Original Research Article

Is There a Relationship Between MMP-9 and Oxidised LDL in a Normal Population?

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Abstract: Oxidised LDL, a marker of systemic oxidative stress, and MMP-9, a collagenase found in vulnerable atherosclerotic plaques have been implicated in the initiation and/or progression of atherosclerosis. Infiltrating inflammatory cells interact with the extracellular matrix and oLDLs, contributing to and increased production of MMP-9 by macrophages. The MMPs contribute to the development of *de novo* atherosclerotic plaques as well as to the rupture of these plaques by degrading the associated extracellular matrix. A study was carried out to determine whether there was an association between these two markers of inflammation in samples from a normal population to see if they could be used as predictors of early coronary heart disease. The results showed that overall, in a normal population there was no association between the plasma levels of oLDL and MMP-9 except when other intrinsic factors were included such as fibrinogen or insulin, then there was a correlation.

Keywords: Atherosclerosis, oxidised low density proteins (oLDL), matrix metallo-proteinase 9 (MMP-9), fibrinogen, insulin.

INTRODUCTION

Atherosclerosis is a chronic inflammatory disease of the arterial wall that arises from an imbalanced lipid metabolism and a maladaptive inflammatory response [1, 2]. Various markers of oxidative stress, such as oxidation of low-density lipoprotein (oLDL), nitrosative stress, lipid peroxidation, and protein oxidation, have been implicated in the initiation and/or progression of atherosclerosis [3]. Studies have shown that the accumulation of low-density lipoproteins (LDL) within the arterial wall undergoes oxidative modification to form oxidised low-density lipoproteins (oLDL) which initiate a complex series of biochemical and inflammatory/ immune-modulatory reactions involving smooth muscle cell (SMC) proliferation and transformation of macrophages into foam cells to form a stable atherosclerotic plaque [4-6]. These plaques consist of a mass of lipid-engorged monocytes covered by a fibrous cap pushed out into the artery lumen by smooth muscle cells partially or totally blocking the blood's flow through the heart, brain, pelvis, legs, arms or kidneys [7].

A key process in the progression of symptomatic atherosclerosis is the rupture of the plaque that predisposes to coronary thrombosis, leading to acute coronary syndromes. Recent studies have shown that the fibrous caps of vulnerable and ruptured atherosclerotic plaques have reduced collagen and glycosaminoglycan content in association with an increased macrophage density and a reduced smooth muscle cell density. The accumulation of oLDLs has been shown to not only have pro-inflammatory properties, but to increase matrix metalloproteinase-9 (MMP-9) secretion by the macrophages [8]. The increased production and release of these matrix-degrading proteases from activated macrophages and foam cellsweaken the fibrous cap, surrounding the plaque making the atherosclerotic lesion more prone to plaque rupture [9-12].

Because of the proposed causal association of oLDLs and MMP-9, Q^2 Solutions (formerly Quest Diagnostics)carried out a study to determine whether there was an association between these two markers of inflammation in samples from a normal population to see if they could be used as predictors of early coronary heart disease.

MATERIALS AND METHODS
Study population
1510 samples were collected from patients of north European origin. They were healthy adults evenly distributed by sex and age. Exclusion criteria were any self-reported disease *eg* cancers, known cardiac problems or severe dementia. Plasma for the oLDL and MMP9 was collected by centrifugation of the blood sample for 15 minutes at 1000 x g within 30 minutes of collection. The plasma samples were then frozen and stored at -20°C before being transported to the laboratory. The samples were kept frozen until testing. Prior to testing the samples were thawed, vortexed to remove layering effects and then centrifuged 15 minutes at 1000 x g. All samples were also tested for fibrinogen, insulin, and free fatty acids using standardised methods.

oLDL Analysis
The concentrations of oLDL were measured in Heparin plasma using a plate assay (Oxidised LDL) supplied by Mercodia (Mercodia AB, Sweden). The assay was a solid phase two-site enzyme immunoassay. Based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the oxidised apo-lipoprotein B molecule. A reference range of 14.4- 102.7 mU/L was used for the statistical analysis [13].

MMP-9 analysis
The concentrations of MMP-9 were measured in Heparin plasma using a plate assay (Quantikine® Human MMP-9 [total] Assay) supplied by R&D Systems (R&D Systems Europe Ltd., Abingdon, UK). The assay was a quantitative sandwich enzyme immunoassay, with a monoclonal antibody specific for MMP9 pre-coated onto the wells. Reference ranges of 14.3-34.6 ng/mL (female) and 19.8-99.5 ng/mL (male) were used for the statistical analysis [14].

Statistical Analysis

A Kolmogorov Smirnov test was used to test for normal distribution of the variables and the assessment of bivariate correlations between variables was examined using Pearson's correlation coefficient for normally distributed data. The Spearman's rank correlation coefficient and Mann-Whitney U-value calculator were used for non-normal data. Variables that exhibited significant correlation at bivariate analysis were included as independent variables in regression analyses. All *P*-values where applicable were two-sided and the level of statistical significance was established at 0.05.

