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Duchenne Muscular Dystrophy (DMD) and Becker Muscular Dystrophy (BMD): A Review on Assessing Measures

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Abstract:- Mutations in the encoding Dystrophin gene lead to lethal, genetic muscular dystrophies such as Duchenne Muscular Dystrophy (DMD), and Becker Muscular Dystrophy (BMD) which have a slower progression than DMD and an intermediate form. Dystrophin gene mutations abolish the production of Dystrophin in body muscles such as skeletal, cardiac, and smooth muscles. The progressive degeneration of muscle tissues and functions will occur. Most often respiratory, orthopaedic, and cardiac-related complications have led to death. These neuromuscular disorders occur at a frequency of about 1 in 5000 newborn males. The objective of this review was to identify and understand the available measures used for assessing muscular dystrophies in DMD and BMD. Review of studies identified from searching medical bibliographic sources relevant to assessing methods and techniques of DMD and BMD between the years of 2002 and 2022. The studies showed measures used to assess the muscles in DMD patients apart from clinical assessments to quantify the pathological changes involved in the muscles as objective parameters. The measures can be categorized into invasive and non-invasive methods. This study has resulted in manual muscle testing methods and methods of assessing the functional ability of the muscles such as muscle biopsies, Ultrasound scans (USS), and Magnetic Resonance Images (MRI) etc. It concludes that the most widely used effective and reliable investigation method has been identified as MRI scans due to various purposes and methods of assessing muscular dystrophies.

Keywords:- *Becker Muscular Dystrophy, Duchene Muscular Dystrophy, Dystrophin, Genetic, Measures, Mutations, Tests.*

I. INTRODUCTION

➢ *Muscular Dystrophy*

The word ‘dystrophy’ can be defined as a disorder in which an organ or tissue of the body wastes away’[1]. Dystrophy of muscles causes weakness and progressively lost muscle mass. Interference of the protein production which is essential to maintain healthier muscles is a cause of the abnormal genes (mutations).

There are more than 30 types of muscular dystrophies caused by genetic mutations [2]. Progression of muscle weakness, involving muscle groups, symptoms, and the ages when diagnosed varies according to genetic variations. Some of the disorders have a slower progression and they are mild; as it is, the symptoms do not affect the person’s daily activities greatly. But some of them have a rapid progression which is making physical disabilities within a shorter period of life and shortening the life expectancy. Most of the muscular dystrophies are diagnosed in childhood and several types of muscular dystrophies can appear in adolescence and adulthood. Other types do not persist until adulthood.

Some of the genetic muscular dystrophies are Duchenne Muscular Dystrophy, Becker Muscular Dystrophy, Congenital Muscular Dystrophy, Myotonic Muscular Dystrophy, Limb-Girdle Muscular Dystrophy, Facioscapulohumeral Muscular Dystrophy, Emery-Dreifuss Muscular Dystrophy, Distal Muscular Dystrophy, Oculopharyngeal Muscular Dystrophy, Collagen Type VI-Related Disorders, etc. [2]. Duchenne Muscular Dystrophy is the most prevalent type of muscular dystrophy [3].

➢ *Duchenne Muscular Dystrophy (DMD)*

Duchenne Muscular Dystrophy is a genetic disorder that is expressed as an X-linked recessive trait. This disorder occurs at a frequency of about 1 in 5000 newborn males [4]. Cannot find evidence of the muscular weakness of carrier females; but it has been described that the symptomatic female carriers account for 2.5% to 20% [5]. If the female carriers are associated with Turner syndrome (45X) or mosaic Turner karyotype [5].

