SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY

THIRUVANANTHAPURAM, KERALA

COMPARITIVE STUDY OF MOTOR UNIT NUMBER ESTIMATION IN AMYOTROPHIC LATERAL SCLEROSIS AND POST POLIO PARALYSIS

Thesis submitted in partial fulfilment of the rules and regulations for

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By

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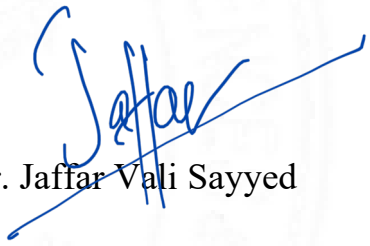
DECLARATION

I, Dr. Jaffar Vali Sayyed , hereby declare that this project was undertaken by me under the supervision of the faculty, Department of Neurology, Sree Chitra Tirunal Institute for Medical Sciences and Technology.



Thiruvananthapuram, Dr. Jaffar Vali Sayyed

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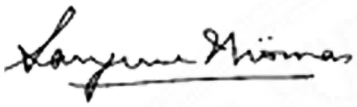

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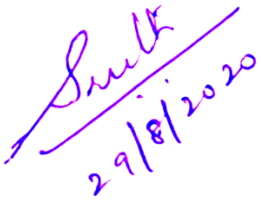

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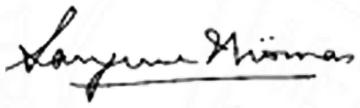

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SYNOPSIS

Amyotrophic lateral sclerosis and polio disease afflict anterior horncells. Each disease differs in their rate of progression. We hypothesized that abnormalities in electrophysiologic motor unit number estimation (MUNE) are more disproportionate to clinical severity in amyotrophic lateral sclerosis compared to post-polio paralysis. We aimed to study the correlation between disease severity and electrophysiologic motor unit number estimation in amyotrophic lateral sclerosis and post-polio paralysis. In this cross-sectional stud, we considered the baseline clinical (ALSFRS - R and MRC Sum score) and electrophysiological motor unit number estimation (MUNE). The relevance of MUNE as a predictor of disease progression in ALS and post-polio paralysis was studied in relation with the already established markers of disease progression like ALSFRS-R and MRC sum score. We found that mean values of individual upper or lower limb MUNE, and summated MUNE were higher in post-polio group than ALS groups, and higher in classic ALS group than non-classic ALS group, but these differences were not statistically significant. There was no statistically significant correlation between clinical severity scores and MUNE in classic and non-classic subtypes of ALS group. A significant positive correlation between lower limb MUNE with MRC sum score in polio group was observed. Though a moderate degree of correlation was noted between ALSFRS-R and lower limb MUNE in polio group, this observation was not statistically significant. We understand that , abnormalities in MUNE preceded the clinical dysfunction in classic and non-classic ALS groups. MUNE is non-specific and has limited diagnostic value. A single MUNE value did not differentiate a progressive disease like ALS from a static or very slowly progressive disease like post-polio paralysis. Individual limb MUNE has same relevance as summated MUNE in ALS. Lower limb MUNE has more relevance than upper limb MUNE in post-polio patients and should be considered in future studies

rather than the more commonly used upper limb MUNE technique. Clinical severity scores, ALSFRS-R and MRC sum scores, were worse in classic ALS group over non-classic ALS group.

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INTRODUCTION

MND is an umbrella term, in routine practical terminology its use was interchangeable with amyotrophic lateral sclerosis (ALS) , however technically speaking , it can be any disease which afflicts motor neurons either of upper motor neuron (UMN) or lower motor neuron (LMN). So, disease effecting motor neurons encompasses lethal, rapidly progressive degenerative disorders (ALS prototype) and also potentially treatable disorders (MND like disease, immune mediated neuronopathies). It is important to differentiate this two forms of disorders as therapeutic implications are different. MND is a disease of middle to late life with a mean age of onset of 58 years . MND is the third commonest neurodegenerative disease after Alzheimer's and Parkinson's diseases. Over all, MND is relatively rare, with an apparently uniform incidence of approximately 2/100 000 (1). Despite its rarity, the disease has attracted a lot of attention, as its devastating course places it at the center of the ethical debate about end of life decision making.

"Let us keep looking, in spite of everything. Let us keep searching. It is indeed the best method of finding, and perhaps thanks to our efforts, the verdict we will give such a patient tomorrow will not be the same we must give this man today."

Charcot (1889)

There has been growing scientific and clinical interest in ALS since the 1990s. Survival in ALS dependent on several factors, including clinical presentation, rate of progression, early respiratory failure, and the nutritional status. Prolonging life expectancy depends on improving understanding of disease pathogenesis, which in turn help in formulation of early and specific diagnostic methods. There is an important need to formulate therapies that not only slow disease progression, but also deal with the secondary consequences of malnutrition and respiratory failure.

At present, no definitive diagnostic test or biomarker for ALS exist, and neurologists rely on clinical criteria for diagnosis. The development of newer biomarkers to objectively assess disease progression helps to a greater extent in refining therapeutic trial design and reducing trial costs. There is an urgent need for a tool to identify the disease , the severity of the disease and its prognostication . This further helps in formulation of treatment plan, monitoring and plan appropriate rehabilitative measures.

REVIEW OF LITERATURE

In Greek the word 'amyotrophic' indicates "no nourishment to the muscle," lateral, refers to the lateral area in the spinal cord i.e., corticospinal tract, whereas sclerosis refers to the scarring of spinal cord occurs once motor neurons degenerate. ALS is most often encountered clinically as a sporadic, progressive, neurodegenerative disorder of unknown etiology that characteristically affects both upper motor neurons (UMNs) and lower motor neurons (LMNs), predominantly somatic motor neurons with sparing of sensory and autonomic systems. Around 10% of cases with ALS are familial(2). In addition, several variants of ALS are well recognized, including progressive bulbar palsy, progressive muscular atrophy (PMA), and primary lateral sclerosis (PLS). Because the prognosis in ALS is uniformly poor compared with other motor neuron disorders, it is essential that the correct diagnosis be reached.

DEMOGRAPHY

The incidence of ALS averages 1.8/100,000 in most of the studies (3). The average age of onset is 56 years for sporadic disease and 46 years for most dominantly inherited forms of the disease (3). Men are affected nearly twice as often as women (4)(5). Incidence increases with each decade of life. Familial forms identified, inherited as an autosomal dominant trait with age-dependent penetrance. The familial cases differ in their symptoms and clinical course from non-familial ones; as a group they have an earlier age of onset, an equal distribution in men and women, and a slightly shorter survival (6).

ETIOPATHOGENESIS

Current hypotheses regarding disease mechanisms in sporadic ALS (sALS) include oxidative damage, accumulation of toxic intracellular protein aggregates, mitochondrial dysfunction, defective axonal transport, growth factor deficiency, and/or glutamate excitotoxicity. These pathophysiological mechanisms may work in series or in parallel with eventual confluence. It is attractive to hypothesize that ALS is a consequence of environmental exposure and genetic risk. Consequently, a significant proportion of ALS research in recent years has focused on disease mechanisms in familial disease with the hope that they are relevant to sporadic disease(2). The recognition that specific gene mutations such as TARDNP, UBQLN2, and C9ORF72 may result in both fALS and apparent sALS, and that their protein products (TDP-43 and ubiquilin 2) can be found within neuronal inclusions in sporadic as well as familial forms of the disease lends support for this construct(7).

CLINICAL FEATURES OF AMYOTROPHIC LATERAL SCLEROSIS

Depending on site of onset of the disease, ALS was divided into 8 phenotypes(8)(9)(10).

AMYOTROPHIC LATERAL SCLEROSIS PHENOTYPES

1)Classic phenotype:	Onset of symptoms in upper or lower limbs with clear, but not dominant, UMN features.
2)Bulbar phenotype:	Onset with bulbar involvement (dysphagia and or dysarthria) and no peripheral spinal involvement in the first 6 months after symptom onset.
3)Flail arm phenotype:	Progressive, predominantly proximal upper limb weakness and wasting with minor (pathological deep tendon or plantar responses) or absent pyramidal signs.

4)Flail leg phenotype:	Progressive distal onset wasting and weakness of lower limbs with minor (pathological deep tendon or plantar responses) or absent pyramidal signs.
5)Pyramidal phenotype (predominant UMN ALS):	Clinical manifestations dominated by pyramidal signs, mainly severe spastic para/quadriparesis, associated with one or more of Babinski or Hoffmann sign, hyperactive reflexes, exaggerated jaw jerk, dysarthric speech and pseudobulbar affect. An onset with a predominant pseudobulbar weakness progressing to a limb weakness later in the course will be included in this phenotype. These patients should have clear evidence of LMN involvement in the form of weakness, wasting and/or active denervation and reinnervation from two or more segments.
6)Respiratory onset phenotype:	Respiratory impairment at onset, defined as orthopnoea or dyspnoea at rest or during exertion, with only mild spinal or bulbar signs in the first 6 months after onset and with signs of UMN involvement.
7)Pure LMN phenotype:	Clinical and electrophysiological evidence of progressive LMN involvement in the absence of clinical UMN signs.
8)Pure UMN phenotype:	Clinical signs of UMN involvement that is, severe spastic para/tetra paresis, Babinski or Hoffmann sign, hyperactive reflexes, clonic jaw jerk, dysarthric speech and pseudobulbar affect in the absence of clinical or EMG evidence of LMN involvement.

Limb involvement is more often distal than proximal leading to weakness of the hands or bilateral foot drop. Patients may complain of clumsiness or impaired mobility. Bulbar involvement

is characterized by weight loss, dysarthria, wasting, fasciculation and slow movement of the tongue, sialorrhoea and dysphagia. Patients complain of difficulty speaking or occasionally hoarseness which usually precedes swallowing difficulties. Clear fluids tend to aspirate causing the patient to cough after drinking. Hyper salivation becomes difficult to clear and leads to the development of drooling and dribbling. Pseudo-bulbar involvement is also associated with pathological emotional lability in which there is excessive uncontrolled laughter or crying and a brisk jaw jerk. Fasciculation is variable but may be prominent after exercise. It is well seen in the tongue and across the back. Head droop may be an early feature because of weakness of the neck extensors and paraspinal muscles. Progressive respiratory muscle weakness may lead to exertional dyspnea and selective diaphragm weakness will cause breathlessness on lying flat and progressive hypoventilation; examination shows paradoxical movements of the abdomen on inspiration. Increasing limb and truncal weakness causes worsening immobility and self-care. Bulbar weakness leads to increasing difficulty with communication and eventual anarthria with worsening dysphagia. Sleep disturbance is common and multi-factorial related to difficulty turning in bed, periodic limb movements, hyper-salivation and inability to clear secretions, difficulty with communication, emotional lability and depression. Sensory, ocular and bladder symptoms are unusual.

In the most typical forms of disease, the onset is perceived by the patient as weakness in a distal part of one limb. This is noted first as an unexplained tripping from slight foot-drop, or by difficulty in tasks requiring fine finger movements (handling buttons and automobile ignition keys), stiffness of the fingers, and slight weakness or wasting of the hand muscles on one side. The earliest manifestation of the lower motor neuron component of this disease is in the form of leg cramps as the patient turns in bed during the early morning hours. As disease progress to opposite side, hand and arm become similarly affected with weakness, stiffness, slowness, atrophy or cramps. Muscle

strength and bulk diminish in parallel or there is a relative preservation of power early in the illness. Babinski and Hoffmann signs are variably present; they may not appear even as the illness progresses. Adductors, abductors, and extensors of fingers and thumb tend to become weak before the long flexors, on which the handgrip depends, and the dorsal interosseous spaces become hollowed, giving rise to the "cadaveric" or "skeletal" hand. The muscles of the upper arm and shoulder girdles are typically involved later. There is a general tendency for adjacent areas to be involved before more distant ones. The affected parts may ache and feel cold, but true paraesthesia, except from poor positioning and pressure on nerves, do not occur or are minor. Sphincteric control is well maintained even after both legs have become weak and spastic, but many patients acquire urinary and sometimes fecal urgency in the advanced stages of the disease. The abdominal reflexes may be elicitable even when the plantar reflexes are extensor. Coarse fasciculations are usually evident in the weakened muscles. The course of this illness, irrespective of its particular mode of onset and pattern of evolution, is progressive. Half the patients succumb within 3 years of onset and 90 percent within 6 years(8).

