MYOTONIC DYSTROPHY: CLINICAL AND MOLECULAR SPECTRUM IN KWAZULU - NATAL

A Thesis submitted to the Nelson R. Mandela School of Medicine
University of KwaZulu-Natal
In fulfillment of the degree of Master of Medicine (MMED) in Neurology

Ayesha Motala

Student number: 943480004

Supervisor: Professor P.L.A Bill
Dissertation Study: 2006

DECLARATION

I hereby declare that this submission is my own work and it has not been submitted to this or any other universities. All sources and references I have used or quoted have been indicated and acknowledged.

This work was supervised by Professor P.L.A Bill (Department of Neurology, Inkosi Albert Luthuli Central Hospital)

Ayesha Motala
Student number : 943480004
November 2006

Acknowledgements

My sincere thanks to **Professor Bill** for supervising this study as well as for his guidance, invaluable advice and constructive criticism throughout the study.

I would like to thank **Professor Bhigjee** for encouraging me to further my studies with an MMed.

I would like to acknowledge and thank **Dr Renu Saxena** for her assistance in the molecular diagnosis. She provided me with a detailed description of the methodology utilized at her laboratory. In addition she provided me with the results of the polymerase chain reaction and southern blot tests performed. She also provided the illustrations of the molecular tests performed on patients in the study and granted me permission for utilising these illustrations in my dissertation.

I would like to thank my sister, **Mariam Motala** for performing the statistical analysis and diagrammatic representations of the results.

I would also like to thank **Kumari Pillay** for assisting me and providing me with results of tests performed at the Neuroscience laboratory.

I would like to acknowledge all **the patients** who participated in this study.

I would like to thank the staff in the Department of Neurology for their assistance especially **Margaret and Veloshnee** who helped me to access previous patient records.

I would also like to thank **Dr Omran el Koha and Dr Faieza Sahid** for their assistance.

Last but not least my great debt of appreciation and thankfulness to **my parents, my sister Farhana and my nephew Muhammad** for their love, care and continuous support.

ABSTRACT

Background:

Myotonic dystrophy is the commonest form of adult muscular dystrophy. Myotonic dystrophy 1 and 2 (DM 1 and DM 2) are autosomal dominant inherited disorders with unusual multisystem clinical features characterized by myotonia, progressive muscle weakness and wasting, cataracts, hypogonadism, frontal balding, cardiac conduction defects and diabetes. Severity varies from asymptomatic to severely affected phenotypes. DM1 presents with predominantly distal weakness whereas DM2 have predominantly proximal weakness.98% of patients identified worldwide present with DM1.

DM 1 is caused by the expansion of an unstable CTG trinucleotide repeat in the 3' untranslated region of the myotonic dystrophy protein kinase gene on chromosome 19q13.3. DM 2 is linked to the long arm of chromosome 3q21. It is caused by a tetranucleotide, CCTG expansion in intron 1 of the zinc finger protein 9(ZNF9) gene that interferes with processing of a variety of RNAs. All DM mutations can be detected using a combination of the Southern Blot and Polymerase Chain reaction (PCR) techniques.

Aim:

This study aims to characterize the clinical spectrum and molecular features of myotonic dystrophy patients in KwaZulu - Natal between 1989 and 2005.

Methodology:

Patients included in this study were obtained from the database of patients diagnosed with Myotonic Dystrophy at the Department of Neurology in KwaZulu-Natal from 1989 to 2005. Patients were subjected to clinical, radiological and neurophysiological assessment. Molecular testing was performed using PCR and Southern blot.

Results:

Thirty-seven patients with Myotonic Dystrophy were identified. Twenty patients consented and were included into the study. Eighty-five percent of patients were of Indian descent and the remaining fifteen percent were White. No African patients were identified. Sixty-five percent were male and thirty-five percent female. Myotonia was clinically present in all patients. Ninety-five percent of patients presented with predominantly distal weakness of which 40% demonstrated mild weakness, 35% moderate weakness and 25 % severe

weakness. No patients were identified with predominantly proximal wasting or weakness. Southern blotting demonstrated expanded CTG repeats (DM1) in all 20 samples analysed. The PCR analysis was unable to demonstrate expanded alleles.

Conclusion:

This study identified patients presenting with Myotonic dystrophy to the Department of Neurology in KwaZulu-Natal and demonstrated that Myotonic Dystrophy Type 1 remains the commonest clinical and molecular presentation. In addition it substantiated previous research findings wherein no South African of African descent was found to be affected by the disease. There have been no reported cases of Myotonic Dystrophy in African Black patients presenting to the Department of Neurology in Durban, no African Black patients have been diagnosed with Myotonic Dystrophy over the past 20 years. However ,the predominance of Indians in this study is more likely a reflection of referral bias than differing incidence amongst sections of the population.

PCR analysis cannot detect trinucleotide repeat expansions beyond 200 repeats and as a result Southern Blotting remains the gold standard in obtaining a molecular diagnosis. A clinical diagnosis is sufficient and molecular confirmation is not an absolute requirement.

TABLE OF CONTENTS

List of Tables and Figures

List of Abbreviations

- **BP -** Blood Pressure
- **CXR -** Chest X Ray
- **CPM -** Calcium , Phosphate , Magnesium
- **DM -** Myotonic Dystrophy
- **DM1-** Myotonic Dystrophy Type 1
- **DM2 -** Myotonic Dystrophy Type 2
- **DM3 -** Myotonic Dystrophy Type 3
- **DMPK -** Dystrophia Myotonica Protein Kinase
- **DNA -** Deoxyribonucleic acid
- **EMG -** Electromyography
- **FBC -** Full Blood Count
- **LFT -** Liver Function Test
- **MMSE -** Mini Mental State Examination
- **mRNA** - Messenger Ribonucleic acid
- **PCR** – Polymerase Chain Reaction
- **RNA** - Ribonucleic acid
- **UE** - Urea and electrolytes
- **VA** - Visual Acuity
- **VF -** Visual Fields
- **ZNF9 -** Zinc Finger Protein 9

Chapter 1: Introduction

Myotonic dystrophy is the commonest form of adult muscular dystrophy. Myotonic dystrophy 1 and 2 (DM 1 and DM 2) are autosomal dominant inherited disorders with unusual multisystem clinical features characterized by myotonia, progressive muscle weakness and wasting, cataracts, hypogonadism, frontal balding, cardiac conduction defects and diabetes. Severity varies from being asymptomatic to severely affected. [1]

DM 1 is caused by the expansion of an unstable CTG trinucleotide repeat in the 3' untranslated region of the myotonic dystrophy protein kinase gene in chromosome 19q13.3. [2,3,4] The postulated disease mechanism is thought to be the result of a reduction of the DMPK gene product, neighbour gene suppression (SIX5) and the processing of RNAs with longer CTG repeats. [4]

The CTG repeat is polymorphic in the general population. Healthy individuals have alleles with repeat lengths ranging from 5 to 35. A repeat length from 35 to 49 is considered a "premutation" allele. Patients with DM 1 have expansions between 50 and several thousand. The size of the repeat is positively correlated with disease severity and inversely correlated with age of onset of symptoms. DM 1 is characterized by anticipation where affected individuals in succeeding generations have an earlier age of onset and a more severe clinical course. [1,5]

Direct analysis of the CTG repeat expansion has a specificity and sensitivity of 100%. All DM1 mutations can be detected using a combination of the Southern Blot and Polymerase Chain reaction (PCR) techniques.[1.2.3.4.6]

PCR is used to identify DM1 repeats of between 5 and 200. Using specifically designed synthetic oligonucleotide primers based on the sequences flanking the triplet repeats, the unstable region can be easily amplified. If run on a 3,5 % metaphor gel along with a size standard, the length of the repeat can be accurately determined. Repeats greater than 500 are not reliably amplified by PCR.

Southern blot analysis of DNA digested with one of several restriction endonucleases (EcoR1, BamH1, Nco1, Bg1) is the procedure of choice for detection of CTG repeats greater than 200. Several probes are available for hybridization. Using southern blot analysis, small-expanded alleles can be detected, which are seen to co-migrate with the normal allele during agarose gel electrophoresis after PCR is performed. This is usually difficult to resolve, hence the use of Southern hybridization for these cases.

These recommendations are based on the new nomenclature and DNA testing guidelines for DM1 produced by the International MD Consortium. [6]

DM 2 is linked to the long arm of chromosome 3q21. [7] It is caused by a tetranucleotide, CCTG expansion in intron 1 of the zinc finger protein 9 (ZNF9) gene that interferes with processing of a variety of RNAs.[7.8.9]

DM 2 closely resembles DM 1 clinically. However, there are important differences [9]

- Absence of a congenital form of DM 2
- DM 2 is generally a milder disease form with predominant proximal weakness and fewer symptoms distally, and less facial and bulbar weakness and muscle atrophy.
- Lack of mental retardation in DM2
- Central hypersomnolence is seen more commonly with DM 1
- Anticipation is generally absent in DM 2

Prior to the discovery of the DM2 gene, 98% of patients with myotonic dystrophy demonstrated expanded CTG repeats on chromosome 19.[2.3] Numerous studies have been performed focusing on characterizing the molecular and clinical spectrum of DM 2 but the frequency with which it occurs in the general population or within the DM group is as yet uncertain. The exact incidence of DM2 will be easier to determine with increasing testing for the ZNF9 gene.

No data has been published regarding the frequency of both forms of DM in clinically affected DM individuals in KwaZulu - Natal.

This study aims to characterize the clinical spectrum and molecular features of myotonic dystrophy patients in KwaZulu - Natal between 1989 and 2005.

Chapter 2: Myotonic Dystrophy - Literature Review

2.1 Incidence

Myotonic dystrophy (DM) is a multisystem autosomal dominant disorder. It is the commonest adult muscular dystrophy worldwide with an incidence of 1 in 8000. [1] The prevalence of DM1 ranges between 2.1 to 14.3 per 100 000 worldwide. DM2 is thought to be equally prevalent. The exact prevalence is unknown in South Africa. A previous study by Lotz and Van Der Meyden[10] in 1985 determined the local prevalence rate for DM in the northern Transvaal as 14.3/100 000.This was a genealogical study performed in the Northern Transvaal. Twenty white kindreds with myotonic dystrophy were identified and studied: thirteen of Afrikaans, five of English and two of German origin. No cases were identified amongst the Black and Indian populations. The local prevalence was calculated for the white population of Northern Transvaal and four kindreds had a common ancestry. [10]

2.2 Myotonic Dystrophy Type 1

2.2.1 Genetics of DM1

The gene lesion in myotonic dystrophy type 1 is due to a trinucleotide (CTG) repeat expansion on chromosome 19q13.3. [2.3.4] The expanded region lies in the

regulatory domain of the 3' end. This region is not involved in direct translation of the gene product but encodes a serine threonine protein kinase called myotonin. [2.3.4] Complex molecular mechanisms have been postulated at DNA, RNA and protein level. At DNA level the expanded CTG repeat may alter the structure of chromatin causing dysfunction in neighbouring genes. At RNA level the expanded repeat in DM protein kinase (DMPK) mRNA may bind to specific proteins and interfere with nuclear function. At protein level the reduced DMPK levels may interfere with signal transduction pathways. [2.3.4.]

Normal individuals have CTG repeat expansions between 5 and 30 with 5 and 13 being the most frequent alleles. [3] Individuals affected with DM1 have expansions greater than 50. Clinical severity correlates with the size of the gene repeat expansion. Individuals with mild signs have a smaller number of repeats compared to individuals who have the typical clinical picture of DM1. Congenital DM children frequently have the greatest number of repeats.

Anticipation is a phenomenon occurring in DM1 where the age of onset decreases and the severity of disease increases in successive generations. The number of CTG repeats is larger in successive generations. This phenomenon occurs as a result of instability in the DNA of the DM1 gene leading to an increase in the CTG repeats. [1.4]

2.2.2 Clinical features of DM1

Symptoms and signs of DM1 involve several organs. The commonest tissue and organs involved are the skeletal muscle, cardiac muscle and smooth muscle. [1] The clinical presentation involves various systems.

2.2.2.1 Muscle weakness

The muscles frequently involved are the facial muscles, levator palpebrae superioris, temporalis muscles, masseters, sternocleidomastoid muscles and the distal muscles of the forearm and dorsiflexors of the feet. As the disease progresses, weakness can involve the proximal muscles, respiratory muscles and bulbar musculature (palatal, pharyngeal and tongue muscles). Extraocular muscle involvement may occur. The clinical course is slowly progressive over many years and patients may remain stable for prolonged periods of time.[1]

2.2.2.2 Myotonia

Myotonia is a delay in relaxation of muscles following contraction. Myotonia is thought to result from alteration in function of chloride or sodium channels, which are responsible for non-dystrophic myotonia. Myotonia is pronounced in the distal muscles of primarily the hands. Affected individuals may be noted to have pronounced myotonia of the grip in the early stages often without specific

complaints relating to myotonia. As weakness progresses the myotonia tends to be less apparent. Percussion of the thenar muscles, forearm extensors and the tongue can elicit myotonia. [1]

2.2.2.3 Involvement of the eye

Cataracts, retinal degeneration, weakness of the extraocular muscles and decreased intraocular pressure are associated with DM1. Cataracts are common and occur in 80 % of adults. Cataracts are uncommon in congenital DM. Typical iridescent lens opacities and posterior cortical lens opacities are highly specific for DM1. The sensitivity is 46.7 % and 50% respectively. The specificity is 100 % for both. [11]

2.2.2.4 Frontal Balding

Frontal balding is generally more prominent in affected females.

2.2.2.5 Endocrine abnormalities

Testicular atrophy occurs in 62 to 86% of male patients. [12] There is impaired responsiveness to follicle stimulating hormone with associated hypogonadism. [13] In females no ovarian dysfunction is noted. Impairment of adrenal androgens and occasionally thyroid dysfunction may occur in patients. [14] Insulin resistance is common and may result from an alteration in signaling function of the insulin

receptor [12]. Diabetes mellitus occurs in 5% of DM individuals, frequently with associated hypersecretion of insulin. [15]

2.2.2.6 Cardiac Involvement

Disturbances of cardiac conduction occur frequently in DM. Atrial arrhythmias and first-degree heart block are the commonest disturbances. Affected individuals may develop complete heart block and require pacemaker treatment. Sudden death may occur and is usually due to cardiac arrhythmias. Severe congestive cardiac failure is unusual in DM. The cardiac muscle disorder takes the form of a dystrophy rather than myotonia and may be responsible for atrial and ventricular arrhythmias. [16] Nguyen et al [17] demonstrated fibrosis, fatty infiltration and atrophy histopathologically involving the cardiac conduction system. Cardiac involvement is an integral part of myotonic dystrophy, affecting the infranodal conduction system more significantly than the sinus node and the myocardium. Annual electrocardiograph monitoring and early pacemaker therapy is effective.

2.2.2.7 Gastrointestinal disturbances

Hypomotility and delayed transit time are prominent features. Affected individuals commonly present with abdominal cramps, distension and constipation. Pseudo - obstruction may occur due to an intestinal myopathy. [18]

The frequency of cholelithiasis / cholecystitis is increased due to impaired contractility of the gall bladder.[1]

2.2.2.8 Abnormalities of the peripheral nervous system

Progression of DM is associated with decrease in the tendon reflexes. A mild peripheral neuropathy may also occur.

2.2.2.9 Abnormalities of the central nervous system

Changes in personality with associated neuropsychiatric abnormalities such as depression may occur in DM. Hypersomnolence with alteration of sleep patterns is common in adults with DM. [1] Intellectual impairment occurs commonly. Studies [19] have noted a decline in the intelligence quotient as the age of onset of signs and symptoms decreased and as the size of the CTG expansion increased. One study [20] found that 84% of myotonic dystrophy patients showed white matter hyperintense lesions on magnetic resonance imaging, compared with 16% of controls. Most of these lesions were bilateral involving all lobes, however in 28% of myotonic dystrophy patients the white matter lesions were prominent in the temporal poles. Myotonic patients also showed significantly more generalized cortical atrophy when compared to the controls. There was no relationship between atrophy or white matter hyperintense lesions and age,

disease duration, or neuropsychological impairment. [20] However decreased cerebral blood flow in the frontal lobe and anterior temporal lobe may play a role in the brain manifestations.

2.2.2.10 Pregnancy related abnormalities

There is an increased incidence of spontaneous abortion in the first three months of gestation. [12] There is an increased incidence of decreased foetal movements, polyhydramnios and increased risk of anaesthetic related complications and of increased muscle weakness. [21] Myotonia and altered smooth muscle contraction power contributes to prolonged labour, increase in instrumental delivery, increase in emergency Caesarian sections and post partum haemorrhage. Perinatal loss is increased due to congenital DM. Preterm labour is also increased if DM affects the foetus. Obstetric complications inversely correlate with age of onset of maternal DM. [21]

2.2.3 Classification of DM1

Three forms have been classified:

- Mild DM
- Classical DM
- Congenital DM

Individuals suffering from a mild form of the disease, present with cataracts and mild distal weakness. Myotonia is present but not disabling. Cardiac abnormalities may occur. Smooth muscle involvement causing gastrointestinal disturbances is not uncommon. In contrast, classical DM individuals have severe myotonia with severe muscle weakness. Non-muscular complications are increased. The disease has a more rapid course in these patients and lifespan is generally decreased. The presence of bulbar and respiratory weakness increases the incidence of aspiration pneumonia, hypoventilation and death. Cardiac arrhythmias are a frequent cause of sudden death.

Congenital DM is the most severe form of DM. Affected infants present with neonatal hypotonia, motor and mental retardation, facial diplegia, and talipes. Respiratory weakness is frequent and often fatal. Cataracts and clinical myotonia is usually absent. If these infants survive, they have a static course with significant mental retardation and develop complications of adult related DM. (1.12)

Congenital DM is exclusively maternally inherited. Infants are usually born to mothers with symptomatic DM. These mothers generally have large expansions of CTG repeats. [22] In an affected female, the risk of having a congenitally affected child is 3 to 9 %. However the risk increases to 20 to 37 % in the subsequent pregnancy if the mother has had one affected child. [23] None of the

women with mild DM had a congenitally affected child in a study by Koch et al. [23] He concluded that the status of the mother during pregnancy had effects on the outcome of the child and that only mothers with multisystem symptoms are at risk of having a congenitally affected child. [23]

2.2.4 Investigations of DM1

Additional investigations can be performed.

- Serum creatinine kinase may be elevated
- Electromyography demonstrates myotonic potentials and myopathic features. Myotonic potentials appear as repetitive bursts of potentials which wax and wane in amplitude and frequency.
- Muscle biopsy is usually non-specific. It demonstrates central nuclei, sarcoplasmic masses and ring fibers. Necrosis, regeneration and increase of collagen are not severe. Seventy percent hypotrophy of Type 1 muscle fibers is observed. Markedly atrophic fibers are less common. [1,24] Repeat expansions can be detected in skeletal muscle samples. [24]

2.2.5 Management of DM1

Treatment is supportive. Physiotherapy, splinting and lightweight ankle orthoses are used for muscle weakness and foot drop. In severe weakness, walking aids

or wheelchairs are required. The myotonia may be treated with drugs such as phenytoin or quinine. Individuals with severe ptosis may require lid-elevating crutches. Lid surgery is usually not indicated. Annual electrocardiographs are required to monitor for arrhythmias and heart block. A PR interval greater than 0.2 seconds indicates an increase risk for severe heart block. If severe, a pacemaker may be inserted. Annual ophthalmology visits are required to detect cataracts. If present, surgery is well tolerated under local anaesthetic. Gastrointestinal symptoms may be treated conservatively. Patients need to avoid high fat foods and maintain bulk in their diet with adequate fluid intake.