The Dystrophin gene which is located in the Xp21 chromosome is responsible for maintaining the healthy muscles of the body such as skeletal, cardiac, and smooth muscles by linking cytoskeletal F-actin with the extracellular matrix via its N-terminal and C-terminal domains by proper following of the ‘reading frame rule [6]. Mutations in the Dystrophin gene (most are deletion and duplications; this accounts for 70% to 80% of the mutations [6]. resulting in the lack or loss of the protein dystrophin. Ultimately the body is unable to regenerate or keep up the repair of muscle tissues. This results in the loss of stability

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of sarcolemma, fiber necrosis, progressive fibrosis, dysfunction of muscle stem cells, fatty tissue replacement, and muscular weakness; it also, secondarily affects the metabolic and inflammatory deregulated pathways. Progressive muscle weakness and loss of muscle mass can be detected in the patients. The disease condition severity depends on the type of mutation. 'Out of frame' mutations do not emulate the 'reading frame rule' and processing of the severe DMD phenotype.

With the rise of inflammatory pathways in the body, the immune system gets activated consequently. Hence the cytotoxic neutrophils, T-lymphocytes, and macrophages drive the muscle invasion and grace muscle apoptosis, muscle atrophy, and eventually muscle cytolysis.

Deregulation of the immune system is a cause of autoimmune diseases, which later on lead to autoreactive T-cell and B-cell responses [7].

This lethal condition is characterized by muscle weakness which is the principal symptom. It can be begun in the early stages of life in 2nd or 3rd year. Initially shows muscular weakness usually in proximal muscle groups and later in distal muscle groups (near the extremities). Generally, the lower external muscles are affected initially, before the upper external muscles. In the early stages, the debility and atrophy of the muscles in the pelvic area occur and it is followed by the shoulder muscles involvement. With the progression of the disease with time, muscles in the trunk area, forearms, and additional muscles of the body are involved gradually [6]. The affected child can be presented with difficulty in walking, jumping, and running. As the other symptoms, are pseudo hypotrophy of the calf regions, lumbar lordosis (inward curve of the lumbar spine), Gower's sign, and waddling gait (myopathic gait). The ongoing weakness of the muscles and the scoliosis condition outcome the impaired pulmonary function and in the end stage is acute respiratory failure [8].

DMD patients are not only exposed to skeletal muscle degeneration but also lead to severe cardiomyopathy in their second decade of life. It is one of the main causes of death of the disorder. The lack or loss of dystrophin protein in cardiac muscles leads to cardiomyocytes having more sensitivity to stretch-induced deprivation. Furthermore, this damage alters intracellular calcium (Ca2+) concentration pathologically, dislocates neuronal nitric oxide synthase (nNOS) alters mitochondrial functions and leads to the development of cardiomyopathy; symptoms associated with inflammation and necrosis [9].

Dystrophin is distributed in the brain and retina and comparatively less than in the muscles. So, some of the manifestations of the disorder related to the central nervous system also can be explained [5]. Muscles are unable to respond to the nerve impulses generated by the brain.

Diagnosis can be done through the clinical signs and symptoms and can be confirmed by raised serum concentration of Creatinine Kinase (CK), absence of Dystrophin protein in muscle biopsy, and mutations in the dystrophin gene on genetic analysis. Normally, the serum CK level goes up till the first 5 years of age until 10 times the normal upper limit and then gradually decreases with age. The muscle biopsy shows necrosis and attempted regeneration of muscle fibers as increased variability of muscle fiber diameter with hypertrophy. Immunohistochemically analysis of the muscle biopsy is usually carried out to confirm the clinical diagnosis. Further genetic analysis is needed when complete absence or severe reduction in dystrophin (less than 5%) staining positive present in immunohistochemical analysis.

Next-generation sequencing (NSG) is an effective diagnostic tool for detecting neuromuscular disorders, as it is hard to decide on a differential diagnosis by considering the high CK levels in neuromuscular disorders [10].

No complete cure in DMD treatment goals; but can support maintaining body functions, prevent contractions, and provide psychological support to the child and family. Mainly steroids have a beneficial effect on muscle force and functions. However, both positive and negative effects of long-term use of steroids are not generally accepted [10].The maximum isometric muscle force is measured by using the Dynamometers to assess whether any muscular weakness is present or any development with time and by evaluating the disorder.

Ultrasound scans (USS) and Magnetic Resonance Images (MRI) are other gold-standard techniques used to assess muscle destruction or evaluation of progress after medications are applied. This study has been conducted to find out the techniques and methods used to quantify muscle strength and assess the muscle pathological changes that follow in DMD patients. That will help to evaluate the therapeutic measures and prognosis to hamper the disease progression.

