Patients and methods

This is a longitudinal observational cohort study conducted at Benha University Hospital and Benha Children Hospital.

The study is carried out on 29 children diagnosed to have neuromuscular diseases (NMDs) who were divided into three groups:

- **Group (I):** Ten children with Duchenne muscular dystrophy (DMD). Diagnosis with DMD was established according to DMD diagnostic criteria *(Table 1)* *(Jennekens et al., 1991)*.

- **Group (II):** Eleven children diagnosed with juvenile dermatomyositis (JDM). Diagnosis with JDM was established by pediatric rheumatologist according to Bohan and Peter diagnostic criteria *(Table 2)* *(Bohan and Peter, 1975)*.

- **Group (III):** Eight children diagnosed with neuropathic disorders.

In groups I & III, final diagnosis was established by the pediatric neurologist using EMG, biochemical and genetic evaluation or muscle biopsy when necessary. The choice of diagnostic instrument depended on the clinical presentation.

All groups were recruited from the inpatients and outpatients' clinic of the Rheumatology, Rehabilitation and Physical Medicine department & Pediatric Department at Benha University Hospital and Benha Children Hospital.

- **Exclusion criteria:**
  a. Patients with age less than 2 years were excluded from the study due to inability to perform manual muscle testing and functional scales.
  b. If no final diagnosis could be established.
  c. The presence of a concomitant illness that may result in peripheral neuropathy or myopathy.

<table>
<thead>
<tr>
<th>Elements</th>
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<tbody>
<tr>
<td>1. Symptoms are present before the age of 5 years.</td>
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<tr>
<td>2. Clinical signs comprise progressive symmetric muscular weakness: proximal limb muscles more than distal muscles; initially only lower limb muscles. Calf hypertrophy is often present.</td>
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<tr>
<td>4. Wheelchair dependency before the age of 13 years.</td>
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<td>5. There is at least a 10-fold increase of serum creatine kinase activity.</td>
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<tr>
<td>6. Muscle biopsy: abnormal variation in diameter of the muscle fibers (atrophic and hypertrophic fibers), foci of necrotic and regenerative fibers, hyalin fibers, increase of endomysial connective and fat tissue.</td>
</tr>
<tr>
<td>7. Muscle biopsy: almost no dystrophin demonstrable, except for an occasional muscle fiber (less than 5% of fibers).</td>
</tr>
<tr>
<td>8. DNA: Duchenne type (frame shift) deletion within the dystrophin gene; identical deletion or identical haplotype, involving closely linked markers, as in previous cases in the family.</td>
</tr>
<tr>
<td>9. Positive family history, compatible with X-linked recessive inheritance.</td>
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</table>

### Assessment of Duchenne Muscular Dystrophy

The diagnosis is definite when:

1. First case in a family:
   a. Age <5 years: (2), 3, 5, 6, 7, (8) all present.
   b. Age 5–12 years: 1, 2, 3, 4, 5 (at least once), 6, 7, 8, or all present.
   c. Age >12 years: (1), 2, 3, 4, 5 (at least once), 8 (or 6 and 7), all present.

2. Another case in the family (according to element 9) complies with the criteria under 1:
   a. Age <5 years: 5 and 9 present.
   b. Age 5–12 years: 1, 2, 3, 5 (at least once) all present.
   c. Age >12 years: (1), 2, 3, 4, 5 (at least once), all present.
Table (2): Bohan and Peter Criteria for Diagnosis of Polymyositis and Dermatomyositis (Bohan and Peter, 1975).

<table>
<thead>
<tr>
<th>Elements</th>
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<tbody>
<tr>
<td>1- Proximal muscle weakness, usually symmetrical.</td>
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<td>2- Elevated serum muscle enzymes (CK, AST, ALT or aldolase).</td>
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<tr>
<td>3- Electromyographic abnormalities: Myopathic potentials (low amplitude, short duration and polyphasic action potentials), fibrillations, positive sharp waves, increased insertional activity and complex repetitive discharges.</td>
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<tr>
<td>4- Muscle biopsy findings typical of PM or DM: Necrosis, phagocytosis, regeneration, inflammation.</td>
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</tr>
<tr>
<td>5- Dermatological features of DM: Gottron’s sign or papules or heliotrope rash.</td>
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**Assessment**

- Definite diagnosis requires four criteria with rash for DM and without rash for PM.
- Probable diagnosis requires three criteria with rash for DM and without rash for PM.

