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Complement activation and vascular complications in type 2 diabetic South Asians

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Abstract

Introduction

South Asian immigrants in Western societies exhibit a high burden of diabetes and subsequent vascular complications. Diabetic vascular complications are associated with vascular inflammation. We hypothesize that enhanced complement activation is involved.

Research desing and methods

Levels of complement C3, SC5b-9 -the soluble end-product of complement activation, and Mannose-Binding Lectin (the recognition molecule of the lectin pathway of complement activation) were measured in 268 South Asians (168 type 2 diabetic and 100 non-diabetic subjects) and compared to an age- and sex matched control-group of native Caucasians. In a cross-sectional study, the association between complement levels, diabetes and albuminuria was assessed. In a longitudinal study, the 168 type 2 diabetic South Asians were followed prospectively for a median duration of 7.66 years. The association of complement levels with future cardiovascular events and progressive renal failure was assessed.

Results

Compared to native Caucasians, South Asians had significantly higher levels of both serum C3 and plasma SC5b-9 and higher levels of MBL. A SC5b-9 level above the median was associated with albuminuria at baseline (univariate OR 2.3, 95%CI 1.18-4.55, $P = 0.015$) and with progressive renal failure (univariate HR 5.45, 95%CI 1.52-19.5, $P = 0.009$) Neither C3 nor SC5b-9 predicted cardiovascular events. Baseline MBL levels were associated with progressive renal failure (multivariate HR 3.59, 95%CI 1.30-9.93, $P = 0.014$)

Conclusion

These results suggest that complement activation is involved in the pathogenesis of microvascular complications in South Asians with type 2 diabetes.

Introduction

South Asian immigrants in Western societies have a high burden of ischemic heart disease, stroke and diabetes [1,2]. In addition to macro-vascular disease, South Asians also have an increased incidence and a faster rate of progression of diabetic nephropathy compared to Caucasians.[3]

Traditional cardiovascular risk factors do not completely explain the increased incidence of cardiovascular disease in South Asians [4]. Hence other factors must be involved.

Atherosclerosis, the pathologic substrate of macro-vascular disease, is recognized to be an inflammatory process [5-7]. Micro-vascular disease such as diabetic nephropathy has also been linked to inflammatory markers [8,9]. As a key-player in the inflammatory response, the complement system has been implicated in this vascular inflammation [10-12]. Deposition of complement components has been demonstrated in atherosclerotic plaques, retinae and kidneys of diabetic subjects [13-15]. Complement activation products also have been detected in urine of subjects with diabetic nephropathy [16].

The complement system can be activated via the classical, alternative or lectin pathway. All three pathways converge into the generation of a C3 convertase, which activates the central molecule C3. The final activation product, C5b-9, exerts lytic and non-lytic harmful effects to its target cells. The lectin pathway is activated when Mannose-Binding Lectin (MBL) binds to its target molecule. MBL binds carbohydrate moieties on microorganisms, but endogenous MBL ligands, such as glycosylated immunoglobulins or cells exposed to oxidative stress, have also been identified [17]. MBL serum levels are primarily determined by 3 polymorphisms (B,C and D genotypes, commonly referred to as O-alleles) in the MBL gene (*mb12*). Subjects with wild type MBL genotype (A/A) have the highest serum MBL levels, subjects with 1 variant allele (A/O) have intermediate levels and subjects with 2 variant alleles (O/O) have the lowest levels. In addition, polymorphisms in the promoter region influence the MBL level [18]. MBL is synthesized in the liver. Although intra-individual levels are relatively stable over time [19], a two- to threefold increase occurs during acute phase reactions [20]. MBL has recently been implicated in the pathogenesis of diabetic vascular complications [21].

We hypothesized that in South Asians the complement system contributes to the increased susceptibility for vascular and renal disease. We therefore examined complement, as judged by the level of the main component C3, the

level of the final activation product SC5b-9 and MBL in South Asian subjects with and without type 2 diabetes living in the Netherlands, and compared these to complement levels in an age- and sex-matched group of native Caucasian volunteers without diabetes. In addition, we examined the predictive value of serum C3, plasma SC5b-9 and serum MBL level for the occurrence of cardiovascular events and progressive renal failure.

