Towards Cardiac Risk Monitoring of Duchene Muscular Dystrophy using Lyfas

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Abstract: Duchene Muscular Dystrophy (DMD) is a progressive neuromuscular disorder affecting male children of a career mother, as seen in more than 70% of cases. It is an X-linked recessive disease that affects 1 in 3600-6000 live male births. As the child grows, muscles are progressively wasted and degenerated in the body. As time passes, it also involves intercostal and heart muscles leading to cardiorespiratory failure and death. Using a smartphone-based application namely, Lyfas that uses the principles of the optical sensor, arterial photoplethysmography, and photochromatography, this report focuses on capturing digital cardiovascular biomarkers at the backdrop of DMD and understanding the physiological aspect of the disorder and its progression, especially the risk of the involvement of cardiac muscles.

Keywords: Duchene muscular dystrophy, Digital biomarkers, Time-series study, Lyfas.

KEY MESSAGE

Monitoring the initiation and extent of involvement of the cardiac muscles is crucial to DMD cases as eventually, patients die due to cardiac failure. A non-invasive and ubiquitous way of monitoring Heart Rate Variability (HRV) and its correlates by studying the Pulse Rate Variability (PRV) from the capillary arteries of the index fingers may be useful in evaluating the myocardial involvement as the illness progresses as the prognostic measure, which is the principal focus of this case study.

INTRODUCTION

DMD is a rare neuromuscular genetic disorder where the skeletal muscle protein, namely dystrophin, is mutated causing 'weak sarcolemma' leading to the wasting of these muscles progressively [1] in about 0.8 million male children in India [2]. Across the world, 1 in 3500 male births is found to be vulnerable to express the disease mostly between 3-6 years of age [3]. Physiotherapy and glucocorticoids remain still the mainstay of the treatment [1].

Mobile health, in short mHealth, is gaining popularity as the extension of the current digital healthcare or eHealth by virtue of the (i) increasing number of smartphone users, (ii) widening network coverage, and (iii) powerful cameras with in-built optical sensors with LED light, which offers the 'reflectant' light source for photoplethysmography (study of blood volume changes) and photochromatography (study of cellular substances and solutes in the blood). PRV surrogates for the Heart Rate Variability (HRV), which reflects Cardiac Autonomic Modulation (CAM) in explaining the mind-body homeostasis. These simple, low-cost, non-invasive, and ubiquitous techniques form the foundation of mHealth.

CASE HISTORY

The report is about a 12-year old Indian boy with 127 cm of height and 28 kgs of weight (BMI: 17.4) who presented with a sudden loss of power to lift his arms in 2018. On clinical examination, bilateral facial palsy, high arch palet, dropping of shoulders, slurred speech, neuromuscular lordosis with hip flexion-contracture, waddling gait, and positive Gower's sign are found. The child was investigated for DMD PCR (30-05-2018), in which 18 exomes were detected but could not rule out exome deletion as it generally happens in the case of DMD. CPK level was tested and found 3489 IU/L (30-04-2018). No abnormalities were detected in MRI of the brain and spine (04-06-2018).

A series of gene sequencing studies showed no deletion or duplication in any of the 79 exomes in the DMD gene (20-07-2018). Later, on 24-07-2018, clinical exome sequencing showed a heterozygous variant of uncertain significance in the TRAPPC11 variants (p.(Pro820Ilefs*5), exon 22), which proves that the patient was a carrier of DMD.

A nerve conduction test on 31-07-2018 showed motor axonopathy involving bilateral median and ulnar nerve affecting biceps, which was corroborating with the very first occurring symptom of the patient. CPK was re-studied on 02-08-21 and its level was 5482IU/L.

