

EVALUATION OF HYPERPOLARIZATION POTENTIALS AND NERVE CONDUCTION PARAMETERS IN AXONAL NEUROPATHIC PATIENTS

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Background: Neuropathies are diagnosed on the basis of some specific parameters of compound motor unit action potential (CMAP). The slowing of conduction velocity along with relatively preserved amplitude considered an indication of demyelination. While, amplitude reduction and slightly slowed conduction velocity, referred to axonal degeneration. Further, CMAP shape and distal latencies are also important for the diagnosis of neuropathies. Although, hyperpolarization is one of the important phase of CMAP but, it is mentioned only in the cases of nerve conduction block and never considered to identify other neuropathies. **Objective:** Therefore, in the present study the hyperpolarization phase of CMAP has been compared and evaluated in terms of its amplitude and duration and its occurrence in axonal neuropathy. **Methods:** The CMAP records of normal subjects and axonal neuropathic patients; randomly selected from OPD cases and evaluated for the occurrence of hyperpolarization, its amplitude, duration, latencies, CMAP amplitude and NCV. The recordings were obtained from the stimulation of median and tibial nerves (both distally and proximally) with recordings of CMAP from Abductor Pollicis Brevis and Abductor Hallucis muscles, respectively. **Results:** The amplitude & duration of hyperpolarization and other parameters measured from both, median and tibial nerves were significantly lesser in neuropathic patients than the normal ones. **Conclusion:** Hyperpolarization reduces significantly in axonal neuropathy and this phase of CMAP may also be considered for the evaluation in other neuropathies. Further, a criteria may be formulated for the diagnosis of various neuropathies using hyperpolarization phase of CMAP.

Keywords: Hyperpolarization, Demyelination, Axonal degeneration, Nerve conduction

INTRODUCTION

Neuropathies are nerve disorders that ultimately results in muscular or sensory deficits in the innervation's area. Nerve conduction studies and needle electromyography are the two important techniques for the evaluation of neuromuscular disorders or neuropathies.^{1,2} Neuropathies are classified in several types. Two important and broad categories are axonal neuropathies and demyelination neuropathies.

Nerve conduction studies are specially used to define that whether the underlying disorder is axonal loss or demyelination.^{1,3} In addition, CMAP is one of the most important record obtained in nerve conduction studies and electromyography which is frequently used for the analysis and diagnosis of neuropathies. It consists of two phases of events, sub threshold and threshold events. Essential characteristics of sub threshold activation include a graded response that produces local changes in transmembrane potential. Sub threshold activation gives rise to a self-limiting local potential that diminishes with distance. However, If, the membrane potential reaches the critical level with about 15-25mV of depolarization, e.g. from -90mV to -65mV, in the case of human muscle cell⁴, the action potential develop in an all-or-none fashion, i.e., the maximal

response occurs through a complex energy dependant process regardless of the kind or magnitude of the stimulus.⁵

However, like sub-threshold and threshold events, the hyperpolarization phase of action potential is not evaluated usually for the indication of neuropathy in general. In fact, the time course for the this phase of CMAP varies considerably from cell to cell, but a typical curve spans about 10-15 milliseconds (ms). However, the farther the recording electrode is from the stimulating electrode, the higher (more positive) will be the hyperpolarization curve. The Distal hyperpolarization is probably due to focal depolarization and that the clinical features of Monofocal motor neuropathy are consistent with a depolarizing/hyperpolarizing lesion.⁶

It is also reported that peripheral fibers with a reduced safety factor for conduction due to demyelination are easily blocked by trains of impulses due to excessive membrane hyperpolarization induced by electrogenic sodium pumping.⁷ In addition, Kaji et al⁸, provided evidence that conduction block in multifocal motor neuropathy is exacerbated by voluntary motor activity, due to hyperpolarization by the electrogenic sodium pump, and that this activity-dependent conduction block can cause muscle fatigue. The relative sparing of sensory

axons may be due, at least in part, to their greater resistance to this type of block.

