Early determinants of atherosclerosis in paediatric systemic lupus erythematosus

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Abstract

Objectives

To assess traditional and non-traditional cardiovascular risk factors and to determine the prevalence and correlates of early vascular markers of atherosclerosis in paediatric systemic lupus erythematosus (pSLE).

Methods

Fifty-four adolescents with pSLE had cardiovascular risk factor assessment, disease activity and vascular testing including carotid intima-media thickness (CIMT), flow-mediated dilatation (FMD), arterial stiffness measures, and myocardial perfusion studies.

Results

The traditional risk factors of hypertension, elevated triglycerides, apolipoprotein B, haemoglobin A1c and insulin levels and non-traditional risk factors of elevated homocysteine and fibrinogen were present (all $p<0.001$). Some arterial stiffness measures, central pulse wave velocity and characteristic impedance were elevated ($p<0.001$), but CIMT, FMD and myocardial perfusion were normal. Cumulative prednisone dose correlated with total cholesterol ($r=0.5790$, $p<0.001$) and elevated LDL-C ($r=0.4488$, $p=0.0012$). Hydroxychloroquine treatment correlated negatively with total cholesterol ($r=-0.4867$, $p=0.0002$), LDL-C ($r=-0.4805$, $p=0.0002$) and apolipoprotein B ($r=-0.4443$, $p=0.0011$). In multivariate analysis LDL-C correlated with cumulative prednisone dose and negatively with hydroxychloroquine treatment ($R^2=0.40$, $p<0.001$).

Conclusions

An increased burden of traditional and non-traditional risk factors and early evidence of insulin resistance and increased central arterial stiffness were present in paediatric SLE. Disease-specific and therapy-related factors are likely modifying these cardiovascular risk profiles warranting prospective longitudinal studies.

Key words

vascular testing, cardiovascular risk factors, lipids, insulin resistance

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Introduction

It is now well recognised that patients with systemic lupus erythematosus (SLE) are at high risk for premature atherosclerosis (1, 2). In adults with SLE, traditional risk factors do not fully explain the increased burden of cardiovascular disease, and it has been suggested that chronic inflammation, disease specific and therapy related factors are involved (1-5). The role of traditional and these non-traditional risk factors and evidence of early atherosclerosis has not been well characterised in paediatric SLE (pSLE). Dyslipidemia is the best studied complication of SLE leading to atherosclerosis with multiple lipid abnormalities described in both adult and paediatric populations (6-9). Insulin resistance has been shown to be present in adults with SLE and is a known independent risk factors for the development of coronary artery disease (10). In adults with SLE it has also been demonstrated that both the inflammation secondary to SLE itself and elevated plasma homocysteine levels may also lead to accelerated atherosclerosis (1, 4, 11, 12). Carotid intima-media thickness (CIMT), endothelial function assessed by flow-mediated dilation (FMD), arterial stiffness assessed by pulse wave velocity (PWV) and abnormalities of myocardial perfusion have been all been used to demonstrate evidence of early atherosclerosis and increased cardiovascular risk in adults with SLE (2, 13-15). Few similar paediatric studies exist and no comprehensive study using all of these modalities has been performed (7, 8, 16-18). Therefore the aims of this study were to assess traditional and non-traditional cardiovascular risk factors and to determine the prevalence and correlates of early vascular markers of atherosclerosis in pSLE.

Materials and methods

Subjects

Patients attending the Lupus Clinic at the Hospital for Sick Children, Toronto between the ages 10 and 18 years of age and who fulfilled the American College of Rheumatology classification criteria for SLE were recruited as part of an ongoing prospective longitudinal cohort study starting in 2002. Participants gave appropriate informed consent, as approved by the Research Ethics Board of the Hospital for Sick Children.

Healthy controls

For laboratory markers, CIMT and FMD, the control group consisted of our previously published data from 60 healthy children aged 10-18 years (30 boys) (24). There was no statistically significant difference in the mean age of the controls at 15.9±1.9 years as compared to our study group at 15.3±1.9 years (p=0.304). For the Echo-Doppler assessment of central arterial stiffness, results were compared with our previously published data in 69 healthy children aged 8-20 years (29 boys) (22). The peripheral PWV results were compared to published data of a group of 155 healthy children aged 6–20 years (81 boys) age-matched over 2-year intervals (23).

