

Epidermal Neurite Density in Skin Biopsies from Patients with Juvenile Fibromyalgia

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Short title: Juvenile Fibromyalgia Neurite Densities

Abstract

Background:

Fibromyalgia is defined by idiopathic, chronic, widespread musculoskeletal pain. In adults with fibromyalgia, meta-analysis of lower-leg skin biopsy demonstrated 45% pooled prevalence of abnormally low epidermal neurite density (END). END <5th centile of the normal distribution is the consensus diagnostic threshold for small-fiber neuropathy. However, the clinical significance of END findings in fibromyalgia is unknown. The prevalence of small fiber pathology has not yet been studied in juvenile fibromyalgia.

Methods: We screened 21 patients aged 13-20y with fibromyalgia diagnosed by pediatric rheumatologists. Fifteen meeting the American College of Rheumatology criteria (modified for juvenile fibromyalgia) underwent lower-leg measurements of END and completed validated questionnaires assessing pain, functional disability, and dysautonomia symptoms. The primary outcome was proportion of fibromyalgia patients with END <5th centile of age/gender/race-based laboratory norms. Cases were systematically matched by ethnicity, race, sex, and age to a group of previously biopsied healthy adolescents with selection blinded to biopsy results. All 23 controls matching demographic criteria were included.

Results: Among biopsied JFM patients, 53% (8/15) had END <5th centile versus 4% (1/23) of healthy controls ($p<0.001$). Mean patient END was 273/mm² skin surface (95% confidence interval: 198-389) versus 413 (95% CI: 359-467; $p<0.001$). As expected, fibromyalgia patients reported more functional disability, dysautonomia, and pain than healthy controls.

Conclusion:

Abnormal END reduction is common in adolescents with fibromyalgia, with similar prevalence to adults with fibromyalgia. More studies are needed to fully characterize the significance of low END in fibromyalgia and to elucidate the clinical implications of these findings.

Introduction:

Juvenile fibromyalgia (JFM) is characterized by persistent, widespread musculoskeletal pain, fatigue, and other somatic symptoms (1). It affects 2-6% of school-aged children, predominantly adolescent females (2). Patients often report impaired physical, school, social and emotional functioning (3). The causes of fibromyalgia are unknown, and the relative contributions of central versus peripheral neurological abnormalities are debated. Brain imaging studies show altered regional blood flow and grey-matter volume, changed activation and connectivity of pain-processing regions (4), and altered levels of several neurotransmitters (5). However, no primary brain pathology has been identified as causal, and similar abnormalities are seen in various acute and chronic pain conditions. These central findings may be non-specific responses to changed peripheral input, pain, deconditioning, mood, or medications (6).

Small-fiber neuropathy (SFN) is a peripheral polyneuropathy that like fibromyalgia, causes chronic widespread pain, exertional intolerance, gastrointestinal symptoms and chronic headache (7, 8). Common causes in mature adults include diabetes, chemotherapy, inflammation, and dysimmunity, with genetic variants and other toxins being rare causes (8). SFN is characterized by electrical hyperactivity and distal degeneration of the peripheral neurons that mediate pain, heat and cold sensation, and internal autonomic functions. Electrophysiologists report excess, spontaneous, and prolonged firing of C and A δ pain-fibers in adult fibromyalgia and SFN (9, 10).

Measuring epidermal neurite density (END) within PGP9.5-immunolabelled lower-leg skin biopsies has become standard for confirming suspected cases of SFN (8). Systematic review of early skin-biopsy studies of adult fibromyalgia generated 45% pooled prevalence of pathological biopsies (95% CI:32-59%) (11). A subsequent study of 117 skin-biopsied adults with

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fibromyalgia identified correlations between severity of multiple fibromyalgia symptoms and extent of cutaneous denervation, and documented abnormal proximal as well as distal skin biopsies in some patients (10). Nerve conduction studies have also identified correlations between low medial plantar and sural nerve amplitudes and low END in adult fibromyalgia (12). Brain imaging studies show reduced functional connectivity between anterior cingulate, amygdala and precuneus in SFN, with severity paralleling skin denervation, supporting the hypothesis that SFN can produce post-synaptic brain effects similar to those reported in fibromyalgia (13). Given the increasing importance of skin-biopsies in adult fibromyalgia, we undertook the first study to examine END in juvenile fibromyalgia.

Patients and Methods:

Fibromyalgia patients aged 13-20y were recruited at Rutgers-Robert Wood Johnson and Columbia University Medical Centers between December 2016-November 2018. Inclusion for screening required clinical diagnosis by a pediatric rheumatologist. Patients with disorders associated with small fiber neuropathy were excluded. This study was approved by the institutional review boards of the participating centers (Rutgers IRB Pro20160000631, Columbia IRB AAAR3311). Participants over 18 provided consent, those under 18 provided assent plus parental permission.