Materials & methods

Study design and study population

This study consists of 2 parts: a cross-sectional case-control study and an observational follow-up study.

Cross-sectional study

At baseline, 268 South Asians were studied. All subjects were participants in a previously conducted study and had been recruited as described earlier [22]. At baseline, 168 subjects had type 2 diabetes according to the ADA 2003 criteria. In addition, a sample of 100 non-diabetic subjects out of the original study population (n=465) was studied. Levels of complement C3, which plays a pivotal role in complement activation, and SC5b-9, the soluble end-product of complement activation, were measured and compared to levels in a group of native Caucasians without diabetes, recruited from healthy personnel from the dialysis ward and laboratory (n=60). Complement levels in South Asians with diabetes were compared to South Asians without diabetes. In the diabetic South Asian subgroup, the association between complement levels and albuminuria was assessed.

Follow-up study

After a median duration of 7.66 (IQR 7.48-8.10) years, follow-up data from the 168 type 2 diabetic subjects were collected. Study-patients were followed up by letter and subsequently by phone. When subjects could not be traced by address or phone number in our database, general practitioners or participating family members were involved.

Follow-up data consisted of medical history with regard to cardiovascular events. Subjects were sent a questionnaire and were invited for a visit to our out-patient clinic. During this visit the questionnaire was reviewed by the

main investigator (M.A.S.). Subjects not willing to visit the out-patient clinic were asked permission to collect medical data from their general practitioner. For subjects who had died during the follow-up period, cause of death and cardiovascular history was retrieved from the general practitioner. All (self-) reported events were verified by contacting the hospital in which the event had occurred. In addition, renal data (serum creatinine, urinary albumin/creatinin ratio) were measured or obtained from the general practitioner. The association of baseline complement levels with cardiovascular events and renal deterioration was assessed.

The study protocol was approved by the Institutional Medical Ethics Committee. All subjects provided informed consent.

Measurement of baseline parameters

The study protocol has previously been published in detail [22]. Briefly, from all subjects morning urine and fasting blood samples were taken and immediately put in ice. Ethylenediaminetetraacetic acid (EDTA) plasma was obtained after centrifugation (10 minutes at 4,000 rpm at 4 degrees Celsius) and the samples were stored in aliquots at -80° Celsius within 1 hour after collection. Serum was prepared by coagulation at room temperature, and after centrifugation the samples were stored at -80° Celsius. All study participants, except known diabetic subjects, were subjected to an oral glucose tolerance test (75 gram glucose load). Diabetes was diagnosed based on the American Diabetes Association 2003 criteria. A brief physical examination (blood pressure, length, weight, hip- and waist circumference) was performed. Clinical information concerning medical history and medication was obtained from a questionnaire. Laboratory measurements including creatinin, fasting lipid profile, HbA1c, and urinary albumin/creatinine (ACR) ratio were measured according to standard methods. High-sensitivity C-reactive protein (hsCRP) was measured with a fully automated Cobas Integra 800, according to the manufacturers protocols (Roche, Almere, the Netherlands).

Quantification of SC5b-9

SC5b-9 levels were assessed by sandwich ELISA. In brief, 96-well ELISA plates were coated with the monoclonal antibody aE11 (mouse IgG2a anti-C5b-9 3mg/ml), described in detail previously [23]. Plasma samples were diluted 1/5 and 1/20 and incubated in the coated wells. Bound SC5b-9 was detected with a biotin labeled monoclonal anti C6 antibody (9C4), followed by detection with

streptavidin-poly horse radish peroxidase (Sanquin, Amsterdam, The Netherlands). Enzyme activity was detected using 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma Chemical Co., St. Louis, MO). The optical density was measured at 415nm using a microplate biokinetics reader (EL312e; Biotek Instruments, Winooski, VT). A calibration line was produced using zymosan activated serum with a known concentration of SC5b-9 of 1000 U/ml.

Quantification of C3

Serum C3 was quantified using radial immunodiffusion according to Mancini, using a polyclonal rabbit anti human C3 antiserum as described earlier [24].

