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Carotid Intima-Media Thickness Is Increased In Patients With Treated Mucopolysaccharidoses Types I and II, And Correlates With Arterial Stiffness

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Abstract

Background—Treatments for mucopolysaccharidoses (MPS) have increased longevity, but coronary artery disease (CAD) and cardiovascular complications cause mortality in a high percentage of patients. Non-invasive measures of sub-clinical atherosclerosis, such as carotid intima-media thickness (cIMT) and arterial stiffness, may be useful for prediction of CAD outcomes in MPS patients.

Objectives—To determine if cIMT and arterial stiffness are abnormal in MPS I and II patients compared to healthy controls.

Methods—MPS patients underwent carotid artery ultrasonography, and electronic wall-tracking software was used to measure cIMT, carotid artery compliance (cCSC), distensibility (cCSD), and incremental elastic modulus (cIEM). Control data from healthy subjects were obtained from a different study that utilized identical testing within the same laboratory.

Results—A total of 406 healthy controls and 25 MPS patients (16 MPS I, 9 MPS II) were studied. All MPS patients had or were receiving treatment: 15 patients (6 MPS I, 9 MPS II) were receiving enzyme replacement therapy (ERT), 9 patients (all MPS I) had received hematopoietic stem cell transplant (HSCT), and 1 patient with MPS I had received HSCT and was receiving enzyme replacement therapy (ERT). MPS patients had significantly higher mean ($\pm$ SD) cIMT (0.56 ± 0.05 mm) compared to controls (0.44 ± 0.04 mm; adjusted $p < 0.001$). MPS patients also

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CONFLICTS OF INTEREST

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had increased stiffness compared to controls, showing significantly lower cCSC (0.14 ± 0.09 mm²/mmHg versus 0.16 ± 0.05 mm²/mmHg; adjusted $p = 0.019$), and higher cIEM (1362 ± 877 mmHg versus 942 ± 396 mmHg; adjusted $p < 0.001$). cCSD in MPS patients was lower than control ($29.7 \pm 16.4\%$ versus $32.0 \pm 8.2\%$) but was not statistically; $p = 0.12$. Among MPS patients, cCSD showed a significant association with cIMT ($p = 0.047$), while the association between cIEM and cIMT approached significance ($p = 0.077$). No significant differences were observed in cIMT, cCSD, cCSC, and cIEM between MPS I and MPS II patients.

Conclusions—Despite treatment, MPS patients had higher cIMT compared to healthy controls, indicating this marker of sub-clinical atherosclerosis may be a useful predictor of CAD outcomes. The association of arterial stiffness measures with cIMT suggests that mechanical and structural changes may occur in concert among MPS patients. Although yet to be confirmed, increased cIMT and arterial stiffness in MPS I and II patients may be a consequence of inflammatory signaling pathways triggered by heparan or dermatan sulfate-derived oligosaccharides. Prospective, longitudinal studies will need to be performed in order to evaluate the usefulness of these carotid measurements as predictors of adverse CAD outcomes in MPS patients.

Keywords

Mucopolysaccharidosis; Hunter; Hurler; Syndrome; intima-media thickness; treatment; marker; vascular disease

1. INTRODUCTION

1.1

The mucopolysaccharidoses (MPSs) are a group of inherited systemic diseases in which glycosaminoglycans (GAGs) are deposited throughout the body resulting in multi-organ dysfunction and shortened life span [1]. GAG deposition occurs in the myocardium, the cardiac valves, the great vessels and coronary arteries in all types of MPS [2]. Diffuse myointimal proliferation and GAG deposition may cause occlusion of the epicardial coronary arteries often resulting in sudden and unexpected death [3–9]. At the present time, therapy includes hematopoietic stem cell transplantation (HSCT) for severe MPS I and enzyme replacement therapy (ERT) for attenuated MPS I, MPS II, and MPS VI; the efficacy of ERT being investigated for post-HSCT MPSI, MPS IIIa, and IV [2]. While HSCT appears to attenuate coronary artery disease in severe MPS I [9,10], the ability of ERT to treat coronary disease is more questionable. Sudden and unexpected death, with evidence for coronary artery stenosis or myocardial infarction, has been reported in patients with both MPS I and II after 1–2 years of ERT [5,11].

