Associated anomalies with neural tube defects in fetal autopsies

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Abstract

Neural tube defects (NTD), the consequences of aberrant neural tube closure during embryogenesis, have been mostly investigated in terms of their high prevalence, rate of mortalities and serious morbidities. A proper prenatal outcome counseling of couples coming across a fetal anomaly necessitates the detection and categorization of the primer abnormality, all the co-existing malformations. The aim of this work is to study the incidence and relevance of associated malformations in order to offer a complete pathology report with a true diagnosis. In this study, among 542 fetal autopsy 62 (%11.4) cases with NTD was recorded by the Akdeniz University Pathology Department between January 2006 and June 2012. Twenty (32.4%) NTD cases were associated with anomaly. Twelve cases of associated groups consisted of a congenital syndrome/association, spondylothoracic dysplasia, amniotic band syndrome, Meckel–Gruber syndrome, schisis association. The frequency of associated NTD was 32%, this result was higher than previous reports. NTDs have a significant genetic component to their etiology that interacts with environmental risk factors, which might pose Turkey to be a country with high prevalence of NTD. We want to emphasize that intensive screening, documentation of co-existent abnormalities of NTD, should be conducted in order to exhibit certain diagnosis, to perform proper prenatal genetic counseling of parents for on-going/future pregnancies.

Keywords

Anencephaly, cranioschisis, genetic, malformation, spina bifida

History

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The two most common forms of NTD are cranioschisis and spina bifida. The former is a congenital failure of the skull closure and contains two subtypes such as anencephaly and cephalocele. The latter is another serious abnormality in which the spinal cord is malformed and lacks its usual protective feature on skeletal and soft tissue. Spina bifida has two subtypes named as meningocoele and meningomyelocele according to characteristics. We performed this study to manifest NTD subtypes and associated abnormalities in Turkey, in which incidence of NTDs is higher compared to that of developed countries [3].

Materials and methods

In our institution all fetal autopsies are performed on fresh material. Before starting the autopsy, we check the autopsy permission of the parents and the type of consent given, the identity of the infant and consult the clinicians to determine the questions that have to be answered by the autopsy. A radiograph of the whole body in mammography dose called baby-gram is performed on all fetuses. Macroscopic photos of fetus are taken. Measurements of crown heel, crown rump, head circumference, foot length and weight are taken for comparison with standard charts. Wigglesworth provides weights and measurements for stillborn and liveborn infants using data from the Women & Infants Hospital, Providence, Rhode Island, USA. Foot length is used to determine
gestational age, which can then be compared with chronological age. The external examination is systematically performed on all fetuses regardless of gestational age. We use a standard autopsy protocol form of Turkish Federation of Pathology Societies, Perinatal and Pediatric Pathology study group (Supplementary material). Timing of the fetal death is determined by the degree of maceration. Cranial, thoracic and abdominal spaces are opened and end-block dissection is performed. The heart is dissected following the blood flow sequentially starting from the right atrium. Before dissection, the outflow tracts, aortic isthmus and ductus arteriosus are measured. In NTD cases for brain and posterior approach is used to preserve the skull or spinal column. Usually we cannot make a complete posterior approach because of small fetus or maceration. Then, we make a modified posterior approach: first of all we make excision of NTD area of spinal column or skull and sample it. Before fixation we measure weights of all organs and examine all of them for macroscopic abnormalities. We make dissections and take at least one sample for each organs and fix these samples in % 10 buffered formaldehyde approximately 24 hours. We also keep the rest of the organs in fixation solution for possible further samplings. We use a special fixation technique for brain: we hang brain and fix it with 10% buffered formaldehyde, every day we renew the fixation solution and check if the brain is fixed. Usually it takes 1 week for brain fixation.