In axonal neuropathies the major change on nerve conduction studies is a reduction in the amplitude of the compound muscle action potential or the compound nerve action potential (or both) with relatively little (or at the most up to 30 percent) slowing in conduction velocity. The amplitude of the evoked responses may be low at all sites of stimulation if there is muscle atrophy or if portions of the muscle are not innervated by the nerve. Indeed, the best way to assess the amount of axonal loss is to compare the amplitude of a potential with either a previous baseline value, a normal control value, or the contra lateral; asymptomatic side.⁹

According to the above mentioned literature it is clear that all phases of CMAP, proximal and distal latencies have been considered for the diagnosis of neuropathies and the phase of hyperpolarization has been mentioned only in case of conduction block or to explain membrane phenomenon. Therefore, the purpose of the present study is to evaluate the hyperpolarization phase of the CMAP in axonal neuropathic condition and to compare this phase of CMAP from the normal subjects. Further, the possibility of indicating a change in this phase in axonal neuropathy will also be taken into account, along with its consistent occurrence.

MATERIAL AND METHODS

Normal and Neuropathic patients, ranging from 10-70 years of age, including both the sexes were engaged for this study from OPD, Department of Neurology, Civil Hospital, Karachi. Any patient having un-established neuropathy who was under observation and testing for neuropathy, was not included in the study. Similarly, the normal subjects having symptoms of neuropathy or muscular weakness and pain were not taken for study.

Neuropack-2 (model No. MEB-7102, Nihon Kohiden) was used for the recording of NCV and CMAP from normal subjects and selected patients.

The subjects were asked to lie down comfortably on couch. Before starting the test, all procedure was explained to the subjects to reduce anxiety and relax them mentally. For Median nerve, abductor pollicis brevis (APB) muscle was used to record CMAP. Stimulation was applied between tendons of Flexor Carpi Radialis (FCR) and Palmaris Longus (PL) muscles at the wrist for distal recording and proximal stimulation was applied in the medial aspect of the antecubital space. For Tibial nerve Abductor Hallucis (AH) muscle was used. Stimulation across the knee and at the ankle was provided to obtain proximal and distal records

respectively. The whole procedure took about 20-25 minutes for Median and Tibial nerve responses when recorded from both the right and left side as no significant difference is already established between them. The stimulus used for nerve conduction experiments was a square-wave pulse of constant duration (0.2 mS) and amplitude. A brief direct current (DC) pulse of 250V was used.

The active recording electrode (G1) was placed over the mid-belly of the muscle being tested and the reference (G2) was placed distally (approximately 4-6cm) to active electrode, G1 for the recording of CMAP on nerve stimulation.

The stimulating electrode, usually on a wand with two rigid electrodes, was placed on the skin over the nerve being tested. Cathode was placed distally to the anode, while the ground was attached on the same limb.

After, all electrodes were in place, the instrument was set to deliver repetitive stimuli at 1 Hz. The stimulus voltage was initially set to zero and then gradually increased. A compound motor action potential (CMAP) gradually appeared and grew larger in size and changed slightly in shape as the stimulating voltage increased. Eventually, further increase in voltage did not cause any change in CMAP amplitude. A stable response was assured if the voltage used was 25% greater than the voltage needed to produce the higher amplitude CMAP.

Measurements of NCV and other CMAP Parameters:

These parameters other than the phase of hyperpolarization were measured to assure that all the cases included in the study belongs to axonal neuropathy.

For the measurement of Motor NCV, two stimulation sites, one distal and one proximal sites were used. The latency values were directly noted from the digital facility given in the Neuropack2. Following parameters were obtained from CMAP records amplitude, Latencies, Duration and amplitude of hyperpolarization.

The data obtained was statistically analyzed using standard statistical tools. The level of significance for the comparison of normal and patient's data was 0.05.

RESULTS

a. Comparison of NCS Parameters of Median Nerve of Normal Subjects and Neuropathic Patients:

i. Latencies:

The Latencies of median nerve recorded at abductor pollicis brevis of neuropathic patients were greater than normal subjects. The difference was found to be significant statistically ($p < 0.0005$) in both distal and

proximal recordings, being 116 and 36% greater than the control, respectively as shown in Table 1a.

