Myotonic Dystrophy Type 1 Clinical, Electrophysiological and Molecular Characterization: Experience at Tertiary Care Centre

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Abstract

Background: Myotonic dystrophy type 1 (DM1) is the most common myotonic disorder. Molecular genetic testing of the Dystrophy Myotonica–Protein Kinase DMPK gene to detect expansion of CTG repeats is confirmatory. TP-PCR (Triplet Primed-Polymerase Chain Reaction) is rapid and effective screening for the CTG repeat expansions in myotonic dystrophy. Indian data regarding clinical and genetic evaluation of DM1 are sparse.

Materials and Methods: This was a prospective observational study at a tertiary neurology centre. It included subjects having clinical and electrophysiological evidence of myotonia with CTG repeat expansion of DMPK gene demonstrated by TP-PCR. Diagnostic molecular assessment was done by two-step procedure; conventional PCR and Fragment length analysis followed by TP-PCR.

Results: Seventeen patients fulfilled the inclusion criteria. There were fifteen males and two females, with age ranging from 19 to 53 years (mean age 33years). In the phenotype, large calves were seen in three patients and ophthalmoparesis and scapular winging were seen in one patient each. Screening of patients by PCR-Fragment analysis identified all 17 cases to be of DM1. Further confirmatory test by TP-PCR also successfully identified the cases to be of DM1. TP-PCR technique using forward combination primers was used successfully in detecting expansion of CTG repeats in 13 cases whereas in remaining 4 cases reverse primer combination was used successfully.

Conclusions: This series establishes that a combination of PCR-Fragment analysis and TP-PCR is simple and cost-effective in determining the diagnosis of Myotonic dystrophy type 1. This study also documents a new clinical observation of calf hypertrophy in genetically confirmed patients with DM1.

Editorial Viewpoint

• TP-PCR is rapid and effective screening for the CTG repeat expansion in myotonic dystrophy.
• This study shows combination of PCR-fragment analysis and TP-PCR is simple and cost-effective in diagnosis of myotonic dystrophy type 1.
• The study has documented a new clinical observation of calf hypertrophy in these patients.

Introduction

Myotonic dystrophy type 1 (DM1) is the most common myotonic disorder having autosomal dominant inheritance. The causative genetic abnormality is expansion of CTG repeats within 3’ untranslated region in the DMPK gene on Chromosome 19. Along with skeletal muscles there is involvement of other systems like eye, heart, endocrine system, and central nervous system. Prevalence of DM1 is 1 in every 8,000 individual worldwide. However, DM1 is less prevalent in certain areas of Japan (1:100,000), Asia (1:18,000), and Iceland (1:10,000), and even rarer amongst Africans.

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In India, limited information is available on DM1.6,7 Gourie-Devi et al found myotonic dystrophies to form 8% of all muscular dystrophies.6 The comparatively infrequent occurrence of myotonic dystrophy in India has been documented7 and is believed to be related to number of repeats in the population.5-10 The repeat numbers are reported to be between 5-30 in India, which is comparatively less than the Caucasians, but more than the Africans.10,11 Basu et al found Indian haplotypes matching with Caucasians in 90% of patients.11

Clinically, myotonic dystrophy is characterised by specific pattern of muscle weakness. Facial weakness, ptosis, bulbar weakness, thin neck due to wasting of sternocleidomastoid muscles, hatchet face (Figure 1a) and distal motor weakness is characteristic of DM1. Patients exhibit clinical and electrical myotonia with elevation of creatine kinase (CK) levels.12 The confirmation of clinical diagnosis of DM-1 involves the molecular genetic testing of the DMPK gene by PCR test. PCR followed by southern blotting is used for the detection of CTG repeats. The TP-PCR (Triplet Primed-Polymerase Chain Reaction) technique was developed by Warner et al13 for screening the CTG repeat expansion in myotonic dystrophy and the utility of TP-PCR in DM1 was shown by Radvansky.14 It provides characteristic peak pattern which confirms the existence of triplet repeats in the DMPK gene. The Indian studies by Ashok et al15 and Bhowmick16 et al also established TP-PCR technique could provide a useful method for screening myotonic dystrophy. But at times TP-PCR gives false negative results in spite of expanded alleles due to interruptions.13,14 To overcome this issue, forward and reverse combined methods were advocated by Radvansky.