In this study, the fetal autopsy records of Prenatal Pathology Department, Akdeniz University, between January 2006 and June 2012 were retrospectively examined. Our institution is a tertiary center on the south coast of Turkey which has an important consultation practice on perinatology and perinatal pathology; this may skew the incidence rates. The analysis was performed by identifying the maternal and gestational ages, macroscopic assessments, the type and exact location of the NTD and accompanying abnormalities and clinical findings for each case in the data set.

The NTDs were first grouped according to their type such as cranioschisis, spina bifida and cranioschisis accompanying spina bifida. Then, we classified the cases under two groups: isolated and associated. If NTD was observed alone in the autopsy, the case was appraised in the isolated group. When one or more additional malformations were present with the NTD, we labelled the case as associated. The associated abnormalities were also grouped according to the main organ system to which they belonged. Statistical analysis was performed with SPSS software (Statistical Package for the Social Sciences, version 16.0; SSPS Inc., Chicago, IL).

Results

Among 542 fetal autopsy recorded by the Akdeniz University Prenatal Pathology Department between January 2006 and June 2012, a total of 62 (11.4%) cases with NTD were identified. In our series 29 of 62 (46.8%) had spina bifida (Figure 1), 31 of 62 (50%) had cranioschisis (Figure 2) and 2 of 62 (3.2%) had both spina bifida and cranioschisis. The total numbers of cases with isolated NTD were 42 (67.6%) and the cases associated with at least one abnormality were 20 (32.4%), respectively. The distribution of all NTD cases according to subtypes is given in Table 1 and detailed associated anomalies are given case by case in Table 2. Additionally, the mean maternal ages was 24.4 (±5.92) in the associated group and 26.6 (±5.63) in the isolated group, no significant difference was obtained between two groups’ mean maternal ages (p > 0.05, Mann–Whitney U-test).

Conclusions

Today, the multidisciplinary approaches to fetal malformations have ever increasing importance in prenatal medicine. Accordingly, fetal and neonatal pathology studies objecting to evaluate the NTDs and the associated malformations may present perspectives not only to understand embryonic development, identify the causes of congenital anomalies, determine recurrence risks and guide expectations for the efficacy of prevention strategies [4], but also to provide proper prenatal recurrence risk counseling at the time of facing these unintended situations. It was previously reported that fetal autopsy was a valued tool and had some advantages over contemporary prenatal diagnostic instruments like ultrasonography and magnetic resonance imaging [5] and in this retrospective study, we disclosed our institution’s archive records of fetal autopsies.

The frequency of NTD associated with congenital anomaly was 32%, in our study. This result was higher than previous reports that manifested a rate of 15–20% [4,6]. We think one of the reasons of this high frequency is our center’s property. Our institution is one of the reference centres for perinatology and perinatal pathology, and mostly complicated cases were...
referred to our institution. Another reason might be the fact that the NTD cases have multifactorial etiology. They have a significant genetic component to their etiology that interacts with a number of environmental risk factors [7,8], which might pose Turkey to be a country with high prevalence of NTD.

The findings of our study also revealed that the most common malformation associated with NTD was urinary system malformations, comprising Meckel–Gruber syndrome (MGS) (8/62; 12.9%). MGS is an autosomal recessively inherited disease with the classical features of NTD, polycystic kidneys and polydactyly [9]. However, only about half of the patients have all the three components of the syndrome and one quarter exhibit other variations such as major central nervous system malformations, microphthalmia, cleft palate, sloping forehead, bile duct dilatation, portal fibrosis, portal fibrous obliteration, genital ambiguity, craniosynostosis, coloboma of the iris, hypoplastic optic nerve and hypoplastic or absent nasal septum, cleft lip, lobulated tongue, cleft epiglottis, prenatal teeth, short webbed neck and short bowed limbs, micrognathia, ear abnormalities, single umbilical artery, patent urachus, omphalocele, malrotation of the gut, accessory spleen, adrenal hypoplasia, imperforate anus and hypoplasia of the urinary bladder [10,11]. There were 5 (5/62, 8.1%) fetuses with MGS in our study: 4 (80%) of these had NTD and cystic renal abnormality and 1 (20%) had all three components of the syndrome. Other components low-set ears and O-bain-shaped lower extremities were other occasional abnormalities, probably due to oligohydramnios. Chromosome bands 17q21-q24, 11q13 and 8q24 were reported to be defective in cases with MGS, and the pleitropy of the MGS genes could be the underlying problem in this wide range of accompanying abnormalities [11,12]. Unfortunately, our data did not contain any information on the genetic analysis. MGS has a 25% of recurrence risk – because of this, if a NTD is associated with MGS then it should be noted in autopsy evaluation for prenatal counseling of the following pregnancies.