Hypersomnolence may be treated with methylphenidate or modafinil. Depression may be treated with antidepressants. Respiratory weakness can be treated with breathing exercises, postural drainage and nocturnal respiratory support. Patients are at an increased risk of developing complications from anaesthetic agents such as thiopentone. DM patients must be well informed and educated about their condition.

2.2.6 Genetic counseling in DM1

In a symptomatic individual, molecular testing for DM is part of a diagnostic evaluation. In genetic counseling, an experienced genetic counselor educates and counsels the patient about the implications of the testing. Patients require emotional support and assistance in dealing with the results. Asymptomatic

family members may request molecular testing to determine their status. Genetic counseling is an essential part of the testing process. Testing is not performed in unaffected children less than eighteen years of age. Prenatal genetic testing may be performed to assess if the foetus is affected. In a complicated pregnancy the presence of a positive test with a large repeat expansion indicates that the foetus is likely to have severe congenital DM. This information can be helpful in preparing for the birth and delivery and some parents may consider termination of pregnancy. However the above raises ethical issues and prenatal testing should be thoroughly discussed with the patient.

2.3 Myotonic dystrophy Type 2

2.3.1 Genetics of DM2

In initial reports [3] the clinical and genetic diagnosis of DM were almost concordant with more than 98 % of affected individuals demonstrating the DM1 gene defect. Subsequently, numerous case reports identified individuals with autosomal dominant myotonia associated with systemic features of DM but with the absence of the DM1 expansion. Clinical differences were observed consisting of mainly proximal weakness, milder disease and the absence of a congenital form. As a result the existence of a second locus was considered. [25]

Thornton et al [26] described patients who presented with multisystem disease associated with myotonia and weakness. The clinical assessment of DM1 was considered however no CTG repeat expansions were detected in the DM1 locus. They reported the lack of anticipation, weakness primarily in the proximal distribution and limb muscles that were large or hypertrophied. [26]

Ricker et al [7] described 17 families with similar findings and proposed that proximal myotonic myopathy was a multisystem disorder similar but distinct from DM. However in 1998, the second DM locus was mapped to chromosome 3q. [7]The DM2 mutation was identified as a CCTG expansion in intron 1 of ZNF9 on chromosome 3q21 and was present in the families previously described with proximal myotonic myopathy. [8.9] The pathophysiological mechanism is thought to be an accumulation of expansions as intranuclear RNA foci, which cause global disruptions in RNA splicing and cellular metabolism. The alteration of splicing of insulin receptors and chloride channel transcripts may cause insulin insensitivity and myotonia respectively. [9]

2.3.2 Clinical features of DM2

The clinical features were defined by Day et al in 2003. [9] One hundred and thirty three families of European descent were identified. Clinical features were similar to DM1 with some exceptions. Common features included progressive weakness, myotonia, cataracts, cardiac involvement, testicular atrophy, and

insulin insensitivity. Fluctuating or episodic muscle pain was common in patients greater than 50 years of age. The characteristic pattern of weakness in DM2 involved the neck flexors, elbow extensors, thumb and deep finger flexors, hip flexors and hip extensors. Dorsiflexion weakness and facial weakness was less common when compared to DM1. Thirty percent of patients who were greater than 50 years of age, presented with hip girdle weakness. Bulbar weakness was uncommon and muscle atrophy was less pronounced. These patients lacked mental retardation and had a decreased incidence of central hypersomnolence. DM2 patients did not demonstrate a congenital form and anticipation was not a prominent feature.[9.28]

2.3.3 Investigations of DM2

- Creatinine kinase may be elevated as in DM1
- Electrocardiography may detect arrhythmias
- Electromyography demonstrates typical myotonia and myopathic features
- Muscle biopsy is similar to DM1 with centrally located nuclei, angulated atrophic fibers and severely atrophic fibers with pyknotic nuclear clumps. Occasional necrosis, fibrosis and adipose deposition is seen.[9] Preferential type 2 fiber atrophy and the absence of type 1 fiber atrophy distinguishes DM2 from DM1.[29] Higher frequency of nuclear clump fibers are also seen in DM2.[29]

2.3.4 Management of DM2

The management of DM2 is similar to DM1. Treatment is supportive and symptomatic. Annual ophthalmology visits and electrocardiography are required to detect cataracts and cardiac arrhythmias respectively.

2.3.5 Genetic Counseling in DM2

As in DM1 all individuals require genetic counseling prior to molecular testing. Prenatal testing is not required in view of the absence of a congenital form.

2.4 Myotonic dystrophy Type 3

In 2004, Hannequin et al [30] described a new disease entity of proximal weakness, clinical myotonia, early bilateral DM1 type cataracts and early onset dementia in individuals from three generations who underwent detailed neurological examination and linkage analysis.

These patients developed slow progressive weakness associated with muscle pain, myotonia, cataracts and mildly increased creatinine kinase. In later stages they developed severe weakness with severe frontotemporal dementia.

Molecular analysis excluded DM1 and DM2. The age of onset was earlier in subsequent generations suggestive of anticipation. Muscle biopsy showed non-specific myopathic features with type 1 and type 2 fibers being equally atrophied, which differs from DM1 and DM2.

Neuropsychological testing, frontal hypoperfusion on single-photon emission computed tomography and neuropathological features of frontotemporal spongiosis, neuronal loss and neuronal and glial tau inclusions were consistent with frontotemporal dementia.

Magnetic resonance imaging demonstrated cortical atrophy without white matter lesions. Genome wide screen analysis strongly suggested a locus at 15q21-24 under a dominant model. This disease entity has been proposed as myotonic dystrophy type 3 (DM3). [30]

Chapter 3:Molecular Diagnosis of Myotonic Dystrophy Type 1

Myotonic dystrophy demonstrates an autosomal dominant pattern of inheritance. [1.4] Phenotypic variability, multisystem involvement and anticipation are important aspects evident in this disorder. Anticipation is an increase in severity of symptoms and a reduction in age of onset that is observed during transmission of the gene within families. [1.2.3.4.5]

Myotonic dystrophy type 1 (DM1) has been mapped to chromosome 19q13.2-13.3 by genetic linkage analysis. [2.3.4.31] In DM1 individuals, a highly polymorphic CTG repeat was identified and found to be unstable with an increased number of repeats.[2.3.4] The mutational mechanism leading to myotonic dystrophy was attributed to a triplet amplification of the CTG repeat residing in the 3' untranslated portion of the protein kinase gene and in the promoter region of the immediately adjacent homeodomain gene, SIX5 .[2.3.4.8] Suggested mechanisms for the cause of the clinical phenotype includes [8]:

a) Haploinsufficiency of the dystrophia myotonica protein kinase protein.
b) Altered expression of neighbouring genes, for example SIX5.
c) Pathogenic effects of the CUG expansion in RNA which accumulates in nuclei and disrupts cellular function.

The extent of the increase in the allele size showed a correlation with increased severity and earlier age of onset. [4] An increase in the number of CTG repeats results in an increase in the size of the polymerase chain reaction products (PCR). Polymerase chain reaction analysis of severely affected individuals reveals only a single band in the normal range. [4] The expanded allele cannot be easily visualized by PCR assay in these patients. [6]

Normal individuals have 5-37 CTG repeats located at the myotonic dystrophy locus. [4,6] The most frequent allele in the general population is 5 repeats (35%), followed by 11, 12 and 13 repeats (50%). [2.3.32] Greater than 30 repeats are rare (less than 2%). [32] Individuals affected with myotonic dystrophy have a minimum of 50 repeats, which is almost double the normal population.[4.6]It is postulated that doubling or tripling in repeat numbers may be the ancestral event that predisposes an allele at the myotonic dystrophy locus to expand into an allele associated with disease expression.[4.6]

Stable transmissions of a minimal CTG repeat expansion may occur by individuals with mild manifestations allowing persistence of the myotonic dystrophy mutation in the population.[33]Stability of the CTG repeat in myotonic dystrophy appears to be a function of size. Once the size of the allele is greater than 80 CTG repeats, stability is unlikely, resulting in anticipation. [33] Stable transmissions have been well documented in individuals with 50 to 80 CTG repeats.[33]

Asymptomatic / minimally affected DM individuals have small expansions known as protomutations (50 to 80). These are inherited in a stable form if transmitted by a female.[32] However if transmitted through male germline they result in a large increase resulting in symptomatic myotonic dystrophy.[32]Repeat sizes of 38 to 50 are called pre mutations and form the pool from which protomutations and full mutations arise.[32]

The incidence of DM1 is constant in the population despite anticipation and the low reproductive fitness in affected individuals. Some mechanism exists to maintain the population incidence of DM1.A common haplotype has been found in European and Asian populations indicating a founder effect.[32] Martorell et al [32] demonstrated mutations involving alleles in the normal range (4 to 37). All were expansions and the majority paternally transmitted. All were greater than 25 repeats in length.[32] These data supported the hypothesis that multiple mutations of alleles within the normal range lead to a gradual increase in CTG repeat lengths eventually generating new premutation size alleles.[32]

Martorell et al [32] also observed the presence of premutations of 38 to 50 repeats in individuals with no clinical symptoms of myotonic dystrophy. Of the transmissions observed, all demonstrated an increase length in the next generation suggesting an increase risk of developing DM1 in future generations.

Paternal transmissions demonstrated larger repeats reflecting male bias in the generation of new DM1 alleles from premutations and protomutations.[32]

Congenital myotonic dystrophy shows exclusively maternal inheritance and large repeat sizes of greater than 2000.[4] In congenital myotonic dystrophy , the paternal allele was shown to be inherited unaltered whilst the maternal allele was unstable suggesting that the triplet repeat becomes unstable during germline meiosis once the repeat number exceeds 50.[2] With the exception of congenital DM , male carriers are more likely than female carriers to give rise to offspring with large expansions.[33]

The molecular diagnosis of DM1 is based on the guidelines of the International Myotonic Dystrophy consortium. [6] The presence of 35 to 49 repeats is classified as premutation alleles and greater than 50 as symptomatic DM. The presence of 50 to 150 repeats is usually associated with mild DM, 100 to 1000 repeats with classic DM and greater than 2000 being found in congenital DM.

The combined southern blot and polymerase chain reaction tests can detect all DM1 mutations without false positives. PCR amplification of the CTG repeat region of genomic DNA revealed that unaffected alleles are readily amplified, while the mutant alleles are not usually visualized.[3] Expanded alleles have a blurred appearance on Southern Blots.[3]

Southern blot analysis of genomic DNA digested by restriction endonucleases is the procedure of choice for detection of repeats greater than 100. However small alleles may co migrate with normal alleles during gel electrophoresis making diagnosis difficult. PCR is then used to detect repeats less than 200 in size. Using synthetic oligonucleotide primers based on sequences flanking the triplet repeat, the unstable repeat is amplified. If the PCR product is run on 3,5% metaphor gel along with size standards, the repeat length can be determined. [6]

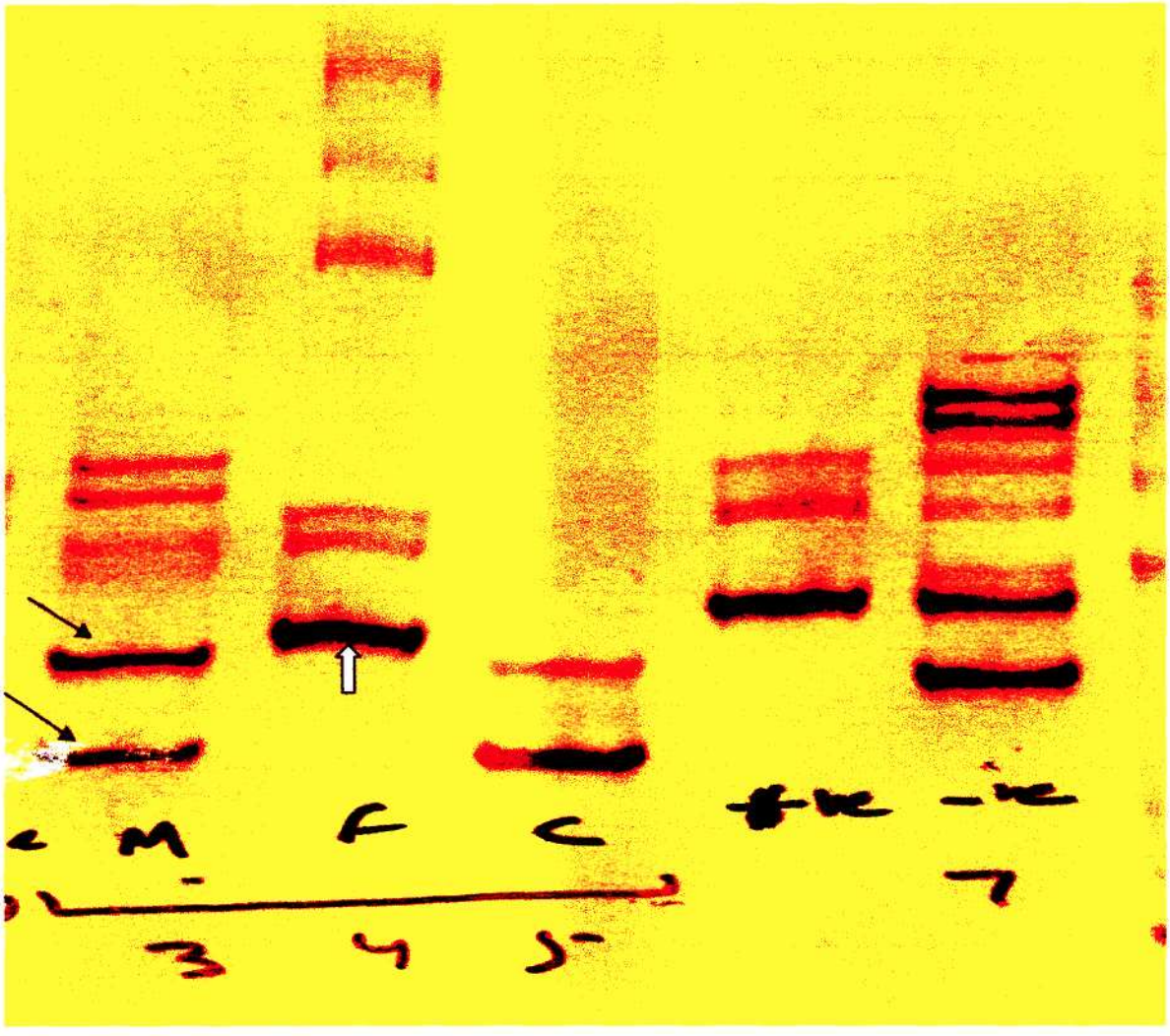


FIGURE 3.1. EXAMPLE OF A PCR ANALYSIS OF A FAMILY WITH A CHILD WITH MYOTONIC DYSTROPHY

In this case, the parents were asymptomatic but the father (F) had an allele in the intermediate range (not fully expanded allele). Mother (M) shows two normal alleles (black arrows) and the father one pre-expanded allele (white arrow). The child (C) has one normal allele from mother and does not have any allele from the father and is symptomatic i.e. the pre-expanded allele has turned into a full expansion in the affected child and is not amplified in the PCR analysis.

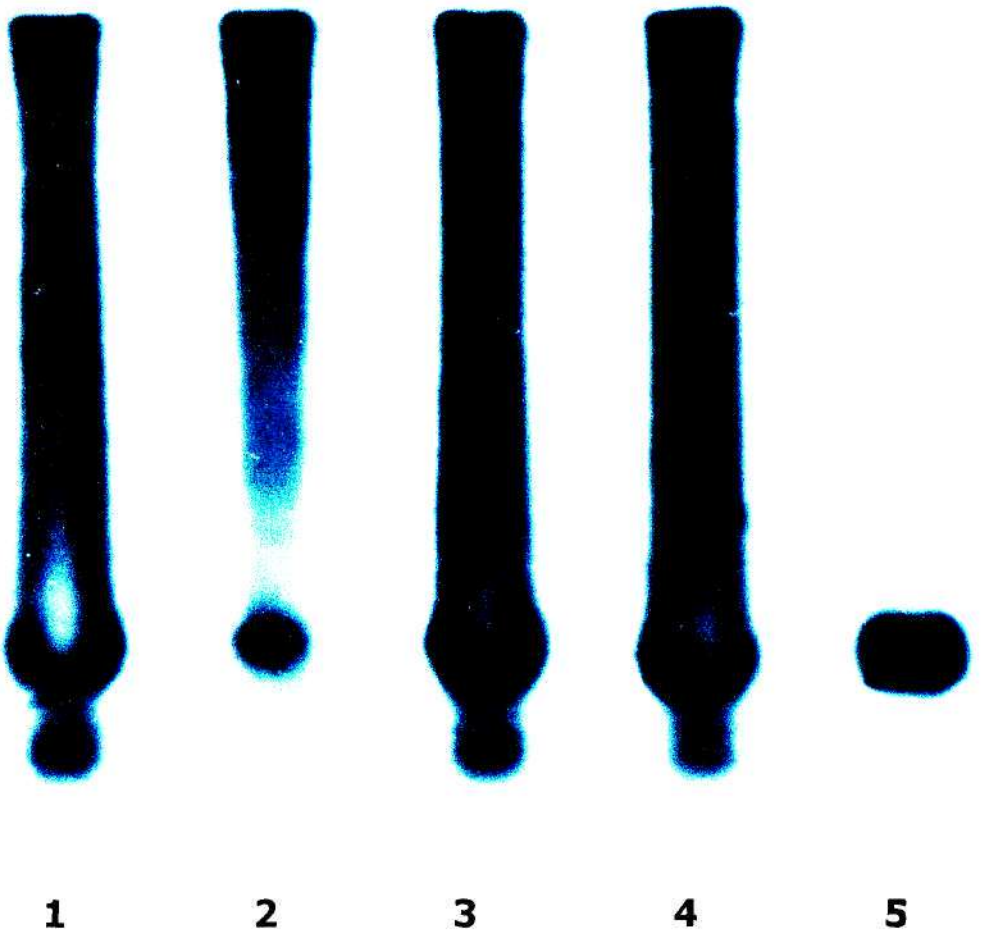


FIGURE 3. 2. SOUTHERN BLOT DEMONSTRATING EXPANSION IN THE FOUR LANES WITH A NEGATIVE CONTROL IN THE FIFTH LANE.

Chapter 4:Molecular Diagnosis of Myotonic Dystrophy Type 2

Ranum et al [7] reported the mapping of a second myotonic dystrophy locus in 1998 after identifying a large family with clinical similarity to DM1 but the absence of chromosome 19 CTG expansions. Linkage analysis was used to determine the disease locus. Based on an autosomal dominant inheritance model, the analysis excluded chromosome 19 as a locus. In addition other loci known to be associated with myotonia were excluded. These were the sodium channel gene (SCN 4A) on chromosome 17q and the chloride channel gene (ClCN1) on chromosome 7q. Further genetic mapping identified a 10-cM region of chromosome 3q as the second locus.[7]

Using positional cloning, Liquori et al [7] identified the myotonic dystrophy type 2 (DM2) mutation as a CCTG expansion in intron 1 of ZNF9 on chromosome 3q21. ZNF9 is referred to as the cellular nucleic acid binding protein gene and is thought to be a RNA binding protein. The accumulation of expansions as RNA foci may cause global disruptions in RNA splicing and cellular metabolism. No congenital form was seen.[7]

Conventional Southern blot techniques have proven unsatisfactory for DM2 due to the extremely large size of expansions.[34] Overall sensitivity of southern blot is 70 to 80%.[34]

Day et al [9] developed a repeat assay, which amplifies the genomic DM2 region containing the repeat expansion followed by southern blot analysis of the PCR products probed with an internal probe. The DM2 repeat assay and repeat – primed PCR has been reported to overcome the sensitivity problem.[19] Day et al [9] demonstrated a sensitivity of 99% for detecting expansions with the repeat assay when compared to 80% detection with Southern blot.

