Pediatric Rheumatology

SHORT REPORT

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Increased vascular deposition of oxidized LDL in untreated juvenile dermatomyositis

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Abstract

Background Juvenile dermatomyositis (JDM) is a systemic vasculopathy associated with metabolic derangements and possible increased risk for premature atherosclerosis. Oxidation of low-density lipoprotein (LDL) in the endothelium is an early step in atherosclerotic plaque formation. It is not known if oxidized LDL is altered in children with untreated JDM. The deposition of oxidized LDL in the vasculature of muscle biopsies (MBx) from patients with untreated JDM and pediatric controls was assessed.

Findings Frozen tissue sections of MRI-directed MBx from 20 female children with untreated JDM and 5 female controls were stained with DAPI and fluorescently labeled antibodies against von Willebrand factor (vWF) and LDL oxidized by copper (oxLDL). Blood vessels were identified by positive vWF staining, and total fluorescence of oxLDL within the vessel walls was measured. Children with untreated JDM had increased deposition of oxLDL in the walls of muscle vasculature compared to healthy children (difference in means ± SEM = 19.86 ± 8.195, $p = 0.03$). Within the JDM cohort, there was a trend towards increased oxLDL deposition with longer duration of untreated disease ($r = 0.43$, $p = 0.06$). There was no significant correlation found between oxLDL deposition and markers of acute JDM disease activity including disease activity scores or muscle enzymes.

Conclusions This study found increased deposition of oxLDL within blood vessels of children with untreated JDM supporting the concern that these children are at increased risk for premature atherosclerosis from chronic exposure to vascular oxLDL. This study highlights the importance of early diagnosis and treatment initiation to ameliorate cardiovascular damage.

Keywords Juvenile dermatomyositis, Cardiovascular disease, Oxidized low-density lipoprotein, Endothelial cells

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Background

Juvenile dermatomyositis (JDM) is the most common type of childhood-onset inflammatory myopathy, characterized by distinct rash, proximal muscle weakness, and vasculopathy [1–3]. While morbidity and mortality of JDM have improved greatly in recent years [4], there remains concern about long term cardiovascular risk in these patients as they transition into adulthood. JDM has been associated with lipodystrophy and resultant abnormalities in serum lipid profiles including elevated triglycerides and decreased circulating high-density lipoprotein [5–9], as well as with other traditional atherogenic risk factors such as hypertension, obesity, and metabolic syndrome [5, 10, 11]. Despite these findings, the data examining risk of premature atherosclerosis in children with JDM is lacking.

One small study comparing adult patients with a history of JDM to healthy controls demonstrated increased carotid intima-media thickness in the JDM patients despite these patients being younger and with lower body mass index which may be an indicator of premature atherosclerosis [12]. A second more recent study, however, showed conflicting evidence with lower endothelial dysfunction in children with JDM compared to healthy controls as measured using Endothelial Pulse Amplitude Testing [13].

Oxidation of low-density lipoprotein (LDL) by reactive oxygen species in the endothelial wall of blood vessels is an early step in atherosclerotic plaque formation [14–16]. Oxidized LDL furthermore enhances inflammation through activation of innate and adaptive immune pathways [17, 18]. It has been well demonstrated that higher levels of circulating oxidized LDL are associated with the development of atherosclerosis [19]; however, data examining levels of oxidized LDL deposition in vessels is more limited. Studies have shown increased levels of oxidized LDL in the walls of atherosclerotic arteries compared to non-atherosclerotic arteries and that higher oxidized LDL levels are associated with higher vulnerability of plaque rupture [20, 21]. It is not known if oxidized LDL deposition is altered in children with untreated JDM. In this study, we sought to compare deposition of oxidized LDL in the vasculature of muscle biopsies (MBx) from patients with untreated JDM compared to pediatric controls.

Findings

Methods

Juvenile dermatomyositis patients and pediatric controls

After informed consent (Ann & Robert H. Lurie Children's Hospital of Chicago IRB# 2010–14,117, 2008–13,457), MRI-directed muscle biopsies were obtained from 20 female children (age 2–10) with definite/probable JDM by Bohan-Peter criteria before the start of therapy. Duration of untreated disease at the time of muscle biopsy (DUDMBx) was calculated, defined as the time from symptom onset to muscle biopsy collection. Disease activity was assessed via disease activity scores (DAS) for skin (S), muscle (M), and total (T) [22]. Nailfold capillary end row loop density was calculated using freeze frame videomicroscopy as previously described [23]. Laboratory values including creatine kinase (CK), aldolase, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), vWF antigen, neopterin, and presence of myositis specific antibodies were recorded. Fasting plasma lipid levels were not available for most patients and thus could not be factored into the analysis. Five control muscle biopsies were obtained from healthy female patients undergoing scoliosis-related surgeries (Lurie Children's IRB# 2001–11,715), after written informed consent. Of note, juvenile idiopathic scoliosis has not been found to be directly associated with increased atherosclerotic risk, and thus these patients were felt to be suitable controls.

