What are The Best Outcome Measures for Persons with Hypermobile Ehlers-Danlos Syndrome and HSD: A Pilot Study?

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ABSTRACT

Background: *Hypermobile Ehlers-Danlos, (hEDs), and Hypermobile Spectrum Disorder (HSD) are connective tissue diseases that are often clinically indistinguishable. The purpose of this study was to assess 3 outcomes for utility for persons with these diseases in terms of intrarater, interrater reliability and intertrial reliability as well as, the Health Assessment Questionnaire Disability Index (HAQ-DI) an assessment of participants perception of pain and quality of life. To assess change in vital signs and cortisol saliva measures before and after testing. Lastly, compare participants to normal.*

Methods: *Participants attended 3 sessions within 2 weeks. Two trained examiners assessed the reliability of the two-minute walk test, (2MWT), five times sit to stand (5XSTS), grip strength on five participants. There were 10 participants, each completed the self-report, (HAQ-DI). 10 trained assessors evaluated between 1-10 of the participants for the stability of the scores between three trials. Vital signs and rate of perceived exertion (RPE) were assessed pre and post 2MWT; saliva samples were taken.*

Statistics: *For 5 subjects the ICC (2,3) was computed for intra and interrater reliability. For 10 subjects (ICC3,3) intertrial consistency was done. Paired t tests assessed grip strength, change after 2MWT for vital signs, changes in cortisol, and assess the HAQ-DI between administrations. Pearson correlations were completed on HAQ-DI variables, RPE, saliva, and vital signs.*

Results: *All intra and interrater reliability, intertrial agreement was found to be good to excellent. Paired t tests indicated that the HAQ-DI was stable and indicated a moderate perception of pain and reductions in quality of life. These participants showed evidence of impairments relative to normal. There were no differences in saliva cortisol levels.*

Conclusion: *These tests appear to have utility in identification of impairments in bodily systems, activity levels and participation in these participants. Further investigation is necessary in order to establish the optimal management.*

Introduction

Hypermobile Ehlers Danlos Syndrome (hEDS) has common characteristics that include multiple subluxations and dislocations that occur spontaneously or with minimal trauma [1]. Hypermobility Spectrum Disorder (HSD) consists of multiple hypermobile joints that can move beyond normal range of motion, with fewer systemic manifestations compared to hEDS. With further investigation, we are learning that the manifestions of these disorders are not limited to the musculoskeletal system. Recently, DiFrancisco- Donoghue et al. found that 100 % of their hEDs/HSD participants had GI symptoms [2]. The most recent classification of EDS, of which hEDS and HSD are included, was revised by the international EDS consortium in 2017 and published by Malfait et al., which defines the syndrome as heterogeneous groups of heritable connective tissue disorders (HCTDs) characterized by joint hypermobility, skin hyperextensibility, and tissue fragility [3]. There have been 13 EDS subtypes established with a wide range of symptoms that overlap among subtypes and with other connective tissue disorders. The syndrome is estimated to affect about 1 in 5,000 people worldwide [1]. Genetically, EDS results from defects in genes involved in collagen biosynthesis or structure. However, at present there has been no genetic defect identified in hEDS or HSD.

Tissue fragility can be seen with poor wound healing, dystrophic scars, easy bruising and in severe cases present with gastrointestinal bleeding or intracranial bleeding [1]. The Beighton scale score is used to determine hypermobility, a score of 5/9 or higher being positive for patients pubertal age to 50 years old, 6 or more for prepubertal and 4 or higher >50 years old. Skin is considered hyperextensible when the skin can be stretched excessively in at least 3 of the following locations: distal forearms, dorsum of hands, neck, elbows, or knees [1].

EDS is considered a rare condition, but as more knowledge of EDS increases, clinicians have agreed that it is underdiagnosed. Symptoms can vary from a mild presentation to a severe and life-threatening presentation. Diagnosing EDS is considered complex due to a lack of comprehensive education on the diagnosis and management of EDS. A diagnosis of hEDS, unlike other subtypes of EDS, must be diagnosed solely on clinical observations included in all three major criteria because the genetic bases are still unknown [1]. This study included hEDS as well as HSD since the two are often clinically indistinguishable from each other according to an international panel of experts [1]. The purpose of this study was to assess the appropriateness of several standardized outcome tools, on persons having hEDS or HSD.