Quantification of MBL

At baseline, serum MBL levels were assessed by sandwich ELISA as described previously [25]. In brief, 96-well ELISA plates (Greiner, Frickenhausen, Germany) were coated with the monoclonal antibody 3E7 (mouse IgG1 anti-MBL at 2.5 µg/ml), kindly provided by Dr. T. Fujita (Fuhushima, Japan). Serum samples were diluted 1/50 and 1/500 and incubated in the coated wells. MBL was detected with Dig-conjugated 3E7. Detection of binding of Dig-conjugated antibodies was performed using HRP-conjugated sheep anti-Dig Abs (Fab fragments, Roche, Mannheim, Germany). Enzyme activity was detected using 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma Chemical Co., St. Louis, MO). The optical density was measured at 415nm using a microplate biokinetics reader (EL312e; Biotek Instruments, Winooski, VT). A calibration line was produced using human serum from a healthy donor with a known concentration of MBL. Earlier studies indicated that this assay primarily detects wildtype MBL in serum and plasma and that there is a direct association with the MBL genotype and with MBL function.

MBL genotyping

DNA was isolated routinely from peripheral blood leucocytes. MBL single nucleotide polymorphisms at codons 52, 54 and 57 of the *mbi2* gene were typed by pyrosequencing. The detailed methodology has been published separately [26]. The MBL genotype of only wild-type allele carriers is designated as A/A and the presence of 1 or 2 variant alleles(s) (B, C, or D) is designated as A/O or O/O, respectively.

Definitions and endpoints

Albuminuria was defined as a urinary albumin/creatinine ratio > 2.5 mg/mmol in men and > 3.5 mg/mmol in women.

Cardiovascular events were pre-defined as the occurrence of either a myocardial infarction, Percutaneous Transluminal Coronary Angioplasty (PTCA), Coronary Artery Bypass Grafting (CABG), stroke, Carotid Artery Desobstruction, peripheral vascular angioplasty, bypass or amputation, or sudden cardiac death. The latter was defined as a witnessed sudden circulatory arrest. The primary end-point was the time to the first cardiovascular event.

Progressive renal failure was arbitrarily pre-defined as a 50% increase in serum creatinine or the initiation of renal replacement therapy (dialysis or kidney transplantation)

Statistical analysis

Normally distributed variables are expressed as mean $\pm$ 1 standard deviation and skewed distributed variables as median and interquartile range. Differences between two groups were assessed by Student's t-test or Mann-Whitney-U test as appropriate. Correlations were assessed by Pearson's or Spearman's correlation as appropriate. Associations between complement levels and albuminuria were assessed with logistic regression. Associations between complement levels, cardiovascular events and progressive renal failure were assessed with Cox proportional hazards regression. All test were two-sided and the level of significance was 0.05. All analyses were performed using SPSS for windows, version 17.

Results

Cross-sectional study

Two hundred sixty-eight South Asians (168 diabetic and 100 non-diabetic subjects) were studied. Of the 168 diabetic subjects, 121 were already known with diabetes at the time of the study, and 47 subjects were newly diagnosed with diabetes based on oral glucose tolerance testing. In the diabetic group, 53 subjects had albuminuria, and in the non-diabetes group 2 subjects had albuminuria. Characteristics of the South Asian study population are shown in Table 1.