Clinical assessments

Patients' heights and weights were measured and body mass index (BMI) calculated. Resting brachial systolic and diastolic blood pressures were recorded in the left arm by auscultation using the standard sphygmomanometry cuff technique. At each clinic visit standard disease activity scores [Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) and European Consensus Lupus Activity Measure (ECLAM)], and damage score [Systemic Lupus International Collaborating Clinics (SLICC)] were calculated and drug usage was recorded. The cumulative prednisone dose for each patient was calculated by obtaining the prednisone dose, as documented in the Toronto HSC SLE database, at the end of the each visit and multiplying by the number of days from the previous visit to the current visit. If a dose had changed between the two visits we took mean of the two doses and multiplied by the number of days between visits. All of the between visits total prednisone dose were then added together to derive the total cumulative prednisone. All prednisone doses were calculated on a mg/kg basis using the weight at the first of the two visits as the denominator.

Laboratory measurements

Serum glucose, haemoglobin A1c, trig-

lycerides, total cholesterol, LDL-C, HDL-C, apolipoprotein A1 (apoA1), apolipoprotein B (ApoB), lipoprotein (a) (Lp(a)), free fatty acids (FFA), homocysteine, fibrinogen, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were measured in all patients after a 12-hour fast. Anti-cardiolipin antibodies were measured at each clinic visit along with other Lupus autoantibodies. Insulin resistance was measured using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) which relates fasting glucose (mmol/L) and fasting insulin levels (IU/ml) using the following formula: *HOMA-IR=fasting insulin x fasting glucose/22.5* (19).

Vascular testing

All vascular tests were performed by experienced vascular sonographers in a quiet room after an overnight fast and 10 minutes of supine rest, using standardised protocols (20-23). Repeated studies and measurements by different observers were performed in five subjects on four occasions, demonstrating acceptable reproducibility, with coefficients of variation for inter- and intra-observer and inter-sessional variability <15% for all vascular indices measured.

a) Carotid intima-media thickness (CIMT)

Using high resolution B-mode vascular ultrasound (ATL 3000 7.5 MHz linear-array transducer, Advanced Technology Laboratories, Bothel, WA) the right and left common carotid arteries were imaged. Intima-media thickness was measured offline using electronic calipers on the far wall, 1 cm proximal to the common carotid bifurcation, on ECG gated end diastolic longitudinal images.

b) Flow-mediated dilatation (FMD)

Using high resolution B-mode vascular ultrasound as above, the right brachial artery was imaged just proximal to the antecubital fossa. Imaging was recorded for 30 seconds baseline before pressure cuff inflation around the mid upper arm to >20 mmHg above resting systolic blood pressure for five minutes and then for three minutes post cuff deflation. Then after a 10 minute interval, imaging was recorded for a further 30 seconds at baseline before a sublingual dose of 400 micrograms glyceryl trinitrate was given and then between the third and fourth minutes post dose. Brachial artery diameters were measured offline using edge detection software (DEA, Vasometrix, Montreal), over a 1 cm segment, on ECG gated end diastolic longitudinal images. Flow-mediated dilatation (FMD, endothelium-dependent vasodilatation) was assessed as the percentage change from baseline to maximal diameter post cuff inflation. Glyceryl trinitrate-mediated dilatation (GTN, endothelium-independent vasodilatation) was assessed as the percentage change from repeat baseline to maximal diameter post dose.

c) Arterial stiffness indices

Central arterial stiffness indices were derived using echo-Doppler (22). Briefly, in the parasternal long axis view, the aortic annulus diameter was measured by 2-D echo and the minimum and maximum ascending aortic diameters by M-Mode. In the suprasternal long axis view, the aortic arch length was measured by 2-D echo and the peak aortic velocity and the transit time between the ascending and descending aortic pulse-wave Doppler envelopes was measured by pulse wave Doppler. Central pulse wave velocity (PWV) was then derived by dividing aortic arch length by the transit time and elastic modulus, stiffness index, input and characteristic impedance were calculated as previously described (22). Brachioradial PWV was measured using photoplethysmography (23). Briefly, two probes, each containing an infrared emitting diode and a phototransistor, were placed over the right brachial and right radial arteries and secured without compression. The transit time was determined from the time delay between the foot of the corresponding brachial and radial pulse waves. Peripheral PWV was derived by dividing the measured distance between the two probes by the transit time.