A collection of outcomes tools is important for clinicians to manage people with these diseases effectively, and accurately measure changes in their condition. In addition to hypermobility, people with HSD/hEDS the most common presentations include a limited number of painful and/or unstable joints and or chronic widespread musculoskeletal pain. The musculoskeletal pain may be due to frequent joint dislocations, subluxations, and joint hypermobility. Patients commonly complain of recurrent subluxations or dislocations of the shoulder, knee, and ankle [4]. Associated impairments and their severity can vary markedly and are not necessarily associated with the degree of joint laxity. These musculoskeletal complications can often lead to physical inactivity and muscle atrophy as well. In addition, higher rates of anxiety and depression have been noted in HSD/hEDS [5,6].

According to Celletti et al., distress, fear of movement and an individuals' coping strategies and behavioral responses to the disease are more likely to predict impairment and quality of life (QoL) rather than intensity of pain [7]. Studies published on HSD/ hEDs indicate that there are no validated outcomes assessment tools that are used for these syndromes. Furthermore, those studies have relied on outcomes assessments that have been used with other diagnoses. The present state is that there is no tool that has been tested specifically for reliability or the ability to measure change for persons with HSD/hEDS. In addition, validating a core set of accurate reliable measurement will enable clinicians to monitor progression and manage this disease with greater efficacy [8]. This study is essential to the formation of a platform upon which other researchers can conduct interventional studies to improve management in this population and to establish evidence-based treatment regimes.

The aim of this study was:
- To identify and assess functional outcomes, HAQ-DI that will be reliable in the management of persons with hEDs /HSD.
- Assess the stress associated with testing for this group utilizing salivary cortisol measurement and vital signs.
- Assess how this group of participant's performance test results align with healthy populations.

Methods

The recruitment for our IRB approved research included 10 subjects from the NYIT-COM clinic and EDS support group. The study was interrupted for over 12 months due to COVID. Originally, after reliability was established, the same 4 examiners were going to complete the testing. All examiners went through the same training process and demonstrated proficiency and fidelity to each outcome before joining the study. Due to the delay, there was a turnover in examiners so that instead of 4 there were 8. In addition to the 2 researchers that did the intra and inter-rater reliability. Post Covid restrictions reduced the number of researchers able to be in the clinic and in the room with each single participant. This resulted in two sets of statistical measures for the ICC, one for intrarater/ interrater and a larger number for intertrial reliability.

The participants were all Caucasian women aging from 22-49 years old, mean age 30.6 SD 8.35. The minimum Beighton scale score was 4 and highest was 9. Seven subjects had hEDS and 3 had HSD. The subjects participated in 3 trials of 3 testing outcome measures, a self-report questionnaire pre and post and saliva

testing last visit.

To be included in the study, subjects had to meet the criteria from the international consortium on Ehlers Danlos Syndromes and related disorders from hEDS; the HSD participants had to have a Beighton score greater than or equal to 4. Subjects needed to be willing to come in for 3 sessions to be measured at the clinic at about 1.5-hour sessions one and 45 minutes for sessions 2 and 3, have ability to walk independently with or without a device for 2 minutes, and be responsible for transportation to and from clinic.

Subjects were excluded if they failed to meet all the criteria for hEDS/HSD, unable to meet the requirements for the tests performed, inability to meet the demands of clinic visits, and those with impaired cognition since this will affect the ability to follow instructions during testing.

The outcome measures were administered in a structured manner and time was permitted between tests to ensure that the participants were not fatigued or in pain.

Clinical Outcome Assessment Measures
2MWT (Two Minute Walk Test)
Walking tests are a safe, easy, and inexpensive measurement tools. The 2MWT measures the distance walked and assesses perceived exertion (RPE), the benefit of a shorter and perhaps less taxing outcome for functional endurance/capacity when compared to the 6MWT. This provided information at the activity level according to the WHO ICF. Test-retest reliability of the 2MWT has been reported having an intraclass correlation coefficient that was .82 (95% confidence interval). Based on a standard error of measurement of 15.3m, the minimum detectable change for the 2MWT is 42.5m [9].

