Increased platelet activation and fibrinogen in Asian Indians

Potential implications for coronary risk

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Aims To determine whether Asian Indians (Indians), a group known to have high rates of coronary heart disease, have increased platelet activation and fibrinogen levels relative to white Americans of European origin (whites).

Methods and Results Forty healthy, non-smoking Indians, aged 25–45, were matched with 40 healthy whites for age (within 3 years) and gender. Platelet activation was tested in blood exiting a bleeding time wound at 1 and 2 min post-incision (wound-induced activation), as well as in venous blood stimulated in vitro with collagen, using whole blood flow cytometry. Other risk factors, including fibrinogen levels, family history of diabetes or coronary heart disease, fasting insulin and lipid levels, and Lp(a) were also assessed. Fibrinogen levels were higher among Indians than whites, even after adjustment for gender or family history of coronary heart disease (P<0·05). Indians had higher levels of wound-induced glycoprotein IIb/IIIa binding and platelet secretion (P-selectin expression) than whites, with the greatest differences found when comparing the upper quintile of activation for each group (Ps<0·05). Indians with a family history of coronary heart disease (n=15) had higher levels of platelet secretion (wound-induced and in vitro) than Indians without a family history (Ps<0·05), while the relationship was reversed among whites. Platelet activation measures were not consistently related to other coronary risk factors, while fibrinogen was related to triglyceride and insulin levels among Indians.

Conclusion Indians have elevated fibrinogen and platelet activation levels relative to whites. These factors may contribute to the increased coronary risk observed in Indians.

See page 685 for the Editorial comment on this article (Eur Heart J 1998; 19: 720–726)

Key Words: India, platelet activation, fibrinogen, coronary disease.

Introduction

Asian Indians, who comprise approximately one-fifth of the world’s population have an increased prevalence of coronary heart disease both in India[1,2] and among immigrants to other countries[3–5]. The prevalence rate of coronary heart disease in India and among Indians in the United States may be three times greater than in the Framingham Heart Study[3,4]. Increased prevalences of adult-onset diabetes, low HDL cholesterol, and increased Lp(a) levels have been cited as partially responsible for this phenomenon[4,6,7] along with other potential risk factors such as low birth weight[8].

However, the increase in coronary heart disease in this community is still poorly understood.

Haemostatic factors, which may increase the risk of thrombosis, may in turn raise the likelihood of coronary heart disease. For example, one study found increased plasma factor VII activity among Indians[9]. Fibrinogen, already an established risk factor[10,11] has been shown to be increased among Indians with increased coronary heart disease risk relative to other Indians[12], but has not been elevated among South Asians relative to other groups[13,14]. One haemostatic factor that has not been tested to date is platelet activation, which is emerging as an important factor in coronary heart disease[15,16]. The methodology for evaluating platelet function has greatly advanced in recent years with the advent of flow cytometric detection of platelet activation[17,18]. Research using whole blood flow cytometry has shown that platelets do not generally circulate in an activated state. However, activation
produced by a vascular injury can be readily detected\[19\], such as in platelets exiting a bleeding time wound (i.e. wound-induced platelet activation)\[17,20\]. In our initial study using this method, we found that wound-induced binding to the fibrinogen receptor glycoprotein IIb/IIIa (GPIIb/IIIa) was increased among individuals who had developed restenosis following coronary stenting\[21\]. We also demonstrated that the method was highly reproducible.

In the course of developing the wound-induced activation method, we noted that some of the Asian Indians we tested had very high levels of activation, and that platelet activation had not been tested previously in this group. We therefore undertook the present study with the primary hypothesis that Asian Indians have increased wound-induced activation relative to white Americans of European origin (whites). We also measured plasma fibrinogen levels, and assessed the potential associations between these haemostatic factors and other coronary heart disease risk factors, particularly those associated with increased risk among Indians. Finally, we assessed the Indians for a polymorphism of the GPIIIa subunit (P\(_{42}^{\text{42}}\)) recently shown to be associated with increased risk of coronary heart disease\[22\].

