RESULTS & DISCUSSION
4.0 RESULTS AND DISCUSSION

4.1 BASIC CHARACTERISTICS

Three hundred patients with known CAD (M:216; F:84, age:25-92) were studied. Mean age of patients was 60.9±12.4 years. There was no age difference between males and females (M: 60.95±12.3; F:61.03±12.9; p =0.10). DM were present in 41.7 % subjects, HTN in 62.7 %, and dyslipidemia in 41.3 % subjects. Subjects with normal weight were 21.7%, 49.7% subjects were overweight, and 28.7% were obese. Metabolic syndrome were detected in 64% subjects and 38% subjects were physical inactive. There were about 5% subjects with CAD who had no traditional risk factors. Basic biochemical parameters of study population are depicted in table-1.

Table-1

Basic Biochemical parameters in study population

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean±SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-fasting (mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>206.2±74.9</td>
<td>126-390</td>
</tr>
<tr>
<td>Non-diabetic</td>
<td>99.2±10.0</td>
<td>72-110</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>28.9±8.2</td>
<td>11-45</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.03±0.17</td>
<td>0.52-1.29</td>
</tr>
<tr>
<td>Total Protein (g/dl)</td>
<td>7.06±0.33</td>
<td>6.42-8.3</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.77±0.33</td>
<td>3.2-4.6</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.80±0.23</td>
<td>0.3-1.2</td>
</tr>
<tr>
<td>Direct bilirubin (mg/dl)</td>
<td>0.40±0.14</td>
<td>0.15-0.85</td>
</tr>
<tr>
<td>SGPT (IU/L)</td>
<td>25.37±8.3</td>
<td>10-40</td>
</tr>
<tr>
<td>SGOT (IU/L)</td>
<td>28.08±4.8</td>
<td>20-39</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>194.04±67.6</td>
<td>65-389</td>
</tr>
<tr>
<td>Uric Acid (mg/dl)</td>
<td>4.76±0.72</td>
<td>3.2-6.0</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.54±0.27</td>
<td>9-10.34</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>3.45±0.34</td>
<td>2.8-4.0</td>
</tr>
</tbody>
</table>

Basic characteristics of subjects are depicted in Table-2. Dyslipidemia was significantly high in males compared to females. Number of subjects with diabetic and hypertensive were higher in males. There was no gender difference in BMI, WHR and physical inactivity. There is no gender difference in fasting, post prandial plasma glucose and HbA1c level. All subjects had normal liver and renal functions. There was no gender
Table-2
Basic characteristic of subjects according to gender

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Male Median (range)</th>
<th>Female Median (range)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60.8±12.3 62(25-92)</td>
<td>61.03±12.9 62(25-89)</td>
<td>0.9205</td>
</tr>
<tr>
<td>Smoking</td>
<td>82(73.9%)</td>
<td>29(26.1%)</td>
<td>0.6738</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>27.65±3.69 27.90(19.3-37.7)</td>
<td>28.5±4.07 27.9(20.02-39.47)</td>
<td>0.0762</td>
</tr>
<tr>
<td>WHR</td>
<td>0.92±0.05 0.92(0.7-1.18)</td>
<td>0.91±0.06 0.92(0.7-1.102)</td>
<td>0.1185</td>
</tr>
<tr>
<td>DM</td>
<td>86(68.8%)</td>
<td>39(31.2%)</td>
<td>0.3613</td>
</tr>
<tr>
<td>HTN</td>
<td>72(75.8%)</td>
<td>23(24.2%)</td>
<td>0.3914</td>
</tr>
<tr>
<td>DM &amp; HTN</td>
<td>59(63.4%)</td>
<td>34(36.6%)</td>
<td>0.038</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>81(65.3%)</td>
<td>43(34.7%)</td>
<td>0.0422</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>177.7±44.4 167.5(91-331)</td>
<td>181.2±48.8 169(98-343)</td>
<td>0.5505</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>198.8±45.4 164.5(64-341)</td>
<td>174.5±48.3 171.5(71-338)</td>
<td>0.428</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>39.3±9.2 39(20-59)</td>
<td>38±9.2 37(20-58)</td>
<td>0.2528</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>33.4±9.6 32.7(2-68.2)</td>
<td>34.1±10.7 33.3(3.4-67.6)</td>
<td>0.6378</td>
</tr>
<tr>
<td>IL6 (pg/ml)</td>
<td>62.3±73.8 25.1(2.0-250.9)</td>
<td>70.1±78.9 25(2.0-253.2)</td>
<td>0.4216</td>
</tr>
<tr>
<td>TNFalpha (pg/ml)</td>
<td>23.8±41.8 9.75(8.0-525.8)</td>
<td>29±38.1 11.4 (8.0-224.9)</td>
<td>0.3242</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>11.8±10.0 11.7(0.1-37.9)</td>
<td>11.1±8.7 12.3(0.11-32.9)</td>
<td>0.5780</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>49.3±41.5 36.4(2.4-254.9)</td>
<td>52.2±48.2 42.4(2.14-274.8)</td>
<td>0.6018</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>16.6±17.01 12.3(0.58-93.7)</td>
<td>22.2±28.6 12.6(0.5-135.7)</td>
<td>0.5029</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.28±0.039 0.27(0.21-0.42)</td>
<td>0.27±0.047 0.26(0.21-0.43)</td>
<td>0.4649</td>
</tr>
<tr>
<td>Vitamin B12 (pg/ml)</td>
<td>226.9±403.5 102.6(60-3056)</td>
<td>135.2±87.5 95.7(60-493)</td>
<td>0.0738</td>
</tr>
<tr>
<td>Homocysteine(µmol/L)</td>
<td>36.5±13.58 32.85(9.1-79)</td>
<td>35.0±18.77 24.35(8.8-80.6)</td>
<td>0.0269</td>
</tr>
<tr>
<td>Folic acid (ng/ml)</td>
<td>8.0±7.1 4.3(3.5-31.7)</td>
<td>7.8±6.7 4.51(3.5-32.6)</td>
<td>0.7980</td>
</tr>
<tr>
<td>Magnesium (mg/dl)</td>
<td>1.72±0.58 1.51(1-4.18)</td>
<td>1.58±0.44 1.48(1-3.12)</td>
<td>0.1051</td>
</tr>
</tbody>
</table>
difference in cholesterol, TG, HDL, LDL, VLDL Cholesterol levels. Hypercholesterolemia was present in 230 subjects (76.7%), hypertriglyceridemia in 188 subjects (62.7%) and low HDL in 164 subjects (54.7%). There was no gender difference in inflammatory markers, cytokines, vitamin B12 and folic acid levels. Homocysteine levels were significantly high in males compared to females. Also there was no gender was found in Insulin, HOMA IR and QUICKI Index (Table-2).
VITAMIN B12 AND ITS ASSOCIATION WITH CARDIOVASCULAR RISK FACTORS
4.2 VITAMIN B12 AND ITS ASSOCIATION WITH CARDIOVASCULAR RISK FACTORS

4.2.1 Results

Vitamin B12 and folate deficiency was present in 86.7% and 2.7% subjects respectively. Subjects with hyperhomocysteinemia were 95.3%. Hcy levels were higher in males compared to females (M:36.5±13.5µmol/l, F:35.0±18.7µmol/l, p:0.02). (Table 2)

Table-3
Vitamin B12, homocysteine and Folic acid levels according to cardiovascular risk factors

<table>
<thead>
<tr>
<th></th>
<th>Vitamin B12 (pg/ml)</th>
<th>Homocysteine(µmol/L)</th>
<th>Folic cid (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SD</td>
<td>P value</td>
<td>Mean ±SD</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>200.6±332.4</td>
<td>0.9893</td>
<td>36.0±13.2</td>
</tr>
<tr>
<td>No</td>
<td>201.2±357.3</td>
<td></td>
<td>36.1±16.2</td>
</tr>
<tr>
<td>physical inactivity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>174.9±240.3</td>
<td>0.5423</td>
<td>36.2±15.7</td>
</tr>
<tr>
<td>No</td>
<td>217.4±400.7</td>
<td></td>
<td>36.1±14.9</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>171.8±192.9</td>
<td>0.4456</td>
<td>36.8±17.9</td>
</tr>
<tr>
<td>≥25</td>
<td>209.0±379.5</td>
<td></td>
<td>35.9±14.3</td>
</tr>
<tr>
<td>Central Obesity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>182.7±302.8</td>
<td>0.2174</td>
<td>36.2±15.6</td>
</tr>
<tr>
<td>No</td>
<td>240.5±428.1</td>
<td></td>
<td>35.8±14.1</td>
</tr>
<tr>
<td>DM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>178.2±301.2</td>
<td>0.0014</td>
<td>39.2±15.9</td>
</tr>
<tr>
<td>No</td>
<td>217.2±377.4</td>
<td></td>
<td>33.9±14.2</td>
</tr>
<tr>
<td>DM &amp; HTN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>161.7±274.5</td>
<td>0.0002</td>
<td>40.3±16.0</td>
</tr>
<tr>
<td>No</td>
<td>218.9±341.0</td>
<td></td>
<td>34.2±14.4</td>
</tr>
<tr>
<td>HTN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>189.6±352.0</td>
<td>0.4639</td>
<td>38.1±15.8</td>
</tr>
<tr>
<td>No</td>
<td>220.1±341.0</td>
<td></td>
<td>32.8±13.5</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>87.9±8.5</td>
<td>&lt;0.0001</td>
<td>50.0±12.4</td>
</tr>
<tr>
<td>No</td>
<td>280.6±437.1</td>
<td></td>
<td>26.3±7.2</td>
</tr>
<tr>
<td>Diet Pattern</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetarian</td>
<td>174.2±244.3</td>
<td>0.001</td>
<td>36.8±16.4</td>
</tr>
<tr>
<td>Semi Vegetarian</td>
<td>153.5±286.4</td>
<td></td>
<td>37.9±11.6</td>
</tr>
<tr>
<td>Non-vegetarian</td>
<td>363.0±601.4</td>
<td></td>
<td>31.4±15.0</td>
</tr>
</tbody>
</table>

Vitamin B12 levels were significantly lower in subjects with DM and/or HTN. However, Vitamin B12 levels were comparable in subjects with and without HTN. Subjects
with dyslipidemia also had significantly lower vitamin B12 levels. There was no difference in vitamin B12 and Hcy levels in subjects with or without obesity, central obesity, physical activity, history of smoking or dietary type. Subjects with DM and/or HTN and dyslipidemia had significantly higher Hcy levels. Patients with non-vegetarian diet had significantly higher vitamin B12 levels than semi-vegetarian and vegetarian diet (r=0.05). Folic acid levels were comparable in subjects with and without traditional risk factors. (Table 3)

### Table-4
**Vitamin B12 and Homocysteine with Insulin resistance and inflammatory markers**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vitamin B12 (pg/ml)</th>
<th>Homocysteine (µmol/l)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;100 (N=132) (group-1)</td>
<td>100-200 (N=128) (group-2)</td>
<td>&gt;200 (N=40) (group-3)</td>
</tr>
<tr>
<td>Insulin</td>
<td>65.8±46.03</td>
<td>38.9±39.6</td>
<td>34.4±27.7</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>27.7±25.7</td>
<td>11.2±13.7</td>
<td>9.1±6.2</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.26±0.02</td>
<td>0.29±0.04</td>
<td>0.29±0.03</td>
</tr>
<tr>
<td>IL6</td>
<td>96.4±76.4</td>
<td>38.9±62.9</td>
<td>41.1±86.6</td>
</tr>
<tr>
<td>hsCRP</td>
<td>16.9±8.4</td>
<td>7.8±8.5</td>
<td>6.5±8.3</td>
</tr>
<tr>
<td>TNF-α</td>
<td>34.4±53.6</td>
<td>18.9±26.9</td>
<td>15.3±16.2</td>
</tr>
</tbody>
</table>

* P value between Group-1 and Group-2
# P value between Group-1 and Group-3
¶ P value between Group-2 and Group-3

All subjects were grouped according to vitamin B12 levels as very low (<100 pg/ml), low (100-200 pg/ml) and normal levels (>200 pg/ml). Insulin levels and HOMA-IR decreased from group-1 to group-3; whereas insulin sensitivity assessed by QUICKI improved. Similarly inflammatory markers (hsCRP, IL-6, and TNF-α) also decreased from group-1 to group-3. Subjects with hyperhomocysteinemia were more insulin resistance (HOMA-IR) and had decreased sensitivity (QUICKI). hsCRP levels were significantly higher in subjects with hyperhomocysteinemia. (Table-4)

Inflammatory markers (hsCRP, IL-6, and TNF-α), HOMA-IR and dyslipidemia were inversely related plasma vitamin B12 concentration. There was no correlation between vitamin B12 levels and BMI, WHR, DM, HTN, smoking and physical activity.
Hcy levels were positively correlated with dyslipidemia, insulin levels, HOMA-IR (Figure-1) and inflammatory markers (hsCRP-Figure-2, and IL-6). Insulin sensitivity was negatively

Table-5
Correlation of Vitamin B12, homocysteine and Folic acid with cardiovascular risk factors

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Vitamin B12</th>
<th>Homocysteine</th>
<th>Folic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
<td>P value</td>
<td>r value</td>
</tr>
<tr>
<td>Smoking</td>
<td>-0.001</td>
<td>0.989</td>
<td>-0.003</td>
</tr>
<tr>
<td>Physical inactivity</td>
<td>-0.060</td>
<td>0.302</td>
<td>0.007</td>
</tr>
<tr>
<td>BMI</td>
<td>0.028</td>
<td>0.634</td>
<td>-0.041</td>
</tr>
<tr>
<td>WHR</td>
<td>-0.026</td>
<td>0.659</td>
<td>-0.022</td>
</tr>
<tr>
<td>DM</td>
<td>-0.055</td>
<td>0.339</td>
<td>0.173</td>
</tr>
<tr>
<td>HTN</td>
<td>-0.042</td>
<td>0.464</td>
<td>0.168</td>
</tr>
<tr>
<td>DM &amp; HTN</td>
<td>-0.076</td>
<td>0.190</td>
<td>0.187</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>-0.273</td>
<td>&lt;0.0001</td>
<td>0.770</td>
</tr>
<tr>
<td>IL6</td>
<td>-0.091</td>
<td>0.116</td>
<td>0.296</td>
</tr>
<tr>
<td>hsCRP</td>
<td>-0.102</td>
<td>0.078</td>
<td>0.313</td>
</tr>
<tr>
<td>TNF-α</td>
<td>-0.016</td>
<td>0.784</td>
<td>0.099</td>
</tr>
<tr>
<td>Insulin</td>
<td>-0.102</td>
<td>0.077</td>
<td>0.313</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>-0.124</td>
<td>0.032</td>
<td>0.377</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.105</td>
<td>0.070</td>
<td>-0.365</td>
</tr>
</tbody>
</table>

Figure-1
Regression graph showing relation between homocysteine and HOMA-IR

y = 0.526x - 0.804
R² = 0.142
P<0.0001
correlated with Hcy levels. Hcy levels also showed positive correlation with DM, HT and dyslipidemia, but was not related to BMI, WHR, smoking and physical activity. Folic acid levels did not show any relation with any risk factors. (Table-5)

**Figure-2**

Regression graph showing relation between homocysteine and hsCRP

![Graph showing relation between homocysteine and hsCRP](image)

\[ y = 0.199x + 4.479 \]

\[ R^2 = 0.098 \]

**Table-6**

Step wise multiregression analysis of Vitamin B12 with risk factors

<table>
<thead>
<tr>
<th>Vitamin B12</th>
<th>Beta coefficient</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyslipidemia</td>
<td>-192.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>dyslipidemia+ age</td>
<td>-192.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>dyslipidemia +age+ BMI</td>
<td>-194.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>dyslipidemia +age+ BMI + Diet</td>
<td>-194.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>dyslipidemia +age+ BMI + Diet + sex</td>
<td>-186.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>dyslipidemia +age+ BMI + Diet + sex + physical inactivity</td>
<td>-189.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>dyslipidemia +age+ BMI + Diet + sex + physical inactivity +Smoking</td>
<td>-189.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>dyslipidemia +age+ BMI + Diet + sex + physical inactivity +Smoking+HTN</td>
<td>-194.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>dyslipidemia +age+ BMI + Diet + sex + physical inactivity +Smoking+HTN+DM</td>
<td>-194.5</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
The association of vitamin B12 with dyslipidemia remained unchanged even after adjustment for all other risk factors; age, sex, BMI, WHR, physical inactivity, smoking and diabetes (Table 6).

Serum vitamin B12 was inversely associated with TG (Figure-3), VLDL and positively with HDL (Figure-4) after adjustment with other risk factors. However, there was no association of vitamin B12 with TC and LDL Cholesterol. Hcy was positively associated with TG, VLDL and negatively with HDL after adjustment with all risk factors. However, there was no association of Hcy with TC and LDL cholesterol (Table-7).

**Figure-3**

Regression graph showing relation between homocysteine and triglycerides

\[
y = 1.320x + 123.4 \\
R^2 = 0.188 \\
P < 0.0001
\]
Figure-4
Regression graph showing relation between homocysteine and HDL cholesterol

![Regression graph showing relation between homocysteine and HDL cholesterol](image)

\[ y = -0.267x + 48.65 \]
\[ R^2 = 0.195 \]
\[ P<0.0001 \]

Table-7
Association of Vitamin B12 and Homocysteine with lipids

<table>
<thead>
<tr>
<th>Lipid levels</th>
<th>Univariate analysis with Vitamin B12</th>
<th>Multiple regression with Vit B12 (adjusted with all other risk factors)</th>
<th>Univariate analysis with Homocysteine</th>
<th>Multiple regression with Homocysteine (after adjustment with all other risk factors)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
<td>p value</td>
<td>Beta coefficient</td>
<td>p value</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-0.010</td>
<td>0.855</td>
<td>-0.032</td>
<td>0.9420</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>-0.140</td>
<td>0.015</td>
<td>-0.965</td>
<td>0.031</td>
</tr>
<tr>
<td>HDL</td>
<td>0.136</td>
<td>0.019</td>
<td>5.017</td>
<td>0.030</td>
</tr>
<tr>
<td>LDL</td>
<td>-0.009</td>
<td>0.882</td>
<td>-0.022</td>
<td>0.9570</td>
</tr>
<tr>
<td>VLDL</td>
<td>-5.271</td>
<td>0.015</td>
<td>-4.825</td>
<td>0.031</td>
</tr>
</tbody>
</table>
Hcy was positively correlated with dyslipidemia in stepwise multiple regression analysis even after adjustment with other risk factors: sex, BMI, WHR, physical inactivity, smoking and diabetes. (Table-8)

**Table-8**

**Step wise multiregression analysis of Homocysteine with risk factors**

<table>
<thead>
<tr>
<th>Homocysteine</th>
<th>beta coefficient</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyslipidemia</td>
<td>23.728</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>dyslipidemia + age</td>
<td>23.731</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>dyslipidemia + age + BMI</td>
<td>23.849</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>dyslipidemia + age + BMI + Diet</td>
<td>23.847</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>dyslipidemia + age + BMI + Diet + sex</td>
<td>24.358</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>dyslipidemia + age + BMI + Diet + sex + physical inactivity</td>
<td>24.425</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>dyslipidemia + age + BMI + Diet + sex + physical inactivity + Smoking</td>
<td>24.434</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>dyslipidemia + age + BMI + Diet + sex + physical inactivity + Smoking + HTN</td>
<td>24.424</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>dyslipidemia + age + BMI + Diet + sex + physical inactivity + Smoking + HTN + DM</td>
<td>24.294</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

### 4.2.2 DISCUSSION

Increased plasma Hcy levels are positively associated with new onset CAD, recurrent cardiovascular events, extent of myocardial damage and mortality in patients with IHD (416,475-476,493). However, few studies with small number of subjects were not able to demonstrate this relation (402,423). We evaluated association of Hcy levels and related nutritional markers (vitamin B12, folic acid) with known cardiovascular risk factors in 300 subjects with CAD. Most of the patients are vitamin B12 deficient (86.7%) but a few are folate deficient (2.7%) in this study. Other studies from India also reported high prevalence of vitamin B12 deficiency. Yajnik et al found vitamin B12 deficiency in 87% of urban population from the same region as ours. Refsum H et al also reported high prevalence of Vitamin B12 deficiency (70%) in Indian adults (815). Many researchers from other countries reported varied prevalence of vitamin B12 deficiency from 1.5% in Korea (480), 16.5% from Spain (501), 24.0% in South Asians living in Auckland (499), to 26.6% in Tehran (488). Similar to our study, lower prevalence of folate deficiency was reported from Korea (4.1%) and Tehran (10.7%) compared to vitamin B12 deficiency. The differences in prevalence of serum vitamin B12 deficiency and folate can be explained on the basis of the different cutoff points defined for vitamin B12 deficiency and dietary intake of vitamin B12.
according to dietary differences (481). Hyperhomocysteinemia is present in 95.3% patients in present study. High prevalence of hyperhomocysteinemia was also reported from the same region of India in one study (79% in urban population) (492) and from northern part of India (84%) (481). Lower prevalence of hyperhomocysteinemia was reported from other countries like 6.1% in middle income group Koreans (480), 29.3% in FHS cohort (407), 17.3% in Chinese (485), 32.9% in Slovaksians, and 47.4% in Tehran population (488). Moreover, mean Hcy levels show a geographical pattern with lower levels reported from European countries (Finland -9.99 µmol/l, Norway - 12.7 µmol/l, UK 14.2 µmol/l), which increases on moving to Asian countries (Tehran – 20.3 µmol/l, Pakistan 18.0 µmol/l, healthy urban Indian – 23.2 µmol/l and Bangladeshis 27.5 µmol/l). In this study higher mean Hcy levels is observed because we have selected patients who already had CAD. It has been suggested that inadequate plasma concentration of vitamin B12 is a contributing factor in approximately 2/3 of all cases of hyperhomocysteinemia (407) and low vitamin B12 concentration contributed 28% to the risk of hyperhomocysteinemia (492).

