CHAPTER VI
CONGENITAL MUSCULAR DYSTROPHY
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REVIEW OF LITERATURE

Congenital muscular dystrophy (CMD) encompasses several groups of hereditary, congenital degenerative muscle diseases. They exhibit variable clinical course with or without cerebral malformation and muscle showing dystrophic changes. The hallmark of this heterogenous disorder is progressive muscular weakness and hypotonia within the first two years of life. They are often associated with arthrogryposis. Already recognised as separate entities within the CMD are Fukuyama type congenital muscular dystrophy and the rigid spine syndrome. The congenital muscular dystrophies have an autosomal recessive mode of inheritance.

The first description was by Batten in 1908 who called the disease "myositis fibrosa" due to the presence of excess of connective tissue seen in the histological preparations of the biopsied muscle. However, the term "Dystrophica muscularis Congenita" proposed by Howard (1908) was preferred as the disease was evident since birth and the muscle biopsy showed dystrophic pathology.

CMD, though non-progressive or benign in nature (Turner, 1962), malignant form with rapid progression of the disease and death occurring within the first decade of life has also been reported (De Langa, 1937).
The coexistence of normal intelligence with progressive white matter hypodensity on computed tomography (CT) are the unusual clinical features seen in CMD (Egger et al., 1983; Tanaka et al., 1990). These findings are different from the CNS malformations with intellectual impairment described in CMD by Fukuyama et al., (1960) among Japanese infants.

The histopathological alterations seen in the muscle are characteristic and generally representative of changes seen in dystrophies. The abnormalities seen are variation in fibre diameter, internalisation of subsarcolemmal nuclei, proliferation of the endomysial connective tissue and increase in fat. Necrosis and regeneration are usually not seen.

Fibre typing utilizing histochemical stains showed no selective involvement of the fibre types. However, in the advanced stages of the disease, presence of type-I fibre predominance and type-IIB fibre deficiency have been reported (Dubowitz and Brooke, 1973; Nonaka and Chou, 1979). In the chronic cases, Kihira and Nonaka (1985) noticed predominance of types-I and type-II fibres. The presence of undifferentiated type-IIc fibres were also encountered.

In electron microscopic investigations (Gubbay, et al., 1966; Fidzianska et al., 1982) the fibres showed fusion of myofibrils forming homogeneous mass. Excess of collagen was
seen around each fibre. The changes seen in the muscle fibres were due to dystrophy, while the excess formation of collagen was considered to be the primary event in the disease (Gubbay, et al., 1966).

Although, the presence of small muscle fibres were thought to be due to a dystrophic process, an arrest in the development of the fibres possibly due to compression caused by excess of collagen was suggested (Fidzianska et al., 1982). The excess of collagen was considered to be due to a defect of mesenchymal cells during early stages of development.

The difference in the pattern of the morphological changes in the benign and malignant forms were identified by Afifi et al., (1969). The clinically benign cases were characterised by subsarcolemmal and perinuclear mitochondria and dilatation of sarcoplasmic reticulum. The changes in the contractile filaments were not prominent. In the clinically severe cases, disarray and disintegration of myofilaments was the prominent feature.

The dystrophic process especially in CMD with arthrogryposis was questioned. Dastur et al., (1972) suggested that the disease was due to embryonic failure of muscle development, possibly secondary to defective innervation, rather than due to a degenerative process.
In the recent years, a subsarcolemmal cytoskeletal protein, Dystrophin in the skeletal muscle fibres has been identified (Hoffmann et al., 1987). It is a rod shaped protein, 150 nm in length, containing 3685 aminoacids with a molecular weight of 400 Kd, (Koenig et al., 1988). Monoclonal and polyclonal antibodies were raised against this protein. By the use of immunohistochemical methods, the presence of dystrophin in the normal muscle cell plasma membrane and its absence in the muscle from patients with Duchenne muscular dystrophy was demonstrated (Arahata et al., 1988; Bonilla et al., 1988; Carpenter et al., 1990). In contrast, application of this method on muscle biopsies of CMD showed normal localisation of dystrophin (Arikawa et al., 1991). Thus it has formed an important tool in distinguishing CMD from DMD immunohistochemically.