FUNCTIONAL/DISABILITY SCORES IN ALS

Several disability scores have been proposed for ALS. The ALS functional rating scale (ALSFRS) is the most widely used functional scale for ALS. In a single-centre study done by Kauffman et al., ALSFRS-R (revised form of ALSFRS) was found to be significantly related to outcome, among all sub components of ALSFRS-R, the respiratory sub-score was found to be the single most factor for deciding outcome (11). In another study by Kimura et al., the progression rate of the ALSFRS-R, calculated as differential of score from onset to diagnosis/disease duration, resulted to be significantly related to prognosis (12). There were several other disability scores which have been used for assessing ALS progression in previous studies. In a single centre tertiary ALS

centre study for validating Appel ALS Score (AALSS) by Haverkamp LJ et al., they found that AALSS score was a good predictor for disease progression(13). This finding was confirmed by another study performed at same centre (14).

Motor Unit Number Estimation

Motor unit:

Motor unit consists of an anterior horn cell, its motor axons, NMJs, and innervated muscle fibers. The extracellular needle EMG recording of a motor unit is the motor unit action potential (MUAP). The number of muscle fibers per motor unit differs from 5 to 10 in laryngeal muscles to thousands in the soleus. The transverse territory of a motor unit usually ranges from 5 to 10 mm in adults, with many motor unit territories overlapping with one another(15). Because of this overlap, two muscle fibers from the same motor unit rarely lie adjacent to each other. Transverse motor unit territory increases greatly with age, doubling from birth to adulthood, mostly because of the increase in individual muscle fiber size. The normal aging process results in a slow dropout of motor units(16). Following table summarizes various electrophysiological methods studied in ALS.

Method	Technique	Advantages	Disadvantages	Relevance for ALS
CMAP amplitude(17)	By conventional NCS technique	Non- invasive Can be done at proximal and distal muscles It is a simple, rapid Its underlying methodology is familiar and easily standardized in different laboratories.	CMAP amplitude measures do not enable detection of the effects of collateral reinnervation in masking MUs loss	CMAP amplitude correlates well with muscle strength as determined by maximum voluntary isometric contraction MVIC or as determined by electrical stimulation(18). There was a significant correlation between CMAP amplitude and the motor unit number estimation (MUNE)(19)
F-waves and H-reflex(20)	Conventional NCS techniques	Easy to perform compared to newer techniques	Influenced by the presence of spasticity	F-wave frequency is reduced in weak muscles in ALS, but not closely related to the clinical progression. F-wave frequency was probably influenced influenced by upper motor neuron signs(21).

Method	Technique	Advantages	Disadvantages	Relevance for ALS
Fibre density (22)	Quantification of EMG abnormalities can be achieved by measuring the fibre density (FD) using the technique of single fibre EMG	Early in the course of ALS, collateral reinnervation by surviving MUs can compensate for motor neuron loss, causing increased FD values in muscles not yet weak . This capacity for reinnervation is greatest at a relatively early stage of the disease (23).	Patient discomfort, and the long period of training	Overall in lack of correlation between FD and strength
MacroEMG (24)		Provides a measure of the size of a motor unit and thus of collateral reinnervation.	Macro EMG is a relatively complex and invasive technique	This technique was applied longitudinally for six months in one study, but was not sensitive in detecting change(25).

Motor Unit Number Estimation:

Determination of MUNEs is a quantitative method of assessing loss of anterior horn cells (AHCs) (19)(26)(27)(28). The number of motor units in a muscle is estimated in MUNEs. The method is most conveniently performed in distal muscles that lend themselves to surface stimulation and recording. This techniques requires 5 to 10 min per nerve for full assessment. Reproducibility is now comparable with that for CMAP (25). Reinnervation by collateral sprouting can prevent reduction in strength and CMAP amplitude with loss of up to half the motor units in a slowly progressive ALS(29). The initial period of disease will have rapid loss of motor unit loss, later the drop will be gradual for the remaining motor units. In ALS, some of the S-MUPs seen on MUNEs are much larger than others, indicating much more collateral sprouting and increase in size. Quantifying motor unit number estimates found to be the most reliable method to measure the loss of motor neurons in clinical trials(28)

Considerable loss of motor units may occur without clinically evident weakness. This is particularly true in slowly progressive disorders where collateral sprouting and reinnervation are at least partially compensatory. In patients with ALS, it has been shown that the compound muscle action potential (CMAP) amplitude may not reliably decline until the estimated number of motor units drops below 10% of normal(30). Numerous motor unit number estimation (MUNE) techniques have been developed in an attempt to count the number of viable motor units in a given muscle(30)(31)(29). This has been done with the assumption that MUNE would represent a more accurate means to monitor disease course or to detect a response to treatment than measurements of strength. All techniques attempt to estimate motor unit number by estimating the average size of the amplitude generated by a single motor unit, and then dividing this number into the maximal CMAP amplitude for the entire muscle. MUNE is, in large part, a research technique, with limited application

in the daily practice of neuromuscular disease(25). It can be time consuming and, with some techniques, technically challenging due to unstable neuromuscular transmission that may occur with reinnervation. It has proven to be among the most sensitive measures of progression over time in patients with ALS, outperforming other clinical measures in head-to-head trails (32)(31)(23)(26)(33). MUNE has also been used to demonstrate loss of motor units with normal aging and in patients with SMA, CMT, and post-polio syndrome, among others(34)(31).

Role of MUNE as an indicator for disease progression in ALS:

There is a growing interest and need in methods to monitor disease progression in amyotrophic lateral sclerosis (ALS). A reliable and sensitive method is relevant, for example, as an outcome measure in therapeutic trials. There are well established and reliable clinical methods to monitor disease progression in the course of the illness, this includes revised ALS functional rating scale (ALSFRS- R) and the Medical Research Council (MRC) scale by MRC-SUM score. However quantitative methods, which has important advantage of directly relating to the underlying disease process are of interest. Motor unit number estimation (MUNE) techniques are based on surface electromyography (sEMG) measurements. As this is a non-invasive and, in contrast to the MRC scale and the compound muscle action potential (CMAP), are not influenced by the compensatory reinnervation process following denervation due to lower motor neuron degeneration, this method has extra advantage over the rest. The number of axons innervating a muscle or group of muscles is a critical piece of information in identifying and characterizing a neurogenic disease. Techniques to estimate motor unit number (MUNE) measure the number of functioning motor units in a muscle. Traditional methods include the measurement of amplitude on nerve conduction studies (NCS) and of motor unit potential (MUP) recruitment on needle electromyography (EMG). Both methods provide useful information in clinical diagnostic EMG, but do not provide numerical measures that

can be reliably compared with either normal values or changes over time. Motor unit number estimation (MUNE) attempt to rapidly and reliably measure the actual number of axons that innervate a muscle. In many cases such a measure of the loss of axons in a neuromuscular disease would be more valuable than the standard NCS measures of amplitude, latency and conduction velocity or the standard EMG measures of fibrillation, MUP recruitment and MUP measurements. The following table summarises various methods of estimating MUNE , their advantages and drawbacks .

Modality	Technique	Advantages	Drawbacks	Current state
MUNE by Incremental method(35)(36)	Different axons having differing excitation thresholds. With step-wise increases in stimulus intensity used to recruit additional discrete motor units	Retest reliability	Alternation phenomena : 2 or more different SMUPs are evoked in alternation rather than simultaneously, due to a very similar activation threshold (25)	Sensitive ,reliable out performing other functional clinical measures in demonstrating disease progression (37)
MUNE by Multiple point stmulation (MPS) method (38)(35)	Stimulation at distinct points along the nerve in an attempt to sample different motor axons .	Attempted to circumvent the Alternation phenomena	Tolerability	Preclinical AHC loss
MUNE by Combined method (31)(39)	Combining both techniques MPS+ Incremental	Simple, Rapid ,Reliable		
Statistical method (Stat-MUNE) (28)(31)	Axons of individual MUs fire at near threshold intermittently, there is an inherent variation in the size of a submaximal CMAP	By applying Poisson distribution ,the variance of a number of measurement is equal to the size of the individual components making up each measurement	Standard EMG equipment along with Special software and expertise	Accurately represent size of the SMUPs recorded at different levels Good retest reliability
High-density MUNE(40)	Utilizes a large number of electrode channels to resolve	Feature not offered by most MUNE techniques	Requirement for specific equipment and software	Demanding technical competency limited its use

	alternation, whilst also enabling the measurement of proximal and distal muscles			
Method	Technique	Advantages	Disadvantages	Relevance for ALS
F-wave method (FWM-MUNE) (20)(24)	F-waves consistently repeating with the same morphology represent single motor unit discharges. Identifying those f waves and digitally computing MUNE	Easy for experienced	limited availability of the software programme and the concern that unstable neuromuscular junctions might excessively interfere with accurate template matching	No head to head trials with other modalities
MScanFIT MUNE (MScan)(33)	It is based upon the acquisition of multiple CMAPs at incremental stimulus intensities ranging from subthreshold to supramaximal (CMAP scan)	Provide information on all motor units contributing to the CMAP	Needs technical expertise Needs Bayesian statistical method Expensive	Superior reproducibility, detection of motor unit loss, and disease progression compared to other MUNE methods Preliminary findings appear promising(29)

Evidences for MUNE in diseases of anterior horn cells :

MUNE has its role as a biomarkers in disease progression, in a study by **Kevine J Felice et al.,** 21 ALS patients with mean age of 58 years were studied. Multiple point stimulation technique was used. Thenar muscle MUNE was estimated at baseline , 4, 8, and 12 months. In addition thenar muscle CMAP, isometric hand grip strength, total MRC score, Appel ALS rating scale, and forced vital capacity (FVC) were observed. They concluded an absolute mean MUNE change per month was significantly greater over rate of change in MRC and FVC values(32).

The findings are consistent in another study by **De Carvalho et al.,** they prospectively studied the role of MUNE and the neurophysiologic index NI, they noted that these markers were significantly correlated with abductor digiti minimi muscle (ADM strength). MUNE and the NI were reliable, but the NI showed a lower variation. (25).

In 2004, **Shefner et al.,** prospectively studied reliability of MUNE in a multicentre trial. They studied reliability and sensitivity of MUNE. Test–retest reliability was high while on testing with normal subjects. Among patients, there was a 23% decline in MUNE over 6 months.

Swoboda, K. J. et al., studied MUNE in spinal muscular atrophy (SMA). This was a prospective study where they analysed 89 patients with SMA, correlation between MUNE and maximum CMAP, correlated with SMN2 copy, age, and function. They observed greater motor unit loss in types 1 and 2, and least in type 3. Possibility considered here was, a balance maintained between reinnervation of muscle fibres by the remaining motor neurons with ongoing motor neuron loss presumably allows for the relative maintenance of strength for longer periods of time despite disease progression. (34).

In 2006, **Sorensen et al.,** prospectively studied a cohort of polio survivors over a period of 15 years with respect to MUNE and strength. they identified a significant association between thenar

MUNE and arm strength, extensor digitorum brevis MUNE and leg strength, and the summated MUNE and global strength of the polio survivors. In this study they have avoided sampling bias by choosing upper and lower limb unlike the previous studies , however the age related drop in motor unit numbers was not adjusted , hence the reason for drop in MUNE was age related or disease related was not sure(41).

In an Indian study by **Jagtap et al.,** in 2015, looked into the rate of progression in ALS using multipoint incremental MUNE and compared MUNE, ALSFRS and MRC sum score at baseline and at 6 months during the course of the disease . 29 ALS patients were studied, where predominant group was spinal onset group. They observed a significant drop in MUNE values over ALSFRS and MRC sum scores in both the groups , more in bulbar onset ALS phenotype. They concluded that MUNE had the highest sensitivity for progression of the disease when compared to the ALS FRS and MRC sum score(39).

In a south Korean cross sectional study by **Kollewe et al.,** 143 patients with ALS were studied, here MUNE was estimated by statistical MUNE method, functional status was assessed by ALSFRS-R. By using mean values of MUNE according to disease duration, regression equation between mean MUNE and disease duration was presented as a formula. All patients were classified into 2 groups (MUNE ratio <1 vs. MUNE ratio ≥1) according to the MUNE ratio. Comparison between the 2 groups revealed that the patients in MUNE ratio <1 group or MUNE ratio ≥1 group were respectively assigned to rapid progression or slow progression. This study demonstrates, at a given point of time statistical MUNE highly correlates with disease duration and ALSFRS-R. Following table summarises previous study designs on MUNE , results and conclusion.