Serum Vitamin B12 is higher in male as compared to female but is statistically comparable. A study from Korea among middle income group reported significantly lower levels in males than females (480). Hcy levels are not correlated with age in present study but other studies have shown positive correlation (407,480,485). Hcy levels are higher in males compared to females (M:36.5±13.5µmol/l, F:35.0±18.7µmol/l, p = 0.02) in our study which has been also observed by other studies (416,485). Similar to our study no correlation was detected among Hcy and BMI and WHR by others (478,486), whereas one study reported higher BMI and WHR in the group with high Hcy as compared to group with normal Hcy levels (491). We observed no difference in levels of Hcy, vitamin B12 and folate levels between smokers and non-smokers. Others have reported lower levels of serum folate and higher Hcy in smokers when compared with non-smokers (483).

In present study, vitamin B12 is lower in patients with DM and dyslipidemia but is independently associated with dyslipidemia only. Similar to our study low serum levels of vitamin B12 were observed in Omani adults with T2DM (500). Serum folate levels did not differ among various traditional risk factors in present study. There are contradictory reports of serum folate levels in DM with some studies reporting low levels (484, 500) and other study higher levels in diabetic patients in a Mediterranean population (816). Hcy is
higher in DM and/or HTN subjects in present study. Others have also observed higher Hcy levels in patient with DM (479,484, 486). Moreover treatment of diabetes made no difference in Hcy levels in a study (484). A study reported higher blood pressure in high Hcy group as compared to the group with normal Hcy level (486).

In our study Hcy levels are higher in subjects with lipid abnormalities than without it. An association between hyperlipidemia and hyperhomocysteinemia has been reported by Obeid R et al (817). Higher plasma Hcy was associated with lower high density lipoprotein level, similar to our study. Hcy levels correlated negatively with HDL concentration (r=-0.30, p=0.013) (484). The effect of Hcy on HDL cholesterol is probably related to inhibiting enzymes or molecules participating in HDL particle assembly (817). Hcy was unrelated to serum TG and HDL levels (478). It seems that hypomethylation associated with Hcy is responsible for lipid accumulation in tissues. Decreased methyl group will decrease the synthesis of phosphatidylcholine, major phospholipids required for VLDL assembly and homeostasis. This link is clinically important in management of vascular risk factor especially in elderly people and patients with metabolic syndrome and cardiovascular disease. In one study none of the lipid biomarkers were significantly related to Hcy levels (482).

Vitamin B12 levels are negatively correlated with HOMA-IR and insulin levels in present study. A similar association has been observed in women with polycystic ovary syndrome (496). Contrary to this a cross-sectional study conducted in 135 Asian Indians women found no correlation between serum vitamin B12 and HOMA IR (499). Vitamin B12 treatment improved insulin resistance and endothelial dysfunction, along with decreasing Hcy levels, in patients with metabolic syndrome, suggesting that vitamin B12 has several beneficial effects on cardiovascular disease risk factors (26). However, several studies done with vitamin B12 and folate supplementation failed to decrease coronary artery events (494-495,498,490).

In present study subjects with hyperhomocysteinemia has significantly high HOMA IR index but insulin levels are comparable. Hcy is having positive association with both insulin and HOMA IR. Framingham offspring study demonstrated a modest association between hyperinsulinemia and fasting Hcy levels (477). Giltay et al also showed a positive association between Hcy and insulin resistance (818). In Japanese diabetic patients, insulin
resistance was an independent predictor of total Hcy levels and insulin and HOMA IR was higher in high Hcy group in subjects with type 2 DM group as compared to group with normal Hcy levels (491). However, other studies failed to show correlation between Hcy and insulin sensitivity in male patients (478) and in healthy pre-menopausal South Asian women (819).

In our study Hcy levels are correlated with IL6 and hsCRP levels. A large observational study among women (Nurses’ Health Study) found positive association between total Hcy and cytokines, IL6 and CRP along with soluble TNF receptor (482). A large number of studies have provided evidence of the role of inflammation in the development and progression of atherosclerosis processes (496,499). This may in part explain the observed association between high circulating concentrations of Hcy and cardiovascular diseases described in many observational studies (402,410,488).

In the present study Hcy is inversely associated with plasma vitamin B12 (r= -0.285, p<0.001). Hcy exhibited inverse association with plasma folate (r=-0.3 to -0.37) and vitamin B12 (r=-0.2 to-0.22) in other studies (402,407,480,482-485). Contrary to the most of the studies we were unable to observe an inverse relation between serum folate levels and Hcy. It has been reported that compound heterozygous mutation consisting of cystathione beta-synthase and methylenetetrahydrofolate reductase may be the main cause of mild hyperhomocysteinemia. Among these individuals with 677TT genotype had elevated Hcy with low-normal folate, whereas those with high-normal folate have normal Hcy concentrations (770). However, such mutation was not demonstrated in Indian population (784). This may explain the observed association of folate with Hcy in western population. Folate act indirectly through vitamin B12 as methyl donor to Hcy. Hence, correlation of serum folate and Hcy is evident in studies in population with vitamin B12 sufficiency. In Indian population there is high intake of folic acid and low percentage of folic acid deficiency in presence of high vitamin B12 deficiency; which may have masked the relation between serum folate and Hcy.

4.2.3 CONCLUSION

Vitamin B12 deficiency and hyperhomocysteinemia are associated with traditional and non-traditional cardiovascular risk factors and are independently associated with dyslipidemia even after adjustment with all other risk factors. Thus, vitamin B12 deficiency
causing hyperhomocysteinemia may be a risk factor for cardiovascular disease and important for prediction of future cardiovascular disease.
MAGNESIUM AND RELATION WITH CARDIOVASCULAR RISK FACTORS
4.3 MAGNESIUM AND RELATION WITH CARDIOVASCULAR RISK FACTORS

4.3.1 Results

Subjects were divided in three groups according to serum magnesium levels.

**Table 9**
Cardiovascular risk factor in groups according to serum magnesium levels

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (≤ 1.6 mg/dl) median (range)</th>
<th>Group 2 (&gt; 1.6-2.6 mg/dl) median (range)</th>
<th>Group 3 (&gt; 2.6 mg/dl) median (range)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>62 (25-92)</td>
<td>63.5 (25-89)</td>
<td>58 (32-75)</td>
<td>0.6369</td>
</tr>
<tr>
<td>p value</td>
<td>0.9842*</td>
<td>0.3349†, 0.3904#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male:67.6% (119) Female: 32.4% (57)</td>
<td>Male: 77.5% (79) Female: 22.5% (23)</td>
<td>Male: 81.8% (18) Female: 18.2% (4)</td>
<td>0.1203</td>
</tr>
<tr>
<td>p value</td>
<td>0.1076*</td>
<td>0.2645†, 0.813#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>40.3% (71)</td>
<td>32.4% (33)</td>
<td>31.8% (7)</td>
<td>0.3604</td>
</tr>
<tr>
<td>p value</td>
<td>0.2309*</td>
<td>0.589†, 0.813#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical Inactivity</td>
<td>38.3% (67)</td>
<td>38.2% (39)</td>
<td>45.5% (10)</td>
<td>0.8011</td>
</tr>
<tr>
<td>p value</td>
<td>0.9046*</td>
<td>0.676†, 0.684#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>27.3 (20.02-39.4)</td>
<td>26.8 (19.3-37.7)</td>
<td>27.4 (22.18-35.02)</td>
<td>0.750</td>
</tr>
<tr>
<td>p value</td>
<td>0.476*</td>
<td>0.9170†, 0.6421#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist hip ratio</td>
<td>0.92 (0.70-1.07)</td>
<td>0.92 (0.70-1.18)</td>
<td>0.93 (0.81-1.02)</td>
<td>0.957</td>
</tr>
<tr>
<td>p value</td>
<td>0.76*</td>
<td>0.7818†, 0.7708†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>52.8% (93)</td>
<td>19.2% (24)</td>
<td>6.4% (8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.00001†</td>
<td>0.2181†, 0.3545#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>74.9% (131)</td>
<td>50% (501)</td>
<td>22.7% (5)</td>
<td>0.0040</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.0001†</td>
<td>&lt;0.0001†, 0.0361#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes &amp; HT</td>
<td>40.3% (71)</td>
<td>16.7% (17)</td>
<td>22.7% (5)</td>
<td>0.0001</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.00001†</td>
<td>0.1709†, 0.744#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>173.5 (91-343)</td>
<td>161(91-331)</td>
<td>165(118-234)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.00001†</td>
<td>0.0397†, 0.7593#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>190(71-341)</td>
<td>145(64-298)</td>
<td>146(99-200)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.00001†</td>
<td>&lt;0.00001†, 0.2346#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>33(20-55)</td>
<td>47(26-59)</td>
<td>49(32-59)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.00001†</td>
<td>&lt;0.00001†, 0.0607#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL Cholesterol</td>
<td>98.9(12.6-273.8)</td>
<td>86(14.4-263)</td>
<td>84.5(39-162.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.00001†</td>
<td>0.0001†, 0.8317#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL Cholesterol</td>
<td>38(14.2-68.2)</td>
<td>29(12.8-59.6)</td>
<td>29.2(19.8-40)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.00001†</td>
<td>0.0043†, 0.2058#</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P value between Group-1 and Group-2
‡ P value between Group-1 and Group-3
# P value between Group-2 and Group-3

Number of subjects with DM; and DM with HTN were significantly higher in group-1 compared to group-2. DM in these three groups was 52.8%, 19.2%, 6.4% in group 1, 2 and 3 respectively. TC, TGs, VLDL and LDL cholesterol were significantly higher and
HDL cholesterol significantly lower in group-1 when compared with group-2 and group-3. There was no significant difference between group-2 and group-3. There was no correlation between BMI and WHR with serum magnesium level. Serum magnesium levels were independent of smoking and physical activity. (Table-9)

Table-10
Comparative serum magnesium in relation to cardiovascular risk factors

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Serum Magnesium Level (mean±sd)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.62±0.52</td>
<td>0.1609</td>
</tr>
<tr>
<td>No</td>
<td>1.71±0.56</td>
<td></td>
</tr>
<tr>
<td>Physical inactivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.7±0.57</td>
<td>0.6607</td>
</tr>
<tr>
<td>No</td>
<td>1.66±0.54</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>1.72±0.57</td>
<td>0.6861</td>
</tr>
<tr>
<td>≥25</td>
<td>1.67±0.55</td>
<td></td>
</tr>
<tr>
<td>Central Obesity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.66±0.55</td>
<td>0.4287</td>
</tr>
<tr>
<td>No</td>
<td>1.71±0.55</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.54±0.51</td>
<td>0.0002</td>
</tr>
<tr>
<td>No</td>
<td>1.78±0.56</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.53±0.42</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No</td>
<td>1.93±0.64</td>
<td></td>
</tr>
<tr>
<td>Diabetes &amp; Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.5±0.49</td>
<td>0.0001</td>
</tr>
<tr>
<td>No</td>
<td>1.76±0.55</td>
<td></td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.32±0.13</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No</td>
<td>1.93±0.59</td>
<td></td>
</tr>
</tbody>
</table>

However, subjects with HTN, DM and/or HTN, and dyslipidemia had significantly lower serum magnesium levels than without these risk factors. There was no difference in serum magnesium levels in subjects without obesity, central obesity, physical inactivity and history of smoking (Table-10).
In univariate analysis, serum magnesium levels were negatively correlated with DM and/or HTN and dyslipidemia. Similar negative relation between serum magnesium and various lipid parameters were observed except HDL cholesterol which was positive correlated with serum magnesium levels. There was no relation between serum magnesium levels and age, sex, BMI, WHR and smoking. (Table-11)

Table-11
Correlation of Serum Magnesium with Cardiovascular Risk Factors (Univariate Analysis)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Beta Coefficient</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.003</td>
<td>0.3004</td>
</tr>
<tr>
<td>Sex</td>
<td>0.137</td>
<td>0.0531</td>
</tr>
<tr>
<td>Smoking</td>
<td>-0.093</td>
<td>0.1609</td>
</tr>
<tr>
<td>BMI</td>
<td>0.001</td>
<td>0.9116</td>
</tr>
<tr>
<td>WHR</td>
<td>0.272</td>
<td>0.6063</td>
</tr>
<tr>
<td>Diabetes</td>
<td>-0.243</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>-0.408</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>-0.606</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-0.002</td>
<td>0.0025</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>-0.004</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL</td>
<td>0.038</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL</td>
<td>-0.002</td>
<td>0.0003</td>
</tr>
<tr>
<td>VLDL</td>
<td>-0.022</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table-12
Correlation of Serum Magnesium with cardiovascular risk factors after adjustment with age, sex, BMI and WHR in Multiple regression analysis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Beta Coefficient</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>-0.231</td>
<td>0.0003</td>
</tr>
<tr>
<td>Hypertension</td>
<td>-0.404</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>-0.599</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-0.002</td>
<td>0.0031</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>-0.004</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>HDL</td>
<td>0.038</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>LDL</td>
<td>-0.002</td>
<td>0.0005</td>
</tr>
<tr>
<td>VLDL</td>
<td>-0.017</td>
<td>&lt;0.00001</td>
</tr>
</tbody>
</table>

The significant association between serum magnesium and DM, HT, dyslipidemia, and various lipid parameters were maintained even after adjustment with age, sex, WHR and BMI in multiple regression analysis (Table-12).
**Dietary Magnesium**

All subjects were divided according to dietary intake of magnesium according to recommended daily allowance. TC, TGs, LDL and VLDL were significantly higher and HDL cholesterol significantly lower in the dietary magnesium group1 when compared with group2 (table 13). Dietary magnesium was positively correlated with serum magnesium (beta coefficient: 53.46, p:<0.0001) and this was maintained even after adjustment with age and sex in multiple regression analysis.

**Table-13**

**Lipid levels according to dietary magnesium groups**

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Dietary magnesium intake</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤350 mg/day</td>
<td>&gt;350 mg/day</td>
</tr>
<tr>
<td>Dietary Magnesium Intake (mg/day)</td>
<td>286.7±35.6</td>
<td>399.3±40.1</td>
</tr>
<tr>
<td>Serum magnesium (mg/dl)</td>
<td>1.48±0.44</td>
<td>2.0±0.57</td>
</tr>
<tr>
<td>Total Cholesterol(mg/dl)</td>
<td>185±49.07</td>
<td>168.5±37.4</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>182.4±48.2</td>
<td>152.6±36.1</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dl)</td>
<td>35.7±8.7</td>
<td>44.2±7.41</td>
</tr>
<tr>
<td>LDL Cholesterol (mg/dl)</td>
<td>112.7±54.8</td>
<td>93.7±40.05</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>36.4±9.64</td>
<td>30.5±7.23</td>
</tr>
</tbody>
</table>

When dietary magnesium was correlated with risk factors, subjects with HTN, DM and DM with HTN had significantly lower dietary magnesium levels than without these risk factors (diabetic; Yes= 314.8±58.6 mg/day, No= 339.9±69.4, p= 0.001;DM & HTN; Yes= 306.7±56.04 mg/day, No= 339.7±68.04 mg/day, p<0.0001). Patients with dyslipidemia had significantly low dietary magnesium levels as compared to patients without dyslipidemia (Dyslipidemia; Yes=293.8±53.3 mg/day, No= 354.6±62.9 mg/day, p<0.0001).
DM and DM with HTN and dyslipidemia were negatively correlated with dietary magnesium levels in univariate analysis and even after adjustment with age, sex and dietary variables (sodium, potassium, carbohydrate, energy, total dietary fiber, protein intake, vitamin B12 and folic acid) in multiple regression analysis (table 14&15). However, no correlation was found between smoking, HTN, physical activity and dietary magnesium levels.

Patients in group-1 also had decreased intake of protein, dietary fibers, calcium, potassium and folic acid compared to other groups. Intake of dietary calcium, potassium, total dietary fiber, proteins, sodium and folic acid increased with increased intake of dietary magnesium (P<0.0001, P<0.05). Significance remained after adjustment for age and sex. However, there was no correlation between dietary fat, carbohydrate, vitamin B12, saturated and polyunsaturated fatty acids intake with dietary magnesium level. (Table-16)
### Table-16
Dietary differences between groups according to magnesium levels

<table>
<thead>
<tr>
<th>Dietary ingredients</th>
<th>Group 1 (≤ 1.6 mg/dl) median (range)</th>
<th>Group 2 (&gt; 1.6-2.6 mg/dl) median (range)</th>
<th>Group 3 (&gt; 2.6 mg/dl) median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate (g/day)</td>
<td>225(130.6-737.2)</td>
<td>213.7(102.7-397.9)</td>
<td>228.7(126.2-378.4)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.0441*</td>
<td>0.5145¶</td>
<td>0.6239#</td>
</tr>
<tr>
<td>Protein (g/day)</td>
<td>41.4(24.6-89.9)</td>
<td>53.1(24.8-135.6)</td>
<td>49.8(25.3-71.2)</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.0001*</td>
<td>0.0014¶</td>
<td>0.2201†</td>
</tr>
<tr>
<td>Fat (g/day)</td>
<td>72.8(46.7-142.7)</td>
<td>78.8(49.5-142.7)</td>
<td>73.2(51.4-138.5)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.0704*</td>
<td>0.6666¶</td>
<td>0.6021#</td>
</tr>
<tr>
<td>Minerals (g/day)</td>
<td>8.1(4.3-14.3)</td>
<td>10.13(5.95-14.8)</td>
<td>9.5(6.2-14.2)</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.0001*</td>
<td>0.0006¶</td>
<td>0.0963#</td>
</tr>
<tr>
<td>Total dietary fiber (g/day)</td>
<td>10.7(2.5-29.4)</td>
<td>15.1(3.03-39.4)</td>
<td>12.5(7.7-36.7)</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.0001*</td>
<td>0.1036¶</td>
<td>0.2183#</td>
</tr>
<tr>
<td>Calcium (mg/day)</td>
<td>568.1(162.05-1772.8)</td>
<td>632.3(314.5-1668.2)</td>
<td>638.6(334.5-1072.5)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.0077*</td>
<td>0.5831¶</td>
<td>0.3622#</td>
</tr>
<tr>
<td>Potassium (mg/day)</td>
<td>1083.2 (463.8-1991.9)</td>
<td>1366.1(552.8-2176.9)</td>
<td>1387.7(632.3-2176.9)</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.0001*</td>
<td>0.0004¶</td>
<td>0.8339#</td>
</tr>
<tr>
<td>Folic Acid (µg/day)</td>
<td>118.5(39.1-279.8)</td>
<td>151.2(60.5-471.6)</td>
<td>154.1(41.4-283.8)</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.0001*</td>
<td>0.0437¶</td>
<td>0.5053#</td>
</tr>
<tr>
<td>Vitamin B 12 (µg/day)</td>
<td>0.224(0-1.87)</td>
<td>0.224(0.07-4.12)</td>
<td>0.240(0.14-0.462)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.1686*</td>
<td>0.4212¶</td>
<td>0.9789#</td>
</tr>
</tbody>
</table>

* P value between Group-1 and Group-2
¶ P value between Group-1 and Group-3
# P value between Group-2 and Group-3

### 4.3.2 DISCUSSION

This is the first study conducted in angiographically proved cardiovascular patients analyzing role of serum and dietary magnesium from India. In present study more than half of patients (58.6%) are having low magnesium level and 62% are taking dietary magnesium below recommended dietary allowance (350 mg/day). Others have documented hypomagnesaemia 19-53% in patients with cardiovascular disease and acute myocardial infarction (820,821).
Few studies have shown that hypomagnesaemia plays an important role in modifying risk factors of cardiovascular disease like dyslipidemia, DM and HTN (553,535,822). In present study there are significantly higher numbers of subjects with DM, HTN and dyslipidemia in the group with the lowest serum magnesium; indicating hypomagnesaemia is common in these conditions. All these risk factors have lower serum magnesium level except in subjects with isolated HTN. Other studies have also reported no differences in serum magnesium levels and dietary magnesium in hypertensive patients (823,824). Serum magnesium has significantly negative correlation with cardiovascular risk factors which persisted after adjustment with age, sex, and anthropometric parameters in multiple regression analysis. None of the subjects are hypertensive in group-3 with normal magnesium levels, which suggest that serum magnesium level may be protective in development of HTN. Many epidemiological and clinical studies have supported the hypothesis that increased magnesium intake contribute to prevention of HTN and cardiovascular disease (552,554,566, 583,825-826). In a study conducted in 615 Japanese men living in Hawaii without cardiovascular disease or HTN, found that minerals (magnesium, calcium, phosphorus, potassium), fiber, vegetable protein, starch, and vitamins (vitamin C and vitamin D) intakes were inversely associated with BP. It suggested that foods such as vegetables, fruits, whole grains, and low-fat dairy items are major sources of nutrients that may be protective against HTN (552). An inverse association was observed between BP and dietary magnesium in both men and women, in Dutch men and women (20,921), aged 20-59 years (554). Dietary Intervention Study in Children who had elevated low-density lipoprotein cholesterol and were aged 8 to 11 years, using 24-hour dietary recalls showed inverse associations of systolic diastolic BP with dietary magnesium (826). In the Atherosclerosis Risk in Communities (ARIC) Study done in four US communities aged, 45-64 years, serum magnesium levels were significantly lower in participants with prevalent CVD, HTN than in those free of these diseases and dietary magnesium intake was also inversely associated with systolic and diastolic BP (553). Sharma A et al in cross sectional Indian study found a strong association between hypomagnesaemia and HTN (1.75 ±0.07, p<0.0001) (566). A Meta analysis showed that magnesium supplementation appears to achieve a small but clinically significant reduction in BP (827). However, in a six year follow up ARIC (Atherosclerosis Risk in
Communities) Study, in which healthy individual were taken and followed up for incident HTN, there was no significant association between dietary magnesium intake and incident HTN in male and female (556).

