RESEARCH

Gestational outcomes of pregnant women who have had invasive prenatal testing for the prenatal diagnosis of spinal muscular atrophy

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Background. Spinal muscular atrophy (SMA) is a neurodegenerative disease which is characterised by progressive degeneration of motor neurons in the anterior horns of the spinal cord. It is a mainly chromosome 5-linked genetic disorder, with recessive inheritance and it can be diagnosed prenatally.

Objective. To communicate the importance of prenatal diagnosis of spinal muscular atrophy (SMA) and to demonstrate the gestational outcomes of disease carrier pregnant women who have had invasive prenatal testing (IPT).

Methods. We retrospectively evaluated 113 pregnancies of 76 patients who were referred to the Division of Perinatal Medicine at Haçettepe University in Ankara, Turkey for the prenatal diagnosis of SMA between 2000 and 2015. We evaluated the screening results and gestational outcomes of the patients. The pregnancy outcomes were compared with a control group of 179 patients. The Beksac Obstetrics Index (BOI) was used for the comparison of obstetrical histories/backgrounds of the study and control groups.

Results. Chorionic villus sampling (CVS) and amniocentesis (AC) were performed in 83 (73.5%) and 30 (26.5%) cases, respectively. In 24 cases (21.2%), the fetuses were found to be disease-positive and 23 of them were terminated. The median gestational day at birth ($p<0.001$), median birthweights ($p=0.002$) and median BOI ($p=0.001$) of the study and control groups were compared and the differences were statistically significant.

Conclusion. Prenatal diagnosis of SMA is very important and a nationwide special antenatal care programme must be established for better diagnosis and eradication of this genetic disorder.

Spinal muscular atrophy (SMA) is a neurodegenerative disease which is characterised by progressive degeneration of motor neurons in the anterior horns of the spinal cord; the prevalence of SMA is 1 in 6 000 heterozygotes and 1 in 40 homozygotes.[1] SMA is a mainly chromosome 5-linked genetic disorder, with recessive inheritance which results from genetic mutations in the survival motor neuron (SMN) genes.[2] Telomeric and duplicated centromeric genes (*SMN1* and *SMN2*, respectively) play a role in the occurrence of SMA types 1 to 4 . There are also autosomal dominant (chromosome 14-linked) and X-linked forms of SMA.[3]

SMA is associated with a wide spectrum of symptoms in terms of the age of onset and severity. The amount of functional SMN protein is critical to the extent of the symptoms, including hypotonia, muscle atrophy, paralysis and even death.[4] Normally, *SMN1* genes produce fully functional SMN protein but this production is insufficient in the case of SMA. *SMN2* genes also produce SMN protein but in different forms, and only a small percentage of this production is functional. Thus, the level of impaired *SMN1* gene production and the contribution of the *SMN2* gene determine the severity and progress of the disease.[5]

As mentioned above, SMA has a wide spectrum of variability in phenotype with subtypes depending on the age of onset and clinical severity:[5,6] SMA type 0 fetuses are inactive in utero, the infant has little ability to move and the prognosis is poor; SMA type 1, or Werdnig-Hoffmann disease, is the severe variant which represents 50 - 70%[7] of childhood onset cases (age of onset between birth and 6 months) – these individuals may not sit and usually die within 2 years; SMA type 2 is the intermediate variant and usually begins between 6 and 18 months of age – children with this variant may be able to sit but never stand, and death usually occurs after 2 years of age;[8] SMA type 3 is a mild variant with onset at ~18 months of age, most patients eventually require use of a wheelchair and death occurs in adulthood; and SMA type 4 is the adult variant and presents during the second or third decade – individuals walk during adulthood and death occurs in adult life.[9]

SMA is a disease that most often presents with protracted difficulties for affected individuals, their families and for health systems. For this reason, prenatal diagnosis (PD) is very important, especially in severe forms.[4] A polymerase chain reaction-restriction

fragment length polymorphism (PCR-RFLP) assay has been established for the diagnosis of SMA and it distinguishes the base differences in exons 7 and 8 *SMN1* from *SMN2* and is used to define homozygous deletions of *SMN1* exon 7 and 8.[10] Deletions of the exons 7 and 8 of *SMN1* is the most common form; however, point mutations and compound heterozygous cases have also been reported.

