Measurement of Oxidized LDL and Remnant Lipoprotein Cholesterol Associated with High-Sensitive C-Reactive Protein in Coronary Heart Disease

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ABSTRACT

Introduction: LDL-C undergoes modification to form Oxidized LDL a crucial occurrence in the oxidation hypothesis of atherogenesis. Oxidized LDL and RLP-C stimulates the immune and inflammatory reactions and promotes atherosclerosis. Because of its lesser size along with high cholesterol content, and increased residence period in blood the remnant lipoproteins are highly atherogenic. Remnant lipoproteins enter into the arterial wall easily and taken up directly by macrophages lead to the formation of foam cells, thus initiating the lipid-laden plaque. Oxidized LDL along with remnant lipoprotein cholesterol and hs-CRP play a crucial role in the progression of coronary heart disease.

Objective: The objective of the study is to measure the level of circulating Oxidized LDL and Remnant Lipoprotein Cholesterol associated with High-Sensitive C-Reactive Protein in Coronary Heart Disease

Materials and Methods: This cross-sectional study was conducted on 91 CHD patient and 91 healthy control in the age group of 25 to 55 years and were age and sex-matched. After overnight fasting body fluid samples were collected for analysis for Lipid Profile, oxidized LDL and hs-CRP. ox-LDL and hs-CRP measured by ELISA method and Lipid Profile are measured using Auto Analyzer AU480.

Results: The mean level of oxidized Low-Density Lipoprotein level in CHD was elevated (40.89±8.69) and statistically significant (p-value <0.001) compared to the normal healthy controls (16.6±3.54). The mean RLP-C showed a significant increase (35.65±16.11) in the CHD group when compared to controls (15.69±12.15) (p <0.001). hs-CRP level in CHD was (3.80±1.35) and also shows a significant (p-value <0.001) increase compared to the normal healthy controls (1.92±0.47).

Conclusion: Addition to conventional parameters, the estimation of ox-LDL, RLP-C and Hs-CRP can prove to be a valuable tool in risk assessment of population and management of the disease. Our results suggesting the link between the level of high ox-LDL along with remnant lipoprotein cholesterol and hs-CRP may consistent with atherogenesis in subject with CHD.

Key Words: Oxidized Low-Density Lipoprotein, Remnant lipoprotein cholesterol, Coronary Heart Disease, Glycosylated Hemoglobin, high-sensitivity C-reactive protein

INTRODUCTION

In developed and developing countries the prevalence of CHD is the most important cause of death in India by 2020. In the adult population, more than 7 million deaths are caused by CHD (21.9 % of total deaths, expected to increase to 26.3 % by 2030 worldwide.1 Remnant lipoprotein cholesterol which is mostly found in fasting and non-fasting triglyceride-rich lipoproteins (TRLs). In fasting state as VLDL-C and IDL-C along with the non-fasting state as VLRLD, IDL and chylomicron remnants.2 Like LDL-C, Remnant cholesterol promoting inflammatory reaction and atherogenic process along with accumulating and infiltrating the endothelial barrier in the arterial wall. Low-density lipoprotein cholesterol along with remnant cholesterol are causally accompanying coronary heart disease.

Remnant particles are larger than LDL and carry ≤40 times as much cholesterol per particle and more atherogenic than...
Increased oxidative stress and formation of superoxide anion in vascular cells stimulate the alteration of LDL-C to atherogenic oxidized LDL. In the vessel wall, uptake of scavenger receptor on monocyte-derived macrophages caused by the elevated level of RLP-C and LDL oxidation, leads to foam cells accumulation along with fatty streak formation. In the Pathogenesis of coronary heart disease CRP plays a significant role. C-reactive protein is a pentraxin family of protein, an acute phase reactant with a molecular weight of 23kDa. It is a highly sensitive marker for inflammation. The level of CRP rises drastically during the inflammatory process. The elevated concentration of hs-CRP directly implies subclinical inflammation in individual.

Remnant lipoprotein cholesterol along with oxidation of LDL associated with local and systemic inflammatory reaction linked to the risk of CHD along with low-grade inflammation.