FUKUYAMA CONGENITAL MUSCULAR DYSTROPHY

Congenital central nervous system malformations and dystrophic features in muscle were reported among Japanese infants by Fukuyama et al., (1960). This was later referred to as Fukuyama congenital muscular dystrophy (FCMD). It is a disorder with autosomal recessive pattern of inheritance (Fukuyama et al., 1981). Since then patients with identical clinical and pathological features have been reported among non-Japanese infants (Fowler and Manson, 1973; Krijgsman et al., 1980; Mc Menamin et al., 1982; Stern et al., 1990).
The clinical characteristics included hypotonia, severe mental retardation, microcephaly, seizures and slowly progressive muscle weakness and wasting. Pseudohypertrophy of calf and elevated serum CPK values were the other features noticed. The pathological changes in the brain comprise severe gyral malformations. The muscle biopsy showed typical features of myopathy similar to that seen in CMD except for the presence of severe inflammatory infiltrates.

Immunohistochemical staining using antibodies to dystrophin showed conflicting results in FCMD. Sugino, et al., (1991) found normal positive staining in the plasma membrane of the myofibre, while partial staining was shown in the studies of Arikawa, et al., (1991). Its absence in the membrane was reported by Beggs et al., (1992).

MATERIAL

The material included 13 cases of congenital muscular dystrophy (CMD). The patients showed hypotonia, with onset of symptom since birth in all. A brief summary of clinical features is given in the Table 5.

OBSERVATIONS

Light microscopic observations on the muscle biopsies revealed three distinct groups.
<table>
<thead>
<tr>
<th>Cases</th>
<th>Age at Biopsy</th>
<th>Consig.</th>
<th>Positive family history</th>
<th>Onset</th>
<th>Hypotonia</th>
<th>Delay in motor milestones</th>
<th>Mental milestone</th>
<th>Contractures</th>
<th>Hypertrophy</th>
<th>Functional disability</th>
<th>EMG</th>
<th>CK</th>
<th>CT</th>
<th>Follow</th>
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<tbody>
<tr>
<td>1.</td>
<td>6 yrs/F</td>
<td>+</td>
<td>Birth</td>
<td>+</td>
<td>+</td>
<td>Mild delay</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Myopathic</td>
<td>5</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>4 yrs/M</td>
<td>+</td>
<td>Birth</td>
<td>+</td>
<td>+</td>
<td>N</td>
<td>+</td>
<td>-</td>
<td></td>
<td>Myopathic</td>
<td>1</td>
<td>599 ul</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>7 1/2 yrs/M</td>
<td>+</td>
<td>Birth</td>
<td>+</td>
<td>+</td>
<td>N</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Myopathic</td>
<td>2</td>
<td>413 ul</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>10 yrs/F</td>
<td>+</td>
<td>Birth</td>
<td>+</td>
<td>+</td>
<td>N</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Myopathic</td>
<td>5</td>
<td>205 ul</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>7 yrs/M</td>
<td>-</td>
<td>Birth</td>
<td>+</td>
<td>+</td>
<td>N</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Myopathic</td>
<td>1</td>
<td>413 ul</td>
<td>-</td>
<td>White matter hypodensity Deteriorated</td>
</tr>
<tr>
<td>6.</td>
<td>14m/F</td>
<td>+</td>
<td>Birth</td>
<td>+</td>
<td>+</td>
<td>Delay</td>
<td>-</td>
<td>-</td>
<td></td>
<td>Myopathic</td>
<td>1</td>
<td>314 ul</td>
<td>-</td>
<td>Improved the form sitting</td>
</tr>
<tr>
<td>7.</td>
<td>2 yrs/M</td>
<td>+</td>
<td>Birth</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Myopathic</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>8m/M</td>
<td>-</td>
<td>Birth</td>
<td>+</td>
<td>+</td>
<td>N</td>
<td>-</td>
<td>-</td>
<td></td>
<td>Myopathic</td>
<td>3</td>
<td>3050 ul</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>2 yrs 6m/F</td>
<td>+</td>
<td>Birth</td>
<td>+</td>
<td>+</td>
<td>Delay</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Myopathic</td>
<td>2</td>
<td>7150 ul</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>2 yrs 6m/M</td>
<td>-</td>
<td>Birth</td>
<td>+</td>
<td>+</td>
<td>N</td>
<td>-</td>
<td>+</td>
<td>1</td>
<td>Myopathic</td>
<td>5</td>
<td>2130 ul</td>
<td>-</td>
<td>Walking without support</td>
</tr>
<tr>
<td>11.</td>
<td>4 yrs/F</td>
<td>-</td>
<td>Birth</td>
<td>+</td>
<td>+</td>
<td>N</td>
<td>+</td>
<td>-</td>
<td></td>
<td>Myopathic</td>
<td>5</td>
<td>153 ul</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12.</td>
<td>7 yrs/M</td>
<td>+</td>
<td>Birth</td>
<td>+</td>
<td>+</td>
<td>N</td>
<td>-</td>
<td>-</td>
<td></td>
<td>115 ul</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13.</td>
<td>7m/M</td>
<td>+</td>
<td>Birth</td>
<td>+</td>
<td>+</td>
<td>N</td>
<td>-</td>
<td>-</td>
<td></td>
<td>Myopathic</td>
<td>1</td>
<td>934 ul</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Quadricipps muscle was biopsied in all.