Table 1a: Comparison of latencies obtained from CMAP records of normal subjects and neuropathic patients. The stimulation was done at both the distal and proximal sites of median nerve.

Stimulation site	Latency (mS)		Significance Level
	Normal	Neuropathic	
Distal	3.167±0.087 (20)	6.859 ± 0.274 (7)	P<0.0005
Proximal	6.569 ± 0.243 (20)	8.974 ± 0.391 (7)	P<0.0005

Table 1b: Comparison of depolarization amplitude obtained from CMAP of normal subjects and neuropathic patients. The stimulation was done at both the distal and proximal sites of median nerve.

Stimulation site	Depolarization Amplitude (mV)		Significance Level
	Normal	Neuropathic	
Distal	8.304 ± 0.500 (20)	2.964 ± 0.854 (7)	P<0.0005
Proximal	7.970 ± 0.487 (20)	2.640 ± 0.799 (7)	P<0.0005

Table 2a: Comparison of latencies obtained from CMAP records of normal subjects and neuropathic patients. The stimulation was done at both the distal and proximal sites of tibial nerve.

Stimulation site	Latency(ms)		Significance Level
	Normal	Neuropathic	
Distal	4.551 ± 0.2 (22)	6.369 ± 0.362 (11)	p< 0.0005
Proximal	12.013 ± 0.514 (22)	16.018 ± 0.645 (11)	p< 0.0005

Table 2b: Comparison of depolarization amplitude obtained from CMAP of normal subjects and neuropathic patients. The stimulation was done at both the distal and proximal sites of tibial nerve.

Stimulation site	Amplitude (mV)		Significance Level
	Normal	Neuropathic	
Distal	9.039 ± 0.696 (22)	0.724 ± 0.180 (11)	p< 0.0005
Proximal	7.332 ± 0.66 (22)	0.524 ± 0.138 (11)	p< 0.0005

Table 3: Comparison of nerve conduction velocity obtained from median and tibial nerves between normal subjects and neuropathic patients.

Nerve	Normal	Neuropathic	P*
Median	58.805 ± 1.387 (20)	46.457±1.829 (7)	P<0.0005
Tibial	48.300 ± 1.579 (22)	39.845 ± 2.439 (11)	p<0.005

ii. Amplitude of Depolarization:

The amplitude of depolarization was also lesser than normal subjects in neuropathic patients. This difference was highly significant statistically (p<0.0005) in both the distal and proximal recordings, being 64 and 66% less than control, respectively as shown in Table 1b.

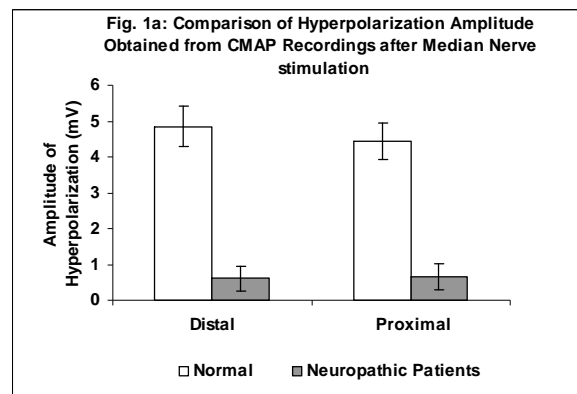
iii. Amplitude of Hyperpolarization:

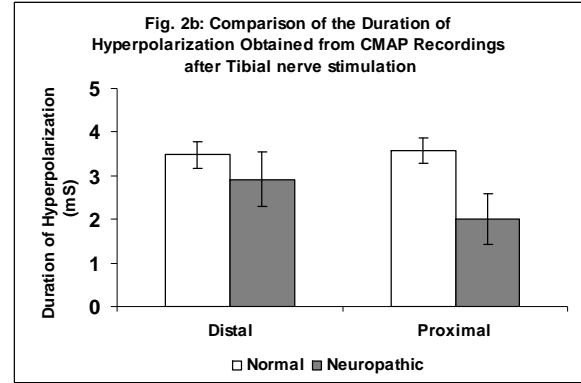
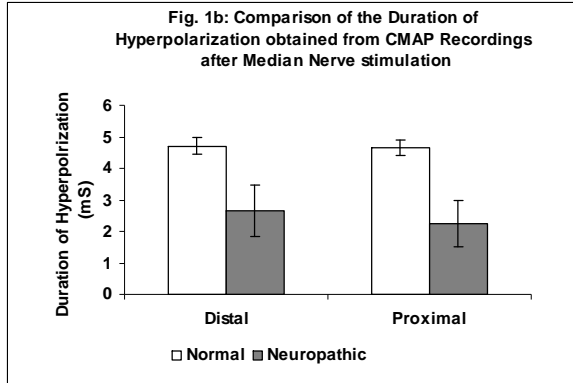
The amplitude of hyperpolarization also markedly decreased in neuropathic patients when compared with normal subjects. The difference among them was also found to be highly significant (p<0.0005) in both distal and proximal recordings, being 87 and 82% of control, respectively as shown in Fig. 1a.

iv. Duration of Hyperpolarization:

The Duration of Hyperpolarization was also lesser in Neuropathic patients than normal subjects and this difference was statistically significant (p<0.05) when

recorded from both the distal and proximal sites, being 34 and 33% of control respectively, as shown in Fig. 1b.





b. Comparison of Different NCS Parameters of Tibial Nerve of Normal Subjects and Neuropathic Patients:

i. Latencies:

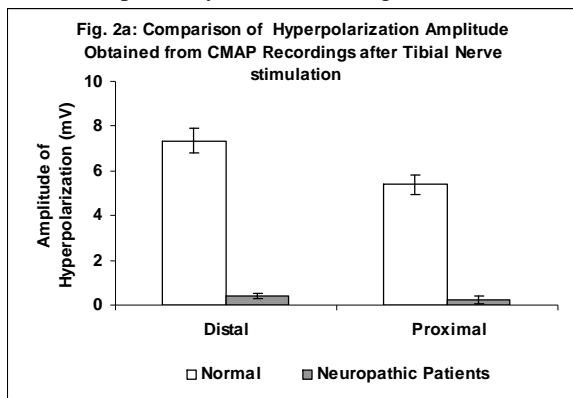
The latencies obtained from tibial nerve recorded at abductor hallucis muscle of neuropathic patients was greater than normal subjects. This difference was found to be highly significant statistically in both distal and in proximal ($p < 0.0005$) recordings, being 40 and 33% greater than control respectively, as shown in Table 2a.

ii. Amplitude of Depolarization:

Marked decrease in the Amplitude of Depolarization was observed in Neuropathic patients. The difference was highly significant ($p < 0.0005$) both in distal and proximal recordings, being 92 and 93% lesser than the control respectively, as shown in Table 2b.

iii. Amplitude of Hyperpolarization:

The Amplitude of Hyperpolarization was markedly decreased in neuropathic patients. The difference was highly significant ($p < 0.0005$) in both distal and proximal recordings, being 94% lesser than the control, respectively as shown in Fig. 2a.



Comparison of Nerve Conduction Velocity of Median and Tibial Nerves Between Normal Subjects and Neuropathic Patients:

The nerve conduction velocity slows down in neuropathic patients as compared to the normal subjects. The difference was also found to be significant statistically ($p < 0.0005$), being reduced by 21% and 18% of the control, in median and tibial nerves as shown in Fig. 3.

d. Comparison of Compound Motor Unit Action Potentials Obtained from Median and Tibial Nerves of Normal Subjects and Neuropathic Patients.

The representative records of compound motor unit action potential has been presented in Fig. 4a & b. The amplitude of CMAP is significantly lesser than normal in both the median and tibial records obtained from neuropathic patients. Similarly, the amplitude of hyperpolarization is also significantly less in neuropathic patients than the normal subjects (Fig. 4a & b).