- *Becker Muscular Dystrophy (BMD)*

Becker Muscular Dystrophy is also caused by the mutations of the dystrophin gene which is an X-linked neuromuscular disorder such as DMD. It is a milder disease compared to DMD with a later onset with age and also has a slower progression than DMD. When 'out of frame' mutations produce severe DMD conditions, 'in-frame' mutations cause less severe BMD by generating the partial functional dystrophin protein [11]. Mutations are more commonly deletions (65% - 70%) or duplications (5% to 10%) [12].

These mutations lead to progressive muscle degeneration and proximal muscle wasting as DMD. As the condition has a late onset, the manifestation of the symptoms can vary widely between the ages of 5 to 60 years almost exclusively in males [12].

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The prevalence of BMD related to all groups varies as 0.26 per 10000 males according to the research conducted in the United States in 2010 and it has been found more common in Non-Hispanic whites than Non-Hispanic blacks [12].

Progression of the disorder causes complications such as cardiomyopathy, loss of liver and pulmonary functions progressively, bone fractures, loss of ambulation, and cognitive impairment. Postoperative chest infections are some of the conditions that have more chances. Rhabdomyolysis causes myoglobinuria and leads to kidney failure [12]. (Thada., *et al* ., 2023)

- *Objective*

To identify and understand the available measures and investigations used for assessing the muscular dystrophies in Duchenne Muscular Dystrophy and Becker Muscular Dystrophy.

II. METHODOLOGY

From 2002 to 2022, studies of assessing methods and techniques that have been used to assess the muscles in DMD and BMD patients were predominantly searched using electronic databases such as Google Scholar, Medline, PubMed, and Indexed journals.

III. RESULTS

The 90 research articles have been referred to and identified including measures and investigations used to assess muscular dystrophy in DMD and BMD patients apart from clinical assessments to quantify the pathological changes involved in muscular dystrophy as objective parameters. The methods identified can be categorized into invasive and non-invasive methods.

Manual muscle testing methods and methods of assessing the functional ability of the muscles

This study has resulted in there are manual muscle testing methods and methods of assessing the functional ability of the muscles.

Running a 9 m distance, rising up from the floor, and acting on lower and upper limbs also validated functional scale for body motor functions are the timed functional tests [13]. Motor skills are assessed using the upper two dimensions (standing/walking, running & jumping) of the Gross Motor Function Measure (GMFM 88) and Timed Motor Tests (TMTs) (10-meter run, sit to stand, supine to stand, climb 4-stairs) [14].

There are reliable measures to assess functional ability widely used in DMD clinical trials such as timed motor performance evaluations (time to walk or run 10 m distance, time to climb four standard stairs, and time to rise from a supine position). It is difficult to assess to evaluate the maximum time for each measure as some patients have lost ambulation and theoretically the maximum time should be infinite, which cannot be done in statistical analyses. Alternatively, as an arbitrary, the allowable maximum time score is about 120 seconds to complete a 10m distance walk [15].

North Star Ambulatory Assessment (NSAA) is a validated unidimensional functional scale for ambulant subjects with DMD [16]. It is one of the robust scales used internationally, widespread, and is suitable for multicentric studies in clinical studies. It is a well-developed scale that has followed modern psychometric analysis to provide a better interpretation of clinically meaningful changes. The scale is consisting 17 items, ranging from 1 to 17. Item 1 is ‘standing’, item 17 names ‘running’ and other items such as head raise, standing on heels, hopping, or running are there to assess the functional abilities which should be necessary to present to maintain the ambulant. Some timed items in this scale include the 10m walk or run test, time to rise from the floor, or the Gower test. Each item is scored on a 3-point scale with simple criteria; 2- Normal achieves goal without any assistance; 1- Modified method but achieves goal independent of physical assistance from another person; 0- Unable to achieve independently [15]. Then the scores for each item are summed and a total score is achieved. The score ranges from 0 (all activities are failed) to 34 (all the activities are achieved). The assessment is usually completed within 15 minutes.