All patients were evaluated at baseline, at 4-months & at 8-months follow up using:

(A) **Clinical evaluation**

1. **Complete history taking:**
   - **Personal history:** Name, gender, age and residence.
   - **Complaint:** Taken in the parents’ or caregivers’ own words.
   - **Present history:**
     Inquiry with careful details as regards:
     - Age of patient at onset of disease.
     - Weakness:
       - Anatomic distribution (arms versus legs, proximal versus distal, symmetric versus asymmetric).
- Presence of focal wasting or hypertrophy of muscle groups.

- Course of weakness:
  - Acute onset (days to weeks)
  - Chronic (months to years)
  - Episodic
  - Is the weakness getting worse, staying the same or getting better?
  - Ascertain the duration and rate of progression of weakness (days, weeks, months or years).

  o Pain (site, character, onset, course, duration, radiation, exacerbating and relieving factors)
  o Fatigue, lack of endurance, frequent falls, difficulty ascending stairs or arising from the floor.
  o Muscle cramps or stiffness.
  o Abnormal muscle movements e.g. muscle twitches.
  o Absence or presence of sensory loss, presence of paresthesia.
  o Abnormal gait: e.g. Toe walking, excessive lordosis, Trendelenburg or gluteus maximus lurch.
  o Functional difficulties:
    - Ambulatory distances
    - Frequency of falls
    - Transitions from the floor to standing
    - Problems climbing stairs
    - Problems dressing
    - Problems reaching overhead
    - Difficulty lifting
    - Running ability
  o History of recent illnesses (e.g. recent viral illnesses, respiratory difficulties, pneumonia and pulmonary infections)
  o Constitutional symptoms:
    - Loss of appetite, weight loss, fatigue, fever and malaise.
    - Symptoms of any concurrent infections.
  o Feeding difficulties, dysphagia, and nutritional status.
  o Cardiac symptoms (dizziness, syncope, chest pain, orthopnea, dyspnea with exertion)
  o Pulmonary symptoms (breathing difficulties)
  o Present medications: Drugs used, dosages and duration of use.
• **Developmental history:**
  o At what age did child:
    - Hold up head
    - Roll over
    - Sit unsupported
    - Stand alone
    - Walk
  o History as regarding language acquisition and mental development.

• **School history:**
  o Year in school
  o School problems
  o Behavior problems
  o Attend special classes
  o Ever seen by psychologist, speech therapist or special teacher

• **Prenatal history:**
  o History of any obstetric complications, drug intake or infections during pregnancy.

• **Birth history:**
  o History of prolonged labor, any medication used during labor, method of delivery, child gestational age at time of delivery.
  o Did the child had any of the following postnatal:
    - Antibiotic treatment
    - Blue spells
    - Convulsions
    - Jaundice
    - Skin rash

• **Immunizations.**

• **Past history:** past history of the following:
  o Serious diseases, treatments and fate.
  o Previous trauma or operations.
  o Previous admission to hospital (date and reason).
  o Medications: types, dosages, duration of administration, drug allergy or drug intolerance.
• **Family history:**
  - History of similar conditions in the family.
  - History of other rheumatologic diseases.
  - History of other chronic or metabolic diseases.

• **Socioeconomic history.**

2. **Thorough clinical examination:**

• **General examination:**
  - General appearance, mentality.
  - Body built, weight, height.
  - Temperature.
  - Pulse.
  - Respiratory rate.
  - Blood pressure.
  - Examination of the skin for
    - Skin rash (heliotrope rash).
    - Gottron's papules.
    - Shawl sign, V-sign.
    - Skin thickening.
    - Calcinosis.
    - Hyperpigmentation and hypopigmentation.
    - Digital pitting ulcers, scars.
    - Vasculitic skin lesions (periungual infarcts, splinter hemorrhage, palpable purpura, livedoreticularis, urticaria and gangrene).
  - Examination of lymph nodes groups, consistency and tenderness.