1 - Neck holding, Can't sit
2 - Can sit with support
3 - Can sit without support
4 - Can stand with support
5 - Can walk with support
Group I (Cases 1, 2, 3, 5, 6, 7 and 13)

**HISTOLOGY**: The muscle sections showed marked distortion of fascicular architecture due to excessive fibrofatty tissue (Fig 84). Marked increase in adipose tissue was seen in 3 cases (case nos. 1, 2 and 3) (Fig 85). The muscle fibres were rounded and showed marked variation in fibre diameter. Internal nuclei were seen in several fibres. An occasional bluish fibre, probably the regenerating fibre was noticed. The features are illustrated (Figs 79 & 81).

**ENZYME HISTOCHEMISTRY**: The muscle sections showed darkly stained myofibres in NADH-TR and SDH reactions. Fibre type distinction could be faintly discerned in ATPase stain (Figs 80 & 82).

**IMMUNOHISTOCHEMISTRY**: Immunohistochemical method using monoclonal antibodies to dystrophin carried out on case 13 revealed positive staining along the sarcolemma in all the fibres (Fig.83).

Group II (cases 4, 11 and 12)

**HISTOLOGY**: The sections of muscle showed moderate distortion in the fascicular architecture. The myofibres were rounded and varied in their cross sectional diameter. There was mild increase in endomysial and perimysial connective tissue as seen in MAT stained sections. The nuclei were
placed internally in a few fibres. Hypertrophic fibres were seen in a few number (Fig 86): These fibres were also seen undergoing splitting. Necrosis and myophagocytosis was seen at a few foci. Basophilic fibres were also noticed.

**ENZYME HISTOCHEMISTRY**: The cryostat sections of muscle stained for enzyme reactions showed clear distinction of fibre types as opposed to that seen in group-I. However, both fibre types were equally involved (Figs 87 & 88).

**IMMUNOHISTOCHEMISTRY**: Immunohistochemical staining using antibodies to dystrophin (cases 11 & 12) showed positive staining along the membrane in all the fibres.

**Group III (cases 8, 9, 10)**

**HISTOLOGY**: The muscle sections showed mild distortion of fascicular architecture. The fibres were rounded and opaque. The nuclei were placed internally in a moderate number of fibres. Necrosis and myophagocytosis were prominent. Regenerating fibres were noticed. Increase in connective tissue was seen (Figs 89 & 90).

**ENZYME HISTOCHEMISTRY**: The muscle sections stained for enzyme reactions viz, NADH-TR, SDH and ATPase showed clear distinction into two major fibre types. Both fibre types were equally involved (Figs 91, 92 & 93).

**ELECTRON MICROSCOPY**: The fine structural observations of muscle biopsies in the three groups described above showed
the plasma membrane of myofibres to be normal in all the cases, while the basement membrane was merged with the excessive endomysial collagen in 7 cases (Fig 94), confirming the increase in connective tissue and fat seen by light microscopy. In the other cases the basement membrane was normal even in the presence of mild to moderate increase in endomysial and perimysial collagen. There was marked disarray of filaments causing displacement of the mitochondria and T-tubular system (Fig 96). This feature was seen in all except in group-2 which showed mild myopathic changes. Dilatation of the sarcoplasmic reticulum was also seen. Varying number of hyalinised fibres with pyknotic nuclei were noticed. The myofilaments in these appeared as homogeneous masses. Peripheral vacuolation, dilatation of reticulum and aggregates of a few mitochondria were also observed (Fig 97). In 3 cases (cases 8, 9 & 10) hyalinised fibres were seen in greater numbers. Myofibres with very small diameter containing large nucleus and prominent nucleolus were seen in between the larger fibres showing pathological changes. These probably represent regenerating fibres (Fig 95). The other features included the presence of satellite cells, and a large number of fibroblasts. The fibroblasts showed prominent RER and had long processes. A few intracytoplasmic filaments were also seen in these cells. The histological and histochemical observations in the three groups of CMD is given in the Chart-3.
### Histological and histochemical observations

