hsCRP, F2-ISOPROSTANE & HOMOCYSTEINE IN PRIMARY HYPERCHOLESTEROLAEMIA

We conducted a cross-sectional study to investigate the levels of these biomarkers in primary hypercholesterolaemic patients. Fifty-six primary hypercholesterolaemic patients, 29 familial hypercholesterolaemia (FH) and 27 non familial hypercholesterolaemia (NFH) age and gender matched control subjects were recruited for this study. Blood samples were taken for the analysis of serum fasting lipid profile, serum hsCRP, plasma Hcys and plasma 8-isoprostane.

Serum Hcys levels were higher in all patients compared with control (mean + SD: 11.3 + 6.4 vs 8.6 + 3.3, p<0.05) with Hcys values being significantly higher in NFH group (mean + SD: 11.9 + 7.0 vs 8.6 + 3.3, p<0.05) compared with control. Serum hs-CRP were higher in NFH group (p<0.05) compared to control. Plasma 8-isoprostane levels of the primary hypercholesterolaemic patients were higher (mean + SEM: 1357.0 + 214.7 vs 398.0 + 20.5, p<0.05) compared to control and they were also higher in FH and NFH subgroups compared to control (mean + SEM: 1395.8+313.3 vs 398.0+20.5, p<0.05 and 1322.8+300.7 respectively). A significant correlation was seen between serum total cholesterol with serum Hcys and plasma isoprostane (r=0.6, p<0.001 and r=0.5, p<0.05 respectively). Correlation was also noted between serum LDL-c with serum Hcys (r=0.6, p<0.001) and plasma 8-isoprostane (r=0.5, p<0.05)

In summary, this study has demonstrated that there is a significant association between hypercholesterolaemia with serum hsCRP, Hcys and plasma 8-isoprostane levels.

Keywords: hs-CRP, homocysteine, 8-isoprostane, familial hypercholesterolaemia.

UDC: 616.1

Introduction

Coronary Artery Disease (CAD) has become the leading cause of death in most developed and developing countries. There have been significant advances in our understanding of the ways in which traditional cardiovascular risk factors such as standard lipid (eg. total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol) and nonlipid (eg. hypertension and smoking) risk factors interact to initiate atherosclerosis and promote the development of cardiovascular disease and how these factors have enhanced the ability to assess risk in individual patients (Fruchart et al., 2004). The major risk factors determining the 10-year risk of CAD are age, gender, smoking habit, systolic blood pressure (BP), total cholesterol (TC) and high density lipoprotein (HDL). Although risk scoring systems that additionally evaluate traditional risk factors greatly improved risk prediction, multiple studies demonstrate that 20-25% of all future events occur in individuals with only one of these factors (Ridker et al, 2004; Khot et al., 2003). Studies have also shown that these conventional risk factors predict less than fifty percent of future cardiovascular events as shown in a recent large-scale study of more than 27000 healthy American women whereby 77% of all future events occurred in those with LDL-c levels of <4.14 mmol/L and 45% of all events occurred in those with LDL-c values <3.36 mmol/L (Ridker et al., 2002, 2004; Khot et al., 2003; Kullo, Gau, Tajik, 2000; Heller, 1984; Wald, 1994). Furthermore, these risk factors may not have similar causal effect in different ethnicity. As an example, there is lack of association between the prevalence of
diabetes and coronary artery disease (CAD) in Afro-Caribbeans (Chaturvedi, McKeigue, Marmot, 1994) and low rates of CAD in Chinese and Japanese despite high smoking rates (Yao, Wu, and Wu, 1993). These recent findings suggest that factors other than established risk factors may contribute to the development of atherosclerosis.

The biomarker will have broader acceptance if reduction of the biomarker leads to reduced vascular risk. Several established and emerging novel biomarkers for cardiovascular risk meet these criteria although few are rarely used in clinical practice. They include markers of inflammation (eg hsCRP, sICAM-1, IL-6), altered thrombosis (eg tPA/PAI-1, fibrinogen, homocysteine), oxidative stress (oxidized LDL, F2-isoprostanes) and altered lipids [eg lipoprotein (a), LDL particle size] (Ridker et al., 2004)

This study is designed to investigate the levels of these newer biomarkers of cardiovascular risk in patients with familial hypercholesterolaemia (FH) and compare them to those of age and gender matched normolipaemic control group. It is also aimed to identify any significant correlation between these novel risk markers and established risk factors (eg. blood pressure, HDL-c, LDL-c, triglycerides (TG), smoking habit, age and gender) in an attempt to determine their significance in the clinical utility of cardiovascular risk assessment.

