Complement C3 and C-Reactive Protein Are Elevated in South Asians Independent of a Family History of Stroke

Riyaz Somani, MRCP; Peter J. Grant, FRCP, MD; Kirti Kain, MRCP, MD; Andrew J. Catto, MRCP, PhD; Angela M. Carter, PhD

Background and Purpose—Complement components are emerging risk factors for cardiovascular disease. In this study, we examined the relation among C3, C-reactive protein (CRP), factor B, and features of the insulin resistance (IR) syndrome in 143 first-degree relatives of South Asian subjects with ischemic stroke, 141 South Asian controls, and 121 white controls.

Methods—C3, CRP (high-sensitivity assay), and factor B levels were measured by ELISAs, and their relation to features of the IR syndrome were assessed. Data are presented as geometric mean (95% CI).

Results—There was no significant difference in the levels of C3 between South Asian relatives (1.25 [1.21, 1.29] g/L) and South Asian controls (1.20 [1.15, 1.24] g/L, \(P = 0.2\)). Levels in both South Asian groups were significantly higher than in white controls (0.95 [0.92, 0.98] g/L; \(P < 0.001\) for both comparisons). These differences remained significant after adjustment for covariates. Similarly, levels of CRP were not different between the 2 South Asian groups, but levels in both South Asian groups, after adjustment for covariates, were significantly higher than in white controls. There was no difference in the levels of factor B among the 3 groups. South Asian subjects with elevated C3 levels clustered risk factors associated with IR to a greater extent than those with high CRP.

Conclusions—These results suggest that South Asians have a greater level of chronic subclinical inflammation than do whites, independent of a family history of stroke. In addition, C3 is more likely to cluster with features of the IR syndrome compared with CRP in South Asians. (Stroke. 2006;37:2001-2006.)

Key Words: atherosclerosis • ethnic groups • inflammation • insulin resistance • risk factors

Chronic subclinical inflammation is now recognized to play a central role in the pathogenesis of insulin resistance (IR), type 2 diabetes mellitus (T2DM), and cardiovascular disease (CVD).\(^1\)\(^2\) Specifically, C-reactive protein (CRP) and complement are emerging as inflammatory components involved in atherogenesis. Several studies have shown that CRP is correlated with features of the IR syndrome,\(^1\) and elevated levels thereof precede and predict the development of atherothrombotic disease.\(^2\)\(^3\) Data also implicate complement in the pathogenesis of atherothrombosis. In particular, complement C3, which is produced by the liver\(^4\) and cultured adipocytes,\(^5\) is correlated with features of the IR syndrome\(^6\) and predicts the development of diabetes.\(^7\) Furthermore, serum C3 concentration was a powerful predictor of future myocardial infarction (MI) in white men\(^8\) and new vascular events (including MI and stroke) in women with preexisting coronary artery disease.\(^9\) This has led to the suggestion that elevated C3 may indicate progression of atherosclerosis and act as a specific marker of chronic inflammation.\(^9\)

In this study, we have analyzed levels of C3, CRP, and complement factor B in a cohort derived from the migrant South Asian population residing in the United Kingdom. Compared with whites, South Asians are at increased risk for developing T2DM and CVD, which may in part be attributable to a greater level of IR,\(^10\) and associated risk factor clustering.\(^11\)\(^12\) Little is known regarding the role of inflammation in the “at-risk” South Asian population, and in particular, the role of complement has not been investigated in this group. The aims of this study were therefore to (1) determine whether levels of C3, factor B, and CRP are elevated in 2 South Asian groups (subjects with and without a family history of ischemic stroke) compared with age- and sex-matched healthy white subjects; (2) assess the differential relation of C3 and CRP with other well-recognized metabolic and hemostatic features of the IR syndrome in South Asian subjects; and (3) determine whether C3 and CRP together are associated with risk factor clustering to a greater extent than either inflammatory factor alone in South Asian subjects.
Subjects and Methods

One hundred forty-three healthy first-degree relatives of South Asian patients with confirmed ischemic stroke (1 relative for each patient with stroke) and 141 age- and sex-matched South Asian control subjects were recruited as previously described. One hundred twenty-one age- and sex-matched white subjects free of a personal or family history of stroke were also recruited from local Family Health Authority general practice registers. All subjects gave written, informed consent according to a protocol agreed by the local research ethics committees. All subjects underwent a full medical examination and provided a fasting blood sample for measurement of glucose, insulin, tissue plasminogen activator (tPA), plasminogen activator inhibitor antigen (PAI-1), fibrinogen, and a full lipid profile, as previously described. IR was determined by the Homeostasis Model Assessment (HOMA-IR).