We assessed each correlation between remnant lipoprotein cholesterol and ox-LDL associated with hs-CRP in the CHD subject.

**MATERIALS AND METHODS**

This cross-sectional study was conducted from Jun 2019 to Dec 2019 at SRM Medical College Hospital and Research Centre, Chennai, Tamil Nadu, India on subjects attending the Cardiology and medicine outpatient. Totally 182 subjects were included who were age and sex match in the age group 25-55 years. 91 CHD subjects and 91 normal healthy subjects were selected as control. The control subjects were also taken from Master health check-up Programme and medicine OP in SRM Medical College Hospital and Research Centre, Chennai, Tamil Nadu, India. This study follows the Declaration of Helsinki and was approved by the institutional ethical committee at SRM Medical College Hospital and Research Centre (ECN: 1513/ICE/2018). Written informed consent was collected from all participants at the time of enrollment. Based on coronary angiography, the CHD patients were selected. Serum glucose value is above the normal and below the diabetic level diagnosed both males and females coronary heart disease patient and patients with chest pain, ECG and ECHO, changes, increased cardiac markers such as creatinine phosphokinase (CPK-MB) and troponin level. The control group consists of Normal healthy individuals. The subjects who were on treatment for renal failure, cancer, autoimmune diseases, surgery, fever, alcoholics, smokers, pregnancy and patients with corticosteroids, estrogen, antiretroviral drugs psychotropic medications. Thyroid, arthritis, rheumatoid arthritis, acute/chronic infection patients were excluded.

We did the study on both NGT & IGR subjects.

**Statistical Methods**

By using a statistical package for social service (SPSS 16.0) the Data were analyzed. The data from the study were shown as mean and standard deviation. p-value was <0.05 were considered as statically significant if Statistical significance for study group and control was analyzed by Student’s ‘t’s test. To find out the correlation between different parameter Pearson’s correlation coefficient was calculated. By simple linear regression analysis, the correlation between the two parameters was determined.

**OBSERVATION AND RESULTS**

**Demographic characteristics of the study subjects**

Totally 182 subjects were included who were age and sex match in the age group 30-55 years 91 CHD subject (45 males...
After modification of Apo B, oxidized LDL and triglyceride-rich lipoprotein cholesterol are positively correlated with various biochemical parameters in Coronary Heart Disease subject.

Pearson's correlation analysis between ox-LDL with various biochemical parameters in Coronary Heart Disease subject
ox-LDL positively correlated with BMI (r = 0.111), Waist Circumference (r = 0.035), Waist Hip Ratio (r = 0.296). Total Cholesterol (r = 0.029), Triglyceride (r = 0.145), LDL-C (r = 0.096), VLDL-C (r = 0.144), TC/HDL ratio (r = 0.634), LDL/HDL ratio (r = 0.743)

And ox-LDL negatively correlated with FBG (r = -0.106), HDL-C (r = -0.148), HbA1c (r = -0.030) [Table 4].

Pearson's correlation analysis between remnant lipoprotein cholesterol with Risk Marker in Coronary Heart Disease subject
Remnant lipoprotein cholesterol are positively correlated with ox-LDL-C (r = 0.424) and hs-CRP (r = 0.330) [Table 7].

**DISCUSSION**

In our study WC and BMI were directly linked with RLP-C, ox-LDL along with CRP showed that blood pressure and BMI, Waist Circumference, waist-to-hip ratio were significantly elevated in CHD subjects when compared with healthy controls. The study done by INTERHEART stated that compared to BMI the waist-hip ratio is a more significant predictor for myocardial infarction. Similarly, our study showed that the high BMI values subjects have a higher RLP cholesterol concentration. Holvoet et al stated that for evaluating the concentrations of circulating ox-LDL, BMI act as the strongest predictor.

This study shows the significant difference in TC, LDL-C, HDL-C and triglyceride, however, the plasma level of LDL-C cholesterol, RLP-C, ox-LDL and hs-CRP were significantly elevated in CHD patients compared to controls as an essential risk factor in the progression of atherosclerosis.

