Prenatal diagnosis of spinal muscular atrophy: clinical experience and molecular genetics of SMN gene analysis in 36 cases

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Summary

Introduction: **prenatal diagnosis in families at risk for spinal muscular atrophy (SMA) mainly of type 1 is often applied due to the high incidence, most severe and newborn outcome of the disease.**
Case: **we present our clinical experience for 36 families with history of having at least one child with homozygous deletions of the SMN1 gene between. Seventeen families requested for prenatal prediction and of these cases, 8 fetuses were diagnosed to be at risk of developing the disease and the parents decided to terminate the pregnancy. Nine fetuses were detected with no homozygous deletion of the SMN1 and reached to full term delivery. Follow-up of live born children and abortion products never led to false or negative result.**
Conclusion: **therefore, application of SMN1 deletion detection by simple PCR assay in families with homozygous deletion of the SMN1 gene could be suggested for prenatal prediction in such families.**

Key words: spinal muscular atrophy, SMN gene, prenatal diagnosis, PCR-RFLP.

Introduction

Spinal muscular atrophy (SMA) is a neurodegenerative disease presented clinically by progressive degeneration of motor neurons in the anterior horn of the spinal cord, resulting to hypotenia, muscle atrophy, paralysis, and even death (1, 2). It is a genetically heterogenous disorder, mostly having a recessive inheritance; however, autosomal dominant and X-linked inheritance has been reported (3, 4). The incidence is about 1 in 10,000 live births with a carrier frequency of 1/40 - 1/60 (5, 6). SMA has extreme variability in phenotype with four clinical subtypes depending on age of onset and clinical severity. SMA type I or Werdnig-Hoffman, is the most severe variant with age of onset between birth and six months, may not sit and usually die by two years. SMA type II is intermediate variant and usually begins at 6 and 18 months of age; children with type II may able to sit but never stand and death usually occurs after 2 years of age (7). Types I and II are at increased risk for complications from respiratory infections. SMA type III or Kugelberg-Welander is mild variant with age of onset ~18 months of age and most patients eventually need to use a wheelchair and death happens in adult life. SMA type IV is adult variant and presents in the second or third decade, individuals walk during the adulthood and death occurs in adult life (8). SMA type 0 or embryonic form is lethal variant, characterized by reduced fetal movements between 30-36 weeks of pregnancy with a very short life expectancy (9).
Survival motor neuron (SMN) is the SMA-determining gene and is located on the 5q 11.2-13.3 region which has two homologues copies: SMN1 and SMN2 which is nearly identical copy of the SMN1 gene (10). The SMN1 gene lies in telemetric and SMN2 in centromeric side of chromosome. In normal individuals, at least one SMN1 copy exists and in the vast majority of chromosomes, one SMN2 copy, although, chromosome with intact SMN1 gene may lack SMN2. Homozygous deletion of exons 7 and 8 of SMN1 is the most common mutation (more than 95% of SMA patients); however, point mutation and compound heterozygous cases have been reported (6).
A PCR-RFLP assay has been established for diagnosing of SMA and it distinguishes the base differences in exons 7 and 8 SMN1 from SMN2 and is currently used by most clinical laboratories to detect homozygous deletions of SMN1 exon 7 (and exon 8) (11).
SMA is an autosomal recessive and consanguineous marriage increases the risk of the disease in the family. High prevalence of SMA has been reported in developing countries like Pakistan, Egypt, Iran, and Saudi Arabia where consanguineous marriage rates are high (12-15). Iran has one of the highest reported consanguineous marriage rates in the world (1). With this high-rate consanguineous marriage, high mortality of SMA in Iranian neonatal is expected. In this study, we present our experience for 36 families with history of

having at least one child with homozygous deletion of SMN1 (exon 7) gene between October 2009 and December 2012 and the results of next pregnancy for SMA in these families.

Patients and Methods

Patients

Our cohort was selected only based on existence of homozygous deletion of the SMN1 gene. These families with a SMA child were referred to outpatients clinic of Tabriz University of Medical Sciences within three years (October 2009 to December 2012). SMA in these families was diagnosed based on electrodiagnostic assay (electromyographic-EMG) and SMA gene analysis results; muscle biopsy was not done in any of the patients. The homozygous deletion of the SMN1 gene in one child of these families was diagnosed using molecular test. This mutation in some families has been previously detected.

Prenatal biopsy and DNA extraction

Chorionic villus sampling (CVS) and amniocentesis were used for fetal biopsies. CVS samples were dissected under the microscope and fetal tissue was selected for DNA extraction. DNA was isolated from dissected CVS and amniotic fluid samples immediately after sampling in order to obtain the best results using Proteinase K (PK) standard protocols. Briefly, due to the low cellularity of the amniotic fluid, before DNA extraction, the samples of amniotic fluid were centrifuged (2,000 rpm, 5 min); the resulting supernatant (approximately 9 ml) was removed, the remaining sedimented fetal cells being resuspended in about 1 ml remaining fluid. These procedures aimed at concentrating fetal cells in the amniotic fluid and increasing the DNA quantity obtained following extraction. Dissected tissues, in the case of CVS, and extracted fetal cells from amniotic fluid were incubated by PK over night at 50°C and followed DNA extraction protocol next day. The blood samples taken from the parents were either immediately processed or preserved at -20°C for later analysis.