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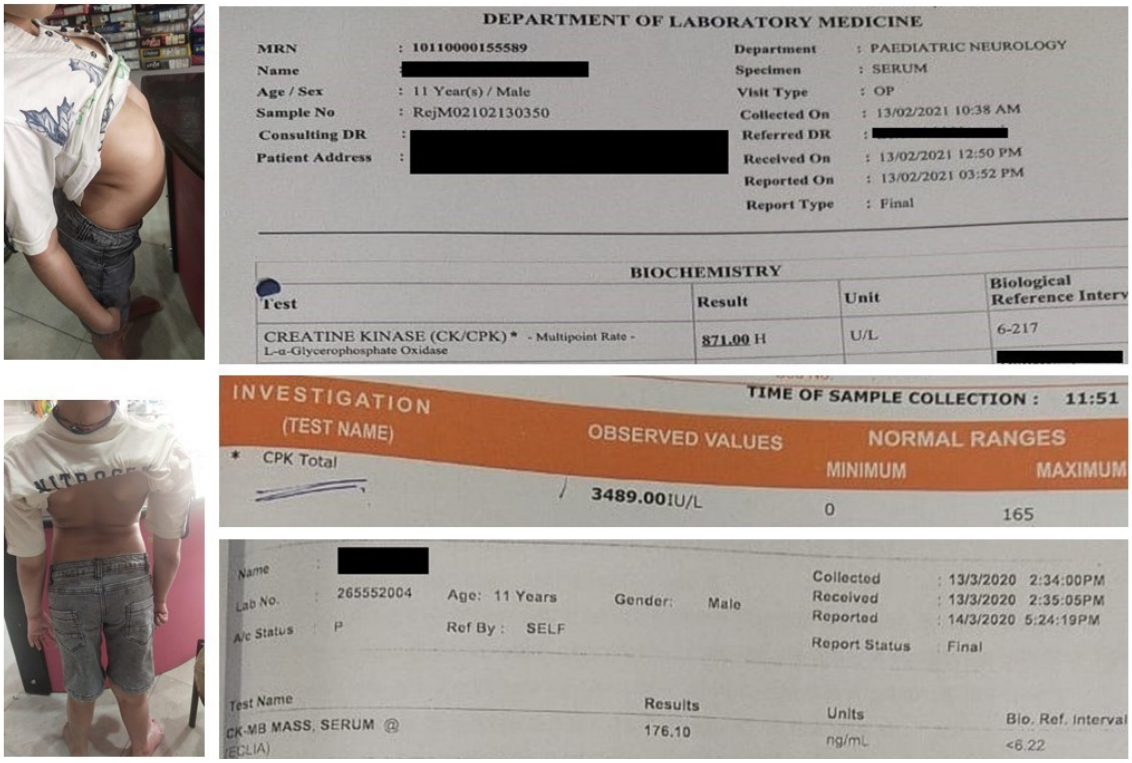


DEPARTMENT OF LABORATORY MEDICINE

MRN : 10110000155589
Name : [redacted]
Age / Sex : 11 Year(s) / Male
Sample No : RejM02102130350
Consulting DR :
Patient Address :

Department : PAEDIATRIC NEUROLOGY
Specimen : SERUM
Visit Type : OP
Collected On : 13/02/2021 10:38 AM
Referred DR : [redacted]
Received On : 13/02/2021 12:50 PM
Reported On : 13/02/2021 03:52 PM
Report Type : Final

BIOCHEMISTRY

Test	Result	Unit	Biological Reference Interv
CREATINE KINASE (CK/CPK) * - Multipoint Rate - L-α-Glycerophosphate Oxidase	871.00 H	U/L	6-217

INVESTIGATION | OBSERVED VALUES | TIME OF SAMPLE COLLECTION : 11:51 | NORMAL RANGES MINIMUM | MAXIMUM

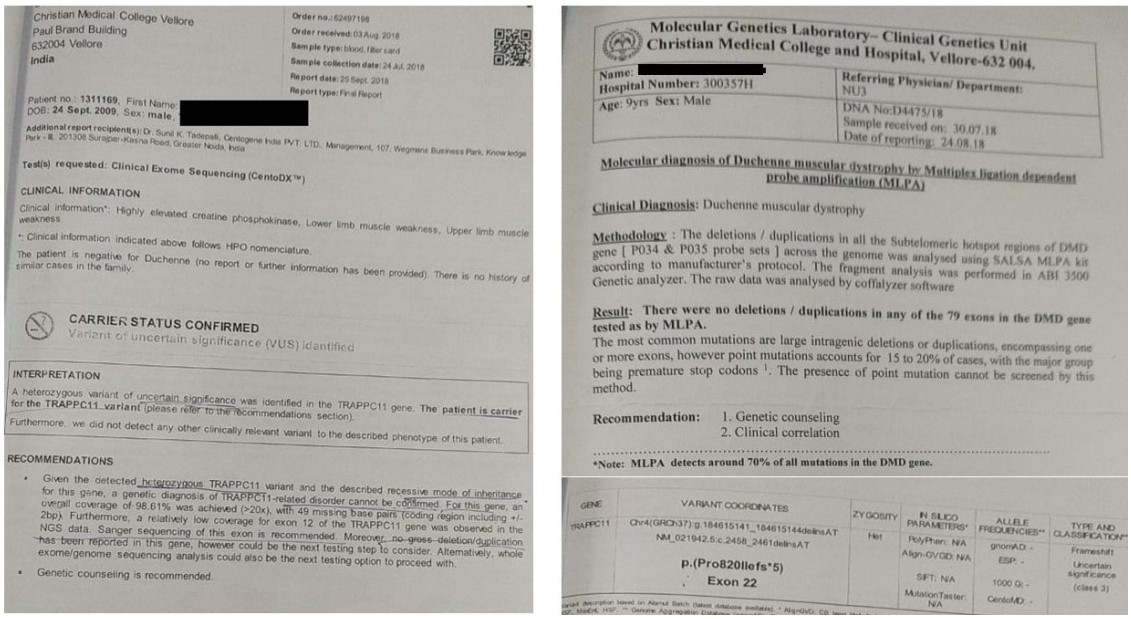
* CPK Total — 3489.00 IU/L — 0 — 165

Name : [redacted]
Lab No. : 265552004 Age: 11 Years Gender: Male
A/c Status : P Ref By : SELF

Collected : 13/3/2020 2:34:00PM
Received : 13/3/2020 2:35:05PM
Reported : 14/3/2020 5:24:19PM
Report Status : Final

Test Name	Results	Units	Bio. Ref. Interval
CK-MB MASS, SERUM @ (ECLIA)	176.10	ng/mL	<6.22

Figure 1: Morphology of the patient and the snapshots of the CK reports.



