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## Original Article

# A study on serum concentrations of oxidized low-density lipoprotein $\beta$ 2-glycoprotein I complex in patients with normal and dyslipidemic lipid profiles

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## ABSTRACT

**Aim/Objective:** We correlated serum levels of oxidized low-density lipoprotein  $\beta$ 2-glycoprotein I complex (oxLDL/ $\beta$ 2GPI) with normal and dyslipidemic subjects with described familial CVD histories.

**Material and methods:** A total of 188 subjects were enrolled [dyslipidemic ( $n=103$ ) and normal ( $n=88$ )]. Clinical assessment, lipid profile and serum ELISA analysis of oxLDL/ $\beta$ 2GPI were measured.

**Results:** A statistically significant difference ( $P < 0.01$ ) in mean serum concentration of oxLDL/ $\beta$ 2GPI was observed for normal (2.29 U/ml–6.48 U/ml with a mean of 4.32 U/ml) vs dyslipidemic (3.98 U/ml–7.99 U/ml with a mean of 6.04 U/ml) The percent of normal lipid subjects with a family history of CVD was 21.6% whereas it was 25.2% for dyslipidemic subjects, which was not statistically different ( $P < 0.05$ ). The mean oxLDL/ $\beta$ 2GPI concentrations for subjects without a family history of CVD were 4.94 U/ml vs 6.28 U/ml for subjects with a family history of CVD history which were statistically different ( $P < 0.05$ ). **Conclusion:** The oxidative marker oxLDL/ $\beta$ 2GPI was significantly elevated in dyslipidemic subjects and correlated with family history of CVD increasing its potential as an additional independent marker for predicting cardiovascular disease in India.

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## 1. Introduction

Cardiovascular diseases are one of the fastest growing public health problems in India.<sup>1</sup> Numerous serologic markers of inflammation such as C-reactive protein<sup>2</sup> and phospholipase A2<sup>3</sup> have been associated with coronary artery disease progression and outcomes. However, the usefulness of many of

these markers in routine patient management remains difficult<sup>4,5</sup> and not well standardized in the Indian subpopulation. More independent biomarkers to predict CVD are needed to address the increasing public health cost and to treat patients before expensive surgical intervention is needed.

The oxidative modification of low-density lipoprotein (oxLDL) has been shown to play a key role in the initiation and

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progression of atherosclerosis.<sup>6–9</sup> OxLDL, unlike unoxidized LDL, binds to  $\beta$ 2-glycoprotein I protein ( $\beta$ 2GPI) in a time-dependent manner and leads to the formation of covalently bound stable oxLDL/ $\beta$ 2GPI complexes.<sup>10,11</sup> which have been shown to be a very relevant predictor for CAD severity with adverse outcomes directly correlated to cardiac catheterization scores.<sup>12</sup>

The physiologic relevance of oxLDL/ $\beta$ 2GPI complexes in atherogenesis is further emphasized by the co-localization of  $\beta$ 2GPI in human atherosclerotic lesions.<sup>13</sup> Patients with acute coronary syndrome (ACS) and high anti- $\beta$ 2GPI and anti-oxLDL/ $\beta$ 2GPI antibodies, show a significantly higher rate of adverse outcomes as compared to patients without these antibodies.<sup>14</sup>

In this present study, we evaluated the association of oxLDL/ $\beta$ 2GPI complexes with total lipid profiles in 188 randomly selected patients reporting into a wellness clinic. We examined the range of serum concentrations of oxLDL/ $\beta$ 2GPI in this normal population, classified the patients between normal and dyslipidemic and examined the relationship between oxLDL/ $\beta$ 2GPI levels, lipid profile and familial history of CVD.

## 2. Methods

### 2.1. Study design and participants

Patient data and serum samples for the study were obtained from patients enrolled in the wellness program at Spectrum Clinical Research P. Ltd., Mumbai, India.

Men and women, between the ages of 16 and 90 years with no vocalized illness were selected to participate. All patients underwent the standard diagnostic procedures in the wellness program which includes the collection of blood for fatty acid lipid profiling. Clinical and laboratory investigators remained blind to each other's data throughout the course of the study. The study protocol was approved by Spectrum Clinical's Institutional Review Board, and before study enrollment, all patients signed informed consent as part of their wellness assessment protocol.