In our study, we found a negative correlation between oxidized LDL and HDL-C. The levels of HDL cholesterol is conversely related to the risk of CHD because it prohibits atherosclerosis by degrading the stimulatory action of oxidized LDL on monocyte aggression.

**Pearson’s correlation analysis between Remnant lipoprotein cholesterol with various biochemical parameters in Coronary Heart Disease subject**
Remnant lipoprotein cholesterol is positively correlated with FBG (r = 0.2008), BMI (r = 0.183), Waist Circumference (r = 0.050), Waist Hip Ratio (r = 0.195). Total Cholesterol (r = 0.721), Triglyceride (r = 0.277), LDL-C (r = 0.416), VLDL-C (r = 0.278), TC/HDL ratio (r = 0.483), LDL/HDL ratio (r = 0.477)

And Remnant lipoprotein cholesterol are negatively correlated with HDL-C (r = -0.395), HbA1c (r = -0.007) [Table 6].

**Remnant Lipoprotein Cholesterol related with Oxidized Low-Density Lipoprotein along with Lipid Profile**
Circulating oxidized LDL-C and RLP-C were positively correlated with Total Cholesterol, Triglyceride, LDL-C, VLDL, TC/HDL Ratio and LDL/HDL Ratio and negatively correlated with levels of HDL-C cholesterol. More studies stated that Ox-LDL is the major cause for hyperlipidaemia which triggers CHD. Nishi et al stated that LDL undergoes oxidation in plaque and that high concentrations of ox-LDL and triglyceride. After modification of Apo B, oxidized LDL and triglyceride-rich lipoprotein undergoes lipolysis by lipoprotein lipase and release of cholesterol-rich remnant lipoprotein. Sampson et al., found that there is still a considerable residual risk of reoccurring cardiovascular events even after reduction in LDL cholesterol to suggested concentration Although stains lowers...
LDL-C and it has a small effect on triglycerides. Therefore the concentration of triglycerides increased thus increase remnant cholesterol. Compared to LDL-Cholesterol the remnant lipoprotein cholesterol are two times more associated with coronary heart disease. RLP-C crosses the barrier of endothelium and uptake by macrophages leads to the formation of foam cell. As a result, remnant lipoproteins might cause dysfunction in the endothelial vasomotor and increase the threat of coronary heart disease in patients with hypertriglyceridemia. Hypertriglyceridemia are significantly related to endothelial dysfunction. Kugiyama et al., stated that RLP-C levels were linked with abnormal endothelium-dependent vasomotor stated that for inhibitory effects of remnant lipoproteins the reduction in the bioactivity of coronary nitric oxide might be responsible for the progression of coronary heart disease.

Remnant Lipoprotein Cholesterol and Oxidized Low-Density Lipoprotein-associated with hs-CRP

The innovative finding of our present study shows remnant lipoprotein cholesterol and ox-LDL was positively correlated with inflammatory biomarker high sensitivity C-reactive protein. In our study elevated hs-CRP is significant compared to the control group. Ridker et al. reported that healthy individuals with elevated hs-CRP values are 4 times possible to have coronary heart disease. Ndrepepa et al., stated that raised hs-CRP level is linked with the threat of future adverse cardiovascular events in healthy persons and subjects with stable coronary heart disease. The fasting serum revealed that increased levels of remnant lipoprotein cholesterol can predict the progression of clinical coronary actions in patients with CHD independently along with other risk factors such as ox-LDL and hs-CRP. In our study, we observed a positive correlation between RLP-C and hs-CRP. Recent genetic studies done with very large samples by Varbo et al. found that elevated remnant cholesterol and ox-LDL is related to low-grade inflammation, but the elevated level of LDL-C causes ischemic heart disease without inflammation. Zhang et al found thatOX-LDL and hs-CRP were positively correlated and it can directly lead to the occurrence of inflammatory reaction. Study by Hong et al., stated that remnant lipoprotein cholesterol was positively associated with the most important inflammatory biomarker hs-CRP. Toward our information, there are no previous studies on defining whether the baseline levels of remnant lipoprotein cholesterol along with ox-LDL is qualified as a useful independent predictor as traditional prognostic variables for adverse outcomes.