Author	Design	Technique	Comparison parameter	Result	Conclusion & Remarks
Kevin J. Felice(32)	Prospective at 4,8,12 months N 21	MUNE using MPS (APB)	Isometric hand grip strength, MRC score, Appel ALS rating scale, and FVC	The absolute mean rate of change per month was significantly greater for MUNE values than for MRC, FVC values.	MUNE values were the most sensitive index for documenting changes in disease progression over time
Eric C. Yuen et al.,(23)	Prospective at 0,3 and 6 months	Fibre density, CMAP amplitude, and MUNE of the ADM and grip strength measured at 0,3 and 6 months.		A significant decrease in MUNE and increase in fibre density were observed at months 3 and 6 compared with baseline . Mean CMAP and grip strength declined, but not significantly. The decrease in MUNE over 6 months was significantly greater than that of CMAP and grip strength .	MUNE and fibre density are more sensitive than CMAP and grip strength in detecting progression of ALS.
Shefner et al.,(31)	Prospective; 50 healthy subjects were evaluated twice and 71subjects with ALS were studied repeatedly for up to 500 days.	Combined MUNE technique		Subjects with ALS showed clear decrements over time, with an overage rate of decline of approximately 9% per month	Multipoint incremental MUNE has number of attributes that make it attractive as an outcome measure in ALS and other diseases characterized by motor unit loss
Timothy J. Doherty et al.,(26)		Motor unit estimate (MUE)	Multiple point stimulation (MPS) as a method of estimating the numbers of motor units in the median innervated thenar muscles of young and older control subjects	In 37 trials from 17 younger subjects (20-40years), the mean MUE was 288 ± 95 SD based on negative peak area and, in 33 trials from 20 older subjects, mean values were 139 ± 68.	Drop in MU with age
Kevin J. Felice(32)	Performed by the same examiner either on separate days or on the same day with new electrode placements. 20 patients with ALS and 16 normal subjects	MUNE by MPS to check reproducibility		The test-retest correlation coefficient (r) was 0.99 for ALS patients and 0.85 for normal subjects	MUNE by MPS has good reproducibility

Kollewe et al., (42)	143 ALS patients	Statistical MUNE MUNE ratio <1 vs. MUNE ratio ≥1; Compared with ALSFRS-R.		Patients in MUNE ratio <1 group or MUNE ratio ≥1 group were respectively assigned to rapid progression or slow progression.	
Nandedkar et al.,(43)	Prospective study	MUNIX MUSIX	To check reproducibility And to estimate its role in disease progression	In healthy subjects, MUNIX showed good reproducibility. In serial studies, healthy subjects showed no change in the CMAP amplitude and MUNIX. ALS patients with minimal change in CMAP amplitude had a significant drop in MUNIX and increase in MUSIX, indicating MU loss compensated by reinnervation. When the CMAP changed significantly (>30%) in 1 year, the CMAP and MUNIX decreased in parallel.	MUNIX would be useful to study MU loss in degenerative diseases of motor neurons.
A.B. Jacobsen et al., (33)		Mscan	MUNE -MPS MUNIX	The mean Coefficient of variation for MScan was significantly lower than for MPS or MUNIX . MScan and MUNIX were significantly quicker to perform than MPS . MScan and MPS were significantly better at discriminating between patients and healthy controls than MUNIX.	MScan was more consistent than MPS or MUNIX and better at distinguishing ALS patients from healthy subjects

HYPOTHESIS, AIMS AND OBJECTIVES

HYPOTHESIS

Abnormalities in electrophysiologic motor unit number estimation are more disproportionate to clinical severity in amyotrophic lateral sclerosis compared to post-polio paralysis.

AIMS OF THE STUDY

We aimed to study the correlation between disease severity and electrophysiologic motor unit number estimation in amyotrophic lateral sclerosis and post-polio paralysis.

OBJECTIVES

1. To estimate the MUNE in patients with ALS and post-polio paralysis and study the correlation with ALSFRS-R functional disability score and MRS sum score.
2. To compare the cross-sectional MUNE between ALS and post-polio paralysis.
3. To study the cross-sectional MUNE in ALS in relation to disease duration and clinical presentation.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board **(SCT/IEC/1340/FEBRUARY 2019).** Study was carried from February 2019 to February 2020. The patients with ALS, as defined by the modified El Escorial Criteria and post polio paralysis were included in this study. Those patients were excluded if they had another disease that could impact assessment of peripheral motor neuron loss due to ALS. Modified ALS functional rating scale as well as the Medical Research Council (MRC) sum score were calculated at baseline. Subjects were recruited from the neurology wards, outpatient department and EMG lab with the disease under study.

Baseline data Is defined as the data which was recorded at the time of first visit to our hospital which included basic demographics, clinical severity by ALSFRS-R score, MRC SUM score and electrophysiological data. Electrophysiological follow up could not be done in the patients due to restrictions in travel and non-essential procedures related to the pandemic in the latter part of the project.

DEFINITIONS OF CASES

ALS -Fulfilling definite or probable criteria by Modified El Escorial Score

Diagnostic categories: Clinically definite (1) UMN plus LMN signs in the bulbar and two spinal regions OR (2) UMN plus LMN signs in three spinal regions Clinically probable: Clinical evidence of UMN plus LMN signs in at least two regions with UMN signs rostral to LMN signs.

Polio – patients who had Prior paralytic poliomyelitis with evidence of motor neuron loss, as confirmed by history of the acute paralytic illness, signs of residual weakness and atrophy of muscles on neurologic examination, and signs of denervation on electromyography.

ELIGIBILITY CRITERIA

Inclusion criteria :

1.Age more than or equal to 18 years.

2.Fulfilling diagnostic criteria for the individual conditions.

3.Consenting for the procedure

Exclusion criteria:

1.No other co-existing lower motor neuron or muscle disease (like neuropathy or myopathy).

2.Abnormal sensory nerve action potentials in nerve conduction tests.

CLINICAL METHODS

Disease severity was monitored at baseline such as the ALS functional rating scale (ALSFRS-R) and the Medical Research Council (MRC) scale was used.

1.Medical Research Council (MRC) sum score, the total MRC sum score ranges from 0 (total paralysis) to 60 (normal strength). The score is the sum of the MRC score of 6 muscles (3 at the upper and 3 at the lower limbs) on both sides, each muscle graded from 0 to 5.The following muscles were examined: Deltoid, Biceps, Wrist extensors, Iliopsoas, Quadriceps femoris, Tibialis anterior.

2. The Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS) is an instrument for evaluating the functional status of patients with Amyotrophic Lateral Sclerosis. It can be used to monitor functional change in a patient over time. It measures (1) speech,(2) salivation,(3) swallowing ,(4) handwriting ,(5) cutting food and handling utensils (with or without gastrostomy) ,(6) dressing and hygiene , (7) turning in bed and adjusting bed clothes , (8) walking ,(9) climbing stairs ,(10) breathing. One weakness of the ALSFRS as originally designed was that it granted disproportionate weighting to limb and bulbar, as compared to respiratory, dysfunction. A revised version of the ALSFRS incorporates additional assessments of dyspnoea, orthopnoea, and the need for ventilatory support.

3. Electrophysiological testing:

We used Viking select neurodiagnostic version-11 system, NCS/ EMG machine for the study. MUNE was estimated by using the standard belly-tendon montage. The recording was done from one upper limb and one lower limb . The better functioning limb was taken for the study. Recording electrodes were placed on the median nerve innervated abductor pollicis brevis (APB) muscle and

peroneal innervated tibialis anterior muscle (TA). There were 3 stimulus locations used for each muscle; for the median nerve, stimulus locations was at 2 cm proximal to the wrist crease, 4 cm proximal to the first stimulation site, 6cm from wrist crease. For lower limb at fibular head, 2 cm proximal to fibular head, 4 cm proximal to fibular head. Filter settings were kept at 2 Hz–10 KHz. Sensitivity for CMAP was kept at 5 milli volts and SMUAP measured at 200 micro volts. Time base was at 5 milli second per division. At destined proximal stimulation sites , we identified better response by moving the stimulator, at better site we gave supramaximal stimulation to get CMAP. The location was marked, and stimulating electrodes were applied; self-adhesive circular motor electrodes were used. For the most distal site, a maximal response was obtained. Subthreshold stimuli was applied at a rate of approximately 1/second, with stimulus intensity increased slowly until an all-or-nothing initial response obtained. Baseline to peak amplitude was measured. For both initial and subsequent incremental responses, the minimum negative peak amplitude considered to be acceptable for recording was at 25 μV. Tracings with an initial positive component was measured from baseline to negative peak as well, disregarding the positive portion of the response. The initial response was recorded on trace 1, after which stimulus intensity was increased until obtaining a clearly defined incremental response (of more than 25 μV incremental amplitude). This response was recorded on trace 2, and a second increment was obtained with further slight increase in stimulus intensity. The final potential was recorded on trace 3. The negative peak amplitude of the third response was recorded. These three potentials were recorded at each site using replicate method. Procedure of getting stimulation at the second and third location were identical to the first location.

Calculation of MUNE and single motor unit action potential amplitude (SMUAP):

The amplitude of the third response at each site were summated , and the result value was divided by 9 to yield the average single motor unit action potential (SMUP) amplitude. The average single motor unit action potential amplitude was divided by the maximum Compound muscle action potential (CMAP) amplitude to yield the MUNE.

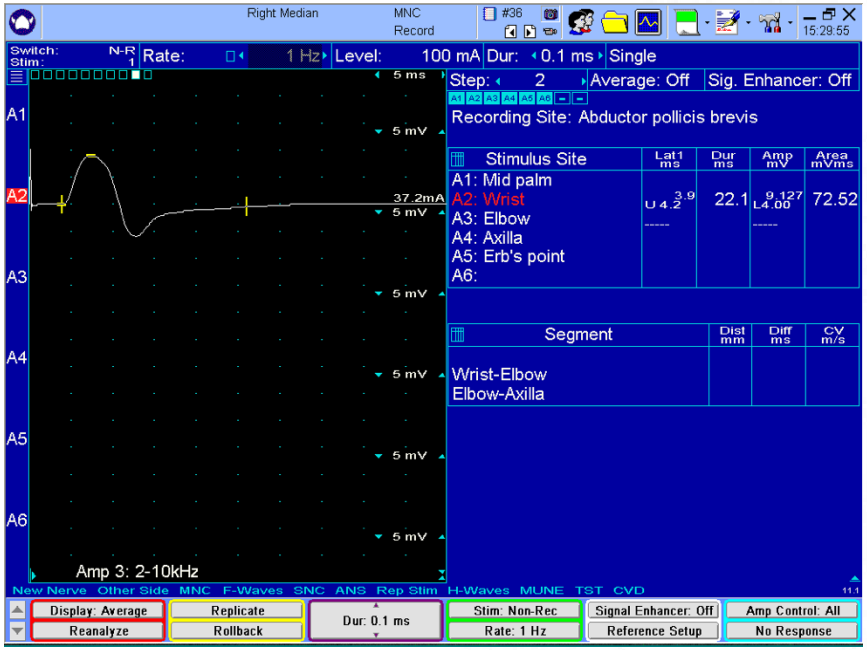
Note : For the hand, motor and sensory nerve conduction studies of the median nerve were performed using standard techniques, to rule out the presence of median neuropathy at the wrist. If a median neuropathy was detected sensory and motor studies of the ulnar nerve were performed. If a significant ulnar neuropathy at the elbow or wrist was detected, or the CMAP amplitude was less than 5 mV, the other hand was studied in similar fashion.

The underlying goal was to choose a nerve/muscle not affected by focal neuropathy and with a CMAP amplitude in the low normal range. If all nerves studied had CMAPs reduced in amplitude, the nerve with the largest motor response was chosen for study. The reason being to have a comparative baseline value during the follow up assessment.

STATISTICAL METHODS:

Statistical analysis was done using SPSS, Windows, Version 21.0 (IBM, Armonk, New York). Variables were analysed by independent samples t test and Pearson correlation studies were done for bivariate analysis. $p < 0.05$ was taken as significant.