Several studies have addressed the question of the relation between hypomagnesaemia and DM. Although studies in small groups of patients have yielded conflicting results (560,828), overwhelming evidence from human and animal studies indicates that both plasma and tissue magnesium levels are reduced in DM (828,829). A study by Yajnik CS et al showed that in all diabetics, plasma magnesium concentrations were inversely related to plasma glucose values and magnesium concentration was lowest in the insulin treated group (829). An inverse association was found between magnesium and prevalence of diabetes (562,565,566). Elderly Nutrition and Health Survey in Taiwan showed an inverse association between plasma magnesium concentration and the prevalence of diabetes. However, no association was found between diabetes and low dietary magnesium (562). In meta-analysis, magnesium intake was found to be inversely associated with incidence of type 2 diabetes. This suggests that increased consumption of magnesium-rich foods such as whole grains, beans, nuts, and green leafy vegetables may reduce the risk of type 2 DM (565). In an Indian study, serum magnesium levels in diabetic population (50 type 1 and type 2 diabetic patients with or without complications and 40 normal healthy persons) was significantly low (1.93 ± 0.282 meq/l) in comparison to control (2.25 ± 0.429 meq/l). Duration of diabetes and serum magnesium was inversely related. The change in serum magnesium level may have a bearing on the complication and morbidity in patients of DM (566). In a study from Switzerland also found significantly lower mean plasma magnesium concentration than that of the control group (0.77± 0.08 and 0.83 ± 0.07 mmol/L, respectively (p <0.001). Plasma magnesium concentrations were below the normal reference range in 37.6% of the diabetic patients and 10.9% of the control subjects (560). In contrast, another study from Kashmir, northern part of India reported that magnesium levels were comparable between diabetic and non-diabetic subjects. It was suggested that magnesium levels are not altered in DM (555). Other study has also reported no association between diabetes and magnesium (570).

Hypomagnesemia was reported to be one of the strongest predictors of gain in left ventricular mass over the following 5 years in “Study of Health in Pomerania” (830). In
addition, experimental hypomagnesaemia inhibits prostacyclin receptor function, producing an imbalance between prostacyclin and thromboxane effects; such imbalance has been suggested to be of aetiological importance for the development of cardiovascular disease (831,832).

In present study TC, TGs, VLDL and LDL cholesterol are significantly higher and HDL cholesterol is significantly lower in subjects with hypomagnesaemia compared with those with normomagnesemia. All lipid parameters is having negative correlation with serum magnesium level except HDL; which is positively correlated with serum magnesium level. Similar observations have made by other studies (553,535). In a double-blind, placebo-controlled study among 47 patients with IHD and acute myocardial infarction magnesium supplementation decreased triglyceride, and thereby very-low-density lipoprotein concentrations by 27% (from 2.41 to 1.76 mmol/L, and from 1.1 to 0.79 mmol/L, respectively) as compared with much smaller decrements in the placebo group. There was tendency toward an increase in high-density lipoprotein cholesterol and in high-density lipoprotein cholesterol ratio after magnesium treatment. It suggested magnesium deficiency might be involved in the pathogenesis of IHD by altering the blood lipid composition in a way that disposes to atherosclerosis (535).

An equal numbers of studies have failed to demonstrate such association (568,822). In a cross sectional study performed in 122 patients (82F, 40M) with mean patient’s age 63 (±10) years, and mean serum magnesium was 2 (± 0.4) mg/dl reported non significant correlations of serum magnesium with serum HDL and TG; but found significant inverse correlations of serum magnesium with serum cholesterol and LDL (568).

Studies in patients with metabolic syndrome or DM have shown that individuals with low levels of magnesium have lower levels of HDL-cholesterol (557,558,563) but higher levels of TGs (558,563) and TC (558). In a study by Guerrero-Romero F et al serum HDL-cholesterol value showed significant graded increase with serum magnesium levels irrespective of glucose values. This suggested that hypomagnesemia decrease HDL-cholesterol by glucose independent pathway (557). Other studies examining serum magnesium levels have shown a positive correlation with HDL-cholesterol, TGs, LDL Cholesterol and TC when general population was studied (569). Among subjects with metabolic syndrome a potential relationship between low ionized magnesium
and total serum magnesium (557,558) and an atherogenic lipid profile, involving low serum HDL-cholesterol (820,821); high TC (558,833) and high TGs (557,558,563) have been reported, proposing a potential role for magnesium in the pathogenesis of CVD (557,558). Similar association was found between dietary magnesium with TC, TG, LDL, HDL and VLDL. TC tended to decrease with increasing magnesium intake across the ranges of magnesium (834) and was inversely related to HDL Cholesterol (553). There was no association of age, gender on serum magnesium in type 2 DM similar to the present study (570). Significant inverse correlation of serum magnesium and ages of the patient was found in studies contrary to our study (568,569).

A population survey focused on the relationship between dietary magnesium intake and serum magnesium level. The magnesium intake correlated with serum magnesium concentrations similar to our study (567). Patients with hypomagnesaemia also has decreased intake of protein, dietary fibers, calcium, potassium and folic acid compared to other groups, which may also contributed to various risk factors. Magnesium is correlated significantly with insulin levels. In a study mean serum magnesium level was inversely associated with insulin level (553).

4.3.3 CONCLUSION:

Half of cardiovascular disease patients have hypomagnesaemia. There is a strong correlation between serum and dietary magnesium. Serum and dietary magnesium are strongly related to cardiovascular risk factors like dyslipidemia, DM and HTN. There are few studies indicating improvement in atherogenic lipid profile with magnesium supplementation (552). Hence, magnesium supplementation in our population may help in cardiovascular disease.
DIFFERENCES IN RISK FACTORS WITH CORONARY ARTERY DISEASE WITH OR WITHOUT DIABETES MELLITUS AND ASSOCIATION WITH INSULIN RESISTANCE
4.4 DIFFERENCES IN RISK FACTORS WITH CORONARY ARTERY DISEASE WITH OR WITHOUT DIABETES MELLITUS AND ASSOCIATION WITH INSULIN RESISTANCE

4.4.1 RESULTS

BMI and WHR were comparable in both groups. Obesity was present in 28% subjects with diabetes and 26.3% without diabetes (p = 0.84). Central obesity was observed in 84% cases and 81.1% controls (p=0.62). Number of subjects with HTN and dyslipidemia were significantly higher in diabetic subjects compared to non diabetic subjects. TG, VLDL and HbA1C were significantly higher and HDL was significantly lower in patients with diabetes compared to those without diabetes. There was no significant difference in serum TC and LDL cholesterol between two groups. Serum LDL levels (>100 mg/dl) were comparable in both groups (40% vs. 38.9%; p=0.93). Hypertriglyceridemia (69.6% vs. 58.3%; p=0.02) and low HDL (68% vs. 45.1%; p=0.0001) were more common in diabetics than non diabetics. Diabetic patients had more severe coronary artery disease than non-diabetics (Table-17).

Table-17
Basic Parameters of study population

<table>
<thead>
<tr>
<th>Basic Parameters</th>
<th>Diabetes (N=125)</th>
<th>Non-diabetic (N=175)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>52(41.6%)</td>
<td>59(33.7%)</td>
<td>0.2028</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.2±3.9</td>
<td>27.6±3.7</td>
<td>0.241</td>
</tr>
<tr>
<td>WHR</td>
<td>0.92±0.06</td>
<td>0.93±0.06</td>
<td>0.5373</td>
</tr>
<tr>
<td>Hypertension</td>
<td>93(74.4%)</td>
<td>95(54.3%)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>67(53.6)</td>
<td>57(32.6%)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>179.3±47.7</td>
<td>178.3±44.2</td>
<td>0.857</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>106.7±53.8</td>
<td>104.7±48.1</td>
<td>0.7391</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>179.9±42.7</td>
<td>164.8±47.7</td>
<td>0.0051</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>36.6±8.88</td>
<td>40.6±9.09</td>
<td>0.0002</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>35.9±8.55</td>
<td>32.9±9.55</td>
<td>0.0051</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>8.8±2.6</td>
<td>5.08±0.34</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Single vessel disease</td>
<td>4%</td>
<td>35.4%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Double vessel disease</td>
<td>16%</td>
<td>32.0%</td>
<td></td>
</tr>
<tr>
<td>Triple vessel disease</td>
<td>80%</td>
<td>32.6%</td>
<td></td>
</tr>
</tbody>
</table>
**Insulin resistance and inflammation**

Diabetic patients were more insulin resistance (HOMA IR) than non-diabetics though insulin levels were comparable. Sensitivity index (QUICKI) was significantly lower in cases compared to controls. Patients with diabetes had significantly high levels of inflammatory markers - IL6, hsCRP and TNF-α compared to non diabetic patients. hsCRP was more significantly elevated compared to IL-6 and TNF-α (Table-18). HOMA-IR showed positive correlation with Hcy levels and inflammatory markers (IL-6 and hsCRP) in total study population and non-diabetic population; but this relation was only evident with Hcy in diabetic subjects (Table-18). This association persisted even after adjustment for age, sex, BMI in multiple regression analysis (Table-19). HbA1C had significant positive correlation with Hcy (Figure-5), HOMA-IR and inflammatory markers (TNF-α, IL-6 and hsCRP) (Table-19-20) (Figure-6).

**Table: 18**

**Insulin resistance, inflammatory and nutritional markers**

<table>
<thead>
<tr>
<th>Basic Parameters</th>
<th>Diabetes (n=125) mean±SD (Median)</th>
<th>Non-diabetic (n=175) mean±SD (Median)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/ml)</td>
<td>78.2±78.4 (34.4)</td>
<td>54.7±71.5 (17.1)</td>
<td>0.0073</td>
</tr>
<tr>
<td>hs CRP (mg/L)</td>
<td>14.4±10.2 (14.7)</td>
<td>9.6±8.7 (6.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>25.7±28.5 (11.9)</td>
<td>24.9±47.8 (8.9)</td>
<td>0.0118</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>51.7±51.2 (26)</td>
<td>50.7±39.7 (49.8)</td>
<td>0.8007</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>26.8±28.8 (14)</td>
<td>12.03±9.2 (11.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.26±0.027 (0.26)</td>
<td>0.29±0.046 (0.27)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vitamin B 12 (pg/ml)</td>
<td>178.2±301.2 (96.1)</td>
<td>217.2±377.4 (103.7)</td>
<td>0.0014</td>
</tr>
<tr>
<td>Homocysteine (µmol/L)</td>
<td>39.2±15.9 (37.7)</td>
<td>33.9±14.2 (30.4)</td>
<td>0.0026</td>
</tr>
<tr>
<td>Folic acid (ng/ml)</td>
<td>7.6±6.3 (4.36)</td>
<td>8.2±7.4 (4.30)</td>
<td>0.5056</td>
</tr>
</tbody>
</table>
Figure-5
Scatter plot with trendline showing correlation between HbA1C and HOMA-IR

\[ y = 4.223x - 9.828 \]
\[ R^2 = 0.25 \]

\[ P<0.0001 \]

Figure-6
Scatter plot with trendline showing correlation between HbA1C and hsCRP

\[ y = 0.081x + 5.691 \]
\[ R^2 = 0.097 \]

\[ P<0.0001 \]
Table 19
Correlation of Vitamin B12 and homocysteine, HOMA-IR and inflammatory markers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Homocysteine</th>
<th>HOMA-IR</th>
<th>HsCRP</th>
<th>IL-6</th>
<th>TNF-α</th>
<th>HbA1C</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>-0.285</td>
<td>-0.124</td>
<td>-0.102</td>
<td>-0.091</td>
<td>-0.016</td>
<td>-0.085</td>
</tr>
<tr>
<td></td>
<td>(&lt;0.0001)</td>
<td>(0.032)</td>
<td>(0.078)</td>
<td>(0.116)</td>
<td>(0.784)</td>
<td>(0.142)</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>-</td>
<td>0.377</td>
<td>0.313</td>
<td>0.296</td>
<td>0.099</td>
<td>0.341</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA IR</td>
<td>-</td>
<td>0.214</td>
<td>0.163</td>
<td>0.035</td>
<td>0.500</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(&lt;0.0001)</td>
<td>(0.005)</td>
<td>(0.540)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hs CRP</td>
<td>-</td>
<td>-</td>
<td>0.564</td>
<td>0.262</td>
<td>0.312</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.205</td>
<td>0.267</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(&lt;0.0001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.087</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.135)</td>
<td></td>
</tr>
<tr>
<td>DIABETIC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>-0.375</td>
<td>-0.188</td>
<td>-0.008</td>
<td>0.027</td>
<td>0.120</td>
<td>-0.120</td>
</tr>
<tr>
<td></td>
<td>(&lt;0.0001)</td>
<td>(0.036)</td>
<td>(0.926)</td>
<td>(0.767)</td>
<td>(0.182)</td>
<td>(0.181)</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>-</td>
<td>0.386</td>
<td>0.174</td>
<td>0.137</td>
<td>0.045</td>
<td>0.435</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(&lt;0.0001)</td>
<td>(0.052)</td>
<td>(0.068)</td>
<td>(0.618)</td>
<td>(&lt;0.0001)</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>-</td>
<td>0.115</td>
<td>0.114</td>
<td>0.029</td>
<td>0.410</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.200)</td>
<td>(0.205)</td>
<td>(0.752)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hs CRP</td>
<td>-</td>
<td>-</td>
<td>0.550</td>
<td>0.340</td>
<td>0.261</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.333</td>
<td>0.306</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(&lt;0.0001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.234</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.009)</td>
<td></td>
</tr>
<tr>
<td>NON DIABETIC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>-0.226</td>
<td>0.045</td>
<td>-0.149</td>
<td>-0.144</td>
<td>-0.062</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>(0.003)</td>
<td>(0.552)</td>
<td>(0.050)</td>
<td>(0.052)</td>
<td>(0.418)</td>
<td>(0.912)</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>-</td>
<td>0.372</td>
<td>0.385</td>
<td>0.311</td>
<td>0.130</td>
<td>0.226</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td>(&lt;0.0001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA IR</td>
<td>-</td>
<td>0.262</td>
<td>0.176</td>
<td>0.145</td>
<td>0.158</td>
<td>0.158</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(&lt;0.0001)</td>
<td>(0.020)</td>
<td>(0.056)</td>
<td>(0.037)</td>
<td>(0.037)</td>
</tr>
<tr>
<td>hs CRP</td>
<td>-</td>
<td>-</td>
<td>0.550</td>
<td>0.252</td>
<td>0.192</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(&lt;0.0001)</td>
<td>(0.001)</td>
<td></td>
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</tr>
<tr>
<td>IL-6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.158</td>
<td>0.208</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.036)</td>
<td>(0.006)</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.085</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.261)</td>
<td></td>
</tr>
</tbody>
</table>

* all value are given in r value (p-value)
Table 20
Interrelation of Vitamin B12 and homocysteine; HOMA-IR and inflammatory markers after adjustment with age, sex, BMI and hypertension in multiple regression analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vitamin B12</th>
<th>Homocysteine</th>
<th>HOMA-IR</th>
<th>HbA1C</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>-</td>
<td>-0.013 (&lt;0.0001)</td>
<td>-0.006 (0.0673)</td>
<td>0.000 (0.246)</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>-6.72 (&lt;0.0001)</td>
<td>-</td>
<td>0.473 (&lt;0.0001)</td>
<td>0.054 (&lt;0.0001)</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>-1.84 (0.067)</td>
<td>0.266 (&lt;0.0001)</td>
<td>-</td>
<td>0.058 (&lt;0.0001)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>-0.075 (0.881)</td>
<td>0.028 (0.1974)</td>
<td>-0.008 (0.7857)</td>
<td>0.005 (0.171)</td>
</tr>
<tr>
<td>IL6</td>
<td>-0.389 (0.1508)</td>
<td>0.058 (&lt;0.0001)</td>
<td>0.032 (0.037)</td>
<td>0.008 (&lt;0.0001)</td>
</tr>
<tr>
<td>hs CRP</td>
<td>-3.668 (0.083)</td>
<td>0.458 (&lt;0.0001)</td>
<td>0.352 (0.003)</td>
<td>0.075 (&lt;0.0001)</td>
</tr>
<tr>
<td>DIABETIC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>-</td>
<td>-0.019 (&lt;0.0001)</td>
<td>-0.015 (0.0832)</td>
<td>-0.001 (0.20)</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>-6.59 (&lt;0.0001)</td>
<td>-</td>
<td>0.642 (&lt;0.0001)</td>
<td>0.070 (&lt;0.0001)</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>-1.68 (0.0832)</td>
<td>0.200 (&lt;0.0001)</td>
<td>-</td>
<td>0.038 (&lt;0.0001)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.641 (0.0950)</td>
<td>0.000 (0.9996)</td>
<td>-0.078 (0.3973)</td>
<td>0.019 (0.024)</td>
</tr>
<tr>
<td>IL6</td>
<td>0.163 (0.6443)</td>
<td>0.045 (0.0541)</td>
<td>0.032 (0.3300)</td>
<td>0.010 (0.001)</td>
</tr>
<tr>
<td>hs CRP</td>
<td>-0.531 (0.8458)</td>
<td>0.265 (0.0630)</td>
<td>0.368 (0.1500)</td>
<td>0.064 (0.006)</td>
</tr>
<tr>
<td>NON DIABETIC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>-</td>
<td>-0.010 (0.0004)</td>
<td>-0.001 (0.2968)</td>
<td>0.000 (0.941)</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>-7.203 (0.00004)</td>
<td>-</td>
<td>0.166 (&lt;0.0001)</td>
<td>0.005 (0.007)</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>-5.134 (0.2968)</td>
<td>0.919 (&lt;0.0001)</td>
<td>-</td>
<td>0.004 (0.391)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>-0.657 (0.2905)</td>
<td>0.031 (0.1712)</td>
<td>0.009 (0.3283)</td>
<td>0.001 (0.179)</td>
</tr>
<tr>
<td>IL6</td>
<td>-0.923 (0.0238)</td>
<td>0.058 (&lt;0.0001)</td>
<td>0.004 (0.5192)</td>
<td>0.001 (0.019)</td>
</tr>
<tr>
<td>hs CRP</td>
<td>-7.616 (0.0230)</td>
<td>0.596 (&lt;0.0001)</td>
<td>0.111 (0.0344)</td>
<td>0.07 .029</td>
</tr>
</tbody>
</table>

* all value are given in beta-coefficient (p-value)

**Nutritional factors**

Serum vitamin B12 levels were significantly lower and Hcy was significantly higher in diabetic group compared to non-diabetic patients (Table-18). Folic acid was comparable in both groups. Vitamin B12 deficiency was detected in 86.7% of patients (cases 90.4% vs. control 84%; p=0.15). Hyperhomocysteinemia was observed in 95.3% subjects with no significant difference between cases and controls (97.6% vs. 93.7%);
Only 2.5% subjects had folic acid deficiency (cases 3.2% vs. control 2.3%; p=0.90).