Sallinen et al recently described in situ hybridization to detect both the genomic expansion and the mutant transcripts in muscle biopsy sections. Flourescence in situ hybridization on extended DNA fibers was used to directly visualize the DM2 mutation and estimate expansion size. [34] Chromogenic in situ hybridization may provide a convenient and reliable method for diagnosis whilst fluorescence in situ hybridization is useful as a research tool.

Currently the proposed three step molecular diagnostic procedure includes [34]:

a) PCR based allele sizing for the exclusion of DM2 in individuals with two normal amplifiable alleles.
b) Southern Blot analysis
c) Repeat assay

Chapter 5: Research methodology

5.1 Aim

The main objective of this study is to describe the clinical and molecular spectrum of myotonic dystrophy patients in KwaZulu – Natal.

5.2 Patient identification

Patients included in this study were identified from the database of patients diagnosed with Myotonic Dystrophy at the Department of Neurology in KwaZulu - Natal from 1989 to 2005. The research and ethics committee of the University of KwaZulu-Natal approved the study.

5.3 Data Collection Method

All patients were informed about the study (appendix 1). Only those who gave informed consent were enrolled (appendix 2). The study was performed at Inkosi Albert Luthuli Central Hospital (IALCH), the major tertiary health care referral hospital of the province of KwaZulu-Natal. The study involved the collection of both prospective and retrospective data. Data was collected on a Myotonic Dystrophy Data sheet (appendix 3).

Data collected included:

5.3.1. Epidemiological data

5.3.1.1 Age

5.3.1.2 Gender

5.3.1.3 Ethnic group.

5.3.2. Clinical data

5.3.1.1 **History:** age of onset of symptoms and associated family history

5.3.1.2 **General examination:** including Blood pressure, pulse, frontal balding and testicular atrophy

5.3.1.3 **Neurological examination:** mini mental state examination, associated hypersomnolence, cranial nerve examination including the presence of cataracts, distribution of wasting, presence of myotonia in forearm extensor groups and thenar muscles, motor strength determined using the standard scale of 0 –5, reflexes (quantified 0 – 4) (0 = absent 1 = depressed 2 = normal 3= brisk 4 = clonic), sensation, co ordination and gait (ambulant, walks with aid, unable to walk).

5.3.3. Haematological data

- Full blood count

5.3. 4. Biochemical data

- Urea and electrolytes
- Liver function tests
- Glucose, calcium, phosphate, magnesium and cholesterol
- Creatinine Kinase

5.3.5. Radiographic data

- Chest x ray

5.3.6. Electrocardiography data

5.3.7. Nerve conduction studies and electromyography data

- Looking for an associated neuropathy
- Myopathic features and myotonic discharges.

5.3.8. Molecular diagnostic data

- Polymerase chain reaction and Southern Blot analysis.

5.4. Methodology for molecular diagnostic testing

Consent for DNA analysis was obtained from each patient attending the neurology or neuromuscular clinic. Blood samples were collected in EDTA tubes. DNA extractions were performed using conventional procedures and PCR analysis of the patients DNA was performed at the Neuroscience Laboratory, University of KwaZulu - Natal. Samples were sent to the Department of Genetic Medicine, Sir Ganga Ram Hospital, Rajinder Nagar, New Delhi for further analysis by PCR and southern hybridization.

The following primers were utilized:

- The DM1 CTG repeat expansion was amplified using the primers for the DM1 locus. Primer sequences were as follows:
    - **101F: 5′ CTT CCC AGG CCT GCA GTT TGC CCA TC 3′**
    - **102R: 5′ GAA CGG GGC TCG AAG GGT CCT TGT AGC 3′**
- The DM2 repeat region was amplified from the genomic DNA using the following primer sequences:
    - **CL3N58-D F (5′ GCC TAG GGG A CAA AGT GAG A 3′)**
    - **CL3N58-D R (5′ GGC CTT ATA ACC ATG CAA ATG 3′)**

5.4.1 PCR analysis at University of KwaZulu - Natal Neuroscience Laboratory

PCR analysis in University of KwaZulu - Natal Neuroscience Laboratory was performed as follows:

- Optimised PCR procedures using the primers specific for DM1 were performed on all patient samples.
- A PCR assay was optimized using primers specific for DM2, and all patient's negative for the DM1 expansion were subjected to the PCR assay for DM2.
- PCR products were electrophoresed on a 2% agarose gel and analysed using the GelDoc software from Biorad.
- The PCR products were subsequently purified using the HighPure kit from Roche.
- The purified products were subjected to DNA sequence analysis using the 3100 Genetic Analyser.
- The results were analysed using the Biotools DNA sequence analysis software.
- Those patients' samples found not to have a DM1 or DM2 duplication using the PCR assays were subjected to Southern hybridization using specifically designed probes.

5.4.2. DNA analysis at Department of Genetic Medicine, Sir Ganga Ram Hospital

DNA analysis at Department of Genetic Medicine, Sir Ganga Ram Hospital was done using the methods described in Current Protocols in Human Genetics. ([11])

5.4.2.1 Materials

- 10 mM dNTP mix
- 10 x PCR amplification buffer with 15mM $MgCl_2$
- 10 ng/µl PCR primer 1a (5 ' GG AGG ATG GAA CAC GGA CGG 3 ')or primer 1 b (5 ' CAG AGC AGG GCG TCA TGC ACA 3 ')
- 10 ng/µl PCR primer 2 : (5 ' GAA GGG TCC TTG TAG CCG GGA A 3 ')
- 5 U/µl Taq DNA polymerase
- DNA sample: 50ng of purified genomic DNA
- Mineral oil
- 10 x gel loading buffer
- 1.8 % agarose gel
- 1 x TBE buffer
- 10 mg/ml ethidium bromide

5.4.2.2. Preparation of PCR reaction

0,5 ml PCR tubes are labeled. One for each DNA sample , plus positive and negative controls. DNA with a known large DM expansion should be used as a positive control for PCR and a sample with water in place of the DNA should be used as a negative control. For each sample a PCR reaction mix is prepared using:

- 4 µl 10 mM dNTP (200 µM)
- 2.5 µL 10 x PCR amplification buffer with 15 mM $MgCl_2$
- 5 µl 10 ng / µl PCR primer 1
- 5 µl 10 ng/µl PCR primer 2
- 7.25 µl autoclaved water
- 0.25 µl 5 U/µl Taq DNA polymerase
- PCR primer product is 64 base pairs larger when using the primer 1bcompared to primer 1a.
- An aliquot of 1 µl DNA sample is added into the tube using a separate DNA pipette. The reactions are covered with mineral oil.

5.4.2.2.1. Preparation of PCR reaction- Electrophoresis of PCR products

- 73 ml of a 1,8% agarose gel in 1 x TBE buffer is prepared.

- 10 mg/ml of ethidium bromide is added to 0.5 µg/ml and the gel is poured in a 15 x 10 cm tray with a 0.8 mm thick comb.
- 10 x gel loading dye is added to 5 µl of PCR product to achieve a final concentration.
- This is applied to the gel.
- One lane of appropriate molecular sized markers is applied.
- The gel is electrophoresed at 80 V, 90 mA in a 1 x TBE buffer that does not contain ethidium bromide.
- When the bromophenol blue dye front has traveled 3,5 cm from the wells , the gel is photographed.
- The normal allele is usually within the ethidium bromide front.
- The DM mutant alleles are usually heterogenous in size and are not typically visualized by ethidium bromide staining.
- Electrophoresis must be stopped when the bromphenol blue dye front has traveled 5.5 cm from the wells and the xylene cyanol dye front has traveled 2cm.

5.4.2.2.2. Preparation of PCR reaction -Transfer of PCR products to nylon membrane

The CTG-PCR product can be transferred overnight by capillary transfer to a positively charged nylon membrane. DNA can be fixed to the membrane with baking or ultraviolet cross-linking.

5.4.2.2.3. Preparation of PCR reaction - Hybridize the membrane

- The membrane is pre-hybridized in a pre-hybridization /hybridization buffer for at least 1 hour and 30 minutes to 12 hours at 50^0 C.
- The buffer is then discarded.
- 20 µl alkaline Phosphatase – conjugated $(CTG)_{10}$
- Oligonucleotide is added to 20 ml fresh pre-hybridization / hybridization buffer and is hybridized to membrane for 1hour and 30 minutes to 2 hours at 50^0 C.
- Wash buffers 1 and 2 are pre-warmed to 50^0 C.
- The membrane is removed from the pre-hybridization / hybridization buffer and excess liquid is drained.
- The membrane is washed using the following regimen:
    - Wash buffer1 – two times, 5 minutes each at 50^0 C
    - Wash buffer 2 – two times, 5 minutes each at 50^0 C
    - Wash buffer 3 – one time, 5 minutes at room temperature

5.4.2.2.4. Preparation of PCR reaction- Develop Signal

- The membrane is placed on a clean surface.
- Lumi- Phos 530 substrate solution is sprayed sparingly from a height of 12 to 18 inches.

- The membrane is covered completely and evenly.
- The membrane is wrapped in plastic wrap or acetate protective sheets.
- The edges are sealed well to prevent excess Lumi – Phos 530 from leaching out and producing artifacts.
- The blot is then exposed to Kodak X- AR autoradiographic film 3 hours to overnight at 37^0 C and viewed.
- Myotonic Dystrophy expansions appear as diffuse smears.

5.5 Statistical Analysis

Data were captured in Microsoft excel and exported into SPSS version 11.5 (SPSS inc. Chicago, Illinois, USA) for statistical analysis and simplifications. Simple one-way frequencies, bar charts and Pie charts were used to describe categorical variables. Quantitative variables were described using descriptive statistics e.g. means.

Chapter 6: Results

6.1. Introduction

This chapter reports on the results of the study. Twenty-five patients were identified from previous referrals to the neurology department. A further twelve relatives were identified from their records. From the thirty-seven individuals identified, twenty were included in the study. The remaining seventeen patients had been diagnosed clinically with myotonic dystrophy type 1 but were not included as they were either deceased (5), refused consent (5) or could not be contacted (7). Thirteen of these patients were first-degree relatives of patients included into the study. Clinical data, follow up visits and molecular results were available in the 20 patients included in the study.

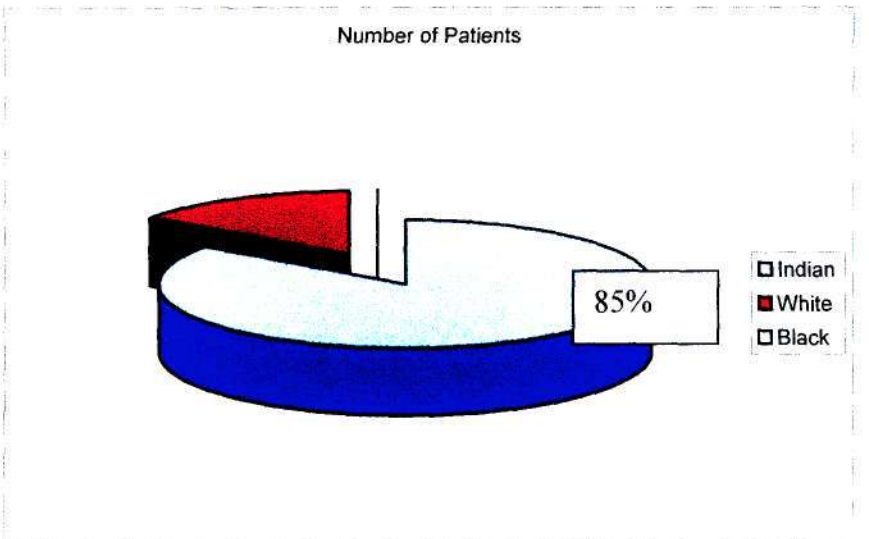
6.2. Findings

6.2.1. Demographic Information

6.2.1.1 Race

From the following pie chart it can be seen that 85 % of patients were of Indian descent and the remaining 15% were White. No African patients were identified in both the included and excluded patients.

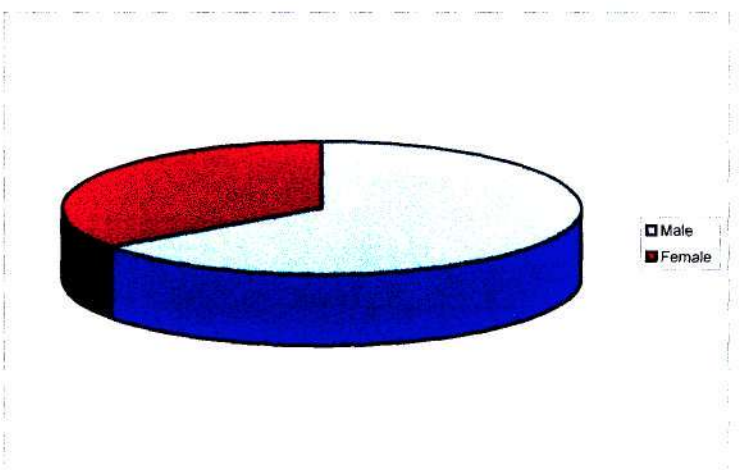
FIGURE 6.1. PIE CHART DEPICTING BREAKDOWN OF RACE



6.2.1.2 Gender

Results showed that 65% were male and 35% female

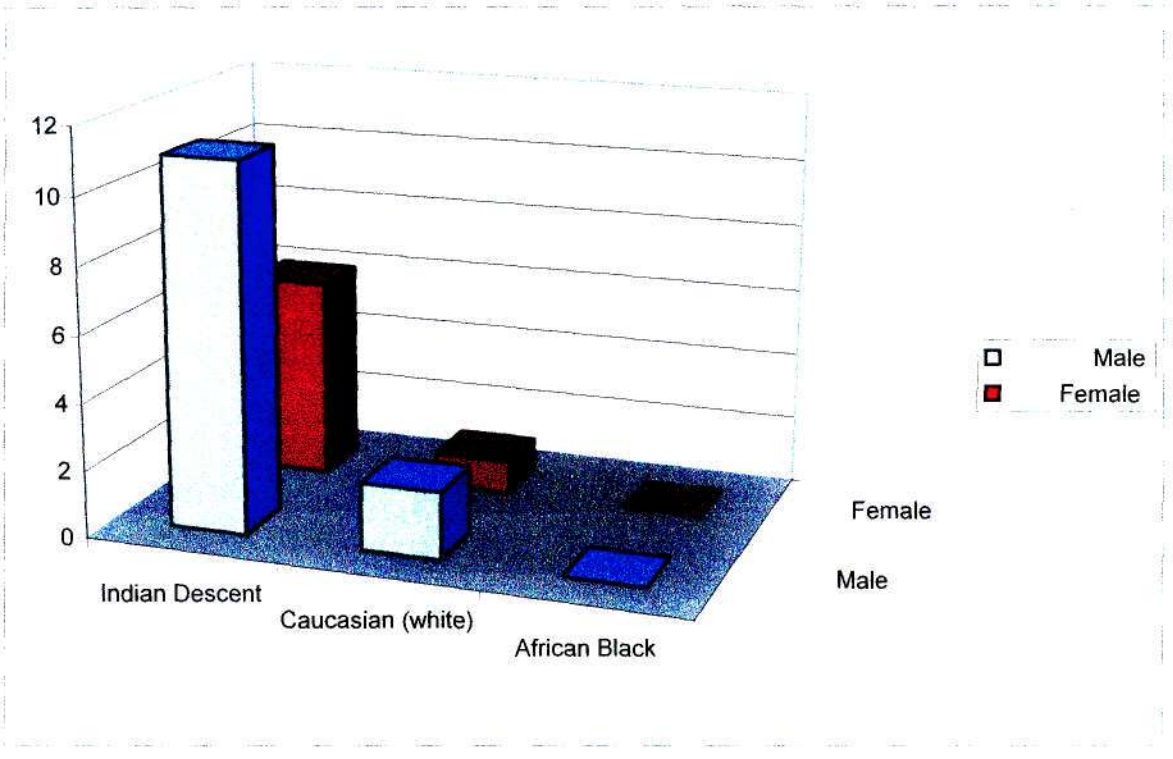
FIGURE 6.2. PIE CHART DEPICTING BREAKDOWN OF GENDER



6.2.1.3 Gender and Race

The following chart highlights the breakdown in terms of both race and gender.

FIGURE 6.3. BAR CHART PRESENTING BREAKDOWN OF BOTH RACE AND GENDER



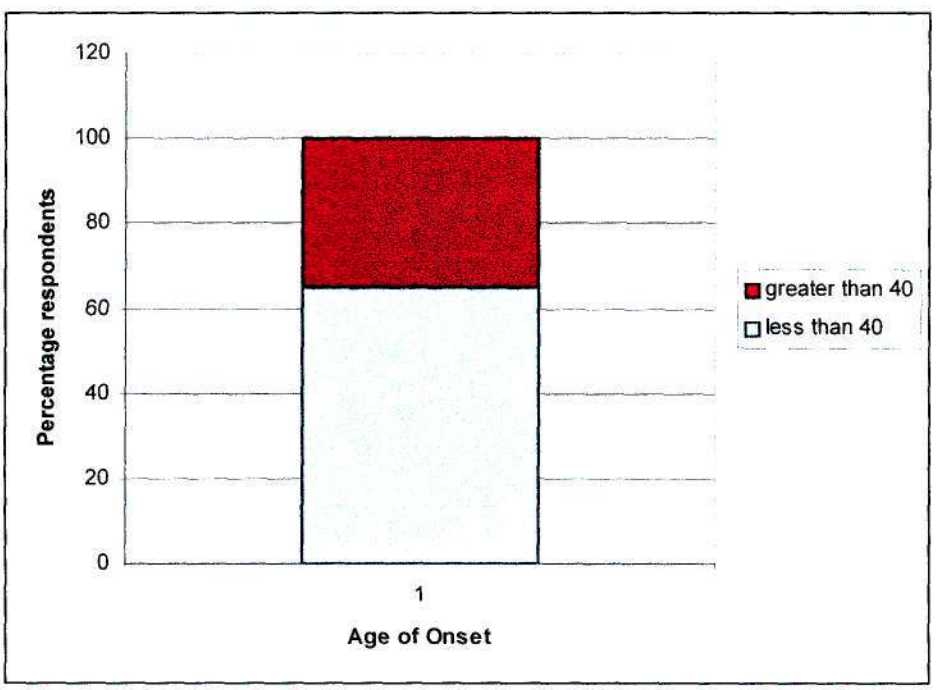
6.2.1.4. Age

Table 6.1. Displays the age demographics. In the majority (65%) the age of onset of clinical symptoms was below the age of 40 years. The mean age of onset of symptoms for the sample was 31.35 years. The average age of the sample was 40.80 years with a range of 17 to 65 years.