### 2.2. Lipid analysis methods

All lipid analysis was carried out using a Johnson & Johnson's VITROS 250 analyzer (Dry Chemistry). The specific methods used were:

Enzymatic detection (cholesterol ester hydrolase, c oxidase & peroxidase) was used to measure total serum cholesterol concentration.

Non-HDL precipitation method followed by enzymatic detection was used to measure HDL cholesterol concentration.

Enzymatic detection using lipase/glycerol kinase was used to measure triglyceride concentration.

LDL and VLDL cholesterol were reported based on calculations using standard diagnostic formulae:

LDL cholesterol = Total cholesterol – (HDL + triglycerides/5).  
VLDL cholesterol = triglycerides/5.

### 2.3. ELISA assay for oxLDL/ $\beta$ 2GPI complexes

All serum samples were blind tested for oxLDL/ $\beta$ 2GPI complex concentrations at the Hofseth Biocare AS laboratory and based on the ELISA method described by Greco et al<sup>12</sup>

Monoclonal antibody (3H3) against human  $\beta$ 2GPI was coated onto microplates by incubating 100  $\mu$ L per well. The plate was blocked overnight at 8 °C with phosphate-buffered saline, treated with 100  $\mu$ L of patient serum and incubated for 1 h. The microwells were washed, fixed with IgG murine monoclonal anti-human ApoB-100 and incubated for 30 min, followed by treatment with horseradish peroxidase–streptavidin. The color was developed using tetra-methylbenzidine/hydrogen peroxide and the optical density read at 450 nm. The intra-assay precision (coefficient of variation %) for the assay ranged from 2.9 to 6.4, and the functional sensitivity was 0.05 U/mL. Serum oxLDL/ $\beta$ 2GPI complex concentration (expressed in U/mL) was calculated against a reference curve. The specific interaction between oxidized LDL and  $\beta$ 2GPI and the specificity of the assay for oxLDL/ $\beta$ 2GPI complexes has been previously reported.<sup>15</sup>

## 3. Results

### 3.1. Patient classification

The patients were classified as normal lipid profile or dyslipidemic using the lipid profile ranges as defined in *The National Cholesterol Education Program (NCEP) Adult Treatment Panel III Report*.<sup>16</sup>

The demographic data on the 188 patients is shown in Table 1.

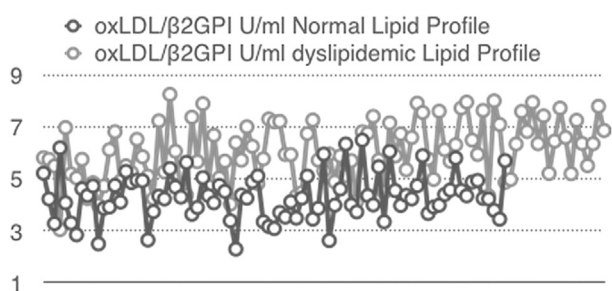
Additional data was collected on the history of CVD in the family and smoking/non smoking status for each patient in the study. The age of the patients ranged from 16 to 90 years with an average age of 46.96 years in the normal lipid profile group and 48.76 years in the dyslipidemic lipid profile group which was comparable and the difference was not statistically significant. There was also no statistical difference in the

**Table 1 – Demographic data.**

| Parameters               | Normal lipid profile | Dyslipidemic lipid profile |
|--------------------------|----------------------|----------------------------|
| Number of cases          | 85                   | 103                        |
| Age <sup>a</sup> (years) |                      |                            |
| Mean                     | 46.96                | 48.76                      |
| SD                       | 14.62                | 10.58                      |
| Range                    | 16–84                | 20–90                      |
| Gender <sup>b</sup> (%)  |                      |                            |
| Male                     | 49 (57.6)            | 60 (58.3)                  |
| Female                   | 36 (42.4)            | 43 (41.7)                  |
| Profile of history (%)   |                      |                            |
| CVD <sup>a</sup>         | 19 (22.3)            | 26 (25.2)                  |
| Smoking                  | 02 (02.0)            | 06 (04.7)                  |

a By ANOVA,  $P > 0.05$ , not significant.

b By Chi-square test, not significant.



**Fig. 1 – Graphical representation of the serum oxLDL/β2GPI concentrations for 188 patients.**

number of male and female patients between the normal and dyslipidemic lipid profile groups.

Significantly, there was no statistical correlation between familial history of CVD and lipid profiles.