Subjects and methods

Subjects

Forty healthy Indians, aged 25–45, were matched with 40 healthy whites for age (within 3 years) and gender. Each group, recruited from the Birmingham community, comprised 23 men and 17 women. The study was conducted at the University of Alabama, Birmingham, U.S.A. No subject was a current smoker; medication that affected platelet function (e.g. aspirin) was withheld for at least 10 days. A call for volunteers was followed by enrolling the first 40 eligible volunteers. Informed consent was obtained from all subjects, and the study was approved by the Institutional Review Board of the University of Alabama at Birmingham.

Design

Subjects were tested following an overnight fast of at least 10 h. Subjects completed questionnaires regarding their health, family history of coronary heart disease (defined as onset of coronary heart disease before age 65 in a parent or grandparent), family history of diabetes, and dietary questions, including number of days a week garlic was consumed and (for the Indians) the percentage of meals that included Indian food. Indians were also asked from which state in India they or their family originated. Because psychological factors may influence platelet function\[23\], subjects completed the Beck Depression Inventory and a modified form of the Cohen Perceived Stress scale\[24\]. Indians additionally completed the American-International Relations Survey\[25\] to assess for potential difficulties in assimilation into the U.S.

Height and weight were recorded, and then a venous blood sample was taken, using a 19 G butterfly needle and a two-syringe technique, for lipids, fasting insulin, fibrinogen levels, platelet studies, and P\(_{42}^{\text{42}}\) polymorphism (for the Indians only). After blood pressure was recorded, a modified bleeding time procedure was performed, as described previously\[21\], with samples taken for activation levels at 1 and 2 min post-incision. After the samples were taken, the wound was allowed to clot as in the usual bleeding time procedure, and the bleeding time was recorded.

Platelet activation measures

Platelet function testing was performed as described previously\[21\]. Whole venous blood, collected in 3·8% sodium citrate, was added to tubes containing Walsh’s buffer, a saturating solution of the anti-CD42b antibody SZ2-FITC (Gentrak, Inc.), and saturating solutions of one of the three antibodies for activation detection: AC1.2-PE (Becton Dickenson) for detection of P-selectin expression (an indication of platelet secretion); biotinylated PAC-1 (Cell Center, University of Pennsylvania), specific for the activated form of GPIIb/IIIa; and biotinylated Anti-LIBS-1, specific for GPIIb/IIIa (ligand) binding, which was graciously provided by Dr Mark Ginsberg. Negative and positive control tubes were prepared for each subject, as previously noted\[21\], and additional tubes were prepared using 100 µg ml\(^{-1}\) of equine collagen, Type I (Chronolog Co., Haverton, PA). For the bleeding time samples, whole blood collected in heparinized capillary tubes was added immediately to similar tubes with one of the three activation antibodies. All tubes were mixed gently and incubated for 15 min. Five microliters of a saturating solution of phycoerythrin-streptavidin (Southern Biotechnology Associates, Birmingham, AL) was then added to the tubes containing biotinylated PAC-1 and biotinylated anti-LIBS-1, followed by a second incubation of 10 min. The reaction was then stopped and the platelets fixed by the addition of 450 µl of 0·25% paraformaldehyde. Flow cytometric acquisition and analysis was performed within 6 h by a trained operator blinded to subject status. Acquisition was limited to include only particles with the characteristic properties of platelets, and stained for the SZ2 antibody. Analytical markers were used to determine the percentage of activated platelets (see\[21\]) for more detail).

Fibrinogen measures

Blood was collected in citrated tubes and centrifuged to obtain plasma, which was stored at −70 °C. Fibrinogen levels were determined using mechanical clot detection.

Other measures

Complete lipid measures including total, LDL, HDL, and Lp(a) cholesterol were obtained using methods described previously\cite{26}. Triglycerides and fasting insulin levels were also tested by a local laboratory. Determination of the P_{Ia}2 polymorphism among the Indians was obtained using methods described previously \cite{22}. Five samples had insufficient DNA extraction, leaving 35 samples for analysis.

Data analysis

Student t-tests were used to compare continuous variables in Indians and whites. Further comparisons were made by identifying quintile levels for each group and performing t-tests for each quintile level. General linear modelling was used to adjust for potential confounders and to assess interactions between groups (Indian/white) and family history of coronary heart disease (positive/negative). Linear regression was used to assess relationships between haemostatic factors and other risk factors.