• **Neurological examination:**
  - Inspection At Rest:
    - Posture: e.g. foot drop, scoliosis and kyphosis
    - Skeletal deformity: e.g. pes cavus
    - Focal or diffuse muscle wasting
    - Focal enlargement of muscles (pseudohypertrophy)
    - Trophic changes: fall of hair, brittle nails, thin skin, trophic ulcers and loss of subcutaneous fat
    - Involuntary movements e.g. fasciculations
  - Muscle tone: normotonia or hypotonia.
  - Motor power examination: Manual muscle testing (MMT)
Co-ordination examination: finger to nose, finger to finger and heel ankle tests.
Superficial reflexes: e.g. plantar reflex, abdominal reflex
Deep reflexes: biceps reflex, brachioradialis reflex, triceps reflex, knee reflex and ankle reflex.
Superficial sensation: normal or hypothesia
Cranial nerves examination.
Gait examination: toe walking, excessive lordosis, Trendelenburg gait, gluteus maximus lurch, high steppage gait or stamping gait.

Musculoskeletal examination:
- Joints of the body are examined thoroughly with the routine articular system examination including:
  - Inspection: for swelling, redness, deformity, nodules and rash.
  - Palpation: for warmth, swelling and tenderness.
  - Range of motion.
- Para-articular structures (tendons, ligaments, etc.) are also examined for tenderness, laxity and calcification.
- Muscles are examined:
  - Inspection: for muscle wasting, pseudo hypertrophy.
  - Palpation: for tone, calcinosis, wasting.
- Deformity: pes cavus, inverted champagne bottle appearance of lower legs.

Chest examination:
Inspection, palpation, percussion, auscultations for pleurisy (pleural rub, pleural effusion), interstitial pulmonary fibrosis.

Cardiovascular examination:
Inspection, palpation, percussion, auscultations for murmurs, thrill, tachycardia, lower limb edema.

Abdominal examination:
Hepatomegaly, splenomegaly.
3. **Body mass index (BMI) assessment:**
BMI of all patients was measured as follows: *(Niu et al., 2009)*
- Height (cm) using a stadiometer.
- Weight (kg) using a digital scale.
- BMI calculated according to the following formula:

\[
\text{Weight (kg)} / \text{Height (m}^2)\]

4. **Manual muscle testing (MMT):**
Patients underwent manual muscle testing (MMT) evaluation at baseline, 4 and 8 months follow up using Kendall’s 0 – 10 point scale. The strength of each muscle group was given a score. The following muscles were tested bilaterally: the biceps brachii muscle (BB), the forearm flexors (FF), the rectus femoris muscle (RF), the tibialis anterior muscle (TA) *Table (3) (Kendall et al., 1993).*

*Table (3):* Kendall’s manual muscle testing grading scale. *(Kendall et al., 1993)*
**Biceps Brachii**

- **Position of the patient:** With the patient sitting, the elbow is flexed at a right angle, with forearm in supination.
- **Position of the examiner:** The examiner should stand in front of and at the testing side of patient. The hand giving resistance is contoured over the flexor surface of the forearm just proximal to the wrist. The other hand is applied to the humerus to provide a counterforce.
- **Test:** Patient flexes elbow against the examiner-applied force.
- **Sample instructions to the patient:** “Bend your elbow, hold it. Don’t let me pull it down.”

**Biceps Brachii: Gravity-Eliminated Position (for weaker patients only)**

- **Position of the patient:** Side lying and the elbow is fully extended.
- **Position of the examiner:** The examiner should stand at test side of the patient and support arm behind the elbow and wrist if necessary.
- **Test:** Patient attempts to bend the elbow with the hand supinated.
- **Sample instructions to the patient:** “Bend your elbow.”

**Fig (1):** Manual muscle testing for biceps brachii. A, against gravity position; B: gravity-eliminated Position.
**Forearm flexors**

- **Position of Patient:** With the patient sitting with the elbow and forearm supported and forearm is in full supination.

- **Position of the examiner:** The examiner should stand or sit at a diagonal in front of the patient.

- **Test:** Support the patients forearm under the wrist while the other hand used for resistance is placed over the palmar surface of the metacarpals.

- **Sample instructions to the patient:** “Bring your wrist up, hold it. Don’t let me push it down”

**Forearm flexors: Gravity-Eliminated Position (for weaker patients only)**

- **Position of the patient:** With the patient sitting, the elbow and forearm are supported and the forearm is in neutral position.

- **Position of the examiner:** The examiner should stand or sit at a diagonal in front of the patient.