The 2MWT is a valid measure of functional endurance in persons with pain, fatigability [10] or cases where there are limitations in space for testing [11]. We performed the 2MWT over a 50' measured course, as described by Anderson. [12]. Participants were instructed to walk as fast as possible, until asked to stop. They were told not to worry if they had to slow down or rest, but that if they stop, they should start walking again as soon as they felt ready to do so. When 1 minute was elapsed, they were told, "You are doing well; you have 1 minute left." Participants stopped walking and sat immediately at 2 minutes; vital signs and the distance completed were then documented.

5XSTS (Five Times Sit to Stand)
The second outcome measure was the five times sit to stand, this was used to assess functional lower extremity strength, transitional movements balance and fall risks. A test with a time of greater than 15 seconds increases the risk for recurrent falls, slow gait speed, and deficits in other ADLs [13].

The 5XSTS has been used as a performance tool to capture LE strength. The test involves standing up from a sitting position 5X as quickly as possible with the arms folded across the chest.

The time is recorded. This is a reliable and valid clinical tool frequently used to assess LE strength and functional mobility. This tool requires little space and training [14]. Makizako found cutoff for development of disability with this tool to be >/= 10 sec in a healthy community dwelling population (mean age 71) [15]. Goldberg et al. found when assessing absolute and relative reliability between the 5XSTS and TUG, that there was excellent relative reliability, SEM was .9 sec and MDC 95 was 2.5 sec for 5XSTS [16]. Normative values, reliability and validity have been established [17].

Handgrip strength
The third outcome measure was grip strength, used to measure the amount of peak force generated by the subjects and can be correlated to overall body strength [18]. Grip strength was measured using a handheld dynamometer. The procedure is widely employed, not just as an indicator of grip strength itself, but as an indicator of overall strength as well. Grip strength has been described as a vital sign [19]. Strength measures provide measurable details at the bodily systems level of our participants. Handgrip strength tested with a dynameter has many applications for the outcomes of people as they age. Weak grip strength has predictive validity for numerous untoward outcomes, including mortality, postoperative complications, hospital length of stay, discharge disposition, hospital readmission, fractures, and physical functioning [20].

Participants elbows were positioned at 90° of flexion with neutral hand grip. Our participants did 3 trials of grip strength beginning with the dominant hand, with 1 minute rest before the next trial on the same hand. Subjects were encouraged during the test with the words, "squeeze, squeeze, squeeze" each time for consistency. The rest period between trials decreases the chance of fatigue although current research displays no significant difference between 15s, 30s and 60s, the decline in strength was lower in the 60 second measure [20]. A rest period of 1 minute was to address fatigue which is common among our subject population.

The test-retest reliability of grip is well-established. Bohannon systematically reviewed the topic and found that for older adults the reliability coefficient ranged from 0.41 to 1.00, but that in more than 90% of the studies the coefficient was at least 0.80 [21]. The responsiveness of handgrip strength measured with a dynamometer has been described using the minimal detectable change and minimal clinically important difference. Values for minimal detectable change from 3 different diagnostic groups range from 2.7 to 5.2kg [22]. Grip strength norms are typically presented as summary statistics for by gender, side, and age-group but may also be presented using regression equations. We assessed peak dominant grip strength and compared dominant to nondominant peak grip strength.

Health Assessment Questionnaire-Disability Index (HAQ-DI)
The Health Assessment Questionnaire- Disability Index is a well validated outcome measure as it is sensitive to change and predictive of long-term outcome [23,24]. The HAQ-DI is a generic self-report

that asks questions related to the participants appreciation of the effects of hEDs/HSD on their lives. Our participants completed the questionnaire the first and last visits. Higher scores on a scale of 0-100 are indicative of greater impairments perceived by the participant.

In terms of quality of life, or participation, the HAQ-DI is a valid measure [25]. Although this tool was developed for persons with Rheumatoid arthritis [26] it was selected because it has been used with our population [27]. The 2-page shortened version, which includes the visual analog pain scale, and VAS global health scale was used. The HAQ-DI has face and content validity, construct validity, convergent and predictive validity, and sensitivity to change [25]. The two areas highlighted in this study were quality of life and pain.

Cortisol levels

Cortisol levels rise independently of circadian rhythm in response to stress. We assessed for changes in the level of cortisol in response to the stress of continued effort over 2 minutes. Saliva was collected immediately before the 2MWT and then after vitals are taken upon completion of the 2MWT on the last session. These deidentified samples, having different colored labels for before and after, were frozen at until shipped to University of California, Irvine Institute for Interdisciplinary Salivary Bioscience Research for analysis.