Participants who did not meet the 2010 American College of Rheumatology (ACR) fibromyalgia diagnostic criteria (modified for juvenile fibromyalgia) were excluded (1). They completed validated surveys of pain, functional disability, and dysautonomic symptoms. Self-reported pain during the prior week was assessed by 0-10 numeric rating scale (0=none; 10=worst pain). We administered the Functional Disability Inventory (FDI), a 60-point self-

report scale with higher scores indicating more disability (14). Dysautonomia symptoms were assessed by Composite Autonomic Symptom Score 31 (COMPASS-31) whose 0-100 range encompasses orthostatic, vasomotor, secretomotor, gastrointestinal, bladder, and pupillomotor symptoms with higher scores indicating worse symptoms (15). COMPASS-31 is validated in adolescent as well as adult SFN (16). Symptom duration was calculated as months between symptom onset and skin biopsy.

Participants underwent 3mm skin punch biopsy from the standard site above the lateral malleolus. Biopsies mailed in Zamboni's fixative to Massachusetts General Hospital (MGH) clinically accredited neuropathology lab were processed, PGP9.5 immunolabeled, and analyzed according to clinical standards. MGH is one of very few diagnostic labs with skin biopsy data from healthy children and adolescents, providing pediatric normative values. All biopsies were blindly evaluated by the same morphometrist. MGH reports ENDs as per mm² skin surface area to control for section thickness variability between labs; the correction factor is 20 for comparing to reports from labs that use 50 μ m sections but report linear ENDs.

We constructed the control group by screening the 65 previously biopsied healthy controls between 13-20y. Thirteen were excluded for unobtainable ethnicity. The remaining 52 were evaluated for inclusion using a non-biased algorithm that matched race, ethnicity, sex and age of the JFM cohort. Matching was blind to all other results including skin-biopsies. To match race and ethnicity, every eligible Black, Hispanic and Asian control was included, then all other males excluded to match the 95% female JFM group, which yielded 23 healthy controls. With MGH IRB approval (1999P009042/MGH), all 23 healthy controls were re-contacted and invited to complete the study questionnaires.

The primary outcome was END < 5th centile of age/sex/race-based norms. Survey question

outcomes were secondary. After verifying normality, categorical outcomes were summarized by proportions and continuous outcomes by means. Fisher exact test compared the prevalence of abnormal results of categorical variables, Student's T-test compared means of continuous variables, and Wilcoxon rank sum test compared non-normal variables.

Results:

Twenty-one adolescents with clinical JFM completed the surveys, and the 18 meeting ACR diagnostic criteria were offered skin biopsies. Fifteen underwent uncomplicated biopsies, 3 declined. The JFM and control samples were well matched by sex (7% vs. 9% male; $p=0.99$), age (17.2 vs. 17.9; $p=0.13$), ethnicity (4 Hispanic vs. 3 Hispanic; $p=0.4$), and race (2 Black vs. 3 Black; $p=0.99$; 1 Asian vs. 0 Asian). Ten of the 23 healthy controls completed surveys, 13 did not respond.

Fifty-three percent (8/15; 95% CI: 26%-79%) of JFM participants had END $<5^{\text{th}}$ centile of the age/gender/race-based normal distribution versus 4% (1/23, 95% CI: 0%-22%) of healthy controls ($p<0.01$; Figure 1). Mean END among participants with fibromyalgia was 273/ mm^2 skin surface (95% CI: 198-389) versus 413 (95% CI: 359-467) for controls ($p<0.001$). JFM participants had markedly higher median pain scores (7 vs. 1), FDI (33 vs. 1), and COMPASS-31 (49 vs. 14) than healthy controls (Table 1) and no controls had scores consistent with fibromyalgia. There were no evident differences between JFM participants with END above vs. below the 5^{th} centile with respect to median age (17 vs. 17 years), symptom duration (26 vs. 21 months), pain scores (7 vs. 7), FDI (33 vs. 33), or COMPASS-31 scores (43 vs 53).

Discussion:

This case-control study provides the first prevalence data for small fiber pathology in adolescents with fibromyalgia, with the 53% prevalence of abnormally low END comparable to the 45% pooled prevalence in adult patients (11). Juvenile and adult fibromyalgia share many clinical characteristics, and most adolescents and young adults continues to experience symptoms into adulthood, suggesting a common spectrum of disease.

These objective neuropathologic findings may have potential for guiding treatment and measuring post-treatment axonal regeneration (8). Some investigators hypothesize that undiagnosed SFN is a cause of fibromyalgia symptoms(10), whereas others believe peripheral changes in fibromyalgia to be epiphenomena of centralized chronic pain (17). Many patients with SFN have presentations that do not resemble fibromyalgia, including exclusively distal or autonomic symptoms, and small-fiber pathology can develop in other neurodegenerative conditions, suggesting that decreased END in patients with fibromyalgia could be an incidental, non-specific finding (18).