Results

Coronary heart disease risk factors among Indians and whites

The characteristics and coronary heart disease risk factors of the two groups are shown in Table 1. On average, Indians had lower systolic blood pressures, lower HDL cholesterol, and higher triglycerides than the whites. Bleeding times did not differ between the groups.

Platelet activation levels among Indians and whites

As shown in Table 2, Indians had higher mean levels of platelet activation for all measures, and t-tests demonstrated significant differences for wound-induced GPIIb/IIIa binding at 1 and 2 min \((P<0.05)\), while there was a non-significant difference for wound-induced GPIIb/IIIa activation at 1 min \((P=0.07)\). Figure 1 displays levels of activation for each group by quintiles; while there were small differences in the lower quintiles, Indians in the upper quintile of the Indian distribution had significantly higher levels of activation than the whites in the upper quintile of the white distribution. These differences were significant for both wound-induced measures of P-selectin expression (Fig. 1(a) and (b)) and GPIIb/IIIa binding ((c) and (d)). Similar trends were seen for other measures, although the differences in the upper quintile were not significant \((P=0.07)\) for collagen-induced GPIIb/IIIa activation in vitro, \(P=0.09\) for wound-induced GPIIb/IIIa activation at 2 min; data not shown).

Differential effect of family history of coronary heart disease on platelet activation among Indians and whites

An interaction was found for collagen-induced P-selectin expression in vitro \((P=0.02)\) between family...
Among the Indians, those with a family history had higher P-selectin activation than those without ($P=0.01$), while the relationship was reversed among the whites. Significant interactions, with the same pattern, were seen for collagen-induced GPIIb/IIIa binding in vitro, and for wound-induced P-selectin at 1 min ($P<0.05$); in all other measures except collagen-induced GPIIb/IIIa activation in vitro, similar patterns were seen, although the interactions were not significant. In addition, after adjusting for family history, wound-induced GPIIb/IIIa activation at 1 min was significantly higher among Indians than whites ($P<0.05$), and similar trends were seen for wound-induced GPIIb/IIIa activation at 2 min ($P=0.07$) and collagen-induced GPIIb/IIIa activation in vitro ($P=0.08$).

Table 2  Mean (SD) platelet activation levels of Indians and whites*

<table>
<thead>
<tr>
<th>Activation measure</th>
<th>Indians</th>
<th>Whites</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-selectin expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wound-induced (1 min)</td>
<td>53.3 (19.8)</td>
<td>47.9 (18.6)</td>
</tr>
<tr>
<td>Wound-induced (2 min)</td>
<td>53.8 (19.9)</td>
<td>50.7 (17.3)</td>
</tr>
<tr>
<td>Collagen-induced in vitro</td>
<td>45.6 (24.1)</td>
<td>41.9 (22.1)</td>
</tr>
<tr>
<td>GPIIb/IIIa activation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wound-induced (1 min)†</td>
<td>16.0 (12.8)</td>
<td>11.2 (10.7)</td>
</tr>
<tr>
<td>Wound-induced (2 min)†</td>
<td>14.5 (12.1)</td>
<td>10.7 (9.2)</td>
</tr>
<tr>
<td>Collagen-induced in vitro</td>
<td>7.9 (8.6)</td>
<td>5.5 (6.1)</td>
</tr>
<tr>
<td>GPIIb/IIIa binding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wound-induced (1 min)†</td>
<td>12.8 (14.3)</td>
<td>7.8 (5.6)</td>
</tr>
<tr>
<td>Wound-induced (2 min)†</td>
<td>13.6 (13.2)</td>
<td>9.0 (6.0)</td>
</tr>
<tr>
<td>Collagen-induced in vitro</td>
<td>9.9 (7.0)</td>
<td>9.3 (4.7)</td>
</tr>
</tbody>
</table>

*Values are percent platelets activated.
†$P<0.05$  ‡$P=0.07$.