C3, CRP, and factor B were measured in cold citrated plasma by in-house ELISAs. C3 was measured with anti-C3c antibodies from Dako; the interassay and intra-assay coefficients of variation (CVs) were 5.6% and 4.9%, respectively. CRP was measured with a high-sensitivity ELISA with antibodies from Dako, with an interassay CV of 5.8% and an intra-assay CV of 2%. Factor B was measured with antibodies from Quidel, with an interassay CV of 10.1% and an intra-assay CV of 3.7%.

Statistics

Based on the results of our previous study of C3 and CRP in white subjects with coronary artery disease and healthy control subjects, the sample size of the present study was sufficient to detect a difference of at least 0.06 g/L in C3 and a 1.55-fold difference in the sample size of the present study was sufficient to detect a difference of at least 0.06 g/L in C3 and a 1.55-fold difference in CRP with 80% power at a difference of at least 0.06 g/L in C3 and a 1.55-fold difference in the sample size of the present study was sufficient to detect a difference of at least 0.06 g/L in C3 and a 1.55-fold difference in CRP with 80% power at a difference of at least 0.06 g/L in C3 and a 1.55-fold difference in CRP with 80% power at a difference of at least 0.06 g/L in C3 and a 1.55-fold difference in

Results

Baseline characteristics of the 3 groups are presented in Table 1. Only insulin and HOMA-IR were significantly higher in South Asian relatives compared with South Asian controls; they were also significantly higher than in white controls. Both South Asian groups had higher triglyceride levels, higher waist-hip ratio (WHR), lower HDL cholesterol, and fewer smokers compared with white controls.

There was no significant difference in the levels of C3 between the 2 South Asian groups (relatives: 1.25 [1.21, 1.29] g/L; controls: 1.20 [1.15, 1.24] g/L; P = 0.2); however, levels were significantly higher in both South Asian groups compared with healthy whites (0.95 [0.92, 0.98] g/L; P < 0.001 for both comparisons). After accounting for WHR, BMI, insulin, HOMA-IR, HDL, triglyceride levels, and history of smoking, C3 remained significantly higher in both South Asian groups (adjusted mean: relatives, 1.19 [1.14, 1.24] g/L; controls, 1.17 [1.13, 1.22] g/L) compared with white controls (adjusted mean: 0.91 [0.86, 0.96] g/L; P < 0.001 for both comparisons). Similarly, levels of CRP were similar between the 2 South Asian groups (relatives: 1.15 [0.96, 1.38] mg/L; controls: 0.91 [0.74, 1.13] mg/L; P = 0.25), significantly higher in South Asian relatives (P < 0.001), and of borderline significance in South Asian controls (P = 0.057) when compared with healthy whites (0.64 [0.51, 0.8] mg/L). After accounting for WHR, BMI, insulin, HOMA-IR, HDL, triglyceride levels, and smoking, CRP levels remained significantly higher in both South Asian groups (adjusted mean: relatives, 1.07 [0.84, 1.36] mg/L; controls, 0.86 [0.68, 1.07] mg/L) compared with white controls (adjusted mean: 0.59 [0.43, 0.8] mg/L; P = 0.002 and P = 0.045, respectively). There were no significant differences in factor B levels between the 2 South Asian groups (relatives: 179.1 [171.4, 186.8] mg/L; controls: 187.4 [177.4, 197.0] mg/L; P = 0.38) or the whites (180.1 [173.2, 187.0] mg/L; P = 0.99 and P = 0.51, respectively).