- **Test:** Support the patient’s wrist. This elevates the hand from the table and removes friction. The patient flexes the wrist.

- **Sample instructions to the patient:** “Bend your wrist toward your body.”

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**Fig (2):** Manual muscle testing for forearm flexors. A, against gravity position; B: gravity-eliminated Position.
**Rectus femoris**

- **Position of the patient:** With the patient sitting with the trunk approximately perpendicular to the floor, the leg is extended, but not locked in extension at the knee.
- **Position of the examiner:** The examiner stands at the side of the tested limb and the testing hand is placed over anterior surface of distal leg just above the ankle. The other hand is placed under the distal thigh.
- **Test:** The patient extends the knee through available range of motion.
- **Sample instructions to the patient:** “Straighten your knee and hold it, don’t let me bend it.”

**Rectus femoris: Gravity-Eliminated Position (for weaker patients only)**

- **Position of the patient:** Side lying with test limb superior to the supporting limb. Lower limb can be flexed for stability. Hold test limb in about 90° of knee flexion with the hip in full extension.
- **Position of the examiner:** The examiner stands behind patient at knee level. One arm cradles test limb around thigh with hand supporting underside of knee. The other hand holds leg at the ankle.
- **Test:** The patient extends the knee through range of motion and the therapist neither assists nor resists the patient’s voluntary movement.
- **Sample instructions to the patient:** “Straighten your leg.”

Fig (3): Manual muscle testing for rectus femoris. A, against gravity position; B: gravity-eliminated Position.
Tibialis anterior

- **Position of the patient:** With the patient sitting, the knee is flexed at 90°.
- **Position of the examiner:** The examiner sits in front of testing limb and supports the leg just above the posterior aspect of the ankle joint.
- **Test:** The patient dorsiflexes the ankle joint foot. Pressure is applied on the dorsum of the foot (in the direction of plantar flexion and eversion).
- **Sample instructions to the patient:** “Pull your foot up to the ceiling.”

Tibialis anterior: Gravity-Eliminated Position (for weaker patients only)

- **Position of the patient:** Side lying with test limb superior to the supporting limb. Lower limb can be flexed for stability. Hold test limb in terminal knee extension with the hip in full extension.
- **Position of the examiner:** The examiner stands near the patient’s feet. The supporting arm supports the test limb just proximal to the malleoli.
- **Test:** The patient moves the foot from plantarflexion to dorsiflexion; the therapist neither assists nor resists the patient’s voluntary movement.
- **Sample instructions to the patient:** “Keep your hip and knee their current position, and move your foot towards your head.”

Fig (4): Manual muscle testing for tibialis anterior. A, against gravity position; B: gravity-eliminated Position.
5. **Functional grading (Vignos and Brooke scales):**

The Vignos scale and the Brooke scale are the commonest functional scales for grading disease severity in NMDs. Both scales were initially designed for DMD, and nowadays are used in many neuromuscular diseases. The Vignos scale was devised to assess the lower extremity function *(Table 4)* *(Vignos and Archibald, 1960)*. The Brooke scale was devised to assess the upper extremity function *(Table 5)* *(Brooke et al., 1981)*. Both scales were used to evaluate the patients at baseline, 4 and 8 months follow up.

*Table (4):* Grading system for the Vignos scale *(Vignos and Archibald, 1960).*

<table>
<thead>
<tr>
<th>Grade</th>
<th>Functional Description</th>
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<tbody>
<tr>
<td>1</td>
<td>Walks and climbs stairs without assistance</td>
</tr>
<tr>
<td>2</td>
<td>Walks and climbs stairs with aid of railing</td>
</tr>
<tr>
<td>3</td>
<td>Walks and climbs stairs slowly with aid of railing (over 25 seconds for eight standard steps)</td>
</tr>
<tr>
<td>4</td>
<td>Walks unassisted and rises from chair but cannot climb stairs</td>
</tr>
<tr>
<td>5</td>
<td>Walks unassisted but cannot rise from chair or climb stairs</td>
</tr>
<tr>
<td>6</td>
<td>Walks only with assistance or walks independently with long leg braces</td>
</tr>
<tr>
<td>7</td>
<td>Walks in long leg braces but requires assistance for balance</td>
</tr>
<tr>
<td>8</td>
<td>Stands in long leg braces but unable to walk even with assistance</td>
</tr>
<tr>
<td>9</td>
<td>Is in a wheelchair</td>
</tr>
<tr>
<td>10</td>
<td>Is confined to a bed</td>
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</table>