Vitamin B12 levels were negatively correlated with Hcy levels in total study population and both groups separately. It was also negatively correlated with HOMA-IR in diabetic patients only. There was no direct correlation between vitamin B12 levels and inflammatory markers. Hcy levels were positively correlated with inflammatory markers in total study population and non-diabetic patients; and there was no correlation in diabetic

### Table-21
Comparison of HOMA-IR and QUICKI in relation to CVD risk factors

<table>
<thead>
<tr>
<th></th>
<th>HOMA IR</th>
<th>P value</th>
<th>Insulin levels</th>
<th>P value</th>
<th>QUICKI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SD</td>
<td></td>
<td>Mean ±SD</td>
<td></td>
<td>Mean ±SD</td>
<td></td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>18.1±21.9</td>
<td>0.9401</td>
<td>45.7±38.3</td>
<td>0.0676</td>
<td>0.27±0.038</td>
<td>0.8782</td>
</tr>
<tr>
<td>No</td>
<td>18.2±20.5</td>
<td></td>
<td>52.7±46.1</td>
<td></td>
<td>0.28±0.044</td>
<td></td>
</tr>
<tr>
<td><strong>Physical inactivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>17.5±17.5</td>
<td>0.3513</td>
<td>48.6±35.7</td>
<td>0.9322</td>
<td>0.27±0.037</td>
<td>0.3036</td>
</tr>
<tr>
<td>No</td>
<td>18.6±23.04</td>
<td></td>
<td>51.1±47.7</td>
<td></td>
<td>0.28±0.044</td>
<td></td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>19.0±21.2</td>
<td>0.6993</td>
<td>49.6±41.5</td>
<td>0.7397</td>
<td>0.28±0.048</td>
<td>0.9043</td>
</tr>
<tr>
<td>≥25</td>
<td>17.9±21.01</td>
<td></td>
<td>50.3±44</td>
<td></td>
<td>0.27±0.039</td>
<td></td>
</tr>
<tr>
<td><strong>Central Obesity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>19.7±21.7</td>
<td>0.069</td>
<td>52.9±41.6</td>
<td>0.1086</td>
<td>0.27±0.03</td>
<td>0.056</td>
</tr>
<tr>
<td>No</td>
<td>14.9±19.5</td>
<td></td>
<td>44.2±46.7</td>
<td></td>
<td>0.29±0.04</td>
<td></td>
</tr>
<tr>
<td><strong>DM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>26.3±28.7</td>
<td>0.0001</td>
<td>51.7±51.2</td>
<td>&lt;0.0001</td>
<td>0.26±0.028</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No</td>
<td>12.4±9.5</td>
<td></td>
<td>49.0±37.0</td>
<td></td>
<td>0.29±0.046</td>
<td></td>
</tr>
<tr>
<td><strong>HTN</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>19.1±7.2</td>
<td>&lt;0.0001</td>
<td>64.6±43.3</td>
<td>&lt;0.0001</td>
<td>0.25±0.009</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No</td>
<td>17.7±24.9</td>
<td></td>
<td>25.8±31.3</td>
<td></td>
<td>0.29±0.047</td>
<td></td>
</tr>
<tr>
<td><strong>DM &amp; HTN</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>28.2±30.6</td>
<td>0.0004</td>
<td>54.0±53.3</td>
<td>&lt;0.0001</td>
<td>0.26±0.029</td>
<td>0.0004</td>
</tr>
<tr>
<td>No</td>
<td>13.7±12.5</td>
<td></td>
<td>48.4±38.2</td>
<td></td>
<td>0.28±0.044</td>
<td></td>
</tr>
<tr>
<td><strong>Dyslipidemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>29.1±25.7</td>
<td>&lt;0.0001</td>
<td>69.3±45.7</td>
<td>0.3613</td>
<td>0.25±0.025</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No</td>
<td>10.4±12.06</td>
<td></td>
<td>36.6±36.2</td>
<td></td>
<td>0.29±0.043</td>
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</tr>
</tbody>
</table>
subjects (Table-19). This correlation persisted even after adjustment for age, sex, BMI in multiple regression analysis (Table-20).

### Table- 22
Cardiovascular risk factors according to HOMA-IR

<table>
<thead>
<tr>
<th></th>
<th>HOMA IR≤2.5</th>
<th>HOMA IR&gt;2.5</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8(27.6%)</td>
<td>103(38%)</td>
<td>0.3668</td>
</tr>
<tr>
<td>No</td>
<td>21(72.4%)</td>
<td>168(62%)</td>
<td></td>
</tr>
<tr>
<td><strong>Physical inactivity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8(27.6%)</td>
<td>108(40%)</td>
<td>0.2699</td>
</tr>
<tr>
<td>No</td>
<td>21(72.4%)</td>
<td>162(60%)</td>
<td></td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>10(14.5%)</td>
<td>59(85.5%)</td>
<td>0.1888</td>
</tr>
<tr>
<td>≥25</td>
<td>19(8.2%)</td>
<td>212(91.8%)</td>
<td></td>
</tr>
<tr>
<td><strong>Central Obesity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>16(7.6%)</td>
<td>194(92.4%)</td>
<td>0.1052</td>
</tr>
<tr>
<td>No</td>
<td>13(14.4%)</td>
<td>77(85.6%)</td>
<td></td>
</tr>
<tr>
<td><strong>DM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
<td>125(46.1%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No</td>
<td>29(100%)</td>
<td>146(53.9%)</td>
<td></td>
</tr>
<tr>
<td><strong>HTN</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
<td>95(35.1%)</td>
<td>0.0002</td>
</tr>
<tr>
<td>No</td>
<td>29(100%)</td>
<td>176(64.9%)</td>
<td></td>
</tr>
<tr>
<td><strong>DM &amp; HTN</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
<td>93(34.3%)</td>
<td>0.0003</td>
</tr>
<tr>
<td>No</td>
<td>29(100%)</td>
<td>178(65.7%)</td>
<td></td>
</tr>
<tr>
<td><strong>Dyslipidemia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
<td>124(45.8%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No</td>
<td>29</td>
<td>147(54.2%)</td>
<td></td>
</tr>
<tr>
<td><strong>FBS</strong></td>
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</tr>
<tr>
<td>Yes</td>
<td>93(72-120)</td>
<td>115(72-390)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No</td>
<td>154(109-238)</td>
<td>170(91-343)</td>
<td>0.0558</td>
</tr>
<tr>
<td><strong>Cholesterol</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>135(64-231)</td>
<td>173(39-341)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No</td>
<td>48(35-59)</td>
<td>37(20-59)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>HDL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>82.8(41.6-179.4)</td>
<td>94.4(12.6-273.8)</td>
<td>0.0322</td>
</tr>
<tr>
<td>No</td>
<td>27(2-46.2)</td>
<td>34(3.4-68.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>LDL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.7(0.1-19.4)</td>
<td>12.72(0.11-37.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>No</td>
<td>5.1(0.5-206)</td>
<td>29.8(0.4-253.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>HsCRP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7.8(0.5-81.7)</td>
<td>10.9(0.5-525.8)</td>
<td>0.0016</td>
</tr>
<tr>
<td>No</td>
<td>0.38(0.33-0.43)</td>
<td>0.26(0.21-0.33)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Insulin resistance and traditional risk factors

Subjects with DM and/or HT, and dyslipidemia were more insulin resistance (HOMA IR), and had lower insulin sensitivity (QUICKI). HOMA IR was significantly high but QUICKI was significantly low in subjects with diabetes. Similar observations were there in subjects with diabetes with HTN. However, patients with HTN had significantly high HOMA IR but significantly lower insulin sensitivity (QUICKI). Subjects with dyslipidemia had high HOMA IR and low insulin sensitivity. There was no difference in HOMA-IR and QUICKI in subjects with or without obesity, central obesity, physical inactivity and history of smoking. (Table-21)

When HOMA IR was divided into two groups; HOMA IR ≤2.5 (group 1) and HOMA IR>2.5 (group 2); subjects with DM were higher in group 2 compared to group 1. Similar results were obtained for HTN, DM, DM with HTN, dyslipidemia. Smoking, physical activities, BMI, WHR were comparable in both groups (Table 22).

Table-23
Univariate analysis of the parameters with HOMA-IR and QUICKI

<table>
<thead>
<tr>
<th></th>
<th>HOMA IR</th>
<th>INSULIN</th>
<th>QUICKI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
<td>p value</td>
<td>r value</td>
</tr>
<tr>
<td>Age</td>
<td>0.015</td>
<td>0.796</td>
<td>0.002</td>
</tr>
<tr>
<td>Smoking</td>
<td>-0.009</td>
<td>0.877</td>
<td>-0.078</td>
</tr>
<tr>
<td>Less physical activity</td>
<td>-0.033</td>
<td>0.570</td>
<td>-0.029</td>
</tr>
<tr>
<td>BMI</td>
<td>0.033</td>
<td>0.563</td>
<td>0.032</td>
</tr>
<tr>
<td>WHR</td>
<td>0.016</td>
<td>0.784</td>
<td>0.036</td>
</tr>
<tr>
<td>DM</td>
<td>0.345</td>
<td>&lt;0.0001</td>
<td>0.030</td>
</tr>
<tr>
<td>HTN</td>
<td>0.325</td>
<td>&lt;0.0001</td>
<td>0.432</td>
</tr>
<tr>
<td>DM&amp;HTN</td>
<td>0.328</td>
<td>&lt;0.0001</td>
<td>0.059</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>0.438</td>
<td>&lt;0.0001</td>
<td>0.371</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.031</td>
<td>0.594</td>
<td>0.052</td>
</tr>
<tr>
<td>TG</td>
<td>0.260</td>
<td>&lt;0.0001</td>
<td>0.236</td>
</tr>
<tr>
<td>HDL</td>
<td>-0.344</td>
<td>&lt;0.0001</td>
<td>-0.322</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.260</td>
<td>&lt;0.0001</td>
<td>0.236</td>
</tr>
<tr>
<td>LDL</td>
<td>0.043</td>
<td>0.459</td>
<td>0.063</td>
</tr>
</tbody>
</table>

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IL6, TNF-α, hsCRP and Insulin levels were significantly high in group with high insulin resistance. Among the lipid parameters, TG, HDL, LDL, VLDL were significantly high in group 2, except cholesterol which was comparable in both groups (Table 18).

In univariate analysis HOMA IR showed negative association with HDL, positive association with dyslipidemia, TG, VLDL, DM, DM with HTN and Insulin. There was no association between HOMA IR, age, BMI, WHR, Cholesterol, HTN, smoking and physical activity (Table-23). Insulin sensitivity (QUICKI) in univariate analysis showed positive association with TG and HDL and negative association with VLDL, DM, DM with HTN and Dyslipidemia.

**4.4.2 DISCUSSION**

In this study there are significant differences between diabetics and non-diabetics in known cases of CVD compared to population based observational and prospective studies (39,319). Among traditional risk factors, there is no difference in BMI, WHR and number of subjects with obesity and central obesity between two groups. A similar observation was made by recently published study. (835) Obesity and central obesity are strong risk factor for type 2 DM and CVD (836,837), hence we expect this difference will persist even after onset of CVD. It may due to similar risk conferred by obesity and central obesity to CVD in subjects with or without diabetes. HTN is more common in diabetic subjects as compared to non-diabetic patients. There is a strong relationship between diabetes and HTN (838). Several studies showed that prevalence of hypertensive in type 2 diabetes is between 50 and 73% (39,839).This high prevalence of HTN in type 2 DM is associated with the presence of insulin resistance syndrome due to activation of sympathoadrenal system, rennin angiotensin system and sodium retention(838). Among lipid abnormalities; only significant difference is observed for serum TG and HDL levels between two groups; which reflects underlying higher insulin resistance in diabetic subjects and its effect. There is no difference in TC and LDL cholesterol levels between two groups. Hence, it may be inferred that LDL cholesterol confers similar risk in diabetics and non-diabetics as also reported by Strong Heart Study where hazard ratio (1.07) was equal in both group (835).

In present study, diabetic subjects has higher insulin resistance, decreased insulin sensitivity and beta cell function compared to subjects without diabetes, which is in accordance with current understanding of pathogenesis of diabetes (840). However, if we
consider a cut-off of 2.6 for HOMA-IR as indicator of insulin resistance in subjects with normoglycemia (841) and 3.8 in subjects with diabetes (842), both group had evidence of underlying severe insulin resistance. Insulin resistance and its associated abnormalities may have important role in development and progression of atherosclerosis and cardiovascular disease in Indian population (843). Exact cause of insulin resistance is not known. Genetic, nutritional, and environmental factors have been implicated to explain insulin resistance. In present study HOMA-IR is positively correlated with Hcy levels and inflammatory markers in subjects without diabetes only. In subjects with diabetes HOMA-IR is positively correlated with glycemic control. It suggests that in diabetic patients, glycemic status play more important role in insulin resistance than Hcy levels or inflammatory markers. This further emphasizes association of insulin resistance with nutritional factor (844) and subclinical inflammation (845).

In present study, inflammatory markers are significantly higher in subjects with diabetes compared with those without diabetes. Several studies have reported higher levels of inflammatory markers in diabetic compared to non-diabetics in subjects without underlying CVD (845,846) or with CVD (629). Inflammatory markers (hsCRP and IL-6) are positively associated with insulin resistance in subjects without diabetes and with glycemic control in subjects with diabetes in this study. In a study among U.S. adults aged 17 years and over, elevated CRP concentrations increased with increasing HbA1c levels and suggested an association between glycemic control and systemic inflammation in people with established diabetes (847). Increase in inflammatory markers has been associated with progression of coronary artery disease (848) and diabetes (617) in prospective population based studies. However, in present study, though TNF-α level is higher in diabetic subjects than non-diabetics; it does not show any association with insulin resistance. TNF-α levels are positively related with glycemic control. Larger studies had shown positive correlation of TNF-α with insulin resistance (617,848). TNF-α is also associated with amount of adipose tissue (846). There is no difference in BMI in both groups in this study which may explain our finding. Inflammation and inflammatory markers increase expression of adhesion molecules in vascular endothelium and may play an active role in initiation, progression and ultimately the thrombotic complications of atherosclerosis (849).
In present study, a high percentage of subjects have vitamin B12 deficiency and hyperhomocysteinemia; which is more marked in subjects with diabetes. Dietary vitamin B12 deficiency is a severe problem in India due to vegetarianism and causes hyperhomocysteinemia (850,851). Similarly high prevalence of hyperhomocysteinemia has been reported in Indian population even without coronary artery disease (481,489) and also with coronary artery disease (497). Yajnik et al (489) reported vitamin B12 deficiency and hyperhomocysteinemia in 81% and 79% of urban middle class population without coronary artery disease. Vitamin B12 deficiency is associated with coronary artery disease in Indian population (852). In this study, all subjects have underlying coronary artery disease which can explain high percentage of vitamin B12 deficiency. Serum vitamin B12 levels are significantly lower and Hcy levels are significantly higher in subjects with diabetes than those without diabetes in present study. Several studies have reported similar finding (479, 500,816). However, another study from India did not find any difference in serum vitamin B12 and homocysteine levels in subjects with coronary artery disease with or without diabetes (815). Serum vitamin B12 levels had significant negative association with Hcy levels in this study and also reported by others from India (489,497,852). Vitamin B12 acts as a coenzyme while folic acid provides the methyl essential for the reactions to take place. Therefore, folic acid and vitamin B12 deficiency can cause reduction in methylene tetrahydrofolate reductase activity, leading to decrease in methionine synthesis and Hcy accumulation (853). A meta-analysis of 27 observational studies (854) and population based prospective studies (855) have shown Hcy to be independent risk factor for CAD independent of other cardiovascular risk factors. Though supplementation of vitamin B12 has shown to reduce Hcy levels, (498,492) meta-analysis of several trials did not reveal any cardiovascular benefit (856).

In this study, though Hcy is positively correlated with insulin resistance and inflammatory markers in study population; serum vitamin B12 levels are not. This suggests vitamin B12 does not have direct association with non-traditional cardiovascular risk factors, but may plays role indirectly through increasing Hcy levels. This is also supported by a cross-sectional study in Asian Indians, which reported no correlation between serum vitamin B12 and HOMA-IR (499). Framingham offspring study demonstrated a modest association between hyperinsulinemia and fasting Hcy levels (477). Some studies have
observed positive association between Hcy and insulin resistance (477,818) whereas others were unable to document such association (478,491). Similar findings have been noted for association of Hcy and inflammatory markers (482). These differences can be explained by different subset of population studies, differences in anthropometric parameters and associated comorbidities. Several mechanisms have been proposed to explain the atherogenic actions of Hcy, which include vascular endothelial dysfunction, direct cytotoxic effects to vascular endothelial cells, proliferation of vascular smooth muscle cells, lipid peroxidation, platelet activation and induction of inflammation (430).

HOMA IR was higher in these patients with CAD (392) and it is associated with the occurrence of major cardiovascular events and CHD (382,857). HOMA IR was not found to be significant risk factor for CHD (400). There is no association between HOMA IR with age, sex, BMI, WHR in the present study. A study done Korea showed that, there was no association between of HOMA IR with age, BMI, WHR, Smoking and physical activity in univariate analysis in obese male adolescents (624). HOMA-IR was more strongly correlated with BMI and waist-to-hip ratio in a study by Song et al (858). In another study HOMA IR was positively associated with BMI and WHR (393). Insulin resistance, sensitivity and Insulin levels are not associated with any of the traditional risk factors. For communities in a transition phase of lifestyle, encouraging physical activity may help prevent insulin resistance and its adverse consequences. The prevalence of physical inactivity increased linearly with increasing HOMA-IR quintiles (390). In the present study, group with high insulin resistance have more subjects with DM, HTN, DM with HTN and dyslipidemia. Subjects with HTN, DM with HTN, Dyslipidemia has more Insulin resistance (HOMA-IR). Esteghamati A et al showed that HOMA IR is associated with HTN in Iranian diabetic and non-diabetic subjects (387). High HOMA-IR was independently and consistently associated with an increased diabetes risk in a multiethnic cohort of U.S. postmenopausal women. As expected, women with diabetes had significantly higher levels of baseline levels of HOMA-IR (858). There were no significant interactions between HOMA-IR and ethnicity, sex, HTN, dyslipidemia, glucose tolerance (impaired glucose tolerance versus normal glucose tolerance), or obesity. The magnitude and direction of the relationship between insulin concentration and incident CVD were similar (382). Furthermore, waist circumference was not a significant variable in
multivariate analysis, suggesting that IR increases CVD risk independent of central adiposity (382).

We observed correlation of HOMA IR with DM, HTN, dyslipidemia including triglycerides, VLDL, except cholesterol and HTN in univariate analysis. Insulin resistance contributes to the development of HTN, a well established risk factor of CVD (339,343). A number of prospective and cross sectional studies have documented a correlation among insulin resistance, hyperinsulinemia and elevated BP (379,338). In univariate regression analysis, there is positive association of HOMA IR with TG, VLDL dyslipidemia and negatively with HDL Cholesterol. In Chennai Urban Rural Epidemiology Study (CURES) an epidemiological study in a representative population of Chennai, in South India, HOMA IR was found to be significantly associated with systolic BP, diastolic BP, TC, serum TG, LDL Cholesterol and HDL Cholesterol (396). Subjects with DM, HTN, HTN with DM, dyslipidemia had low insulin sensitivity. Insulin sensitivity in the present study has negative association with VLDL, DM, HTN, DM with HTN, dyslipidemia, insulin, and positively associated with TG, HDL. Insulin sensitivity index showed a significant inverse association with carotid artery atherosclerosis that was independent of insulin concentration and other CVD risk factors (859).