SAMPLE ELECTROPHYSIOLOGICAL STUDY DONE ON ONE OF OUR PATIENT WITH CLASSIC ALS



A. CMAP wave form , while at wrist stimulation, at a 5 milli volts sensitivity, time base 5 milli seconds , filter setting at 2-10 Khz. was note the sensitivity for SMUAPs was set at 200microvolts

B. SMUAP wave form, three traces at A1, A2, A3 with three replicates at each stimulation location. Note : Sensitivity at 200 mic volts, time base at 5 milli seconds per division. Filter setting at 2-10 Khz.

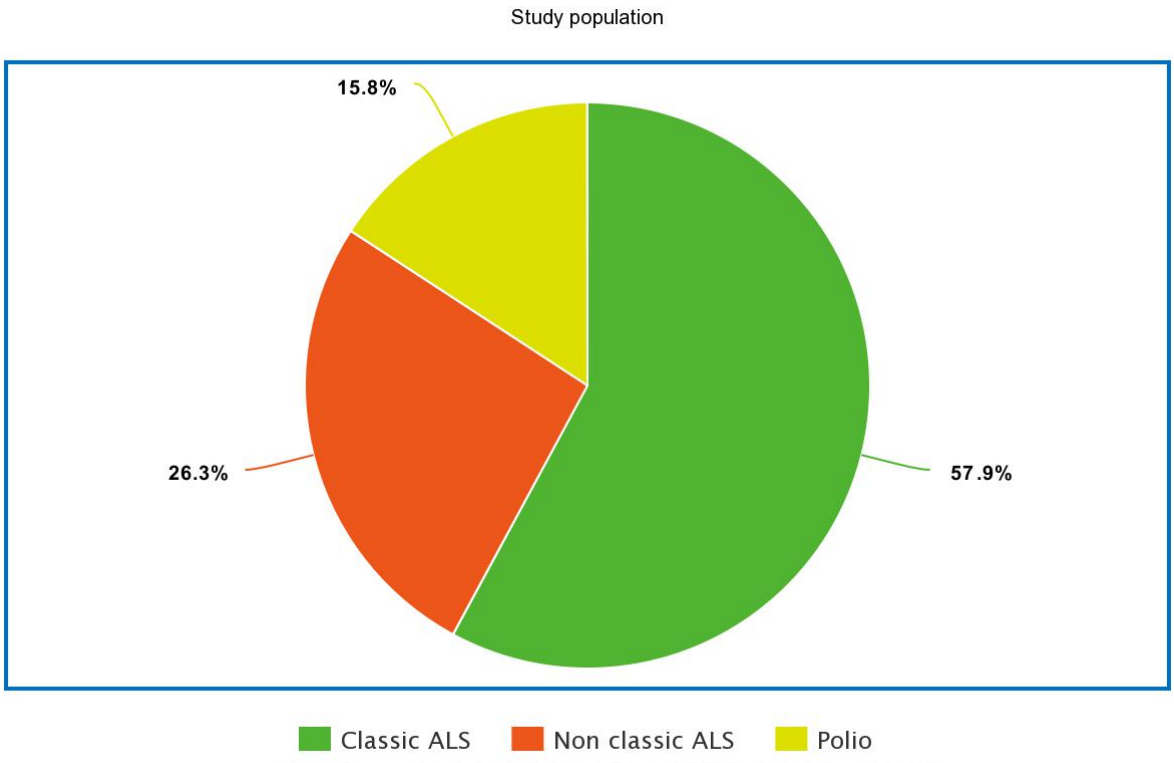
RESULTS

DEMOGRAPHIC DATA

We recruited 38 patients for the study, 32 with a diagnosis of ALS and 6 with diagnosis of post-polio paralysis.

The ALS group was divided into classic ALS (spinal onset group) and non-classic ALS (other subtypes), with 22 subjects in classic and 10 in non-classic groups (figure 1). Among the 10 patients in non-classic ALS group , one patient had pure LMN phenotype, one respiratory onset phenotype, and the rest of the patients were bulbar onset ALS phenotype.

Figure 1 : Study population



AGE AND GENDER

The mean age of the study population was 54 (± 10.7) years (figure 2). The patient distribution across the age groups was comparable in the 3 groups. Mean age of classic ALS group was 54.2 (±9.69) years, non-classic ALS group 53.6 years (±11.01) and post-polio group was 53.8 (±13.08) years.

Figure 2 : Age of study population

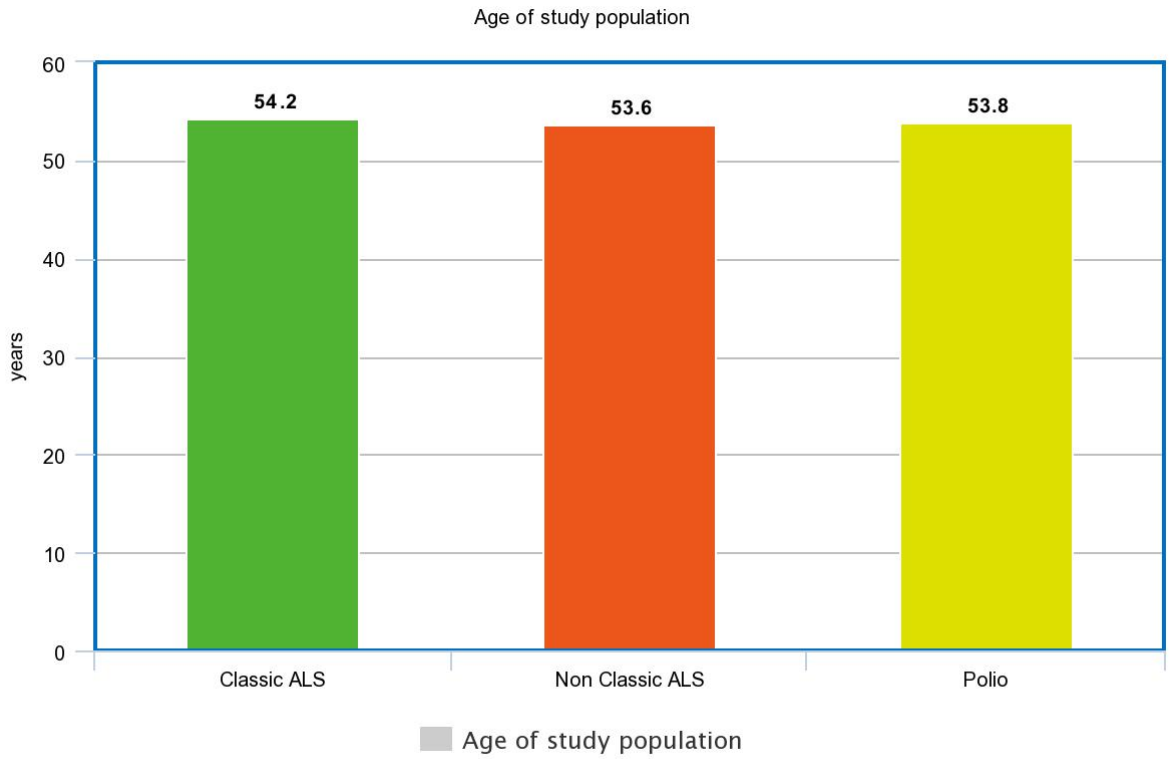
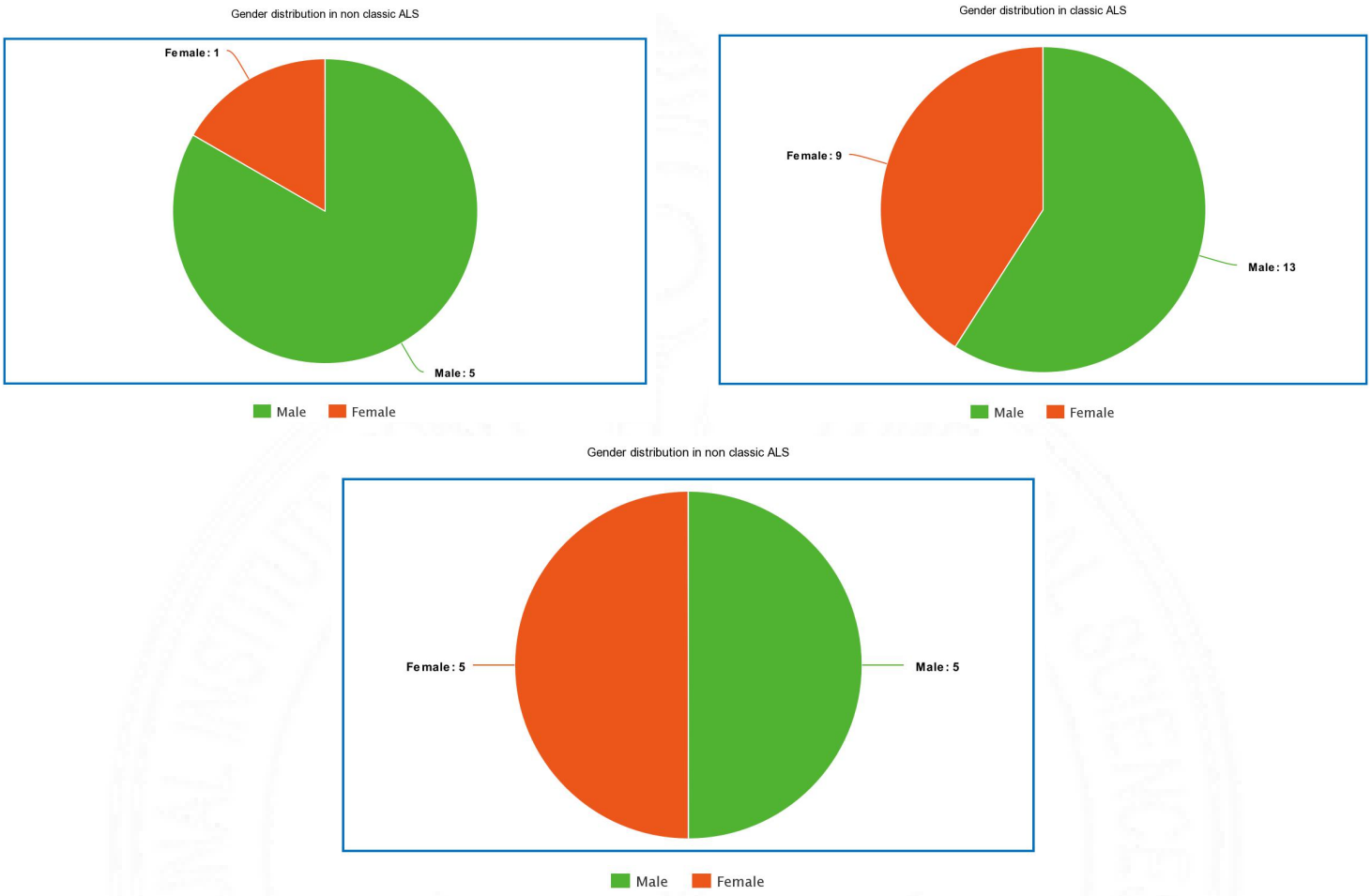


Figure 3 : Gender distribution in different groups



Classic ALS patients group was predominantly composed of male population whereas gender distribution in non-classic ALS patients was equal. Only one patient was female among 6 post-polio patients (figure 3) .

DISEASE DURATION

At the time of presentation, mean duration into the illness in classic ALS group was 16.22 (±11.38) months, and non-classic ALS group was 13.5 (±7.33) months (figure 4). When compared, non-classic ALS phenotypes had presented 2 months early, earliest being respiratory onset phenotype who presented at the 6th month into the illness with significant orthopnea. In the post-polio group, mean age of acute polio myelitis was 4.6 (± 0.94) years. Mean duration of presentation from acute poliomyelitis was 564 (±155.69) months.