Christian Medical College Vellore
Paul Brand Building
632004 Vellore
India

Order no.: 62497198
Order received: 03 Aug. 2018
Sample type: blood, filter card
Sample collection date: 24 Jul. 2018
Report date: 25 Sept. 2018
Report type: Final Report

Patient no.: 1311169, First Name: [redacted]
DOB: 24 Sept. 2009, Sex: male,

Additional report recipient(s): Dr. Sunil K. Tadepalli, Centogene India P/T. LTD., Management, 107, Wegmans Business Park, Know ledge Park - II, 201308 Surajpur-Kasna Road, Greater Noida, India

Test(s) requested: Clinical Exome Sequencing (CentoDX™)

CLINICAL INFORMATION

Clinical information*: Highly elevated creatine phosphokinase, Lower limb muscle weakness, Upper limb muscle weakness

*: Clinical information indicated above follows HPO nomenclature.

The patient is negative for Duchenne (no report or further information has been provided). There is no history of similar cases in the family.

CARRIER STATUS CONFIRMED
Variant of uncertain significance (VUS) identified

INTERPRETATION

A heterozygous variant of uncertain significance was identified in the TRAPPC11 gene. The patient is carrier for the TRAPPC11 variant (please refer to the recommendations section).

Furthermore, we did not detect any other clinically relevant variant to the described phenotype of this patient.

RECOMMENDATIONS

- Given the detected heterozygous TRAPPC11 variant and the described recessive mode of inheritance for this gene, a genetic diagnosis of TRAPPC11-related disorder cannot be confirmed. For this gene, an overall coverage of 98.61% was achieved (>20x), with 49 missing base pairs (coding region including +/- 2bp). Furthermore, a relatively low coverage for exon 12 of the TRAPPC11 gene was observed in the NGS data. Sanger sequencing of this exon is recommended. Moreover, no gross deletion/duplication has been reported in this gene, however could be the next testing step to consider. Alternatively, whole exome/genome sequencing analysis could also be the next testing option to proceed with.
- Genetic counseling is recommended.

Molecular Genetics Laboratory– Clinical Genetics Unit
Christian Medical College and Hospital, Vellore-632 004.

Name: [redacted]
Hospital Number: 300357H
Age: 9yrs Sex: Male

Referring Physician/ Department: NU3
DNA No:D4475/18
Sample received on: 30.07.18
Date of reporting: 24.08.18

Molecular diagnosis of Duchenne muscular dystrophy by Multiplex ligation dependent probe amplification (MLPA)

Clinical Diagnosis: Duchenne muscular dystrophy

Methodology : The deletions / duplications in all the Subtelomeric hotspot regions of DMD gene [P034 & P035 probe sets] across the genome was analysed using SALSA MLPA kit according to manufacturer's protocol. The fragment analysis was performed in ABI 3500 Genetic analyzer. The raw data was analysed by coffalyzer software

Result: There were no deletions / duplications in any of the 79 exons in the DMD gene tested as by MLPA.
The most common mutations are large intragenic deletions or duplications, encompassing one or more exons, however point mutations accounts for 15 to 20% of cases, with the major group being premature stop codons [1]. The presence of point mutation cannot be screened by this method.

Recommendation: 1. Genetic counseling
2. Clinical correlation

*Note: MLPA detects around 70% of all mutations in the DMD gene.

GENE	VARIANT COORDINATES	ZYGOSITY	IN SILICO PARAMETERS*	ALLELE FREQUENCIES**	TYPE AND CLASSIFICATION***
TRAPPC11	Chr4(GRCh37):g.184615141_184615144delinsAT NM_021942.5:c.2458_2461delinsAT p.(Pro820Ilefs*5) Exon 22	Het	PolyPhen: N/A Align-GVGD: N/A SIFT: N/A MutationTaster: N/A	gnomAD: - ESP: - 1000 G: - CentoMD: -	Frameshift Uncertain significance (class 3)

Figure 2: The snapshots of the past genetic studies.

The patient had a high CPK-MB value (176ng/ml on 14/03/2021, while the normal value is less than 6.22), which is a biomarker for the involvement of the heart muscles. Meanwhile, the patient was advised to take calcium, multivitamins, occupational therapy, and physiotherapy.

Parents of the patient consulted Acculi Labs Pvt. Ltd. Bangalore for Lyfas testing (which is non-invasive and ubiquitous) to understand the underlying physiological state of the child, performed at the home of the patient from 24-08-2021 to 27-08-2021. Among many digital biomarkers, in this case, Heart Rate (HR), HR changes, SDNN, RMSSD, a 50 msec time-domain

measure of HR variability (pNN50), low vs high-frequency domain of HRV (LF/HF) are considered. Each of these biomarkers has been discussed concerning its clinical significance, in the following section. Figure **1**-**3** respectively shows the snapshots of the patient's morphology and CPK enzyme levels, results of the genetic studies, and the Lyfas biomarker scores. It is worth noting that we have shown the plots of only abnormal biomarkers.