*Table (5):* Grading system for the Brooke scale *(Brooke et al., 1981).*

<table>
<thead>
<tr>
<th>Grade</th>
<th>Functional Description</th>
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<tbody>
<tr>
<td>1</td>
<td>Starting with arms at the sides, the patient can abduct the arms in a full circle until they touch above the head</td>
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<tr>
<td>2</td>
<td>Can raise arms above head only by flexing the elbow (shortening the circumference of the movement) or using accessory muscles</td>
</tr>
<tr>
<td>3</td>
<td>Cannot raise hands above head, but can raise an 8-oz glass of water to the mouth</td>
</tr>
<tr>
<td>4</td>
<td>Can raise hands to the mouth, but cannot raise an 8-oz glass of water to the mouth</td>
</tr>
<tr>
<td>5</td>
<td>Cannot raise hands to the mouth, but can use hands to hold a pen or pick up pennies from the table</td>
</tr>
<tr>
<td>6</td>
<td>Cannot raise hands to the mouth and has no useful function of hands</td>
</tr>
</tbody>
</table>
6. **Childhood Myositis Assessment Scale:**

The Childhood myositis assessment scale (CMAS) is a quantitative evaluation tool to assess the severity of muscle involvement in children with JDM. The complete CMAS scoring sheet is shown in *appendix (1)*. The scores for the 14 items are summated to give a total score ranging from 0 (very poor physical function and strength) to 52 (normal physical function and strength). We used CMAS to evaluate all patients at baseline, 4 and 8 months follow up (*Huber et al.*, 2004).

7. **Childhood Health Assessment Questionnaire (CHAQ):**

The Childhood Health Assessment Questionnaire-Disability Index (CHAQ-DI) is a parent- or patient-reported questionnaire for assessment of functional ability in childhood rheumatic diseases and has become a primary outcome tool in the management of juvenile dermatomyositis. This questionnaire has advantages over other assessment tools of physical disability due to its multidimensionality. It assesses eight domains of physical function: dressing and grooming, arising, eating, walking, hygiene, reach, grip, and activities. The CHAQ has shown high validity, reliability, and good responsiveness in rehabilitation trials (*Singh et al.*, 1994).

Each item is scored from 0 - 3 (0 = no difficulty; 1 = some difficulty; 2 = much difficulty; 3 = unable to do). If the item is not applicable e.g. because of the young age, this item is not calculated in the score. If help from another person or aiding devices are used in a certain domain the minimum score is 2. For calculating the final score of CHAQ, the highest scores of every domain are summed and divided by eight giving a final score ranging from 0 – 3 with higher scores meaning higher disability. The CHAQ also evaluates presence of pain using VAS visual analogue scale 0–100 mm and a VAS 0–100 mm for the evaluation of overall well-being (with 0 being the best possible score) (*Lam et al.*, 2004).

We used the cross culturally adapted arabic version of the Childhood Health Assessment Questionnaire (CHAQ) proposed by El Miedany et al. The CHAQ is shown in *appendix (2)*. CHAQ was used to evaluate all patients at baseline, 4 and 8 months follow up (*El Miedany et al.*, 2003).
Patients and methods

(B) **Laboratory assessment:**

All patients were subjected to the following measurements at baseline, 4 and 8 months follow up:

- **Serum creatine kinase (CK) levels:** CK was measured by (ELISA) technique using Bovine Creatine Phosphokinase (CPK) ELISA Kit supplied from (My biosource, San Diego, California, USA). The assay procedures were followed according to the manufacturer’s instructions. The reference values are (for males 46-171U/L and for females 34 – 145U/L).

- **Serum Lactate dehydrogenase (LDH) levels:** LDH levels were measured by lactate dehydrogenase (LDH) Pyruvate kits supplied from (BioSystems S.A. Costa Brava, Barcelona, Spain). The assay procedures were followed according to the manufacturer’s instructions. The reference value is (105 - 414 IU/L).