Table 1 baseline characteristics of 268 South Asians

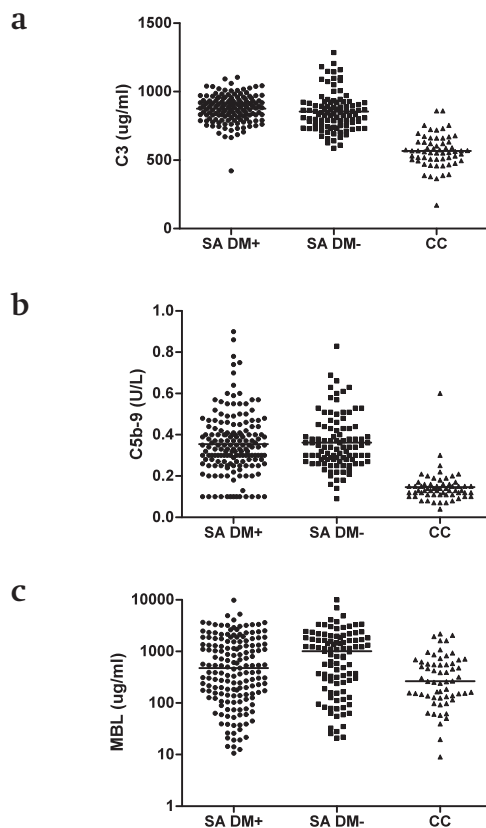
	Diabetic SA (n=168)	Non-diabetic SA (n=100)	P-value
Age (yrs)	50.5 ± 11.3	46 ± 7.3	< 0.001
% male sex	46	47	0.97
Diabetes duration (yrs)	7.0 (0.0-13.0)	-	
HbA1c (%)	7.7 ± 1.9	-	
Current or former smoker (%)	44	31	0.037
Body Mass Index	28.1 ± 4.5	26 ± 4.0	< 0.001
Waist circumference (cm)	97.2 ± 14.5	91.0 ± 10.3	< 0.001
Waist-to-hip ratio	0.98 ± 0.08	0.92 ± 0.08	< 0.001
Systolic blood pressure (mm Hg)	139 ± 25	128 ± 17	< 0.001
Diastolic blood pressure (mm Hg)	84 ± 11	81 ± 10	0.016
Total cholesterol (mmol/L)	5.1 ± 0.97	5.3 ± 0.97	0.082
Fasting triglycerides (mmol/L)	1.5 (1.2-2.2)	1.2 (0.84-1.88)	< 0.001
Ratio total: HDL cholesterol	4.33 ± 1.22	4.17 ± 1.24	0.318
Cockcroft-Gault creatinin clearance (ml/min)	93 ± 31	86 ± 17	0.012
Urinary albumin/creatinine ratio (mg/mmol)	1.0 (0.39-4.75)	0.32 (0.20-0.63)	< 0.001
hs CRP (mg/L)	3.5 (1.8-8.3)	Not available	
Serum C3 (µg/ml)	875 ± 97	854 ± 139	0.184
Plasma SC5b-9 (U/ml)	0.35 ± 0.15	0.36 ± 0.13	0.708
Serum MBL (µg/L)	476 (143-1563)	1009 (239-1847)	0.043

Age and sex were not significantly different between the whole South Asian and Caucasian group (mean age 48.8 ± 10.2 versus 46 ± 8 , P = 0.10 and % male sex 46 versus 45, P = 0.9, respectively) .

Complement levels in South Asians compared to native Caucasians

Serum C3 in the whole South Asian group was significantly higher compared to native Caucasians (867 ± 115 µg/ml versus 580 ± 128µg/ml, P<0.001) (Figure 1). When considering only non-diabetic South Asians, they still had higher C3 levels than native Caucasians (854 ± 139 µg/ml versus 580 ± 128 µg/ml, P<0.001).

Figure 1



Serum concentration of C3 (upper panel), plasma concentration of SC5b-9 (middle panel) and serum concentration of Mannose-Binding Lectin (lower panel, Logarithmic scale) in South Asians with type 2 diabetes (SA DM+, n=168), South Asians without type 2 diabetes (SA DM-, n=100), and a control group of native non-diabetic Caucasians (CC, n=60). Horizontal bars represent mean (C3, SC5b-9) and median (MBL)

South Asians also had significantly higher plasma levels of SC5b-9, the soluble end-product of complement activation (mean 0.358 ± 0.14 U/ml versus 0.149 ± 0.07 U/ml, $P < 0.001$) (Figure 1b). This was also the case when considering only non-diabetic South Asians (0.362 ± 0.13 U/ml versus 0.149 ± 0.07 U/ml, $P < 0.001$). MBL levels in the whole South Asian group were higher than in the Caucasian

group (median 558 (IQR 156-1680 $\mu\text{g/L}$ versus 263 (IQR 133-613, $P = 0.002$). Non-diabetic South Asians had even higher MBL levels compared to Caucasians (1009 (IQR 239-1847) versus 263 (IQR 133-613), $P < 0.001$)