Myocardial perfusion studies

Technetium-99m-labelled exercise stress-sestamibi single-photon emission computed tomographic imaging using a modified Bruce treadmill protocol was performed as previously described (18). The result of the scan was considered abnormal if a segmental reduction in activity was seen in two views. Reversible ischaemia was considered present if a defect that was present after exercise was not present at rest. A fixed defect was defined as one that was not reversible.

Questionnaires

Thirty-eight patients completed a family history questionnaire which is routinely used in the HSC lipid clinic (website URL: http://www.sickkids.ca/cardiacref/lipid.asp), and a three-day food diary provided by the Division of Nutrition and Dietetics at HSC. Data collected from the three-day food diary were analysed using Food Smart Millenium Edition software® (Sasquatch Software Corporation, Canada) to provide information regarding total calories consumed as well as dietary intake of protein, fat (total fat, saturated fat, polyunsaturated fat, monounsaturated fat), carbohydrates, fibre, total cholesterol, vitamins A, D, E, K and B12, folic acid and calcium.

Statistical analysis

Clinical, dietary, laboratory and vascular indices (as indicated above) were converted to z-scores from previously published historical healthy control data and tested for normality using single sample *t*-tests (22-24). Pearson correlations were performed to assess associations between traditional (serum lipid levels, prevalence of insulin resistance, hypertension, smoking, diabetes mellitus and BMI in study patients and prevalence of cardiovascular disease in a first-degree relative) and non-traditional risk factors (ESR, CRP, homocysteine, fibrinogen, anticardiolipin antibody status, SLEDAI, ECLAM and SLICC scores) and early vascular markers of atherosclerosis. Due to multiple statistical comparisons, the following *p*-value interpretation was used: *p*-values <0.005 were considered statistically significant, *p*-values between 0.005 and 0.05 were considered marginally significant and worth further consideration and *p*-values >0.05 were not con-

sidered to be statistically significant. Multivariable linear regression analysis was used to evaluate the independent effect of selected variables on parameters of interest. Models with an R^2 value <0.40 and with a *p*-value <0.001 were considered to be significant in the regression analysis.

Results

Clinical characteristics of study subjects

All consecutive patients seen in the Toronto SLE clinic during the study were approached to enter into the study. Initially 58 of 103 patients agreed to participate, however, 4 families subsequently declined to participate prior to commencement of the study having initially provided informed consent. Therefore, 54 patients were in the study cohort and 11 of these were male. The mean age at diagnosis of SLE was 15.3±1.9 years (range 10-18 years) and the mean disease duration 2.6±2.3 years at study entry. The mean cumulative prednisone dose at the time of testing was 339±319 mg/kg (median 216 mg/kg). A total of 45 patients in the Toronto SLE clinic did not participate in the study. The only statistically significant difference between the recruited group and the unrecruited group was the age at diagnosis (Table I). This was not surprising as patients <10 years are unable to perform all the vascular testing required reliably and therefore were excluded from the study (see *Methods* section).

Table I. Demographic data, clinical features and medication usage in both recruited and unrecruited patients (mean±SD).

Parameter	Recruited (n=54)	Unrecruited (n=45)	*p*-value*
Age at diagnosis (years)	15.3 ± 1.9	10.9 ± 3.9	<0.0001
Disease duration (years)	2.57 ± 0.32	3.15 ± 0.35	0.23
Male:female ratio	11:43	11:34	0.63
Cumulative prednisone dose (mg/kg)	339 ± 319	NA**	NA**
Body mass index (kg/m^2)	24.0 ± 0.8	22.2 ± 0.9	0.12
SLEDAI	4.2 ± 0.4	4.4 ± 0.8	0.66
ECLAM	2.4 ± 0.2	2.6 ± 0.4	0.60
SLICC	0.37 ± 0.10	0.29 ± 0.08	0.52
Lupus nephritis	34	20	0.07
CNS manifestations	17	8	0.16
Prednisone	47	36	0.41
Azathioprine	27	19	0.54
Mycophenolate mofetil	4	4	1.0
Hydroxychloroquine	39	31	0.86
Cyclophosphamide	6	3	0.50
Methotrexate	2	4	0.41
Antihypertensive(s)	14	10	0.67