Triple immunofluorescence staining

The Zenon technique was used to couple a mouse monoclonal antibody against vWF (1:100, Abcam ab20435) with Fab-Alexa Fluor 568 (Invitrogen Z-25,006) following the manufacturer's instructions [24]. To immunolabel frozen muscle on glass slides, the tissues were fixed with Pipes buffer and 3.7% paraformaldehyde in a 2.1:1 ratio. The slides were blocked with 10% donkey serum and incubated for three hours in a humidified chamber at room temperature with the primary antibody rabbit anti-human LDL oxidized by copper (oxLDL, 1:400, Abcam ab14519). The slides were subsequently washed with PBS and incubated with the secondary antibody, Alexa Fluor 488-conjugated anti-rabbit Ig (1:200, Invitrogen A21206), for one hour at room temperature. After washing, the slides were incubated with Alexa Fluor 568-conjugated anti-vWF Fab for 30 min at room temperature. The slides were washed and then incubated with DAPI (1:2000 in PBS, Invitrogen D1306) for 30 min. The slides were washed, mounted with Fluorosave, and cover-slipped.

Image capture

Images of the stained muscle biopsies were acquired within 48 hours using Openlab computer software 4.04 (Improvision Inc., Lexington, MA) and a Leica DMR-HC microscope (Leica Microsystems GmbH, Wetzlar, Germany) coupled to a Photometric Cool Snap charge-coupled device camera. ImageJ (NIH, Bethesda, MD) was used to identify blood vessel areas, defined by the presence of vWF, and measure the fluorescence of the area (microns^2) and intensity (pixels) of oxLDL within the vessel walls. For each vessel visualized, the intensity of

oxLDL was calculated by multiplying the mean intensity of oxLDL in the vessel by the area of the vessel and then subtracting the mean background intensity multiplied by the same area. The total fluorescence for a muscle biopsy was calculated by dividing the sum of the intensities of the individual vessels by the sum of area of the vessels.

Data analysis

Data was analyzed using t-tests and Pearson correlation using GraphPad Prism 8.

Results

Clinical characteristics of study participants

Table 1 lists the demographic data of the study participants at the time of muscle biopsy. All samples were obtained from female children with mean age of 6.3 +/- 2.2 years (median 6.3 years, range=2.4–9.7) in the untreated JDM group and 14.4 +/- 1.8 years (median 13.9 years, range=12.2–17.0) in the healthy control group. All of the JDM patients identified as White, non-Hispanic. Three of the healthy controls identified as White non-Hispanic, one as African American/Black non-Hispanic, and one as White Hispanic. JDM patients had a mean DUDMBx of 7.6 +/- 7.2 months (median 6.2 months, range=0.9–32.0). All patients were untreated. Patients with untreated JDM had moderate to high disease activity (mean DAS-T=12.0 +/- 3.7; median DAS-T=13, range=4–17). Nailfold capillary end row loop density was decreased in 90% of patients. Muscle enzyme testing (CK, aldolase, LDH, AST) were elevated in 80% of patients. vWF antigen was elevated in 20%, and neopterin was elevated in 80%. Fifteen of the JDM patients tested positive for a myositis specific antibody, most commonly p155/140 which was present in 12 patients.

Table 1 Clinical characteristics and demographics of untreated patients with JDM ($n=20$) and healthy controls ($n=5$)

	Healthy controls ($n=5$)	JDM ($n=20$)
Age (years), mean ± stdv	14.4 ± 1.8	6.3 ± 2.2
Duration of untreated disease at time of biopsy (months), mean ± stdv	NA	7.6 ± 7.2
Race, n (%)		
White	4 (80%)	20 (100%)
African American/Black	1 (20%)	0 (0%)
Ethnicity, n (%)		
Hispanic	1 (20%)	0 (0%)
Non-Hispanic	4 (80%)	20 (100%)
Clinical Measures of Disease Activity, mean ± stdv		
DAS-S (0–9)	NA	6.2 ± 1.5
DAS-M (0–11)	NA	5.8 ± 2.8
DAS-T (0–20)	NA	12.0 ± 3.7
End row capillary loop density (#/1 mm)	NA	4.73 ± 1.36
Lab Values, mean ± stdv		
CK (IU/L)	NA	2218 ± 4497
Aldolase (U/L)	NA	24.5 ± 33.8
LDH (IU/L)	NA	430 ± 217
ALT (IU/L)	NA	126.7 ± 148.3
vWF antigen (%)	NA	158.2 ± 64.8
Neopterin (nmol/L)	NA	19.4 ± 9.5
Myositis Specific Antibodies, n (%)		
P155/140	NA	12 (60%)
Mi-2	NA	4 (20%)
MJ	NA	2 (10%)
Negative	NA	4 (20%)
Not available	NA	1 (5%)