Diabetic versus non-diabetic South Asians

The diabetic South Asian group was older than the non-diabetic South Asian group (mean age 50.5 ± 11 versus 46 ± 7 , $P < 0.001$). Sex distribution was not different (% male sex 46 versus 47, $P = 0.97$)

Both C3 and SC5b-9 levels were not different in diabetic South Asians compared to non-diabetic South Asians ($875 \mu\text{g} \pm 97$ versus 854 ± 139 , $P = 0.184$ and 0.355 ± 0.15 versus 0.362 ± 0.13 , $P = 0.708$, respectively).

Median MBL level was higher in non-diabetic South Asians compared to diabetic South Asians (1009 (IQR 239-1847) versus 476 (IQR 143-1536), $P = 0.043$). Genotype distribution was not different between non-diabetic and diabetic South Asians (A/A 63 versus 57%, A/O 31 versus 35%, O/O 6 versus 8%, $P = 0.58$)

Correlations of complement levels and clinical parameters in type 2 diabetic South Asians

In the 168 type 2 diabetic South Asians, serum C3 levels correlated with age, sex, hsCRP, Body Mass Index, hip circumference, waist circumference and Cockcroft-Gault creatinine clearance. SC5b-9 correlated only with sex and with fasting triglycerides. MBL level correlated with HbA1c and hip circumference but not with C3, SC5b-9, and hsCRP (table 2).

Complement levels and albuminuria in diabetic South Asians

To investigate whether increased complement levels are associated with renal damage, complement levels in diabetic South Asians with albuminuria ($n=53$) were compared to diabetic South Asians without albuminuria ($n=112$). A SC5b-9 level above the median was associated with the presence of albuminuria (sex adjusted OR 2.31, 95% CI 1.18-4.55, $P = 0.015$) whereas a C3 level above the median was not (OR 0.83, 95% CI 0.42-1.65, $P = 0.60$).

MBL level above the median was not associated with albuminuria (OR 1.2, 95%CI 0.63-2.13, $P = 0.580$). There was no statistically significant difference in median MBL level between diabetic subjects with and without albuminuria ($528 \mu\text{g/L}$ (193-1308) versus $388 \mu\text{g/L}$ (130-1713), $P = 0.740$). Genotype distribution was also not different between diabetic South Asians with or without albuminuria (A/A 60 versus 55%, A/O 32 versus 37%, O/O 8 versus 8%, $P = 0.873$)

Table 2 Association between baseline clinical parameters and complement levels in 168 type 2 diabetic South Asians

	C3		SC5b-9		MBL [#]	
	Pearson's r	P-value	Pearson's r	P-value	Pearson's r	P-value
Age	-0.285	< 0.001	0.139	0.078	-0.21	0.785
Sex	0.463	< 0.001	0.213	0.006	-0.141	0.071
Serum C3	1		0.097	0.247	-0.059	0.474
Plasma SC5b-9	0.97	0.247	1		0.027	0.731
Serum MBL [#]	-0.059	0.474	0.027	0.731	1	
High-sensitivity C-reactive protein [#]	0.479	< 0.001	0.006	0.936	0.042	0.596
Diabetes duration [#]	-0.79	0.430	0.079	0.403	-0.055	0.562
HbA1c	0.049	0.554	0.046	0.564	0.165	0.034
Systolic blood pressure	-0.061	0.463	0.078	0.325	-0.148	0.059
Diastolic blood pressure	-0.033	0.689	-0.012	0.875	-0.133	0.090
Body Mass Index	0.264	0.001	0.079	0.319	-0.155	0.470
Waist circumference	0.180	0.029	0.009	0.913	-0.124	0.113
Hip circumference	0.218	0.008	0.038	0.627	-0.169	0.030
Waist-to-hip ratio	-0.050	0.547	-0.037	0.641	0.042	0.594
HOMA-IR [†]	0.014	0.862	0.015	0.853	0.141	0.072
Total cholesterol	0.035	0.675	0.084	0.287	-0.108	0.170
Fasting triglycerides [#]	0.026	0.752	0.168	0.033	-0.081	0.304
Urinary albumin/creatinine ratio [#]	0.072	0.382	0.093	0.236	-0.001	0.986
Cockcroft-Gault creatinine clearance	0.307	< 0.001	-0.016	0.843	-0.084	0.286

