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GAVIN PUBLISHERS

International Journal of Cerebrovascular Disease and Stroke

Skjelland M, et al. Int J Cerebrovasc Dis Stroke 2: 114.

DOI: 10.29011/IJCDS-114.100014

Brief communication

Increased Endothelial Activation in Patients with Mixed Connective Tissue Disease

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Citation: Skjelland M, Zamani M, Hetlevik S, Lilleby V, Halvorsen B, et al. (2019) Increased Endothelial Activation in Patients with Mixed Connective Tissue Disease. Int J Cerebrovasc Dis Stroke 2: 114. DOI: 10.29011/IJCDS-114.100014

Received Date: 7 May, 2019; **Accepted Date:** 22 May, 2019; **Published Date:** 28 May, 2019

Abstract

Objective: Juvenile-onset Mixed Connective Tissue Disease (JMCTD) is a chronic inflammatory disease. We have previously demonstrated preclinical atherosclerosis in these patients, now exploring this further by assessing markers of endothelial dysfunction.

Methods: Thirty-three patients with JMCTD and 33 age-and sex-matched controls were included. sICAM-1, Il-6 and vWf were assayed from blood taken at the time of carotid ultrasound.

Results: Our major findings were: (i) Levels of sICAM-1 ($p<0.001$), IL-6 ($p=0.004$) and vWf ($p=0.001$) were higher, whereas (ii) HDL cholesterol (<0.01) and ApoA1 ($p<0.01$) were lower in the patient group compared to controls.

Introduction

Mixed Connective Tissue Disease (MCTD) is a chronic inflammatory, systemic rheumatic, autoimmune disease where up to 23 % of patients present in childhood [1,2]. Patients with chronic inflammatory rheumatic disease manifest endothelial dysfunction, and have an increased risk of Premature Cardiovascular Disease (CVD) such as stroke and myocardial infarction [3,4]. We have recently documented pre-clinical atherosclerosis with increased carotid intima-media thickness for patients with Juvenile Mixed Connective Tissue Disease (JMCTD) independent of traditional risk factors for cardiovascular disease [5]. The relative contribution of systemic inflammation and disease-specific factors of rheumatologic disease in the development of atherosclerotic vascular disease in this patient group remains unclear. The majority of the reported increased cardiovascular risk is from adult patient populations making it difficult to explore the atherogenic mechanisms in this patient group, and a younger, juvenile population might therefore be better suited. The long-term risk for patients with JMCTD disease is uncertain [1,2,6], and the connection between endothelial cell damage and atherosclerosis in JMCDT, has not been described previously.

Endothelial Cell Dysfunction (ECD) is a hallmark for both initiation and maintenance of atherosclerosis resulting in detectable lesions of Atherosclerotic Cardiovascular Disease (CVD), possibly serving as a marker for future risk of cardiovascular events [7]. Dysfunctional endothelial cells express intercellular adhesion molecules on their surface allowing migration and recruitment of circulating leukocytes and accumulation of Low Density Lipoprotein (LDL) across the vascular endothelium into the intimal space [8]. High Density Lipoprotein (HDL) has been

reported to have anti-atherogenic effect by countering this process with Reverse Cholesterol Transport (RCT). Membrane bound Intercellular Adhesion Molecule-1 (ICAM-1) is cleaved and released to form soluble ICAM-1 (sICAM-1) into the bloodstream where it acts as a marker of ICAM-1 and is upregulated during autoimmune activation [9]. Concentration of sICAM-1 in serum/plasma has been associated with cardiovascular disease in several prospective epidemiological studies of mature adults [10,11], and associations have been found between sICAM-1 and cardiovascular mortality in both healthy individuals [11,12], and populations at high risk including pulmonary fibrosis and SLE [11,13]. Previous studies examining the relationship between sICAM-1 and subclinical atherosclerosis have produced mixed results with the positive association of sICAM-1 to preclinical atherosclerosis attenuated after adjustment for traditional cardiovascular risk factors [14,15].

In this juvenile patient group with sub-clinical atherosclerosis we therefore aimed to assess endothelial activation, assuming that increased activation would most likely be attributable to the inflammatory burden of the disease itself rather than cardiovascular risk factors.

Materials and Methods

Patients and Control Subjects

Data on the patients and controls included in this study as well as methods used for patient and control identification is reported previously [16]. Thirty-three patients had serum collected and represented the total sample available for analyzing markers of endothelial dysfunction.

The study was approved by the Regional Ethics Committee for Medical Research (Id 2012/1721) and written informed consent was obtained from all patients.