Evaluation of oxLDL deposition in vascular endothelium

Representative images of muscle biopsies of (A) healthy children and (B) children with untreated JDM are shown in Fig. 1A and B. Children with untreated JDM demonstrated significantly higher fluorescence of oxLDL (green) in the walls of muscle vasculature compared to healthy children (difference in means ± SEM = 19.86 ± 8.195, $p=0.03$; Fig. 1C). Within the JDM cohort, there was a trend towards increased oxLDL fluorescence with longer duration of untreated disease at time of muscle biopsy with correlation that bordered on significance ($r=0.43$, $p=0.06$, Fig. 1D). There was no significant correlation found between oxLDL fluorescence and markers of acute disease activity including DAS scores, capillary end row loop density, muscle enzymes, vWF antigen, or neopterin (Table 2).

Conclusions

JDM is a systemic inflammatory muscle disorder and has been previously considered a vasculopathy associated with increased risk of adult atherosclerosis. In this study, we assessed parameters of atherosclerosis early in life in children with untreated JDM and found increased deposition of oxLDL within blood vessels of children with untreated JDM compared to healthy controls. This seemingly begins early on at onset of JDM before treatment is initiated. There was also a trend towards association between increased oxLDL deposition and months of untreated JDM disease. There was no significant correlation between oxLDL deposition and measures of acute disease activity including DAS scores, capillary end row loop density, muscle enzymes, vWF antigen, or neopterin. This study has several limitations that may potentially impact results. First, the control patients were notably older than the patients with JDM. Puberty is known to alter lipid metabolism and can result in lower circulating LDL-cholesterol [25], which may furthermore lead to reduction in endothelial deposition of LDL and oxLDL. Second, no data was available for other clinical risk factors that may impact lipid metabolism such as body mass index, comorbid healthy conditions, or family