RESULTS
1131 of the original 1510 patients were included in the final analysis as a normal population, with an average age of 67.5 (median 69) ranging from 21-80 years of age. There was a male to female ratio of 1.0: 0.8. There were 379 patients excluded because of abnormal results from one or more the other analytes. Of these 260 had normal levels of MMP-9 and oLDLbut included 50 patients with abnormal insulin levels and 210 patients with raised fibrinogen levels. And finally 119 patients were excluded from the 'normal population' analysis because the MMP-9, oLDL or both levels fell outside of the agreed reference range intervals.

Figure 1 shows that there was no correlation between the levels of oLDL and MMP-9 in our normal population group (r^2 =-0.55). The data were further evaluated and gave a Pearson coefficient p- value of 0.051. The Mann-Whitney test showed that there was an approximately normal distribution of results (U-value of 259446) with a Z-score of 1.959(p- value of 0).This result was further compared to those 119 samples that had had abnormal levels of oLDL, MMP-9 or both (Figure 2) and a definite relationship (r^2 =0.9583) became apparent. This was a good correlation, where high oLDL results were linked with high MMP9 results (and vice versa).
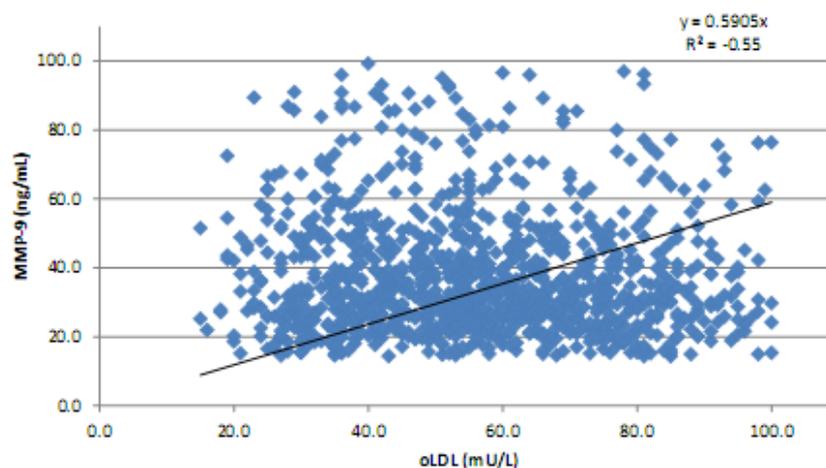

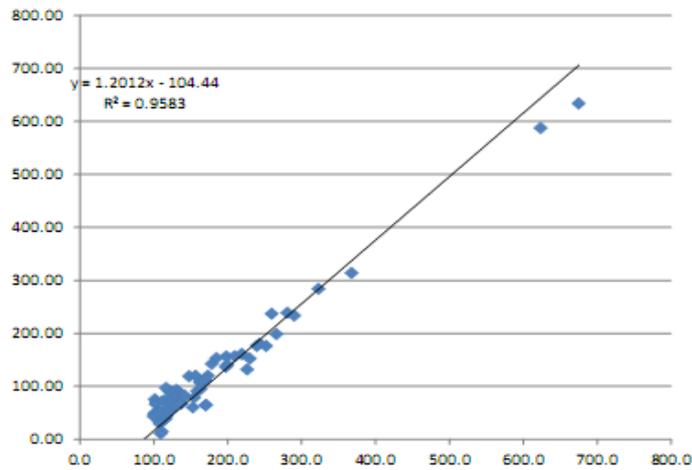**Fig-1: Correlation between oLDL and MMP-9 in a Normal Population**

Fig-2: Raised levels of MMP9, oLDL or both

For the 251 patients with raised fibrinogen or insulin levels (9 patients had low insulin levels and was too small a group for a definitive analysis) but normal oLDL and MMP-9 results, these were plotted on regression plots and the r^2 values of 0.593 and 0.910 respectively (Figure 3 and 4).
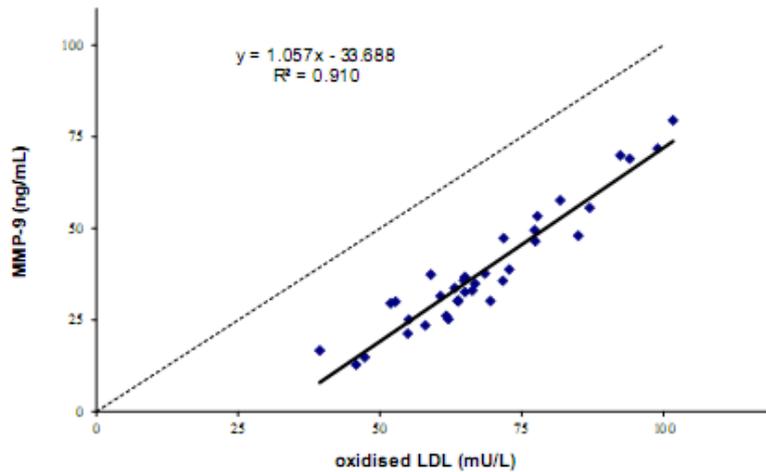