Materials and method

This was a cross-sectional, observational study conducted between Universiti Malaya Medical Centre and Medical Faculty, Universiti Teknologi MARA (UiTM). A total of 56 patients attending the Specialists’ Clinics in both centres who fulfilled the set criteria (29 FH and 27 NFH: 27 males, 29 females, mean ± SD age = 45.4 ± 10.3 years) and 52 age and gender matched normolipaemic controls were recruited for this study. All patients were screened through a protocol consisting of medical history, physical examination and laboratory tests including fasting glucose, serum lipids, renal profile, liver function and thyroid function tests. Diagnosis of FH was made based on the Simon Broome’s criteria. Inclusion criteria for NFH were total cholesterol level of >6.5 and/or LDL-c >3.8 mmol/L and those who did not fulfill Simon Broome’s criteria for definite or possible familial hypercholesterolemia. Patients with diabetes mellitus, renal, liver, endocrine diseases or any other causes of secondary hypercholesterolemia, those with recent febrile illness, concomitant neoplasm, inflammatory disease or immunosuppressive therapy including steroid usage and those taking vitamin supplements were excluded from this study. This study complies with the declaration of Helsinki and the protocol was approved by the Research Ethics Committee of the academic institution. All patients gave written informed consent for participation in this study.

Blood pressure (BP), body mass index (BMI), waist hip ratio (WHR), smoking habits and history of personal coronary artery disease (CAD) were documented. Presence of CAD was assessed based on the clinical history, previous medical records and exercise tolerance test reports.

Overnight fasting venous blood samples were collected following non-traumatic venepuncture in the morning between 08.00 and 10.00 h. Serum which was separated within 1 hour were analyzed for total cholesterol (TC), HDL-cholesterol (HDL-c), triglycerides (TG), fasting glucose, renal profile, liver function, thyroid function, hsCRP and homocysteine levels.

Demographic variables are presented as mean ±1 standard deviation (SD) for continuous normally distributed variables, as mean ± SEM for continuous non-normally distributed data, and as percentages for categorical data. Analysis of normality was performed with the Kolmogorov-Smirnov test. For continuous normally distributed variables, comparisons between the two groups were performed by use of Student’s t-test. While non-normally distributed data were compared between the groups by using Mann-Whitney test. Categorical data and proportions were analyzed using Chi-square test. Pearson’s or Spearman’s correlation coefficient was used to analyze correlation between
two variables with normal distribution or non-normal distribution respectively. A P value <0.05 was considered statistically significant. The statistical analysis was performed on the Statistical Package for Social Sciences (SPSS version 12.0) software on an IBM-compatible computer.

Results

Demographic data of the patients and control group that participated in this study is shown in Table 1. A total of 56 primary hypercholesterolaemic patients were recruited into this study and they were categorized as follows - FH (mean ± SD age = 41.8±11.6, 12 males and 17 females) and NFH (mean ± SD age = 49.3±7.1, 15 males and 12 females). There was a significant difference between FH, NFH and control groups with regards to serum TC (mean ± SD: 9.3±2.1 vs 4.9±0.5, p<0.0001 and 7.2±0.9 4.9±0.5, p<0.0001 respectively), TG (mean ± SEM: 1.6 ± 0.1 vs 1.0±0.05, p<0.0001 and 2.0±0.3 vs 1.0 ± 0.05, p<0.05 respectively) and LDL-c (mean ± SD: 7.3±2.1 vs 3.2±0.5, p<0.0001 and 5.0±0.8 vs 3.2±0.5, p<0.0001 respectively). There were more smokers among the patients compared to the control group (14.3% vs 0.0%, p<0.05) and systolic BP was higher in patients compared to control (mean ± SD: 135.2±23.1 vs 120.3±12.2, p<0.05).

There were no significant difference between groups regarding gender, race, BMI and serum HDL-c.