Indian data regarding clinical and genetic evaluation of DM1 are sparse. This study was undertaken to study the clinical and laboratory features of a cohort of myotonic dystrophy patients and use of conventional PCR- Fragment analysis and TP-PCR as rapid tests for diagnosing DM1.

Materials and Methods

This was a prospective observational study carried out from August 2013 to December 2015, at a tertiary neurology centre of Grant Government Medical College, Mumbai. The study was approved by institutional ethics committee. Before enrolment, informed consent was obtained from all individuals participating in the study.

Inclusion criteria

• Clinical and electrophysiological evidence of myotonia
• Expansion of CTG repeats in DMPK gene by PCR test

Exclusion criteria

• Secondary causes of myotonia
• Neuromyotonia
• Absence of electrophysiological evidence of myotonia
• Absence of expansion of CTG repeats in DMPK gene

Data collection

We did detailed clinical examination to note pattern of muscle weakness, muscle power charting17 and systemic features of DM1. Examination of sensory system and reflexes was performed. Family history and pedigree charts were recorded. Family members were examined when possible. Severity score (0-70) calculated for each patient as per score devised by Gourie-Devi et al.6

Investigations

Serum CK was estimated using the Diamension method. The patients underwent nerve conduction study and electromyography using standard protocol.18 Slit lamp examination of eyes, ECG and Echocardiography, fasting blood sugar, thyroid function test was done in all patients.

5 ml of whole blood was collected in EDTA vacutainer tubes for molecular genetics assessment for diagnostic genetic testing after the written informed consent.

DNA Extraction

Extraction of DNA from whole blood was done by commercially available kit (QIAmp Blood kit, Qiagen Hilden, Germany). Qualitative, quantitative and integrity analysis was performed for all DNA samples using agarose gel electrophoresis and spectrophotometer.

Genotyping DMPK

Molecular genetic analysis of DMPK gene for DM1 was done as per recommended best practice guidelines.19

A two-step procedure as described by Dryland PA et al20 was used in DM1 genetic testing. The first step was conventional PCR followed by Fragment length analysis, which identifies and sizes alleles within normal range. The second step employed Triplet-repeat primed PCR (TP-PCR) technique which differentiates between individuals who are homozygous for an allele within normal range and DM1 individuals carrying one allele within normal range and one unamplifiable expanded allele.
Table 1: Primers used in the genotyping DMPK gene

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<th>ID</th>
<th>5' - 3' nucleotide sequence</th>
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Primer (Eurofins Genomics India, Bangalore, India) used for the genotyping DMPK gene is given in Table 1.

PCR- Fragment analysis

Each 20 μL PCR mixture was comprised of 10 μl KAPA2G Fast Hot start (2x) Reaction Buffer (KAPA Biosystems, Boston, Massachusetts, USA), 10 μM Fluorescent labelled forward (FAM’DMIF) and reverse (DMIR) primers and 50 ng of genomic DNA. The PCR amplification conditions were initial denaturation of 95°C for 3 minutes then 35 cycles of denaturation at 95°C for 15 seconds, annealing at 64°C for 15 seconds with extension at 72°C for 5 seconds and a final extension at 72°C for 2 minutes on 2720 Thermal cycler (Applied Biosystems, Foster city, USA). PCR products for fragment analysis were subjected to capillary electrophoresis using an Applied Biosystems model 3130xl Genetic Analyzer, Foster city, USA and the data was analysed using GeneMapper version 4.0 software (Applied Biosystems Foster city, USA).

Triplet Repeat Primed PCR

TP-PCR protocol was followed as reported by Dryland et al with modifications in the PCR procedure.25 Due to the CTG interruptions observed at the 3’ and 5’ end of the DMPK gene two primer combinations were used viz. forward combination and reverse combination.