Figure **1** shows the morphological photograph of the patient and the reports of CPK and CPK-MB. Snapshots of the genetic studies can be seen in Figure **2**, where Figure **3** shows the scores of the Lyfas cardiovascular biomarkers and its time-series plotting for the visualization.

DISCUSSION

DMD is an X-linked genetic disorder and caused by mutation of the DMD gene which is located on the short arm(p) of the X chromosome(Xp21.2) and the gene encodes for Dystrophin protein which regulates skeletal and cardiac muscles and is attached to the inner membrane of muscle fiber, so any mutation in the DMD gene leads to loss of dystrophin protein which further leads to degeneration of muscle fiber.

Studies suggest that mutation in the recessive TRAPPC11 gene causes a spectrum of diseases like Limb-Girdle muscular dystrophy, myopathy with a movement disorder, and intellectual disability. [4] The patient's report, in this study, shows the presence of the TRAPPC11 gene with variant c.2458_2461delinsAT p.(Pro820Ilefs*5) creates a frameshift at codon 820 which is suggestive of muscular dystrophy as seen in this case.

Creatine phosphokinase (CPK) or Creatine kinase is the enzyme that is used to supply ATP to tissue and cells of the brain, skeletal muscles, and the heart. Increased CPK levels are indicative of muscular damage as in this case of Duchenne muscular dystrophy. When the patient was first tested of CPK the value was 3489 IU/L on 30/04/2018 but reduced to 871 U/L on 13/02/2021 because the degeneration of muscle is more initially and later when there is a decline in muscle mass the CPK value is also low. There are 4 isoenzymes of CPK which is used to detect damage to a particular muscle. CK-MB is present in the cardiac muscles and can detect acute myocardial infarction [5]. CK-MB value of the patient is 176ng/ml (on 14/03/2021), which was very high and suggested the involvement of cardiac muscles [6].

Gower's sign is a classical feature of DMD that signifies weakness in the proximal hip muscles and patients, therefore, climb on their thighs while standing up from the sitting position and it can be seen when the disorder has advanced enough [7]. Genetic studies and

No	Date	Time (hr.)	HR	HR change	SDNN	RMSSD	pNN50	LF/HF	SD1/SD2
1	24-08-2021	21:24	94	44	53	56	25	1	4
2	25-08-2021	10:21	111	19	43	50	19	0.9	1.2
3	25-08-2021	14:09	102	19	38	49	20	1.6	1.4
4	25-08-2021	21:57	107	14	42	52	29	2.1	2.5
5	26-08-2021	10:32	97	15	47	56	23	2.3	1.8
6	26-08-2021	13:50	91	36	36	52	18	1.3	2.6
7	26-08-2021	19:14	96	17	17	62	16	1	1
8	27-08-2021	10:15	101	43	43	50	25	1.7	1.4
		AVERAGE	**99.9**	**25.9**	**39.9**	**53.4**	**21.88**	**1.49**	**1.99**
		SD	**6.73**	**12.86**	**10.62**	**4.37**	**4.36**	**0.53**	**1.00**
		Nomal_val	**79.6±14.4**	**10**	**50**	**44**	**23**	**1.3**	**0.25**

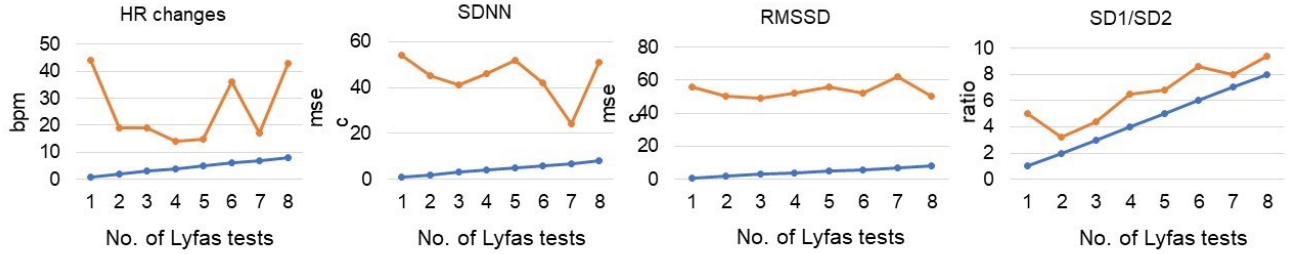


Figure 3: Snapshots of the digital cardiovascular biomarkers were obtained from a series of Lyfas tests.

Abbreviations mentioned in Figure **3** and its full forms:
HR, Heart Rate as bpm or beats per minute; **SDNN**, Standard deviation of N-N intervals (in mse or millisecond); **RMSSD**, Root Mean Square of successive R-R interval difference (in mse); **SD1/SD2**, the ratio of Poincaré plot standard deviation perpendicular to the line of identity).