*Chi-squared, Fisher's exact test and Student's *t*-test were used as appropriate.
**NA: not available as the prednisone dose was not recorded with sufficient accuracy in all of the unrecruited patients to determine the mean cumulative prednisone dose for these patients.

Traditional risk factors

Fourteen SLE subjects were being treated for hypertension. However, there were no significant differences between the cases and controls with respect to the prevalence of diabetes mellitus, smoking, elevated BMI or family history of cardiovascular disease in a first degree relative. There were no significant correlations found between these traditional risk factors and any of the measured parameters.

a) Serum lipid levels

The mean serum triglyceride and ApoB levels were significantly higher in SLE patients than in healthy controls ($p<0.001$ for both) (Table II). Free fatty acid ($p=0.024$) and Lp(a) levels ($p=0.025$) were only marginally elevated compared to controls. There was no significant difference in levels of total cholesterol, LDL-C, HDL-C, and ApoA1 levels.

Univariate analysis showed a positive correlation between cumulative prednisone dose and total cholesterol ($r=0.5790$, $p<0.0001$), LDL-C ($r=0.4488$, $p=0.0012$) and ApoB levels ($r=0.4400$, $p=0.001$) but not with FFA, triglyceride, HDL-C, ApoA1 or Lp(a) levels. The daily prednisone dose (mg/kg) at the time of the study was positively correlated with total cholesterol ($r=0.5590$, $p<0.001$) but with none of the other lipid measurements. Treatment with hydroxychloroquine was negatively correlated with total cholesterol ($r=-0.4867$, $p=0.0002$) and LDL-C ($r=-0.4805$, $p=0.0002$) and ApoB ($r=-0.4443$, $p=0.0011$). None of the other therapies demonstrated correlations with serum lipid levels. In the multivariate analysis, only the positive association of total cholesterol level with the cumulative prednisone dose and the negative association with treatment with hydroxychloroquine remained statistically significant. Together, these parameters explained 40% of the variance in total cholesterol levels between cases and controls ($R^2=0.040$, $p<0.001$). None of the other measurements (ESR, CRP, homocysteine, fibrinogen, anticardiolipin antibody status, SLEDAI, ECLAM and SLICC scores) correlated with lipid levels.

b) Insulin resistance

Mean haemoglobin A1c and insulin C-peptide were significantly higher in the study population ($p<0.001$ for both) and there was also a trend towards higher insulin levels in SLE patients ($p=0.09$) (Table II). However, there were no significant difference in the mean HOMA-IR values between study patients and healthy controls and HOMA-IR values were normal in the majority of lupus patients. Paradoxically, serum glucose levels were lower in SLE patients (z-score difference 0.92, $p<0.001$).

Non-traditional risk factors

Mean serum homocysteine (z-score difference +1.16, $p<0.001$) and fibrinogen levels (z-score difference 0.48, $p<0.001$) were significantly higher in SLE subjects compared with healthy controls. In addition, mean ESR was elevated at 70±63.7 mm/hour but mean CRP value was within normal limits. Both the mean SLEDAI score at 4.2±3.3 (median 4, range 0–15) and ECLAM score at 2.5±1.8 (median 2,

Table II. Serum lipids and markers of insulin resistance.

