The present study was conducted in the Department of Cardiology, PGIMER, Chandigarh, on 160 subjects during the period of November 2011 to August 2013.

**Study Design**

This was an Observational Case-Control study

**Study period and duration**

The present study of 22 months was conducted during the period of November 2011 to August 2013.

**Methods of Collection of data**

**Source of data**

Patients attending the OPD or admitted in the wards of cardiology department, PGIMER, Chandigarh, with history and clinical symptoms suggestive of coronary heart disease including unstable and stable angina were included.

**Sample Size**

160 subjects (110 patients and 50 controls) were selected for the study

**Patient group**

Patients were divided into two groups:

1. Young CHD individuals (<40 years)
2. Higher age group (individuals > 40 years of age)

**Control**

50 healthy individuals free of any symptoms of coronary heart disease were taken as controls.

**Selection Criteria**

**Inclusion Criteria**

Coronary heart disease patients (aged 20-60 years) of either sex with a history of acute chest pain, non ST-segment elevation, unstable and stable angina were included for this
study. Patients suffering from hypertension were included in the study. Healthy individuals free of any symptoms of coronary heart disease were taken as controls.

**Exclusion Criteria**

Patients with diabetes mellitus, renal disease, respiratory disease, thyroid disorders, acute infection or any other systemic illness and on lipid lowering drugs for the past 3 months were excluded. Tobacco and alcohol abusers were also excluded. The same exclusion criteria were also applied for the selection of controls.

**Procedure**

Patients attending OPD or admitted in the wards of department of Cardiology, PGIMER, Chandigarh, were evaluated based on the selection criteria. The selected patients were briefed about the nature of study and a written consent was obtained (Annexure-).

Demographic data like gender and age were collected along with relevant history and recorded on predesigned proforma (Annexure-). A thorough clinical examination was conducted and the findings were also recorded. Physical examination was conducted to assess body mass index. Body mass was calculated based on formula;

\[
\text{Body Mass Index} = \frac{\text{Weight (Kg)}}{\text{Height}^2 (m)}
\]

Informed consent was obtained from all subjects before drawing blood. Routine investigations such as haemogram (haemoglobin, total count, differential count, erythrocyte sedimentation rate), renal, liver function test, urine routine and microscopy were done. Each patient was subjected to detailed clinical history and detailed clinical examination (by a cardiologist) and was subjected to ECG, Colour Doppler Echocardiography and Angiography.

Special tests such as cardiac enzymes, fasting lipid profile, apolipoprotein A (by Immunonephelometry method), apolipoprotein B (by Immunonephelometry method) and Lp(a) (by latex immunoturbidimetric method) were conducted and recorded. Non-HDL-C was calculated by subtracting HDL-C from total cholesterol. The various lipid and lipoprotein ratios (Ratio T-C/HDL, LDL/HDL, TG/HDL, apoB/apoA-I and non-HDL/HDL) were then calculated.
**METHODS:**

1. **Estimation of Serum Cholesterol**

The VITROS CHOL Slide method is performed using the VITROS CHOL Slides and the VITROS Chemistry Products Calibrator Kit 2 on VITROS 250/350/950/5, 1 FS and 4600 Chemistry Systems and the VITROS 5600 Integrated System. The VITROS CHOL Slide is a multilayered, analytical element coated on a polyester support. The method is based on an enzymatic method similar to that proposed by Allain et al.167

**Test Type and Conditions**

<table>
<thead>
<tr>
<th>Test Type</th>
<th>VITROS System</th>
<th>Approximate Incubation Time</th>
<th>Temperature</th>
<th>Wavelength</th>
<th>Reaction Sample Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorimetric</td>
<td>5600, 4600, 5,1 FS, 950, 250/350</td>
<td>5 minutes</td>
<td>37 °C (98.6 °F)</td>
<td>540 nm</td>
<td>5.5 μL</td>
</tr>
</tbody>
</table>

**Reaction Scheme**

\[
\text{Lipoprotein} \xrightarrow{\text{TX100}} \text{cholesterol + cholesterol esters + proteins}
\]

\[
\text{cholesterol esters + H_2O} \xrightarrow{\text{cholesterol ester hydrolase}} \text{Cholesterol + fatty acids}
\]

\[
\text{cholesterol + O}_2 \xrightarrow{\text{cholesterol oxidase}} \text{Cholest-4-en-3-one + H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 + \text{leuco dye} \xrightarrow{\text{peroxidase}} \text{dye + 2H}_2\text{O}
\]

**Calculations**

Cholesterol in sample (mg/dl) = \( \frac{\text{Ab sample} - \text{Ab Blank}}{\text{Ab standard-Ab Blank}} \times \text{Conc. Of standard (mg/dl)} \)
2. dHDL

VITROS Chemistry Products dHDL Slides are used to quantitatively measure HDL cholesterol (HDLC) concentration in serum and plasma using VITROS 250/350/950/5, 1 FS and 4600 Chemistry Systems and the VITROS 5600 Integrated System.