Most of the NTDs are associated with omphalocele, diaphragmatic defects, and cleft lip. It was first described in 1981 by Czeizel and named as ‘‘schisis association (SA)’’ [13]. Polydactyly, conotruncal septation defects, exotrophy of bladder/cloacae, limb abnormalities and, extremely rarely oligodactyly were reported to be associated minor manifestations of SA [13,14]. One of our SA cases had syndactyly, and to the best of our knowledge, this case is the first reported association of NTD with syndactyly.

Another common association is amniotic band syndrome (ABS). ABS is a group of congenital malformations that

| Type                      | Cases           | Isolated | Associated |
|---------------------------|-----------------|----------|------------|
| Craniostenosis            | 31 50           | 16 25.8  | 6 9.7      |
| Spina bifida              | 29 46.8         | 3 4.8    | 5 8.1      |
| Cranioschisis + Spina bifida | 2 3.2     | 4 6.5    | 4 6.5      |
| Total                     | 62 100          | 42 67.6  | 20 32.4    |

Table 1. Numbers and percentage of associated and isolated NTDs.

Figure 2. (a) Fetus with cranioschisis (anencephaly subtype). (b) Fetus with cranioschisis (encephalocele subtype).
includes the extremities, craniofacial–vertebral regions and body wall defects [15,16]. Its pathogenesis includes germ disc disruption, genetic disruption, vascular disruption and amniotic disruption [17]. We determined one case with ABS which has facial cleft, omphalocele and unilateral upper extremity amputation.

Anencephaly is one of the most common subtypes of NTDs which is not compatible with life. Additionally, the most common co-existent anomaly with anencephaly is extremely small hypoplastic adrenal glands [2,18]. The adrenal gland develops almost normally until about 20 of gestation and then atrophies progressively grow to term [18]. In our study, one case with anencephaly at the 16th week of gestation was associated with adrenal hypoplasia of cytomegalic subtype (Figure 3). Sixteen weeks of gestation was quite early for anencephalic-type adrenal hypoplasia. In our opinion, this case was a primary adrenal hypoplasia rather than a secondary phenomenon due to anencephaly itself. Another finding suggesting that this case as a primary adrenal hypoplasia was histological type. The histologic type of the adrenal hypoplasia was cytomegalic type. Cytomegalic-type adrenal hypoplasia is similar to that seen in adults with primary Addison disease and neonatal adrenoleukodystrophy.

The cortex is shrunken and irregular, cells are arranged nodularly. Fetal zone cells are enlarged with eosinophilic cytoplasm. Scattered groups of nuclei are moderately enlarged with pseudoinclusions similar to, but less marked than in classical adrenal cytomegaly [19]. Weiss and Mellinger described an X-linked ressesive trait in their case with cytomegalic-type adrenal hypoplasia [19]. On the other hand, a few cytomegaly and adrenal hypoplasia cases can be sporadic [20]. In the light of literature and autopsy findings, our case seems to be an association of anencephaly and primary adrenal hypoplasia of cytomegalic type. Genetic consultation is suggested for this case.

Besides these entities, pulmonary artery atresia, macroglossia, hypoplastic left heart, micrognathia, congenital adenomatoid malformation, absent uterus and vagina and bifid costa were the other associated malformations of NTD in our study. The main limitation of this study is poor genetic data. On the other hand, plenty of morphological data are available.