<table>
<thead>
<tr>
<th>GROUP - 1</th>
<th>GROUP - 2</th>
<th>GROUP - 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histology</strong></td>
<td><strong>Histology</strong></td>
<td><strong>Histology</strong></td>
</tr>
<tr>
<td>Marked fallout of myofibres, excessive</td>
<td>Mild fallout of myofibres, mild increase in endo</td>
<td>Rounded and hyalinised fibres, mild increase</td>
</tr>
<tr>
<td>endo and perimysial fibrofatty tissue,</td>
<td>and perimysial connective tissue,</td>
<td>in endo and perimysial connective tissue,</td>
</tr>
<tr>
<td>necrosis and myophagocytosis seen to a lesser degree.</td>
<td>presence of hyper-trophic fibres and fibre splitting, necrosis and phagocytosis seen to a mild degree.</td>
<td>presence of necrosis and myophagocytosis to a significant degree.</td>
</tr>
<tr>
<td><strong>Histochemistry</strong></td>
<td><strong>Histochemistry</strong></td>
<td><strong>Histochemistry</strong></td>
</tr>
<tr>
<td>Loss of fibre type distinction.</td>
<td>Fibre type distinction preserved, involvement of both fibre types.</td>
<td>Fibre type distinction preserved, involvement of both fibre types.</td>
</tr>
</tbody>
</table>
DISCUSSIONS

In a survey of 13 cases of congenital muscular dystrophy we found a heterogenous nature in their morphological features. The common finding was variation in fibre size and fibrosis (Gubbay et al., 1966; Afifi et al., 1969; Fidzianska et al., 1982). In the present study the degree of endomysial fibrosis varied, with marked increase seen in the clinically severe cases (Group-I). Three of these cases also revealed excessive infiltration of adipose tissue. This feature is in contrast to the finding of Afifi et al., (1969) who noticed increase in adipose tissue in the clinically benign cases.

Histochemical studies has shown type-I fibre predominance and type-IIB fibre deficiency to be a common finding in the clinically severe cases (Dubowitz and Brooke, 1973, Nonaka and Chou, 1979). However, in our study majority of the surviving fibres showed dark reaction to all enzyme stains. These fibres may probably represent the degenerated fibres.

The other significant observation was the presence of hypertrophic fibres accompanied with fibres splitting and moderate myopathic changes (Group-II). This explains the functional ability to walk at the time of presentation in these patients despite degenerative process spread over many years. Unlike the typical form of CMD (the severe form) patients with CMD of this type are rarely reported (Nonaka
et al., 1972; Kihira and Nonaka, 1985). Kihira and Nonaka (1985) assumed such changes to reflect long standing dystrophic process similar to that seen in limb girdle dystrophy. In the present study, although the morphological features of muscle biopsy resembled that of limb girdle dystrophy, these cases were included under congenital muscular dystrophy as the onset of disease was at birth and the serum CPK values were not elevated.

The ultrastructural findings correlated well with the morphological features seen at light microscopy. The presence of small fibres in between the fibres showing pathological changes were considered arrested myotubes caused due to compression by excessive collagen (Fidzianska et al., 1982). However, we regard such fibres to be regenerating fibres and not arrested myotubes due to their basophilic nature and absence of myotubes as seen in foetal life.

Most prominent feature noticed was increase in endomysial collagen and number of fibroblasts in the clinically severe cases and to a lesser degree in the other forms. Abnormal fibroblasts - myofibroblasts as described by Fidzianska et al., (1982) were not observed in any of our cases. The above authors consider increase in collagen as the cardinal pathology in CMD. We feel that the degenerative changes observed in the muscle appears to be the basic pathogenetic mechanism and that fibrosis is due to degeneration and loss of myofibres.
Atypical features seen in this study (Group-3) were the presence of large number of hyalinised fibres, necrosis, myophagocytosis, regenerative activity and elevated serum CPK values, similar to that seen in DMD. These cases however were included under CMD as the patients presented clinically as floppy babies with onset of symptoms at birth. Their family history did not suggest X-linked inheritance.

Necrosis, myophagocytosis and elevated serum CPK values are also the features of Fukuyama congenital muscular dystrophy (Fukuyama et al., 1960). The FCMD is usually associated with congenital cerebral malformation. Such malformations were not seen in our cases. The possibility of these cases to be a variant of FCMD has been entertained. A rare possibility of these being early onset Duchenne dystrophy depends upon the demonstration of dystrophin along the muscle sarcolemma.