Figure 1  Wound-induced platelet activation for Indians (■) and whites (●). For each group, quintiles were determined for each measure. (a) and (b) show the average percent of platelets expressing P-selectin (platelet secretion) for each group at 1 and 2 min, respectively, while (c) and (d) show the average percent of platelets with GPIIb/IIIa ligand binding for each group at 1 and 2 min. For all four measures (wound-induce activation at 1 and 2 min for both activation markers), the average activation among the top quintile for the Indians was markedly higher than the top quintile for the whites ($P<0.05$ for P-selectin at one minute, $P<0.01$ for other measures).
Table 3  Mean (SD) plasma fibrinogen levels for Indians and whites by gender*

<table>
<thead>
<tr>
<th>Gender</th>
<th>Indians</th>
<th>Whites</th>
<th>Total†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>271·3 (62·2)</td>
<td>250·9 (44·3)</td>
<td>260·9 (54·1)</td>
</tr>
<tr>
<td>Women</td>
<td>318·8 (57·5)</td>
<td>261·1 (89·9)</td>
<td>288·2 (80·8)</td>
</tr>
<tr>
<td>Total†</td>
<td>291·8 (64·0)</td>
<td>255·5 (67·9)</td>
<td></td>
</tr>
</tbody>
</table>

*Values are mg. dl⁻¹ n=40 for whites, 37 for Indians.
†P=0·02 ‡P=0·08.

Fibrinogen levels among Indians and whites

Table 3 shows the fibrinogen levels by gender for Indians and whites. Indians had significantly higher fibrinogen levels than whites (P<0·05), while women had non-significantly higher levels than men (P=0·08). There was no relationship between fibrinogen levels and family history of coronary heart disease in either group (data not shown).

Relationships between fibrinogen and other risk factors

Among the entire cohort, fibrinogen was positively correlated with triglyceride and insulin levels (rs=0·37 and 0·34, respectively, P<0·005), while HDL was inversely related to fibrinogen (r=0·27, P<0·05). When analysed by group, fibrinogen remained significantly related to triglycerides and insulin levels, but only among the Indians (rs=0·36 and 0·48, respectively, P<0·05 and 0·01, respectively). Otherwise, fibrinogen was unrelated to other variables, including platelet activation measures.

Relationships between platelet activation and other risk factors

For the entire cohort, wound-induced GPIIb/IIIa activation at 1 min was greater among those with a family history of diabetes than those without (16·8 ± 13·3 vs 11·2 ± 10·2%, P<0·05); in addition, an interaction similar to that described previously was found between Indian/white group and family history of diabetes for collagen-induced GPIIb/IIIa binding in vitro (P=0·02). However, no other significant relationships between family history of diabetes and activation measures were found. For the whole cohort, collagen-induced GPIIb/IIIa activation in vitro was related to perceived stress (r=0·38, P<0·005). Among the Indians only, this measure was significantly related to both perceived stress (r=0·48, P<0·005) and Beck Depression scores (r=0·36, P<0·05) and these relationships were stronger among Indians with a family history of coronary heart disease (rs=0·57 and 0·66, respectively, P<0·05). Only four of the 35 Indians tested had the PI42 polymorphism associated with thrombosis (whites were not tested; see above), and this was not significantly related to any of the activation measures. Among whites only, LDL cholesterol was positively related to wound-induced GPIIb/IIIa activation at 1 and 2 min (rs=0·39 and 0·36, respectively, P<0·05) and age was positively correlated with wound-induced GPIIb/IIIa binding at 1 min (P=0·06) and 2 min (r=0·40, P=0·01), and with wound-induced GPIIb/IIIa activation 2 min (P=0·06). Bleeding time among the entire cohort was inversely related to several platelet activation measures, but the relationship was significant only for wound-induced P-selectin expression at 1 and 2 min (rs=−0·25 and −0·28, respectively, P<0·03). Other factors, including percent of Indian meals among Indians, garlic intake, acculturation, family lineage from Northern vs southern India, and other factors listed in Table 1, were not consistently related to platelet activation measures, either in the whole cohort or in either of the two groups alone.