Parameter	Median	Z-score difference	*p*-value
Free fatty acids (mmol/L)	0.57	+0.38	0.024
Triglycerides (mmol/L)	1.23	+0.62	<0.001
Total cholesterol (mmol/L)	3.99	+0.26	0.199
LDL-C (mmol/L)	2.37	+0.03	0.856
HDL-C (mmol/L)	1.11	+0.10	0.583
ApoA1 (g/L)	1.35	+0.32	0.082
ApoB (g/L)	0.75	+0.58	<0.001
Lp(a) (g/L)	15.9	+0.39	0.025
Insulin (picomol/L)	64.6	+0.16	0.09
Glucose (mg/L)	4.5	-0.92	<0.001
C-peptide (picomol/L)	1.9	+0.33	<0.001
Haemoglobin A1c	0.05	+0.64	<0.001
HOMA-IR	1.84	-0.02	0.488

Apo A1: apolipoprotein A1; ApoB: apolipoprotein B; Lp(a): lipoprotein A; HOMA-IR: Homeostasis Model-Insulin Resistance (Insulin x Glucose / 22.5).

Table III. Vascular markers.

Parameter	Median	Z-score difference	*p*-value
R-CIMT (mm)	0.43	-0.119	0.267
L-CIMT (mm)	0.43	-0.103	0.300
FMD (% change)	8.56	0.0009	0.964
GTN (% change)	14.57	0.135	0.614
Central PWV (m/s)	4.3	+1.046	<0.001
Elastic modulus (mmHg)	210	-0.424	0.143
Stiffness index	2.3	-0.617	0.011
Input impedance (dyne•s/cm^5)	170	-0.063	0.750
Characteristic impedance (dyne•s/cm^5)	149	+0.746	<0.001
Peripheral PWV (m/s)	8.1	-0.047	0.874

FMD: Flow-mediated dilatation; GTN: glyceryl trinitrate-mediated dilatation; PWV: pulse wave velocity.

range 0–7) showed mildly active disease. The SLICC scores (0.37±0.10) indicated low disease damage in our cohort. Thirty-four patients were anticardiolipin antibody positive. There were no statistically significant correlations found between these non-traditional risk factors (ESR, CRP, homocysteine, fibrinogen, anticardiolipin antibody status, SLEDAI, ECLAM and SLICC scores) and subjects' clinical characteristics, drug usage or traditional risk factors (including serum lipid levels and insulin resistance).

Food diaries

Patients with SLE had a significantly lower dietary intake of vitamin E (z-score difference =-0.366, p=0.001). However, no differences existed between the two populations in regard to dietary intake of calories, cholesterol, fibre, fat, protein, carbohydrate, vitamins A, D, K and B12, folic acid or calcium.

Vascular markers of premature atherosclerosis

Of the 31 SLE subjects who completed vascular testing, mean right (p=0.267) and left CIMT (p=0.103), FMD (p=0.964) and GTN (p=0.614) responses were no different in SLE subjects compared with healthy controls (Table III). There was also no correlation between any of these measures and any of the clinical features (lupus nephritis, CNS involvement, thrombosis) or laboratory measures or with any treatment regime. Regarding the arterial stiffness indices, mean central PWV and characteristic impedance were higher, but paradoxically the stiffness index was lower in SLE subjects compared with healthy controls. A significant correlation was found between central PWV and azathioprine treatment (r=0.5393, p=0.0017), but there were no other significant correlations between central PWV and any of the other of the other traditional or non-traditional risk factors. Central PWV was abnormal (z-score >2 SD) in 4/31 patients. Despite the small sample size there was a trend toward significance in correlations of abnormal CPWV and the SLICC index (r=0.44, p=0.02), the cumulative prednisone dose (r=0.47, p=0.02) and treatment with azathioprine (r=0.42, p=0.03). There was no difference between these two sub-groups for any other clinical or laboratory variables including disease duration (p=0.26).

Myocardial perfusion studies

Of the 22 SLE subjects who underwent myocardial perfusion studies, two patients had suboptimal heart rate elevation during the testing due to fatigue and five patients had suboptimal exercise endurance. All sestamibi scans, both resting and post-exercise were normal.

Discussion

SLE patients are at high risk for the development of premature atherosclerosis (1, 2, 4, 5, 7, 11-14) likely the result of both traditional and non-traditional risk factors (1, 2, 4, 5, 7, 11-14, 25). However, prior to this study, no study has comprehensively examined for evidence of early atherosclerosis or determined the role of both traditional and non-traditional risk factors in the development of premature atherosclerosis in patients with pSLE. We have shown that pSLE patients have the traditional risk factors of increased serum triglyceride levels, hypertension and insulin resistance (elevated insulin and insulin C-peptide levels) but not increased BMI or diabetes mellitus. These findings are of potential long-term importance as insulin resistance and traditional risk factors of atherosclerosis have been associated with an increased risk of coronary artery disease in later life even in children and adolescents (26).