Molecular genetic analysis of SMN1 exons 7 and 8

Genomic DNA was extracted from blood and fetal (Amniocensis and CVS) samples using standard DNA extraction protocol. For preventing of contamination of fetal and mothers' tissues, three DNA samples were extracted separately from different parts of each fetal sample. Deletion of exon 7 and 8 of SMN1 gene was examined by PCR amplification followed by restriction endonuclease digestion according to the method of (11). Briefly, PCR was performed in a 50 μl reaction mix containing 100 ng DNA, 75 ng of each primer, 200 µM of dNTPs, 1.5 mM of Mg Cl2, 2.5 U Taq polymerase. PCR amplification was carried out at 95°C for 5 min followed by 35 cycles of denaturation for 30 sec at 95°C, annealing for 30 sec at 55°C, and elongation for 2 min at 72° C, followed by a final extension step for 10 min at 72° C. PCR reaction was set up from three CVS fetal different samples. PCR products of exon 8 were digested with DdeI (fermentas, USA), and that of exon 7 with Dra I (fermentas, USA). The digestion pattern of the PCR products was evaluated using 3% agarose gels (fermentas, USA) and visualized by ethidium bromide staining. Maternal contaminations were rule out using AG1 and CATT polymorphic intragenic markers in the SMA critical region (16).

Results

Consanguinity was detected in 31 out of 36 families (75%). The SMN genes, SMN1 and SMN2, were analyzed by the PCR-RFLP method. Restriction enzyme Dra I does not cleave the PCR-amplified fragments of SMN 1 exon 7 or Dde I does not cleave the PCR-amplified fragments of SMN 1 exon 8. On the contrary, Dra I cleave the PCR-amplified fragments of SMN 2 exon 7 and Dde I cleave the PCR-amplified fragments of SMN 2 exon 8. Thus, PCR-amplified fragments of SMN1 exon 7 and 8 can be separated from those of SMN2 exons 7 and 8 after the restriction enzyme-digestion procedures.

We tested deletion of SMN1 exons 7 and 8 in 17 fetuses for prenatal prediction of SMA and of these cases, 8 fetuses were diagnosed to be at risk of developing the disease (homozygous deletion of SMN1 exons 7). The parents decided to terminate the pregnancy. Nine fetus were detected no homozygous deletion of the exon 7 SMN and develop normally at term pregnancy. The recombination events in the investigated markers were not observed and the AG1 marker in 100% and CATT marker in 80% cases were informative. Follow-up of the abortion products and live born children from these pregnancies never led to a false-positive or negative result.

Discussion

The SMN1 and SMN2 genes and the neuronal apoptosis inhibitory protein (NAIP) gene on the 5q11.2-13.3 region were suggested to play an important role in SMA diseases. While loss of SMN1 gene function is essential for pathogenesis of SMA, disruption of SMN2 and NAIP contributes to disease severity (17). According to our results, homozygous deletion is the most common mutation in SMN1 gene in Iranian SMA patients and is similar the observed frequency in the Saudi Arabia, Pakistan, Oman, India and Egypt (12-15).

Consanguinity is the important factor incidence of autosomal recessive inheritance disorders, and it is very high in Iranian society, thus, the prevalence, morbidity and mortality of SMA are high in Iran (1). In this study, the consanguinity rate is 75% (31 of 36) and almost

similar to the observations in other studies (4).
Currently, there is no available treatment for SMA; the availability of genetic testing it is now possible to diagnose these children early so that appropriate counseling can be given to the family on the risk of future pregnancies (10). Some families denied genetic testing due to various reasons, and possibility by educating the population could be to reduce the incidence of SMA.
In our study all families had at least one affected child or affected infants died at the time of test and SMA disorder was molecularly diagnosed in these families. Prenatal diagnosis can prevent birth of the SMA offspring in couples at risk. We have detected accurately homozygous deletion of the SMN1 gene in all affected fetuses by using this simple and reliable test. Genetic testing for SMA in our University was started from 2006, and this is the first study from North West of Iran that reports clinical experience and genetic testing of SMA prenatal diagnosis.

Conclusion

In conclusion, this study highlights the need for genetics counseling and performance of prenatal diagnosis in high risk consanguineous populations like Iran to reduce the incidence of the SMA disease. Meanwhile, the accuracy rate of the simple explained assay for prenatal diagnosis is almost 100% and could be suggested for prenatal prediction in families with homozygously deleted ex on 7 of SMN1 gene in developing countries.

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