Clinical and Blood Sample Assessment

All patients and controls underwent clinical examination at Oslo University Hospital (OUH) from March 2013 to June 2015. Details on physical examination as well as assessment of disease activity and clinical remission is reported previously [16]. Fasting blood samples were analysed by routine methods at OUH and included; Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), Triglycerides (TG), Apolipoprotein A1 (ApoA1), Apolipoprotein B (ApoB), fasting glucose, glycosylated Haemoglobin (HbA1c). Serum samples were separated from whole blood and were kept at -80 ˚C until biochemical analysis. Serum levels of sICAM, was measured by Meso Scale Diagnostics V-plex. Rockville, USA, and IL-6 was ELISA (Bio-Techne, Minneapolis, USA) and von Willenbrand factor (vWf) quantified by in house analysis using antibody obtained from R&D Systems, Abingdon, UK).

Cholesterol efflux capacity was measured as previously described [17]. Percentages efflux was calculated using the equation; $[DPM_{medium}/(DPM_{(cell+medium)})]x100$.

Statistical Analysis

Categorical variables were compared with the Mann-Whitney u test (two groups) and Student`s t-test/paired samples t-test was used for comparison of normally distributed data. Patients and controls were compared using paired testing. The Chi square test was used for analyzing categorical data. All calculations were performed with SPSS for Windows statistical software (Version 21.0; SPSS Inc, Chicago, IL).

Results

Baseline Characteristics of Patients and Controls

Thirty-three patients had blood tests at the same time as carotid ultrasound and were all included for analysis of markers of endothelial dysfunction. Patient characteristics are shown in Table 1. Twenty-eight patients were female. The mean age of diagnosis in the patient group was 13 years, whereas mean duration of disease was 16.9 years. In the control group there were 29 female patients (88%) and the mean age was 28.7 years (SD 9.4).

Characteristics	Patients with JMCTD, n=33
Female gender*	28 (84.8)
Age at inclusion, years	27.9 (9.7)
Age at diagnosis, years	13.3 (3.6)
Disease duration	16.9 (10.1)
PGA at inclusion, cm VAS**	17 (4-58)
Current medication at inclusion*	
Steroids per os	12 (36)
Azathioprine	4 (12)
Methotrexate	10 (30)
Hydroxycloroquine	19 (57)
Mycophenolate Mofetil	1 (3)
Rituximab	1 (3)
The values are given as mean (SD) or *number (percentage) or **median (range). PGA: Physician Global Assessment.	

Table 1: Patient Characteristics.

Cardiovascular risk factors for patients and controls are shown in Table 2. Patients had significantly lower levels of HDL cholesterol compared with controls (1.3 vs 2.8 mmol/l, p <0.01), as well as lower levels of Apolipoprotein A at 1.4 vs 1.69 mmol/L, p <0.01). Patients had higher levels of both triglycerides and ESR compared with controls (1.1±0.8 vs 0.8±0.3 mmol/l, p=0.13) and (11.4±8.6 vs 5.8±3.9, p<0.01), respectively. Intima media thickness remained significantly higher in patients compared with healthy controls (0.58±0.08 vs 0.53±0.07, p=0.025) Figure 1.

Variables	Patients, n=33	Controls, n=33	P
BMI, kg/m^2	22.9±3.9	23.5±3.1	0.44
Cigarette smoking, yes n (%)	6 (18.2)	6 (18.2)	0.70
Diabetes, n (%)	1 (3.0)	0	0.32
Systolic blood pressure, mmHg	109±13.9	115±14.7	0.12
Diastolic blood pressure, mmHg	63±16.9	68±9.4	0.19
HDL, mmol/L	1.3±0.4	2.8±0.9	<0.001
LDL, mmol/L	2.5±0.8	2.8±0.9	0.14
Triglycerides, mmol/L	1.1±0.8	0.8±0.3	0.13
ESR mm	11.4±8.6	5.8±3.9	0.003
HbA1c, %	5.3±0.2	5.4±0.9	0.56
CRP, mg/L	2.3±3.2	1.4±1.9	0.19
Apolipoprotein A1	1.42±0.29	1.69±0.27	0.001
Apolipoprotein B	0.79± 0.24	0.85±0.22	0.36

Values are mean ± SD or *median (range). BMI: Body Mass Index; HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein; HbA1c: glycosylated Hemoglobin; CRP: C-Reactive Protein; ESR: Erythrocyte Sedimentation Rate; IMT: Intima Media Thickness. °Smoke occasionally or every day.

Table 2: Cardiovascular risk factors in patients with juvenile onset MCTD and controls.