4.4.3 CONCLUSION

Subjects with diabetes have more traditional risk factors (HTN, dyslipidemia) than subjects without diabetes in known patients with CAD. However, there is no difference in BMI and WHR. Diabetic patients have higher levels of insulin resistance, inflammatory markers and Hcy compared to non-diabetics. Vitamin B12 deficiency is common and subjects with diabetes have lower levels of vitamin B12. Nutritional factors are inter-related with insulin resistance and inflammation and may play an important role in pathogenesis of diabetes and cardiovascular disease. Though prospective trials were unable to show beneficial effects of vitamin supplementation (856); long term prospective studies are required in our population with underlying high prevalence of nutritional deficiency to show beneficial effect of nutritional supplementation on non-communicable diseases. The result of our study suggest that decrease in insulin sensitivity, increase in insulin resistance with high insulin is the main determinants of progression to CVD.
INFLAMMATORY MARKERS AND ITS ASSOCIATION WITH CARDIOVASCULAR RISK FACTORS
4.5 INFLAMMATORY MARKERS AND ITS ASSOCIATION WITH CARDIOVASCULAR RISK FACTORS

4.5.1 RESULTS

hsCRP levels were higher in subjects with dyslipidemia, diabetes and/or HTN compared to non diabetics and normotensives. There was no significant difference in IL-6 levels between sexes, smokers and non-smokers; and subjects with or without obesity or central obesity. IL-6 levels were higher in subjects with dyslipidemia, diabetes and/or HTN compared to those without these risk factors. TNF-α level was also significantly higher in smokers, diabetes and/or HTN, dyslipidemia compared to subjects with absence of these risk factors (Table-24).

Table-24
Association of risk factors with inflammatory markers

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>hsCRP (mg/L)</th>
<th>IL6 (pg/ml)</th>
<th>TNF-α (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SD P value</td>
<td>Mean ±SD P value</td>
<td>Mean ±SD P value</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤65</td>
<td>11.4±9.6 0.5784</td>
<td>61.7±74.6 0.4169</td>
<td>27.3±49.2 0.9565</td>
</tr>
<tr>
<td>&gt;65</td>
<td>12.0±9.8</td>
<td>69.0±76.3</td>
<td>21.9±21.3</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11.8±10.02 0.5780</td>
<td>62.3±73.8 0.4216</td>
<td>23.8±41.8 0.3242</td>
</tr>
<tr>
<td>Female</td>
<td>11.1±8.72</td>
<td>70.1±78.9</td>
<td>29.0±38.1</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12.7±9.6 0.1295</td>
<td>70.4±74.7 0.2965</td>
<td>32.6±56.3 0.0095</td>
</tr>
<tr>
<td>No</td>
<td>11.0±9.6</td>
<td>61.0±75.5</td>
<td>20.9±27.2</td>
</tr>
<tr>
<td>Physical inactivity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11.4±9.2 0.7293</td>
<td>65.4±75.6 0.8645</td>
<td>25.1±34.2 0.9928</td>
</tr>
<tr>
<td>No</td>
<td>11.8±9.9</td>
<td>63.9±75.2</td>
<td>25.3±44.6</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>10.8±9.7 0.4251</td>
<td>54.6±70.2 0.2328</td>
<td>24.2±65.5 0.1799</td>
</tr>
<tr>
<td>&gt;25</td>
<td>11.9±9.6</td>
<td>67.2±76.4</td>
<td>25.5±30.9</td>
</tr>
<tr>
<td>Central Obesity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12.3±9.6 0.0878</td>
<td>64.8±73.8 0.9180</td>
<td>27.6±47.0 0.6182</td>
</tr>
<tr>
<td>No</td>
<td>10.2±9.5</td>
<td>63.8±78.6</td>
<td>20.2±21.4</td>
</tr>
<tr>
<td>DM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14.4±10.2 &lt;0.0001</td>
<td>78.2±78.4 0.0073</td>
<td>25.7±28.5 0.0118</td>
</tr>
<tr>
<td>No</td>
<td>9.6±8.7</td>
<td>54.7±71.5</td>
<td>24.9±47.8</td>
</tr>
<tr>
<td>HTN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>13.1±9.3 0.0005</td>
<td>73.1±77.6 0.0097</td>
<td>29.2±48.6 0.0002</td>
</tr>
<tr>
<td>No</td>
<td>9.1±9.6</td>
<td>50.0±68.9</td>
<td>18.6±20.8</td>
</tr>
<tr>
<td>DM &amp; HTN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14.9±9.71 0.0001</td>
<td>79.2±77.5 0.0235</td>
<td>28.4±31.0 0.0009</td>
</tr>
<tr>
<td>No</td>
<td>10.1±9.2</td>
<td>57.9±73.4</td>
<td>23.8±44.4</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>17.3±8.3 &lt;0.0001</td>
<td>99.4±76.5 &lt;0.0001</td>
<td>34.8±54.8 &lt;0.0001</td>
</tr>
<tr>
<td>No</td>
<td>7.6±8.4</td>
<td>39.9±63.9</td>
<td>18.5±25.0</td>
</tr>
</tbody>
</table>
Figure-7
Scatter plot with trendline showing correlation between hsCRP and triglycerides

\[ y = 1.762x + 150.5 \]
\[ R^2 = 0.135 \]
\[ P < 0.0001 \]

Figure-8
Scatter plot with trendline showing correlation between hsCRP and HDL cholesterol

\[ y = -0.487x + 44.66 \]
\[ R^2 = 0.261 \]
\[ P < 0.0001 \]
hsCRP was positively correlated with total and LDL cholesterol, TGs (Figure-7), insulin levels, HOMA-IR and negatively correlated with insulin secretion and HDL cholesterol (Figure-8) in univariate analysis. IL-6 was positively correlated with DM (r=0.155, p=0.007), HTN (r=0.149, p=0.01) and dyslipidemia (r= 0.390; p=<0.0001); but after adjustment for other risk factors in multiple regression analysis, it showed only association with dyslipidemia. It was positively correlated with all lipid parameters except HDL cholesterol with which it had significant negative correlation. IL-6 showed positive correlation with insulin levels, HOMA-IR and negative correlation with insulin sensitivity assessed by QUICKI. IL-6 also did not show any correlation between BMI and WHR, it was independent of physical activity and smoking. Similar to other inflammatory markers, there was no correlation between BMI and WHR with TNF-α and it was independent of physical activity. However, it was negatively correlated with age and positively with smokers. (Table-25)

**Table 25**

**Correlation of inflammatory markers with risk factors**

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>hsCRP r (p value)</th>
<th>IL-6 r (p value)</th>
<th>TNF-α r (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>0.059(0.310)</td>
<td>0.064(0.269)</td>
<td>-0.147(0.011)</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.088(0.130)</td>
<td>0.060(0.296)</td>
<td>0.138(0.017)</td>
</tr>
<tr>
<td>Physical inactivity</td>
<td>-0.020(0.729)</td>
<td>0.010(0.864)</td>
<td>-0.003(0.961)</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>0.014(0.805)</td>
<td>0.076(0.191)</td>
<td>-0.028(0.633)</td>
</tr>
<tr>
<td>WHR</td>
<td>0.037(0.526)</td>
<td>-0.011(0.849)</td>
<td>0.043(0.461)</td>
</tr>
<tr>
<td>DM</td>
<td>0.244(&lt;0.0001)</td>
<td>0.155(0.007)</td>
<td>0.010(0.857)</td>
</tr>
<tr>
<td>HTN</td>
<td>0.201(&lt;0.0001)</td>
<td>0.149(0.010)</td>
<td>0.126(0.029)</td>
</tr>
<tr>
<td>DM, HTN</td>
<td>0.230(&lt;0.0001)</td>
<td>0.131(0.023)</td>
<td>0.052(0.369)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>0.492(&lt;0.0001)</td>
<td>0.390(&lt;0.0001)</td>
<td>0.196(0.001)</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>0.150(0.009)</td>
<td>0.188(0.001)</td>
<td>-0.024(0.681)</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>0.368(&lt;0.0001)</td>
<td>0.257(&lt;0.0001)</td>
<td>0.135(0.019)</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>-0.511(&lt;0.0001)</td>
<td>-0.392(&lt;0.0001)</td>
<td>-0.198(0.001)</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>0.162(0.005)</td>
<td>0.195(0.001)</td>
<td>-0.010(0.859)</td>
</tr>
<tr>
<td>VLDL(mg/dl)</td>
<td>0.368(&lt;0.0001)</td>
<td>0.257(&lt;0.0001)</td>
<td>0.135(0.019)</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>0.153(0.008)</td>
<td>0.105(0.070)</td>
<td>0.044(0.449)</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>0.214(&lt;0.0001)</td>
<td>0.163(0.005)</td>
<td>0.035(0.540)</td>
</tr>
<tr>
<td>QUICKI</td>
<td>-0.298(&lt;0.0001)</td>
<td>-0.222(&lt;0.0001)</td>
<td>-0.090(0.119)</td>
</tr>
</tbody>
</table>
However, after adjustment with other risk factor the association between hsCRP and HTN was lost but the significance was maintained with dyslipidemia and DM (HTN; beta coefficient 1.475, p=0.15, DM; beta coefficient 2.539, p=0.012, Dyslipidemia; beta coefficient 9.013, p<0.0001). hsCRP was divided into quartiles and it also confirmed the above finding regarding dyslipidemic, hypertensive and diabetic subjects. TC, TG, LDL, and VLDL increased significantly with quartiles of hsCRP except HDL which decreased (Table-26).

Table-26
Association of risk factors with quartiles of hsCRP

<table>
<thead>
<tr>
<th>hsCRP (mg/L)</th>
<th>≤2.4</th>
<th>&gt;2.4-12</th>
<th>&gt;12-18.9</th>
<th>&gt;18.9</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>24(31.2)</td>
<td>27(36)</td>
<td>27(37.5)</td>
<td>33(43.4)</td>
<td>0.4740</td>
</tr>
<tr>
<td>P value</td>
<td>0.6463</td>
<td>0.5214</td>
<td>0.1615</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical inactivity</td>
<td>29 (37.7)</td>
<td>34 (45.3)</td>
<td>23 (31.9)</td>
<td>30 (39.5)</td>
<td>0.4184</td>
</tr>
<tr>
<td>P value</td>
<td>0.4265</td>
<td>0.5756</td>
<td>0.9489</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight/Obesity</td>
<td>60 (77.9)</td>
<td>53 (70.7)</td>
<td>55 (76.4)</td>
<td>63 (82.9)</td>
<td>0.3558</td>
</tr>
<tr>
<td>P value</td>
<td>0.8208</td>
<td>0.9780</td>
<td>0.5680</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>25 (32.5)</td>
<td>22 (29.3)</td>
<td>34 (47.2)</td>
<td>44 (57.9)</td>
<td>0.0009</td>
</tr>
<tr>
<td>P value*</td>
<td>0.9301</td>
<td>0.9780</td>
<td>1.70(1.19-2.43)</td>
<td>0.0027</td>
<td></td>
</tr>
<tr>
<td>DM&amp;HTN</td>
<td>16(20.8)</td>
<td>16(21.3)</td>
<td>27(37.5)</td>
<td>34(44.7)</td>
<td>0.0017</td>
</tr>
<tr>
<td>P value</td>
<td>1.0101</td>
<td>1.36(0.96-1.92)</td>
<td>1.76(1.28-2.4)</td>
<td>0.0006</td>
<td></td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>8(10.4)</td>
<td>16(21.3)</td>
<td>46(63.9%)</td>
<td>54(71.1%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P value</td>
<td>0.9082</td>
<td>0.0384</td>
<td>0.0028</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>4.37±0.98</td>
<td>4.64±1.18</td>
<td>4.73±1.24</td>
<td>4.81±1.26</td>
<td>0.1047</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.73±0.46</td>
<td>1.75±0.42</td>
<td>2.04±0.49</td>
<td>2.2±0.54</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.16±0.21</td>
<td>1.08±0.24</td>
<td>0.91±0.18</td>
<td>0.86±0.16</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>2.4±1.06</td>
<td>2.71±1.2</td>
<td>2.88±1.36</td>
<td>2.93±1.43</td>
<td>0.0555</td>
</tr>
<tr>
<td>VLDL(mmol/l)</td>
<td>0.79±0.20</td>
<td>0.80±0.19</td>
<td>0.93±0.22</td>
<td>1.01±0.24</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>WHR</td>
<td>0.92±0.06</td>
<td>0.92±0.06</td>
<td>0.92±0.06</td>
<td>0.92±0.05</td>
<td>0.7853</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>256.2±231.9</td>
<td>320.1±250</td>
<td>428.5±407.6</td>
<td>392.3±267.3</td>
<td>0.002</td>
</tr>
<tr>
<td>HOMAIR</td>
<td>11.1±13.4</td>
<td>14.5±14.2</td>
<td>23.8±27.8</td>
<td>23.5±23.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.3±0.04</td>
<td>0.28±0.04</td>
<td>0.27±0.03</td>
<td>0.26±0.03</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*P value between lowest quartile and the column of quartile
Diabetic, hypertensive and dyslipidemic subjects showed similar findings after division into tertiles. All lipid parameters increased significantly with quartiles of IL-6 except HDL which decreased (Table-27).

### Table-27
Association of risk factors with quartiles of IL6

<table>
<thead>
<tr>
<th>IL-6 (pg/ml)</th>
<th>≤6.6</th>
<th>&gt;6.6-25</th>
<th>&gt;25-107</th>
<th>&gt;107</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value*</td>
<td>0.2999</td>
<td>1.000</td>
<td>0.6643</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical inactivity</td>
<td>22(29.7%)</td>
<td>35(45.5%)</td>
<td>34(43.9%)</td>
<td>25(33.3%)</td>
<td>0.0881</td>
</tr>
<tr>
<td>P value</td>
<td>0.0680</td>
<td>0.0622</td>
<td>0.8610</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight/Obesity</td>
<td>53(71.6%)</td>
<td>62(80.5%)</td>
<td>53(71.6%)</td>
<td>63(84%)</td>
<td>0.1695</td>
</tr>
<tr>
<td>P value</td>
<td>0.0707</td>
<td>0.0622</td>
<td>0.8610</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>22(29.7%)</td>
<td>29(37.7%)</td>
<td>33(44.6%)</td>
<td>41(54.7%)</td>
<td>0.0160</td>
</tr>
<tr>
<td>P value</td>
<td>0.1708</td>
<td>0.0444</td>
<td>0.0036</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM&amp;HTN</td>
<td>14(18.9%)</td>
<td>22(28.6%)</td>
<td>27(36.5%)</td>
<td>30(40%)</td>
<td>0.0274</td>
</tr>
<tr>
<td>P value</td>
<td>0.0442</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>10(13.5%)</td>
<td>15(19.5%)</td>
<td>47(63.5%)</td>
<td>52(69.3%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P value</td>
<td>0.0442</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>4.46±1.11</td>
<td>4.48±1.02</td>
<td>4.62±1.12</td>
<td>4.93±1.32</td>
<td>0.0510</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>1.28(0.93-1.78)</td>
<td>1.63(1.19-2.24)</td>
<td>1.65(1.22-2.27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.1708</td>
<td>0.0444</td>
<td>0.0036</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.75±0.45</td>
<td>1.76±0.44</td>
<td>2.08±0.48</td>
<td>2.13±0.58</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>1.28(0.93-1.78)</td>
<td>1.63(1.19-2.24)</td>
<td>1.65(1.22-2.27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.1708</td>
<td>0.0444</td>
<td>0.0036</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.13±0.20</td>
<td>1.11±0.25</td>
<td>0.91±0.17</td>
<td>0.86±0.18</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>1.28(0.93-1.78)</td>
<td>1.63(1.19-2.24)</td>
<td>1.65(1.22-2.27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.1708</td>
<td>0.0444</td>
<td>0.0036</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>2.52±1.2</td>
<td>2.56±1.13</td>
<td>2.75±1.33</td>
<td>3.09±1.48</td>
<td>0.0285</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>1.28(0.93-1.78)</td>
<td>1.63(1.19-2.24)</td>
<td>1.65(1.22-2.27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.1708</td>
<td>0.0444</td>
<td>0.0036</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL (mmol/l)</td>
<td>0.80±0.20</td>
<td>0.80±0.20</td>
<td>0.95±0.22</td>
<td>0.97±0.26</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>1.28(0.93-1.78)</td>
<td>1.63(1.19-2.24)</td>
<td>1.65(1.22-2.27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.1708</td>
<td>0.0444</td>
<td>0.0036</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td>0.93±0.06</td>
<td>0.91±0.06</td>
<td>0.92±0.06</td>
<td>0.92±0.05</td>
<td>0.1892</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>281.9±268.0</td>
<td>325.7±328.4</td>
<td>396.5±308.3</td>
<td>388.2±288.9</td>
<td>0.0652</td>
</tr>
<tr>
<td>Odds ratio</td>
<td>1.28(0.93-1.78)</td>
<td>1.63(1.19-2.24)</td>
<td>1.65(1.22-2.27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.1708</td>
<td>0.0444</td>
<td>0.0036</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>12.1±14.6</td>
<td>15±20.1</td>
<td>23.2±24.9</td>
<td>22.5±21.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.29±0.04</td>
<td>0.28±0.04</td>
<td>0.27±0.03</td>
<td>0.26±0.03</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
| *P value between lowest quartile and the column of quartile

There was progressive increase in percentage of subjects with diabetes, HTN and dyslipidemia with increasing levels of TNF-α (quartiles). TC, TG, LDL, and VLDL also increased progressively with quartiles of TNF-α except HDL which decreased (Table-28).
Table 28