Primers for forward combination were fluorescent labelled FAM’DMIF and DMICAG and for Reverse combination were Fluorescent labelled FAM’DMIR and DMICTG (Table 1). Each patient was initially analysed by forward combination primers. Patients with negative results using forward combination primers were further analysed by reverse combination primers.

Each PCR consisted of KAPA2G Fast Hotstart Ready mix (2x) (KAPA Biosystems, Boston, Massachusetts, USA), 10 μM forward or reverse combination primers and 100 ng of DNA. PCR conditions were initial denaturation at 95°C for 3 min followed by 35 cycles of denaturation at 95°C, annealing at 60°C for 15 sec, extension at 72°C for 20 sec and final extension at 72°C at 2 min. The amplified PCR product was subjected to capillary electrophoresis using ABI3130XL genetic analyser (Foster city, USA) and the data generated was analysed on GeneMapper version 4.0 software from Applied Biosystems (Foster city, USA).

Statistical Analysis

Data was entered in MS Excel 2010, responses were coded and entered. Descriptive statistics was expressed in terms of actual numbers and frequency.

Results

Seventeen patients fulfilled the inclusion criteria. There were fifteen males and two females, with age ranging from 19 to 53 years (mean age 33 years). Mean age of disease onset was 27 years and average duration from onset of complaints to disease diagnosis was 6.4 years. Description of clinical features of all studied patients is depicted in Table 2.

Three patients had large calves (Figure 1b), ophthalmomoparesis and scapular winging was seen in one patient each (Figure 1c). Mean CK value was 266 [150-543].

Genetic analysis

Conventional PCR- Fragment analysis was performed on control and DM1 patients. Control subjects revealed two fragments corresponding to two heterozygous CTG repeats in DMPK gene (Figure 2a) whereas in all 17 DM1 patients only one fragment was detected(Figure 2b). As the conventional PCR- Fragment analysis is capable of detecting the repeat lengths up to 150, it picked up only the normal allele whereas expanded allele remained undetected in DM1 patients. Following CTG repeats were detected in DM1 patients, 5 CTG repeats in 9 cases, 11 CTG repeats in 4 cases, 12 repeats in 2 cases and 13 and 14 repeats in 1 case each.

To detect expanded allele, patients then underwent TP-PCR which showed expanded allelic ladder pattern for all 17 cases confirming the expansion of the CTG repeats in the DMPK gene (Figure 2c, 2d) and also confirming the diagnosis of DM1. TP-PCR technique using forward combination primers was used successfully in detecting expansion of CTG repeats in 13 cases whereas in remaining 4 cases reverse primer combination was used successfully.

Discussion

In this cohort, one new clinical feature was documented.

We observed 3 patients having enlargement of calves (Figure 1b) which not been previously reported in patients of DM1. Out of the three patients having calf enlargement, two had weakness of sternocleidomastoid and distal hand muscles, favouring the diagnosis of DM1. The third patient was most unusual, as this 21 year old male (Figure 1b) had calf hypertrophy without weakness or extra muscular manifestations; a phenotype which would be more consistent with myotonia congenita or DM2. Moreover,
Table 2: Description of the clinical features (N=17)

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n = 17, SCM = Sternocleidomastoid muscle, EMG = Electromyography