1.2

Determining the extent of coronary artery disease in MPS by coronary angiography is often difficult due to its diffuse nature and risk of adverse outcomes [12]. Although intracoronary ultrasound has been successful in detecting similar intimal proliferation in adults with Fabry's disease [13], it is an invasive method and its use has not been reported in children with MPS. We have previously reported the use of non-invasive testing with ultrasound measurement of carotid intima media thickness (cIMT) and vascular function (digital reactive hyperemic index) as surrogates for coronary artery risk in a small group of children with MPS I and II [14,15]. Here, we report the results of combined cIMT and three complementary carotid-ultrasound derived measurements of arterial stiffness, cross-sectional distensibility (cCSD), cross-sectional compliance (cCSC), and incremental elastic modulus (cIEM), in a larger, multi-center cohort of MPS I and II patients.

2. METHODS

2.1 Study Design and Participants

2.1.1—This was a dual-center, cross-sectional study of 25 children and adolescents with biochemically and/or molecularly confirmed MPS types I and II and 406 healthy controls. Individuals with MPS I or II who received clinical care at the University of Minnesota (Minneapolis, MN) or Children's Hospital of Orange County (Orange, CA) were recruited. Vascular data from healthy controls were obtained from a study of insulin resistance and cardiovascular risk at the University of Minnesota that utilized an identical testing protocol. Clinical data (demographic information, ethnicity, confirmation of diagnosis, treatment status) from the MPS patients were obtained from chart review. The protocol was approved by the respective Institutional Review Boards at the University of Minnesota and the Children's Hospital of Orange County.

2.2 Measurement of Carotid Artery Intima-Media Thickness and Stiffness

2.2.1—Following informed consent/assent, vascular studies were performed in a quiet, temperature-controlled environment. Vascular images were obtained with conventional ultrasound scanners with participants in the supine position. Images were digitized and stored on electronic media (Children's Hospital Orange County scans) or a personal computer (University of Minnesota scans) for later off-line analysis by one reader at a central laboratory (University of Minnesota) using an electronic wall-tracking software program (Vascular Research Tools 5, Medical Imaging Application, LLC, Iowa City, IA, USA).

2.2.2—Following at least 10 minutes of quiet rest in the supine position, vascular images were obtained from the common carotid artery with a 15-8 MHz linear array probe held at a constant distance from the skin and at a fixed point over the imaged artery, approximately 1 cm proximal from the carotid bifurcation bulb, to capture the lumen diastolic and systolic diameters (for the calculation of compliance and distensibility). Depth and gain settings were set to optimize images of the lumen/arterial wall interface. Systolic and diastolic blood pressures were recorded with an automated blood pressure sphygmomanometer during the 10-second carotid measurements. Images were collected at 20 frames per second for 10 seconds (200 frames) to ensure the capture of full arterial diameter change during a cardiac cycle. Carotid intima-media thickness was measured on the far wall, and the left carotid values were used for all subjects except for one, who had values only for the right carotid.

2.3 Description of cCSD, cCSC, and cIEM measurements

2.3.1—cCSD, defined by the percent change in carotid lumen area from diastole to systole, has the unit of % and is defined by the equation:

$$cCSD = \frac{sD^2 - dD^2}{dD^2} \cdot 100\%$$

where sD is the maximum systolic carotid diameter, dD is the minimum diastolic carotid diameter. Increasing cCSD reflects high carotid distensibility and low stiffness, and vice versa.

2.3.2—cCSC, defined by the relative change in carotid lumen area from diastole to systole for a given change in blood pressure, has the unit mm²/mmHg and is defined by the equation:

$$cCSC = \frac{\pi(sD^2 - dD^2)}{4 \cdot PP}$$

Where PP is pulse pressure (or systolic blood pressure – diastolic blood pressure). Increasing cCSC, similar to cCSD, reflects high carotid distensibility and low stiffness, and vice versa.

2.3.3—cIEM, the elasticity constant of the carotid vessel, has the unit mmHg and is defined by the equation:

$$cIEM = \frac{3(1 + \frac{sD^2}{dD^2})}{cCSC}$$

In contrast to cCSC and cCSD, increasing cIEM reflects low carotid distensibility and high stiffness.

2.4 Statistical Analysis

2.4.1—Descriptive statistics were tabulated separately for the healthy control and MPS groups, which included the mean and standard deviation for continuous variables and frequency for categorical variables. Comparisons between group means used the t-test with unequal variances and Welch degrees of freedom for confidence intervals and P-values. Age- and gender-adjusted results and associations of stiffness measures with cIMT used linear regression and the t-distribution with corresponding model degrees of freedom for confidence intervals and p-values. Paired analyses among a subset of the MPS group evaluated means of the rate of change between measurements using the t-test. All analyses were conducted using R v2.15.2 [16].