Association of risk factors with quartiles of TNF-α

<table>
<thead>
<tr>
<th>TNF-α (pg/ml)</th>
<th>≤8.0</th>
<th>&gt;8.0-10</th>
<th>&gt;10-25.3</th>
<th>&gt;25.3</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>32(30.2%)</td>
<td>17(37.8%)</td>
<td>26(35.1%)</td>
<td>36(48%)</td>
<td>0.1057</td>
</tr>
<tr>
<td>Odd ratio (95% CI)</td>
<td>1.11(0.87-1.40)</td>
<td>1.09(0.83-1.44)</td>
<td>1.39(1.04-1.85)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.4708</td>
<td>0.5915</td>
<td>0.0225</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Physical inactivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical inactivity</td>
<td>42(39.6%)</td>
<td>15(33.3%)</td>
<td>31(41.9%)</td>
<td>28(37.3%)</td>
<td>0.8106</td>
</tr>
<tr>
<td>Odd ratio (95% CI)</td>
<td>0.92(0.75-1.13)</td>
<td>1.03(0.80-1.33)</td>
<td>0.96(0.74-1.23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.5852</td>
<td>0.8801</td>
<td>0.8755</td>
<td></td>
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</tr>
<tr>
<td><strong>Overweight/Obesity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overweight/Obesity</td>
<td>80(75.5%)</td>
<td>32(71.1%)</td>
<td>53(71.6%)</td>
<td>66(88%)</td>
<td>0.0614</td>
</tr>
<tr>
<td>Odd ratio (95% CI)</td>
<td>0.93(0.72-1.19)</td>
<td>0.91(0.68-1.23)</td>
<td>1.35(1.06-1.73)</td>
<td></td>
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<tr>
<td>P value</td>
<td>0.7213</td>
<td>0.6846</td>
<td>0.0559</td>
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<td></td>
</tr>
<tr>
<td><strong>DM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>35(33%)</td>
<td>17(37.8%)</td>
<td>39(52.7%)</td>
<td>34(45.3%)</td>
<td>0.0535</td>
</tr>
<tr>
<td>Odd ratio (95% CI)</td>
<td>1.06(0.84-1.33)</td>
<td>1.41(1.07-1.86)</td>
<td>1.24(0.95-1.64)</td>
<td></td>
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</tr>
<tr>
<td>P value</td>
<td>0.7071</td>
<td>0.1272</td>
<td>0.0225</td>
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</tr>
<tr>
<td><strong>HTN</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>HTN</td>
<td>52(49.1%)</td>
<td>25(55.6%)</td>
<td>57(77%)</td>
<td>54(72%)</td>
<td>0.0033</td>
</tr>
<tr>
<td>Odd ratio (95% CI)</td>
<td>1.08(0.87-1.33)</td>
<td>1.59(1.25-2.01)</td>
<td>1.46(1.15-1.86)</td>
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</tr>
<tr>
<td>P value</td>
<td>0.5804</td>
<td>0.0033</td>
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</tr>
<tr>
<td><strong>DM&amp;HTN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM&amp;HTN</td>
<td>22(20.8%)</td>
<td>11(24.4%)</td>
<td>32(43.2%)</td>
<td>28(37.3%)</td>
<td>0.0053</td>
</tr>
<tr>
<td>Odd ratio (95% CI)</td>
<td>1.06(0.81-1.39)</td>
<td>1.63(1.15-2.3)</td>
<td>1.45(1.03-2.04)</td>
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</tr>
<tr>
<td>P value</td>
<td>0.7744</td>
<td>0.0221</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Dyslipidemia</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>10(9.4%)</td>
<td>23(51.1%)</td>
<td>46(62.2%)</td>
<td>45(60%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Odd ratio (95% CI)</td>
<td>2.68(1.58-4.53)</td>
<td>4.33(2.45-7.66)</td>
<td>4.19(2.3-7.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cholesterol (mmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>4.39±1.03</td>
<td>4.5±1.1</td>
<td>4.92±1.31</td>
<td>4.74±1.22</td>
<td>0.0160</td>
</tr>
<tr>
<td>P value</td>
<td>0.5340</td>
<td>0.0150</td>
<td>0.0388</td>
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<td></td>
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<tr>
<td><strong>Triglyceride (mmol/l)</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>1.74±0.41</td>
<td>1.91±0.67</td>
<td>2.0±0.46</td>
<td>2.05±0.53</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P value</td>
<td>0.0479</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HDL (mmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>1.16±0.21</td>
<td>0.99±0.24</td>
<td>0.87±0.15</td>
<td>0.91±0.20</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>LDL (mmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL</td>
<td>2.42±1.13</td>
<td>2.62±1.20</td>
<td>3.09±1.46</td>
<td>2.88±1.34</td>
<td>0.0046</td>
</tr>
<tr>
<td>P value</td>
<td>0.3156</td>
<td>0.0053</td>
<td>0.0142</td>
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</tr>
<tr>
<td><strong>VLDL (mmol/l)</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>0.79±0.18</td>
<td>0.87±0.30</td>
<td>0.95±0.21</td>
<td>0.94±0.24</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P value</td>
<td>0.0479</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>WHR</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td>0.92±0.06</td>
<td>0.91±0.05</td>
<td>0.92±0.06</td>
<td>0.92±0.05</td>
<td>0.5938</td>
</tr>
<tr>
<td>P value</td>
<td>0.2896</td>
<td>0.3926</td>
<td>0.9712</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Insulin (pmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>284.7±256.2</td>
<td>368.7±331.2</td>
<td>433.3±361.1</td>
<td>341.6±258.3</td>
<td>0.0112</td>
</tr>
<tr>
<td>P value</td>
<td>0.2277</td>
<td>0.0007</td>
<td>0.1444</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HOMA-IR</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>12±13.3</td>
<td>19±21.7</td>
<td>25.2±26</td>
<td>19.5±22.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P value</td>
<td>0.1241</td>
<td>&lt;0.0001</td>
<td>0.0012</td>
<td></td>
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</tr>
<tr>
<td><strong>QUICKI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.29±0.04</td>
<td>0.28±0.04</td>
<td>0.26±0.03</td>
<td>0.27±0.03</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P value</td>
<td>0.2741</td>
<td>&lt;0.0001</td>
<td>0.0037</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P value between lowest quartile and the column of quartile

Levels of inflammatory markers were compared with subject with and without metabolic syndrome. IL6 (77.6±79.4 vs. 41.2±60.7, p<0.0001), TNF-α (28.0±47.4 vs. 20.4±24.5, p<0.0001) and hsCRP (14.3±9.9 vs. 7.0±7.1, p<0.0001) levels were significantly high in subjects with metabolic syndrome compared to non metabolic syndrome. Subjects were also categorized into subject with modifiable risk factor and without modifiable risk factors. Subjects with modifiable risk factors had significantly
higher inflammatory levels (hsCRP; 12.8±9.56 vs.4.8±7.2, IL6; 72±77.1 vs. 21.0±42.5, TNF-α; 27.4±43.5 vs.12.5±13.9) compared to without modifiable risk factors.

4.5.2 DISCUSSION

Atherosclerosis is a chronic inflammatory state and many studies have demonstrated association of inflammatory markers with angiographically proven CAD (611, 620,627); elevated levels of these markers as a predictor of future ischemic events (616) and mortality from CAD (632). In present study, we have measured inflammatory markers in three hundred patients with angiographically proven coronary artery disease. Among inflammatory markers, hsCRP has been extensively studied and was part of two metanalysis of several prospective studies (860,861). Mean hsCRP levels are 11.7±9.7mg/L (median 12 mg/L) in present study. These levels are higher than those reported in the literature in subjects with coronary artery disease except a study from Brazil, which reported higher levels of hsCRP (Male 18.2±2.3mg/L and female 12.0±2.1mg/L) in angiographically proven patients with CAD (637). Lower levels of hsCRP in known case of CAD were reported from Japan (2.42±0.52mg/L) (862), Pakistan (4.26±0.521.42mg/L) (628); Saudi Arab (5.0±4.4mg/L, Habib 2008; 5.2±7.1mg/L and 8.2±7.8mg/L without/with DM respectively (638), and from Turkey (8.2 mg/L) (625). Similarly high level of hsCRP was also reported in a study among patients with atrial fibrillation with underlying CAD, DM and HT (14.0mg/L) from USA (631). Many studies have suggested as cut-off point of >3.0 mg/L for hsCRP for high risk for CAD (615,845,861). In the present study 73.6% of the patients has hsCRP levels >3.0 mg/L whereas other study reported 50% patients of CAD above this level (615). Elevated CRP (>3 mg/L) is found in >10% of normals, in >20% of patients with chronic stable or variant angina, but in >65% of patients with unstable angina, and in >90% of patients with acute infarction proceeded by unstable angina, but in >50% of those in whom the infarction was totally unheralded (576,577). This suggests that individuals may vary in their response to inflammatory stimuli; however, hsCRP levels were highest in Indians compared to other population. There are dietary differences among various populations. Dietary differences as high carbohydrate - fat diet (863); vitamin B12 deficiency and hyperhomocysteinemia (497) and hypomagnesemia (864) are associated with inflammation whereas increase intake of fish has been negatively associated with inflammatory markers in subjects with CAD (613). Insulin resistance and
associated metabolic syndrome is also associated with increased levels of inflammatory markers (616,627) and Indians have higher insulin resistance than other population (613,865).

Similar to our study, hsCRP was positively related with DM, HTN, and dyslipidemia in other studies (609,645). But in contrast to our study, other studies have shown positive relation between hsCRP and BMI, WHR and smoking (608,618,623,624,639,647). Most of patients in our study has high BMI and WHR on the background of already existing CAD, which may have masked the relation, observed by other studies. hsCRP levels are also positively correlated with TC, TG, LDL, plasma insulin levels, HOMA-IR, QUICKI and negatively with HDL levels. Similar association has been described by others (28,618,623,627). The odds of having elevated hs-CRP levels were approximately 4 times higher in participants with metabolic syndrome than in those without it (633).

Patients with features of metabolic syndrome have higher hsCRP level than those without MS in present study. Several studies have reported association of MS with elevated hsCRP levels (616,618,845). In this study, patients without modifiable risk factors (smoking, DM, HT and dyslipidemia), has higher hsCRP levels than reported in healthy young Indian population (845). This may suggest role of inflammation in patients without modifyable risk factors (615,866). Low grade inflammation in these patients may be related to subclinical infection (609); dietary factors (863) or environmental factors (867). Genetic and epigenetic factors have also been implicated in inflammation. DNA methylation is emerging as a primary regulator of inflammation. Methylation has been shown to control leukocyte functions related to cardiovascular risk, including the expression of soluble mediators and surface molecules that direct, adhesion, and migration of blood leukocytes in vascular tissues. DNA methylation states may vary over an individual’s lifetime, and have been shown to regulate biological processes underlying cardiovascular disease, such as atherosclerosis, inflammation, HTN, and diabetes (868,869,870). DNA methylation has been suggested to reflect hyperproliferation and altered functions of cell types involved in immune or inflammatory responses during atherosclerosis (871). Altered DNA methylation may be related to dietary factors like sufficiency of folic acid in presence of vitamin B12.
deficiency in Indian population (872). CRP itself has been implicated in genesis of atherosclerosis (873,874), however other suggests no direct role in causation of CAD (634).

IL-6 is main inducer of hsCRP production and secretion from liver. It has been reported to predict future risk of CAD (875,615) and mortality in patients with CAD (876). Mean IL6 level in the present study is 64.55±75.25 pg/ml (median 25.0 pg/ml) and those with diabetes has higher level (78.2±78.4 pg/ml). Almost similar level has been reported in a study from Pakistan, in patients with CAD with diabetes (73.1±2.57 pg/ml) and without diabetes (66.2±2.08 pg/ml) (643). Another study from Iran has also reported higher level of IL6 in subjects with stable (102.4±1.9 pg/ml) and unstable (224.6102±3.6 pg/ml) angina pectoris (642). Other studies have also reported higher level of IL6 in patients with CAD (608,629,620).

Serum IL6 levels are significantly higher and have showed positive correlation in patients with dyslipidemia, DM and/or HTN. Serum IL6 levels are increased in diabetic (660) and hypertensive subjects (635) compared to those without these morbidities. In a nested case control study elevated IL6 levels were strong independent predictor of type 2 DM (617). Bautista et al reported that after adjusting for other risk factors; age, sex, and BMI, IL-6 were not significantly associated with hypertension which is similar to present study (876).

Serum IL6 levels are not correlated with age, BMI, WHR and smoking in our study. There are contradictory reports of relation of IL6 with age (610,609); BMI (609,624,660,635) and smoking (608,609).

Similar to hsCRP, serum IL-6 levels has positive correlation with insulin resistance and negative correlation with insulin sensitivity. Among lipid parameters, TC, LDL and TGs has positive correlation and HDL has negative correlation with IL6. There was progressive increase in percentage of subjects with diabetes, HTN and dyslipidemia with increasing levels of inflammatory markers, further strengthening association between them. Odd ratio for DM, HT and dyslipidemia between highest and lowest quartile are 1.73, 1.65 and 4.5. Similar association has been reported in the literature with lipid parameters (609,635). It has been suggested that IL6 is causally related with insulin resistance (878); however contrary view also exist (619).
TNF-α is a pro-inflammatory cytokine which is thought to be pre-equisite for initiation of inflammation and production of other cytokine (879). TNF-α has been associated with recurrent MI and cardiovascular death after a first MI (875) and also correlate with the burden of atherosclerosis healthy middle-aged men (630), but Sukhija et al reported no correlation with severity of CAD (626). Mean TNF-α level in present study is 25.3±40.9 pg/ml (median 10.0pg/ml). TNF-α is correlated negatively with age and was positively correlating with smoking. Smokers have significantly higher TNF-α than non smokers. Similar observation has been made by others (880,881). The effect of aging per se on TNF-α secretion under basal conditions has not been clearly established. A lower level of TNF-α secretion has been observed in the older compared with the younger group (882) other study reported no relation with age (609). Elevated plasma TNF-α level have been observed in obese compared to lean subjects (609,624) which is in contrast to our study. Physical activity was not related to any inflammatory markers, a meta-analysis of twenty three studies concluded that exercise training was associated with reduced inflammatory activity in patients with CAD (641).

TNF-α levels are higher in diabetic subjects, but there is no correlation with insulin levels, HOMA-IR and QUICKI in present study. However, these all parameters showed positive graded response according to quartiles of TNF-α. Another study from India also failed to show any significant difference in TNF-α level between diabetic and non-diabetic groups (883). In present study hsCRP and IL6 are positively correlated with markers of insulin resistance, whereas TNF-α is not. Yudkin et al have proposed that IL-6 might be the major trigger culminating in the various manifestations of the metabolic syndrome, including insulin resistance, HTN, dyslipidemia, endothelial dysfunction, and a procoagulant state and that the IL-6 levels could be driven by psychosocial stress, smoking, central obesity, and genotypic programming (884). However all inflammatory markers act by triggering secondary intracellular pathway (JNK and IKKβ pathways) that promote the development of insulin resistance (866). TNF-α level were higher in hypertensives and was positively correlated with it in this study. Khan et al found no significant correlation between levels of TNF- alpha and stage of HTN in essential hypertensive patients (635).

In present study, TNF-α level is significantly higher in subjects with dyslipidemia compared to those without it. TNF alpha levels have shown positive association with
increased TG and reduced HDL, but not with TC and LDL. Mandall et al also observed similar association (609). In contrast Khan et al reported a strong association of TNF-α with LDL but not with TG and HDL (635). The reason for strong association of TNF-α with a reduced serum level of HDL is uncertain. It has been speculated that the reduced HDL seen in the inflammation result from increased serum concentration of serum amyloid A protein replacing apo A1 as an apolipoprotein in HDL particles and that this leads to increased catabolism (885). In experimental studies TNF-α administration to mice and human increased TG concentration by ~85% (886,887). TNF-α level increases plasma TG by increasing the concentration of free fatty acids, substrate for TG synthesis and by diminishing the clearance of TG rich lipoproteins (VLDLs) from the circulation (887,888).

4.5.3 CONCLUSION

In conclusion, the present study showed that inflammatory markers (hs-CRP, IL6 and TNF-α) have a strong association with diabetes, dyslipidemia, HTN, and insulin resistance in subjects with underlying CVD. The findings of this study thus suggest that many Indians have an underlying proinflammatory state that may contribute to increased cardiovascular risk and predispose them to CVD.
DIETARY FACTORS AND ITS ASSOCIATION WITH CARDIOVASCULAR RISK FACTORS
### 4.6 DIETARY FACTORS AND ITS ASSOCIATION WITH CARDIOVASCULAR RISK FACTORS

#### 4.6.1 RESULTS

Table 29

Correlation of dietary parameters with insulin resistance

<table>
<thead>
<tr>
<th>Dietary Parameters</th>
<th>HOMA IR</th>
<th>HOMA-IR (adjusted for age, sex, BMI and smoking)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
<td>p value</td>
</tr>
<tr>
<td>Energy [Kcal/day]</td>
<td>0.071</td>
<td>0.223</td>
</tr>
<tr>
<td>Carbohydrates [gms/day]</td>
<td>0.136</td>
<td>0.019</td>
</tr>
<tr>
<td>Protein [gms/day]</td>
<td>-0.180</td>
<td>0.002</td>
</tr>
<tr>
<td>Fat [gms/day]</td>
<td>0.138</td>
<td>0.016</td>
</tr>
<tr>
<td>Palmitic acid [gms/day]</td>
<td>0.206</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total Dietary fiber [gms/day]</td>
<td>-0.134</td>
<td>0.021</td>
</tr>
<tr>
<td><strong>VITAMINS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choline [mgs/day]</td>
<td>-0.101</td>
<td>0.082</td>
</tr>
<tr>
<td>Folic Acid [µg/day]</td>
<td>-0.057</td>
<td>0.329</td>
</tr>
<tr>
<td>Niacin [mgs/day]</td>
<td>-0.147</td>
<td>0.011</td>
</tr>
<tr>
<td>Riboflavin [mgs/day]</td>
<td>-0.150</td>
<td>0.009</td>
</tr>
<tr>
<td>Thiamine [mgs/day]</td>
<td>-0.147</td>
<td>0.011</td>
</tr>
<tr>
<td>Total B6 [mgs/day]</td>
<td>-0.155</td>
<td>0.007</td>
</tr>
<tr>
<td>Vitamin A [µg/day]</td>
<td>-0.100</td>
<td>0.082</td>
</tr>
<tr>
<td>Vitamin B12 [µg/day]</td>
<td>-0.046</td>
<td>0.428</td>
</tr>
<tr>
<td>Vitamin C [mgs/day]</td>
<td>-0.103</td>
<td>0.076</td>
</tr>
<tr>
<td>Beta carotene [µg/day]</td>
<td>-0.042</td>
<td>0.466</td>
</tr>
<tr>
<td><strong>MINERALS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minerals [gms/day]</td>
<td>-0.162</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Macronutrients</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium [mgs/day]</td>
<td>-0.056</td>
<td>0.333</td>
</tr>
<tr>
<td>Potassium [mgs/day]</td>
<td>-0.074</td>
<td>0.203</td>
</tr>
<tr>
<td>Calcium [mgs/day]</td>
<td>-0.056</td>
<td>0.332</td>
</tr>
<tr>
<td>Magnesium [mgs/day]</td>
<td>-0.180</td>
<td>0.002</td>
</tr>
<tr>
<td>Phosphorous [mgs/day]</td>
<td>-0.185</td>
<td>0.001</td>
</tr>
<tr>
<td>Sulfur [mgs/day]</td>
<td>-0.137</td>
<td>0.018</td>
</tr>
<tr>
<td><strong>Micronutrients</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron [mgs/day]</td>
<td>-0.171</td>
<td>0.003</td>
</tr>
<tr>
<td>Chromium [mgs/day]</td>
<td>-0.059</td>
<td>0.308</td>
</tr>
<tr>
<td>Copper [mgs/day]</td>
<td>-0.117</td>
<td>0.043</td>
</tr>
<tr>
<td>Manganese [mgs/day]</td>
<td>-0.036</td>
<td>0.531</td>
</tr>
<tr>
<td>Molybdenum [mgs/day]</td>
<td>-0.036</td>
<td>0.533</td>
</tr>
<tr>
<td>Chlorine [mgs/day]</td>
<td>-0.107</td>
<td>0.064</td>
</tr>
<tr>
<td>Zinc [mgs/day]</td>
<td>-0.118</td>
<td>0.041</td>
</tr>
</tbody>
</table>
Dietary carbohydrate and fat were positively, and protein and dietary fiber intake were negatively correlated with HOMA-IR and IL-6 (Table 29 & 30).

Positive association of carbohydrate with HOMA-IR (beta coefficient 0.044, p =0.027) and IL-6 (beta coefficient 0.147, p =0.037) persisted in multiple regression analysis even after adjustment for age, sex, BMI and smoking. However, association of fat with HOMA-IR and inflammatory markers became non significant after adjustment for age, sex, BMI and smoking. Protein intake had significant negative association with HOMA-IR (beta coefficient -0.262, p =0.0023) and all inflammatory markers: hsCRP (beta coefficient -0.175, p <0.0001); IL-6 ((beta coefficient -1.419, p <0.0001) and TNF-α (beta coefficient -0.445, p= 0.006). There was no correlation of individual amino acids with HOMA-IR but showed strong negative correlation with inflammatory markers (hsCRP; IL-6 and TNF-α). This relation also persisted even when adjusted for age, sex, BMI and smoking. Among fats only palmitic acid was strongly correlated with insulin resistance and inflammatory markers (Table 29-30).