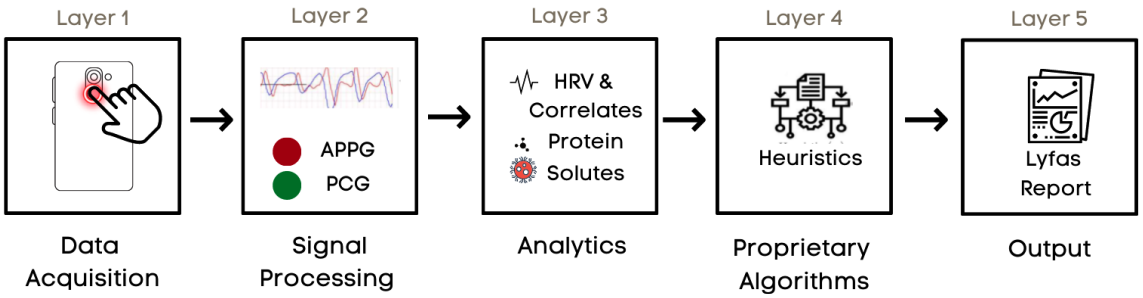


Figure 4: The working principle of Lyfas.

blood CPK levels (the biomarker of the destruction of muscle mass) are for the diagnosis of DMD [8]. It eventually becomes a life-threatening condition due to progressive weakening of the myocardium leading to death due to cardiorespiratory failure and digital biomarkers such as Heart Rate Variability (HRV) and its correlates can provide physiological clues to the grade of such deterioration and set an alarm to the neurologists, as there is a strong correlation between myocardial fibrosis and diminished HRV [9].

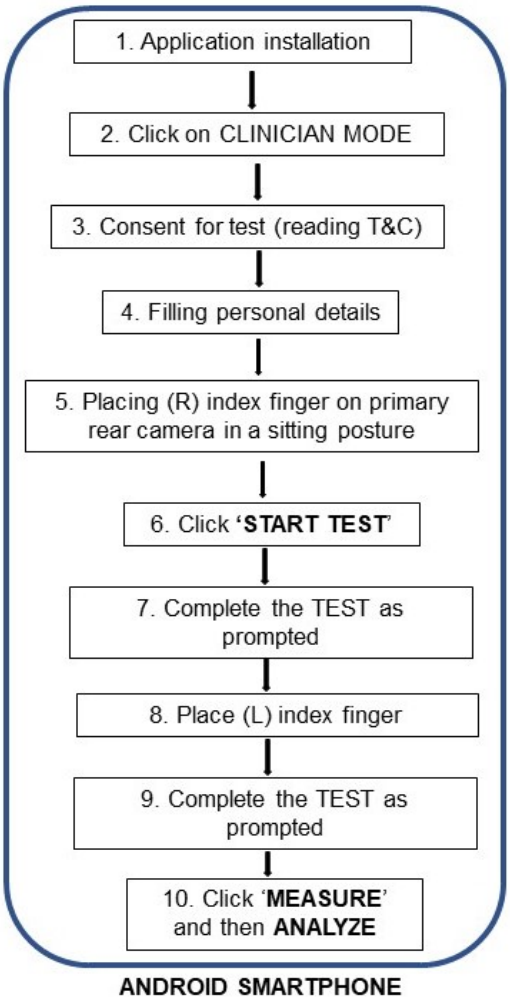
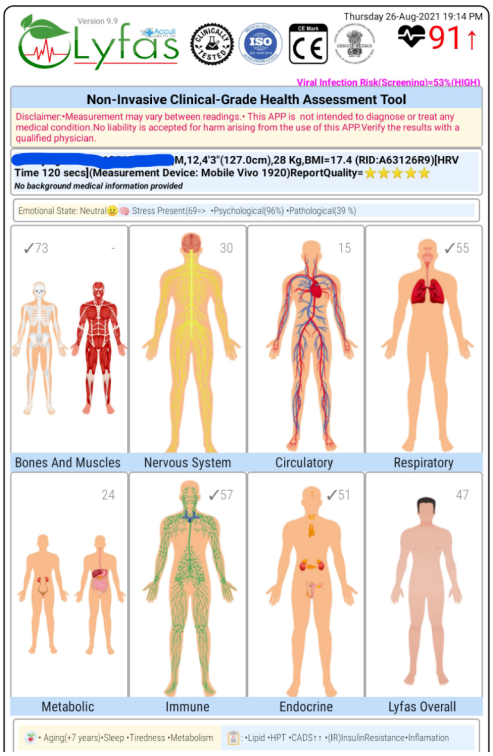