Not normally distributed parameters([#]) were log-transformed

[†]HOMA-IR = Homeostatic Model Assessment for assessing insulin resistance

Follow-up study

Out of 168 type 2 diabetic subjects at baseline, 21 could not be traced and 13 subjects refused to participate. Eighty-six subjects visited the out-patient clinic, 31 subjects did not visit the out-patient clinic but medical information was

obtained from the general practitioner, and 17 subjects had died (see below). The median duration of follow-up was 7.66 (IQR 7.48-8.10) years. Participants lost to follow-up did not differ in baseline characteristics from participants in whom follow-up data were available. Renal follow-up data were missing in 4 subjects which were excluded from analysis with respect to renal endpoints.

Complement and cardiovascular events

During follow-up, 39 cardiovascular events occurred in 30 subjects (16 men, 14 women): 3 sudden cardiac deaths, 2 fatal and 5 non-fatal myocardial infarction, 13 percutaneous coronary interventions, 8 coronary artery bypass graft procedures, 2 fatal and 5 non-fatal strokes, 1 lower extremity amputation. Eleven of these 30 subjects had already experienced a cardiovascular event at baseline. Ten subjects died due to non-cardiovascular causes. Two of these subjects reached the primary end-point before they died, eight subjects did not and these were censored.

Baseline levels of complement did not predict cardiovascular events during follow-up (table 3). Serum C3 above the median was not associated with cardiovascular events (HR 1.28, 95% CI 0.58-2.84, $P = 0.538$). A SC5b-9 level above the median was also not associated with cardiovascular events (HR 1.79, 95% CI 0.84-3.78, $P = 0.132$). In addition, complement levels were not associated with total cardiovascular events at the end of follow-up. Baseline MBL levels were not associated with cardiovascular events (data not shown). The association between MBL genotype and cardiovascular events is described in more detail in chapter 3 of this thesis.

Complement and progressive renal failure

At the end of follow-up, 14 subjects had progressive renal failure (11 subjects had a 50% increase in serum creatinine level, 1 initiated hemodialysis and 2 had a kidney transplantation).

A baseline SC5b-9 level above the median was associated with progressive renal failure (sex-adjusted HR 6.18, 95%CI 1.69-22.64, $P = 0.006$). After adjusting for MBL level, a SC5b-9 level above the median was still associated with progressive renal failure (HR 5.24, 95% CI 1.44-19.05, $P = 0.012$). After adjusting for age, sex, systolic blood pressure, HbA1c, fasting triglycerides, and urinary ACR, the association was statistically insignificant (multivariate HR 4.38, 95%CI 0.88-22.0, $P = 0.072$).

Serum C3 was not associated with progressive renal failure.

Table 3 Association between clinical parameters, cardiovascular events and progressive renal failure