Persons with HSD/hEDS are subject to fatigue, anxiety, and depression. Administering this on the third visit would eliminate much of the anxiety associated with an unknown task. We hypothesized, that by this time the participants were be familiar with the testing protocol, thus reducing performance stress. Transfer of cortisol from blood to saliva takes place in 2-3 minutes [28]. Therefore, our results should have reflected any change in physical stress from the 2MWT. We assessed consistency measures between the saliva test and the HAQ-DI in the pain and quality of life domains. Likewise, the RPE measure obtained pre and post 2MWT from was compared these responses. This assisted in assessing the calibration between perceived effort/fatigue and changes is cortisol levels. The saliva biomarker enabled us to address two of our aims related to stress from having HSD/hEDS related to 2 minutes of activity, and also if the stress levels were reflected in any of the self-reported items, lastly, allowed the assessment of consistency between cortisol levels and perceived performance exertion.

All of our performance outcome measures are in the NIH toolbox. The completion of each test was recorded by the researchers at the time of testing.

Results

Our ten subjects completed the study with no adverse outcomes. Each subject completed the 2MWT, 5XSTS, and grip strength testing during each trial. The subjects also received the HAQ-DI which was used to assess pain, and VAS global health (QoL) scale

during their first and last trial. The saliva was tested for cortisol levels the last visit before and after the 2MWT.

Table 1 demonstrates the intra-rater and interrater reliability, of the performance tests mean, SD, ICC (2,3), that was determined by two trained researchers when applied to 5 of the participants. COVID precautions forced the modifications as earlier introduced. All of the performance assessments demonstrated good to excellent reliability.

Table 1: Performance tests, intra-rater reliability, Interrater reliability.

Outcome	N	Mean/ SD Chronbach Rater 1		Mean /SD Rater 2	Chronbach	Interrater-Reliabilty ICC (2,3)
2MWT	5	190/37.33	.913	198.40/29.63	.813	.954
STS	5	11.27/4.34	.969	9.55/2.54	.892	.886
DGS	5	58.81/13.35	.911	64.41/10.22	.800	.900
NDGS	5	53.74/14.08	.798	57.18/10.40	.811	.726

Table 2 demonstrates the intertrial consistency of the 10 participants including the results of the performance tests mean, SD, ICC (3,3). These results were obtained by 10 researchers on the full complement of 10 participants. Each participant had 2 or more (when restrictions were reduced) researchers administering and documenting each trial simultaneously.

Table 2: Intertrial reliability.

Outcome	N	Mean	SD	Icc (3,3) Intertrial Reliability
2MWT	10	203.55	31.15	.989
STS	10	10.44	3.75	.956
DGS	10	47.32	17.15	.957
NDGS	10	48.56	14.33	.905

The 2MWT distances were in keeping with women in their age group. For the 5XSTS, our subjects performed below the norm, when compared to healthy controls. There were no significant differences in grip strength nondominant vs dominant, 48.56# ND, 47.32# dominant t=-.093, p=.926.

Descriptive combined scores for all the variables included in the paired t tests can be found in table 4. Paired Samples tests can be viewed in table 5. There were no significant differences noted in pre and post 2MWT heart rate and saliva cortisol levels. However, there were differences in pre to post RPE, Systolic BP. In addition, concerning perceived activity limitations, HAQ-DI are depicted, which demonstrate non-significance between the 2 administrations.

Pearson correlations were computed on the post values RPE, HR, SBP, Saliva, HAQ pain, HAQ QOL; there was a strong correlation between HAQ2 pain and QOL2. r= .780, p.008. In addition, there was also a strong correlation between RPE after 2MWT and post 2MWT Systolic BP r = .849, p. 000. There were no significant correlations between HR and saliva samples pre and post 2MWT with pain, QOL, RPE, or Systolic BP.

Table 3: Combined pre and post scores on paired variables.

Descriptive Statistics			
	Mean	Std. Deviation	N
HAQQOL1	69.0000	13.90444	10
HAQQOL2	60.5000	17.03754	10
HAQPAIN1	68.5000	12.03005	10
HAQpain2	57.5000	20.17286	10
PRERPEALL	9.0833	3.17543	10
POSTRPEALL	13.2917	1.63009	10
COM sys pre	119.0000	13.60481	10
Com Sys post	131.5000	21.91098	10
hr1	83.2000	11.97961	10
hr2	81.7000	6.56675	10
Saliva1	.2785	.23787	10
Saliva2	.2079	.11212	10

Table 4: Combined performance outcomes.