The 6-Min Walking Test (6 MWT) is one of the functional abilities assessing tests which is a modified version of the American Thoracic Society (ATS) guidelines. A short orientation video is taken before the test with the consent of the subject, continuous encouragement and chases behind the walking subject during the test for his safety are some modifications which are included. The test is usually completed within 15-20 minutes. The specified trainee is working as the examiner for this test. In this 6MWT, subjects are asked to walk steadily and quickly without running, within a 15m distance. Encourage the subject throughout the test and every remaining minute is informed. If patients are necessary to stop, allow them to stop and begin it again within 6 minutes [15].

Measures to assess the lungs and respiratory muscle functions. For the early identification of the progression of the DMD disorder and to improve the quality of life and longevity through providing an optimal intervention, it is very important to measure the lungs and respiratory muscle functions. Assessing the respiratory functions is beneficial for calculating or quantifying the feasible effects to innovate therapeutic trials.

Measures to assess the lungs and respiratory muscle functions can be classified as non-invasive / non-volitional, non-invasive/ volitional, invasive/non- volitional, and invasive/ volitional according to the encroachment and to the cooperation required of the patients. These measures can be identified as ‘specific’ or ‘global’ according to the involvement and contribution of the various respiratory muscles such as intercostal muscles, diaphragm, abdominal muscles, and accessory inspiratory muscles [17].

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These tests can be assessed by EMG (electromyography); the level of muscle activation is measured by the electric activity of the muscle, measuring the developed pressure of the muscle to assess the force developed by the muscle, measuring the flow and/ or volume to assess the contraction of the muscle and using varies imaging techniques to assess the muscular morphology [18].

- *Non-Invasive / Non-Volitional Tests;*

Transcutaneous EMG (electromyography) is one of the non-invasive tests measured by using surface electrodes to measure the electric activity of the respiratory muscles. Usually, the signal/noise ratio is very poor at rest as weak muscles are due to the 'crosstalk' of other muscles and cardiac activity [19],[20].

The ventilator pattern is measured by a pneumotachograph, during quiet breathing at rest when the mouth is on a breath-by-breath basis, using a mask other than a mouthpiece to perform the test accurately without leakage of as the weakened cheek muscles and macroglossia, which are the possible characteristics of the progressed disease [21]. These can be performed for any age in various postures such as supine, sitting, and standing.

Reconstruction of the shape of the diaphragm can be resolute excellently by using Magnetic Resonance Imaging (MRI). This technique is still limited due to the high cost and it takes more time to acquire; so, the patient needs to keep in the supine position for a prolonged time and it is uncomfortable for the patient [22].

Ultrasound (US) imaging is one of the non-invasive measures used to study the diaphragm. The replacement of the dome of the diaphragm, the length apposition of the diaphragm, and the thickness of the diaphragm can be assessed through USS [23], [24], [25].

- *Non-Invasive/ Volitional Tests;*

All these non-invasive/ volitional tests should be performed with maximum effort and should be repeated to achieve an accurate measurement. So, it makes the weakness of the muscles of DMD patients difficult to conduct for the children. Usually, children above 7 years old can be done with full cooperation to achieve a reliable manoeuvre.

Maximal Inspiratory Pressure (MIP) and Maximum Expiratory Pressure (MEP) are measured at Residual Volume (RV) and Total Lung Capacity (TLC), respectively, when inspiratory and expiratory muscles are maximally stretched, at the mouth, by supplying a global index of inspiratory and expiratory muscle strength. This strength is dependent on the lung volume. Sniff nasal inspiratory pressure (SNIP) is an easier test to perform. The needed manoeuvre is more natural and measured at the nostrils. strength of the diaphragm is measured by a specific index, by considering the outward movement of the abdomen. In the case of nasal obstruction, the pressure is underestimated than the normal pressure [18], [26], [27].Tension Time Index (TTI) is used to assess the inspiratory muscle fatigue and it is measured or calculated as P0.1/MIP· duty cycle; P0.1 is the pressure during the first 100milliseconds of occlusion when breaths quickly, the duty cycle is the ratio between inspiratory time and the total time duration of the breath. When inspiratory muscles are fatigued, the TTI values are high [28], [29], [30].Peak Expiratory Flow (PEF) is measured during a maximal exhalation and Cough Peak Flow (CPF) is measured during a maximal cough at the mouth to get some clues on the expiratory muscles [31]. FVC Forced Vital Capacity (FVC) and Forced Expiratory Volume in 1 second(FEV1) are some of the traditional Spiro metric parameters to assess respiratory functions.