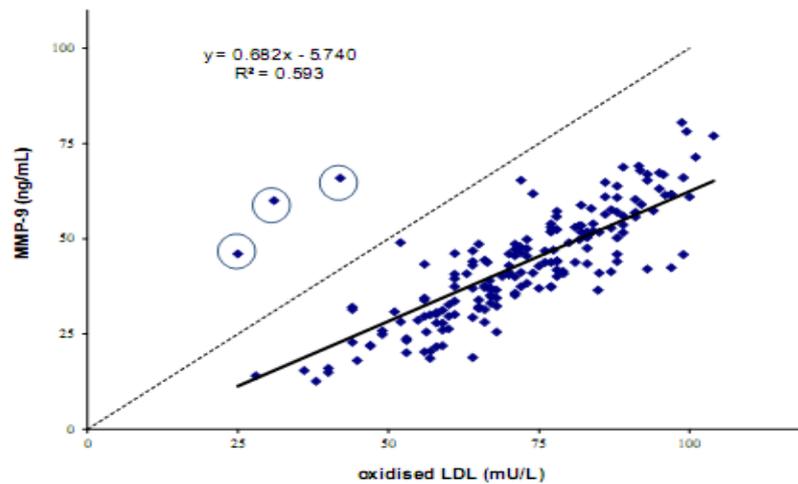**Fig-3: Normal levels of MMP-9 and oLDL with raised insulin levels (*n= 41*)**

Fig-4: Normal levels of MMP9 and oLDL with raised Fibrinogen levels (*n =210*)

DISCUSSION

This study investigated the relationship between oLDL, a marker of systemic oxidative stress, and MMP-9, a collagenase found in vulnerable atherosclerotic plaques. Overall, in a normal population there was no association between the plasma levels of oLDL and MMP-9 but this data does not provide insight into the signaling pathways through which oLDL regulates MMP-9 expression. Studies have shown that infiltrating inflammatory cells interact with the extracellular matrix and oLDLs, contributing to increased production of MMP-9 by macrophages [15]. The presence of increased macrophages provides stimulus for synthesis or activation of MMPs by neighbouring cells facilitating further structural changes and advancement of atherosclerotic lesions [16]. The MMPs contribute to the development of *de novo* atherosclerotic plaques as well as to the rupture of these plaques by degrading the associated extracellular matrix [17].

While there was no correlation in the normal population, the two analytes do seem to begin to correlate in cases where another intrinsic factor was involved. Certainly studies have shown that the activation of MMP-9 is involved in inflammatory agent fibrinogen (Fg) induced enhanced transcytosis through endothelial cells (ECs) [18]. It is therefore possible that at an elevated level, Fg enhances the formation of functional caveolae through activation of MMP-9 [19]. The results from this study would seem to support this to some extent as both the oLDL and MMP-9 start to increase in value proportionally in raised levels of fibrinogen. What was interesting was that in general, the oLDL result was always greater than the MMP9 result but there were three samples which were outside of the normal analysis where there was a reversal of results, the MMP9 result was greater than the oLDL. Was there anything unusual about these patients? They were all male and in their 70s and the only other similar trait was a decreased insulin level. But, studies have shown that patients with low insulin levels could have elevated MMP-9 levels due to the increase in neutrophil activation rather than stress response or chronic hyperglycemia [20]. So, it is possible that these three patients fell into this category and the oLDL result was not being affected.

There has however, been an increasing interest in the role of oxidative stress in common metabolic disorders such as type 2 diabetes(T2D) and obesity in recent years. Certainly, studies have shown that oxidative stress and inflammatory processes play an important role in the development of vascular pathologies, T2D and the metabolic syndrome which is characterized by insulin resistance, central obesity, hypertension and dyslipidemia [21-23]. For instance, the levels of oLDL, have been shown to be elevated in individuals with the metabolic syndrome compared to those without. And oLDL level, not LDL-cholesterol, was also found to be predictive of incident T2D. This suggests that oLDL may be a marker of metabolic changes preceding or accompanying the onset of T2D. Furthermore, oxidative stress is also associated with some risk factors of these clinical diseases. While a number of studies show that the LDL particles of diabetic subjects are more susceptible to oxidative modification in vitro than those of control subjects, and oLDL has been positively associated with obesity and inflammation, and among individuals with elevated abdominal fat, the effect of oxidative stress was even greater on cardiovascular disease (CVD) risk [24-26].

CONCLUSION

In our normal population there was no correlation between the two analytes, oLDL and MMP9 but as soon as either was challenged by another factor *ie* fibrinogen or insulin they began to show a slight to high correlation. Certainly, if either oLDL or MMP-9 were elevated it would warrant further investigation to include other cardiac markers.

STUDY LIMITATIONS

The limitation of this study is that there was only the laboratory data available and does not provide insight into the clinical details of the patients. Although the focus of the study was on evaluating the association between oLDL and MMP-9 it could not show if other members of the MMP family were influenced.

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