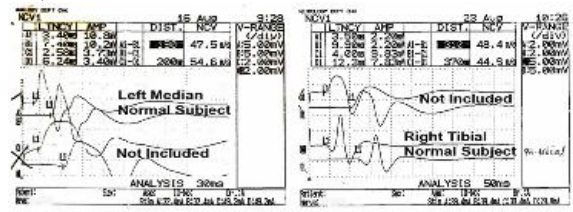


Fig. 4a

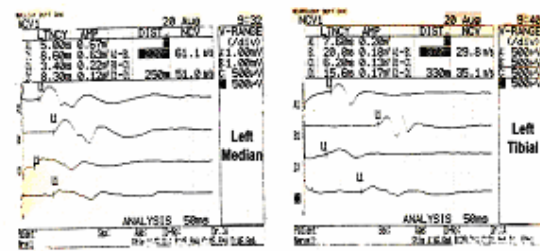


Fig. 4b

iv. Duration of Hyperpolarization:

The difference in the duration of hyperpolarization between normal subjects and neuropathic patients was found significant statistically ($p < 0.025$) in proximal recordings, i.e., 12% lesser than control. However, in the case of distal recordings, it was non-significant ($P > 0.05$) as shown in Fig. 2b.

DISCUSSION

In the present study the amplitude of hyperpolarization has been found to decreased markedly in axonal neuropathic patients than normal subjects and this decrease was found to be highly significant ($p < 0.0005$) in both median and tibial nerves and both the distal and proximal stimulation sites, (Fig. 1a and 2a). This significant reduction in the amplitude of hyperpolarization in both the median and tibial nerves indicate lesser refractoriness in nerve or muscle and thus probably reduced the threshold level. Although such reduction of threshold level should increase the NCV. But, in the present study the NCV has been found to reduce significantly in axonal neuropathic patients. In our opinion, the reduction in the hyperpolarization amplitude was due to compensatory membrane phenomenon to reduce threshold level towards normal. However, the axonal loss was extreme which dominated over the compensation and thus NCV reduced significantly. It had been reported earlier that the muscle and nerve fiber membrane become more excitable after denervation, while in the present study, decreased NCV is actually associated with pure axonal pathology^{10,11,12,13}. In addition, another possibility of reduction in NCV may be the gross reduction in internodal distances¹⁴ due to regeneration phase of axonal neuropathy. However, we expect that instead of this regenerative reduction in internodal distance, large fiber were lost with preservation of smaller one in the axonal neuropathic patient who showed reduced NCV as also reported in acrylamide neuropathy by Gilliat¹⁰.

In addition, the duration of hyperpolarization which was significantly reduced probably indicate a rapid Na^+ , K^+ exchange across the axonal membrane in the remaining axons of the axonal neuropathic patients evaluated in the present study. It has been reported that greater frequency of stimulation (8Hz) induces greater hyperpolarization in motor axons¹⁵. On these basis, it is quite possible that axonal degeneration must have reduced the firing rate of the motor neuron present in spinal cord which has resulted in the reduced amplitude and duration of hyperpolarization in the present study. Such reduction in hyperpolarization is also reported by Kuwabara et al¹⁶, i.e., following maximum voluntary contraction of a normal muscle the refractory period of transmission is impaired distal to the stimulus site causing axonal hyperpolarization. Since axonal degeneration patients exhibit less voluntary contraction therefore, it may have also resulted in reduced amplitude and duration of hyperpolarization in our study.

Further, significantly higher latencies and reduced CMAP amplitude justifies the selection of

neuropathic cases as axonal one in the present study. The parameters like latencies & NCV were recorded additionally to confirm that the selected patients were suffering from axonal degeneration.

Conclusion

On the basis of obtained result specially the duration and amplitude of hyperpolarization, it is confirmed that axonal loss is also associated with hyperpolarization as reported for conduction block. However, this association of hyperpolarization is opposite in the case of axonal degeneration being reduced instead of increased, reported in conduction block.

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