Discussion

It is becoming increasingly evident that a number of traditional coronary heart disease risk factors among American or European whites and blacks (blood pressure, LDL cholesterol) do not contribute to our understanding of the increased prevalence of coronary heart disease among Asian Indians. In addition, risk factors that are more prevalent among Indians (low HDL cholesterol, insulin resistance, Type II diabetes) do not fully explain the increased rates of coronary heart disease in this group. The results of the present study indicate that fibrinogen and platelet activation may play a role in coronary heart disease among Indians.

The present study is the first to find increased fibrinogen levels among Indians relative to whites. A larger study in England found no difference in fibrinogen levels between Bangladeshis and a non-Asian control group[13], and another study of myocardial infarction patients found lower fibrinogen levels in south Asian patients relative to patients with a predominantly Arabic background[14]. In the present study, differences in fibrinogen levels between Indians and whites were primarily found among women, for reasons that are unclear. In most studies of Western populations, fibrinogen has been independently predictive of coronary heart disease[10,11], even though it has been associated with...
other risk factors\textsuperscript{[27–32]}. In the present study, consistent with other studies\textsuperscript{[4,6,13]}, Indians had lower HDL cholesterol and higher triglycerides than whites. In addition, associations of fibrinogen with HDL, triglycerides, and insulin were found, and the association with insulin and triglycerides remained among Indians only when the groups were considered separately. These associations indicate that fibrinogen may be associated with the already-established risk factor (among Indians) of insulin resistance. Increased fibrinogen levels have been associated with an increased coronary risk index among Indians in a previous study\textsuperscript{[12]}; future studies must establish whether fibrinogen is independently predictive of coronary heart disease among Indians, or whether it is part of a developing insulin resistance.

Platelet activation, unlike fibrinogen, is not an established risk factor for coronary heart disease, but two findings support the possibility that it may be a risk factor for Indians. First, the largest differences between Indians and whites were at the top quintiles of the distributions, for the two wound-induced activation measures which were the most reliable in our previous testing\textsuperscript{[21]}. Indeed, these levels were, on average, the highest we have seen in our laboratory in any white or black group, including smokers or those with coronary heart disease (unpublished results). Second (and perhaps more importantly), a number of activation measures were higher among Indians with a family history of coronary heart disease than those without, while the reverse was true for whites. These results, together with the finding that activation was not related to other coronary heart disease risk factors among Indians, suggest that platelet activation may constitute an independent risk factor among Indians.

The mechanisms responsible for increased platelet activation among the Indians are not clear at this point. The number of Indians with the $P\text{IIa}^A$ polymorphism, associated with thrombosis in a previous study\textsuperscript{[21]}, was low in this study, and did not significantly contribute to explaining the increased activation levels. This prevalence was higher than the prevalence among Koreans, but lower than that found among white and black Americans\textsuperscript{[33]}. The only factor related to platelet activation measures among Indians was perceived stress, which was primarily related to collagen-induced GPIIb/IIIa activation in vitro among Indians with a family history of coronary heart disease. This finding is consistent with other evidence linking psychological stress with platelet activation\textsuperscript{[23,34]}.

The present study is somewhat limited by a small sample size and the relative youth and good health of the subjects. Until future studies confirm the present findings with older subjects and/or coronary heart disease patients, it is premature to make firm clinical recommendations to Indians on the basis of this study. It may not be unreasonable, however, to recommend aspirin for Indians over the age of 40 with a family history of coronary heart disease, given aspirin’s effects on platelet activation\textsuperscript{[35,36]} in order to reduce the risk of thrombotic events.

In summary, fibrinogen and platelet activation are increased among Indians relative to whites, and activation is increased particularly among Indians with a family history of coronary heart disease. Insulin resistance may explain the increased fibrinogen levels among Indians, while coronary heart disease risk factors do not fully explain the increased platelet activation. Future studies must explore haemostatic risk factors among Indians with coronary heart disease, and must further examine the mechanisms that may explain increased haemostatic factor levels in this group. Platelet activation measures may also be useful in prospective studies of coronary heart disease among Indians to determine their value in predicting thrombosis.

Supported by Grant 1K08HL02975-01 from the National Heart, Lung, and Blood Institute, USA. The authors would like to thank William Shear for his technical assistance in the performance of the $P\text{IIa}^A$ polymorphism determinations.

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