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Qualitative Morphological Analysis of Muscle Biopsies Using Neural Networks

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Abstract

Qualitative data from human muscle biopsies have been extracted and analyzed by artificial neural network (ANN) models trained with the Kohonen's self-organizing feature maps algorithm to provide an automated medical diagnosis. Data from 6 distinct groups of neuromuscular disorders were examined. Training and evaluation were carried out on 80 and 25 cases respectively. The diagnostic performance of models investigated varied form 87 to 95%, and 88 to 92% for the training and evaluation. Furthermore, the diagnostic usefulness of the self-organizing feature map models was tested on 11 muscle biopsies with no specific diagnostic findings that gave encouraging results.

1. Introduction

Neuromuscular diseases is a group of disorders that involve the motor nuclei of the cranial nerves, the anterior horn cells of the spinal cord, the nerve roots and spinal nerves, the peripheral nerves, the neuromuscular junction and the muscle itself [1]. These disorders cause muscular weakness and/or wasting. Histopathology is one of the many techniques applied for the diagnosis of neuromuscular disorders. Human muscle biopsies are employed and histochemical and immunocytochemical tests are carried out [2]. Qualitative morphometric analysis of muscle biopsies is desirable in order to provide a more precise diagnosis. Although computer assisted measurements to quantify muscle fibre size and type were reported by several workers [2]-[4], the issue of automated diagnosis based on muscle biopsy findings received limited attention. In this study qualitative features extracted from light microscopical examination of muscle sections have been supplied to artificial neural network (ANN) models trained with the Kohonen's self-organizing feature maps algorithm [5] to obtain an automated diagnosis. The data include specific features of the muscle fibres and fascicles under normal and pathological conditions. The system aims to provide an independent and unbiased diagnosis in order to assist the histopathologist in reaching a more reliable diagnosis.

2. Materials and Methods

Open muscle biopsies of quadriceps muscle of men, women and children aged between 5 months to 82 years old have been investigated. They were all clinically suspected to be having a neuromuscular problem. The muscle samples were frozen in liquified Arcton 12 at about -150 C and 10 μm frozen sections were cut in a cryostat. Histochemical and immunocytochemical tests were performed on the frozen sections. The remaining muscle samples were stored in liquid nitrogen for future use. Qualitative analysis of the muscle sections was carried out using a binocular light microscope in order to examine the various characteristics (features) of the muscle sections [2]. Qualitative microscopical analysis of these sections was based on specific features exhibited by muscle fibres and the general appearance of the fascicles, during the course of the disease. Features extracted for input to the ANN system are briefly analyzed under the following

four groups.

A. Muscle fibre morphology (features examined: polygonal, round, angular, atrophic, hypertrophic, hyaline, necrotic, regenerating, central nuclei, ring fibres, inside core, and splitting)
Muscle fibre size and shape is a good indicator of muscle health state. Gross deviations hypertrophy or atrophy from normal size (50 μm diameter in normal adults) shows a pathological condition. Also the disappearance of the normal polygonal shape of the fibre giving rise to various shapes such as rounded, angular, ringed, and other shapes indicates pathological states. Furthermore, presence of splitting, necrotic, regenerating, hyaline fibres, and presence of internal cores and central nuclei are features of myopathic and/or neuropathic state.

B. Fibre type distribution (features examined: checkerboard, fibre type predominance, tendency or obvious grouping).
Muscle fibres are of two types, type I (slow twitch) and II (fast twitch). Their numbers being approximately equal in the quadriceps muscle. However, their numbers can vary in other skeletal muscles depending on the type of work performed. Large deviations of numbers from normality is pathognomonic. Fibre types have a checkerboard distribution in normal muscle whereas grouping of either or both types is indicative of a pathological condition.

C. Connective tissue and inflammation (features examined: normal presence, increased presence, absence).
Increase in connective tissue between the muscle fibres and/or replacement of muscle fibres by connective tissue is related with neuromuscular disease. Focal inflammatory areas are also indicative of an inflammatory muscle state.

D. Dystrophin test (features examined: present being strong and continuous, scarce, present being weak and patchy).
Complete absence of the dystrophin protein from the muscle cell membrane is specific of Duchenne Muscular Dystrophy.

Myopathic, neuropathic and normal muscle states were examined paying special attention to specific neuromuscular disorders such as Duchenne Muscular Dystrophy (DMD), Motor Neurone Disease (MND), and Spinal Muscular Atrophy (SMA). Biopsies that showed generalised pathological changes are classified as Myopathic non specific (MyoNS) and neuropathic non-specific (NeuroNS) according to where these changes lie. Thus DMD and MyoNS represent a myopathic state whereas MND, SMA and NeuroNS represent a neuropathic state. The three specific neuromuscular disorders that have been employed for analysis are briefly discussed.

• *DMD* is a sex-linked slowly progressive recessive disorder due to a hereditary biochemical disorder of the muscle fibre membrane, typically seen in young boys. In childhood the biopsy shows a plethora of pathological features including hyaline fibres, necrotic and regenerating fibres. The progressive breakdown of muscle fibres leads to eventual fibro-fatty replacement. Also specific immunocytochemical tests using antidystrophin monoclonal antibodies show complete absence of the dystrophin protein on the muscle fibre membrane of DMD patients.
• *MND* is a rapidly progressive denervating disorder due to degeneration and loss of motor neurones. This is a disease of middle aged to older aged groups. The earliest signs of denervation in the muscle biopsy are small groups of angular atrophic fibres.
• *SMA* is a slowly progressive hereditary disease of the motor neurone characterised by degeneration of the anterior horn cells, encountered in infants, children and young adults. The age

of onset, progression and severity are variable according to the sub-type of the disease. The key pathological feature of the muscle biopsy is grouped atrophy against a background of normal size or grossly hypertrophied fibres.