### 3.2. Comparison of oxLDL/β2GPI with lipid profiles

We evaluated 85 patients with normal lipid profiles and 103 patients with dyslipidemic lipid levels. The normal ( $n = 85$ ) lipid patient serum concentration range of oxLDL/β2GPI was 2.29 U/ml–6.48 U/ml with a mean of 4.32 U/ml while the dyslipidemic lipid ( $n = 103$ ) patient serum concentration range of oxLDL/β2GPI was 3.98 U/ml–7.99 U/ml with a mean of 6.04 U/ml.

Statistical analysis by ANOVA method showed that the mean serum concentrations of oxLDL/β2GPI in the two groups was significantly different ( $P < 0.01$ ) demonstrating that oxLDL/β2GPI is directly correlated to standard lipid markers for predicting CVD in an Indian subpopulation.

The serum oxLDL/β2GPI complex concentration for the entire 188 patient pool is graphically shown in Fig. 1.

### 3.3. Comparison of CVD familial history with lipid profiles and oxLDL/β2GPI

We analyzed familial history of CVD (as defined using the wellness clinical questionnaire) against the measured lipid profiles.

Out of the 188 patients tested, 45 described an immediate family member with clinically observed/treated CVD.

In the normal lipid profile group, 19 of these patients described an immediate family member with clinically observed/treated CVD whereas in the dyslipidemic lipid

profile 26 of these patients described an immediate family member with clinically observed/treated CVD. These two numbers were not statistically different (ANOVA,  $P < 0.05$ ) and hence lipid profile concentration was not correlated with familial history of CVD in this study.

We then analyzed the familial history data against serum concentrations of oxLDL/β2GPI. As shown in the Table 2, the comparison of the mean oxLDL/β2GPI levels between positive vs negative familial CVD history within each lipid group (normal vs dyslipidemic) separately, also showed significant ( $P < 0.05$ ) differences between the oxLDL/β2GPI concentration for positive and negative familial CVD. In the normal lipid profile group, the mean concentration of oxLDL/β2GPI that had a positive CVD familial history was 5.33 U/ml which was significantly higher than the negative CVD familial history group at 4.03 U/ml ( $P < 0.05$ ). A similar result was seen in the dyslipidemic lipid profile group.

## 4. Discussion

Our study shows that patients with a dyslipidemic lipid profile also exhibit elevated levels of oxLDL/β2GPI concentration, demonstrating that oxLDL/β2GPI is directly correlated with standard lipid markers for predicting CVD in the Indian subpopulation. This supports previous publications that show the oxLDL/β2GPI serum marker may have a central pathogenic role in atherosclerosis mechanisms.<sup>17</sup>

We have also shown, for the first time, that genetics may play an important role in increasing the downstream incorporation of the oxLDL/β2GPI complex into arterial walls, where they can be taken up by macrophages to form foam cells and/or plaque. Patients with a positive family history of CVD had increased serum concentrations of oxLDL/β2GPI in both the normal lipid and dyslipidemic groups showing that this biomarker has a potential additive role in predicting CVD, rather than using a standard lipid profile alone.

Early serum detection of elevated levels of oxLDL/β2GPI now linked to increased familial CVD potential for plaque formation, rather than a vague connection to family history, could induce patients into earlier adoption of simple dietary to lower the risk of CVD, without the cost of expensive drugs that are difficult to afford and distribute within the rural Indian population.

The ELISA assay used to measure serum levels of oxLDL/β2GPI needs to be developed into a fluorometric assay to facilitate more wide spread use in rural diagnostic laboratories and this work is on-going in our laboratories. Further

**Table 2 – Comparison of mean oxLDL/β2GPI levels between familial CVD positive and negative groups and lipid profile analysis.**

|              | Normal lipid profile                     |                                          | Dyslipidemic lipid profile               |                                          |
|--------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|
|              | Positive familial history for CVD (U/ml) | Negative familial history for CVD (U/ml) | Positive familial history for CVD (U/ml) | Negative familial history for CVD (U/ml) |
| oxLDL/β2GPI* | 5.33                                     | 4.03                                     | 6.98                                     | 5.72                                     |

By ANOVA, \* $P < 0.05$ , Significant.

studies to increase the clinical accuracy of the predictive value of circulating oxLDL/ $\beta$ 2GPI concentrations in the development of cardiovascular disease are also planned.

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### Conflicts of interest

All authors have none to declare.

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