In addition to elevated triglycerides, we found elevated ApoB, and Lp(a) but not total cholesterol, LDL-C, HDL-C and ApoA1 levels. We have previously shown that HDL-C are low in active, untreated SLE and then rise with steroid therapy (9) and therefore differences in disease activity and prednisone doses may explain the dif-

ferences between our study and previous studies (7, 8, 17, 27). We found that LDL-C levels were associated with the cumulative prednisone dose but not the daily prednisone dose, suggesting that long-term prednisone therapy had a negative effect on LDL-C levels. We have also shown that, similar to adult patients with SLE (3), treatment with hydroxychloroquine in pSLE had the beneficial effects of decreased total cholesterol and LDL-C levels. The effect of hydroxychloroquine appeared to be independent of its effect on improving the primary disease symptoms of SLE and is likely secondary to its inhibition of hepatic cholesterol biosynthesis and LDL-C degradation (28). In this study, we have for the first time shown elevated free fatty acids in patients with SLE.

We have confirmed elevation of homocysteine levels in patients with pSLE (17, 29) and also shown elevated fibrinogen and ESR levels (shown to be associated with premature atherosclerosis). These results demonstrate that pSLE, similar to adult SLE, patients have multiple non-traditional risk factors for atherosclerosis. Although, we did not find any association of these non-traditional risk factors with any of the multiple measures of early atherosclerosis, they may be important in long-term follow-up of these patients.

In this study of pSLE of relatively short duration, we were able to detect early changes in central arterial stiffness, which have been associated with early atherosclerosis and the extent of atherosclerosis (30). Previous adult studies have demonstrated increased arterial stiffness in SLE patients and most importantly that these changes may be reversible with better disease control (14, 31). We found a correlation between central arterial stiffness and the use of azathioprine but not with any other risk factor, measures of disease activity or therapy. We did not find any other changes consistent with early evidence of atherosclerosis as measured by CIMT, FMD or myocardial perfusion studies. Our FMD findings are in agreement with the only other reported paediatric study although they differ from studies in adult SLE (9, 16). The results of our current study demonstrating normal myocardial perfusion scans are consistent with our previous study (18). Our finding of normal CIMT is in contrast to abnormal results reported in adult patients with SLE and in the only other paediatric study (7, 12, 14, 25). However, in the studies of adults, most young patients had normal CIMT. The difference between our results and the previous paediatric study may reflect a difference in the two populations studied (7). Unlike the APPLE study, we did not find an association with azathioprine use and CIMT (32).

One of the limitations of this study is that the sample size is relatively small. In addition, a potential source of variability may be the vascular testing methods, but the coefficients of variation obtained in our reproducibility study demonstrated acceptable range for testing of physiological measures. The historical control data for each of the vascular tests were derived from 3 separate populations and it would have been preferable to have a single contemporary population for comparison.

Conclusion

In summary, this study has provided comprehensive data regarding the prevalence of risk factors and early vascular markers of atherosclerosis in pSLE. We have demonstrated that both traditional risk factors (abnormalities of lipid and glucose metabolism including early evidence of insulin resistance) and non-traditional risk factors (evidence of chronic inflammation as demonstrated by elevated ESR and fibrinogen levels and elevated homocysteine levels) are present early in the course of pSLE. We have also shown that hydroxychloroquine was associated with a beneficial effect on serum lipids. We demonstrated early atherosclerosis as measured by central arterial stiffness was present in pSLE with relatively short disease duration and low disease activity/good disease control. We suggest that pSLE patients are at risk for the development of premature atherosclerosis as a result of both traditional and non-traditional risk factors and that all of these risk factors should be considered when therapeutic interventions are designed to prevent early atherosclerosis in pSLE. Longitudinal studies will help to determine the natural history of these abnormalities and to determine the rate and incidence of progression of atherosclerosis in this population of high-risk adolescents.

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