A quantitative study of intermediolateral column cells in motor neuron disease and the Shy-Drager syndrome

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SUMMARY The intermediolateral column neurons in the lateral horns of the grey matter of the thoracic spinal cord were counted in five patients who had died of motor neuron disease, two of the Shy-Drager syndrome and three of other neurological diseases not affecting the spinal cord or roots. The number of intermediolateral column cells in all the motor neuron disease cases was slightly reduced compared with the control cords, this difference being apparent both when the whole thoracic sympathetic outflow was assessed as well as its upper, middle and lower thirds. The difference, however, was not statistically significant. By contrast, in Shy-Drager cases there was a highly significant reduction in intermediolateral column cells compared with the normal cords.

The intermediolateral columns of the thoracic spinal cord contain the preganglionic neurons of the sympathetic nervous system. Cell loss in these columns has been reported in diseases in which autonomic failure occurs such as the Shy-Drager syndrome¹⁻⁶ and diabetic autonomic neuropathy.⁷ Although previous studies have stated that intermediolateral column nerve cells seem not to be depleted in the spinal cords of patients dying of motor neuron disease,⁸⁻¹⁰ a recent report has documented mild but significant impairment of autonomic function in patients with motor neuron disease.¹¹ Moreover, in addition to the loss of anterior horn cells and degeneration of the corticospinal tracts frequently seen in motor neuron disease,^{8,10} degeneration of the anterolateral and posterior white columns⁸ and loss of cells in Clarke's column¹² have also been described in some cases. The neurons in the anterior horns and intermediolateral columns are cholinergic, and it seems reasonable to suppose that both groups of cholinergic neurons might be affected by motor neuron disease. In view of these considerations, and the importance of estimating cell numbers accurately in the intermediolateral columns we have

used the quantitative histological techniques of Oppenheimer¹ to assess possible loss of neurons in this region of the spinal cord in motor neuron disease. We have also studied cases with the Shy-Drager syndrome where cell loss in the intermediolateral columns has been reported and compared them with spinal cords from cases in whom pathology was limited to the cerebral hemispheres.

Methods and cases studied (see table)

(a) Cases with normal spinal cords

Case 1 was a man who died at the age of 58 years following a subarachnoid haemorrhage from a ruptured cerebral aneurysm.

Case 2 was a man who died at the age of 49 years of a malignant cerebral glioma.

Case 3 was a woman who died at the age of 61 years of a malignant cerebral glioma.

In all these cases the spinal cords and roots were macroscopically normal.

(b) Motor neuron disease cases

Case 4 was a man who died at the age of 68 years. His symptoms started with difficulty in swallowing and then breathing, and he subsequently required artificial ventilation. On examination he had jaw weakness, absent palatal movements, a reduced gag reflex and wasting and fasciculation of the tongue. There was widespread wasting, fasciculation and pyramidal signs in all limbs. There was no sensory loss. He died 1 year after the onset of symptoms.

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Received 15 February 1985. Accepted 23 March 1985.

Table Number of cells in the intermediolateral columns of thoracic spinal cord

Case	Age (yrs)	Sex	Diagnosis	Mean cell counts in:		
				Upper third (T2-4)	Middle third (T6-8)	Lower third (T10-12)
1	58	Male	Cerebral haemorrhage	5.8 ± 0.8 (95)*	3.5 ± 0.8 (94)	3.1 ± 1.0 (97)
2	49	Male	Cerebral glioma	5.6 ± 1.5 (79)	3.8 ± 1.6 (80)	2.9 ± 1.0 (100)
3	61	Female	Cerebral glioma	5.3 ± 1.0 (82)	3.7 ± 1.0 (81)	3.1 ± 0.7 (98)
4	68	Male	Motor neuron disease	4.2 ± 1.0 (148)	3.4 ± 1.1 (99)	2.8 ± 1.3 (95)
5	38	Female	Familial motor neuron disease	4.2 ± 1.1 (98)	3.3 ± 1.0 (96)	2.6 ± 1.1 (95)
6	50	Male	Motor neuron disease	5.6 ± 1.7 (164)	4.2 ± 1.1 (150)	3.3 ± 1.1 (162)
7	62	Male	Motor neuron disease	3.9 ± 1.2 (96)	2.7 ± 0.9 (94)	1.8 ± 0.9 (94)
8	58	Male	Motor neuron disease	5.2 ± 1.7 (191)	3.4 ± 1.2 (141)	2.7 ± 0.9 (150)
9	43	Female	Shy/Drager syndrome	1.6 ± 0.7 (90)	1.3 ± 0.9 (81)	1.1 ± 0.6 (95)
10	58	Male	Shy-Drager syndrome	1.4 ± 0.8 (101)	1.2 ± 0.6 (98)	1.0 ± 0.7 (93)

*Mean number of neurons ± standard deviation in one lateral horn in a 10 µm section. The figure in brackets shows the number of cell counts performed, each section providing 2 cell counts.