Descriptive Statistics					
	N	Minimum	Maximum	Mean	Std. Deviation
Combined 2MWT	30	136.26	252.80	203.31	31.15
Combined 5XSTS	30	5.52	25.25	10.4440	3.75277
Combined DGS	30	18.00	78.00	47.3200	17.15852
Combined NDGS	30	25.70	78.00	48.5633	14.33312
Valid N (listwise)	30				

Table 5: Paired samples on pre and post variables.

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	PRERPEALL - POSTRPEALL	-4.20833	2.57133	.74228	-5.84208	-2.57459	-5.669	11	.000**
Pair 2	COMsysV1pre - ComSysV2post	-12.50000	14.29876	4.12770	-21.58500	-3.41500	-3.028	11	.011*
Pair 3	hr1 - hr2	1.50000	9.69822	3.06685	-5.43769	8.43769	.489	9	.636
Pair 4	Saliva1 - Saliva2	.07060	.16839	.05325	-.04986	.19106	1.326	9	.218
Pair 5	HAQQOL2 - HAQQOL1	-6.50000	19.86762	6.28269	-20.71244	7.71244	-1.035	9	.328
Pair 6	HAQpain2 - HAQPAIN1	-9.00000	17.60682	5.56776	-21.59516	3.59516	-1.616	9	.140

** Significant at .001; *Significant at .05

We need a toolbox from which to accurately measure these constructs. Having a toolbox and assessment set such as this will enable clinicians to manage hEDS more efficaciously both medically and economically. This study shows that the selected outcome tests and measures provide reliable tools for this toolbox.

Discussion

This pilot study utilized 10 participants, all female, and who had confirmed diagnosis of hEDS or HSD. Three of the subjects were confirmed to have a diagnosis of HSD, while the rest of the subjects had a confirmed diagnosis of hEDS. The reliability of the 2MWT, 5XSTS, Grip strength, cortisol levels in response to testing, and HAQ-DI were assessed.

These results indicate that the assessments/ outcomes selected are reliable and stable over the two-week study period with multiple examiners in this group.

The 2MWT is a reliable endurance test for patients. The normal values of the 2-minute walk test for females from the ages 20-

29, 30-39, and 40-49, are 193.24m, 181.36m, and 180.75m, respectively [9]. Results from our data (Table 1) demonstrate that most of our subjects scored around the normal values of the 2 minute walk test with 2-3 subjects scoring above average results.[10] However, each subject demonstrated an significantly elevated RPE score of 4+ points from the baseline after performing the 2MWT. According to the BORG RPE 6-20 scale mean increase of 4 points on the scale should be equivalent to 40 beats per minute of the heart. In addition, there was a significant increase in Systolic blood pressure (11.5mmHg), but not exertion. This increase in RPE may be indicative of muscle fatigue felt by the participants rather than an increased heart rate. In fact, Englebert et al had that fatigue has been recognized as the most disabling complaint and these findings may suggest that fatigue plays a large part in their performance[8]. This would also align with the 5XSTS scores.

The 5XSTS is a reliable measure of lower extremity strength. Additionally, the 5XSTS is affected by motor control, balance, and sensation. Individuals with HSD/hEDS present with joint

instability and pain, decreased proprioception, and decreased strength, leading to increased time to complete the 5XSTS [27].

The normal values of the 5XSTS for females from the ages 20-29, 30-39, 40-49 was 6 seconds, 6.1, 7.6 seconds respectively [17]. Results from our data demonstrate that all of our subjects required more time, with the mean score being 10.44 seconds [14]. In a study from Bohannan et al, the mean time for the 5XSTS for the age group of 80-85 years old was 10.8 seconds [17]. Our participants were not engaged in any outside physical activity or sport due to musculoskeletal issues mostly. Since 5XSTS is a measure of lower extremity strength, these values appear to identify the present physical condition of these impaired young adults.