In conclusion, we want to emphasize that intensive screening and documentation of co-existent abnormalities of NTD, either with ultrasonography/MRI or fetal autopsy should be conducted in order to exhibit certain diagnosis

Table 2. Detailed presentation of NTD cases with associated malformations.

| Type                  | Associated malformations                                                                 | Recognizable syndromes                      | Fetal external genitalia | Gestational week | Maternal age |
|-----------------------|------------------------------------------------------------------------------------------|---------------------------------------------|--------------------------|------------------|--------------|
| Anencephaly           | Pulmonary hypoplasia, anal atresia                                                       | Spondylothoracic dysplasia                 | Female                   | 12               | 27           |
| Anencephaly           | Single umbilical artery                                                                  |                                             | Female                   | 21               | 18           |
| Anencephaly           | Pulmonary artery atresia, hypoplastic right heart, macroglossia                          |                                             | Female                   | 17               | 19           |
| Anencephaly           | Cleft palate, omphalocele, syndactyly, rudimentary 5th finger of feet, short tibia, agenesis of diaphragma, transposition of great arteries | Schisis association                         | Female                   | 17               | 35           |
| Anencephaly           | Adrenal gland hypoplasia (cytomegalic subtype)                                          |                                             | Female                   | 16               | 18           |
| Anencephaly           | Polycystic kidneys                                                                       | Meckel–Gruber syndrome                      | Male                     | 17               | 20           |
| Anencephalocele       | Single umbilical artery, unilateral hypoplasia of 3rd and 4th hand fingers               |                                             | Female                   | 21               | 19           |
| Encephalocele         | Renal microcystic tubular dilatation                                                     | Meckel–Gruber syndrome                      | Female                   | 20               | 30           |
| Encephalocele         | Multicystic kidneys, polydactyly, micrognathia, low-set ears, O-bein shaped lower extremities | Meckel–Gruber syndrome                      | Female                   | 12               | 20           |
| Encephalocele         | Hypoplastic left heart, micrognathia, hydrops fetalis                                    |                                             | Female                   | 21               | 17           |
| Meningocele           | Unilateral renal agenesis, unilateral diffuse cystic dysplasia of kidney, anal atresia, bilateral congenital pulmonary adenomatoid malformation type 3, absent uterus and vagina | Meckel–Gruber syndrome                      | Female                   | 20               | 27           |
| Meningocele           | Omphalocele                                                                               |                                             | Male                     | 14               | 33           |
| Meningocele           | Pelvicalycal dilatasy (ureteropelvic junction obstruction), internal hydrocephalus       |                                             | Female                   | 20               | 20           |
| Meningomyelocele      | Internal Hydrocephaus                                                                     |                                             | Male                     | 19               | 29           |
| Meningomyelocele      | Congenital diaphragmatic hernia, agenesis of unilateral kidney and adrenal gland          | Schisis association                         | Female                   | 22               | 20           |
| Meningomyelocele      | Polydactyly, bilateral polycystic kidneys                                                | Meckel–Gruber syndrome                      | Male                     | 17               | 25           |
| Meningomyelocele      | Unilateral renal agenesis, fusion of adrenal glands                                      | Caudal regression syndrome                  | Male                     | 18               | 21           |
| Meningomyelocele      | Bifid costa, fibrous band at ileocaecal valve                                            | Anniotic band syndrome                      | Male                     | 20               | 33           |
| Meningomyelocele      | Facial cleft, omphalocele, unilateral upper extremity amputation                          |                                             | Male                     | 14               | 33           |
| Meningomyelocele      | Pelvicalycal dilatasy (ureteropelvic junction obstruction), chorangiosis of placenta     |                                             | Male                     | 25               | 32           |

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and to perform proper prenatal genetic counseling of parents for on-going and future pregnancies.

Declaration of interest

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Supplementary material available online