TABLE 6.1. AGE DEMOGRAPHICS OF PATIENTS

	Age in years
Mean	**40.80**
Range	**17 - 65**
Mean age of onset of symptoms	**31.35**

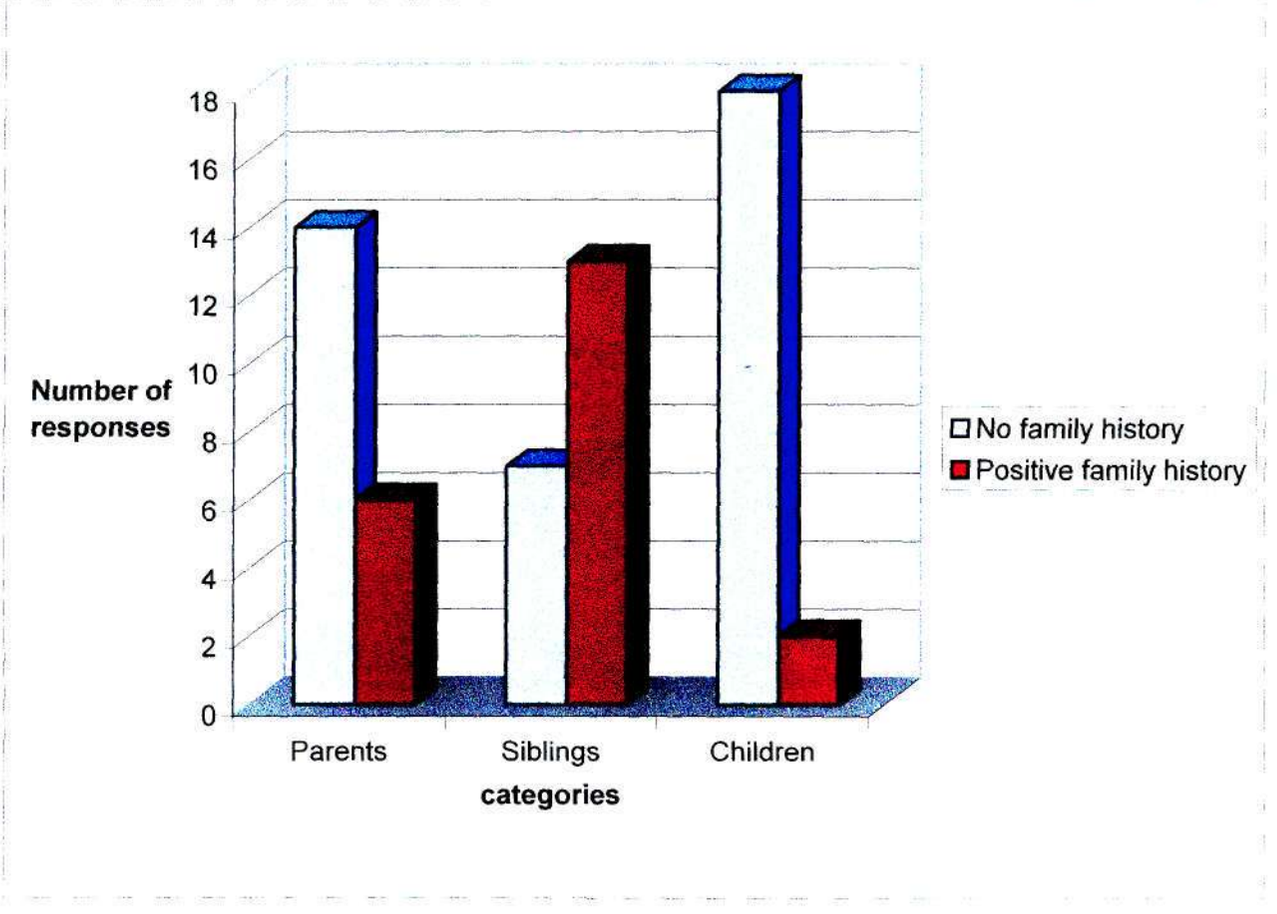
FIGURE 6.4. BAR CHART PRESENTING BREAKDOWN OF THE AGE OF ONSET OF SYMPTOMS



6.2.1.5 Family History

Twenty Five percent had no family history of note. Of the remaining 75%, positive parental history was noted in 30%, 65% in siblings and 10% in offspring.

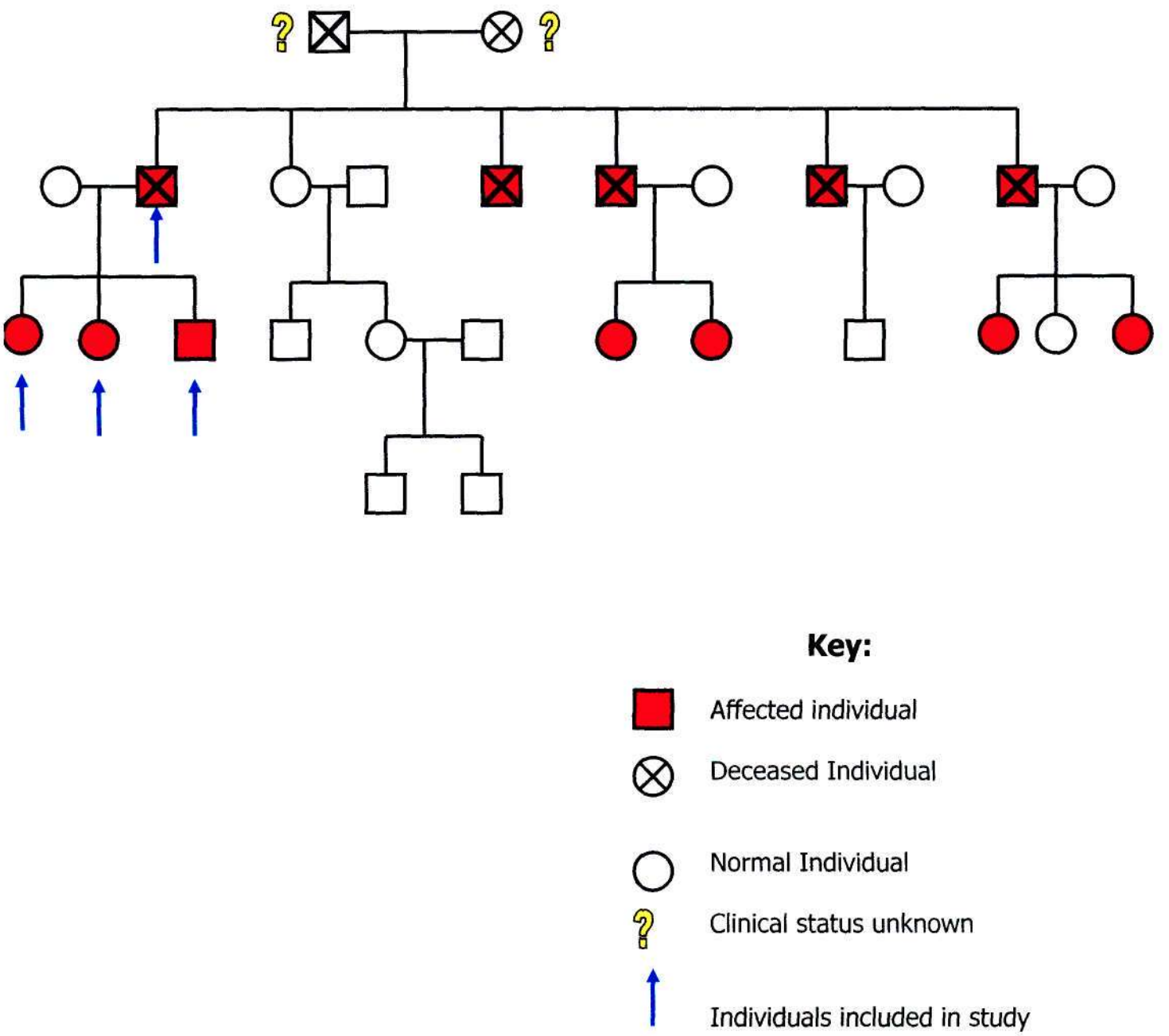
FIGURE 6.5. BAR CHART DEPICTING BREAKDOWN OF FAMILY HISTORY



Four patients were part of a large family and their pedigree analysis demonstrated an autosomal dominant pattern of inheritance.

The four affected individuals who were not included into the study were diagnosed on history. They had been evaluated elsewhere and molecular studies reportedly confirmed their diagnosis. The following Family tree highlights this:

FIGURE 6.6. FAMILY TREE DISPLAYING HEREDITY IN AN AUTOSOMAL DOMINANT PATTERN

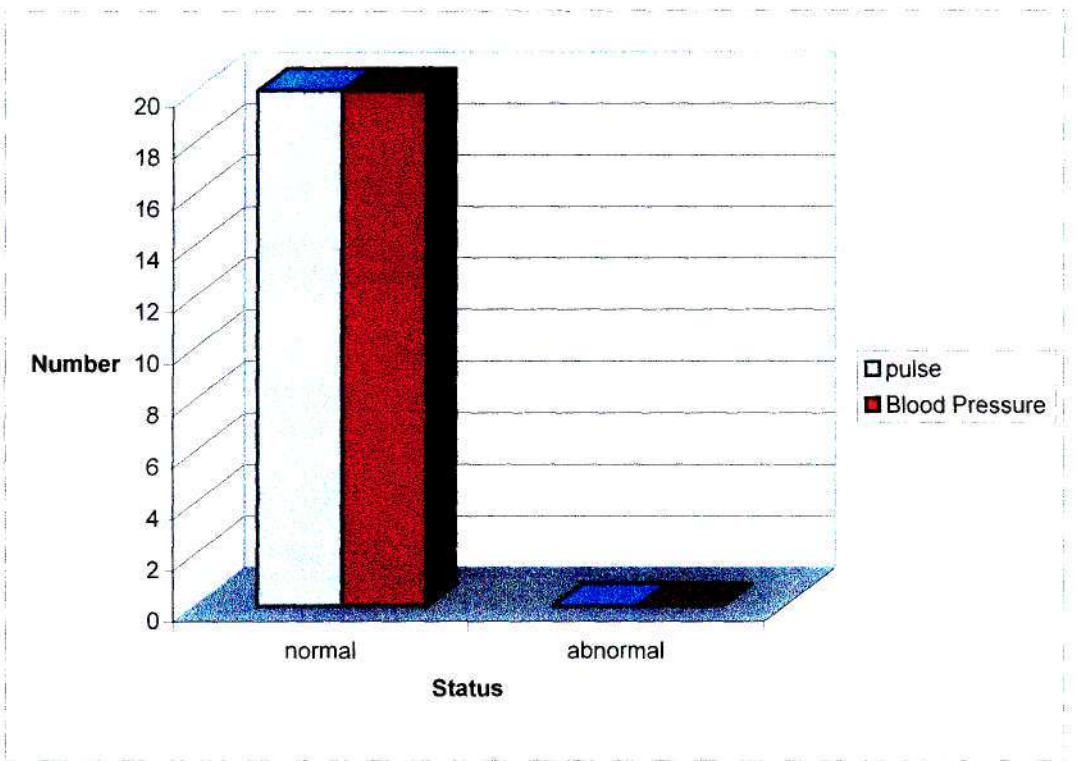


6.2.2. Clinical findings

6.2.2.1. Blood Pressure and Pulse

No abnormalities were noted in the vital signs. One patient was on treatment for hypertension.

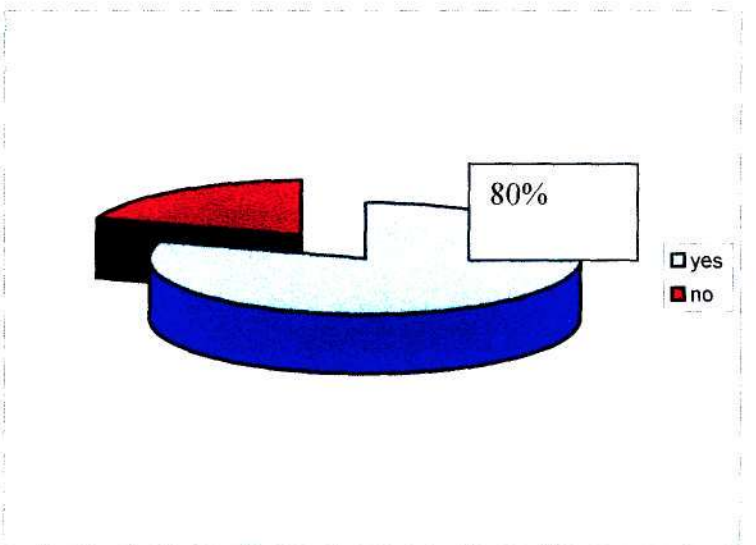
FIGURE 6.7. BAR CHART DEPICTING STATUS OF BLOOD PRESSURE AND PULSE



6.2.2.2. Frontal Balding

Frontal balding was present in 80% of patients.

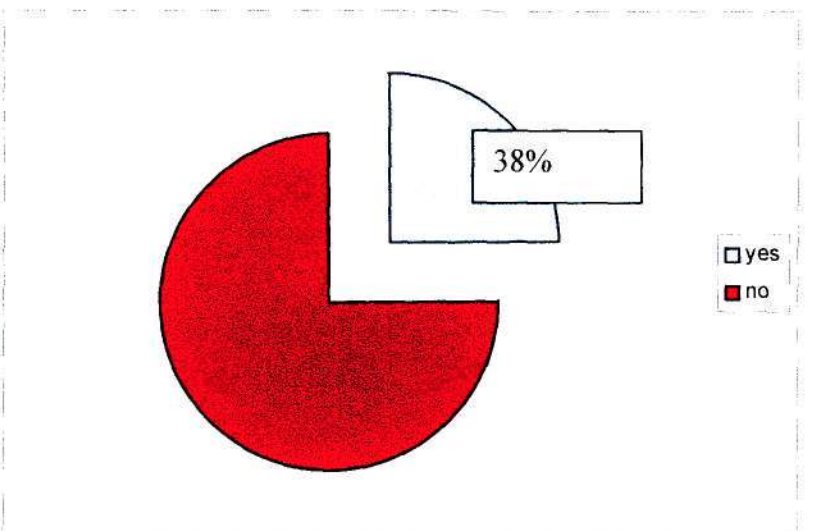
FIGURE 6.8. PIE CHART SHOWING PRESENCE OF FRONTAL BALDING



6.2.2.3. Testicular atrophy

Testicular atrophy was present in 38% of patients.

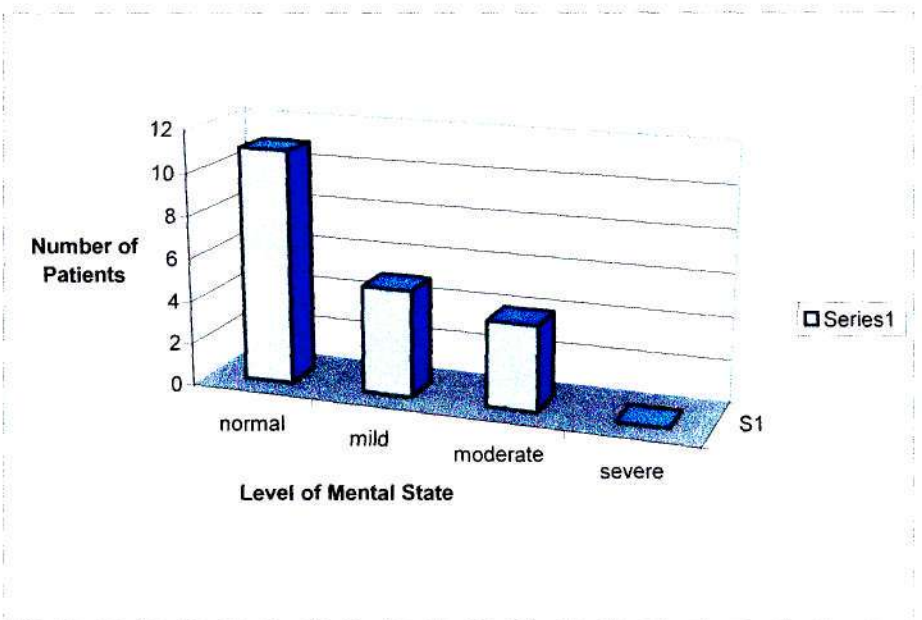
FIGURE 6.9. PIE CHART SHOWING PRESENCE OF TESTICULAR ATROPHY



6.2.2.4. Mini mental state examination

Fifty five percent of the sample demonstrated a normal MMSE of 30 whilst 25% demonstrated mild cognitive impairment of between 26-29 and 20% moderate impairment (20-25).

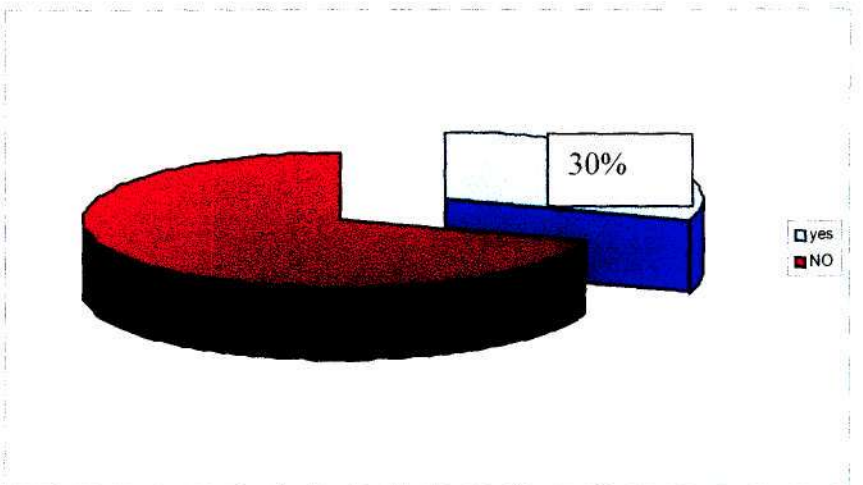
FIGURE 6.10. BAR CHART SHOWING MENTAL STATE EXAMINATION



6.2.2.5. Hypersomnolence

Symptoms of hypersomnolence were found in 30% with 5% requiring treatment with methylphenidate.

FIGURE 6.11. PIE CHART DEPICTING PREVALENCE OF HYPERSOMNOLENCE



6.2.2.6. Cataracts

Forty percent of the sample presented with either unilateral or bilateral cataracts at bedside ophthalmoscopy.