	Cardiovascular events		Progressive renal failure	
	Univariate HR	95% CI	Univariate HR	95%CI
Serum C3	1.002	0.997-1.006	0.997	0.991-1.003
Serum C3 > median	1.28	0.58-2.84	0.66	0.235-1.861
Plasma SC5b-9	1.31	0.12-14.22	16.6	1.15-240
Plasma SC5b-9 > median	1.78	0.84-3.80	5.45	1.53-19.5
Serum MBL [§]	1.18	0.68-2.05	3.19	1.23-8.23
Serum MBL > median	1.48	0.71-3.09	2.61	0.90-7.54
Age	1.02	0.99-1.06	1.037	0.993-1.083
Male sex	1.51	0.74-3.12	0.79	0.29-2.11
Diabetes duration	1.03	0.99-1.07	1.043	0.994-1.094
HbA1c	1.22	1.02-1.46	1.26	0.993-1.607
RR systolic	1.01	0.999-1.027	1.027	1.008-1.045
RR diastolic	1.02	0.989-1.058	1.029	0.983-1.077
Cockcroft-Gault creatinin clearance	0.99	0.98-1.006	0.973	0.951-0.996
Urinary albumin/creatinine ratio [§]	1.97	1.36-2.83	4.44	2.61-7.54
Current or former smoking	1.27	0.61-2.62	1.04	0.384-2.814
Total cholesterol	1.20	0.81-1.80	1.28	0.74-2.21
Waist circumference	1.032	0.999-1.067	1.034	0.991-1.08
Hip circumference	0.96	0.94-0.99	1.012	0.957-1.070
Waist circumference > median	2.99	1.33-6.75	1.67	0.61-4.56
Waist-to-hip ratio > median	3.84	1.62-9.01	2.69	0.93-7.77
Body Mass Index	0.995	0.914-1.08	1.043	0.947-1.148
Fasting Triglycerides [§]	1.84	0.37-9.19	7.08	1.25-40.13
Hing-sensitivity C-reactive protein [§]	1.37	0.60-3.13	1.53	0.52-4.51
Previous cardiovascular event	5.09	2.33-11.14	1.74	0.49-6.19
			Multivariate HR[#]	95% CI
Serum SC5b-9 > median			4.38	0.88-22.0
Serum MBL [§]			3.59	1.30-9.93

[§] skewed-distributed variables are log-transformed[#] included as covariates are age, sex, systolic blood pressure, ACR, triglycerides, HbA1c.

Baseline MBL levels were significantly associated with progressive renal failure (univariate HR 3.19, 95%CI 1.23-8.23, $P = 0.017$), while MBL genotype was not. Adjustment for SC5b-9 did not change this association (HR 3.1, 95%CI 1.26-7.64, $P = 0.014$), nor did adjustment for hsCRP (HR 3.01, 95%CI 1.17-7.77, $P = 0.023$). In a multivariate analysis with sex, age, systolic blood pressure, hsCRP, HbA1c, fasting triglycerides, and urinary ACR as covariates, MBL levels remained associated with progressive renal failure (multivariate HR 3.59, 95%CI 1.30-9.93, $P = 0.014$). Baseline Cockcroft-Gault creatinine clearance, urinary albumin/creatinine ratio, systolic blood pressure and fasting triglycerides were also associated with progressive renal failure (table 3).

Discussion

South Asians have a high incidence of diabetes and subsequent vascular complications. Since traditional cardiovascular risk factors do not completely explain the increased incidence of vascular disease in South Asians, other factors must be involved. There is increasing evidence that the complement system is involved in both macro- and micro-vascular complications [10-12]. Complement activation products are detected in atherosclerotic aortic plaques and in kidney biopsies and urine of subjects with diabetic nephropathy. In addition, experimental evidence supports a pathophysiologic role for SC5b-9 in the progression of atherosclerosis [27,28]

We hypothesized that increased activity of the complement system might be involved in the enhanced rate of vascular complications in diabetic South Asians. In the cross-sectional study, we found that not only C3 – the central molecule in complement activation – is increased in South Asians compared to native Caucasians, but also SC5b-9 – the effector phase of complement activation –, supporting our hypothesis that complement activation in South Asians is occurring at a higher level of activity. In addition, a high SC5b-9 level was associated with albuminuria, suggesting that complement activation is related to renal damage.

C3 levels were closely related to measures of adipose tissue mass (BMI, waist circumference, hip circumference). Indeed, C3 mRNA expression has been observed in –mainly omental– adipose tissue [29]. Adipocyte-derived C3 serves as a precursor of C3adesarg, also called Acylation Stimulating Protein (ASP) [30]. C3adesarg is generated through interaction of C3 with complement factor

B and the enzyme adipsin (also known as complement factor D). C3adesarg is inactive with respect to complement activation. Its main function is to increase triglyceride synthesis in fat-storing cells through stimulation of fatty acid incorporation. Increased C3 levels in South Asians thus might simply reflect increased central adipose tissue mass, although it is currently unknown to what extent adipocyte-derived C3 contributes to serum total C3 levels. Secondary mechanisms involving hepatic C3 production might also be involved.

