed by lipid peroxidation and glutathione peroxidase activity.

Levels of the proinflammatory cytokine IL-6 were significantly higher in the hyperc-

holesterolaemic group compared to healthy controls. There was a positive correlation with cholesterol, LDL, oxidative stress and endothelial function. This result agreed with that of Nawawi et al., who observed that hypercholesterol-aemia caused endothelial dysfunction, leading to increased production of adhesion molecules and cytokines (IL-6) [*14*]. Desai et al. concluded that the inflammatory cytokine IL-6 was an important mediator of increased endothelial permeability via alterations in ultra-structural distribution of tight junctions and morphologic changes in shape causing endothelial barrier dysfunction [*15*].

Blood levels of inflammatory markers have been associated with hypercholesterolaemia [*16*]. Cytokines (IL-6, Il-1B) and soluble adhesion molecules have been associated with both hypercholesterolaemia and atherosclerotic diseases. Soluble intercellular adhesion molecule-1 and IL-6 have been found to reflect endothelial dysfunction in patients with primary hypercholesterolaemia [*17*].

IL-6 has been found to induce oxidative stress and endothelial dysfunction by over-expression of the angiotensin II type I receptor [*18*]. Pro-inflammatory cytokines such as tumour necrosis factor alpha and IL-6 are important mediators of immune response, associated with endothelial dysfunction [*19*].

Conclusion

Hypercholesterolaemia causes endothelial microinflammation. IL-6 and P-selectin were also identified as risk factors for cardiovascular disorders.

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