6.2.2.7. Levator palapebrae superioris and extraocular muscle weakness

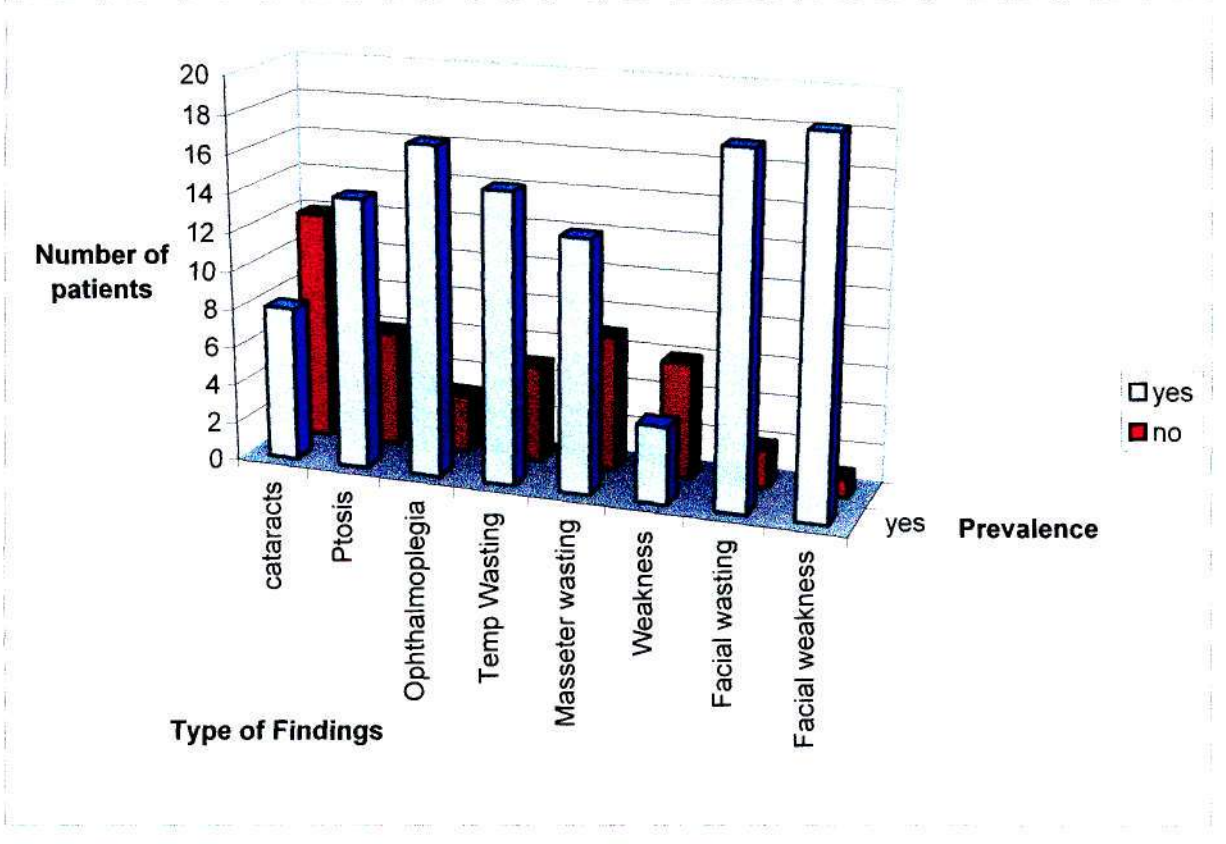
Ptosis was found in 70% with 15% having additional ophthalmoplegia.

6.2.2.8. Temporal, masseter and facial muscle wasting and weakness

Temporal muscle wasting was found in 75% of patients. Masseter muscle wasting was found in 65% with 20% demonstrating masseter weakness. Facial wasting was seen in 90% and 95% had demonstrable facial weakness.

The following Bar chart highlights the Clinical Findings of Cataracts, Ptosis, Ophthalmoplegia, Temporal Wasting , Masseter Wasting, Weakness, Facial Wasting and Facial Weakness.

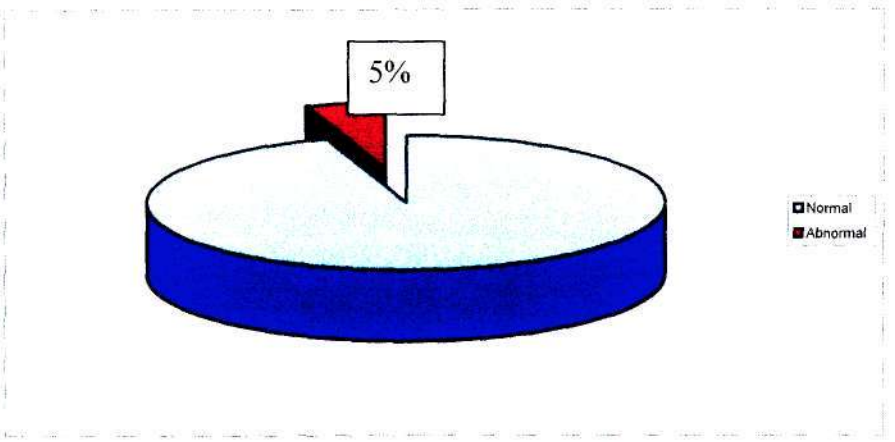
FIGURE 6.12. BAR CHART DEPICTING PREVALENCE OF CLINICAL CONDITIONS



6.2.2.9 Auditory examination

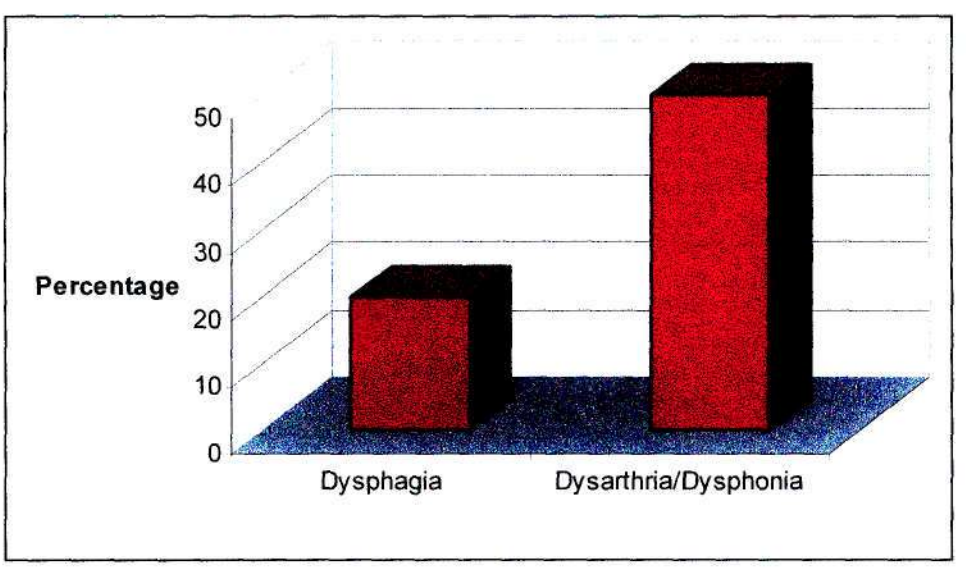
Only 5 % (1 patient) of the sample was found to have hearing impairment on clinical examination. The patient demonstrated bilateral sensori-neural hearing loss.

FIGURE 6.13. PIE CHART DEPICTING STATUS OF AUDITORY EXAMINATION



6.2.2.10. Bulbar muscle involvement

FIGURE 6.14. BAR CHART HIGHLIGHTING PREVALENCE OF BULBAR MUSCLE INVOLVEMENT

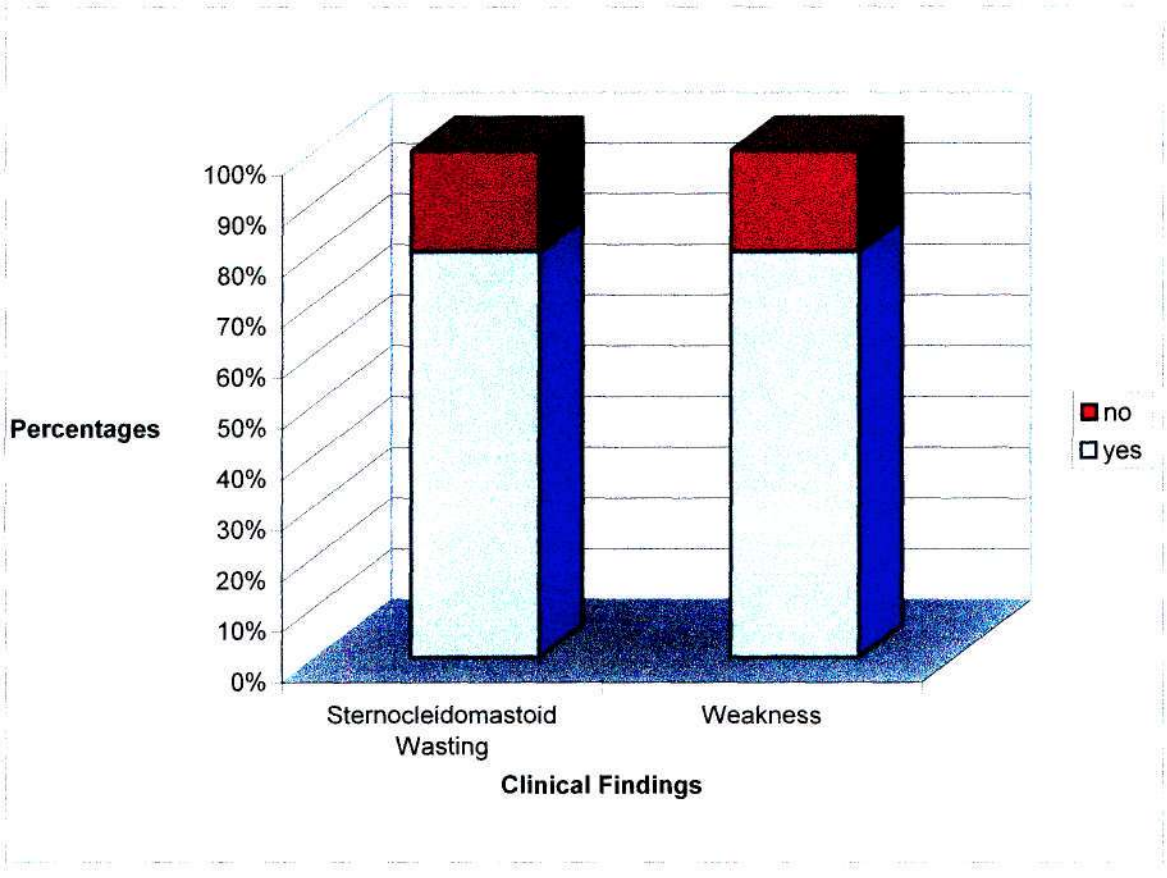


Dysphagia was uncommon (20%). Dysarthria / dysphonia was present in 50 % of the patients.

6.2.2.11 Sternocleidomastoid Involvement

Sternocleidomastoid wasting and weakness was seen in 80% of patients.

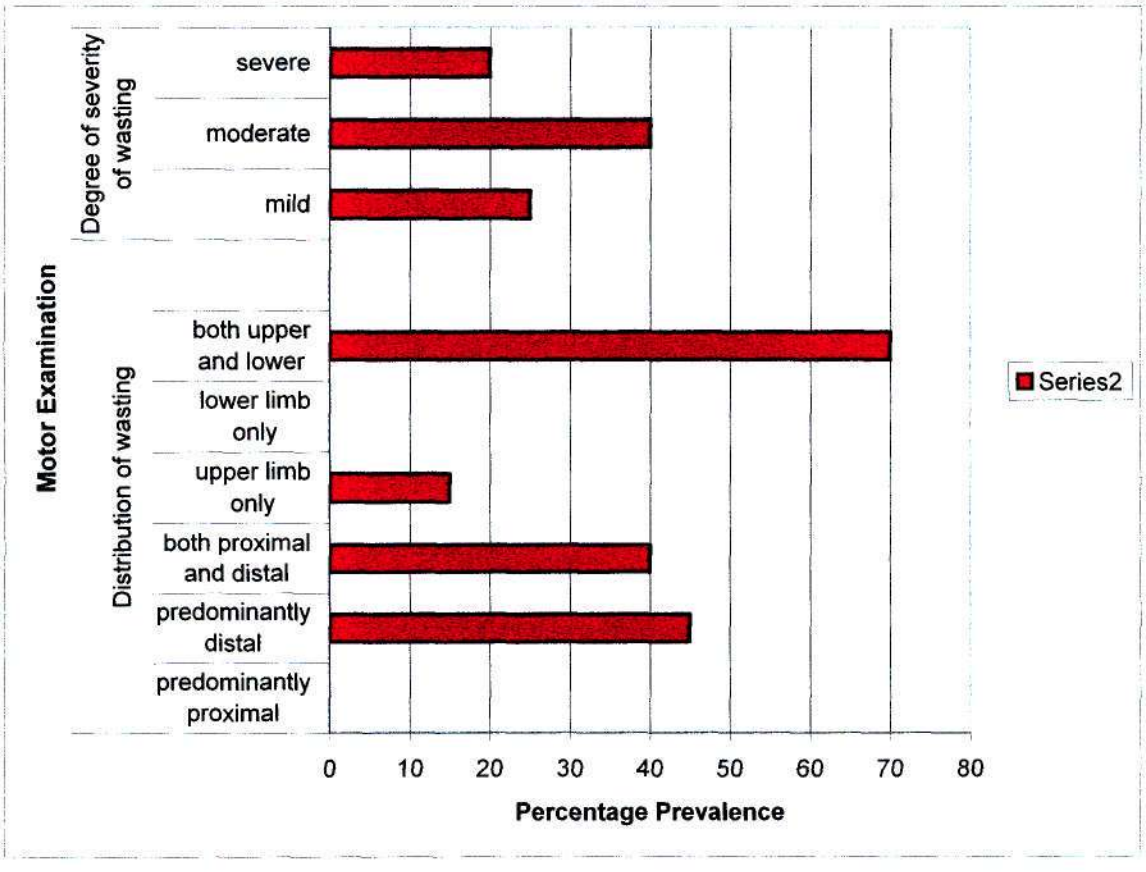
FIGURE 6.15. BAR CHART HIGHLIGHTING STERNOCLEIDOMASTOID INVOLVEMENT



6.2.2.12 Distribution and severity of wasting in limbs

Forty five percent of patients presented with predominantly distal wasting whereas 40% had wasting of both proximal and distal muscle groups. No patients were identified with predominantly proximal wasting. Wasting was evident in both upper and lower limbs in 70% of patients. 40% had evidence of moderate wasting with 20% demonstrating severe wasting.

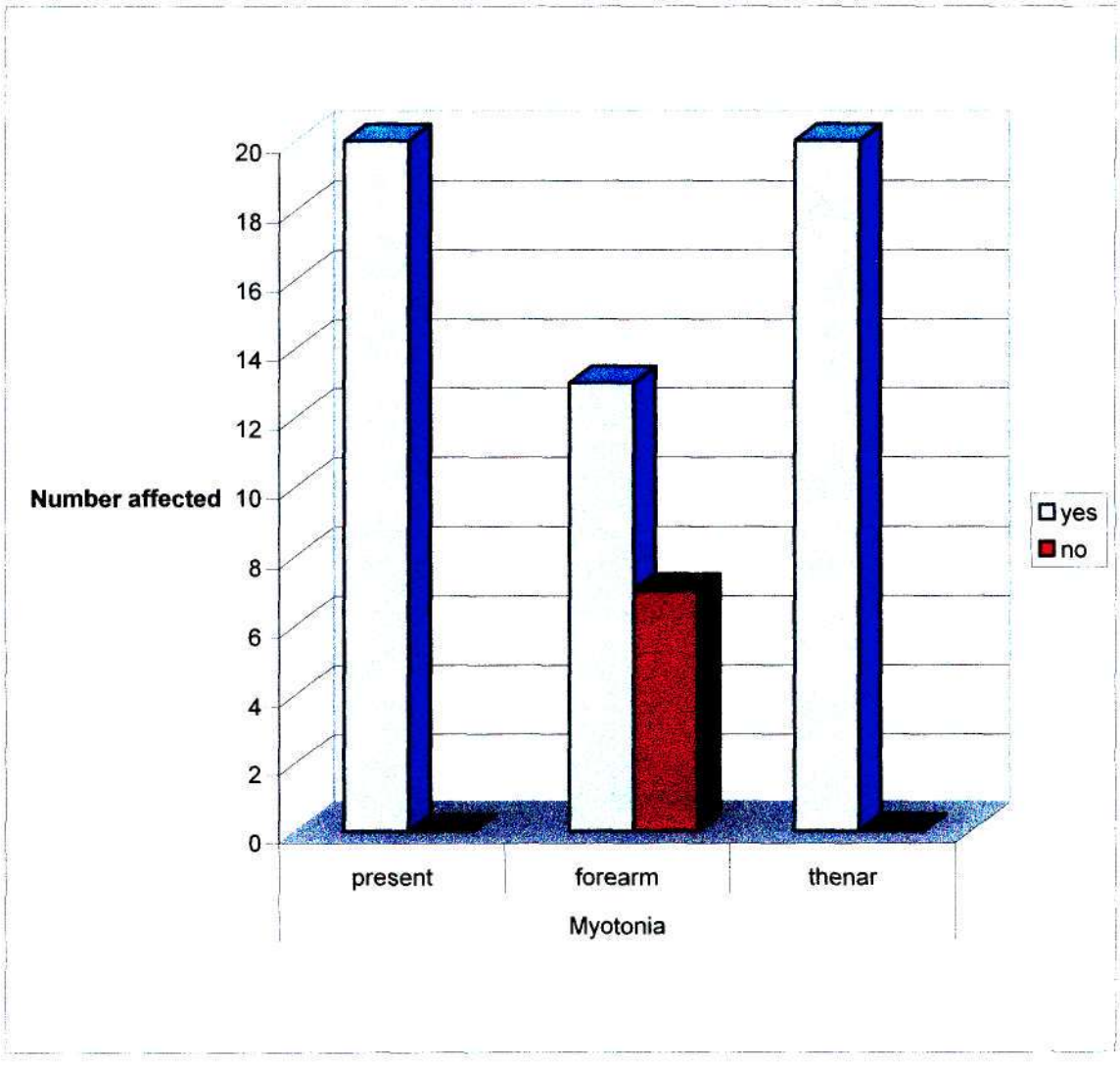
FIGURE 6.16. BAR CHART DEPICTING MOTOR EXAMINATION



6.2.2.13 Myotonia

Myotonia was clinically evident in all patients. All patients demonstrated percussion myotonia in thenar muscles with an additional 65% in the forearm extensors.