A key question is whether an increased C3 level predisposes to enhanced complement activation. As C3 did not correlate with SC5b-9, we did not find evidence that increased C3 levels result in enhanced complement activation, although enhanced complement activation at tissue level cannot be ruled out.

In the prospective study, C3 level did not predict the occurrence of cardiovascular events, which is in contrast with observations in other populations [31]. However, the predictive value of a certain risk marker depends on the population being studied. Given the increased C3 level in the South Asian population, the inter-individual predictive value with respect to cardiovascular disease might be attenuated, which has also been observed with other cardiovascular risk markers [32].

Baseline SC5b-9 levels also failed to predict cardiovascular events. Although this does not rule out a role for complement activation in atherosclerosis, plasma SC5b-9 level is not sensitive enough to serve as a risk marker for cardiovascular events.

There is firm evidence that the complement system is involved in the progression of chronic renal failure. Experimental studies in various proteinuric models show complement activation on tubular cell brush border [33-37]. Inhibition of complement, either by administration of a complement inhibitor or by knock-out of complement components, results in attenuation of tubulointerstitial damage [33,36,37]. Complement activation also has been linked to the induction of renal fibrosis [38,39].

In our study, a high baseline SC5b-9 level was associated with both albuminuria and progressive renal failure. In contrast to the above mentioned experimental studies, which studied urinary complement activation in overtly proteinuric animals, we measured blood levels of complement activation in subjects with various degrees of proteinuria. An association between blood levels of complement activation products and renal failure has not been reported previously. However, progressive renal damage has been linked to serum levels

of other inflammatory markers such as interleukines and Tumor Necrosis factor [40], suggesting that inflammation plays an important role in the pathogenesis of diabetic nephropathy. Whether increased SC5b-9 level in progressive renal failure reflects leakage of intra-renally formed SC5b-9, or whether it reflects systemically formed SC5b-9 due to generalized vascular inflammation is unknown.

More recently, the MBL pathway of complement activation has come into focus as a contributor to diabetic vascular complications [10]. Cross-sectional studies found increased MBL levels in type 1 diabetic subjects compared to healthy controls [41]. In contrast, we found lower MBL levels in our type 2 diabetic South Asians compared to non-diabetic South Asians. This is probably explained by hormonal and metabolic differences between type 1 and type 2 diabetes, since MBL levels are lowered by insulin [42], and type 2 diabetes but not type 1 diabetes is characterized by hyperinsulinemia.

In type 1 diabetic Caucasians, MBL levels predict future development or progression of diabetic nephropathy [43, 44]. Experimental data show that MBL deficient streptozotocin-treated mice develop less diabetic nephropathy [45], which strongly suggests a pathophysiological role of MBL in the pathogenesis of diabetic nephropathy. Although earlier studies suggested that a high MBL genotype conferred an increased risk to diabetic nephropathy [10, 42], later studies with greater patient numbers could not confirm this [46]. In line with this, we found that high MBL levels at baseline were associated with progressive renal failure, whereas MBL genotype was not. High MBL levels thus may reflect an inflammatory state. In experimental ischemia/reperfusion models, high MBL levels exacerbate tissue damage [47]. Oxidative stress induces a change on the cellular surface [48], which results in binding of MBL leading to enhanced complement mediated injury. Since in our study the association of MBL and progressive renal failure was independent of plasma SC5b-9, we found no evidence that high MBL levels result in increased complement activation, although enhanced complement activation at tissue-level cannot be ruled out. Whether increased MBL levels itself results in enhanced complement activation, or whether MBL levels are just a marker of inflammation thus remains to be resolved.

In contrast to progressive renal failure, baseline MBL levels were not associated with cardiovascular events (see chapter 3 of this thesis).

In conclusion, C3 levels are increased in South Asians compared to Caucasians, but do not predict future cardiovascular events or progressive renal failure.

MBL levels and a high SC5b-9 were associated with progressive renal failure (micro-vascular disease) but not with cardiovascular events (macro-vascular disease), suggesting that complement activation primarily affects the micro-circulation.

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