3. RESULTS

3.1 General cohort characteristics

3.1.1—A total of 406 healthy controls and 25 MPS patients (16 MPS I, 9 MPS II) were studied. Demographic information regarding the patients can be found in Table 1. All of the MPS II patients were male. All MPS patients had or were receiving treatment: 9 patients (all MPS I) had received HSCT; 1 patient with MPS I had received HSCT and was receiving ERT; and 15 patients (6 MPS I, 9 MPS II) were receiving ERT.

3.2 Carotid measurements

3.2.1—MPS patients had significantly higher mean ($\pm$ SD) cIMT (0.56 ± 0.05 mm) compared to controls (0.44 ± 0.04 mm; adjusted $p < 0.001$). MPS patients also had increased stiffness compared to controls, showing significantly lower cCSC (0.14 ± 0.09 mm²/mmHg versus 0.16 ± 0.05 mm²/mmHg; adjusted $p = 0.019$), and higher cIEM (1362 ± 877 mmHg versus 942 ± 396 mmHg; adjusted $p < 0.001$). MPS cCSD was lower than controls ($29.7 \pm 16.4\%$ versus $32.0 \pm 8.2\%$) but did not reach statistical significance (adjusted $p = 0.12$). There were subtle, but significant trends in the MPS cohort between age and cIMT (increase of 0.01 mm/10 years, adjusted $p = 0.003$), cCSD (decrease of 5.33%/10 years, adjusted $p < 0.001$), cCSC (decrease of 0.03 mm²/mmHg/10 years, adjusted $p < 0.001$), and cIEM (increase of 314 mmHg/10 years, adjusted $p < 0.001$). These findings are summarized in Table 2. There were also trends in the MPS cohort between cIMT and measurements of stiffness: for every 0.05 mm increase in cIMT, cCSD showed a significant (adjusted $p =$

0.047) decrease of 6.35%, while cIEM increased by 305 mmHg (adjusted $p = 0.077$), and cCSC decreased by 0.01 mm²/mmHg (adjusted $p = 0.62$). These findings are summarized in Table 3. Between MPS I and MPS II patients, there were no significant adjusted differences in cIMT ($p = 0.50$), cCSD ($p = 0.61$), cCSC ($p = 0.13$), or cIEM ($p = 0.51$).

3.3 Longitudinal cIMT assessment

3.3.1—As the MPS cohort included seven individuals who previously underwent cIMT measurement in an earlier study [14], matched-pair analysis was performed to determine if significant longitudinal changes in cIMT could be observed. Possibly because of the short interval (mean 2.4 years) between cIMT measurements and inability of the ultrasonogram to detect changes less than 0.01 mm, no significant changes (−0.001 mm/year) were observed in the MPS patients with longitudinal cIMT measurements.

4. DISCUSSION

4.1

While patients with MPS types I and II are surviving longer as a result of HSCT and ERT, they continue to experience progressive symptoms in refractory organ systems. Cardiovascular complications, especially congestive heart failure, valvular thickening and dysfunction, and coronary artery occlusion continue to occur in stably treated patients, often resulting in death [5,11,17]. Development of validated, predictive biomarkers to identify individuals at-risk for severe cardiovascular disease is imperative to guide medical decision-making for clinicians and patients alike, and eventually to measure the efficacy of novel therapeutic agents. Carotid intima-media thickness correlates well with carotid histology [18], has been repeatedly validated as a predictive risk factor for myocardial infarction and cerebrovascular accident [19], and is also used as an end point in interventional studies. The finding of increased cIMT, independent of ERT, prior HSCT-associated corticosteroid exposure, or degree of aortic insufficiency, in a cohort of 12 MPS I and II patients [14] indicated its potential utility as a biomarker for MPS cardiovascular disease. The results from that study suggested that the plaques and GAG storage observed in post-mortem coronary and aortic specimens [4] reflected a pan-arterial process that could be visualized *in vivo* with non-invasive ultrasound imaging.

4.2

This study corroborates the findings of our original study utilizing a larger, multi-institutional cohort of MPS I and II patients. The cIMT of MPS patients from this study, 0.56 ± 0.05 mm, was comparable to the cIMT of MPS patients from the first study, which was 0.54 ± 0.07 mm [14]. Similar to findings from other pediatric cIMT studies [20], there was a small but significant correlation (0.01 mm/10 years) between age and increasing cIMT in the MPS population. There were also similar correlations between age and reduced carotid artery compliance, distensibility, and increased incremental elastic modulus, all three of which reflect increasing stiffness. This is consistent with other studies that have demonstrated decreased arterial distensibility with increasing age [21,22].