Figure 4 : Distribution of ALS patients according to average disease duration

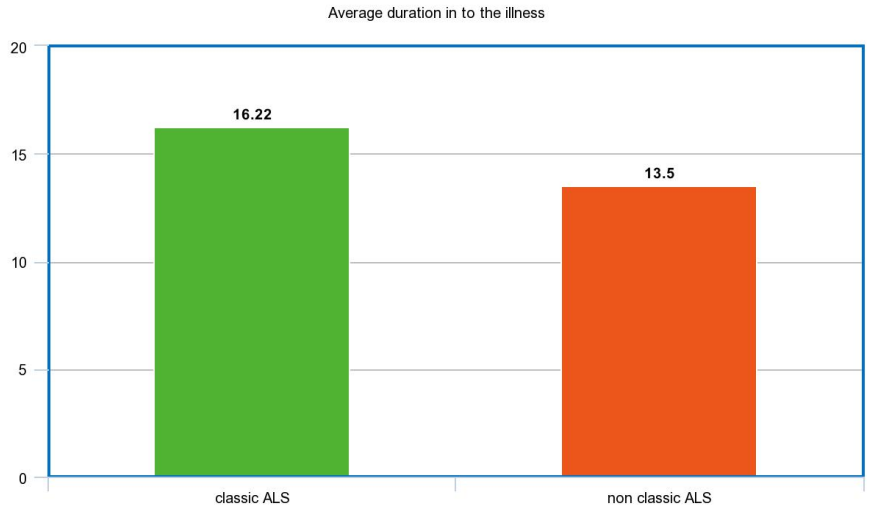
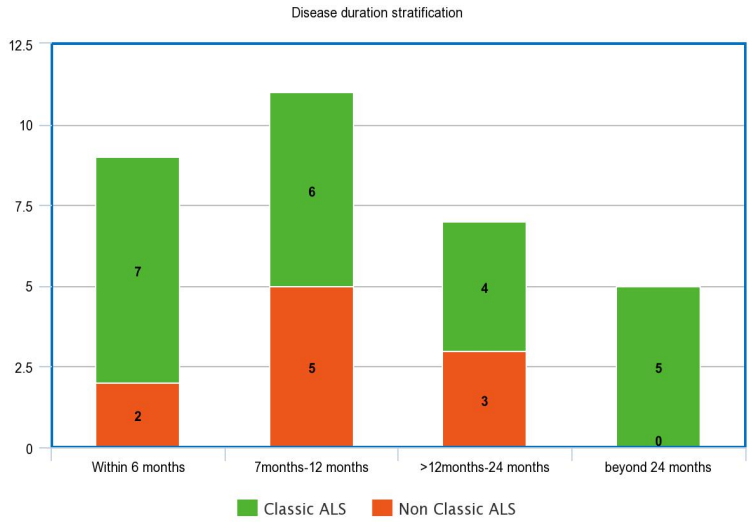


Figure 5 : Disease duration stratification ALS



Majority of the patients in the classic and non-classic groups presented within 12 months from the onset of the illness. Five patients who presented beyond 2 years had a classic presentation (figure 5) . Nearly 85% of the patients with limb-onset phenotype who crossed average disease duration of bulbar onset phenotype also had bulbar symptoms.

CLINICAL GRADING

Two clinical grading systems were considered for analysis: Revised amyotrophic lateral sclerosis functional rating scale (ALSFRS-R) and the medical research council (MRC) sum score (figure 6). The normal value of ALSFRS-R score is 48 and lower values indicate higher levels of disability. The mean ALSFRS-R score was 37.7 (±5.6) in classic ALS group and 38.2 (±6.04) in non-classic ALS group; this difference was not statistically significant. We applied the same score in the post-polio group to estimate the disability status, and the mean score was 46.5 (±1.25) in this group. The difference between mean ALSFRS-R score between ALS group and post-polio groups was significant.

Figure 6: ALSFRS-R among classic ALS, non-classic ALS and post-polio groups

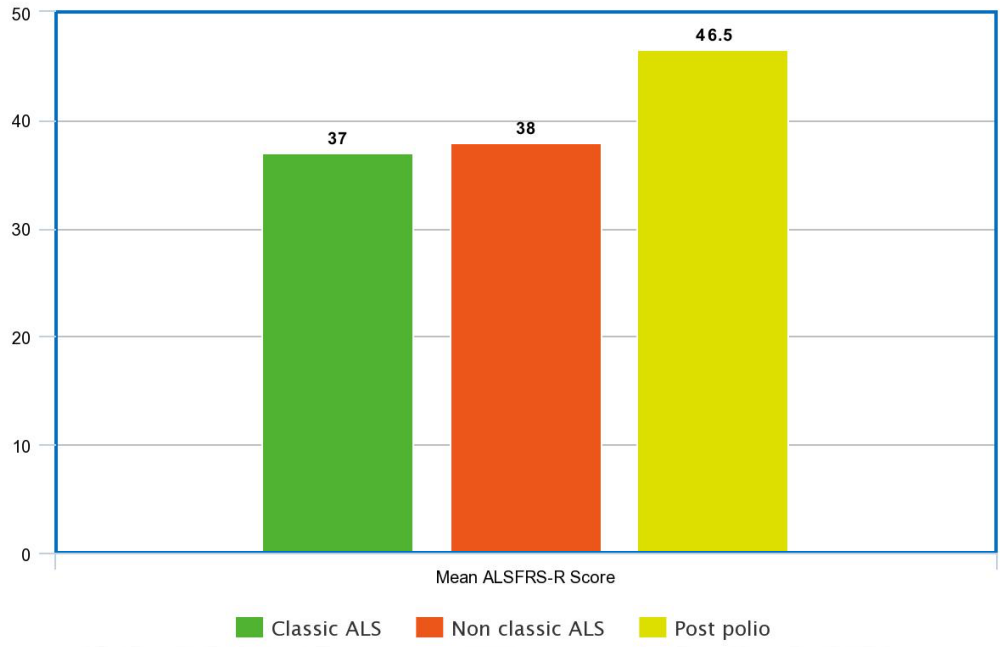
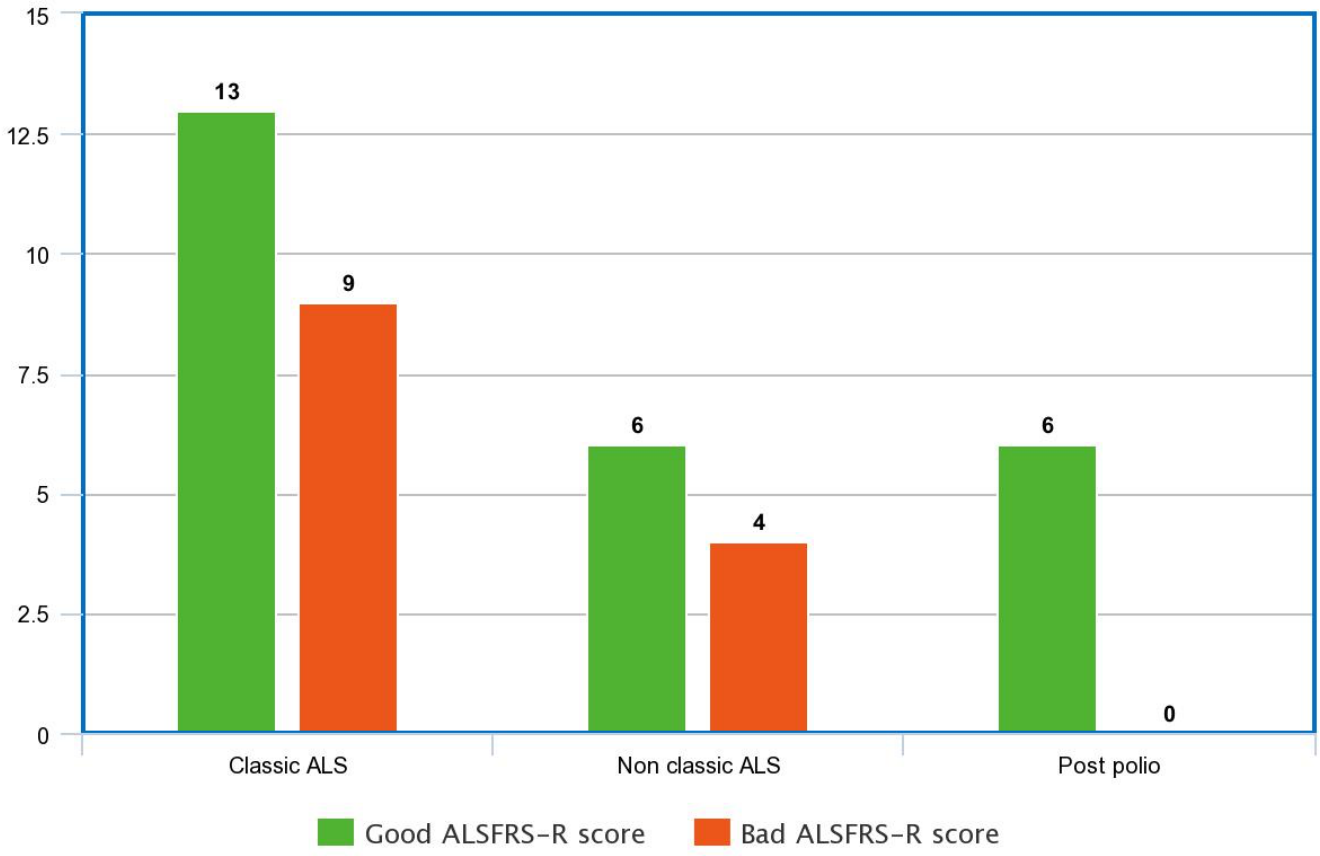
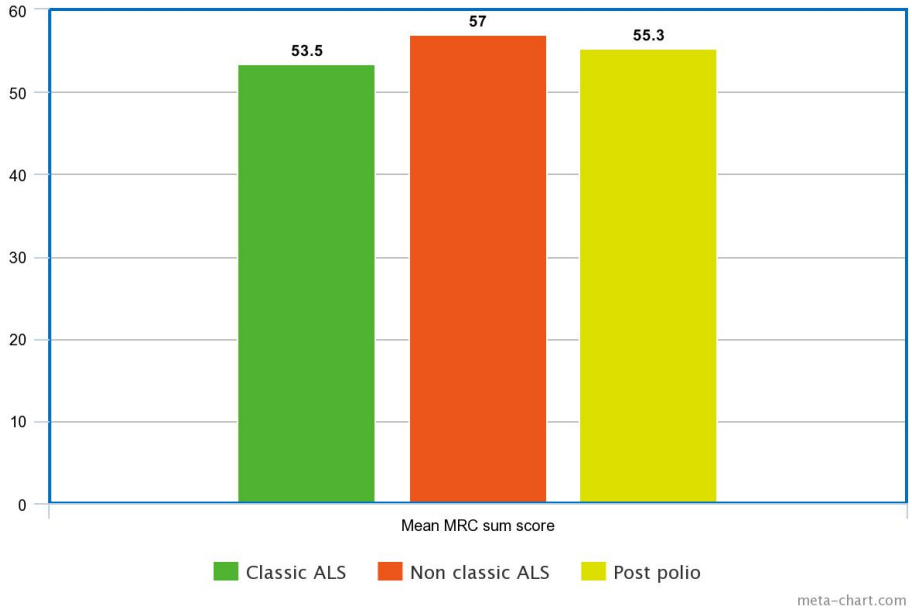


Figure 7: Stratification by ALSFRS-R among classic ALS, non-classic ALS and post polio groups



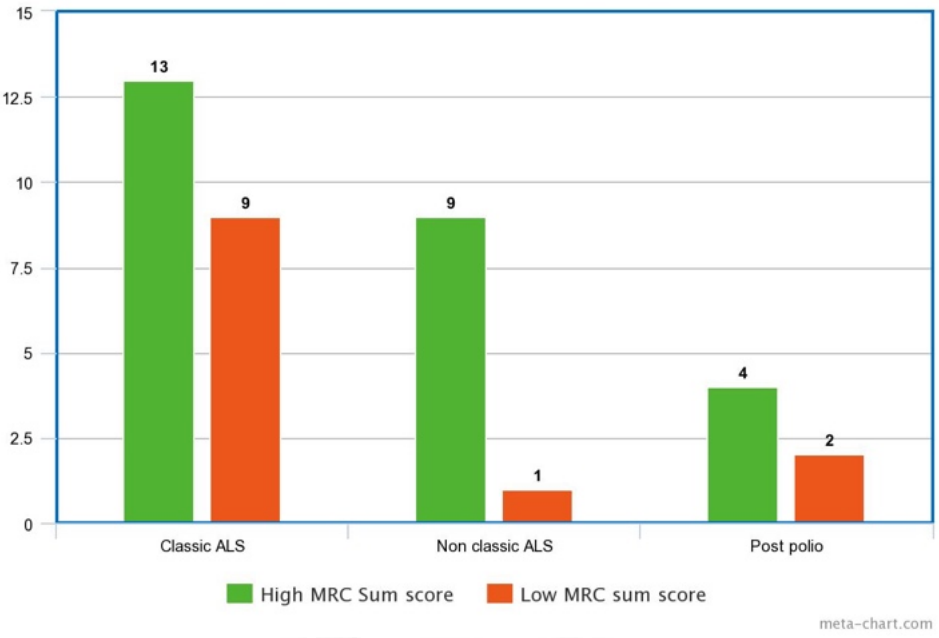
The MRC sum score is calculated as the sum of the power (MRC scoring) of shoulder abduction, elbow flexion, wrist extension, hip flexion and knee flexion and toe dorsiflexion each side. The total score is 60 points with a maximum of 5 each for individual task. In study population, mean MRC sum score was 53.54 (± 5.92) in classic ALS group , 57.4 (±3.55) in non-classic ALS group, 55.3 (±4) in polio group (figure 8). We divided each disease subgroup into high MRC and low MRC. Those whose total MRC sum score above the average score of study population was taken as high MRC and those score below the average MRC sum score of study population was taken as low MRC.