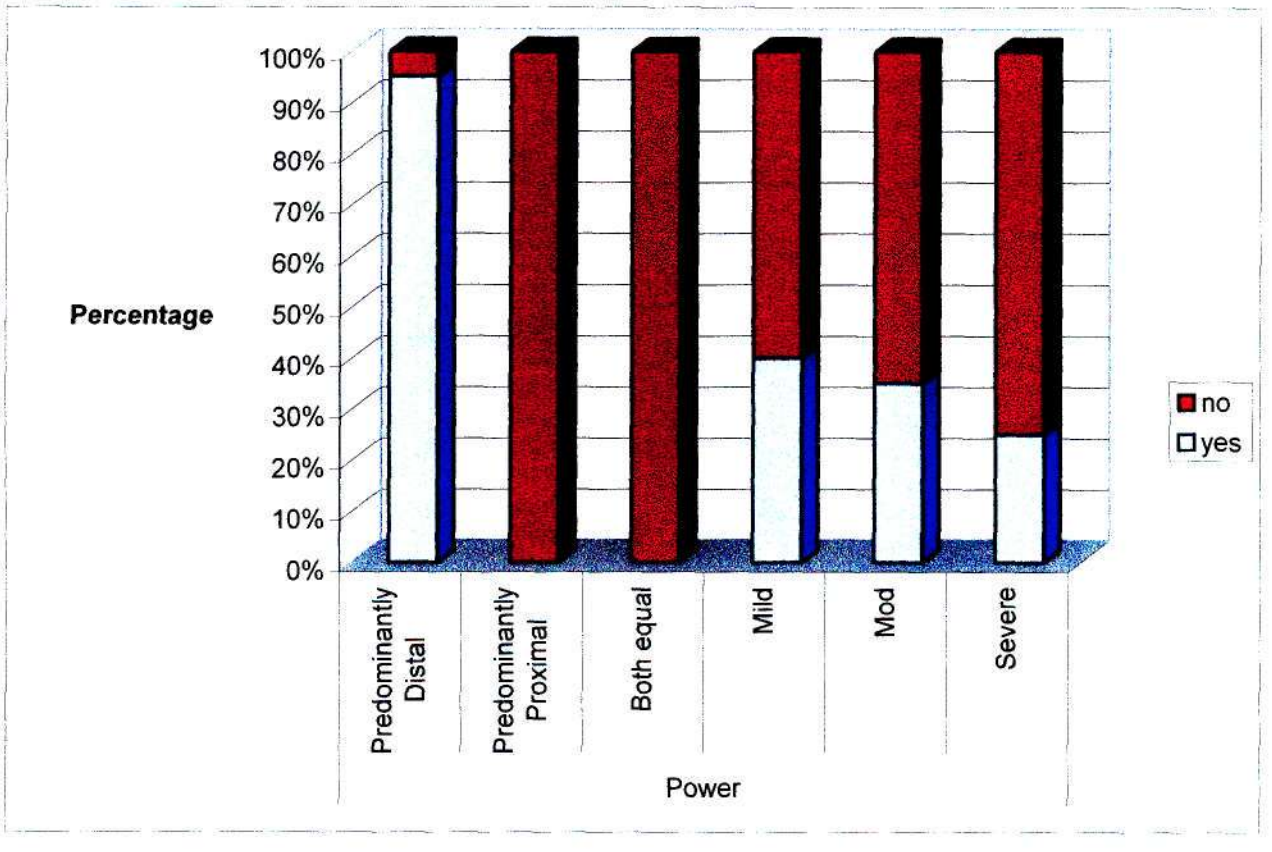
FIGURE 6.17. BAR CHART DEPICTING PREVALENCE OF MYOTONIA



6.2.2.14 Distribution and severity of weakness

Ninety five percent of patients presented with predominantly distal weakness of which 40% demonstrated mild weakness (power grading of greater than 4), 35% moderate weakness (power grading of 4 and 4 -) and 25 % severe weakness. (power grading of 3 or less).

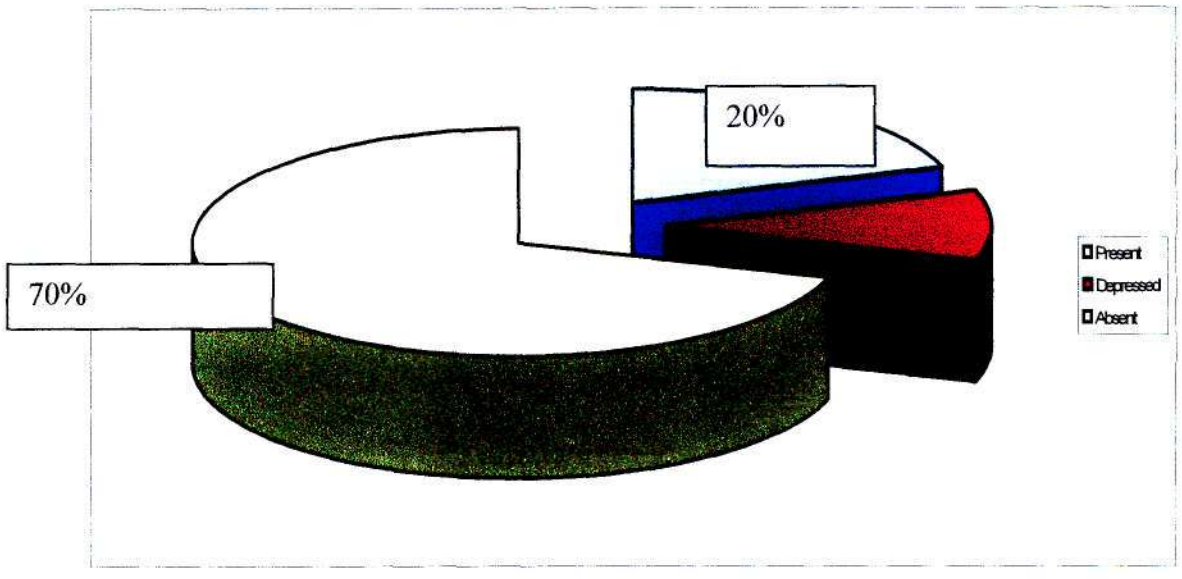
FIGURE 6.18 BAR CHART DEPICTING STATUS OF POWER



6.2.2.15 Tendon reflexes

Reflexes were depressed in 10% and absent in 70%.

FIGURE 6.19. PIE CHART HIGHLIGHTING TENDON REFLEXES



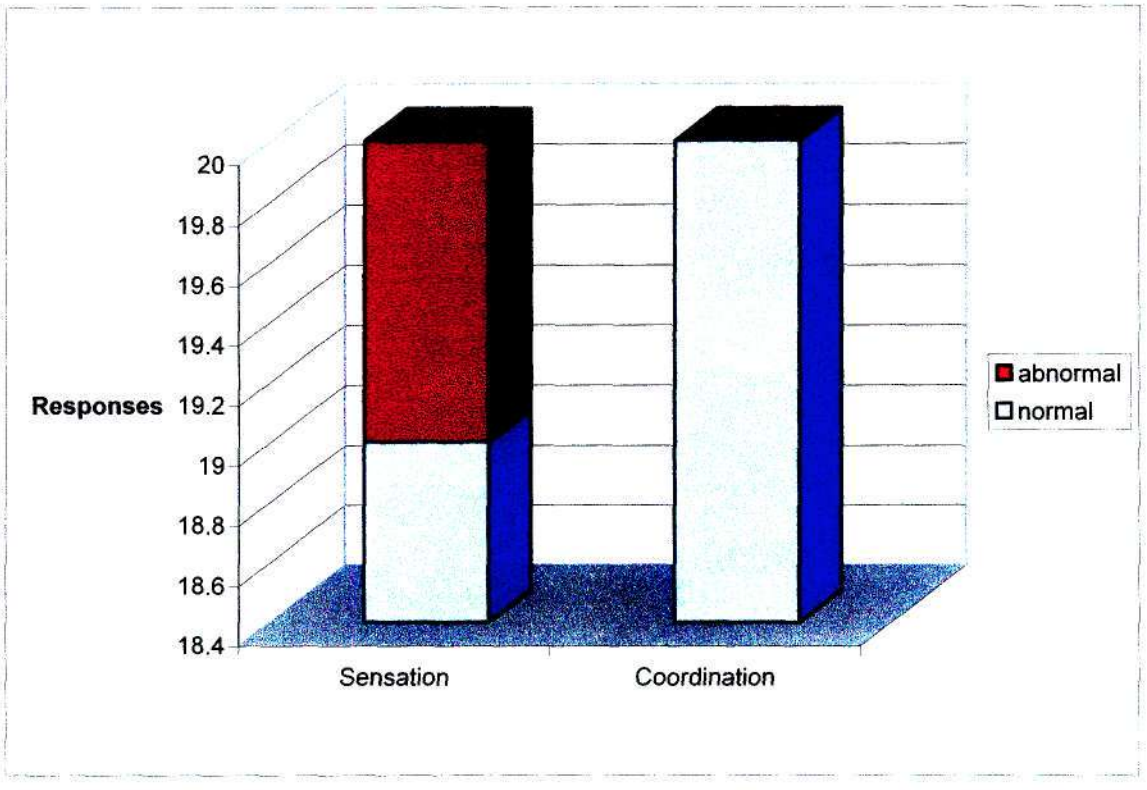
6.2.2.16. Sensory examination

Sensory examination was normal in 95 % with one patient demonstrating mild glove and stocking sensory loss.

6.2.2.17. Co ordination

Co ordination was preserved in all patients.

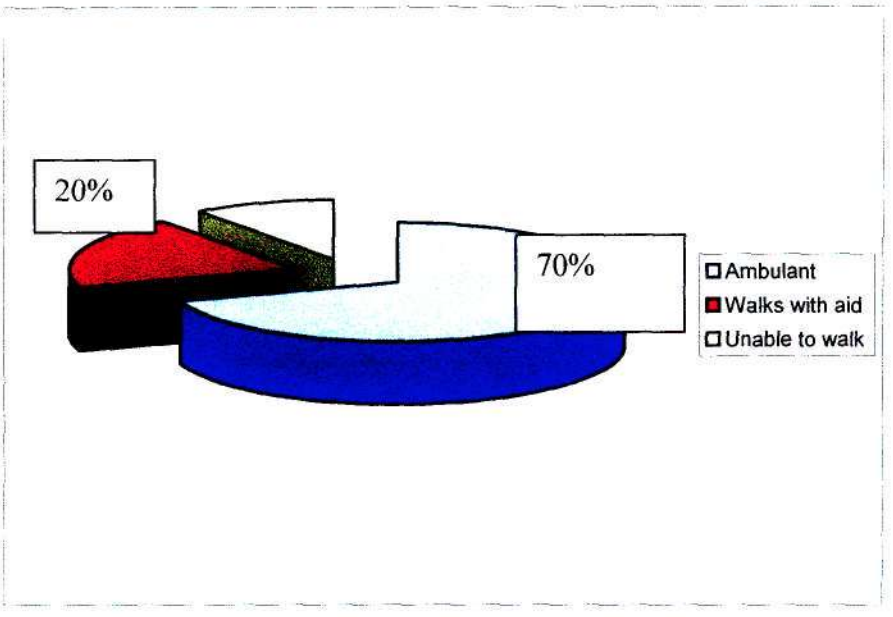
FIGURE 6.20. BAR CHART HIGHLIGHTING SENSORY EXAMINATION AND CO ORDINATION



6.2.2.18. Gait

Seventy percent of patients remained ambulant while 20% required a walking aid and the remaining 10% were wheelchair bound.

FIGURE 6.21. PIE CHART HIGHLIGHTING EXAMINATION OF GAIT



6.2.3. Laboratory investigations

Basic blood investigations such as full blood count, renal function, liver function and electrolytes were all normal.

- Elevated cholesterol was noted in 30%.
- One patient was on treatment for hypothyroidism.
- Three (15%) patients were being treated for diabetes mellitus type 2.
- Creatinine Kinase was mildly elevated in 50% .The highest level obtained was 899. Twenty percent had normal values. Thirty percent had no available creatinine kinase results.

6.2.4. Radiographic investigations

The chest radiograph was normal in 80% with the remaining 20% having no available CXR.

6.2.5. Electrocardiography

Electrocardiography was performed in 75% of patients. 55% were in sinus rhythm. Three patients (15%) had evidence of left bundle branch block. One patient (5%) had a right bundle branch block. PR interval was not measured in this study.

6.2.6. Nerve conduction studies

Nerve conduction studies were obtained in 60% of patients and were normal in 55%. One patient demonstrated an abnormality. Unilateral studies were performed. The upper limb motor and sensory studies were normal. The lower limb study demonstrated a decrease in the amplitude (60% of the lower limit of normal) of the compound muscle action potential on stimulation of the common peroneal nerve when recording from the extensor digitorum brevis. The latency and conduction velocity were normal. The sural sensory potential was normal.

6.2. 7. Electromyography

Elelectromyography was performed in 85 % of patients with all demonstrating typical myotonic changes while 40% had evidence of myopathic findings as well.

6.2.8. Management

All patients were managed supportively with physiotherapy and occupational therapy.

6.2.9. Follow up

At the end of the study date two patients (10%) had demised and one (5%) was lost to follow up. The cause of death in the two patients was unknown. The remaining patients were followed up either telephonically or with a recent visit to our clinic.

6.2.10 Molecular Analysis

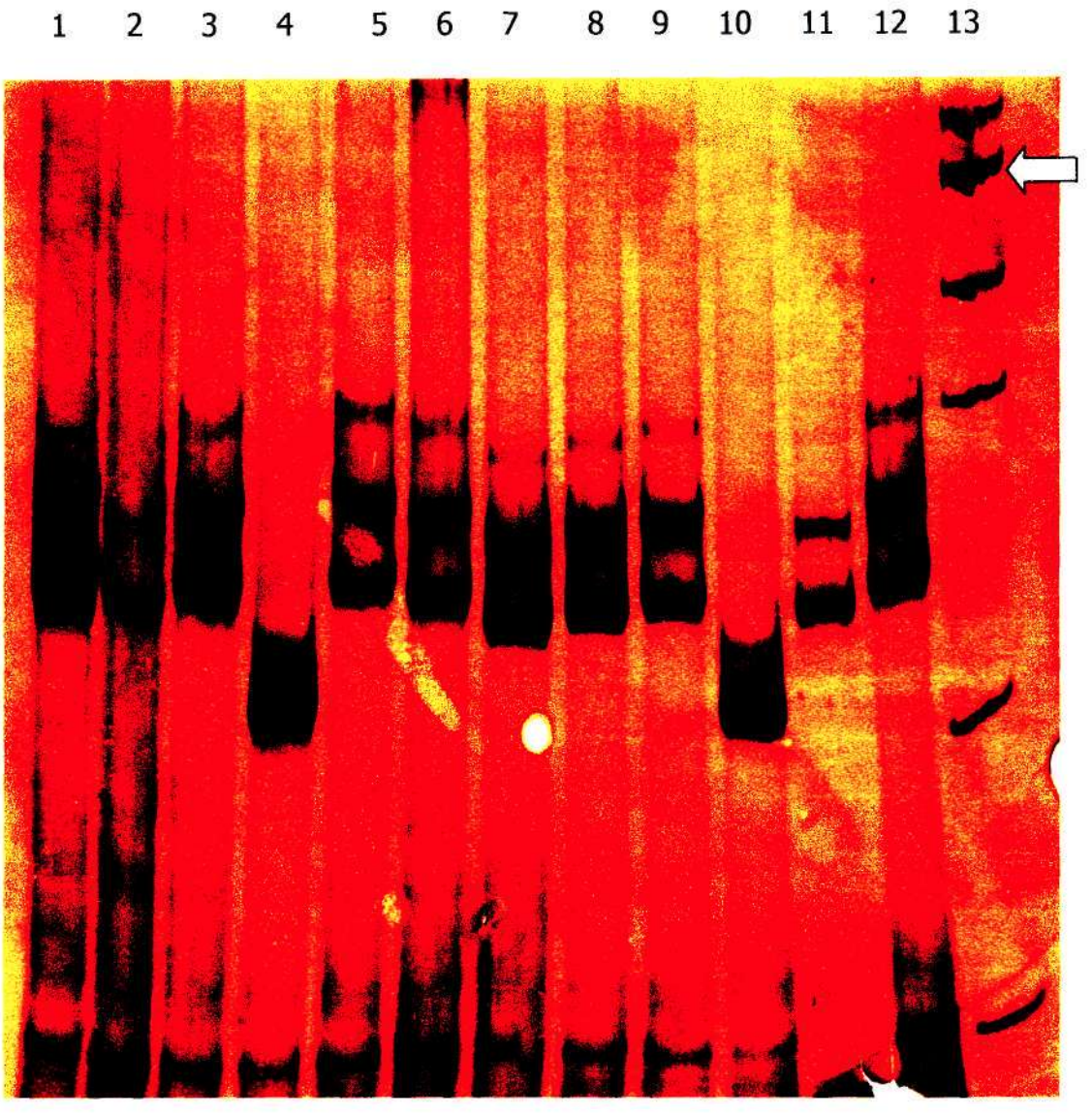
The PCR analysis performed at the Sir Ganga Ram Hospital Genetics Department and the University of KwaZulu Natal Laboratory , was unable to demonstrate expanded alleles in all 20 samples. PCR analysis is sensitive for determining trinucletide repeat expansions of less than 200 in size.

Southern blotting performed at Sir Ganga Ram Hospital demonstrated expanded CTG repeats in all 20 samples. Expanded trinucleotide repeats that are only detected on southern blot testing imply trinucleotide repeat sequence expansions of greater than 200 in size.

The evaluation of southern blotting performed at Sir Ganga Ram Hospital could not quantify the exact number of trinucleotide repeat expansions.

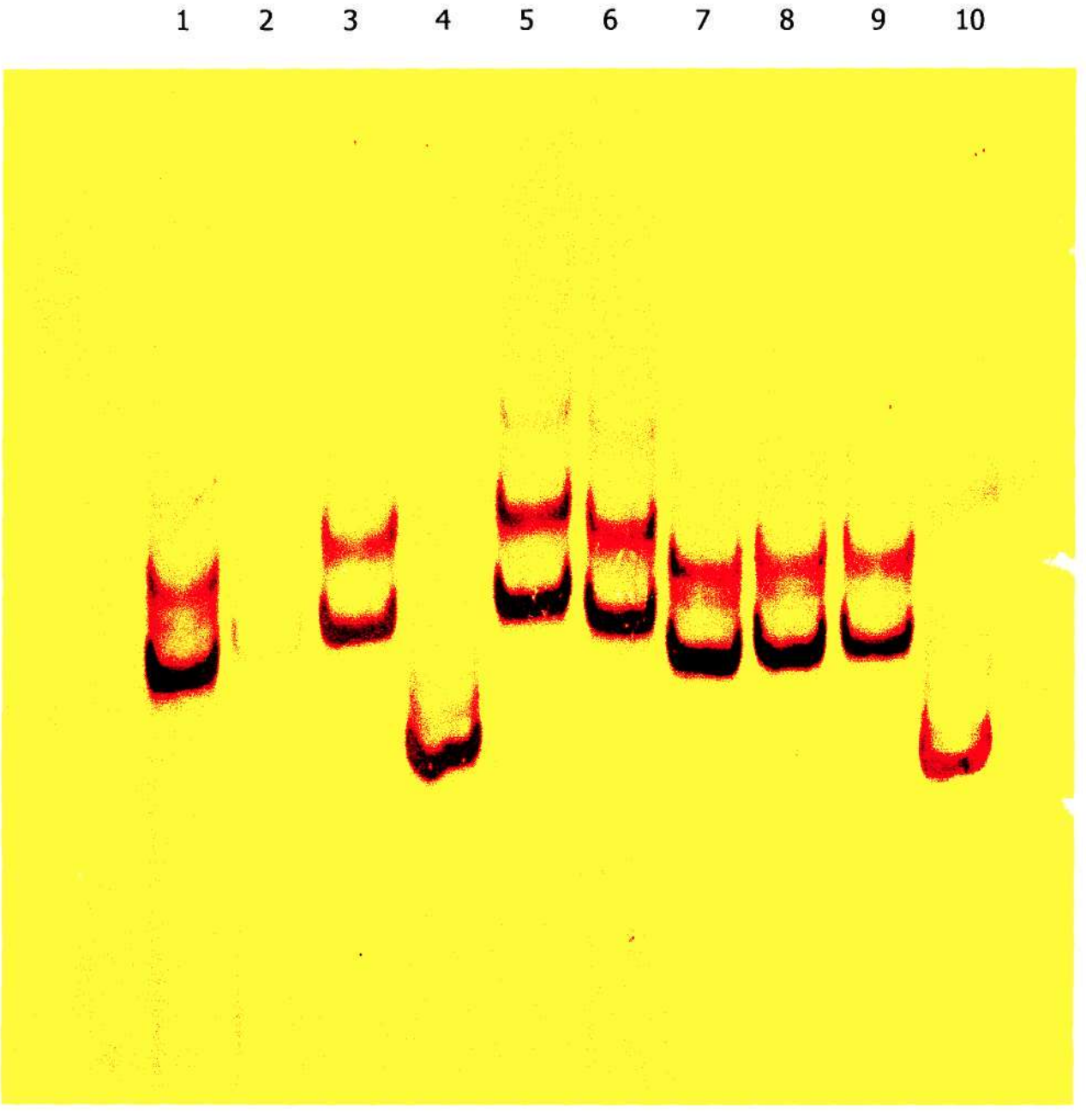
However the findings were sufficient to indicate that all patients demonstrated trinucleotide repeat expansions beyond 200.