In the condition of restrictive lung disease, FEV1/FVC ratio is above 70% and the TLC value is reduced. In obstructive lung diseases, FEV1/FVC ratio is less than 70% [32].

Absolute lung volumes such as Functional Residual Capacity, TLC, and RV measuring techniques needed either nitrogen washout technique or body plethysmography as they cannot be measured by simple spirometry. But it is difficult to perform plethysmography for DMD patients with wheel-chair bound [31],[33],[34] [35].

- *Invasive/Non- Volitional Tests;*

These tests are performed by using oesophagal and/or gastric unpleasant balloon-catheter systems and are used to assess the various respiratory muscles. But the catheter systems are not recommended for patients with severe DMD as they have difficulty swallowing to avoid aspiration and regurgitation [31].

The oesophagal pressure (POES) and the gastric pressure (PGA) can be measured by transducer-catheter-balloon-system and the trans-diaphragmatic pressure (PDI) can be measured through PGA- POES [31].

To evaluate the diaphragm dysfunction, various dynamic imaging, and static imaging methods are used. Some of the dynamic imaging techniques are ultrasonography, dynamic MRI, and fluoroscopy. Computed tomography, brightness mode ultrasound, static MRI, and chest radiography are some examples of static imaging techniques [36].

- *Invasive/ Volitional Tests;*

Measuring POES, PGA, and PDI during maximal activities such as cough and sniffing are the tests coming under these tests and these tests provide information on the strength of global inspiratory muscles and diaphragm [37], [38], [39], [40].

- *Muscle biopsies*

Muscle biopsies are one of the invasive surgical procedures used. Short and long-term health impacts are related to this technique. As it makes painful and temporary

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cessation of daily activities, general anaesthesia for the caregivers is recommended. Scarring is one of the long-term impacts of muscle biopsies with varied sizes, as relatively small incisions are done in the needle and Concho tome biopsies (smaller than 1cm), otherwise several centimetres in open biopsies [41]. Usually, open biopsies are currently used for most of the clinical trials in DMD which is a technique to quantify dystrophin [42]. The purposes of muscle biopsies in clinical trials in DMD are to identify any restoration of protein expression or to determine any improvement in muscle quality through histological studies as well as for the development of therapies for DMD. The biopsy analysis is a pharmacodynamics biomarker [43].

The restoration of dystrophin protein can be measured by biopsies taken in smooth muscle layers of the skin [44], [45]. So it is possible to perform skin biopsies than muscle biopsies in the future, but a disadvantage of this is, that the distribution of therapeutic compounds varies between skeletal muscles and the skin, which could provide both false positive and false negative results [41].

- *Serum Creatine Kinase (CK) Levels*

Serum Creatine Kinase levels are one of the invasive techniques used. Measuring the CK concentration from dried blood spots is one of the newborn screening studies for DMD which has been done in several countries, but most of the countries have been discontinued. The samples with increased CK levels, then undergo the dystrophin gene mutation tests [46].

CK is released from muscle fibers when sarcolemma increases its permeability of it. So, the CK level goes up and it is an assay mark of the muscular damage. CK level is markedly high in DMD patients when compared with the normal value and it is used as a diagnostic value in DMD. Normally, the maximal value of serum CK levels is identified in the ages of 2 to 5 years of DMD patients and then gradually decreases value with the disease progression [42]. Between the development of the dystrophic process and the serum CK level lacking has a stronger association than age [42].