4.3

Adjusted for gender and age, the MPS cohort had reduced cCSC, and a trend toward reduced cCSD and increased cIEM compared to controls. The three indices concordantly indicate that the MPS cohort has increased arterial stiffness compared to the unaffected control cohort, beyond that which can be accounted for by co-variables alone. Taken together, our findings indicate impaired arterial structure and function/mechanics in MPS patients, corroborating previous reports of endothelial dysfunction in MPS I and II patients as

measured by digital peripheral arterial tonometry following forearm ischemia [15,17] and by aortic elastic indices acquired via echocardiogram [23]. Moreover, cIMT correlated with increasing carotid stiffness in the MPS patients, indicating a link between thicker arterial intima/media in MPS patients and reduced arterial elasticity. To our knowledge, this is the first report of increased carotid artery stiffness, and positive correlation between increasing cIMT and carotid artery stiffness, among patients with MPS types I and II.

4.4

Several potential mechanisms linked to GAG storage and inflammation may be responsible for the increased cIMT and abnormal arterial function observed in our MPS cohort. Similar to the severe lesions noted in untreated MPS I humans [4], the MPS I canine model demonstrates large, eccentric luminal “plaques” composed not only of proteoglycans and collagen, but also of proliferating myofibroblasts, vascular smooth muscle cells, and CD68+ activated macrophages resembling what is seen in human atherosclerotic atheromas [24]. Expansion of the intima caused by simple accumulation of undegraded glycosaminoglycan cannot account for these observations. Studies have demonstrated that GAGs and GAG-derived oligosaccharides trigger a pro-inflammatory signaling cascade ending with macrophage activation, via the Toll-like receptor 4 (TLR4) and its downstream mediator, activated nuclear factor κ -B (NF- κ B). In MPS IIIb rat brain, HS-oligosaccharides resulted in TLR4-mediated overexpression of the pro-inflammatory cytokines tumor necrosis factor α (TNF α), macrophage inflammatory protein 1 α , and interleukin 1 β mRNAs, subsequent microglial activation, and neuroinflammation [25]. MPS VII mice demonstrated high serum levels of TNF α , abnormal growth plate architecture, and poor linear growth that were not present in the MPS VII/TLR4 double knockout [26]. Activation of TLR4 by GAGs may explain the observation of increased transforming growth-factor β and activated NF- κ B within canine MPS I aortic plaque, with subsequent proliferation of extracellular matrix and recruitment of VSMCs, myofibroblasts, and activated macrophages [24]. Other studies have measured increased levels of TNF α , matrix metalloproteinases, and cathepsin proteases in aortas of MPS VII mice, and dogs with MPS I and VII [27,28]. Multiple studies have demonstrated dilatation of the aortic root and elastin fiber fragmentation in human MPS VII [27], canine MPS I and VII [24,27], feline MPS I and VI [29], and murine MPS I and VII [28,30,31]. Since elastin is a substrate of both metalloproteinase and cathepsin protease families [32,33], the loss of vascular elastin integrity and subsequent increase in arterial stiffness in MPS may be a result of GAG-TLR4 mediated inflammatory signaling. We are currently studying the roles played by HS or DS-derived oligosaccharides, TLR4, and downstream inflammatory mediators, in the aortic phenotype of the MPS I murine model. Because aortic histopathology from MPS III, IV, and VI patients [7,34,35] have demonstrated lesions similar to those described in MPS I, we are planning additional studies to determine if cIMT is increased in patients with those MPS types. Although longitudinal studies in MPS patients are vital to correlate biomarker measurements to actual cardiovascular events, cIMT and arterial stiffness measurements have both been validated as independent risk factors for cardiovascular disease in many other conditions. Our study supports the premise that carotid ultrasonographic measurements can be used as cardiovascular risk predictors for MPS [36].

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Highlights

- Carotid artery structure and function were studied in 25 MPS I and II patients
- MPS patients had significantly higher carotid intima-media thickness versus controls
- MPS patients demonstrated increased carotid stiffness versus controls
- MPS carotid intima-media thickness was positively associated with carotid stiffness
- No differences seen in carotid intima-media thickness or stiffness between MPS I and II

Table 1

Descriptive characteristics and unadjusted carotid measurements of the study cohorts. Values presented are mean (sd) or N (%) where indicated; superscripts indicate the number of patients with missing data.