Figure 1A demonstrates the striking difference in the distribution of C3 levels between the 2 South Asian groups.

| TABLE 1. Characteristics of First-Degree Relatives of South Asian (SA) Subjects With Ischemic Stroke, SA Controls, and White Controls |
|-----------------|-----------------|-----------------|
|                  | SA Controls     | SA Relatives    | White Controls  |
|                  | (n = 141)       | (n = 143)       | (n = 121)       |
| Age, y           | 41 (33, 49)     | 41 (33, 50)     | 42 (36, 47)     |
| Sex, F:M         | 71:75           | 68:75           | 60:61           |
| Smoker, %        | 14*             | 8*              | 36              |
| BMI, kg/m²       | 25.4 (24.6, 26.2) | 26.0 (25.2, 26.8) | 25.9 (25.1, 26.8) |
| WHR              | 0.89 (0.88, 0.91)* | 0.92 (0.90, 0.94)* | 0.84 (0.82, 0.86) |
| Insulin, μIU/mL  | 8.5 (7.3, 7.8)  | 12.0 (10.9, 13.3)** | 8.4 (7.2, 9.8)  |
| HOMA-IR          | 1.94 (1.64, 2.28) | 2.73 (2.45, 3.05)** | 1.86 (1.56, 2.21) |
| Triglyceride, mmol/L | 1.4 (1.3, 1.5)*      | 1.5 (1.4, 1.7)      | 1.1 (1.0, 1.2)    |
| HDL, mmol/L      | 1.1 (1.1, 1.2)*  | 1.1 (1.0, 1.2)*   | 1.5 (1.4, 1.6)   |
| Glucose, mmol/L  | 5.12 (4.93, 5.32) | 5.10 (4.92, 5.28) | 4.90 (4.77, 5.03) |
| Hypertension, %  | 12               | 11              | 5               |
| Diabetes, %      | 8                | 8               | 2               |

Data are presented as mean or geometric mean (95% CI) unless otherwise stated. *P < 0.05 compared with white controls; †P < 0.01 compared with SA controls.
compared with white controls, with median C3 levels in South Asians approaching the 97.5th percentile of C3 levels observed in white controls. A similar trend was seen with levels of CRP (Figure 1B), although the difference was much less pronounced. To evaluate risk factor clustering of C3 and CRP in South Asians, both South Asian groups were combined, and C3 and CRP levels were dichotomized above and below the median (1.25 g/L and 1.13 mg/L for C3 and CRP, respectively). As shown in Table 2, compared with subjects with both C3 and CRP levels below the median (n=98), subjects with only CRP levels above the median (n=45) had a significantly higher prevalence of hypertension and higher levels of tPA, factor B, and fibrinogen; subjects with only C3 levels above the median (n=44) had significantly higher insulin, HOMA-IR, triglyceride, PAI-1, tPA, and factor B levels. Subjects with both C3 and CRP above the median (n=97) had a higher prevalence of hypertension, higher BMI, WHR, insulin, HOMA-IR, triglyceride, fasting glucose, PAI-1, tPA, fibrinogen and factor B. Finally, comparing subjects with both C3 and CRP above the median with subjects with only one raised inflammatory variable, subjects with raised C3 and CRP had significantly higher HOMA-IR, BMI, and fibrinogen, as shown in Figure 2.

Discussion

Atherosclerosis is the chief underlying cause for CVD, and given its associated morbidity and mortality, the search for new risk markers and preventive strategies is paramount. It is now widely accepted that atherosclerosis is an inflammatory process that results in plaque development, plaque progression, and finally plaque degeneration. Prospective studies have established CRP as a powerful predictor of atherothrombotic disease, and it may also play an active role in atherogenesis. Similarly, the presence of activated complement components observed in atherosclerotic plaques, often colocalizing with CRP, has led to the hypothesis that complement activation may play an essential role in atherogenesis.