In terms of reliability concerning grip strength, one of the subjects had wrist symptoms that developed between trials and resulted in intertrial differences in response. Overall, in all of the performance tests, there is more variance in grip strength. In terms of grip strength, the normative values for grip strength of 20-29-, 30-39- and 40–49-year-old was 66, 68 and 63 pounds respectively [9]. The participants in this study had a mean dominant grip strength of 47.32 pounds which was approximately 10 pounds less than the norm. Shiratori et al, states that the dominant hand has a 10% increase in strength over nondominant [20]. Non-Dominant grip strength norms for the ages 20-29, 30-39, 40–49-year-old are 62, 64, 62 pounds respectively [29]. The mean non dominant grip strength in this study was 48.56 pounds. Our group did not demonstrate those differences between the dominant and nondominant grip strength. The dominant grip strength was less that non dominant.

These reductions in grip strength, also recognized as vital sign, are indicative of reductions in strength, endurance, and functional capabilities. Decrease in grip strength may suggest generalized decline in strength and overall health [30,31]. This is a significant finding based on the value of these reliable grip strength measures in terms of general health and wellness.

The HAQ-DI remained stable in the domains of pain and quality of life over the two-week trial. There were no differences noted on paired t tests. Table 3 includes the descriptive statistics for all of the combined pairs. Considering the scales used for Qol and Pain are from 0 (best) – (worst) 100% the participants indicated from questionnaire one to questionnaire two, the significant impact of hEDS-HSD on their pain and quality of life.

This study demonstrates the need for continued assessment. A larger clinical trial with is warranted with greater ethnic and gender diversity, to see if these results can be repeated and improve the confidence in these tools. Based on our findings, these tools are appropriate and stable over two-week periods for this group of persons with hEDS and HSD.

This is among the first studies to measure the effects of the outcomes on the population. Given that hEDS/HSD leads to pain, weakness, and balance issues in patients [27] it is evident that grip strength, HAQ-DI, and the 5 times sit to stand would indicate below average results compared to the norms based on age and gender in these participants.

Conclusion

This study sets a platform for a combination of outcomes, that appear to accurately and reliability measure the bodily systems, activity, and participation levels in persons with hEDs and HSD. In addition, it is important to note that this disease is present throughout the lifespan. The investigation of outcomes tools such as these performance outcomes that cross the lifespan will enable future valid longitudinal study. Trials such as this will develop a means of validly quantifying change. Without validated tools to measure accurately, study results are suspect.

Further investigation is necessary in order to establish the best possible management for hEDS/HSD and other rare diseases.

References

1. Miller E, Grosel JM. A review of Ehlers-Danlos syndrome. JAAPA. 2020; 33: 23-28.

2. DiFrancisco-Donoghue J, Southard V, Margulies M, et al. Assessment of gastrointestinal function and its' effect on bone mineral density and body composition in hypermobility spectrum disorder and hypermobile Ehlers-Danlos syndrome. J Clin Densitom. 2022; 28: S1094-6950(22)00058-0.

3. Malfait F, Francomano C, Byers P, et al. The 2017 international classification of the Ehlers-Danlos syndromes. Am J Med Genet C Semin Med Genet. 2017; 175: 8-26

4. Tobias JH, DeereK, PalmerS, et al. Joint hypermobility isa risk factor for musculoskeletal pain during adolescence. Findings of a prospective cohort study. Arthrit Rheumat. 2013; 65:1107-1115.

5. Lumley MA, Jordan M, RubensteinR, et al. Psychosocial functioning in the Ehlers-Danlos syndrome. 1994; 53: 149-152.

6. Mallorqui-Bague N, Bulbena A, Roe-Vellve N, et al. Emotion processing in joint hyper- mobility: A potential link to the neural bases of anxiety and related somatic symptoms in collagen anomalies. Eur Psychiatry. 2015; 30: 454- 458.

7. Celletti C, Castori M, La Torre G, et al. Evaluation of kinesiophobia and its correlations with pain and fatigue in Hypermobility Spectrum Disorder/Ehlers-Danlos syndrome hypermobility type. Biomed Res Int. 2013; 2013: 580460.

8. Engelbert RH, Juul-Kristensen B, Pacey V, et al. The evidence-based rationale for physical therapy treatment of children, adolescents, and adults diagnosed with Hypermobility Spectrum Disorder/hypermobile Ehlers Danlos syndrome. Am J Med Genet Part C Semin Med Genet 2017; 175C: 158-167.

9. Bohannon RW. Normative references values for the two-minute walk derived by meta-analysis. Journal of Physical Therapy Science.2017; 12: 2224-2227.