Chorion villus sampling (CVS) and amniocentesis (AC) are invasive prenatal tests (IPTs) used for the prenatal diagnosis of SMA.[10] Due to the recessive inheritance of types 1 to 4 SMA cases, up to 25% of prenatal diagnoses are expected to be SMA and pregnancies are terminated. The other important issue is the follow-up of the remaining pregnancies on whom IPT has been performed.

In this study, we have reported PD results and evaluated the gestational outcomes of the remaining pregnant women who gave birth at our medical centre.

Methods

We retrospectively evaluated 113 pregnancies of 76 patients who were referred to the Division of Perinatal Medicine at Haçettepe University in Ankara, Turkey, for the PD of SMA between 2000 and 2015. Prenatal diagnosis was performed on couples who were both carriers of the disease or had at least one child with SMA in their families. Fetuses with a homozygous *SMN1* deletion were terminated after genetic counselling with ethical and legal support. The study protocol was approved by the institutional ethics committee of Haçettepe University (ref. no. GO 16/690) and written informed consent was obtained from the patients.

In the second step of the study, we evaluated the gestational outcomes of the remaining pregnant women whose fetuses were normal or heterozygous for SMN1 deletion and who gave birth at our institution. Patients who gave birth at other hospitals and patients with missing data were excluded from the second step of the study. The pregnancy outcomes were compared with a control group of 179 patients which was randomly chosen from the Antenatal Care Program patients who gave birth to their babies at our hospital between January 2000 and December 2015, without any history suggestive of SMA.

CV specimens were dissected under a microscope to remove

maternal tissue and were transferred to the Department of Medical Biology for molecular genetic analysis. Fetal DNA was extracted from uncultured CV and amniotic fluid by the phenol-chloroform method. Deletion analysis of the *SMN1* gene was performed by PCR-RFLP as previously described. Briefly, exons 7 and 8 of *SMN1* were amplified with PCR using specific primers to the corresponding exons through 35 cycles at the following conditions: 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute. Exon 7 and 8 deletions were analysed via overnight digestion of the amplicons at 37°C with DraI and DdeI restriction endonucleases, respectively. Digestion products were electrophoresed in ethidium bromide-stained 4% agarose gel and the results were evaluated according to the digestion pattern. Genomic DNA of the mother was extracted from peripheral blood and multiple polymorphic markers were used in both maternal and fetal samples to rule out the risk of maternal cell contamination.

Tables 1 and 2 show the personal/clinical characteristics of the patients, Beksac Obstetric Index (BOI) scores and the PD data together with pregnancy outcomes. BOI is an obstetric index for the assessment of risk levels by using the following formula: number of alive children + $\pi/10$/gravida.[11] Chorionic villus sampling (CVS) or amniocentesis was performed in 83 (73.5%) and 30 (26.5%) cases, respectively.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 22 (IBM Corp., USA). The variables were investigated using visual (histograms and probability plots) and analytical methods (Shapiro-Wilk's test) to determine whether they were normally distributed. Descriptive analyses were presented using medians and minimum-maximum values for the non-normally distributed and ordinal variables. Since the age, gestational day at birth, BOI, and first-minute APGAR values were not normally distributed, nonparametric tests were conducted to compare these parameters, as well as to compare ordinal variables. The Mann-Whitney U-test was used to compare age, gestational day at birth, BOI, and APGAR 5 values between the groups. A *p*-value <0.05 was considered to show statistical significance.

Results

In this retrospective study, 113 pregnancies of 76 patients, who had undergone PDs, were evaluated for the risk of SMA. Among the 113 pregnancies, 24 (21.2%) of the fetuses were found to be disease-positive. Twenty-three of the 113 fetuses were found to have both exon 7 and 8 deletions and one was found to have only exon 7 deletion in the *SMN1* gene. Twenty-three of the cases underwent termination of pregnancy. The remaining patient delivered prematurely at 26 weeks' gestation and the fetus died on the first day.

We also evaluated the frequency of consanguinous marriages and found that 96 of the cases had consanguinous marriages (85%). Eighty patients had a family history of SMA type 1 (70.8%), 28

Table 1. Demographical features of the patients

	Mean (SD)
Age (years)	29.65 (5.19)
Gravida	3.50 (1.67)
Parity	1.65 (0.94)
APGAR 1	8.26 (1.44)
BOI	0.15 (0.04)
Prenatal test week	13.2 (2.45)

SD = standard deviation; BOI = Beksac Obstetrics Index.