FIGURE 6.22. EXAMPLE OF A SILVER STAINED PAGE PCR PICTURE OF SAMPLES ALONGSIDE A MARKER (ARROW)



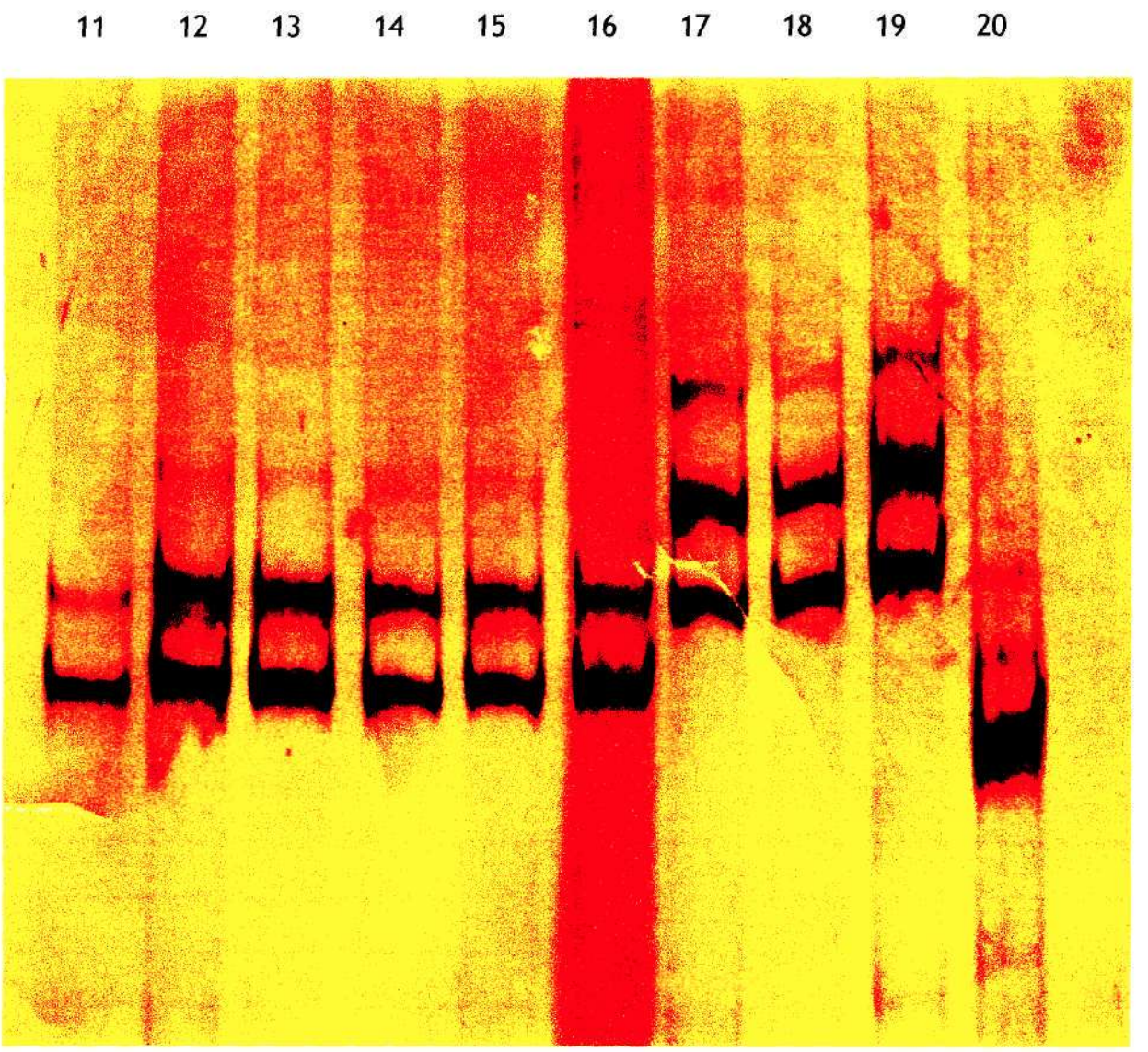
The above example demonstrates how PCR samples are compared to a marker to determine the size of the alleles. Lanes 1 to 12 demonstrate PCR products which are normal. Lane 13 contains markers representing different size of alleles.

FIGURE 6.23. PCR OF PATIENTS 1 TO 10. SILVER STAINED PAGE PICTURE SHOWING PRESENCE OF ONLY ONE ALLELE, WHICH IS IN THE NORMAL RANGE



Lanes 1 to 10 demonstrates PCR product of 10 patients showing 1 allele in the normal range.

FIGURE 6. 24. PCR OF PATIENTS 11 TO 20. SILVER STAINED PAGE PICTURE SHOWING PRESENCE OF ALLELE IN THE NORMAL RANGE.



Lanes 11 to 20 demonstrates PCR product of 10 patients showing 1 allele in the normal range

FIGURE 6.25. SOUTHERN BLOT DEMONSTRATING A NEGATIVE CONTROL IN LANE 3 AND POSITIVE CONTROLS IN LANES 1 AND 2. SAMPLES OF PATIENTS 1 TO 10 IN LANES 4 TO 13 DEMONSTRATE EXPANSION.

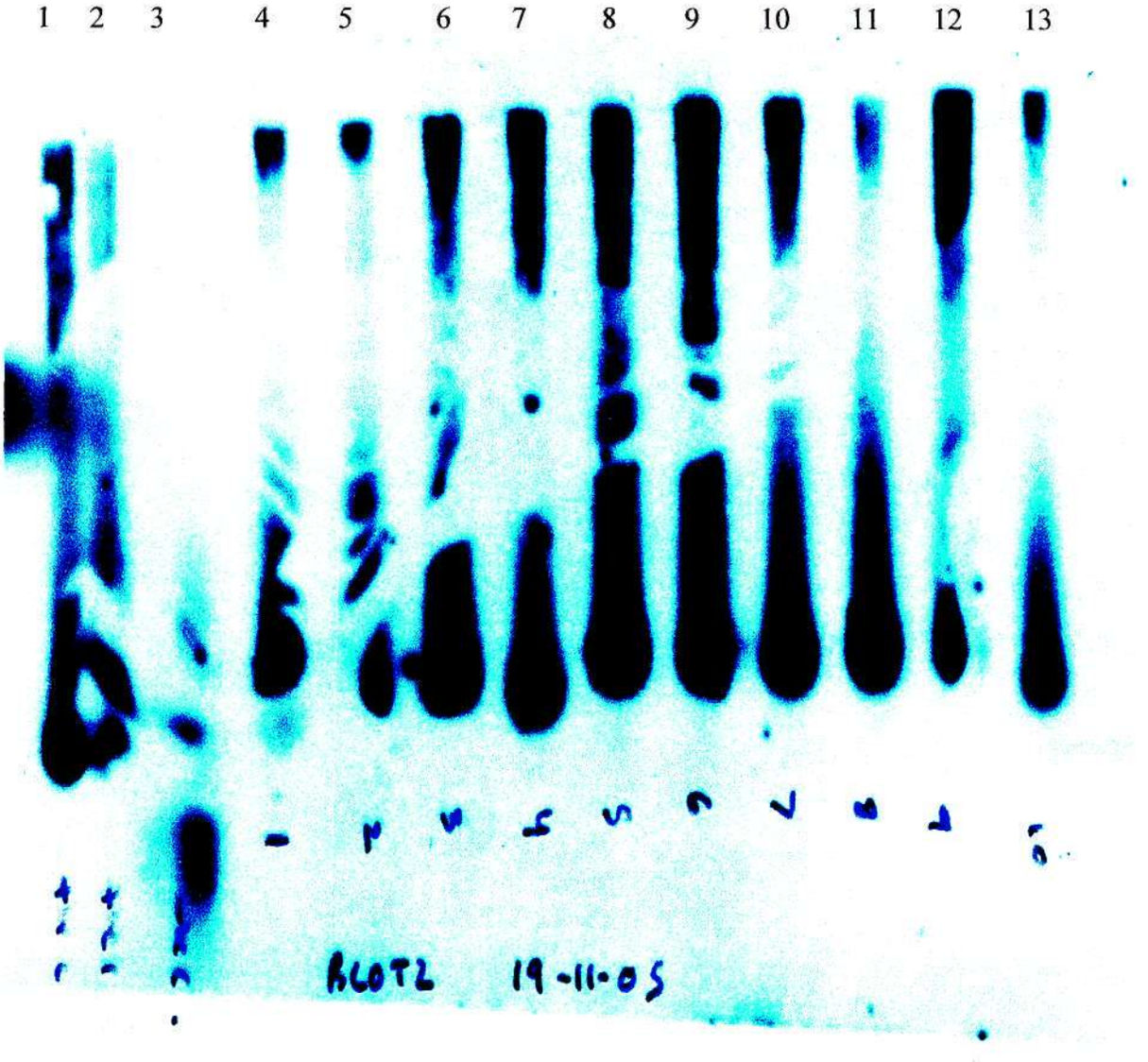
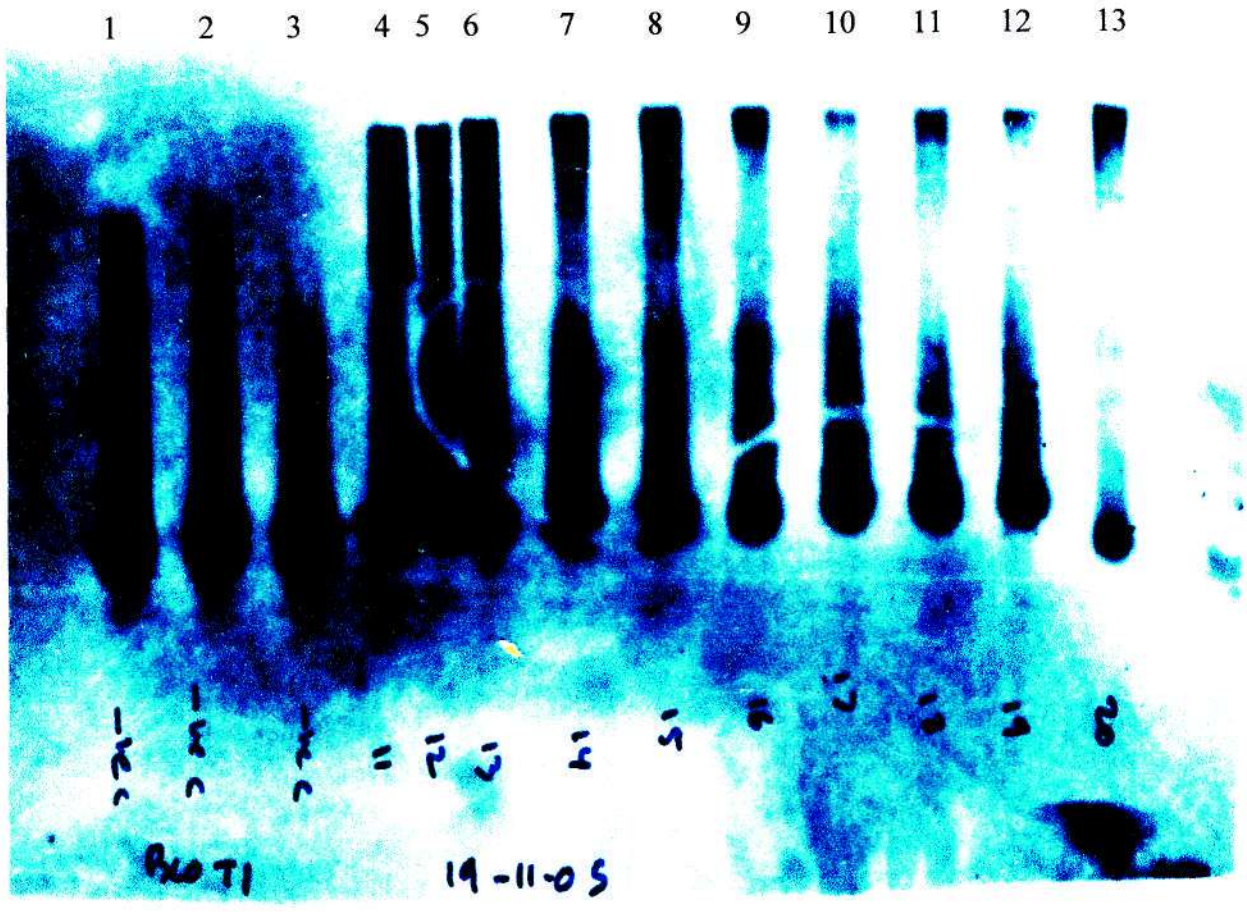


FIGURE 6.26. SOUTHERN BLOT DEMONSTRATING NEGATIVE CONTROL IN LANES 1,2 AND 3. SAMPLES OF PATIENTS 11-20 DEMONSTRATE EXPANSION.



Southern blot studies confirmed the presence of an expansion in all 20 samples for the CTG trinucleotide repeat found in myotonic dystrophy type 1.

Chapter 7. Discussion

All patients included in this study presented with predominantly distal weakness and a clinical assessment of type1 myotonic dystrophy was made.

The molecular diagnosis confirmed the presence of an expanded repeat for myotonic dystrophy type 1 supporting the clinical diagnosis.

The study revealed that the majority of patients referred to the Department of Neurology in KwaZulu - Natal who were affected by Myotonic Dystrophy were of Indian Descent. No patients of African origin were identified. The reasons for the small number of White patients may be explained by referral bias. The incidence of DM1 in South Africa is unknown and it is not known if a differing incidence exists between the Indian and White sections of the population. A previous study by Lotz and Van Der Meyden in 1985 identified only white patients in the northern Transvaal and no patients of Indian or African origin were documented. [10.36] Goldman [37] in addition documented no South African Negroid patients with myotonic dystrophy.

In addition Goldman [37] found that South African blacks have significantly fewer large repeat lengths than do white and Japanese populations and suggested that the occurrence of fewer large CTG repeats in the normal range may, in part, explain the absence of DM in Southern African blacks.

In addition Goldman [38] suggested that DM mutations in the Afrikaans population might have originated from a common initial founder who introduced one of the European ancestral mutations. The genealogical study by Lotz and Van Der Meyden [10] found common ancestry amongst four of the twenty White kindreds studied.

One of the limitations identified in this current study is that it evaluates a cohort of patients and family members referred to the Department Of Neurology. Patients with Myotonic Dystrophy that presented to private Neurologists would not have been included. As a result the actual prevalence and incidence of the disease could not be determined amongst the general population and one could not answer questions relating to the incidence of disease amongst the different racial groups.

In this study the majority of patients demonstrated typical clinical features of myotonic dystrophy. EMG was diagnostic in those patients in whom they were performed.

A clinical study was performed by Lotz and Van Der Meyden [36] in 1980. Ninety six patients were identified. Seventy patients were classified as Adult Myotonic Dystrophy with an average age of 36.8. Eighteen patients were classified as Early Adult Myotonic Dystrophy with an age varying between 5 and 12 years. Eight patients were classified as Congenital Myotonic Dystrophy. In the current study we did not identify any patients with Congenital Myotonic Dystrophy or Early Adult Myotonic Dystrophy. The average age was similar to the average age in the Adult Myotonic Dystrophy subgroup of Lotz and Van Der Meydens study. [36]

Comparison of clinical findings with those of the subgroup of Adult Myotonic Dystrophy from the study in South Africa by Lotz and Van Der Meyden [36] revealed similar findings. Mental retardation was detected in 17% of patients. Hypersomnolence was common but not quantified. Facial weakness was present in 87 % of patients. Neck flexor weakness was present in 71% of patients. Ptosis was noted in 40 % of patients. Extra ocular movement abnormalities were noted in 7% of patients. Cataracts were evaluated with bed side ophthalmoscopy and were present in 60% of patients. Frontal balding was common and noted in 70 % of patients. Testicular atrophy was not documented. Wasting of the limb muscles was not documented but weakness was noted to be severe distally. Percussion induced myotonia was elicited in 90% of patients over the thenar eminence and in 60% of patients in the forearm muscles.

With regards to the severity of disease, 50 % demonstrated no or mild disabilities , 30 % moderate disability and 20% were wheelchair bound. Cardiac involvement was assessed clinically. Electrocardiography was not performed. Seven (10%) patients demonstrated clinical cardiac involvement. Two patients were known with cardiac pacemakers , two with right sided cardiac failure and three with extrasystoles.No laboratory,electrophysiological or molecular investigations were performed. Figure 6.27 A and B demonstrates the comparison between the two studies.