Among vitamins – niacin; riboflavin; thiamine and B6 were negatively correlated with HOMA-IR and inflammatory markers. In addition; folic acid and vitamin B12 were also negatively correlated with inflammatory markers; but did not show any relation with insulin resistance. Same association persisted in multiple regression analysis when adjusted for age, sex, BMI and smoking. (Table 29-30)

Dietary intake of minerals was negatively correlated with HOMA-IR and inflammatory markers. Among minerals magnesium; phosphorus; sulfur; iron and zinc showed significant relationship with insulin resistance and inflammatory markers; whereas potassium; chromium; copper and molybdenum were only related to inflammatory markers. This relation remained unaltered in multiple regression analysis after adjustment with age, sex, BMI and smoking.(Table 29-30)

Diabetic patients had similar energy and carbohydrate intake, but had significantly lower protein and total dietary fiber intake as compared to non diabetics. There were no significant differences in individual amino acid or fat intake between two groups.
**Table-30**  
Correlation of dietary parameters with inflammatory markers

<table>
<thead>
<tr>
<th>Dietary Parameters</th>
<th>IL-6</th>
<th>hsCRP</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
<td>p value</td>
<td>r value</td>
</tr>
<tr>
<td>Energy [Kcal/day]</td>
<td>0.081</td>
<td>0.162</td>
<td>0.067</td>
</tr>
<tr>
<td>Carbohydrates [gms/day]</td>
<td>0.129</td>
<td>0.025</td>
<td>0.104</td>
</tr>
<tr>
<td>Protein [gms/day]</td>
<td>-0.278</td>
<td>&lt;0.0001</td>
<td>-0.264</td>
</tr>
<tr>
<td>Fat [gms/day]</td>
<td>0.060</td>
<td>0.302</td>
<td>0.016</td>
</tr>
<tr>
<td>Palmitic acid [gms/day]</td>
<td>0.104</td>
<td>0.072</td>
<td>0.261</td>
</tr>
<tr>
<td>Total Dietary fiber [gms/day]</td>
<td>-0.208</td>
<td>&lt;0.0001</td>
<td>-0.245</td>
</tr>
<tr>
<td>AMINO ACIDS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine [mgs/day]</td>
<td>-0.212</td>
<td>&lt;0.0001</td>
<td>-0.171</td>
</tr>
<tr>
<td>Cystine [mgs/day]</td>
<td>-0.206</td>
<td>&lt;0.0001</td>
<td>-0.181</td>
</tr>
<tr>
<td>Histidine [mgs/day]</td>
<td>-0.201</td>
<td>&lt;0.0001</td>
<td>-0.161</td>
</tr>
<tr>
<td>Isoleucine [mgs/day]</td>
<td>-0.204</td>
<td>&lt;0.0001</td>
<td>-0.168</td>
</tr>
<tr>
<td>Leucine [mgs/day]</td>
<td>-0.212</td>
<td>&lt;0.0001</td>
<td>-0.186</td>
</tr>
<tr>
<td>Lysine [mgs/day]</td>
<td>-0.203</td>
<td>&lt;0.0001</td>
<td>-0.152</td>
</tr>
<tr>
<td>Phenylalanine [mgs/day]</td>
<td>-0.220</td>
<td>&lt;0.0001</td>
<td>-0.176</td>
</tr>
<tr>
<td>Threonine [mgs/day]</td>
<td>-0.214</td>
<td>&lt;0.0001</td>
<td>-0.174</td>
</tr>
<tr>
<td>Tryptophan [mgs/day]</td>
<td>-0.202</td>
<td>&lt;0.0001</td>
<td>-0.178</td>
</tr>
<tr>
<td>Valine [mgs]</td>
<td>-0.213</td>
<td>&lt;0.0001</td>
<td>-0.170</td>
</tr>
<tr>
<td>VITAMINS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic Acid [µg/day]</td>
<td>-0.226</td>
<td>&lt;0.0001</td>
<td>-0.167</td>
</tr>
<tr>
<td>Niacin [mgs/day]</td>
<td>-0.238</td>
<td>&lt;0.0001</td>
<td>-0.216</td>
</tr>
<tr>
<td>Riboflavin [mgs/day]</td>
<td>-0.242</td>
<td>&lt;0.0001</td>
<td>-0.185</td>
</tr>
<tr>
<td>Thiamine [mgs/day]</td>
<td>-0.269</td>
<td>&lt;0.0001</td>
<td>-0.243</td>
</tr>
<tr>
<td>Total B6 [mgs/day]</td>
<td>-0.143</td>
<td>0.013</td>
<td>-0.204</td>
</tr>
<tr>
<td>Vitamin A [µg/day]</td>
<td>-0.003</td>
<td>0.965</td>
<td>-0.012</td>
</tr>
<tr>
<td>Vitamin B12 [µg/day]</td>
<td>-0.107</td>
<td>0.065</td>
<td>-0.123</td>
</tr>
<tr>
<td>Vitamin C [mgs/day]</td>
<td>0.055</td>
<td>0.338</td>
<td>-0.009</td>
</tr>
<tr>
<td>Beta carotene [µg/day]</td>
<td>0.010</td>
<td>0.864</td>
<td>-0.030</td>
</tr>
<tr>
<td>MINERALS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minerals [gms/day]</td>
<td>-0.288</td>
<td>&lt;0.0001</td>
<td>-0.280</td>
</tr>
<tr>
<td>Macronutrients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium [mgs/day]</td>
<td>-0.131</td>
<td>0.023</td>
<td>-0.059</td>
</tr>
<tr>
<td>Potassium [mgs/day]</td>
<td>-0.237</td>
<td>&lt;0.0001</td>
<td>-0.198</td>
</tr>
<tr>
<td>Magnesium [mgs/day]</td>
<td>-0.285</td>
<td>&lt;0.0001</td>
<td>-0.404</td>
</tr>
<tr>
<td>Phosphorous [mgs/day]</td>
<td>-0.294</td>
<td>&lt;0.0001</td>
<td>-0.286</td>
</tr>
<tr>
<td>Sulfur [mgs/day]</td>
<td>-0.094</td>
<td>0.094</td>
<td>-0.147</td>
</tr>
<tr>
<td>Micronutrients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromium [mgs/day]</td>
<td>-0.190</td>
<td>0.001</td>
<td>-0.240</td>
</tr>
<tr>
<td>Copper [mgs/day]</td>
<td>-0.223</td>
<td>&lt;0.0001</td>
<td>-0.228</td>
</tr>
<tr>
<td>Manganese [mgs/day]</td>
<td>-0.285</td>
<td>&lt;0.0001</td>
<td>-0.075</td>
</tr>
<tr>
<td>Chorine [mgs/day]</td>
<td>0.015</td>
<td>0.796</td>
<td>-0.062</td>
</tr>
<tr>
<td>Zinc [mgs/day]</td>
<td>-0.294</td>
<td>&lt;0.0001</td>
<td>-0.356</td>
</tr>
</tbody>
</table>
Among vitamins diabetic patients had lower intake of vitamin A; riboflavin and vitamin B12. There was significantly lower intake of minerals (magnesium; phosphorus; iron and zinc) by diabetic patients (Table-31).

**Table-31**

<table>
<thead>
<tr>
<th>Dietary Parameters</th>
<th>Diabetic (n=125)</th>
<th>Non Diabetics (n=175)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy [Kcal/day]</td>
<td>1891.3±408.6</td>
<td>1837.2±371.2</td>
<td>0.2334</td>
</tr>
<tr>
<td>Carbohydrates [gms/day]</td>
<td>238.2±67.9</td>
<td>229.5±57.5</td>
<td>0.1458</td>
</tr>
<tr>
<td>Protein [gms/day]</td>
<td>45.3±13.31</td>
<td>50.4±14.63</td>
<td>0.0024</td>
</tr>
<tr>
<td>Fat [gms/day]</td>
<td>76.4±16.43</td>
<td>79.3±19.62</td>
<td>0.2868</td>
</tr>
<tr>
<td>Total Dietary fiber [gms/day]</td>
<td>12.67±5.17</td>
<td>14.12±6.39</td>
<td>0.0839</td>
</tr>
<tr>
<td><strong>VITAMINS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choline [mgs/day]</td>
<td>150.16±117.27</td>
<td>139.98±108.53</td>
<td>0.4397</td>
</tr>
<tr>
<td>Folic Acid [µg/day]</td>
<td>136.91±53.42</td>
<td>138.79±62.53</td>
<td>0.7857</td>
</tr>
<tr>
<td>Niacin [mgs/day]</td>
<td>8.59±2.34</td>
<td>9.04±2.53</td>
<td>0.1179</td>
</tr>
<tr>
<td>Riboflavin [mgs/day]</td>
<td>0.68±0.2</td>
<td>0.737±0.205</td>
<td>0.0248</td>
</tr>
<tr>
<td>Thiamine [mgs/day]</td>
<td>1.08±0.27</td>
<td>1.13±0.28</td>
<td>0.0981</td>
</tr>
<tr>
<td>Total B6 [mgs/day]</td>
<td>20.6±12.61</td>
<td>21.22±13.37</td>
<td>0.7186</td>
</tr>
<tr>
<td>Vitamin A [µg/day]</td>
<td>96.44±82.56</td>
<td>121.89±122.97</td>
<td>0.0343</td>
</tr>
<tr>
<td>Vitamin B12 [µg/day]</td>
<td>0.28±0.24</td>
<td>0.36±0.50</td>
<td>0.0291</td>
</tr>
<tr>
<td>Vitamin C [mgs/day]</td>
<td>53.80±45.14</td>
<td>57.87±49.01</td>
<td>0.4645</td>
</tr>
<tr>
<td>Beta carotene [µg/day]</td>
<td>1689.9±2857.4</td>
<td>1678.0±3266.4</td>
<td>0.9740</td>
</tr>
<tr>
<td><strong>MINERALS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minerals [gms/day]</td>
<td>8.72±1.85</td>
<td>9.28±1.91</td>
<td>0.0126</td>
</tr>
<tr>
<td><strong>Macronutrients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium [mgs/day]</td>
<td>123.5±61.9</td>
<td>129.0±71.6</td>
<td>0.4909</td>
</tr>
<tr>
<td>Potassium [mgs/day]</td>
<td>1178.6±326.0</td>
<td>1234.5±351.5</td>
<td>0.1626</td>
</tr>
<tr>
<td>Calcium [mgs/day]</td>
<td>650.7±293.3</td>
<td>683.2±269.3</td>
<td>0.3227</td>
</tr>
<tr>
<td>Magnesium [mgs/day]</td>
<td>314.85±58.67</td>
<td>339.97±69.47</td>
<td>0.0013</td>
</tr>
<tr>
<td>Phosphorous [mgs/day]</td>
<td>1064.75±232.44</td>
<td>1157.99±268.38</td>
<td>0.0019</td>
</tr>
<tr>
<td>Sulfur [mgs/day]</td>
<td>275.93±93.9</td>
<td>287.45±92.24</td>
<td>0.2908</td>
</tr>
<tr>
<td><strong>Micronutrients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron [mgs/day]</td>
<td>14.83±7.29</td>
<td>17±7.69</td>
<td>0.0146</td>
</tr>
<tr>
<td>Chromium [mgs/day]</td>
<td>0.036±0.015</td>
<td>0.036±0.012</td>
<td>0.0701</td>
</tr>
<tr>
<td>Copper [mgs/day]</td>
<td>1.55±0.38</td>
<td>1.645±0.447</td>
<td>0.0601</td>
</tr>
<tr>
<td>Manganese [mgs/day]</td>
<td>4.37±2.32</td>
<td>4.83±3.34</td>
<td>0.0123</td>
</tr>
<tr>
<td>Molybdenum [mgs/day]</td>
<td>0.298±0.0207</td>
<td>0.298±0.1200</td>
<td>0.7442</td>
</tr>
<tr>
<td>Chlorine [mgs/day]</td>
<td>90.62±71.2</td>
<td>106.2±99.5</td>
<td>0.3726</td>
</tr>
<tr>
<td>Zinc [mgs/day]</td>
<td>5.08±1.22</td>
<td>5.56±1.37</td>
<td>0.0021</td>
</tr>
</tbody>
</table>
4.6.2 DISCUSSION

This cross sectional study in Indian population revealed that dietary factors are inversely correlated with inflammatory markers and insulin resistance in subjects with CAD. In present study there is significant difference in diet of diabetic subjects as compared to non diabetic subjects. Diabetic subjects have diet which is low in protein, fibers, carotene and minerals. This will further help to understand rising prevalence of diabetic in Indian Population.

In present study carbohydrate is positively correlated and has positive association with insulin resistance and inflammatory markers. Glucose ingestion in normal subjects is associated with increased superoxide generation in leukocytes and mononuclear cells, and increased activity of nuclear factor-kB (NF-kB), a transcriptional factor regulating the activity of at least 125 genes, most of which are pro-inflammatory (670). This leads to increased circulating levels of inflammatory cytokines, such as tumour necrosis factor-a (TNF-a), interleukin 6 (IL-6), and interleukin 18 (IL-18) (671,672). A similar relation has been observed in present study. Evidence from prospective observational studies supports the hypothesis that this may increase the risk of CVD. Among 75,521 women free of known diabetes or cardiovascular disease at baseline, higher glycemic load was associated with greater risk of CVD over 10 years of follow-up (889). Among postmenopausal women, a positive association between carbohydrate intake and progression of coronary atherosclerosis appeared more pronounced among women with lower physical activity (890). A recent meta-analysis observed increased relative risk of diabetes with increased white rice consumption particularly in Asians (706). A study from Southern part of India also reported a positive association of refined grain intake with the risk of type 2 diabetes (891). Yajnik et al reported increase in inflammatory markers in urban slum dwellers, which was associated with increased insulin resistance (892). Most of calories in low income group is consumed in the form of rice or wheat contents of which are mainly carbohydrate and may explain this observation.

Effects of saturated fat intake on non-traditional CVD risk factors, such as insulin sensitivity, inflammation, or endothelial function, are less well established. In animal studies saturated fat intake worsens insulin resistance, effects in humans may depend on type of fat consumed and associated other dietary factors. (893,894). In present study,
dietary fat intake has positive association with insulin resistance but not with inflammatory markers. Similar observation has been made by another study from India (892). This was particularly significant for saturated fat (Palmitic acid). The effect of hyperglycemia, hypertriglyceridemia, and raised free fatty acids levels, both fasting and post-prandial, on endothelial function may be mediated through the generation of an oxidative stress. Both FFA and inflammatory markers have been shown to predict type 2 DM independent of known risk factors (676,677). Both free fatty acids and TNF-α have also been shown to activate IKKβ in adipocytes and hepatocytes, which can then increase the serine phosphorylation of IRS-1, with subsequent reduction in insulin-dependent tyrosine phosphorylation of IRS-1, and ultimately glucose transport (678). IKKβ is a serine kinase that controls the activation of NF-κB, a transcription factor associated with inflammation. This causes insulin resistance both locally in liver and systemically.

Effects of fats and carbohydrates on CVD risk may also vary depending on underlying insulin resistance or predisposition to the metabolic syndrome. Older age, female gender, adiposity, and physical inactivity are each powerful determinants of insulin sensitivity, and thus each of these may alter the relations between diet and CVD risk (895). In view of this, we performed multiple regression analysis after adjustment of these factors and a positive association between insulin resistance, inflammation and carbohydrate was maintained but association with fat became insignificant. A study from Sweden also failed to show relation between saturated fat and inflammatory marker hsCRP (896).

Dietary fiber intake is negatively associated with insulin resistance and inflammatory markers in present study. Several cross-sectional epidemiologic studies reported inverse associations of serum insulin with fiber intake (897,685). In the present study, dietary fiber intake is negatively correlated with insulin levels but is not statistically significant. Dietary fiber intake had lower risk of developing diabetes (891). In contrast, other studies have not found association between diabetes risk and total dietary fiber intake (691,692). The 20-year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study yielded no association of dietary fiber with impaired glucose tolerance or diagnosed diabetes (898). However, dietary fiber intake improves cardiovascular risk factors including insulin sensitivity (25). Recently other studies have also documented a negative association between dietary fiber intake and markers of inflammation and insulin resistance (764,899).
Dietary protein intake is negatively associated with insulin resistance and inflammatory markers in present study. Low protein diet impairs the development of fetal pancreas and has long-term consequences on its secretory capacity (714). We also found that protein intake has positive association with insulin sensitivity as assessed by QUICKI. Similar observation has been reported by a study, where low protein intake was associated with islet cell dysfunction (900). In present study all amino acids including arginine, cysteine and lysine were negatively associated with inflammatory markers but not with insulin resistance. Intake of arginine rich food is associated with decrease in inflammatory markers (901). The question whether amino acids contribute to the pathogenesis of insulin resistance and type2 DM in functional manner remains unsettled (902). Dietary composition is likely to influence both metabolite levels and the development of insulin resistance in a causal manner (903,904), yet they did not find concomitant associations with dietary measures and HOMA-IR. Glutathione plays important roles in antioxidant defense, nutrient metabolism, and regulation of cellular events, which is synthesized from cysteine and lysine (905).

Micronutrients are vitamins and minerals are required in small quantities for specific functions such as essential coenzymes and cofactors for metabolic reactions and required to maintain energy production and life (906). In present study dietary vitamins has showed diverse pattern, but essentially a negative correlation with insulin resistance and inflammatory markers. Fruit and vegetables are main source of vitamins. There are several studies which showed negative correlation of intake of fruit and vegetables with insulin resistance and inflammatory markers (779).

Dietary trace elements are associated with heart disease risk. In present study dietary mineral intake is negatively associated with insulin resistance and inflammatory markers. The mechanisms by which particular elements or their compounds may affect heart disease risk are not clear, but it is likely that they involve effects on enzymes, hormones and messenger molecules. More than 27% of known enzymes contain mineral elements and/or require minerals for activity (907). Among minerals magnesium and zinc has significant relationship with both parameters, whereas chromium is only related to inflammatory markers. Many epidemiological and clinical investigations that supported the hypothesis that increased magnesium intake contribute to prevention of HTN and
cardiovascular disease (552,825). Serum and dietary magnesium intake also correlates with various cardiovascular risk factors (864). There is strong inverse relationship between dietary magnesium and inflammation (858).

4.6.3 CONCLUSION

Increased consumption of high-density and low-quality foods, such as those rich in refined starches, sugar, and unhealthy lipids and poor in fiber, vitamins and minerals may facilitate activation of the innate immune system, most likely by an excessive production of pro-inflammatory cytokines associated with a reduced production of anti-inflammatory cytokines. This imbalance may favour the generation of a pro-inflammatory milieu, which in turn may produce insulin resistance in the peripheral tissues and endothelial dysfunction at the vascular level, and ultimately predispose susceptible people to an increased incidence of diabetes and cardiovascular disease. This may explain partly recent epidemic of obesity, DM and cardiovascular disease.

Because changes in dietary habits are relatively low risk, low cost, and widely available, even small effects on risk are important on a population level. Thus, dietary recommendations, together with other lifestyle modifications such as smoking cessation and increased physical activity must play a central role in the prevention and treatment of CVD. Additional carefully designed, prospective, observational studies and randomized clinical trials of effects of different fats and carbohydrates on CVD risk factors, particularly non lipid risk factors will further elucidate the optimal dietary habits to minimize the health burden of CVD.
ASSOCIATION OF CONSTITUTIONAL TYPE OF AYURVEDA (PRAKRITI) WITH CARDIOVASCULAR RISK FACTORS
4.7 ASSOCIATION OF CONSTITUTIONAL TYPE OF AYURVEDA (PRAKRITI) WITH CARDIOVASCULAR RISK FACTORS

4.7.1 RESULTS

Constitution types which were identified in this study were Kapha, Pitta Kapha, Vata Kapha and Vata Pitta. VK was more prevalent (62.3%) compared to others; K-5%, KP-15.7%, VP-17%. A comparison of cardiovascular risk factors with different constitution types (prakriti) are given in table-27. Anthropometric parameters were comparable in all constitution types. There was no correlation between BMI, WHR and physical activity with prakriti (table-32).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Kapha n=15</th>
<th>Pitta Kapha n=47</th>
<th>Vata kapha n=187</th>
<th>Vata pitta n=51</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age,</td>
<td>60±12.5</td>
<td>58.8±11.3</td>
<td>61.2±12.1</td>
<td>61.9±14.5</td>
<td>0.5995</td>
</tr>
<tr>
<td>Sex, % (n)</td>
<td>M-86.7%(13)</td>
<td>F-13.3%(2)</td>
<td>M-69% (129)</td>
<td>M-74.5% (38)</td>
<td>0.3771</td>
</tr>
<tr>
<td>Smoking</td>
<td>46.7%(7)</td>
<td>29.8%(14)</td>
<td>40.6%(76)</td>
<td>27.5%(14)</td>
<td>0.1944</td>
</tr>
<tr>
<td>Physical inactivity % (n)</td>
<td>40% (6)</td>
<td>40.4% (19)</td>
<td>39.8% (74)</td>
<td>33.3% (17)</td>
<td>0.8616</td>
</tr>
<tr>
<td>BMI (Kg/m^2)</td>
<td>28±2.9</td>
<td>27.6±4.2</td>
<td>27.9±3.8</td>
<td>27.7±3.5</td>
<td>0.9352</td>
</tr>
<tr>
<td>WHR</td>
<td>0.94±0.03</td>
<td>0.92±0.05</td>
<td>0.92±0.06</td>
<td>0.92±0.06</td>
<td>0.3805</td>
</tr>
<tr>
<td>DM, % (n)</td>
<td>26.7%(4)</td>
<td>36.2% (17)</td>
<td>48.1% (90)</td>
<td>27.5% (14)</td>
<td>0.0241</td>
</tr>
<tr>
<td>HTN, % (n)</td>
<td>53.3% (8)</td>
<td>48.9% (23)</td>
<td>70.6% (132)</td>
<td>49% (25)</td>
<td>0.0038</td>
</tr>
<tr>
<td>DM &amp; HTN, % (n)</td>
<td>26.7% (4)</td>
<td>27.7% (13)</td>
<td>36.9% (69)</td>
<td>13.7% (7)</td>
<td>0.0145</td>
</tr>
<tr>
<td>Dyslipidemia % (n)</td>
<td>6.7% (1)</td>
<td>8.5% (4)</td>
<td>62% (116)</td>
<td>5.9% (3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>160±23</td>
<td>174.7±44.9</td>
<td>18.6±49.1</td>
<td>166.4±32.5</td>
<td>0.0661</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>144.8±35.7</td>
<td>146.6±31.5</td>
<td>184.7±47.6</td>
<td>152±35.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>47.4±5.8</td>
<td>44.9±8.3</td>
<td>35±7.6</td>
<td>45.3±8.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>83.9±21.1</td>
<td>100.4±48.6</td>
<td>112.6±54.6</td>
<td>90.7±35.8</td>
<td>0.0355</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>28.9±7.1</td>
<td>29.2±6.3</td>
<td>36.9±9.5</td>
<td>30.4±7.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>91.8±74</td>
<td>6.3±4.5</td>
<td>92.5±77.9</td>
<td>7.3±4.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TNF-α, pg/ml</td>
<td>49.6±24.2</td>
<td>8.1±0.61</td>
<td>32.3±48.8</td>
<td>8.1±0.24</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>16.8±2.5</td>
<td>2.3±2.1</td>
<td>16.0±9.0</td>
<td>2.6±2.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin, mU/L</td>
<td>26±24.8</td>
<td>41.5±37</td>
<td>57.3±46.1</td>
<td>38.8±36.9</td>
<td>0.0002</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>7.5±6.03</td>
<td>11.3±10.7</td>
<td>22.9±24.4</td>
<td>10.4±10.6</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

DM and DM with HTN has significant association with VK prakriti, it was highest in VK group compared to VP. There was no association between K and PK group. HTN was highest in VK group compared to PK and VP group but not associated with K group. 26.7%, 36.2%, 48.1% & 27.5% in K, PK, VK, and VP types were diabetics respectively.
Cytokines (IL6) and inflammatory markers (TNF-\(\alpha\) and hsCRP) were analysed, IL6 and hsCRP was highest in VK but TNF-\(\alpha\) had significantly highest values in K prakriti (Table 33 & 34).