Electrophysiological studies documented myotonia in all and myopathic potentials in 16/17 patients with DM1. However, myopathic features were absent in one patient (case 8), who presented with calf hypertrophy without weaknesses. Another feature of the electrophysiology was the absence of axonal neuropathy. Axonal neuropathy has been described with varying frequency in different studies ranging from 17 to 33 percent. Studies documenting axonal neuropathy were more frequent in patients with DM1. Electrophysiological studies confirmed the diagnosis of DM1, which was clinically difficult. Particularly, in case 3, ophthalmoparesis helped to confirm the diagnosis of DM1. Molecular studies helped to confirm the diagnosis of DM1, which was clinically difficult. Particularly, in case 3, ophthalmoparesis helped to confirm the diagnosis of DM1. Myopathic features were absent in one patient (case 8), who presented with calf hypertrophy without weakness. Another feature of the electrophysiology was the absence of axonal neuropathy. Axonal neuropathy has been described with varying frequency in different studies ranging from 17 to 33 percent. Studies documenting axonal neuropathy were more frequent in patients with DM1. Electrophysiological studies confirmed the diagnosis of DM1, which was clinically difficult. Particularly, in case 3, ophthalmoparesis helped to confirm the diagnosis of DM1. Myopathic features were absent in one patient (case 8), who presented with calf hypertrophy without weakness. Another feature of the electrophysiology was the absence of axonal neuropathy. Axonal neuropathy has been described with varying frequency in different studies ranging from 17 to 33 percent. Studies documenting axonal neuropathy were more frequent in patients with DM1. Electrophysiological studies confirmed the diagnosis of DM1, which was clinically difficult.
higher prevalence of neuropathy have included older patients with longer disease duration and having abnormalities of blood sugars and lipids. In the present cohort, none of the patients had diabetes and patients were younger with lesser disease duration, which may explain the absence of neuropathy in them.

Detection of expanded CTG repeats is the hallmark for diagnostic confirmation of DM1. In traditional method, the combination of conventional PCR and southern blotting is used for detection of expanded alleles. Conventional PCR based on fragment analysis is used as a first step in detecting and measuring the DMPK CTG repeat size. A heterozygous allele pattern with CTG repeat sizes in the range of 5-34 repeats is considered to be normal whereas homozygous allelic pattern is considered to be positive for DM1. Figure 2a and Figure 2b depict the heterozygous allelic pattern in normal control and in DM1 positive case respectively. In our study, of the 17 cases analysed by conventional PCR – fragment analysis, all cases presented homozygous allelic pattern indicating positive screening test for DM1. The CTG repeat length >150 is considered as classical and between 50 to 150 repeats is considered as mild DM1. Traditional Conventional PCR – fragment analysis has the capacity to detect the length till 150 CTG repeats. In our study no patient presented with CTG repeats in this range, indicating the classical phenotype and making the TP-PCR necessary.

Conventional PCR is fraught with limitations in determining the true homozygotes from the heterozygotes with one expanded allele leading to false negative findings. To distinguish between true homozygous CTG repeat alleles from heterozygous allele pattern with one expanded allele, traditionally a southern blot technique is applied. However this technique is highly expensive, time consuming, laborious and also this technique requires high amount DNA making its use difficult in the prenatal diagnosis. In addition due to somatic heterogeneity of CTG repeats in DM1, the results of southern blotting are obtained as smear, causing confusion in the measurement of the exact length of the CTG repeats.

Triplet repeat primed PCR (TP-PCR) is recently being used in detecting the expansion of the repeat alleles. With this technique the distinction between
true homozygous from false homozygous is possible based on the presence and absence of the expanded triplet ladder pattern. This technique significantly reduces the need for southern blotting and can also be easily utilized in prenatal diagnosis with quick results. In our study TP-PCR was performed both in the forward and reverse direction of the DMPK gene using fluorescently labelled forward and reverse combination primers. In our study, we observed distinct expanded allelic ladder pattern for all 17 cases confirming the expansion of the CTG repeats in the DMPK gene and also confirming the diagnosis of DM1. TP-PCR technique using forward combination primers was used successfully in detecting expansion of CTG repeats in 33 cases whereas in remaining 4 cases reverse primer combination was used successfully. This showed that interruption in the 5’ and 3’ region of the DMPK gene indeed affects the diagnostic procedure leading the false negative results. Figure 2c and Figure 2d represents normal and positive DM1 case analysed by TP-PCR.

Conclusions

With this series it was observed that a combination of PCR-Fragment analysis and TP-PCR is simple, rapid and cost-effective in determining the diagnosis of Myotonic dystrophy type 1. This small series also documents a new clinical observation of calf hypertrophy in genetically confirmed patients with DM1. This observation is important as muscle hypertrophy is suggestive of other myotonias like the congenital myotonias and DM 2. The sample size needed for this study is 4.99. Inclusion of 17 patients increases the power of present study.

References