Case 5 was a woman who died at the age of 38 years. Her father had died of motor neuron disease at the age of 33 years. Her symptoms started with difficulty swallowing and speaking and neck weakness, and she subsequently developed weakness of her arms. On examination she had dysphonia, wasted neck and jaw muscles, impaired palatal movements and a wasted fasciculating tongue. There was marked wasting, fasciculation and weakness with intact reflexes in the arms, and proximal weakness and pyramidal signs in the legs. There was no sensory loss. She died 1 year after the onset of symptoms.

Case 6 was a man who died at the age of 50 years. His symptoms started with cramps in his calves and then widespread muscle twitching. He soon developed weakness of his arms and legs and then difficulty breathing. On examination there was bilateral jaw and facial weakness, slight impairment of palatal movements and weakness of sternomastoids. There was marked wasting, fasciculation and weakness of all muscle groups with pyramidal signs. There was no sensory loss. He died 7 months after the onset of symptoms.

Case 7 was a man who died at the age of 62 years. His symptoms started with weakness in his legs and then his right hand. After 1 year his speech became slow and difficult. His symptoms then progressed slowly. On examination he had a slurring dysarthria, fasciculation of the tongue and widespread fasciculation and wasting in the limbs with marked pyramidal signs. There was no sensory loss. He died 7 years after the onset of symptoms. There had been no dementia.

Case 8 was a man who died at the age of 58 years. His symptoms started with weakness in the left arm and hand and cramps in both legs, and his disease became progressive. On examination he had widespread weakness and fasciculation but no sensory loss. He subsequently developed breathing difficulty and died two and half years after the onset of symptoms.

(c) Shy-Drager syndrome cases

Case 9 was a woman who died at the age of 43 years. She had a progressive history of urinary incontinence, increasing difficulty walking with stiffness and weakness in the

limbs, and symptoms of severe postural hypotension. On examination she had profound postural hypotension, and marked extrapyramidal and pyramidal signs. She died 8 years after onset of the first symptoms.

Case 10 was a man who died at the age of 58 years. He had a progressive history of urinary incontinence, impotence, stiffness in the limbs, slowness of movement and symptoms of severe postural hypotension. On examination he had profound postural hypotension and marked extrapyramidal signs. He died 5 years after the onset of the first symptoms.

Materials and methods

In each case transverse blocks were made from 6 alternating thoracic cord segments from T2 to T12. Each block was then divided into an upper and lower segment and processed separately that is, a total of 12 blocks was obtained from each cord. The blocks were then embedded in paraffin wax and 20 serial sections 10 µm in thickness were cut from each block. The sections were stained in 0.1% cresyl fast violet in distilled water acidified with 10% acetic acid. Some sections were also stained with luxol-fast blue combined with cresyl fast violet. Nerve cells were counted within the area of the lateral horn on both sides of each cord section as previously described¹ that is, two separate counts were made in each section.

The techniques and limitations of this counting procedure have been discussed in detail by Oppenheimer.¹ The numbers of separate cell counts which were performed on the thoracic segments are shown in the table. Only cells definitely identifiable as neuronal in type which were contained within the lateral horns were counted. Only cells in which a nucleolus was visible were included. Cells which were outside this region or which did not have a clearly visible nucleolus were not counted. No corrections were applied to compensate for shrinkage. All cases were treated in exactly the same way so that the counting technique served only to compare one spinal cord with another and the numbers do not represent absolute counts.¹

The means and standard deviations of the counts were calculated in each case for the upper (T2 and T4), middle

(T6 and T8) and lower (T10 and T12) thirds of the thoracic cords. Student's *t* test was then used to compare the mean number of cells counted in motor neuron disease or Shy-Drager syndrome cases with normal spinal cords.

Results

In all cases of motor neuron disease studied there was unequivocal loss of anterior horn cells although this loss was not quantified. Degeneration of the corticospinal tract was severe in case 7, moderate in cases 4 and 6, slight in case 5 and absent in case 8.