- **Serum Liver enzymes (AST & ALT) levels:** Aspartate aminotransferase (AST – SGOT) was measured using Aspartate aminotransferase (AST/GOT) kits supplied from (BioSystems S.A. Costa Brava, Barcelona, Spain) with reference value (up to 40 U/L). Alanine aminotransferase (ALT – SGPT) was measured using Alanine aminotransferase (ALT/GPT) kits supplied from (BioSystems S.A. Costa Brava, Barcelona, Spain) with reference value (up to 41 U/L).

(C) **Quantitative Electromyography (QEMG) assessment:**

Quantitative electromyography (QEMG) is the process of extracting quantitative information related to the morphology and activation of motor units from EMG signals (*Adel and Stashuk; 2013*).

QEMG assessment for all patients was done at baseline, 4 and 8 months follow up. EMG signals were acquired using TruTrace EMG custom software developed on a Deymed EMG system (Deymed Diagnostic, 7th Ave, Payette, USA). The same operator performed all the QEMG studies. Intramuscular EMG signals were detected using a standard concentric needle electrode (*Fig. 5*).
Patients and methods

Fig. (5): A, Electromyography (EMG) system. B, concentric EMG needle electrode with diameters 30 x 0.45 mm

Patients lay supine on a standard examination table. The following muscle: biceps brachii (BB) and rectus femoris (RF) were studied on the most affected side. The needle was inserted about 0.5–1 cm into the muscle of interest. For BB muscle, with the patient’s forearm supinated, the needle was inserted at the mid-point between biceps tendon and anterior shoulder. Then, the patient was asked to flex the elbow with the hand supinated. For RF muscle, the needle was inserted into the anterior thigh, at the mid-point between the hip and knee. Then, the patient was asked extend the knee while lifting the heel from bed.

Once the needle was inserted, the child was instructed to slightly contract the muscle. Once adequate motor unite potentials (MUPs) were detected, the child was instructed to slowly increases the force of contraction to maximal effort. So that the interference pattern can be detected.

Data from at least 3 contractions were collected for each muscle from superficial, moderate, and deep needle detection sites. A minimum of 20 MUPs per muscle was collected. The MUP trains were then reviewed offline and markers were manually repositioned if needed. The needle-detected EMG signals were decomposed using a series of algorithms provided by the device software. The following MUPs’ parameter were automatically determined: duration (ms), peak-to-peak amplitude (μV), area (μV * ms) and area to amplitude ratio (AAR).

The EMG interference pattern analysis by the device software provided the number of turns per second (NT), the mean amplitude change between successive turns (MA) and NT: MA ratio.
(D) **Quantitative muscle ultrasound assessment:**

A commercially available Logiq e real-time scanner (General Electric Medical Systems, Milwaukee, Wisconsin, USA) was used for the muscle ultrasound (US) examination using a high frequency (8-13 MHz) linear transducer (*Fig. 6*).

Machine settings were optimized to obtain muscle ultrasound images in which healthy muscle and subcutaneous tissue appeared relatively black with strong reflections of fascia and bone. System settings were kept constant throughout the study, using a gain of 70, frequency 12.

*Fig. (6):* A, Logiq e Ultrasound machine. B, Broad spectrum (8-13 MHz) linear transducer.

US examination was performed after suitable period of rest and before EMG evaluation. Patients were examined in the supine position with their arms and legs extended (as much as possible in case of contractures) and their muscles relaxed. The hands were supinated, while the feet kept in a neutral position. Quantitative US measurements were performed to biceps, forearm flexors, quadriceps (rectus femoris) and tibialis anterior. A standard protocol for Quantitative ultrasound measurements was performed as following:

- A generous amount of contact gel was used to minimize the required pressure of the transducer on the skin.
Patients and methods

- All scans were made in the transverse plane with a standard location for the transducer. The transducer is placed longitudinally to the muscle length (corresponding to the muscle belly). The muscles were measured bilaterally at the following anatomical sites as suggested by Jansen et al: *(Jansen et al., 2012)*
  - The biceps brachii muscle (BB) at two-thirds of the distance from the acromion to the antecubital crease.
  - The forearm flexors (FF) at two-fifths of the distance from the antecubital crease to the distal end of the radius.
  - The rectus femoris muscle (RF) midway the distance between the anterior superior iliac spine to the superior aspect of the patella.
  - The tibialis anterior muscle (TA) at one-quarter of the distance from the inferior aspect of the patella to the lateral malleolus.