Figure 8 : MRC Sum score among classic ALS , non-classic ALS and post polio groups



When compared in individual group, majority of patients had higher MRC sum score. 9 out of 10 patients in non-classic ALS group had higher MRC sum score.

Figure 9 : Stratification by MRC Sum score among groups

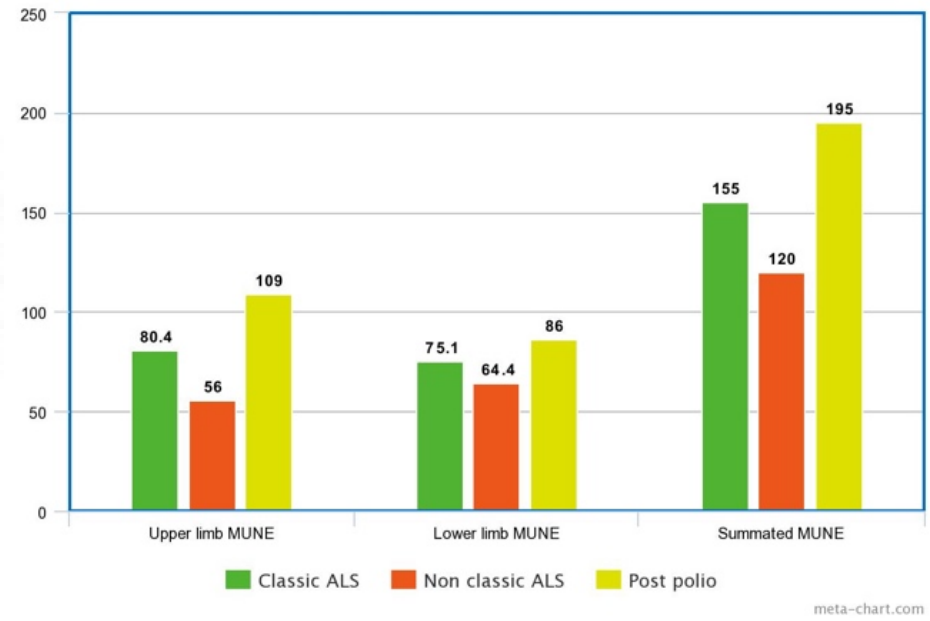


ESTIMATION OF MUNE

In classic ALS group the mean CMAP amplitude in upper limb was 5.62 (±3.66) mV, and in lower limb was 12.9 (±20) mV. In non-classic group the mean CMAP amplitude in upper limb was 5.9 (±4.3) m, and in lower limb was 9.6 (± 16.4) mV. In the post-polio group, mean CMAP amplitude in upper limb was 6.4 (±3.5) mV and lower limb mean CMAP amplitude was 4.5 (±3.6) mV.

We analyzed MUNE by the techniques described previously. In ALS group we analyzed the least affected limb. In the post-polio group we analyzed most affected limb. In classic ALS group, mean MUNE in upper limb was 80.4 (±93.57), lower limb was 75.1(±34.71), and summated MUNE was 155(±128.33). In non-classic ALS group, mean MUNE in upper limb was 56(±52.32), lower limb was 64.4 (±34.64), and summated MUNE was 120 (±17.67). In post polio group, mean MUNE in upper limb was109 (±63.89), lower limb was 86 (±39.103), and summated MUNE was 195(±103.23) (figure 10).

Figure 10 : MUNE among classic ALS , non-classic ALS and post polio groups



COMPARISON STUDIES

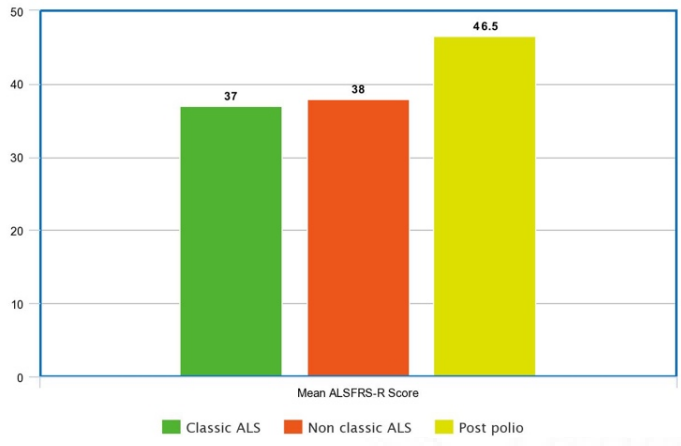
COMPARISON OF ALSFRS -R SCORE & MRC SUM SCORE AMONG GROUPS-

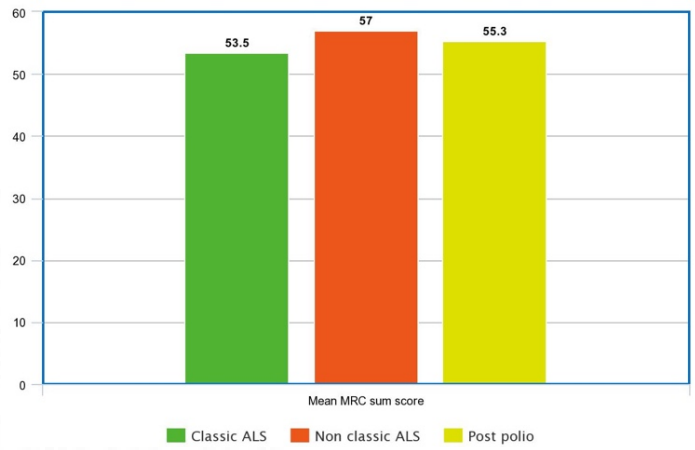
The difference in mean ALSFRS-R score between ALS group and post-polio group (37.8 vs 46.5 ,p=0.001), classic ALS group and post-polio group (37.7 vs 46.5, p=0.001), non-classic ALS group and post-polio group(38.2 vs 46.5, p= 0.008) were statistically significant (figure 11, table 1)

.

The difference in mean MRC sum score between ALS group and post-polio group (54.75 vs 55.33, p=0.84), classic ALS group and post-polio group (53.55 vs 55.33, p=0.5), non-classic ALS group and post-polio group (57.4 vs 55.33 ,p= 0.33) were not statistically significant (figure 11, table 1). The difference in MRC Sum scores between classic ALS and non-classic ALS was not statistically significant (53.55 vs 57.4, p=0.07). The difference between mean MRC sum score in classic vs non-classic ALS groups was nearly 4 points, however the difference was not statistically significant.

Figure 11: Comparison of ALSFRS -R score & MRC sum score among groups





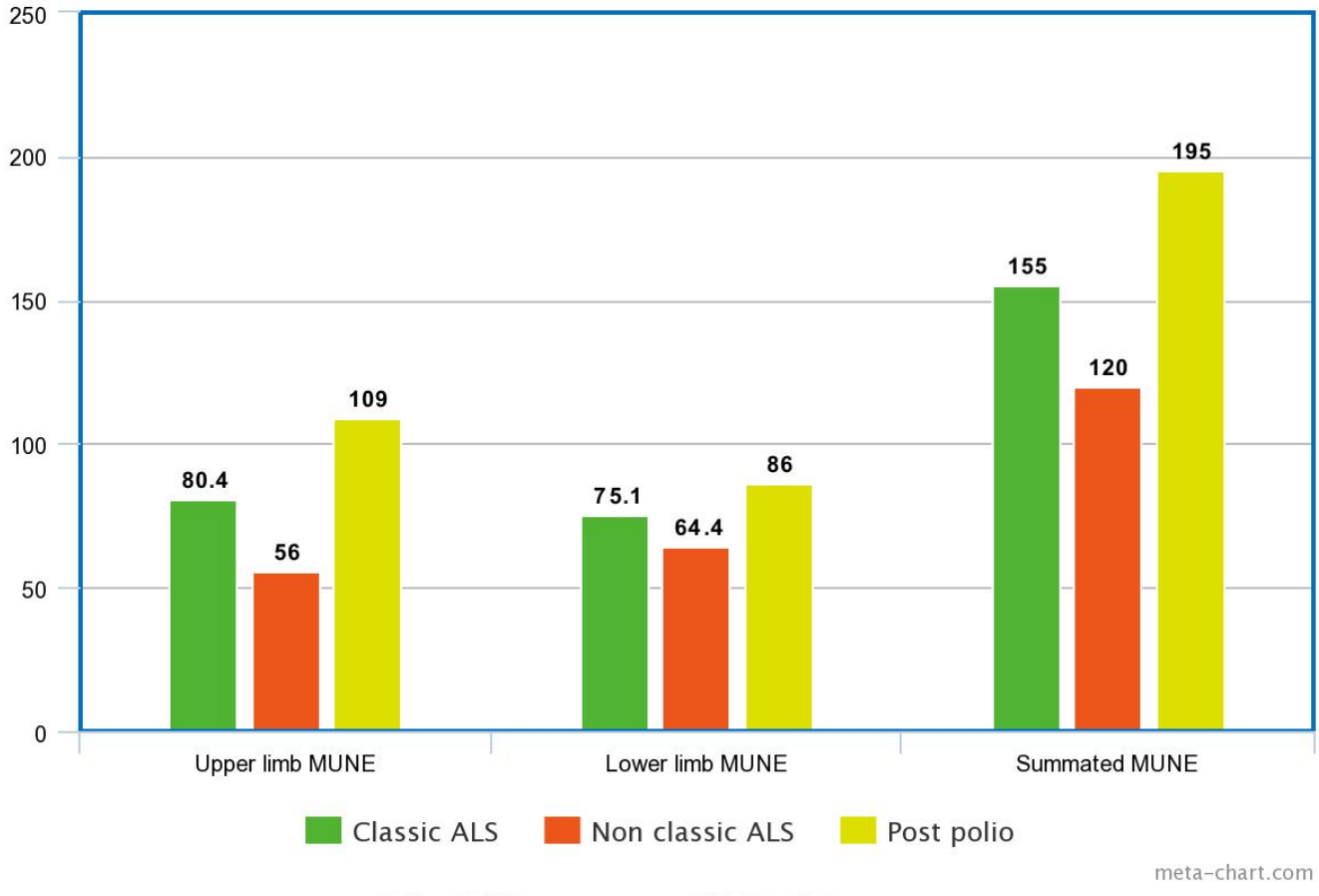
COMPARISON OF MUNE BETWEEN DISEASE SUBTYPES AND CLINICAL SEVERITY SCORE :

Table 1 : Comparison of MUNE, ALSFRS, MRS Sum score among the groups.