The results of the cell counts are shown in the table. The mean number of neurons/lateral horn/section derived from the pooled data for all levels of the thoracic cords were 4.1 in the normal cases, 3.6 in the motor neuron disease cases and 1.3 in the cases of Shy-Drager syndrome. Thus there was a slight overall reduction in the neuronal cells within the lateral horns of motor neuron disease cases compared with normal controls. However, this trend did not reach statistical significance ($p > 0.1$). The reduction in pooled lateral horn cell numbers in the Shy-Drager cases, however, was very marked and this difference was highly significant ($p < 0.001$).

A comparison was also made of the mean number of lateral horn cells derived from the pooled data for each third of the thoracic cords. The results for the different levels of the cord were analysed in this way because the number of intermediolateral cells varies according to the thoracic level, usually being highest in the upper segments.¹ The means of the cells counted in the upper third (T2-4) of the normal controls, motor neuron disease cases and Shy-Drager cases were 5.6, 4.6 and 1.5 respectively. The corresponding figures in the middle third (T6-8) were 3.7, 3.4 and 1.3 respectively, and in the lower third (T10-12) 3.0, 2.6 and 1.1 respectively. Thus, in all three regions of the thoracic cords there was a slight reduction in the number of lateral horn cells in motor neuron disease cases compared with normal controls, the difference being greatest in the upper third. These differences, however, failed to reach statistical significance ($p > 0.05$ for upper third, and $p > 0.1$ for middle and lower thirds). By contrast, in all three parts of the thoracic cord of the Shy-Drager cases there was a highly significant reduction in cell numbers compared with normal controls ($p < 0.001$ for all thirds).

Discussion

It was found that the number of intermediolateral column cells in patients dying of motor neuron disease was slightly but consistently reduced throughout the whole of the thoracic sympathetic outflow.

This reduction, however, did not reach statistical significance but the trend is clear. By contrast, the Shy-Drager cases did show a very significant loss of intermediolateral column cells. Although the number of cases studied was small very many sections of each cord were examined and analysed quantitatively.

The present findings are consistent with a number of previous non-quantitative studies of motor neuron disease cases which have reported the intermediolateral column as being normal.⁸⁻¹⁰ It was important to perform a quantitative study because of the difficulties of estimating accurately such a small number of cells and also because of the marked variation in cell number from one section to another, even in serial sections. For example, in this study, counts in adjacent sections were 2 and 9. Although it has been reported briefly that some cases of progressive spinal muscular atrophy show abnormalities in the intermediolateral columns,¹³ quantitative methods were not employed. It is of interest that the motor neuron disease case with the highest cell counts (case 6) was the patient with the shortest duration of disease (7 months), while the lowest cell counts in motor neuron disease were found in a man with the longest duration of disease (7 years). Moreover, we did not detect abnormalities of cell morphology in these columns in the motor neuron disease cases in this study.

The possibility that shrinkage of the spinal cord of the motor neuron disease cases, which is a consequence of anterior horn cell loss and pyramidal tract degeneration, could cause falsely high cell numbers in the lateral horns should be considered. Although it is certain that cord shrinkage occurs in the transverse plane in this disease we feel that it is very unlikely that longitudinal shrinkage would have occurred. The latter process might be expected to have an effect on cell density in the lateral horns, but the former would be very unlikely to do so. It might also be argued that this study should have been carried out blind. However, this was impractical since the striking loss of anterior horn cells in the motor neuron disease cases and of intermediolateral column cells in the Shy-Drager cases immediately indicated the diagnoses as soon as the sections were examined.

The observations in this study are in keeping with the documented mild clinical autonomic abnormalities seen in motor neuron disease.¹¹ These abnormalities, however, are almost certainly due to defects at multiple anatomical levels¹¹ and are unlikely to be due solely to the slight loss of preganglionic sympathetic cells in the cord. It is not certain whether the mild autonomic abnormalities in motor neuron disease are primary or secondary to chronic

neurological disease.¹¹ It should be emphasised that there was no clinical evidence of autonomic failure in any of the motor neuron disease cases that we studied.

The results which were observed in the Shy-Drager cases confirm those of previous studies including those in which formal cell counts were made which have reported the loss of intermediolateral column cells in this condition.¹⁻⁶ It is not clear whether the autonomic failure is the direct result of the loss of sympathetic preganglionic cells, although it seems likely that this loss is responsible for at least some of the clinical dysautonomic features.

We thank Miss Frances Smith FIMLS for the microscopic preparations.

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