Studies have reported increased levels of CRP in South Asians; however, there have been no previous reports that we are aware of comparing C3 levels between white and South Asian subjects. In the present study, we determined levels of CRP, C3, and factor B in first-degree relatives of South Asian patients with stroke, South Asian controls, and white controls. We found no significant difference in levels of CRP, C3, or complement factor B between the 2 South Asian groups, suggesting that a family history of stroke is not associated with an enhanced inflammatory state in South Asians. However, our data demonstrated significantly higher levels of C3 and CRP in both South Asian groups, confirming that South Asians, who are recognized to be at increased risk of CVD, have a heightened proinflammatory state. These results imply that C3 and CRP are unlikely to contribute to the familial risk of stroke in South Asians but represent generalized vascular risk in this group. The source of the increased C3 and CRP in South Asians remains unclear. There may be increased production of C3 and CRP in the liver secondary to a state of generalized low-grade inflammation. C3 is also known to be produced by adipocytes, and in this study, both South Asian groups had a significantly higher WHR, reflecting greater central adiposity compared with white controls. Similarly, interleukin-6, which is the primary cytokine involved in CRP synthesis, is also produced by adipocytes. Thus, the elevated levels of C3 and CRP may partly be explained by the greater central adiposity found in South Asians, although levels remained significantly higher in South Asians even after adjusting for BMI and WHR, supporting previous findings. The generation of CRP and complement components is reported to occur directly within

Figure 1. The distribution of C3, CRP, and factor B in first-degree relatives of South Asian subjects with ischemic stroke (SAR), South Asian controls (SAC), and white controls (WC). Center horizontal bar=median; upper horizontal bar=97.5th percentile; lower horizontal bar=2.5th percentile; o=outliers.
rupture rising late in the disease process,15 and our results suggest that elevated C3 may be a more sensitive marker for CVD. Although CRP is accepted as an independent risk factor for CVD, studies have failed to establish a clear association between CRP levels and atherosclerotic burden.23 In the present study, C3 clustered with risk factors associated with IR and had the highest levels of HOMA-IR, BMI, and PAI-1, ng/mL.24 It has been hypothesized that elevated C3 may be a more sensitive marker for early atherosclerotic development and progression and that CRP may be a better marker of plaque vulnerability and rupture rising late in the disease process,15 and our results lend support to this suggestion.

Increasing evidence suggests that complement activation may play a functional role in atherogenesis and that increased levels of C3 are more than an epiphenomenon. In animal studies, the terminal C5b-9 complex of the complement cascade was deposited in the aorta of hypercholesterolemic rabbits, preceding foam cell production,25 and atherosclerotic lesions failed to progress beyond the foam cell stage in C3-deficient mice.26 Complement activity is upregulated during the acute phase of both MI27 and ischemic stroke28 and is thought to be an important mediator of ischemia/reperfusion injury.29 In addition, elevated levels of C3, at the time of an ischemic event, have been associated with worse outcome.30 In the context of ethnic differences, South Asians have increased morbidity and mortality after cardiovascular events,31,32 and it is possible that C3 contributes to the development of vascular disease and may also predict poor outcome through enhancement of mechanisms associated with ischemia/reperfusion injury. Taken together, these data suggest that complement in general, and C3 specifically, may play a significant functional role in atherogenesis and tissue injury and that elevated C3 may act as an important risk marker for CVD.

In conclusion, the results of the present study demonstrate that South Asians, who are recognized to be at increased risk for CVD and T2DM, have higher C3 and CRP levels than their white counterparts, in agreement with previous reports19,20 indicating a greater level of chronic subclinical inflammation in this population. Furthermore, the results suggest that elevation of C3 and CRP is independent of a family history of stroke and imply that C3 and CRP do not contribute to the familial predisposition to stroke in South Asians but reflect generalized vascular risk in this population. Elevated C3 levels in South Asians are also more likely to cluster with other well-recognized atherothrombotic risk factors compared with elevated CRP. In view of its potential role in atherogenesis, C3 may serve as a therapeutic target, and further studies are therefore warranted to evaluate the role of C3 and complement activation in CVD, particularly in South Asians.
Figure 2. The association of C3 and CRP with HOMA-IR, BMI, and fibrinogen in South Asian subjects. South Asian subjects were evaluated for differences in cardiovascular and metabolic risk factors dependent on whether levels of C3 and/or CRP were above the median compared with subjects with both C3 and CRP below the median. *P<0.05 compared with [C3<1.25 and CRP<1.13] group and [C3=1.25 and CRP<1.13] group. †P<0.05 compared with [C3<1.25 and CRP<1.13] group.

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Disclosures
None.

References


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