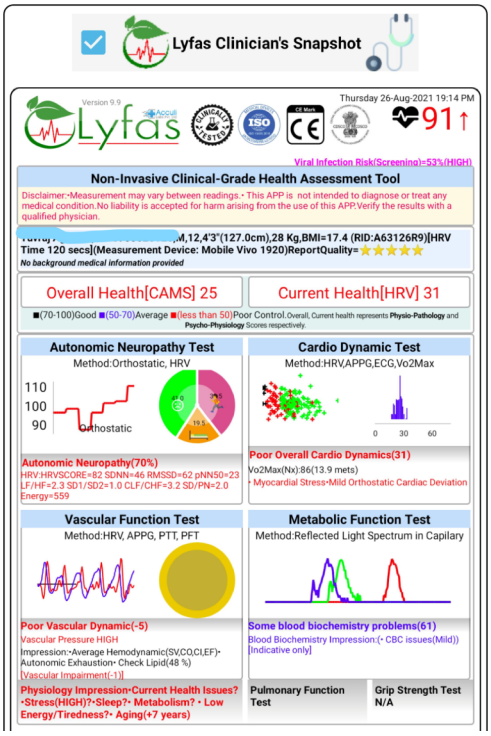
Figure 5: Lyfas test procedure.

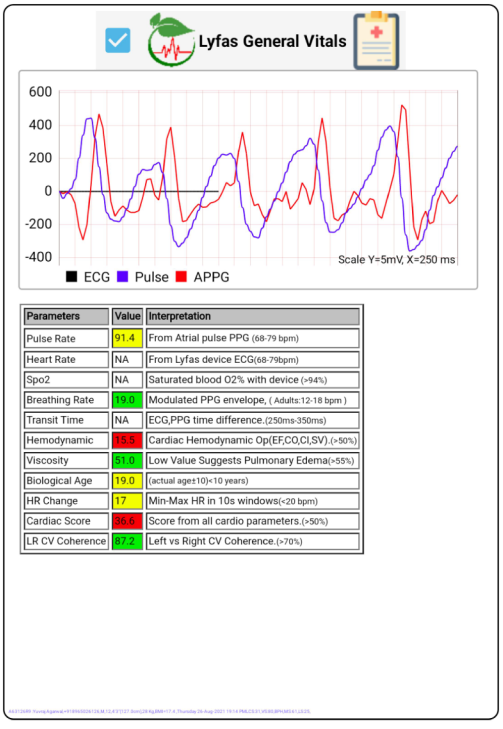
Lyfas is a commercially available smartphone-based non-invasive ubiquitous biomedical application with theISO certification number: 13485:2016 and CE certification number: 29695, dated 01/11/2019) [10]. It can capture several digital biomarkers by studying the capillary blood flow of the index fingers [11]. Using the principles of reflectant Arterial Photoplethysmography it can capture the Pulse Rate Variability (PRV) that surrogates for the HRV and its correlates, which can be classified into the (A) *Time-domain measure* that includes (i) the standard deviation of NN interval or SDNN, which refers to the cardiac risk as values <50 ms indicates the highest risk, while values between 50-100 ms refer to moderate risk, and values over 100 ms refer to no risk, (ii) the percentage of sinus NN intervals that differs over 50 ms *i.e.*, pNN50, a value of 3% or more eliminates the cardiac risk while <3% indicates very high cardiac risk [12] and (iii) the root mean square of successive RR interval difference known as the RMSSD that reflects the vagus nerve mediated autonomic control of the heart and a low RMSSD level (<50 ms) refers to sympathetic overactivity and hence poses for a higher cardiac risk [13]; (B) *Frequency-domain measure*, which is the ratio of Low and High-frequency powers known as the LF/HF score, which signifies the sympathovagal balance in the body, respectively and high LF-HF value signifies the high cardiac risk; and (C) *Non-linear measure* under which lies the ratio of Poincaré plot standard deviation perpendicular to the line of identity, which is termed as the SD1/SD2 score and is also a measure of the autonomic (sympathetic vs parasympathetic) balance and a high ratio refers to the cardiac risk due to sympathetic dominance [11]. Using photochromatography Lyfas can detect the solutes and cellular material in the capillary blood, which reflects the metabolic state of the body. Its novel heuristics collates the above findings into comprehensive mind-body homeostasis analytics with data visualization through graphs and plots, which can be downloaded as a report for clinical correlations, *i.e.*, correlating the values of these biomarkers with that of the chief

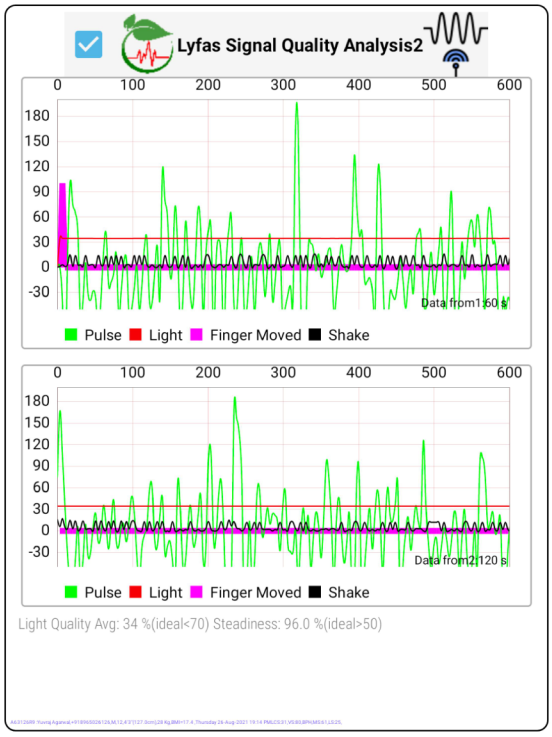
complaints, sign-symptoms, and physical state of the test-takers. Figure **4** - **6** show the snapshots of the Lyfas working principle, the test procedure, and a sample report, respectively.