	DIAGNOSIS	Mean (Std. Deviation)	p Value
MUNE in upper limb	ALS	72.8 (66.8)	.229
	Polio	109.6 (72.8)	
MUNE in lower limb	ALS	71.8 (35.1)	.446
	Polio	86.0(69.4)	
ALSFRS-R SCORE	ALS	37.8 (5.8)	**.001**
	Polio	46.5(1.3)	
MRC Sum Score	ALS	54.7(5.6)	.814
	Polio	55.3(4.4)	
MUNE in upper limb	Classic ALS	80.4(69.2)	.373
	Polio	109.6(72.8)	
MUNE in lower limb	Classic ALS	75.1(39.1)	.614
	Polio	86.0(69.4)	
ALSFRS-R SCORE	Classic ALS	37.7(5.7)	**.001**
	Polio	46.5(1.3)	
MRC Sum Score	Classic ALS	53.5(6.0)	.5
	Polio	55.3(4.4)	
MUNE in upper limb	Non-classic ALS	56.0(60.9)	.135
	Polio	109.6(72.8)	
MUNE in lower limb	Non-classic ALS	64.4(24.49)	.377
	Polio	86.0 (69.4)	
ALSFRS-R SCORE	Non-classic ALS	38.2(6.3)	**.008**
	Polio	46.5(1.3)	
MRC Sum Score	Non-classic ALS	57.4(3.7)	.336
	Polio	55.3(4.4)	
MRC Sum Score	Classic ALS	53.5(6.0)	.07
	Non-classic ALS	57.4(3.7)	

The difference in mean MUNE between ALS group and post-polio group (upper limb 72.8 vs 109, lower limb 71.8 vs 86.0 respectively) , classic ALS group and polio group (upper limb 80.4 vs109.6, lower limb 75.1 vs 86 respectively), non-classic ALS group and polio group (upper limb 56.0 vs 109, lower limb 64.4 vs 86 respectively) were not statistically significant(table 1). The mean MUNE in upper limb was worse in non-classic ALS phenotype over classic ALS Phenotype, and mean lower limb MUNE was worse in non-classic ALS phenotype over classic ALS phenotype, however, these differences were not statistically significant.

Figure 12: Mean upper limb, lower limb and summated MUNES across the groups



The mean upper limb MUNE , lower limb MUNE , summated MUNES were higher in post-polio group over classic and non-classic ALS groups (figure12). These differences were not

statistically significant. Five out of seven patients in classic ALS group , who presented with shorter duration of the illness, within 6 months, had summated MUNE values below the mean MUNE value for ALS patients. We analysed the concordance between patients' summated MUNE value, disease duration and ALSFRS-R score with a presumption of patients with disease duration less than average disease duration of study population would have good MUNE, and ALSFRS-R score, vice versa. However, concordance was seen only in 9 among 36 patients irrespective of type of the disease (ALS or Polio).

Table 2: Correlation between MUNE in upper (UL) and lower limbs (LL) Vs MRC Sum score & ALSFRS-R scores

		Pearson correlation	P value
Classic ALS	MUNE UL Vs MRC	.180	.423
	MUNE LL Vs MRC	.208	.354
Non-classic ALS	MUNE UL Vs MRC	-.105	.77
	MUNE LL Vs MRC	-.086	.84
Polio	MUNE UL Vs MRC	.203	.20
	MUNE LL Vs MRC	.854	**.03**
Classic ALS	MUNE in UL vs ALSFRS-R	.12	.594
	MUNE IN LL vs ALSFRS-R	.22	.315
Non-classic ALS	MUNE in UL vs ALSFRS-R	-.22	.53
	MUNE IN LL vs ALSFRS-R	-.10	.778
Polio	MUNE in UL vs ALSFRS-R	-.049	.926

	MUNE in UL vs ALSFRS-R	.43	.39

We analyzed the correlation between clinical status (quantified by ALSFRS -R score and MRC Sum score) and objective electrophysiological data (MUNE) in ALS vs post-polio groups and subtypes of ALS group vs post-polio groups. There was no statistically significant correlation between ALSFRS-R score or MRC sum score and MUNE of either limb in ALS group, classic and non-classic subtypes of ALS group. There was a statistically significant high degree (0.854) positive correlation between lower limb MUNE vs MRC sum score in polio group ($p < 0.03$). A moderate degree of correlation was noted between ALSFRS-R and lower limb MUNE in post-polio group, however this observation was statistically not significant.

DISCUSSION

In this cross-sectional study, we evaluated the role of MUNE as a biomarker for disease progression in motor neuron disease. We considered the baseline clinical (ALSFRS -R and MRC Sum score) and electrophysiological motor unit number estimation (MUNE) in two different diseases which affect the anterior horn cell at different paces. The relevance of MUNE as a predictor of disease progression in ALS and post-polio paralysis was studied in relation with the already established markers of disease progression like ALSFRS-R and MRC sum score. Mean values of individual upper or lower limb MUNE, and summated MUNE were higher in post-polio group than ALS groups, and higher in classic ALS group than non-classic ALS group, but these differences were not statistically significant. There was no statistically significant correlation between clinical severity scores and MUNE in classic and non-classic subtypes of ALS group. A significant positive correlation between lower limb MUNE with MRC sum score in polio group was observed. Though a moderate degree of correlation was noted between ALSFRS-R and lower limb MUNE in polio group, this observation was not statistically significant.

Motor neuron disease is a devastating and inexorably progressive disease. Although reasonable knowledge was acquired in last decade about its pathogenesis, course of the disease and supportive rehabilitative measures, very few successful therapeutic studies have emerged in this disease (44) (Kiernan et al., 2011). With increasing knowledge about the pathogenesis, new therapeutic measures have evolved targeting specific molecules implicated in disease causations. Tofersen, an antisense oligonucleotide, has been shown to reduce CSF superoxide dismutase1 (SOD1) in a recently published phase I-II trial and is a hopeful therapy for ALS associated with *SOD1* mutations (45). With trials ongoing on this and several similar molecules, it is important to have a

biomarker which is accurate, sensitive, feasible and cost effective (Miller, 2020). Further understanding of the disease in lines of it progress among different subtypes, i.e classic phenotypes and non-classic phenotypes was also looked into in last few years. There are multiple prospective studies in literature, Indian and international studies which have looked into the role of clinical markers and electrophysiological markers which could predict the prognosis in ALS(39)(46)(47). However, a tool to predict disease course at first visit is the need of the moment for the neurologist. This also has implications in predicting survival of study subjects for clinical trials where attrition is a major limitation.

We observed that, the population sizes of ALS and polio was not proportionate in this study likely due to the rarity of a polio patient being attending to a tertiary referral center for the disability per se after such a long course into the illness unless they have a progression in illness (Post polio syndrome) . The age group of study population is consistent with the common age of presentation for the disease, that is 5^{th} decade for sporadic ALS. There was no patient with a family history of ALS. In concordance with previous studies by our study population also has male gender predominance in all groups(3)(48). The reason for male predominance was elusive , a previous study which looked into the role of sex hormones (oestrogen) in ALS had failed to draw conclusions(5)(4). However, in vitro studies with supra- physiological levels of oestrogens were shown to have ability to protect motor neurons(5)(4).

Non-classic ALS subtype comprises 1/3 of population, in which majority are bulbar onset disease. These patients presented earlier compared to classic ALS subtypes, which may be due to the fact that difficulty in speaking and swallowing are very disabling for an individual and would have required earlier medical attention. Another reason could be a rapid course of the illness in some of

the non-classic phenotypes (bulbar and respiratory onset), the shortest average survival being <2 years post diagnosis as observed in previous studies (46)(49). The non-classic phenotypes with a longer mean disease duration than classic ALS, namely pure LMN, pure UMN and flail limb varieties, were poorly represented in our cohort. Irrespective of the focality at onset, if disease progresses to a reasonable extent, demise of the other anterior horn cells is inevitable and that is the possible reason why nearly 85% of the patient with limb onset phenotype who crossed average disease duration of bulbar onset phenotype also had bulbar symptoms. The duration for this progress is just months or very few years. This presents a very different picture from post- polio paralysis which has near static disability in most people and a risk of a slow progressive syndrome after a latency of many years.

We attempted to answer two questions through this study,

1. **Does a single recording of MUNE correlate with clinical disease severity?**

We noted that five out of seven patients in classic ALS group, who presented with short duration of illness of less than 6 months (time from the first subjective disability perceived by the patient to initial presentation to the hospital), had MUNE values below the mean MUNE value for the entire group. Interestingly, in these patients ALSFRS-R score was at or above the average ALSFRS-R of the study population. This observation was shared in patients with bulbar onset ALS with shorter disease duration. This observation was consistent with the assumption that MUNE would be the first parameter to deteriorate prior to the clinical worsening. This was consistent with previous studies by (Kollewe et al., 2010)(42). In one study by Aggarwal et al., (2001) in familial patients with pathogenic genes, they observed a decrease in MUNE over a period of months before clinical manifestation of the illness (50). MUNE as marker for disease progression was inferred in previous prospective studies (32)(25)(31)(39). There have been limited cross-sectional studies so far which

looked into the role of MUNE as a marker for disease severity. Kollewe et al., (2010) in their cross-sectional study, identified that MUNE was a good predictor for disease progression in ALS patients(42). Our study is different from their study with respect to techniques of MUNE estimation and study methodology. They performed MUNE by statistical MUNE technique which require an expensive software. So, evidences are limited to infer whether single MUNE can be considered as predictor for clinical disease severity or not. From our study it is well evident that abnormalities in MUNE precedes clinical worsening.

However, MUNE in patients with disease duration beyond one year was not significantly worse than those who presented within a year of symptom onset. This could be due to (i) those patients being slow progressors and/or (ii) the inherent fallacy in technique. Slow progression is due to significant re- innervation of the existing denervated motor units. Second reason, multiple incremental MUNE may underestimate the true number of motor neurons. This occurs as the axons of individual MUs near threshold fire intermittently leading to an inherent variation in the size of a submaximal CMAP producing different values (this values with Poisson distribution on plots) rather than uniform values. Statistical MUNE method can appropriately overcome this issue as evidenced by Shefner et al., in his study among patients with ALS(31).

2. **Can we use MUNE as a diagnostic tool?**

MUNE is heterogeneous from subject to subject (29). MUNE "estimates" the number of motor units (MUs), not the exact "count" of MUs in a muscle. This drawback occurs as the MUNE is not an estimate of number of motor units in the whole of the muscle, but rather an estimate in that portion of the muscle within the uptake area of the surface electrode (29).

With a presumption that patients with smaller disease duration would have good MUNE and ALSFRS- R score, and vice versa , we had divided the ALS and post-polio patients in the study

population into two different groups. Concordance was seen between disease duration and MUNE only in 9 among 36 patients irrespective of type of the disease (ALS or Polio). This is because motor unit loss is different from fast progressors to slow progressors and very slow progressors. When we consider very slow progressors like patients with polio, their MUNE value might be greater than the MUNE value of ALS patients. So, MUNE value cannot specify type of the disease, rather it indicates loss of anterior horn cells/ motor axons. However, a first MUNE value gives an idea regarding the surviving motor neurons, and a follow-up study would help in estimation of disease progression, provided, the same methodology applied in initial and subsequent studies. Because of heterogeneity in the motor unit numbers which varies from individual to individual in a given motor unit at a given point of time, a MUNE, though with poor diagnostic value , carries its own significance for prognosticating the ongoing disease or herald the onset of disease in asymptomatic carriers in a familial setting.

Strengths of the study :

Multiple studies done previously have evaluated the role of MUNE as a marker for disease severity in ALS. Our study is the first study which compared two different diseases afflicting anterior horn cell to at different pace. This is to understand the correlation between disease severity and electrophysiologic motor unit number estimation, this would help in exploring the options for a good biomarker to predict disease severity at a given point of time. This has many implications in prognostication, related apprehension for the patient and their family. This also has implications in predicting survival of study subjects for clinical trials where attrition is a major limitation.

Limitations:

1. The study population size was too small to give robust results in subgroup analysis. Clinical and electrophysiological follow up could not be done in the patients due to restrictions in travel and non-essential procedures related to the pandemic in the latter part of the project.
2. The utility of MUNE analysis may be improved by combining multiple techniques or by application of statistical MUNE technique.
3. MUNE is an estimation of the motor unit number and is not accurate representation of the exact number of motor units. This inherent fallibility of MUNE can lead to sampling bias. Neither this method nor any other MUNE method has been specifically validated against an objective assessment of motor unit number; indeed, it is hard to conceive of such a study being performed in humans.

CONCLUSION

1. Abnormalities in MUNE preceded the clinical dysfunction in classic and non-classic ALS groups.
2. MUNE is non-specific and has limited diagnostic value. A single MUNE value did not differentiate a progressive disease like ALS from a static or very slowly progressive disease like post-polio paralysis.
3. Individual limb MUNE has same relevance as summated MUNE in ALS.
4. Lower limb MUNE has more relevance than upper limb MUNE in post-polio patients and should be considered in future studies rather than the more commonly used upper limb MUNE technique.
5. Clinical severity scores, ALSFRS-R and MRC sum scores, were worse in classic ALS group over non-classic ALS group.