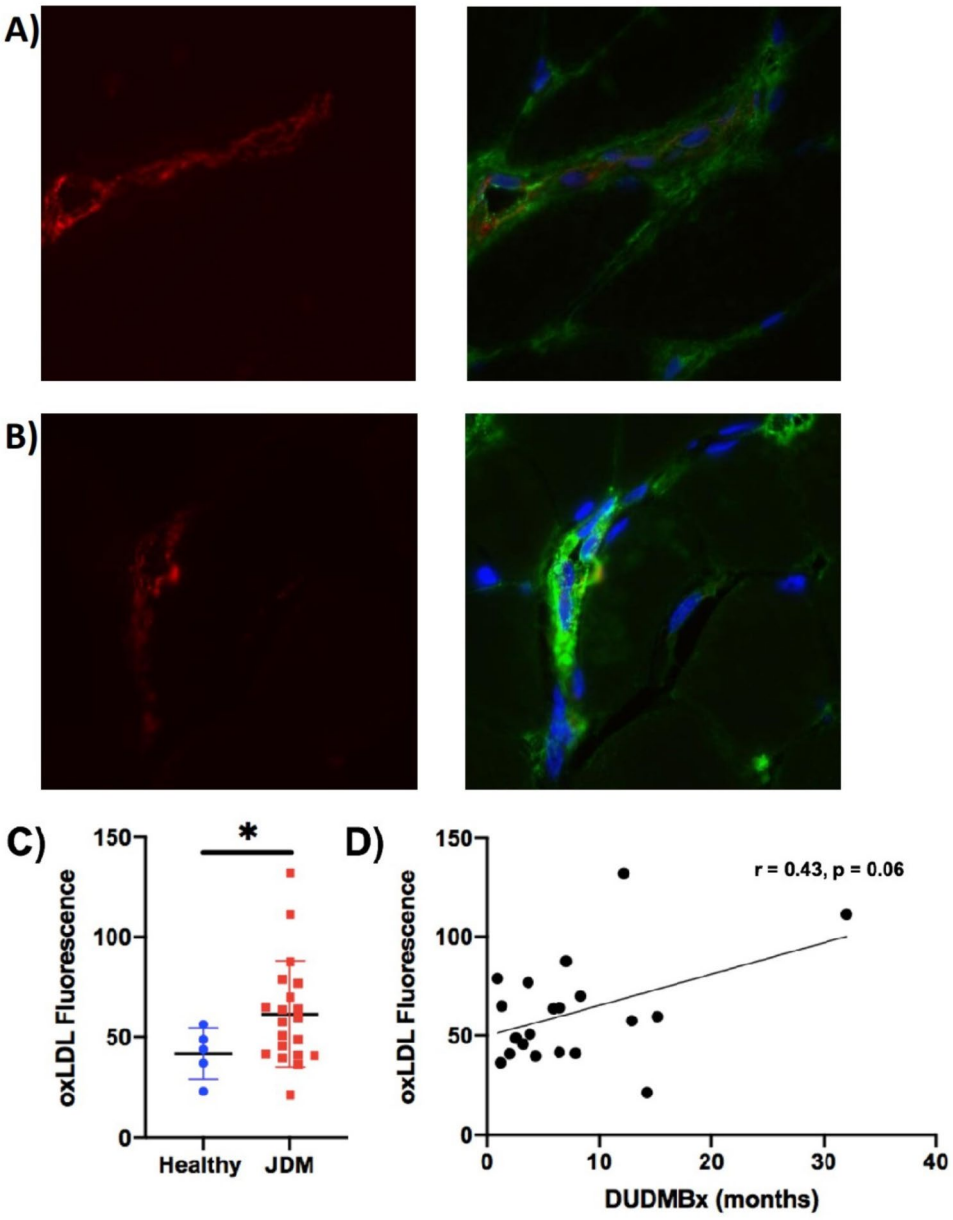

Fig. 1 Fluorescence of oxLDL in the endothelium of muscle vasculature is increased in children with untreated JDM. **Legend**: Immunohistochemistry of muscle biopsies (10X) from (**A**) healthy children and (**B**) children with untreated JDM. Muscle biopsies were stained for DAPI (blue), vWF (red), and oxLDL (green). Blood vessels were identified by the presence of vWF. The fluorescence of the area (microns2) and intensity (pixels) of oxLDL within the vessel walls was calculated. (**C**) Fluorescence of oxLDL (intensity/area) in the walls of muscle vasculature is increased in JDM muscle biopsies compared to healthy controls (difference in means ± SEM = 19.86 ± 8.195, $p = 0.03$). The mean value for each group is indicated by the line, and the standard deviation is indicated by the whiskers. JDM patients ($n = 20$) and healthy controls ($n = 5$). (**D**) Within the cohort of JDM children, there was a trend towards increased fluorescence of oxLDL with longer duration of untreated disease at the time of muscle biopsy.

history of dyslipidemia. Lastly, we did not have fasting plasma lipid profiles on our patients, so were unable to compare circulating lipid and lipoprotein levels with level of oxLDL deposition.

This study highlights the importance of early diagnosis and potentially aggressive upfront treatment in patients with JDM, which may help to ameliorate advancement to cardiovascular damage in the long term. These data suggest that for children with JDM, surveillance of cardiovascular status should be a part of routine assessment as they mature and transition to adult care.

Table 2 Correlation of clinical and laboratory parameters of untreated JDM patients with fluorescence of oxLDL

Clinical/Laboratory Parameter	Correlation with Fluorescence of oxLDL
Age (years)	$r=0.01$, $p=0.96$
Duration of untreated disease at time of biopsy (months)	$r=0.43$, $p=0.06$
Clinical Measures of Disease Activity	
DAS-S	$r=-0.31$, $p=0.18$
DAS-M	$r=-0.005$, $p=0.98$
End row capillary loop density	$r=0.18$, $p=0.44$
Lab Values	
CK	$r=0.15$, $p=0.53$
Aldolase	$r=0.09$, $p=0.73$
LDH	$r=-0.02$, $p=0.92$
ALT	$r=0.07$, $p=0.78$
vWF antigen	$r=-0.24$, $p=0.3$
Neopterin	$r=-0.12$, $p=0.63$

Abbreviations

JDM	Juvenile Dermatomyositis
LDL	Low Density Lipoprotein
MBx	Muscle Biopsy
vWF	von Willebrand Factor
oxLDL	LDL oxidized by copper
DUDMBx	Duration of Untreated Dsease at time of Muscle Biopsy
DAS-S	Disease Activity Score – skin
DAS-M	Disease Activity Score – muscle
DAS-T	Disease Activity Score – total
CK	Creatine Kinase
LDH	Lactate Dehydrogenase
AST	Aspartate Aminotransferase

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12969-024-01001-2.

Supplementary Material 1

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Author contributions

JS performed the immunofluorescence staining, captured the images, analyzed the data, and composed the manuscript. AKO assisted in muscle biopsy preparation, staining, and image capture. JCM assisted in image capture, data analysis, and manuscript preparation. GM consented patients, maintained database, and performed and analyzed nailfold capillaroscopy. LP assisted in project conceptualization and design, data analysis, and manuscript preparation. All authors read and approved the final manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Ann & Robert H. Lurie Children's Hospital of Chicago (IRB# 2010–14117, 2008–13457, 2001–11715). All patients involved in this study signed informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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