3. Results and Discussion

The neural network models in this system were derived using the Kohonen's self-organizing feature maps algorithm. With this algorithm the training process involves the presentation of pattern vectors from the training set one at a time. A winning neuron (node) is selected in a systematic way after all input vectors are presented. A weight adjustment process takes place by using the neighbourhood concept that shrinks over time and a learning coefficient that also decreases with time. After several input vectors are presented, weights will form clusters or vector centres that sample the input space such that the point density function of the vector centres tends to approximate the probability density function of the input vectors [5]. The weights will also be organized such that topologically close output nodes are sensitive to inputs that are physically similar. Thus the output nodes will be ordered in a natural way.

For training the neural networks system 80 biopsies were employed. These were clearly diagnosed into 6 distinct groups, 34 NOR, 7 MyoNS, 3 NeuroNS, 13 DMD, 18 MND, and 5 SMA. The diagnostic performance of the models was evaluated on 25 subjects, 11 NOR, 3 MyoNS, 4 DMD, 5 MND, and 2 SMA. The results of the self-organizing feature map models that were investigated are summarized in Table I. The size of the output grid, and the diagnostic yield for the training (TR%), and evaluation (EV%) sets are given. The initial gain factor was 0.90 and the number of epochs was 1550 for all models. The performance of the models varied from 87 to 95%, and 88 to 92% for the training set and evaluation sets respectively. As shown in Table I, the diagnostic performance of models presented was slightly increased for grid sizes bigger than 7x7.

Furthermore, the diagnostic usefulness of the self-organizing feature maps was tested on 11 muscle biopsies from subjects with no specific diagnostic findings. For each of these cases, the feature vector was applied on the 8 models of Table I. The output of the 8 models is a string expressing the number of models that classified the subject under investigation as NOR, and/or MyoNS, and/or NeuroNS, and/or DMD, and/or MND, and/or SMA. The string can also be expressed in percentage format. Findings of this scheme are shown in Table II. Five biopsies (P07, P09, P23,

Table I ANN models

	Grid Size	Diagnostic yield	
		TR%	EV%
1	5x5	87	88
2	6x6	90	88
3	7x7	91	92
4	8x8	93	92
5	9x9	93	92
6	10x10	95	92
7	12x12	95	92
8	15x15	95	92

Table II Performance of ANN models on 11 test cases

	NOR	MyoNS	NeuroNS	DMD	MND	SMA
P23	8					
P37	8					
P09			8			
P33			8			
P08		1	7			
P07					7	1
P30		2			6	
P05			3		4	1
P12	1	7				
P19		6		1	1	
P14			2	1		5

P33, P37) were classified to specific groups, whereas the other six (P05, P08, P12, P14, P19, P30) were allocated to more than one specific group. Results of these cases are individually, assessed as follows.

- P23 biopsy a 63 year old male showed minor changes which are not pathognomonic.
- P37 biopsy of a 45 year old male showed a generalised muscle fibre hypertrophy which is probably indicative of the Universal Hypertrophy syndrome.
- P09 a 42 year old male although classified as NeuroNS, the muscle changes were due to an osteoarthritic episode in the past.
- P33 a 15 year old boy was classified as NeuroNS due to presence of some atrophic angular fibres although the general muscle appearance considered to be normal.
- P08 a 42 year old male showing neuropathic features classified as NeuroNS (87.5%) by the system.
- P07 a 44 year old female suffering from Myasthenia Gravis, identified as MND (87.5%), impossible to be diagnosed by the muscle biopsy alone.
- P30 a 49 year old female was clinically diagnosed as a myopathic patient due to the plethora of pathological features but the system has failed to identify her as such conclusively.
- P05 a 5 year old male showing neuropathic changes, has been evaluated as NeuroNS (37.5%), MND (50%) and SMA (12.5%), clearly indicating the neuropathic significance.
- P12 a 72 year old female was classified as MyoNS (87.5%) due to the absence of the pathological features. This is quite possible although the pathology could be due to old age.
- P19 a 47 year old female showing myopathic changes and classified as MyoNS (75%).
- P14 a 2 month old baby classified as NeuroNS (25%), DMD (12.5%) and SMA (62.5%) but clinically too young to be assessed conclusively by the muscle biopsy.

Results of this study encourage the use of neural network technology in the diagnosis of neuromuscular disorders. In a similar work, where cluster analysis (Ward's method) was applied to subclassify the histological features of 60 cases of neuromuscular diseases, 33 MND, 13 Peripheral neuropathy, and 14 other; results indicated that there was no clear relationship between clusters and diseases [6].

4. Conclusions

The diagnosis of neuromuscular diseases employing muscle biopsies has been attempted by neural network models trained with the Kohonen's self-organizing feature maps algorithm. The preliminary results have shown that neural network models performed well in the medical diagnosis of muscle biopsies. However more data and more neuromuscular diseases must be introduced into the system in order to improve its performance.

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