CMD is a heterogeneous entity in which muscle biopsy showed variable histomorphological features. Myopathic process starting in the foetal life is the basic pathogenetic mechanism. Increase in collagen appears to be secondary to myofibre degeneration and loss.
Legends to Photomicrographs—Congenital muscular dystrophy

Figure.79: Quadriceps muscle biopsy from a male child aged 7 years (case 7): Transverse section showing marked fallout of muscle fibres with replacement by endomysial and perimysial connective tissue. The fibres are rounded and majority are very small. Note absence of necrosis and inflammation. H and E(cryo)x300.

Figure.80: Biopsy from the child same as above showing intense oxidative enzyme reaction in the muscle fibre. There is loss of fibre type distinction. NADH-TR X300.
Legends

Figure 81: Transverse section of muscle biopsy from a male child aged 7 months (case 12) showing marked distortion of fascicular architecture. The myofibres are rounded and show marked fallout. The nuclei are eccentrically placed. There is marked increase in endo and perimysial connective tissue. H and E(cryo)x300.

Figure 82: Biopsy from the child same as above stained for myosin ATPase reaction. Some of the fibres retain fibre type distinction while other show dark reaction. ATPase (pH9.5) X300.

Figure 83: Transverse section of muscle same as above, immunostained with antibodies to dystrophin shows positive staining along the membrane in all the fibres. Dystrophin X 480.
Legends

Figure 84: Biopsy of muscle section showing marked increase in collagen (blue stain). The surviving myofibres show variable diameter.

MAT X 300.

Figure 85: Transverse section of muscle from a male child aged 7 1/2 years (case 3) showing marked increase in the perimysial fatty tissue. Muscle fibres show variation in diameter. An occasional fibre have central nuclei. MAT X 300.
Legends

Figure 86: Biopsy from a female child aged 10 years (case 4), Transverse section showing marked variation in fibre diameter in the fascicle. Increase in perimysial fatty tissue and mild increase in endomysial fibrous tissue is evident. Note-fall out of fibres is not prominent. MAT X300.

Figure 87: Biopsy from the child same as above stained for oxidative enzyme reaction appears to be normal in many of the fibres. NADH-TR X300.

Figure 88: Biopsy from the child same as above stained for myosin ATPase reaction shows distinct fibre types. ATPase (pH4.6) X300.
Legends

Figure.89: Quadriceps muscle from a child aged 2 1/2 years (case 10). Transverse section shows distortion of fascicular architecture and moderate fallout of muscle fibres. The myofibres are rounded and show variation in diameter. The nuclei are placed centrally in some of the fibres. Myonecrosis and phagocytosis is evident. Increase is endo and perimysial connective tissue is also seen. H and E X300.

Figure.90: Biopsy from case 8 (8 months, male) shows rounding and hyalinization of the fibres, an occasional fibre with central nuclei, necrosis and myophagocytosis and increase in endo and perimysial connective tissue.

H and E (cryo)X300.
Legends

Figure.91: Biopsy stained for oxidative enzyme shows intense reaction in a few fibres. NADH-TR X300.

Figure.92: Muscle section from the same biopsy showing both fibre types involved. ATP (pH-9.5) X300.

Figure.93: Biopsy from the child same as above, myosin, ATPase reaction shows type-I, type-II A and type-II B fibres, both fibre types are involved. ATPase (pH4.6) X 300.
Legends

Figure 94: Electron micrograph of muscle biopsy from a female child aged 10 years (case 4) showing longitudinal section of a portion of muscle fibre and marked increase in endomysial collagen (C). Fibroblastic processes (f) are seen. The basement membrane cannot be distinguished. X 4,070.

Figure 95: Transverse section of muscle biopsy from a child aged 7 years (case 5) shows two small fibres (\). One of the fibres contains a large nucleus (N) and scanty sarcoplasm. The other fibre shows a few scattered myofilaments (M), mitochondria (mt) and triads (\). A portion of fibroblast with nucleus and a few fibroblastic processes (f) are also evident. Excess collagen (C) is seen. X 10,000.
Legends

Figure.96: A portion of muscle fibre in transverse section illustrating disarray of myofilaments and clumping of Z-band (Z). A portion of nucleus is also seen. X 24,000.

Figure.97: Transverse section of muscle biopsy from a male child aged 2 1/2 years (case 9) showing dense mass of filaments (M), vacuoles (V), dilatation of sarcoplasmic reticulum (SR) and aggregates of mitochondria. Nucleus (N) is also seen. X 8000.