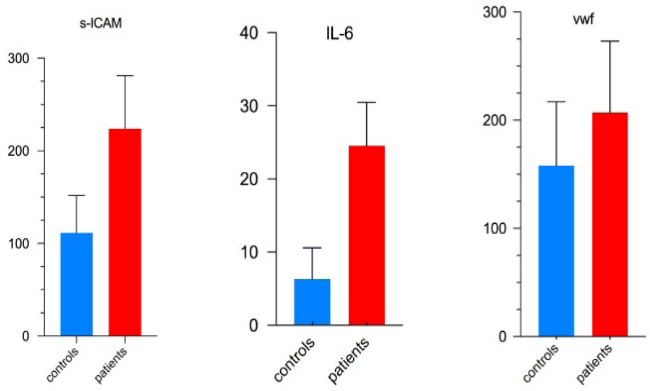

Figure 1: Box plot showing levels of IL-6 (pg/mL), s-ICAM (ng/mL) and vWf (%) in patients and healthy controls. The bottom and the top boxes represent the first and third quartile. Horizontal lines in boxes represent the median value and the whiskers the range limit. Median levels of IL-6, s-ICAM and vWf were significantly higher in patients compared to controls.

Cholesterol Efflux Capacity

Even though within normal reference values both HDL and ApoA1 were significantly reduced within patients as compared to controls, posing the question whether the patients had reduced atherogenic protection. We therefore assessed the cholesterol efflux capacity to exhibit the functional properties of serum as surrogate marker of HDL`s efflux capacity. We, however, found the efflux to be similar in the two groups with mean efflux capacity (%) of 5.5±1.3SD in patients compared to 5.5±0.9SD in controls.

Increased Serum Levels of sICAM, IL-6 and vwf in Patients Compared with Controls

Next, we assessed whether the patient's low HDL could cause altered endothelial function. To study this, we measured 3 classic endothelial markers (sICAM-1, IL-6 and vWF). On paired analysis assessing serum levels we found higher levels of sICAM (256.2±107.4 vs 111.3±40.5, p<0.001), vWf (251.1±165.4 vs 167.0±68.5), p=0.01) and IL-6 (38.3±48.6 vs 17.9±23.9, p=0.04) in patients compared to controls respectively. On regression analysis with adjustment for factors significant on primary analysis (ESR, Apolipoprotein A and HDL) sICAM-1 (p<0.001) and HDL (p<0.001) remained significantly higher in patients compared with controls.

Discussion

This study investigated circulating markers of endothelial dysfunction in patients with juvenile MCTD known to have pre-clinical atherosclerosis. The main findings were that patients had (i) increased levels of sICAM-1, vWf and IL-6, and (ii) lower levels of HDL and ApoA1 compared to healthy age-and sex-matched controls. Elevated sICAM-1 in patients with negligible presence of traditional cardiovascular risk factors, indicates that the driver of endothelial dysfunction and atherosclerosis in this group might be related to the inflammatory burden associated with the underlying disease itself.

Chronic inflammation plays a key role in the development of atherosclerotic CVD and chronic inflammatory diseases such as MCTD, RA and SLE are known to be associated with an increased risk of premature CVD. CVD in patients with adult connective tissue disease, however, has also been associated with an increased prevalence of traditional risk factors and a juvenile population might therefore be better suited to explore the mechanisms involved in the development of premature atherosclerosis in this disease.

IL-6 and vWf were increased in patients compared to controls (although this difference was attenuated when adjusting for ESR), likely reflecting the general inflammatory state of the patients. The increased levels of sICAM-1 in these patients with documented preclinical atherosclerosis are in keeping with previous studies reporting sICAM-1 to be associated with both IMT [18], and change in IMT [19]. Adhesion molecules are key players in the development of atherosclerosis by facilitating monocyte recruitment to the endothelium and migration across the endothelial surface into the intima wall layer. In fact, previous reports have indicated associations between ICAM-1 and stroke with ICAM-1 expression found up-regulated in vascular endothelium of patients with ischemic stroke [20].

In this current study, High Density Lipoprotein (HDL) and ApoA1 were higher in controls compared to patients indicating a possible loss of vascular protection from HDL in the patient group. Cholesterol efflux, however, demonstrated patients to have HDL function similar to control group in its removal of radiolabeled cholesterol from lipid laden macrophages. Even though the efflux capacity was normal, it is tempting to speculate that the reduced HDL level causes reduced protection of endothelium and subsequent activation driven by the inflammatory burden in the patients. Further studies investigating the mechanisms leading to increased subclinical atherosclerosis in this patient group is warranted. Despite the number of patients included in this study being comparable to other published reports on markers of ECD and cardiovascular risk in patients with connective tissue disease, it still remains a small population. Furthermore, measuring endothelium dependent flow-mediated vasodilatation in addition to IMT, would be of interest in this patient group. The strengths of this study are the long-term follow-up of a well-defined cohort, with paired controls, and the presentation of novel data on markers of endothelial dysfunction and pre-clinical atherosclerosis in JMCDT.

Acknowledgment

The author thanks Ellen Lund Sagen for technical assistance. The study is partly funded by Norwegian Research Council RC grant 144245.

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