- In each measurement, the transducer was angled perpendicular to the bone to yield an image with the brightest and narrowest bone reflection. Between measurements, the subject was allowed to move and the transducer was repositioned.

- Three consecutive measurements were obtained for each muscle to minimize variation in echo intensity during analysis. The screen images of these measurements were stored offline in DICOM format. The images were analyzed as exported from the ultrasound machine without any adjustments or manipulations. We did not use zoom or change the depth settings during the study, as this would potentially alter echo intensity. As oblique scanning angles can lead to incorrect muscle thickness and echo intensity measurements, this was avoided by adjusting the angle of the probe until the best bone echo was obtained in every image.

The mean muscle echo intensity (EI) were determined using a computer-assisted grey-scale analysis: *(Fig. 7)*

- The captured images were analyzed offline for echo intensity by means of computer-assisted grayscale histogram analysis, using the standard histogram function of Adobe Photoshop CC 2015 17.0.1 software Supplied by (Adobe Systems Inc, San Jose, CA, USA).
In each of the stored muscle images, region that include as much muscle mass as possible without bone and fascia is manually selected. From the selected region the mean muscle echo intensity was calculated based on a histogram analysis which expresses every pixel as a value between 0 (black) and 255 (white).

Fig. (7): Transverse ultrasound image of biceps brachii muscle of a 6 years old boy with Duchenne muscular dystrophy. The muscle is brighter than normal i.e. increased echo intensity with reduced bony reflection. A region of interest was selected for the muscle and included as much muscle mass as possible without bone and fascia from which the mean echo intensity was calculated as shown in histogram box. The histogram box to the right of the image from computer software shows the corresponding grayscale values (mean 126). SC: subcutaneous tissue; m: muscle (biceps brachii); B: Humerus; ROI: region of interest.
Muscle thickness (MT) was measured during examination with electronic calipers at standardized positions as suggested by Jansen et al: (Jansen et al., 2012) (Fig. 8)

- The biceps brachii muscle was measured between the bone echo of the humerus and the superficial fascia of the biceps (which includes the brachialis muscle).
- The forearm flexors between the interosseous membrane (next to the radius) and the ventral fascia of the most ventral flexors.
- The quadriceps muscle between the bone echo of the femur and the superficial fascia of the rectus femoris (which includes the rectus femoris and vastus intermedius).
- The tibialis anterior muscle between the interosseous membrane (next to the tibia) and the ventral fascia of the tibialis anterior muscle.

Fig. (8): Transverse ultrasound image from right biceps brachii muscle of 6 years old female with SMA type III. The muscle is showing increased echogenicity. Yellow dots represents subcutaneous tissue thickness. Red dots represents muscle thickness. SC: subcutaneous tissue; m: muscle (biceps brachii and brachialis); B: Humerus.
**Statistical analysis**

All statistical analyses were carried out in STATA/SE version 11.2 for Windows (STATA Corporation, College Station, Texas). The collected data were summarized in terms of mean ± Standard Deviation (SD) and range for quantitative data and frequency and percentage for qualitative data.

Comparisons between the different study groups were carried out using the Fisher’s Exact Test (FET) to compare differences between proportions. The One-way Analysis Of Variance (ANOVA; F) and the Kruskal Wallis ($\chi^2$) test were used to compare more than two groups as regard parametric and non-parametric data respectively.

These were followed by post-hoc test using the Bonferroni method to detect differences in pairs. Differences in estimated parameters over time were tested using repeated measure ANOVA and Friedman test as appropriate and differences in matched pairs were examined using paired t-test and Wilcoxon signed-rank respectively.

Pearson correlation coefficient ($r$) and Spearman correlation coefficient (rho; $\rho$) were used to examine the correlation quantitative muscle US evaluation and estimated parameters.

Changes in muscle parameters over time were estimated using the Random-effects Generalized Least Squares regression analyses; univariate and multivariate, and the results were presented as regression coefficient ($\beta$) and 95% Confidence Interval (95% CI).

After the calculation of each of the test statistics, the corresponding distribution tables were consulted to get the “P” (probability value). Statistical significance was accepted at P value <0.05 (S). A P value <0.001 was considered highly significant (HS) while a P value >0.05 was considered non-significant.

**Ethical consideration**

The local ethics committee of Benha Faculty of Medicine approved the study on 15/12/2015 and all participants gave a written informed consent before being enrolled in this study.