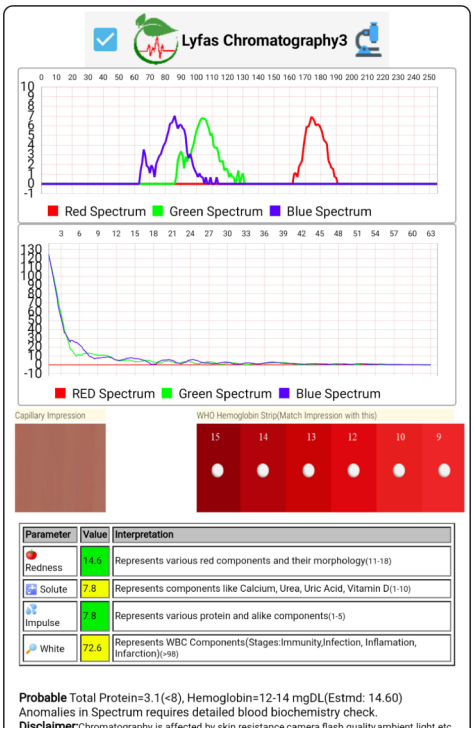
The summary of the time-series Lyfas test reports concerning the HRV correlates shows that the average values of Heart Rate (HR), HR changes, RMSSD, SD1/SD2, and LF/HF are high as seen in Figure **3**; while SDNN and pNN50 are found to be lower than normal. Critical analysis of the overall physiological state of the homeostasis, in this case, shows that the vagal compensatory mechanism (as evident by the high average value of RMSDD and low pNN50) is in-state to prevent the cardiac risk as a result of the

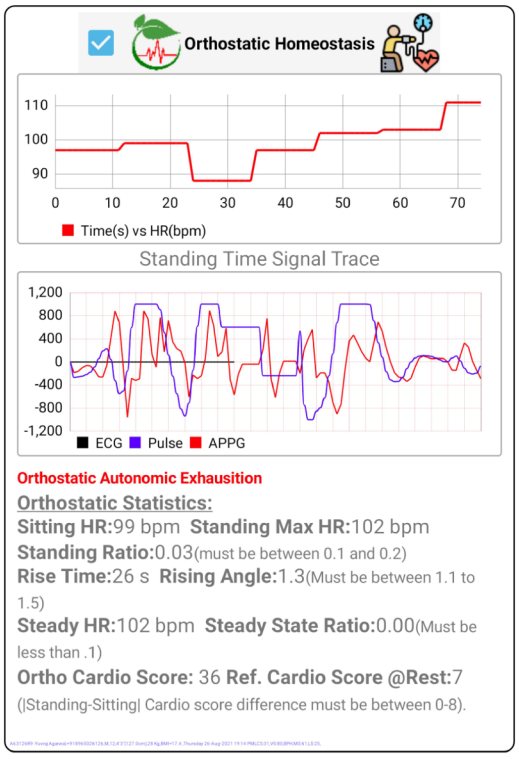


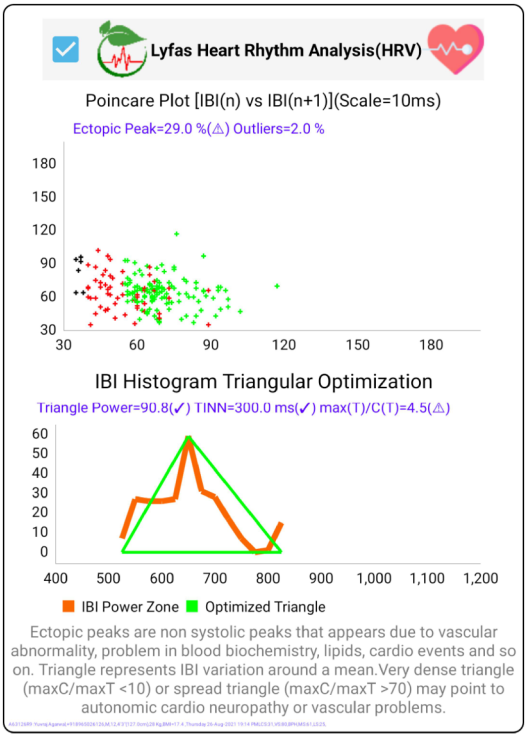












tendency of underlying sympathetic overdrive (as evident by the high LF/HF, HR, HR changes, and SD1/SD2). There is also deterioration of the overall health condition due to the lower value of SDNN with the progression of the disease [14-16]. Hence, the study concludes that Lyfas parameters can predict the cardiac and general physiological health of the patients by studying the cardiovascular biomarkers surrogating for the CAM in his body and thus could be useful to the neurologists to monitor the progress of the illness and assess the risk of cardiac involvement in a case of DMD. The findings conclude that Lyfas can be used as a prognostic tool for monitoring the extent of cardiac involvement in case of a neuromuscular disorder.