CONCLUSION

CHD has been a subject of research for decades. Finding of its relation with remnant lipoprotein cholesterol, ox-LDL, Hs-CRP, dyslipidemia, hypertension, and DM have now become emerging areas of concern.

The current study concludes that assessment of RLP-C, ox-LDL and hs-CRP may contribute to the early finding of coronary heart disease may reduce the morbidity and mortality risk.

LIMITATIONS

A small sample size and more in-depth work on the role of ox-LDL, Remnant Lipoprotein Cholesterol and hs-CRP are needed to evaluate for the prevention of future risk of CHD.

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Ethical Approval: All procedures performed by human participants were involved in this study are performed following the ethical standards. The study protocol was approved by the institutional ethical committee (ECN: 1513/ICE/2018). Informed Consent: Informed consent was taken from all the participants involved in the study.

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Table 1: Demographics and baseline characteristics Coronary Heart Disease Subject and Healthy Controls

| Parameters                          | Controls (n=91) | CHD patient (n=91) | P-Value  |
|-------------------------------------|----------------|-------------------|----------|
| Mean age (years, mean ± S.E.M.)     | 41.8 ± 9.7     | 43.93 ± 9.39      | <0.0001*** |
| Male Sex (%)                        | 40 (37%)       | 45(41%)           | -        |
| Female Sex (%)                      | 51(63%)        | 46(59%)           | -        |
| Body mass index (kg/m²)             | 21.9±0.37      | 23.47±0.35        | <0.0001*** |
| Waist circumference (cm)            | 90.9 ± 10.1    | 93.8 ± 9.6        | <0.0001*** |
| Waist to hip ratio                  | 0.94±0.02      | 1.01±0.01         | <0.0001*** |
| Waist to height ratio               | 0.56±0.01      | 0.62±0.02         | <0.0001*** |
| Systolic blood pressure, mm Hg      | 112.73 ±8.32   | 119.38±16.57      | <0.0001*** |
| Diastolic blood pressure, mm Hg     | 77.69 ±7.95    | 74.58±13.26       | <0.0001*** |
| Family history of CHD               | Yes            | 0                 | 25(14%)  |
|                                    | No             | 91(100%)          | 66(86%)  |

Table 2: Comparison of Lipid profile in Coronary Heart Disease Subject and Healthy Controls

| Parameters             | Controls (n=91) (Mean ± SD) | CHD Patient (n=91) (Mean ± SD) | P-Value  |
|------------------------|-----------------------------|--------------------------------|----------|
| FBG(mg/dl)             | 95.7±7.68                   | 97.29±6.98                     | <0.0001*** |
| Total Cholesterol (mg/dl) | 170.76±16.13              | 227.01±34.27                  | <0.0001*** |
| TG (mg/dl)             | 82.74±28.41                 | 140.19±60.71                  | <0.0001*** |
| HDL (mg/dl)            | 46.96±9.4                   | 37.64±4.12                    | <0.0001*** |
| LDL (mg/dl)            | 106.54±12.45                | 161.3±24.48                   | <0.0001*** |
Table 2: (Continued)

| Parameters          | Controls (n=91) (Mean ± SD) | CHD Patient (n=91) (Mean ± SD) | (P- Value) |
|---------------------|-----------------------------|-------------------------------|------------|
| VLDL (mg/dl)        | 17.26±8.77                  | 28.06±12.14                  | <0.0001*** |
| TC/HDL Ratio        | 3.71±0.70                   | 6.17±1.14                    | <0.0001*** |
| LDL/HDL Ratio       | 2.35±0.53                   | 4.22±0.75                    | <0.0001*** |
| HbA1c (%)           | 4.9±0.17                    | 5.21±0.28                    | <0.0001*** |

FBG- Fasting Blood Glucose; TC- Total Cholesterol; TG- Triglyceride; HDL- High-Density Lipoprotein; LDL-C- Low-Density Lipoprotein; ox-LDL- Oxidized LDL hs-CRP- High sensitive C-reactive protein
Values are expressed in Mean ± Standard Deviation;
*P-value < 0.05 is considered to be significant; NS-Not significant; ***Very Highly significant; **Highly Significant.