With the progression of the disease, the pulmonary functions monotonically decrease and most of them die from respiratory failure as the dystrophic variations affect the respiratory musculature [43],[44],[45],[46]. But it is unknown that any influence of serum CK level on pulmonary functions.

The serum CK level is comparatively higher in DMD patients than in BMD patients. The normal range of serum CK is <200 IU/l and in DMD, the range elevates high between 5000 - 150 000 IU/l [47].

- *Capillary Western Immunoassay (Wes) and Western Blot (WB) Assay*

Capillary Western immunoassay (Wes) is one of the invasive techniques used. Web analysis is comparatively more producible and highly sensitive, it needs only a fraction of the sample to produce a dystrophin signal and can detect a trace amount of dystrophin in the skeletal muscles of DMD patients.[48].

To quantify the dystrophin levels in pre and post-treatments, a simple capillary immunoassay (Wes) method (a gel- and blot-free method requiring less sample, antibody, and time to run than conventional Western blot assay) has been applied.

The Western blot (WB) assay is widely used to measure dystrophin. Usually, the WB assay is more quantitative than the other assays such as Immunofluorescence analysis (IFA), as WB includes a calibration curve. So, the combination of WB and IFA methods is used widely to quantify the dystrophin levels in muscle biopsies of DMD patients [48]. But on the other hand, it has some difficult to perform and some technical challenges as it is needed a large amount of dystrophin protein to assess; due to this requirement, it is difficult to blot and also this method cannot discriminate dystrophin or trace dystrophin [48]. However, Wes has a wider dynamic range to quantify the dystrophin than WB[48].

- *Immunofluorescence Analysis (IFA)*

Immunofluorescence analysis (IFA) is one of the other techniques used to quantify the dystrophin levels in DMD. The standardized IFA technique is sensitive, reproducible, and operator-independent. This technique is based on imaging by confocal microscopy and semi-automated image analysis using computer software. In this method, the dystrophin contribution of each fiber is determined by using a cross-section of a muscle biopsy which is undergone by spectrin co-staining [49].

Usually, Immunofluorescence provides intuition on the presence of functional dystrophin available in the sarcolemma of individual fibers and they are complementary. It can center the dystrophin levels in the muscle fibers by excepting the areas with fibrosis and fatty cells which are present in the muscle cells, due to the progression of the disease. In trials, it is used to differentiate both potential drug effects and trace and revertant fiber dystrophin expression. It is more accurate if compare to two biopsies that have been taken from muscle areas with similar content [49]. It is a disadvantage of IFA; it is needed well-preserved morphology of biopsies relatively, for analyzing the cross-sections. It is a challenge in trials of international and multicenter, to preserve samples with high quality without freeze artifacts [49].

- *Enzyme-Linked Immunosorbent Assay (ELISA)*

One of the assays used in practice [47]. In ELISA for Dystrophin, utilizing a carboxy-terminal capture antibody and detecting antibodies by spanning 65% of the molecule. This arrangement is specific for dystrophin and the possibility of a false diagnosis is reduced, because of losing antigenic indicators by deletion or the existence of truncated products due to frame-shift mutations. This ELISA for dystrophin helps to determine DMD patients from other unrelated disorders and may provide a

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prognostic value for BMD patients. This assay is an achievable and rapid artefact for diagnosing DMD and BMD and a better tool for evaluating therapies that aim to establish dystrophin or increase its expression [50].

ELISA is a beneficial tool for screening muscular dystrophies, observing the progression of these dystrophies, investigating exercise-related sarcomeric disarrangement and their repairing procedure, and assessing the efficacy of therapeutic measures [51].

- *Mass Spectrometry (MS)*

One of the assays used in practice. MS is a mass spectrum analytical process, which is used to evaluate the mass-to-charge ratio of ions. This assay indicates a plot intensity as a role of this ratio; mass-to-charge. MS analytical process is used in various fields and it is applicable for the pure samples and also for the complex mixtures [52].