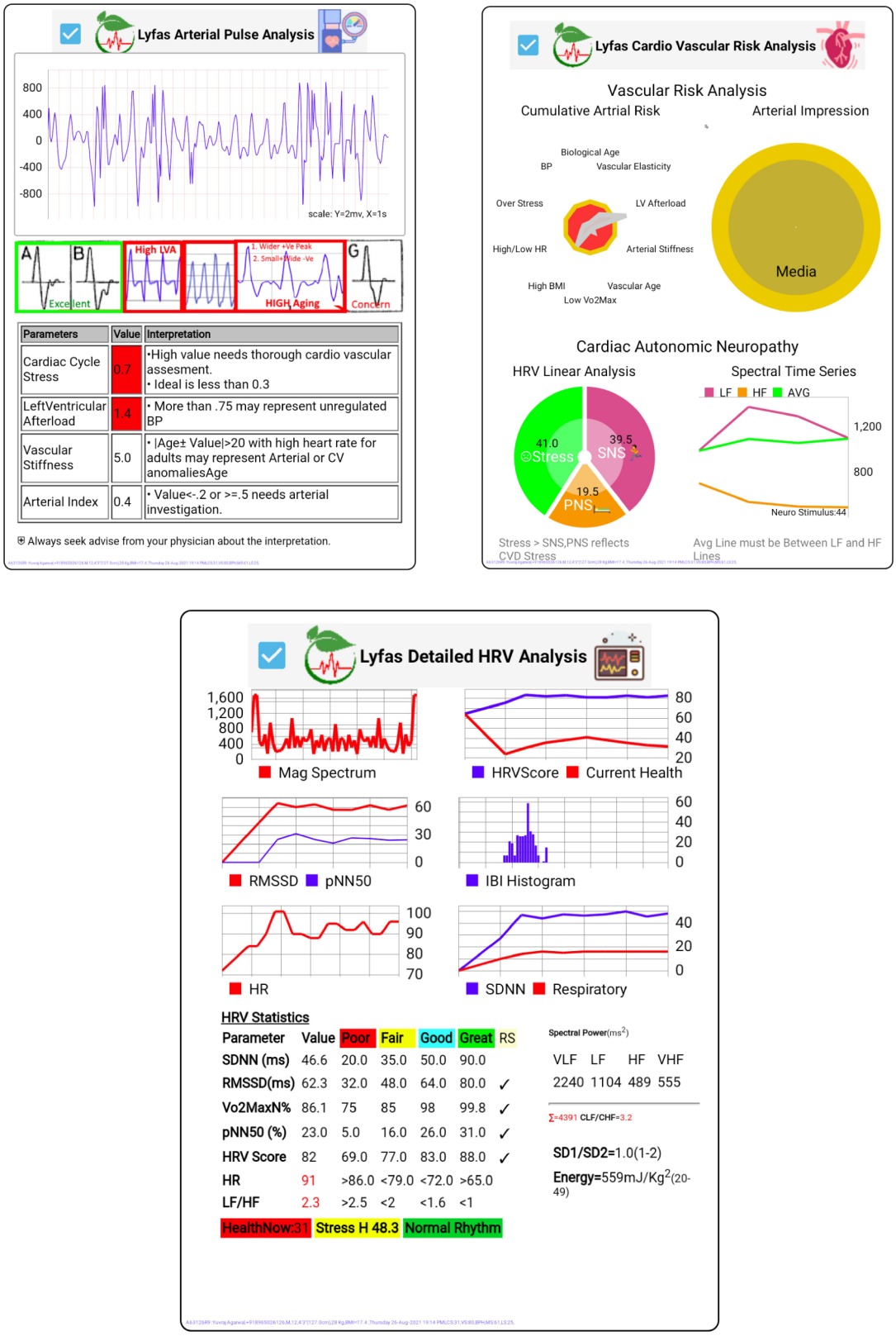


Figure 6: Snapshot of one Lyfas report of the present case.

ETHICAL CLEARANCE NUMBER: ECR/1181/INST/K.A., 2019

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CONFLICT OF INTEREST

The authors declare that there is no personal or organizational conflict of interest with this work.

AUTHOR CONTRIBUTION

SC and **RD** conducted the study. **SC** conceptualized the data, analyzed it, corelated clinically, and written the paper. **RD** developed Lyfas application in smartphone and won the grant of DERBI Foundation, The Ministry of Electronics and Information Technologies, Govt. of Karnataka, India. Certificate number: IN-KA45186921153022T, Dated: 13th July 2021.

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