FIGURE 6.27. A. COMPARISON OF LOTZ ET AL STUDY AND CURRENT STUDY

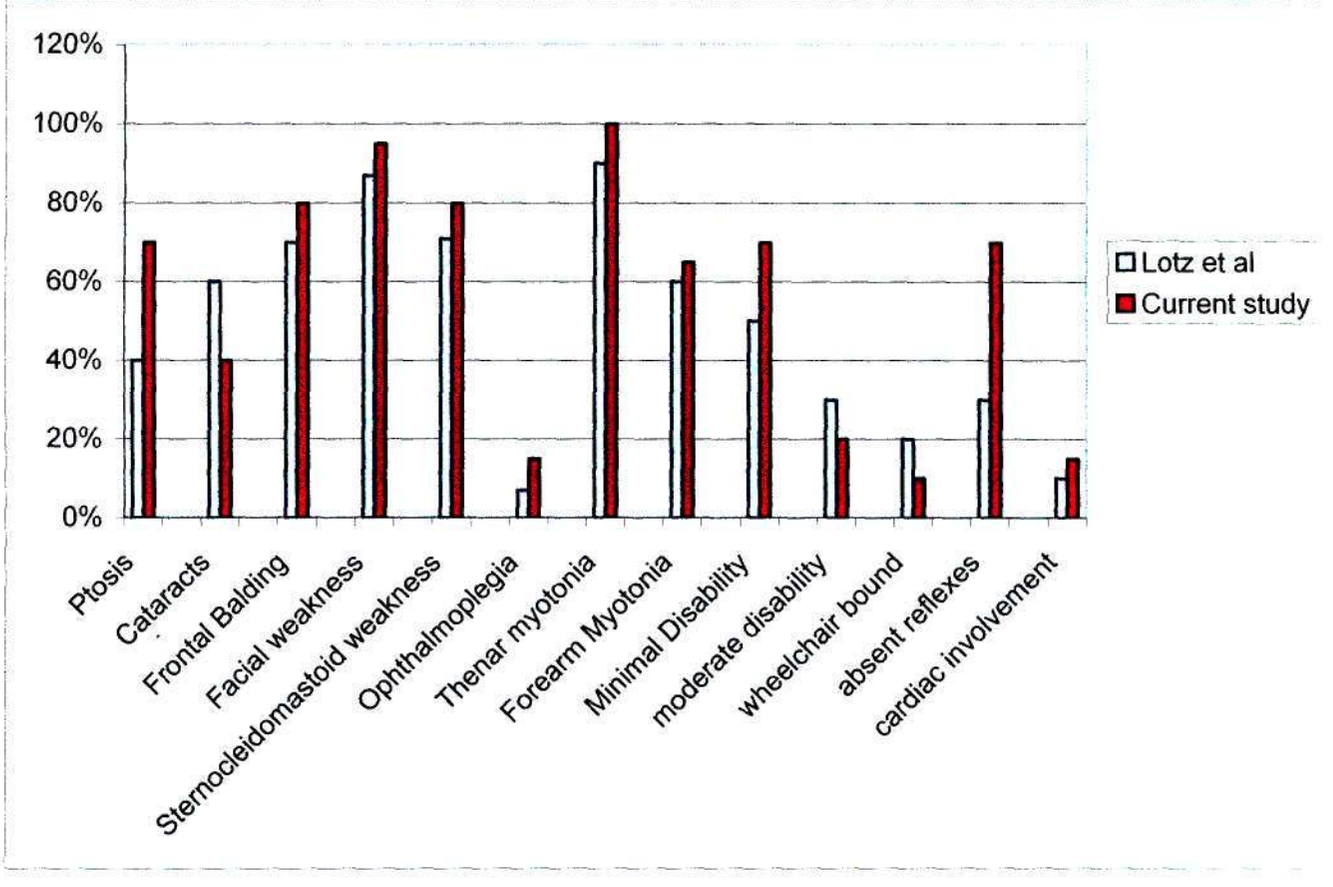
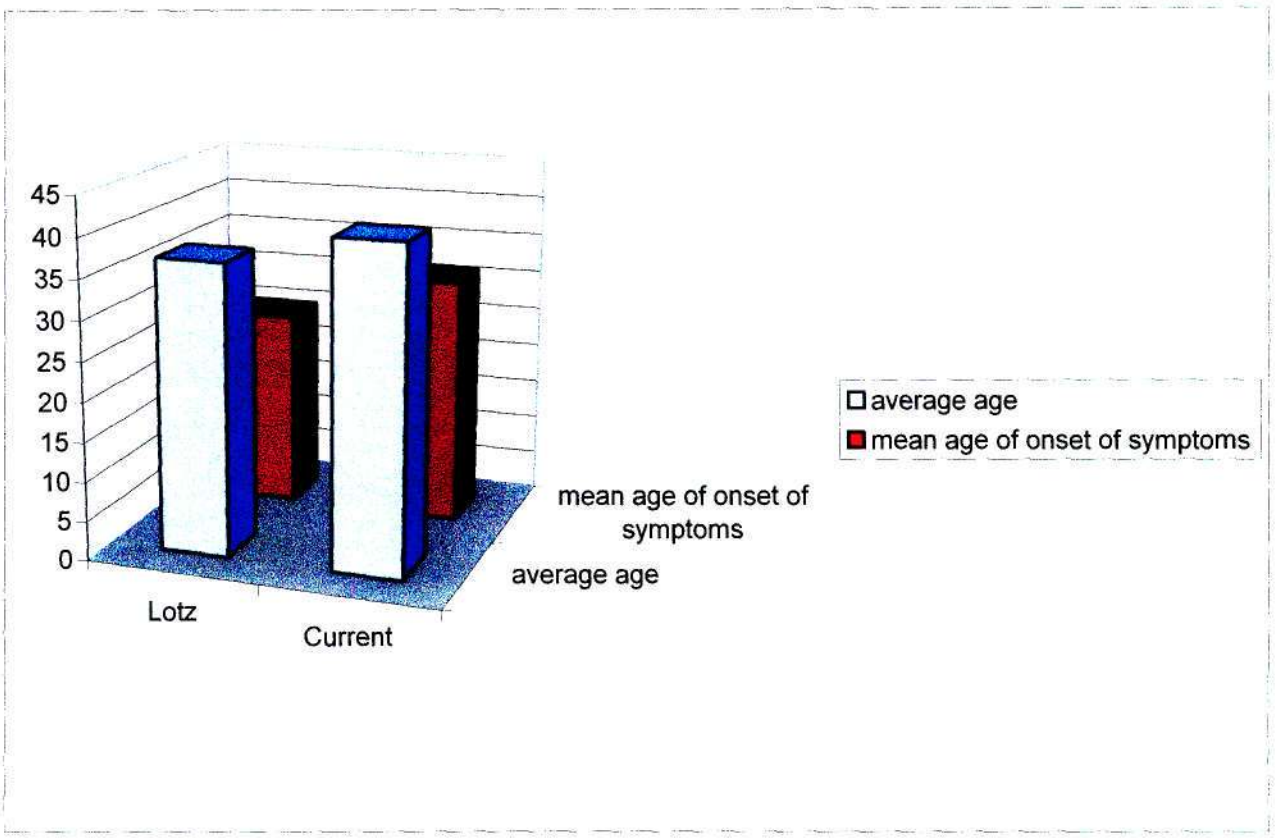


FIGURE 6.27. B. COMPARISON OF LOTZ ET AL STUDY AND CURRENT STUDY



With regards molecular diagnosis in the current study, PCR did not demonstrate any expanded repeats. However the expansions were observed in all samples with southern blotting. While the southern blot expansions could not be quantified, they usually demonstrate expanded repeats when larger than 200.The negative PCR would therefore confirm that PCR can only be used to demonstrate expansions in patients with shorter repeats of between 5 – 200.

All patients demonstrated clinical features typical of myotonic dystrophy type 1. The molecular findings demonstrated markedly expanded trinucleotide repeat expansions which could only be confirmed on southern blotting. PCR should therefore be used as a screening procedure and not as a definitive diagnostic tool.

Mildly symptomatic patients can also be screened with PCR, as they may possess shorter repeats. Further limitations in our study included our inablilty to quantify the expansion size and as a result we were unable to correlate clinical severity with expansion size. A possible focus in future studies would be to quantify expansions and correlate with the clinical severity and to assess trinucleotide expansions in other tissues which would aid in explaining the different disease profiles in different patients.

In conclusion, this study identified patients presenting with myotonic dystrophy to the Department of Neurology in KwaZulu -Natal and demonstrated that Myotonic Dystrophy Type 1 remains the commonest clinical and molecular presentation.

In addition it supported previous findings in which no South African of African descent was found to be affected by the disease.

The molecular studies confirm that PCR is of limited value as only small repeat expansions of less than 200 trinucleotide repeat sequences can be demonstrated by this method. PCR may be important as a screening tool but cannot be relied on to exclude the presence of an abnormal allele. Southern Blotting remains the gold standard in obtaining a molecular diagnosis. This is an important issue in the genetic counseling of patients and their families.

Another important finding is that the clinical diagnosis was confirmed by molecular tests and this would suggest that clinical diagnosis is sufficient and molecular confirmation is not a requirement. The molecular testing would be of value in differentiating between DM1 and DM2 and in prenatal testing.

8. REFERENCES

1. Michael H Brooke. Neurology in clinical Practice: Principles of diagnosis and Management. 3rd edition: Volume 2: 2208-2211.
2. Fu YH, Pizzuti A, Fenwick RG Jr, *et al* .An unstable triplet repeat in a gene related to myotonic muscular dystrophy. Science 1992:255:1256-1258
3. Mahadevan M, Tsisfidis C, and Sabourin L, et al. Myotonic Dystrophy mutation: an unstable CTG repeat in the 3' untranslated region of the gene. Science 1992:255:1253-1255
4. Brook JD, Mc Currach ME, Harley HG, et al. Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at 3' end of a transcript encoding a protein kinase family member. Cell 1992; 68: 799-808
5. Ashizawa T, Dubel J, Dunne PW, et al. Anticipation in myotonic dystrophy. Complex relationships between clinical findings and structure of the CTG repeat. Neurology 1992; 42: 1877-1883
6. Ashizawa T. IDMDC. New nomenclature and DNA testing guidelines for myotonic dystrophy type 1.Neurology 2000; 54: 1218 - 1221.
7. Ranum LP, Rasmussen PF, Benzow KA, Koob MD, Day JW. Genetic mapping of a second myotonic dystrophy locus. Nature Genetics 1998; 19: 196-198

8. Liquori C, Ricker K, Moseley ML, et al. Myotonic Dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. Science 2001; 293: 864- 867

9. Day JW, Ricker K et al. Myotonic dystrophy type 2. Neurology 2003; 60:657-664

10. Lotz BP, van der Meyden CH. Myotonic dystrophy. Part I. A genealogical study in the northern Transvaal. S Afr Med J. 1985 May 17; 67(20): 812-814.

11. Ashizawa, T.; Hejtmancik, J. F.; Liu, J.; Perryman, M. B.; Epstein, H. F.; Koch, D. D. Diagnostic value of ophthalmologic findings in myotonic dystrophy: comparison with risks calculated by haplotype analysis of closely linked restriction fragment length polymorphisms. Am. J. Med. Genet. 1992. 42: 55-60.

12. Harper, P. S. Myotonic Dystrophy. Philadelphia: W. B. Saunders (pub.) (2nd ed.) 1989.

13. Sagel, J.; Distiller, L. A.; Morley, J. E.; Isaacs, H.; Kay, G.; van der Walt, A.: Myotonia dystrophica: studies on gonadal function using luteinising-releasing-hormone (LRH). J. Clin. Endocr. Metab. 1975.40: 1110.

14. Lee, F. I.; Hughes, D. T. D. Systemic effects in myotonia dystrophica. Brain. 1964. 87: 521

15. Barbosa, J.; Nuttall, F. Q.; Kennedy, W.; Goetz, F.: Plasma insulin in patients with myotonic dystrophy and their relatives. Medicine.1974. 53: 307

16. Perloff JK, Stevenson WG, Roberts NK, Cabeen W, Weiss J. Cardiac involvement in myotonic muscular dystrophy (Steinert's disease):a prospective study of 25 patients. Am J Cardiol 1984; 54:1074-1081

17. Nguyen H H, Wolfe JT, Holmes DR, Edwards WD. Pathology of the cardiac conduction system in myotonic dystrophy: a study of 12 cases. J Am Coll Cardiol 1988; 11:662-671

18. Brunner, H. G.; Hamel, B. C. J.; Rieu, P.; Howeler, C. J.; Peters, F. T. M. Intestinal pseudo-obstruction in myotonic dystrophy. J. Med. Genet.1992 29: 79- 793.

19. Turnpenny, P.; Clark, C.; Kelly, K.:Intelligence quotient profile in myotonic dystrophy, intergenerational deficit, and correlation with CTG mplification. J. Med. Genet.1994 31: 300-305.

20. Censori, B.; Provinciali, L.; Danni, M.; Chiaramoni, L.; Maricotti, M.; Foschi, N.; Del Pesce, M.; Salvolini, U. Brain involvement in myotonic dystrophy: MRI features and their relationship to clinical and cognitive conditions. Acta Neurol. Scand.1994 90: 211-217.

21. Rudnik-Schoneborn, S.; Nicholson, G. A.; Morgan, G.; Rohrig, D.; Zerres, K. : Different patterns of obstetric complications in myotonic dystrophy in relationto the disease status of the fetus. Am. J. Med. Genet.1998 80: 314-321.

22. Tsilfidis, C.; MacKenzie, A. E.; Mettler, G.; Barcelo, J.; Korneluk, R. G. Correlation between CTG trinucleotide repeat length and frequency of severe

congenital myotonic dystrophy. Nature Genet.1992 1: 192-195.

23. Koch, M. C.; Grimm, T.; Harley, H. G.; Harper, P. S.: Genetic risks for children of women with myotonic dystrophy. Am. J. Hum. Genet.1991 48: 1084-1091.
24. Meola G. Clinical and Genetic heterogeneity in Myotonic Dystrophies. Muscle and Nerve.2000.23: 1789-1799.
25. Thornton CA, Ashizawa T. Getting a grip on myotonic dystrophies. Neurology.1999.52: 12.
26. Thornton CA, Griggs RC, Moxley RT. Myotonic dystrophy with no trinucleotide expansion. Ann neurology 1994; 35: 269-272
27. Ricker K, Koch MC, Lehmann-Horn F, Pongratz D, Speich N, Reiners K, Schneider C, Moxley RT. Proximal Myotonic Myopathy. Arch Neurology.1995; 52:25-31
28. Day.J, Roelofs R, Leroy B, Pech I, Benzow K, Ranum L.P.W. Clinical and genetic characteristics of a five-generation family with a novel form of myotonic dystrophy (DM2). Neuromuscular Disorders. 1999.9: 19-27.
29. Vihola A et al. Histopathological differences of myotonic dystrophy type 1 and PROMM/DM2. Neurology.2003.60.
30. Hannequin D et al.A non DM1, non DM2 multisystem myotonic disorder with frontotemporal dementia: phenotype and suggestive mapping of the DM3 locus to chromosome 15q21-24. Brain.2004.127: 1979-1992.

31. Johnson K et al. A new polymorphic probe which defines the region of chromosome 19 containing the myotonic dystrophy locus.Am J Human Genetics. 1990. 46: 1073- 1081.

32. Martorell L, Monckton DG, Sanchez A, Lopezde Munain, Baiget M. Frequency and stability of the myotonic dystrophy type 1 premutation. Neurology. 2001.56: 328 – 335

33. Simmons Z, Thornton CA, Seltzer WK, Richards CSR Relative stability of a minimal CTG repeat expansion in a large kindred with myotonic dystrophy. Neurology.1998.50.

34. Sallinen R et al. New methods for molecular diagnosis and demonstration of the CCTG mutation in myotonic dystrophy type 2. Neuromuscular Disorders.2004.14: 274-283.

35. Surh L, Mahadevan M, Korneluk R. Current Protocols in Human Genetics (1998) 9.6.1 – 9.6.13

36. Lotz BP, van der Meyden CH. Myotonic dystrophy. Part II. A clinical study of 96 patients S Afr Med J. 1985 May 17; 67(20): 815-7.

37. Goldman A,Ramsay M, Jenkins T. Absence of myotonic dystrophy in southern African Negroids is associated with a significantly lower number of CTG trinucleotide repeats. J Med Genetics.1994.31: 37-40.

38. Goldman A Krause A Ramsay M Jenkins T. Founder effect and prevalence of myotonic dystrophy in South Africans: molecular studies. Am J Hum Genet 1996 Aug; 59:445-52

9. APPENDICES

9.1. Appendix 1: INFORMATION DOCUMENT

Study title: Myotonic Dystrophy : the clinical and molecular spectrum in Kwa Zulu Natal ref H042/06

Dear Patient

You have been diagnosed previously by the Department of Neurology with Myotonic Dystrophy. This is a muscle disorder that is inherited and may involve other systems in your body. The consulting neurologist, based on your clinical presentation and neurophysiological tests, made this diagnosis.

I am a doctor in the Department of Neurology. **I would like to invite you to participate in our study, which I will be using for my Masters in Medicine Thesis.**

Our study aims to describe the clinical and molecular features of Myotonic Dystrophy in KwaZulu - Natal. If you agree to participate in this study we will examine you during your routine follow up visit at the neuromuscular clinic. We will extract data regarding your age, sex, and history of illness, clinical symptoms and signs.

The blood, radiological (X-rays) and neurophysiological (muscle test) investigations will be extracted from your previous charts. The results of the

genetic testing, which were previously performed at our local laboratory and the laboratory in India, will also be extracted from your charts. You will not be expected to increase your visits to the hospital. Your diagnosis and management will not be affected in any way.

The study will help us to improve the clinical and genetic diagnosis of Myotonic dystrophy in our local population. Your participation is voluntary and refusal to participate will have no penalty in your future follow up and management.

Personal Information will be confidential. Absolute confidentiality cannot be guaranteed. Personal information may be disclosed if required by law. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the Research Ethics Committee.

You can contact me for further information.

Dr Ayesha Motala

Department of Neurology

Tel: 031 240 2359 (secretary)

Tel: 031 240 1991 (office)

Cell : 0829085173

9.2. Appendix 2: CONSENT DOCUMENT

Study title: Myotonic Dystrophy: clinical and molecular spectrum in KwaZulu- Natal

Consent to Participate in Research

You have been asked to participate in a research study. You have been informed about the study by Dr A Motala. You may contact Dr Ayesha Motala at 031 240 2359 at any time if you have questions about the research .You may also contact the Medical Research Administration Office at the Nelson R Mandela School of Medicine at 031-260 4769 if you have any questions about your rights as a research participant.

Your participation in this research is voluntary, and you will not be penalized or lose benefits if you refuse to participate.

If you agree to participate, you will be given a signed copy of this document and the participant information sheet that is a written summary of the research.

The research study, including the above information, has been described to me verbally. I understand what my involvement in the study means and I voluntarily agree to participate.

_____ _____

Signature of Participant Date

_____ _____

Signature of Witness Date

(Where applicable)

_____ _____

Signature of Translator Date

(Where applicable)

9.3. Appendix 3 MYOTONIC DYSTROPHY DATA SHEET

MYOTONIC DYSTROPHY DATA SHEET

1. Demographic details

Name:
Age:
Sex:
Hospital Number:

2. History

Age of onset

Family history

	Affected	Unaffected	Not available
Parents			
Siblings			
Children			

3. General examination

Pulse

BP

Frontal Balding [Y] [N]

Testicular Atrophy [Y] [N]

Neurological Examination

(A) Mental State

1. MMSE score [ ]

2. General comment

[ ]

3. Hypersomnolence [Y] [N]

(B) Cranial Nerves

1. [Abnormal] [Normal]

2. VA Left [ ] Right [ ]

VF [Abnormal] [Normal]

If abnormal [ ]

[Y] [N]

Cataracts

If Y

Fundi Abnormal Normal

If abnormal

3.4.6. Abnormal Normal

ptosis Y N

if Y

5. wasting of temporalis Y N

wasting of masseter Y N

weakness Y N

7. wasting of facial muscles Y N

weakness Y N

8. Abnormal Normal

9. dysphagia Y N

10. palatal movements Abnormal Normal

12. dysarthria Y N

if abnormal

11. wasting of sternocleidomastoids Y N

weakness Y N

(C) Motor System

1. Distribution of wasting

Proximal	Y	N
Distal	Y	N
Both	Y	N
Upper limbs	Y	N
Lower limbs	Y	N
Both	Y	N

Severity: Mild ☐

Moderate ☐

Severe ☐

2. Myotonia

Present ☐ Absent ☐

Forearm extensor group 

Thenar muscles 

3. Power standard grading

Shoulder Abduction	R	L
Shoulder Adduction	R	L
Elbow Flexion	R	L
Elbow Extension	R	L
Wrist Flexion	R	L
Wrist Extension	R	L
Finger Flexion	R	L
Finger Extension	R	L
Finger Abduction	R	L
Finger Adduction	R	L
Hip Flexion	R	L
Hip Extension	R	L
Hip Abduction	R	L
Hip Adduction	R	L
Knee Flexion	R	L
Knee Extension	R	L
Ankle Dorsiflexion	R	L
Ankle Plantarflexion	R	L

Reflexes:

Jaw Jerk		
Biceps	R	L
Triceps	R	L
Supinator	R	L
Knee	R	L
Ankle	R	L
Upper Abdominal	R	L
Lower Abdominal	R	L
Plantar	R	L
Primitive Reflexes	R	L

Sensation:

	Abnormal	Normal
If abnormal		

Coordination:

	Abnormal	Normal
If abnormal		

Gait:

Ambulant
Walks with walking aid
Unable to walk

Investigations

1. FBC
2. UE
3. LFT
4. Blood glucose
5. CPM
6. Creatine kinase
7. Other
8. Chest XRay | Normal | Abnormal |

If abnormal

9. Electrocardiograph | Normal | Abnormal

If abnormal

10. Nerve conduction studies | Normal | Abnormal

If abnormal

11. EMG

Myopathy | Y | N

Myotonia | Y | N