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<tbody>
<tr>
<td>Colorimetric</td>
<td>5600, 4600, 5,1 FS, 950, 250/350</td>
<td>5 minutes</td>
<td>37 °C (98.6 °F)</td>
<td>670 nm</td>
<td>6 μL</td>
</tr>
</tbody>
</table>

**Reaction scheme**

HDL + non-HDL → PTA/MgCl₂ → high density lipoproteins + non-HDL

HDL + Proteins → Emulgen B-46 → cholesterol + HDL-C esters + proteins

HDL-C esters + H₂O → cholesterol ester hydrolase → cholesterol and fatty acids

Cholesterol + O₂ → cholesterol oxidase → cholest-4-en-3 + H₂O₂

H₂O₂ + leuco dye → peroxidase → dye + H₂O₂

**Calculations**

Cholesterol in sample (mg/dl) = \( \frac{\text{Ab Sample-Ab Blank}}{\text{Ab Standard-Ab Blank}} \times \text{Conc. Of standard (mg/dl)} \)

3. Serum triglyceride

VITROS Chemistry Products TRIG Slides quantitatively measure triglyceride (TRIG) concentration in serum and plasma using VITROS 250/350/950/5, 1 FS and 4600 Chemistry Systems and the VITROS 5600 Integrated System.
## Test Type and Conditions

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<td>37 °C (98.6 °F)</td>
<td>540 nm</td>
<td>5.5 μL</td>
</tr>
</tbody>
</table>

## Reaction Scheme

\[
\begin{align*}
\text{lipoproteins} & \xrightarrow{\text{surfactant}} \text{triglycerides + proteins} \\
\text{triglycerides + H}_2\text{O} & \xrightarrow{\text{lipase}} \text{glycerol + fatty acids} \\
\text{glycerol + ATP} & \xrightarrow{\text{glycerol kinase/MgCl}_2} \text{L-α-glycerophosphate + ADP} \\
\text{L-α-glycerophosphate + O}_2 & \xrightarrow{\text{L-α-glycerol-phosphate oxidase}} \text{dihydroxyacetone phosphate + H}_2\text{O}_2 \\
\text{H}_2\text{O}_2 + \text{leuco dye} & \xrightarrow{\text{peroxidase}} \text{dye + 2H}_2\text{O}
\end{align*}
\]

### 4. LDL direct

**Principle of procedure**

The assay is based on a modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol methyl ether (PEGME) coupled classic precipitation method with the improvements in using optimized quantities of PVS/PEGME and selected detergents. LDL, VLDL, and chylomicron (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol oxidase (CHOD) and cholesterol esterase (CHER), whereas HDL reacts with the enzymes. Addition of R₂ containing a specific detergent releases LDL from the PVS/PEGME complex. The released LDL reacts with the enzymes to produce H₂O₂ which is quantified by the Trinder reaction.
**Reaction Scheme**

\[
\begin{align*}
\text{HDL + LDL + VLDL + CM} & \xrightarrow{\text{PVS, PEGME}} \text{HDL + (LDL + VLDL + CM) \cdot PVS / PEGME} \\
\text{HDL} & \xrightarrow{\text{CHOD, CHER}} \text{Fatty Acid + H}_2\text{O}_2 \\
(LDL + VLDL + CM) \cdot PVS / PEGME & \xrightarrow{\text{Detergent}} \text{LDL+ (VLDL+CM) \cdot PVS / PEGME} \\
\text{LDL} & \xrightarrow{\text{CHOD, CHER}} \text{Fatty Acid + H}_2\text{O}_2 \\
2\text{H}_2\text{O}_2 + 4\text{AA+TODB} & \xrightarrow{\text{Peroxidase}} \text{Quinone + 5 H}_2\text{O}
\end{align*}
\]

**Calculation**

\[
LDL = \frac{(\text{Abs. of Sample} - \text{Abs. of Sample Blank})}{(\text{Abs. of Cal.} - \text{Abs. of Cal. Blank})} \times \text{Concentration of Calibrator}
\]

**5. VLDL Cholesterol**

VLDL Cholesterol was estimated by the following formula:

VLDL Cholesterol in sample (mg/dl) = Total Cholesterol-(HDL+LDL)

**6. Apolipoprotein B**

**Test Principle**

Immunonephelometry is applied. This method involves measuring the light scattered by insoluble complexes formed by reaction between specific protein in samples and its respective antiserum, and the amount of scattered light is directly proportional to the concentration of the protein under condition that antiserum is in excess. Concentrations are automatically calculated by reference to a calibration curve stored in the instrument.

**7. Apolipoprotein A-I**

**Test Principle**

Immunonephelometry is applied. This method involves measuring the light scattered by insoluble complexes formed by reaction between specific protein in samples and its respective antiserum, and the amount of scattered light is directly proportional to the
concentration of the protein under condition that antiserum is in excess. Concentrations are automatically calculated by reference to a calibration curve stored in the instrument.

8. Lipoprotein (a)

**Test Principle:**

Lipoprotein (a) assay is based on latex enhanced immunoturbidimetric method. Lp(a) in the sample binds to the specific anti-Lp(a) antibody, which is coated on latex particle, and causes agglutination. The degree of turbidity caused by agglutination can be measured optically and is proportional to the amount of Lp(a) in the sample.

It is done on NEPHSTAR PROTEIN ANALYSIS SYSTEM for quantitative determination of human Apolipoprotein A-1 in serum by kit method (Goldsite Diagnostics Inc.). It is based on the principle of Immunonephelometry. This method involves measuring the light scattered by insoluble complexes formed by reaction between specific protein in samples and its respective antiserum, and the amount of scattered light is directly proportional to the concentration of the protein under condition that antiserum is in excess. Concentrations are automatically calculated by reference to a calibration curve stored in the instrument.

**Statistical analysis**

The comparison of the risk factors in study and control group was done by independent ‘t’ test. All values were calculated as mean ± standard deviation. For statistical significance the p value was calculated. Comparison of the various parameters was analysed in terms of receiver operating characteristic (ROC) curve. A ROC curve is a plot between sensitivity (Y-axis) versus specificity (X-axis), obtained for different cut off points. Areas under the curve (AUC) and their 95% confidence intervals (CI) were evaluated as a measure of diagnostic accuracy. Greater AUC of the ROC curve indicated better markers of the study. The area under the ROC curve was considered a global performance indicator for a prognostic factor. All p-values <0.05 were considered as significant. All analyses were performed using the SPSS computer program version 16.0.