**Table-33**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>k-vk</th>
<th>pk-vk</th>
<th>vk-vp</th>
<th>k-pk</th>
<th>k-vp</th>
<th>pk-vp</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>0.1820</td>
<td>0.1910</td>
<td>0.0131</td>
<td>0.7159</td>
<td>0.6158</td>
<td>0.4777</td>
</tr>
<tr>
<td>HTN</td>
<td>0.2699</td>
<td>0.0084</td>
<td>0.0066</td>
<td>1.000</td>
<td>1.000</td>
<td>0.8461</td>
</tr>
<tr>
<td>DM &amp;HTN</td>
<td>0.6070</td>
<td>0.3097</td>
<td>0.0029</td>
<td>0.6106</td>
<td>0.2100</td>
<td>0.1445</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.6505</td>
<td>0.6532</td>
<td>0.4539</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.1007</td>
<td>0.2102</td>
<td>0.0419</td>
<td>0.5158</td>
<td>0.4789</td>
<td>0.9518</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.0018</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.8704</td>
<td>0.4917</td>
<td>0.4103</td>
</tr>
<tr>
<td>HDL</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.3558</td>
<td>0.4339</td>
<td>0.8223</td>
</tr>
<tr>
<td>LDL</td>
<td>0.0611</td>
<td>0.1652</td>
<td>0.0242</td>
<td>0.5268</td>
<td>0.6298</td>
<td>0.7328</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.0018</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.8704</td>
<td>0.4917</td>
<td>0.4103</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.9715</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.2597</td>
<td></td>
</tr>
<tr>
<td>TNF-(\alpha)</td>
<td>0.0013</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.8579</td>
<td></td>
</tr>
<tr>
<td>hsCRP</td>
<td>0.7151</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.6215</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>0.0014</td>
<td>0.0307</td>
<td>0.0087</td>
<td>0.1362</td>
<td>0.2143</td>
<td>0.7147</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.0005</td>
<td>0.0002</td>
<td>&lt;0.0001</td>
<td>0.3121</td>
<td>0.4218</td>
<td>0.6709</td>
</tr>
</tbody>
</table>

k-vk: p value between kapha and vata kapha
pk-vk: p value between pitta kapha and vata kapha
vk-vp: p value between vata kapha and vata pitta
k-pk: p value between kapha and pitta kapha
k-vp: p value between kapha and vata pitta
pk-vp: p value between pitta kapha and vata pitta

TC, TGs, VLDL and LDL cholesterol were significantly higher and HDL cholesterol significantly lower in VK when compared with other constitution type. For TG, VK had highest and HDL has lowest values compared to all groups (K, PK, VP). Serum cholesterol levels were also significantly high in VK group but in individual comparison VK was significantly high compared to VP only. Similarly, LDL was high in VK type but compared to VP only.

Insulin and HOMA-IR was highest in VK compared to all prakriti types. Insulin resistance showed positive correlation VK (beta coefficient: 13.97, p<0.0001).
related with KV except HDL, which was correlate negatively in univariate analysis. Significance was maintained even after adjustment with age, sex and BMI in multiple regression analysis. (Table 34-35)

### Table-34

**Correlation of prakriti with risk factors**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vata Kapha, n-187</th>
<th>Vata Pitta, n-51</th>
<th>Pitta Kapha, n-47</th>
<th>Kapha, n-15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r value</td>
<td>p value</td>
<td>r value</td>
<td>p value</td>
</tr>
<tr>
<td>DM</td>
<td>0.169</td>
<td>0.003</td>
<td>-0.130</td>
<td>0.024</td>
</tr>
<tr>
<td>HTN</td>
<td>0.211</td>
<td>&lt;0.0001</td>
<td>-0.128</td>
<td>0.027</td>
</tr>
<tr>
<td>DM &amp; HTN</td>
<td>0.164</td>
<td>0.004</td>
<td>-0.169</td>
<td>0.003</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>0.541</td>
<td>&lt;0.0001</td>
<td>-0.326</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.165</td>
<td>0.004</td>
<td>-0.122</td>
<td>0.035</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.378</td>
<td>&lt;0.0001</td>
<td>-0.188</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL</td>
<td>-0.546</td>
<td>&lt;0.0001</td>
<td>0.314</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL</td>
<td>0.180</td>
<td>0.002</td>
<td>-0.133</td>
<td>0.021</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.378</td>
<td>&lt;0.0001</td>
<td>-0.188</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.480</td>
<td>&lt;0.0001</td>
<td>-0.344</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.222</td>
<td>&lt;0.0001</td>
<td>-0.191</td>
<td>0.001</td>
</tr>
<tr>
<td>hscCRP</td>
<td>0.585</td>
<td>&lt;0.0001</td>
<td>-0.425</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.213</td>
<td>&lt;0.0001</td>
<td>-0.118</td>
<td>0.041</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.286</td>
<td>&lt;0.0001</td>
<td>-0.167</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**r value**: Correlation of prakriti with risk factors

All lipids levels were positively correlated with KV except HDL, which was correlated negatively in univariate analysis. Significance was maintained even after adjustment with age, sex and BMI in multiple regression analysis. (Table 34-35)

### Table-35

**Multi-regression analysis of constitutional types with risk factor**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vata Kapha, n-187</th>
<th>Vata Pitta, n-51</th>
<th>Pitta Kapha, n-47</th>
<th>Kapha, n-15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beta coefficient</td>
<td>p value</td>
<td>Beta coefficient</td>
<td>p value</td>
</tr>
<tr>
<td>DM</td>
<td>0.164</td>
<td>0.0553</td>
<td>-0.174</td>
<td>0.0214</td>
</tr>
<tr>
<td>HTN</td>
<td>0.201</td>
<td>0.0004</td>
<td>-0.168</td>
<td>0.0233</td>
</tr>
<tr>
<td>DM &amp; HTN</td>
<td>0.145</td>
<td>0.0082</td>
<td>-0.207</td>
<td>0.0034</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>0.543</td>
<td>&lt;0.0001</td>
<td>-0.423</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>15.122</td>
<td>0.0554</td>
<td>-14.935</td>
<td>0.0334</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>36.048</td>
<td>&lt;0.0001</td>
<td>-22.917</td>
<td>0.0012</td>
</tr>
<tr>
<td>HDL</td>
<td>-10.29</td>
<td>&lt;0.0001</td>
<td>7.659</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL</td>
<td>18.210</td>
<td>0.0024</td>
<td>-18.011</td>
<td>0.0202</td>
</tr>
<tr>
<td>VLDL</td>
<td>7.210</td>
<td>&lt;0.0001</td>
<td>-4.583</td>
<td>0.0012</td>
</tr>
<tr>
<td>IL-6</td>
<td>73.996</td>
<td>&lt;0.0001</td>
<td>-69.120</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TNF-α</td>
<td>18.832</td>
<td>&lt;0.0001</td>
<td>-19.993</td>
<td>0.0012</td>
</tr>
<tr>
<td>hscCRP</td>
<td>11.776</td>
<td>&lt;0.0001</td>
<td>-11.026</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin</td>
<td>18.978</td>
<td>0.0002</td>
<td>-13.576</td>
<td>0.0431</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>12.115</td>
<td>&lt;0.0001</td>
<td>-9.259</td>
<td>0.0042</td>
</tr>
</tbody>
</table>

**Beta coefficient**: Association of prakriti with risk factors
In univariate analysis IL6 was positively correlated with KV but not with K (r: 0.083, p: 0.150). However, TNF-α and hsCRP were positively correlated with both KV and K group. (TNF-α; r: 0.137, p: 0.018, hsCRP; r: 0.123, p: 0.033). (Table-34) Even after adjustment with age, sex & BMI significance level was maintained. (Table-35)

4.7.2 DISCUSSION

The entire description of human physiology in Ayurveda is based primarily in the theory of Tridosha (791-792,794). The “homeostatic mechanisms” as conceptualized in modern biomedicine have a very close resemblance with this theory. On the basis of this understanding, Ayurveda recognizes that a mild disturbance in the balance of Vata, Pitta and Kapha in the heart muscle results in the impairment of the cardiac function, which is usually compensated by augmenting the heart rate and increasing the force of ventricular contraction.

This is possibly the first study to report an association of risk factors, inflammatory markers and insulin resistance with prakriti type in angiographically proved cardiovascular patients. More than half of patients (62.3%) are of Kapha Vata type and it is more prevalent compared to other parkriti; VK>VP>PK>K. CAD have element of cellular proliferation and metabolic abnormalities. Vata contributes to manifestation of shape, cell division; Kapha is responsible for anabolism, growth and maintenance of structure and Pitta is primarily responsible for metabolism. Hence, combined abnormalities will be more prevalent in CAD, which is observed in this study. There have been many studies to provide an evidence base to the traditional systems of medicine (908,909). Investigators have tried to provide interesting genetic, biochemical, hematological or anatomical basis to the concept of Ayurveda constitution (791,797-801). But most of the recent investigations carried out in relation to prakriti have included only those individual who are healthy or belonging to three extreme types of constitution (K,V and P), which is comparatively a rare occurrence.

Prasher B and others observed that individuals from the three most contrasting constitutional types exhibited striking differences with respect to biochemical and hematological parameters and at genome wide expression levels. They also reported that biochemical profiles like liver function tests and lipid profiles and hematological parameters like hemoglobin levels exhibited differences between Prakriti types. Thus, they
concluded that Ayurveda-based method of phenotypic classification of extreme constitutional types may be utilized to uncover genes that may contribute to system level differences in normal individuals (791).

Prakriti fundamentally and dosha as its applied extension, presented themselves as the central dogma of Ayurveda. Fascinated by its possible application to Ayurvedic diagnostics and for its being as an evidence to help decision making for personalized treatment, it has recently evoked the scientific community to look at the issue in their own perspectives (910). The identification of biochemical correlates and whole genome expression to the extreme constitutional types as described in Ayurveda (791). Almost a decade back, prakriti was seriously thought as an important factor determining the final outcome of any therapeutic intervention in a given population. Dahanukar and Thatte in a revealing study were able to correlate the therapeutic outcomes with phenotypical specifications as described in Ayurveda (911). A definitive role of prakriti to the prevalence and prognosis of rheumatoid arthritis (RA) was identified by Rastogi S et al. This report identified a vata-pitta constitutional subtype as more prone yet fairly treatable fraction among the RA population (912). Tripathi PK and others suggest that these basic cardiovascular responses do not vary significantly as per the dual constitutional types and noted a significant fall in the diastolic BP immediately after performing the isotonic exercise for five minutes, in Vata-Kapha individuals in comparison to the other two groups, namely, Pitta-Kapha and Vata-Pitta (801). Udupa KN and others reported that the normal persons with features of Vata, Pitta and Kapha constitutions exhibited a relative preponderance of Blood Cholinesterase, Monoamine oxidase and Histaminase activity, respectively (797). Ghodke Y and others carried out CYP2C19 genotyping in 132 unrelated healthy subjects of either gender, and, observed significant association between CYP2C19 genotype and major classes of Prakriti types. They reported that the extensive metabolizer genotype was found to be predominant in Pitta Prakriti, and the poor metabolizer genotype was highest in Kapha Prakriti when compared with other two Prakriti groups. 76 subjects were evaluated both for their Prakriti and HLA DRB1 types, finding significant correlations in support of it. The study concluded that Ayurveda based phenomes may provide a model to study multigenic traits, possibly offering a new approach to correlating genotypes with
phenotypes for human classification (799). Hence, this study adds to existing knowledge base regarding relation of various prakriti and cardiovascular risk factors.

Dyslipidemia is more common in kapha vata subjects and lowest prevalence of 5.9% is found in vata pitta subjects. TC, TGs, VLDL and LDL cholesterol were significantly higher and HDL cholesterol significantly lower in subjects with VK. All lipid parameters has positive correlation with VK except HDL; which is negatively correlated with VK. Prasher B et al. found high levels of TG, TC, VLDL and low levels of HDL in Kapha individuals in healthy subjects (791).

Vata kapha group has higher number of patients with cardiovascular risk disease than other groups. There are significantly higher number of subjects of DM, HTN and dyslipidemia in vata kapha type indicating VK is common in these conditions. All these risk factors are more prevalent in VK type. There is no correlation between WHR and BMI with type of prakriti, but contrary to our study, Hankey A said that age and BMI correlates with prakriti of individual (913). Most of the population based studies have reported relation between WHR; BMI (914). However; in this study we have taken subjects who underwent coronary angiography and detected CAD. Most of our patients had mean WHR >0.9 and mean BMI >25 which were already higher than normal population according to International and Indian Guideline (811,291). Hence; probably we did not find the association. Perhaps due to higher individual of dual prakriti, larger study may be required.

Cytokines and inflammatory markers IL6, TNF-α and hsCRP are high in kapha prakriti when compared with VK & VP, however in vata kapha inflammatory markers are high compared to vata pitta only. However, HOMA-IR and Insulin are more prevalent in only KV (p<0.0001 for both) prakriti. Insulin resistance is correlated positively with VK. IL6 is positively correlated with KV but not with K. Inflammatory markers; TNF-α and hsCRP are positively correlated with both VK and K group. From an ayurvedic perspective the inflammation (pericarditis) is associated with Pitta, while fluid accumulation (pericardial effusion) with Kapha and stiffness (constrictive pericarditis) with Vata (802).

Ayurveda identifies five distinct kinds of heart diseases as per their clinical description. This disease classification is essentially the etiological classification where the symptoms originating as result of some specific cause are grouped under the heading of disease. As per the doshic distinction of causes, the heart diseases of Ayurveda can either
be caused by independent doshas (vata, pitta, and kapha) or a combination (tridoshaja) or else as a complication (krimija) (915).

The aetiological factors are generally classified as psychological factors, diet, activity, excessive sexual indulgence, suppression of natural urges, alcohol in excess, bacteria, viruses, worms and other toxins, iatrogenic, causes effects of drugs, improper management of disease, abnormal or excess use of emetics, purgatives or enemas, trauma to the heart, complications of other diseases. These will cause abnormal increase or decrease in Vata, Pitta and Kapha and in turn Rasa which enters the heart and gives rise to the Cardiovascular Disease (801). In summary, the eight basic elements that maintain the integrity of the cellular structure and functions of the heart are, Rasa, Rakta, Mamsa, Ojas, Prana vata, Vyana vata, Sadhaka pitta and Avalambaka kapha. Rasa vruddhi and rasa vikruti (vitiated) may lead to kaphaja heart disease. On the other hand congestive cardiac failure can lead to increased blood volume, due to impaired circulation (802).

Ayurveda provides insights into the development of the disease process, showing how the doshas when aggravated by certain aetiological factors affect the dhatus (tissue) and srotas (channel) of the body, eventually manifesting in disease. Degeneration of the blood vessels is caused by increased Vata in the blood vessels, which make them hard, thin, dry and rough. Deposits of lipids and calcium represent deposition of Kapha (water & earth element) in the degenerated vessels resulting in irregular thickening of blood vessels. However, no separate data was collected to find out ahaar (dietary), vihaar (lifestyle), manas (psychological) and hetus (causes). Moreover, Hridya is kapha predominant organ hence not predisposed to pitta vikruti.

It is important to maintain and protect the volume and composition of Rasa, the body fluids, at all times. Any disturbance in Rasa can impair the movement of essential nutrients to our body’s cells and organs. This will then affect all our tissues (dhatus), Rakta (blood), Mamsa (muscle), Meda (fat), Asthi (bone), Majja (nervous tissue), Shukra (reproductive tissue), Ojas (vital fluid) which in turn will effect our sense organs and mind. Any effect to the channel that carries Rasa (Rasa Vaha Srotas) will cause imbalance in Rasa. Rasa can be vitiates (rasa-dusthi), increased (rasa-vruddhi) or decreased (rasakshaya). As per modern understanding meda dhatu is the adipose tissue. It provides support to asthi dhatu and also lubricates the body. The abnormality of meda dhatu leads to
obesity, accumulation of fat, early syndromes of polyuria, glycosuria, undesirable growth of glands, hyperglycemia, excessive sweating, etc. However, attributes like Sthira, manda, guru, snigdha and sheeta which in combination with ruksha, khara, tend to cause a Vata Kapha vikruti in form of CAD. Any prakruti can develop CAD but VK were more predisposed due to aforementioned reasons.

Heart disease occurs as a complication of many diseases: Anemia, infectious fever, rheumatic fever, vatarakta, diabetes, chronic respiratory disease, vomiting, bleeding disorders, worms, alcoholic intoxication, side effects of drugs, neurological disorders. There was no association between Serum bilirubin, ALT, AST, Alkaline phosphatase, total protein, albumin, globulin, calcium, phosphorus, uric acid, sodium, potassium and chloride with individual constitution but study by Prasher B et al. showed elevated levels of serum uric acid in kapha and serum phosphorus in pitta individuals in healthy subjects.

Though, the present study does not suggest any significant association of PK and VP with risk factors and biochemicals but a strong association is found between risk factors (Diabetes, HTN and dyslipidemia), Insulin resistance and serum magnesium with individual having VK type of prakriti. Similarly, association is found of IL6, TNF-α, hsCRP with individual having VK and K type of prakriti. VK is strongly associated with CAD risk factors, whereas other prakriti is not associated and showed reverse association with risk factors, hence may other factors like "meda" may be contributing factor in them.

It may be presumed that dominance of VK group has got some positive relationship with cardiovascular risk factors. Insulin resistance, Cytokines and inflammatory markers has got positive relation with VK and K group both. These factors may be taken as a lead and further studies may be designated to explore this relationship.

Limitation of the study was male predominance and less number of cases in some groups of prakriti. Further, no detailed data was collected to find out ahaar (dietary), vihaar (lifestyle), manas (psychological) and hetus (causes).

4.7.3 CONCLUSION

Half of cardiovascular disease patients have vata kapha constitution type. It may be concluded that as there is dominance of vata kapha prakriti and there is strong correlation with risk factors, insulin resistance, cytokine (IL6) and inflammatory markers. But IL6, TNF-α and hsCRP is positively correlated with Kapha group also. Hence, identifying an
individual with Vata Kapha and Kapha prakriti will help in taking precautionary measures for future risk of cardiovascular disease.