One of the clinical research reviews is that immune-mass spectrometry imaging is an applicable approach for research of pre-clinical to clinical DMD. This assay can quantify the respective dystrophin rapidly, which even in a single tissue segment, application is efficient for the patients and also provides data on the efficacy of medicine for a better therapeutic approach [53].

- *Magnetic Resonance Imaging (MRI)*

One of the Non-invasive imagining techniques. MRI is widely used for various purposes of assessing muscles in DMD ambulant and non-ambulant patients [54]. Loss of muscular structure infiltration of muscle with fibrous tissues, and levels of replaced fibrous tissues in muscles can be determined clearly by imaging. The majority of the articles referred to the use of MRI in assessing muscles in DMD patients. Especially muscle MRI is useful for the diagnosis and evaluation of the Progression of the diseases such as DMD [55]. A lot of research studies show that the MRI can detect changes in skeletal muscle structure and the constitution of the muscles in muscular dystrophies [56],[57],[58].In MRI, no ionizing radiation is used; hence it helps to resolve the involving muscles as well as the connective tissues fat, and bones. As an advantage of MRI, has minimal operator dependence and provides an excellent image of all muscles. Magnetic Resonance Spectroscopy (MRS) is used combined with MRI which is a non-invasive biochemical sampling technique, used to quantify the lipid fraction and metabolic by-products in muscles [59].

- *Ultra Sound Scan (USS) or Sonography*

One of the Non-invasive imagining techniques. Diagnostic musculoskeletal ultrasound is a low-cost modality that provides the characteristics of normal and pathological muscle tissues [60]. USS is a long-term technique to assess the muscular damage due to neuromuscular diseases such as muscular dystrophy and lately, alters in body and tissue composition combined with muscle wasting disorders such as Sarcopenia [60].

Key elements of USS for muscular dystrophy are sonographic measures of echo intensity for estimating tissue composition and digital calliper measures of tissue dimensions for assessing muscular atrophy [60].

The quantitative USS has been recommended frequently for DMD. When measurements of echo intensity are analyzed by using grayscale histogram analysis, provides evidence of pathological muscle changes with increased non-contractile features in DMD [60].

- *Electromyographic Signals (EMG)*

One of the Non-invasive imagining techniques. For the diagnosis of DMD patients and detection the of DMD carriers, the EMG signals have been utilized for decades[61]. To detect the motion intention of the users and maintain the restoration or helping devices; which can retard the progression of the disease and increase the life expectancy of the dystrophies, sEMG (surface-EMG) can be used [62], [63].

- *Electrical Impedance Myography (EIM)*

One of the Non-invasive imagining techniques. It is a potential therapy response biomarker [64]. This technique, uses a weak and high-frequency electrical current across a muscle and the voltages resulting are measured [65]. Alternates in the voltage result provide clues about the health of the muscle and its unity, the influence of the disease, and the therapeutic effectiveness [66].

EIM takes only a few seconds to conduct, is a painless technique, cheaper than other standard imaging techniques, and influences the patient's respiratory status or body deformities. Parameters of the technique, are sensitive to the progression of the dystrophy [67].

- *Non-Hand-Held Dynamometers*

Using for assessing muscle strength and force. Maximum isometric muscle force is the value obtained in measuring muscle strength by using dynamometers. Cybex and Biodex are some examples of non-handheld dynamometers.

A research article reviews that, in a standard clinical assessment, the measurements of muscular strength conclude the probity of the neuromuscular system and permit the setting up of association with the quality of life of the individual [68].

Mechanical or electronic dynamometers have been used in several clinical studies and the manual muscle test to analyze the grip strength has been used simultaneously. Such tests provide accurate data on the progression of particular muscle group weakness in DMD patients and it help to achieve successful therapeutic interventions [69], [70].

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There are some components which are influence muscular strength performance; such as the architecture of the muscular fiber, size of the muscle, length of the muscle when contracts, muscular average, contraction velocity, age, and gender; on the other hand, the emotional and cognitive phases of the child also influencing [71].

Dynamometer's handle's shape and its calibration are also some issues for the measurements of muscle strength [72].