A strength of our study is the strict inclusion criteria requiring clinical diagnosis of JFM plus best-available diagnostic criteria (1). Our results corroborate previous reports of abnormal skin biopsies and autonomic function testing in youth with more loosely defined chronic widespread pain, in whom JFM diagnoses were not captured (19, 20). Given barriers to recruiting healthy children for research requiring invasive procedures, another strength of our study is the availability of comparator biopsies from demographically matched healthy controls. This approach contrasts with a recent publication that used a convenience control sample of younger children undergoing skin biopsies for other chronic neuromuscular diseases (20).

Our study main limitation was lack of neurologists' evaluations to determine which JFM patients met criteria for SFN. Questionnaires and other metrics for diagnosing and tracking SFN

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should be validated in children, along with forthcoming research diagnostic criteria for adult SFN (7, 16). Also, our small sample may not have fully represented the JFM population, providing an inexact population prevalence. Controls were recruited at different times and from a different geographic region than JFM participants, though they were demographically matched. Another limitation is that the healthy controls' participation in surveys was limited, and their surveys were completed at different times than skin biopsies. The survey results from controls, however, reflected values expected from healthy youth. Finally, the small sample size limited our ability to compare JFM patients with vs. without abnormal END. Despite this, we still observed striking differences in END between cases and controls that were highly consistent with studies of adults with fibromyalgia.

In summary, our study offers the first description of epidermal neurite density measurements in juvenile fibromyalgia. Our data demonstrate abnormally low epidermal neurite density in half of juvenile fibromyalgia participants, in marked contrast to the expected 5% prevalence in healthy adolescents. These findings parallel those from adult fibromyalgia patients. More research is needed to fully characterize the significance of epidermal neurite density findings in fibromyalgia and to elucidate the clinical implications of these findings.

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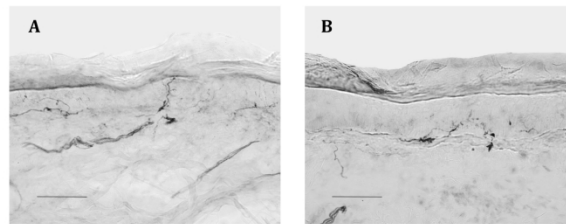
Figure Legends

Figure 1

Representative skin biopsies from 10 cm above the lateral malleolus, bright-field, 40 x. Scale bar =100μm; arrows demonstrate epidermal nerve fibers

A. Healthy control: Biopsy from 17.9-year-old Caucasian female with epidermal neurite density (END) of 500/mm² skin surface area; at the 64.3 percentile of the age, sex, and race-adjusted normal distribution.

B. Juvenile fibromyalgia patient: Biopsy from a 17.8-year-old Caucasian patient with END (242/mm² skin surface area) at the 4.7 centile of predicted.



Standard clinical processing and morphometry of skin biopsies from 10 cm above the lateral malleolus, bright-field, 40 x. Scale bar = 100µm; arrows demonstrate epidermal nerve fibers

A. Healthy control: Biopsy from 17.9-year-old Caucasian female with epidermal neurite density (END) of 500/mm² skin surface area; at the 64.3 percentile of the age, sex, and race-adjusted normal distribution.

B. Juvenile fibromyalgia patient: Biopsy from a 17.8-year-old Caucasian patient with END (242/mm² skin surface area) at the 4.7 centile of predicted.

215x279mm (300 x 300 DPI)

Patient Characteristics		JFM patients (n=15)
Age, y, median (IQR)		17.2 (15.7 - 18.2)
Female sex, n (%)		14 (93)
Hispanic, n (%)		4 (26)
Black, n (%)		2 (13)
Asian, n (%)		1 (7)
Skin Biopsy Data		
END < 5th centile, n (%)		8 (53)
Mean END (neurite/mm2) ± SD		273 ± 149
Surveys		JFM patients (n=15)
Pain Score (median, IQR)		7 (6-8)
FDI (median, IQR)		33 (26-39)
COMPASS-31 (median, IQR)		
Total		49 (41-59)
Orthostatic Intolerance		24 (22-32)
Vasomotor		0 (0-3)
Secretomotor		6 (4-10)
Gastrointestinal		12 (8-16)
Bladder		1 (0-2)
Pupilomotor		3 (2-3)

Healthy controls (n=23) P value

17.9 (16.3 - 19.3)	0.13
21 (91)	0.99
3 (13)	0.4
3 (13)	0.99
0	

1 (4)	<0.01
413 ± 132	<0.001

Healthy Controls (n=10)

1 (0-2)	<0.001
1 (0-4)	<0.001
14 (3-19)	<0.001
12 (0-19)	<0.001
0 (0-0)	0.08
0 (0-0)	<0.001
1 (0-3)	0.001
0 (0-0)	0.03
0 (0-0)	<0.001