Table 3: Comparison of Biochemical Risk Markers in Coronary Heart Disease Subject and Healthy Controls

| Parameters       | Controls (n=91) (Mean ± SD) | CHD Patient (n=91) (Mean ± SD) | (P- Value) |
|------------------|-----------------------------|-------------------------------|------------|
| RLP-C (mg/dl)    | 15.69±12.15                 | 35.65±16.11                  | <0.0001*** |
| Ox-LDL           | 16.6±3.54                   | 40.89±8.69                   | <0.0001*** |
| hs-CRP (mg/dl)   | 1.92±0.47                   | 3.80±1.35                    | <0.0001*** |

RLP-C- Remnant Lipoprotein Cholesterol; ox-LDL- Oxidized LDL; hs-CRP- High sensitive C-reactive protein
Values are expressed in Mean ± Standard Deviation;
*P-value < 0.05 is considered to be significant; NS-Not significant; ***Very Highly significant; **Highly Significant.

Table 4: The Pearson correlation analysis between Ox-LDL [40.89±8.69] with other biochemical parameters in subjects with CHD

| PARAMETERS       | Ox-LDL | P- Value |
|------------------|--------|----------|
| BMI              | 0.111  | <0.0001*** |
| WAIST CIRCUMFERENCE | 0.035  | <0.0001*** |
| WAIST HIP RATIO  | 0.296  | <0.0001*** |
| FBG              | -0.106 | <0.0001*** |
| TOTAL CHOLESTEROL| 0.920  | <0.0001*** |
| TRIGLYCERIDE     | 0.145  | <0.0001*** |
| HDL-C            | -0.148 | <0.0001*** |
| LDL-C            | 0.996  | <0.0001*** |
| VLDL-C           | 0.144  | <0.0001*** |
| CARDIAC RISK RATIO- I | 0.634  | <0.0001*** |
| CARDIAC RISK RATIO- II | 0.743  | <0.0001*** |
| HbA1c            | -0.030 | <0.0001*** |

a- Positive Correlation
b- Negative Correlation

Table 5: Pearson’s correlation analysis between Ox-LDL with Risk Marker in Coronary Heart Disease subject

| Parameters | Ox-LDL | P- Value |
|------------|--------|----------|
| RLP-C      | 0.424  | <0.001** |
| Hs-CRP     | 0.853  | <0.0001*** |

a-Positive Correlation
b-Negative Correlation
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Table 6: The Pearson correlation analysis between Remnant lipoprotein cholesterol [35.65±16.11] with other biochemical parameters in subjects with CHD

| PARAMETERS                  | RLP-C | P- Value         |
|-----------------------------|-------|------------------|
| BMI                         | 0.183 | <0.0001***       |
| WAIST CIRCUMFERENCE         | 0.050 | <0.0001***       |
| WAIST HIP RATIO             | 0.195 | <0.0001***       |
| FBG                         | 0.2008| <0.0001***       |
| TOTAL CHOLESTEROL           | 0.7213| <0.0001***       |
| TRIGLYCERIDE                | 0.2778| <0.0001***       |
| HDL-C                       | -0.3953| NS               |
| LDL-C                       | 0.4169| <0.0001***       |
| VLDL-C                      | 0.278 | <0.0001***       |
| CARDIAC RISK RATIO- I       | 0.483 | <0.0001***       |
| CARDIAC RISK RATIO- II      | 0.477 | <0.0001***       |
| HbA1c                       | -0.007| <0.0001***       |

a- Positive Correlation
b- Negative Correlation

Table 7: Pearson’s correlation analysis between RLP-C with Risk Marker in Coronary Heart Disease subject

| Markers       | RLP-C | P- Value         |
|---------------|-------|------------------|
| Ox-LDL        | 0.424 | <0.001**         |
| Hs-CRP        | 0.330 | <0.0001***       |

a- Positive Correlation
b- Negative Correlation