- *Handheld Dynameters*

Some examples of hand-held dynamometers are Jamar mechanical dynamometer, Sphygmomanometer, Kin-Com dynamometer, Smeldy hand dynamometer, Harpenden hand-grip dynamometer, Martin Vigorimeter, single spring dynamometer, Rotterdam Intrinsic Hand Myometer, BIODEX dynamometer, Fugl-Meyer motor scale, Nicholas Hand-Held Dynamometer (NHHD), Baseline and Grip dynamometers, Penny and Giles myometer, Hand-held electric dynamometer, Spring-loaded device, Chatillon Series D hand-held spring-scale dynamometer, Preston Dynamometer, Therapeutics Instruments dynamometer, RKK grip dynamometer, My Gripper dynamometer, Duffield dynamometer, Kratos ZM dynamometer, Clinifeed/Roussel dynamometer [73].

A research article reviews that the muscle groups (knee extensors and flexors, elbow extensors and flexors) are checked through isometric and isokinetic protocol by using the Kin-Com Robotic Dynamometer and the quantitative analysis has been done. During this evaluation, the subjects are seated on a dynamometer chair and the seat of it can be adjusted to the size of the subject. To limit the compensatory movements of the limbs during performances to maintain a proper position, a seat belt around the hips as well as two around trunks have been used. The subject was asked to cross his chest with arms81. Both axes of the dynamometer and the tested joint (knee or elbow) were aligned by the evaluator. The arm and leg lengths are adjusted proportionally. Kept the knee and elbow joints in a 90-degree position, during isometric tests. It was measured by a goniometer. During the knee isokinetic test, the moving range varied from 90 degrees to 10 degrees. Asked the subject to watch the computer screen for visual feedback within the contraction. The evaluation consisted of two sessions; the first- was the lower limb lasting 40 minutes and the second upper limb approximately 15 minutes. The 30-minute rest period was there in between these two sessions. Asked the subject to do each performance three times with a maximum contraction; held for five seconds. The same evaluator and the same continuous verbal encouragements have been given during the evaluation and asked to perform each exercise as hard as he/she could [74].

IV. DISCUSSION

According to the review done most widely used effective and reliable efficient investigation method has been identified as MRI scans due to various purposes and methods of use in muscle assessment. MRI can be used as a non-invasive, painless technique which is more convenient in handling patients who are younger [75], [76], [77], [78], [79].

MRI also can be used in non-ambulant patients in muscle assessments in which manual muscle testing of functional ability muscles and muscle force assessment have failed [80].

Non-hand-held dynamometers can be used; but due to their size, complicated use, and high costs cannot be used in bedside investigations. The Hand-held dynamometer (HHD) has been used as one of the alternatives for measuring muscle strength in DMD patients' bedside and outdoor patients' investigation. It has the advantages of being portable, affordable, efficient, simple, sensitive, and objective. This instrument is held by the examiner and placed against the patient's limb during a maximal isometric contraction. The device can be used to test both proximal and distal muscles in all extremities. Specific dynamometers are used to test grip strength. The testing positions are standardized to reduce variations in serial measures [81].

There is a disadvantage in the use of hand-held dynamometers which has issues in reliability. It depends on the examiner used to carry out the muscle force assessment. The study examines intra-tester and test-retest reliability using a hand-held dynamometer for the measurement of isometric muscle strength is important. But when the patient's muscle force is less than the examiner's, then an investigation will be independent or less affected by the strength of the examiner strength [82],[83].

V. CONCLUSION

This study concluded the identifying methods in practice to use to assess the muscles in DMD patients. This includes the methods of invasive and non-invasive techniques such as serum CK level, muscle biopsies, immunoassay Wes's method, Immunofluorescence analysis method, assays, manual muscles testing, functional ability testing, Imaging techniques such as MRI, USS, EMG, EIM and muscle force testing using dynameters. In addition to clinical testing, maximum isometric muscle force testing handheld dynamometers have been widely carried out. As well during the study it was identified MRI is the most efficient, effective, and reliable method becoming the most popular in assessing muscles of ambulant and non-ambulant DMD patients

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