Abbreviations: SBP, systolic blood pressure. DBP, diastolic blood pressure. HR, heart rate. BMI, body mass index.

Covariate	Control (N=406)	All MPS (N=25)	MPS I (N=16)	MPS II (N=9)
Male	220 (54.2%)	19 (76.0%)	10 (62.5%)	9 (100%)
Female	186 (45.8%)	6 (24.0%)	6 (37.5%)	0 (0%)
Race				
White	379 (93.3%)	20 (80.0%)	14 (87.5%)	6 (66.7%)
Asian	0 (0.0%)	2 (8.0%)	0 (0.0%)	2 (22.2%)
Unknown	27 (6.7%)	3 (12.0%)	2 (12.5%)	1 (11.1%)
Age at visit (yrs)	12.71 (3.69)	11.74 (4.32)	12.26 (4.86)	10.80 (3.17)
SBP (mmHg)	106 (10.69) ²	105 (11.35)	106 (11.2)	103 (11.96)
DBP (mmHg)	57.58 (7.68) ²	51.64 (12.06)	50.25 (14.2)	54.11 (6.94)
HR (beats/min)	78.59 (62.47) ⁴	88.92 (15.08)	87.19 (16.57)	92.00 (12.3)
Height (cm)	154 (17.91)	134 (14.15)	134 (15.8)	134 (11.48)
Weight (kg)	50.54 (20.65)	37.93 (16.61)	37.87 (18.87)	38.05 (12.65)
BMI (kg/m ²)	20.46 (4.98)	20.24 (4.81)	20.06 (5.54)	20.54 (3.41)
cIMT (mm)	0.44 (0.04)	0.56 (0.05)	0.56 (0.04)	0.55 (0.07)
cCSD (%)	31.98 (8.20)	29.69 (16.4)	28.39 (14.77)	32.01 (19.66)
cCSC (mmHg)	0.16 (0.05)	0.14 (0.09)	0.12 (0.06)	0.18 (0.12)
cIEM (mm ² /mmHg)	942 (396)	1362 (877)	1452 (997)	1203 (633)

Table 2

Age and gender-adjusted comparisons of mean carotid measures between the MPS and control cohorts. Significant comparisons are highlighted in **bold**.

Metric	Covariate	Measurement (mean $\pm$ SD)	Adj. Difference (95% CI)	p-value
cIMT	MPS	0.56 $\pm$ 0.05 mm		
	Control	0.44 $\pm$ 0.04 mm		
	MPS vs. Control		0.12 (0.10, 0.13)	<0.001
	Female		-0.01 (-0.01, 0.00)	0.064
	Age (per 10y)		0.01 (0.00, 0.02)	0.003
cCSD	MPS	29.7 $\pm$ 16.4 %		
	Control	32.0 $\pm$ 8.2 %		
	MPS vs. Control		-2.78 (-6.31, 0.75)	0.122
	Female		0.11 (-1.55, 1.77)	0.895
	Age (per 10y)		-5.33 (-7.53, -3.13)	<0.001
cCSC	MPS	0.14 $\pm$ 0.09 mm ² /mmHg		
	Control	0.16 $\pm$ 0.05 mm ² /mmHg		
	MPS vs. Control		-0.02 (-0.05, 0.00)	0.019
	Female		0.00 (-0.01, 0.01)	0.345
	Age (per 10y)		-0.03 (-0.04, -0.01)	<0.001
cIEM	MPS	1362 $\pm$ 877 mmHg		
	Control	942 $\pm$ 396 mmHg		
	MPS vs. Control		435.16 (263.43, 606.90)	<0.001
	Female		-70.43 (-151.13, 10.27)	0.087
	Age (per 10y)		314.37 (207.20, 421.55)	<0.001

Table 3

Associations within the MPS cohort between cIMT and the three metrics of carotid stiffness: cCSD, cCSC, and cIEM. cCSD showed a significant reduction (highlighted in bold) of -6.35% for every 0.05 mm increase in cIMT.

Metric	Covariate	Adjusted Difference (95% CI)	p-value
cCSD	cIMT (per 0.05 mm)	-6.35 ($-12.60, -0.10$) %	0.047
cCSC	cIMT (per 0.05 mm)	-0.01 ($-0.05, 0.03$) mm ² /mmHg	0.621
cIEM	cIMT (per 0.05 mm)	304.75 ($-36.31, 645.82$) mmHg	0.077