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ABBREVIATIONS

ALS	Amyotrophic lateral sclerosis
UMN	Upper motor neuron
LMN	Lower motor neuron
MUNE	Motor unit number estimation
ALSFRS-R	Amyotrophic lateral sclerosis functional rating scale -revised
MND	Motor neuron disease
PMA	Progressive muscular atrophy
PLS	Primary lateral sclerosis
sALS	Sporadic amyotrophic lateral sclerosis
fALS	Familial amyotrophic lateral sclerosis
MUAP	Motor unit action potential
MU	Motor unit
NMJ	Neuromuscular junction
CMAP	Compound muscle action potential
AHC	Anterior horn cell
EMG	Electromyography
NCS	Nerve conduction study
SMA	Spinal muscular atrophy
FVC	Forced vital capacity
MUP	Motor unit action potential
CMT	Charcot Marie tooth disease
MRC	Medical research council
AALSS	Appel amyotrophic sclerosis score
APB	Abductor pollicis brevis

PROFORMA FOR PATIENTS WITH MOTOR NEURON DISEASE

Identification number ☐

Date of evaluation: ----------

1. Patient biodata

1.1. Age ---------- years

1.2 Sex ---------- 1.Male 2. Female

1.3 Date of registration/admission ----------

1.4 Phone No. ----------

1.5 Address ----------

---

2. Clinical details (1=yes, 2=No)

2.1. Year of symptom onset ----------

2.2 Duration of illness ----------years ---------- months

2.3 Past history of polio

2.4 duration of complete/partial recovery since polio

2.5 Genetic studies

2.5.1SMN1 gene 5q deletion detected ---------(yes/no)

2.5.2 SMN 2 gene copies detected ….(yes /no)

2.5.3 Other genetic studies…..

2.6 Electrophysiological study diagnosis-----

3 ALSFRS-R SCORING

3.1 Speech ----------

3.2 Salivation ----------

3.3 Swallowing ----------

3.4 Handwriting ----------

3.5.a.Cutting food and handling utensils (patients without gastrostomy)
b. Cutting food and handling utensils (alternate scale for patients with gastrostomy)? ----------
---

3.6 Dressing and hygiene ----------

3.7 Turning in bed and adjusting bed clothes ----------

3.8 Walking ----------

3.9 Climbing stairs

3.10 Dyspnoea (new)----------

3.11 Orthopnoea (new) ----------

3 .12 Respiratory insufficiency (new) ----------

3.13 Total ALSFRS R score----------

4 MRC-Muscle Grading Scale

4.1 Biceps

4.2 Wrist extensor

4.3 Iliopsoas

4.4 Quadriceps femoris

4.5 Tibialis anterior

5.Other Electrophysiologic study

5.1 SMUAP----------

5.1.1 1st record ----------

5.1.2 2nd record----------

5.1.3 3rd record ----------

5.1.4 AVERAGE ----------

5.2 CMAP----------

5.3 MUNE ----------

5.4 surface EMG interference pattern ----------

5.4.1 1st record ----------

5.4.2 2nd record----------

5.4.3 3rd record ----------

5.5 MUNIX ----------

6. Current treatment (1 = yes, 2 = no)

6.1 riluzole ----------

6.2 edaravone ----------

6.3 others ----------

ANNEXURE –I

ALS Functional Rating Scale-Revised

1. SPEECH

No change value = 4

Noticeable speech disturbance value = 3

Asked often to repeat words or phrases value = 2 Alternative communication methods value = 1 Unable to communicate verbally value = 0

Q1. Score =

2. SALIVATION

No change value = 4

Slight excess saliva, night-time drooling value = 3 Moderately excessive saliva, minimal drooling value = 2

Marked excess of saliva, some drooling value = 1 Marked drooling, requires constant tissue value = 0

Q2. Score =

3. SWALLOWING

No change value = 4

Occasional choking episodes value = 3 Modified the consistency of foods value = 2 Supplemental tube feedings value = 1

NPO (do not eat anything by mouth) value = 0

Q3. Score =

4. HANDWRITING

No change value = 4

Slow or sloppy, all words legible value = 3 Not all words legible value = 2

Able to hold pen, unable to write value = 1 Unable to hold pen value = 0

Q4. Score =

5a. **CUTTING FOOD AND HANDLING UTENSILS**

(patients without gastrostomy) No change value = 4

Somewhat slow and clumsy, needs no help value = 3 Sometimes needs help value = 2

Foods cut by someone else value = 1 Needs to be fed value = 0

Q5a. Score =

5b. **CUTTING FOOD AND HANDLING UTENSILS**

(patients with gastrostomy)

Uses PEG without assistance or difficulty value = 4 Somewhat slow and clumsy, needs no help value = 3 Requires assistance with closures and

fasteners value = 2

Provides minimal assistance to caregiver value = 1 Unable to perform any manipulations value = 0

Q5b. Score =

6. **DRESSING AND HYGIENE**

No change value = 4

Performs without assistance with increased effort or decreased efficiency value = 3 Intermittent assistance

or different methods value = 2

Requires daily assistance value = 1

Completely dependent value = 0

Q6. Score

7.**TURNING IN BED AND ADJUSTING BEDCLOTHES**

No change value = 4

Slower or more clumsy, without assistance value = 3

Can turn alone or adjust bed clothes value = 2 Can initiate but requires

assistance value = 1 Helpless in bed value = 0

Q7. Score =

7. **WALKING**

No change value = 4

Change in walking, no assistance or devices value = 3

Requires assistance to walk value = 2 Can move legs or stand up,

unable to walk from room to room value = 1 Cannot walk or move legs

value = 0

Q8. Score =

8. **CLIMBING STAIRS** No change value = 4 Slower value =

3

Unsteady and/or more fatigued value = 2 Requires assistance value = 1

Cannot climb stairs value = 0

Q9. Score =

9. **DYSPNEA**

No change value = 4

Occurs only with walking value = 3 Occurs with minimal exertion value

= 2

Occurs at rest, either sitting or lying value = 1 Significant shortness of breath

considering mechanical support value = 0

Q10. Score =

10. **ORTHOPNEA**

No change value = 4

Occasional shortness of breath, does

not routinely use more than two pillows value = 3 Require more than 2 pillows to

sleep value = 2 Can only sleep sitting up value = 1

Require the use of respiratory support (BiPAP®) to sleep value = 0

Q11. Score =

11. **RESPIRATORY INSUFFICIENCY** No respiratory support value = 4

Intermittent use of BiPAP® value = 3

Continuous use of BiPAP® at night value = 2 Continuous use of BiPAP day and night value = 1 Invasive mechanical ventilation value = 0

Q12. Score =

Total Score = ***/ 48***

ANNEXURE –II

MRC Sum score scale			
Muscle		**Score 0-5**	**MRC scale for muscle strength (0-5)**
Shoulder abductors	Left		**Grade 5: Normal** **Grade 4: Movement against gravity and resistance** **Grade 3: Movement against gravity over (almost) the full range** **Grade 2: Movement of the limb but not against gravity** **Grade 1: Visible contraction without movement of the limb (not existent for hip flexion)** **Grade 0: No visible contraction** **MRC grade for each muscle given in full numbers: (4+/4.5 =4) (4- =3) (5- = 4)**
	Right		
Elbow flexors	Left		
	Right		
Wrist extensors	Left		
	Right		
Hip flexors	Left		
	Right		
Knee extensors	Left		
	Right		
Foot dorsiflexors	Left		
	Right		
Total (out of 60)			

श्री चित्रा तिरुनाल आयुर्विज्ञान और प्रौद्योगिकी संस्थान, त्रिवेन्द्रम
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Institutional Ethics Committee

(IEC Regn No. ECR/189/Inst/KL/2013/RR-16)

SCT/IEC/1340/FEBRUARY-2019 02.03.2019

Dr. Jaffar Vali Sayyed
Senior Resident
Department of Neurology
SCTIMST, Thiruvananthapuram

Dear Dr. Jaffar Vali Sayyed,

The Institutional Ethics Committee reviewed and discussed your application to conduct the study entitled "LONGITUDINAL STUDY OF MOTOR UNIT NUMBER ESTIMATION AND INDEX IN AMYOTROPHIC LATERAL SCLEROSIS, POST-POLIO SYNDROME AND SPINAL MUSCULAR ATROPHY (IEC/1340)" on 16th February, 2019.

The following documents were reviewed:

Original submission

1. *Covering letter addressed to the Chairperson, IEC, SCTIMST dated 24.01.2019 with checklist*
2. *Forwarding Letter from the HOD*
3. *TAC Approval Letter*
4. *IEC Application Form*
5. *Project Proposal*
6. *Proforma*
7. *Informed Consent Form and Information Sheet in English and Malayalam*
8. *CV of Principal Investigator and Co- Principal Investigators*

Revised submission

1. *Covering letter addressed to the Chairman, IEC, SCTIMST dated 25.02.2019 with checklist*
2. *Copy of IEC Recommendation Letter dated 18.02.2019*
3. *TAC Approval Letter*
4. *Forwarding Letter from the HOD*
5. *IEC Application Form*
6. *Project Proposal*
7. *Proforma*
8. *Informed Consent Form and Information Sheet in English and Malayalam*
9. *CV of Principal Investigator and Co- Principal Investigators*

The following members of the Ethics Committee were present at the meeting held on 16th February, 2019 at G. Parthasarathi Board Room, AMCHSS, SCTIMST

SL. No.	Member Name	Highest Degree	Gender	Scientific /Non Scientific	Affiliation with Institution(s)
1.	Dr. R V G Menon	M Tech, PhD	Male	Lay Person (Chairman)	No
2.	Dr. Rema M. N	MD	Female	Basic Medical Scientist	No
3.	Dr. Kala Kesavan. P	MBBS, MD	Female	Basic Medical Scientist	No
4.	Dr. Harikrishna Varma PR	Ph.D(Materials Science)	Male	Medical Technology	Yes
5.	Dr. Christina George	MD Psychiatry	Female	Clinician	No
6.	Dr. S S Giri Sankar	LL.M. Ph.D.	Male	Legal Expert	No
7.	Dr. Aneesh V Pillai	BA. LLB (Hons.), LLM, Ph. D, SET (Law)	Male	Legal Expert	No
8.	Dr. P. Manickam	BSMS, MSc (Epid).,PhD	Male	Health Science Expert/ Social Scientist	No
9.	Mr. Satheesh Chandran	MSW, PGDPM	Male	Lay person/ NGO/ Social Scientist	No
10.	Dr. Harikrishnan S	MD, DM (Cardiology) DNB (Cardiology)	Male	Clinician	Yes
11.	Dr. Mala Ramanathan	PhD	Female	Social Scientist (Member Secretary)	Yes

IEC Decision

The IEC approved the conduct of the study in the present form.

Remarks:
The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information/informed consent and asks to be provided a copy of the final report.

There was no member of the study team who participated in voting / decision making process. The ethics committee is organized and operated according to the requirements of Good Clinical Practice and the requirements of the Indian Council of Medical Research (ICMR).

Sincerely,

Mala Ramanathan
Member Secretary, IEC

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SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY THIRUVANANTHAPURAM, KERALA / COMPARITIVE STUDY OF MOTOR UNIT NUMBER ESTIMATION IN AMYOTROPHIC LATERAL SCLEROSIS AND POST POLIO PARALYSIS Thesis submitted in partial fulfilment of the rules and regulations for DM Degree Examination of Sree ChitraTirunal Institute for Medical Sciences and Technology By Dr. Jaffar Vali Sayyed DM Neurology Resident
Month and Year of Submission: August 2020 Department of Neurology
Sree Chitra Tirunal Institute for Medical Sciences and Technology Thiruvananthapuram 2018-2020 DECLARATION I, Dr.

Jaffar Vali Sayyed , hereby declare that this project was undertaken by me under the supervision of the faculty, Department of Neurology, Sree Chitra Tirunal Institute for Medical Sciences and Technology. Thiruvananthapuram, Dr. Jaffar Vali Sayyed Date: 31-08-2020.