Table 2. The results of pregnancy outcomes of prenatally healthy and carrier fetuses

	Study group (*n*=49)	Control group (*n*=179)	*p*-value
Gestation (days), median (range)	267 (231 - 281)	272 (189 - 294)	*p*<0.001
Birthweight (grams), median (range)	3 000 (1 670 - 3 800)	3245 (600 - 4 500)	*p*=0.02
APGAR 1, median (range)	9 (6 - 9)	9 (4 - 10)	*p*<0.001
C/S, *n*	38	66	
Vaginal birth, *n*	11	113	
BOI (median)	0.15 (0.06 - 0.31)	0.20 (0.01 - 0.41)	*p*=0.001

C/S = caesarean section; BOI = Beksac Obstetrics Index.

patients had an SMA type 2 history (24.8%) and 5 patients had an SMA type 3 history (4.4%). There were 69 (61%) male and 44 female (39%) fetuses in our cohort. Among them, 20.2% of the male and 22.7% of the female fetuses had a deletion in the *SMN1* gene.

Forty pregnancies who were delivered at other medical institutions or for which there were missing data were excluded from the study and the pregnancy outcomes of the remaining 49 pregnancies whose fetuses were found to be healthy or carriers in terms of SMA and delivered at our hospital were compared with the control group which consisted of 179 patients. Table 2 shows the results of pregnancy outcomes of prenatally diagnosed deletion-negative fetuses (*n*=49). The median (range) maternal age of the study group was 30 (17 - 42) years, while the median (range) maternal age of the control group was 28 (19 - 44) years. Maternal age was not statistically significantly different between the study and the control groups (*p*=0.297).

The median (range) gestational day at birth of the study and control groups were 267 (231 - 281) and 272 (189 - 294) days, respectively, and the difference was statistically significant (*p*<0.001). The median (range) birthweights of the study and control groups were 3 000 and (1 670 - 3 800) and 3 245 (600 - 4 500) g, respectively, and the difference was also statistically significant (*p*=0.002). The median (range) BOIs of the study and control groups were found to be 0.15 (0.06 - 0.31) and 0.2 (0.01 - 0.41), respectively (*p*=0.001). Thus, gestational day at birth, birthweight and BOI were lower in the study group and the results were statistically significant. The median (range) APGAR 5 scores of the 2 groups were 9 (6 - 9) and 9 (4 - 10) (*p*<0.001).

Discussion

Our study consisted of 113 pregnancies of 76 patients and was retrospective. Multicentre-based studies with greater numbers of patients may reveal more information about gestational outcomes of women who had IPT for SMA. The study was limited by large amounts of missing data from obstetric follow-ups and therefore only limited conclusions could be drawn from existing data for a condition which presents in infancy or beyond.

SMA is a disease with social consequences for the families and the societies in which they live.[12,13] Prenatal diagnosis of SMA is critical for couples who are heterozygous for *SMN1* gene deletion, although screening must be limited to families who already have children with SMA (or SMA presence in the extended family). We must remember that there is a significant *de novo* mutation rate (1.7%).[3,14] The American College of Obstetricians and Gynecologists does not recommend general screening and offers screening only for patients who had preconception or antenatal genetic counselling.[15] Furthermore, the screening tests show if there are homozygous deletions of the telomeric exons 7 and 8 of the *SMN1* gene but they cannot distinguish the different types of the disease. The other important issue is the ethical issue, especially for type 4 SMA as individuals can reach middle age by the help of supportive therapies.[4]

In this study, we have demonstrated that the obstetric histories of the pregnant women who had undergone the PD of SMA were more problematic compared with the control group, but the exact reason for this remains unclear.

We have shown that birthweights and gestational age at delivery were statistically significantly lower in pregnancies which had undergone IPT for the PD of SMA and were delivered at our hospital, but remained within normal/acceptable ranges. The median gestational age at birth was 38 weeks and 1 day

and the median (range) birthweight of the neonates was 3 000 (1 670 - 3 800).

Study limitation

As previously stated, the study is limited, particularly in the interpretation of obstetric outcomes, by a considerable amount of missing data.

Conclusion

We believe that PD of SMA is critical and a nationwide special antenatal care programme must be run for better diagnosis and eradication of this genetic disorder. In our study, almost all families whose fetuses were found to be homozygous for the *SMN1* gene deletion accepted termination of the pregnancy. Nationwide programmes will enable relevant institutions to spend and invest more money in this field.

The very high incidence of consanguinity in the study undoubtedly contributed to the prevalence of SMA. Social and cultural factors in Turkey contribute to the high incidence of marriage between first and second cousins.

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