Serum homocysteine levels were higher in all patients compared with control (mean ± SD: 11.3±6.4 vs 8.6±3.3, p<0.05) as shown in Figure 1, with Hcys values being significantly higher in NFH group (mean ± SD: 11.9±7.0 vs 8.6±3.3, p<0.05) compared with control. Serum hs-CRP were higher in NFH group (p<0.05) compared to control (Figure 2). Figure 3 illustrates that plasma 8-isoprostane levels of the primary hypercholesterolaemic patients were higher (mean ± SEM: 1357±214.7 vs 398.0±20.5, p<0.05) compared to control and they were also higher in FH and NFH subgroups compared to control (mean ± SEM: 1395.8±313.3 vs 398±20.5, p<0.05 and 1322.8±300.7 respectively).

Correlation between Serum Hcys, hsCRP and plasma 8-isoprostane and Serum lipid profile (Figures 4-8)

There was positive correlation between serum total cholesterol with serum Hcys and plasma isoprostane (r=0.6, p<0.001 and r=0.5, p<0.001 respectively) as shown on the scatter plot in Figures 4 and 5. Correlation was also noted between serum LDL-c with serum Hcys (r=0.6, p<0.001) and plasma 8-isoprostane (r=0.5, p<0.05) as illustrated in Figures 6 and 7. A significant negative correlation was also seen between serum HDL and Hcys levels (r= -0.3, p<0.05) as shown in Figure 8. This study did not show any significant correlation between any of the lipid profiles and serum hsCRP results.

Discussion

This cross-sectional observational study involving subjects with primary hypercholesterolaemia demonstrated that serum hsCRP levels were significantly higher in NFH patients compared to those of the control subjects. However, there was no significant difference between FH patients and that of control group. There was no correlation between any of the lipid profiles (TC, LDL-c, TG and HDL-c) and hsCRP level. The lack of correlation between hsCRP and lipid levels is consistent with results from other studies (Ridker et al., 1999; 2001; Musial et al., 2001; Jialal et al., 2001; Albert et al., 2001). Our data also did not find any correlation between hsCRP levels with other established cardiovascular risk factors such as age, gender, blood pressure, and smoking habit.

With regards to serum Hcys, our study showed that these levels were significantly higher in primary hypercholesterolaemic patients (FH and NFH both) compared to controls, particularly in the NFH subgroup. There was also a significant positive correlation between serum TC and LDL-c with Hcys levels which is in agreement with findings from other studies that have shown association between these profiles. There was however, no
correlation between serum Hcys levels with that of other established risk factors such as age, gender, systolic and diastolic BP and smoking habit. These current findings from this study would suggest that Hcys could possibly be a potential reliable marker for cardiovascular risk.

The results of in vivo studies have shown that rodent models of CAD in which hyperlipidaemia has been induced are associated with enhanced oxidative stress as reflected by the increased 8-isoprostane levels (Roberts and Morrow, 2000; Rokach et al., 1997; Famm and Morrow, 2003; Davi, Falco, Patrono, 2004). In our present study, plasma 8-isoprostane was found to be significantly higher in patients with FH and NFH compared to the control group which is in concordance with the results from other studies looking at the association between hypercholesterolaemia and levels of 8-isoprostane. Plasma 8-isoprostane also showed significant correlation with serum TC and LDL-c in our study, but not with other lipid profiles such as HDL-c and TG and other established traditional risk markers such as smoking which have been demonstrated in other studies.

There were some limitations to this study with the first being the small sample size that was recruited over a limited period of time. Secondly, due to the cross-sectional design of this study, relationships between these newer risk biomarkers and other cardiovascular risk factors cannot be deemed causal in nature. Lastly, because our study population consisted of primary hypercholesterlaemic patients caution should be exercised when generalizing these data to other general populations.

Conclusion

In summary, this study has demonstrated that there is an association between hypercholesterolaemia with serum hsCRP, Hcys and plasma 8-isoprostane levels as seen by the significant difference between patients with hypercholesterolaemia and those of normolipaemic control group. The results for serum hsCRP in our study however were not consistent with several other studies that have established the significance of this marker as a cardiovascular risk factor. The findings of this investigation need to be confirmed in a larger study representing the general population and involving other novel cardiovascular risk markers that reflect inflammatory process (eg interleukin-1, interleukin-6 and tumour necrosis factor-alpha), thrombosis (eg fibrinogen and plasminogen activator inhibitor-1) and markers of oxidative stress (eg oxidized LDL). In addition to that, prospective studies need to be carried out in order to evaluate the association of these biomarkers with subsequent cardiac events to further lend an insight into their usefulness in the diagnosis and prognosis of CAD.