10. Scalzitti D, Harwood K, Maring J et al. Validation of the 2-Minute Walk Test with the 6-Minute Walk Test and other Functional measures in person with multiple sclerosis. Int J MS Care. 2018; 20: 158-163.

11. Bohannon RW, Wang YC, Gershon RC. 2015 Two-Minute Walk Test (2MWT) Performance by Adults 18-85 Years: Normative Values, Reliability and Responsiveness. Arch Phys Med Rehabil. 96: 472-477.

12. Anderson L The 2MWT is a potential alternative to the 6MWT to describe walking capability among patients with neuromuscular diseases during clinical trials, 2016 Neurology. 2; 86: 442-445.

13. Duncan R P. Leddy A L. Earhart G M. Five Times Sit to Stand Test Performance in Parkinson Disease. Archives of Physical and Medical Rehabilitation. 2011; 92: 1431-1436.

14. Bohannon RW, Bubela DJ,Magasi SR, et al. Sit-to-stand test: Performance and determinants across the age-span. Isokinetics and exercise science.

15. Makizako H, Shimada H, Doi T, et al. Predictive Cutoff Values of the Five-Times Sit-to-Stand Test and the Timed "Up & Go" Test for Disability Incidence in Older People Dwelling in the Community. Phys Ther. 2017; 1: 417-424.

16. Goldberg A, Chavis M, Watkins J, et al. The five-times-sit-to-stand test: validity, reliability, and detectable change in older females. Aging Clin Exp Res. 2012; 24: 339-344.

17. Bohannon RW. Reference values for the five-repetition sit-to-stand test: a descriptive metaanalysis of data from elders. Percept Mot Skills 2006; 103: 215-222.

18. Bohannon RW. Hand-grip dynamometry predicts future outcomes in aging adults. J Geriatr Phys Ther. 2008; 31: 3-10.

19. Cheung CL, Nguyen US, Au E, et al. Association of handgrip strength with chronic diseases and multimorbidity; a cross-sectional study. Age (Dordr) 2013; 35: 929-941

20. Shiratori AP, Iop Rda R, Borges Júnior NG, et al. Evaluation protocols of hand grip strength in individuals with rheumatoid arthritis: a systematic review. Rev Bras Reumatol. 2014; 54: 140-147.

21. Bohannon RW. Test-Retest Reliability of Measurements of Hand-Grip Strength Obtained by Dynamometry from Older Adults: A Systematic Review of Research in the PubMed Database. J Frailty Aging. 2017; 6: 83-87.

22. Bohannon RW. Minimal clinically important difference for grip strength: a systematic review. J Phys Ther Sci. 2019; 31: 75-78.

23. Greenwood M. Does the Stanford Health Assessment Questionnaire have potential as a monitoring tool for subjects with rheumatoid arthritis? Ann Rheum Dis. 2001; 60: 344-348.

24. Health Assessment Questionnaire: Health Assessment Questionnaire (HAQ)_Prof%20Fries_Stanford.pdf

25. Bruce B. Fries J. The Stanford Health Assessment Questionnaire: Dimensions and Practical Applications. Health and Quality of Life Outcomes. 2003; 1: 20: 1-6.

26. Frederick Wolfe. The determination and measurement of functional disability in rheumatoid arthritis. Arthritis Res 2002, 4 (suppl 2): S11-S15

27. Mark Scheper, Lies Rombaut, Janneke de Vries, et al. The association between muscle strength and activity limitations in patients with the hypermobility type of Ehlers–Danlos syndrome: the impact of proprioception, Disability and Rehabilitation 39; 14: 1391-1397.

28. Bozovic D, Racic M, Ivkovic N. Salivary cortisol levels as a biological marker of stress reaction. Med Arch. 2013; 67: 374-377.

29. Simmonds MJ, Olson SL, Jones S, et al. Psychometric Characteristics and Clinical Usefulness of Physical Performance Tests in Patients With Low Back Pain. Spine. 1998; 23: 2412-2421.

30. Massy-Westropp NM, Gill TK, Taylor AW, et al. Hand Grip Strength: age and gender stratified normative data in a population-based study. BMC Res Notes. 2011; 4:127.

31. Wind, AE, Takken T, Helders, PJ, et al. Is grip strength a predictor for total muscle strength in healthy children, adolescents, and young adults? European journal of pediatrics. 2010: 169: 281-287.