References

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## Appendix

### Table 1. Baseline Characteristics of Primary Hypercholesterolaemic Patients and Control Subjects

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>FH (n=28)</th>
<th>NFH (n=27)</th>
<th>All Patients (n=56)</th>
<th>Control (n=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>41.8 ± 11.6* ##</td>
<td>49.3 ± 7.1</td>
<td>45.4 ± 10.3</td>
<td>40.0 ± 8.0</td>
</tr>
<tr>
<td>Male</td>
<td>12 (41.4%)</td>
<td>15 (55.6%)</td>
<td>27 (48.2%)</td>
<td>12 (33.3%)</td>
</tr>
<tr>
<td>Female</td>
<td>37 (58.6%)</td>
<td>12 (44.4%)</td>
<td>29 (51.8%)</td>
<td>24 (66.7%)</td>
</tr>
<tr>
<td>Malay</td>
<td>23 (79.3%)</td>
<td>18 (66.7%)</td>
<td>41 (73.2%)</td>
<td>28 (77.8%)</td>
</tr>
<tr>
<td>Chinese</td>
<td>5 (17.2%)</td>
<td>8 (29.6%)</td>
<td>13 (23.2%)</td>
<td>6 (16.7%)</td>
</tr>
<tr>
<td>Indian</td>
<td>1 (3.4%)</td>
<td>1 (3.7%)</td>
<td>2 (3.6%)</td>
<td>2 (5.6%)</td>
</tr>
<tr>
<td>Smoker</td>
<td>5 (17.2%)</td>
<td>3 (11.1%)</td>
<td>8 (14.3%)*</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>134.1 ± 21.3*</td>
<td>136.4 ± 23.4*</td>
<td>135.2 ± 23.1*</td>
<td>120.3 ± 12.2</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>76.0 ± 9.1</td>
<td>79.0 ± 10.8</td>
<td>77.4 ± 10.0</td>
<td>78.6 ± 8.0</td>
</tr>
<tr>
<td>BMI</td>
<td>25.0 ± 4.6</td>
<td>26.0 ± 4.6</td>
<td>25.4 ± 4.6</td>
<td>24.3 ± 5.3</td>
</tr>
<tr>
<td>WHR (Waist Hip Ratio)</td>
<td>0.8 ± 0.09</td>
<td>0.9 ± 0.06**</td>
<td>0.9 ± 0.1*</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>9.3 ± 2.1*** ###</td>
<td>7.2 ± 0.9**</td>
<td>8.3 ± 1.9***</td>
<td>4.9 ± 0.5</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.6 ± 0.1 (SEM)**</td>
<td>2.0 ± 0.3 (SEM)*</td>
<td>1.8 ± 1.1 (SEM)**</td>
<td>1.0 ± 0.05</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>7.3 ± 2.1*** ###</td>
<td>5.0 ± 0.8**</td>
<td>6.2 ± 2.0**</td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.3 ± 0.1 (SEM)**</td>
<td>1.3 ± 0.2 (SEM)</td>
<td>1.3 ± 0.1 (SEM)</td>
<td>1.3 ± 0.5</td>
</tr>
</tbody>
</table>

Notes: * P<0.05 compared to control, ** P<0.005 compared to control, *** P<0.0001 compared to control, ### P<0.005 compared to NFH, #### P<0.0001 compared to NFH
**FIGURE 1. SERUM HOMOCYSTEINE LEVELS**

Note: *p < 0.05 compared to control group

**FIGURE 2. SERUM hsCRP LEVELS**

Note: *p < 0.05 compared to control group
**Figure 3. Plasma 8-Isoprostane Levels**

Note: *p < 0.05 compared to control group

**Figure 4. Correlation Between Serum Total Cholesterol (mmol/L) and Serum Hcys (μmol/L)**

**Figure 5. Correlation Between Serum Total Cholesterol (mmol/L) and Plasma 8-Isoprostane (pg/L)**
**Figure 6. Correlation Between Serum LDL-c (mmol/L) and Serum Hcys**

![Graph showing correlation between serum LDL-c (mmol/L) and serum Hcys.]

**Figure 7. Correlation Between Serum LDL-c (mmol/L) and Plasma 8-Isoprostane (pg/L)**

![Graph showing correlation between serum LDL-c (mmol/L) and plasma 8-isoprostane (pg/L).]

**Figure 8. Correlation Between Serum HDL-c (mmol/L) and Serum Hcys**

![Graph showing correlation between serum HDL-c (mmol/L) and serum Hcys.]