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Original Article

Evaluations of Pregnancies Diagnosed with Fetal Neural Tube Defects in our Center

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ABSTRACT

Objective: To evaluate the risk factors, chromosomal abnormalities and additional anomalies of cases diagnosed with fetal neural tube defects (NTDs).
Material and methods: The data of cases diagnosed with fetal NTDs between January 2016 and August 2020 with fetal NTD were retrieved from the hospital database. Only patients whose diagnosis confirmed after pregnancy termination and have a genetic test were included in the study. The family and antenatal history of patients included maternal age, maternal education level, diabetes mellitus, exposed to teratogenic drugs, smoking, and folic acid intake before or during the first trimester, siblings with a congenital abnormality, consanguinity, and AFP MOMs (alpha-fetoprotein, multiple of median) was obtained. Also, the type and level of NTDs and additional anomalies were noted. The data were evaluated using version 23.0 (SPSS Inc., Chicago, IL, USA).
Results: During the study period, a total of 68 patients who met the study criteria were included in the study. A total of 27 patients diagnosed with spina bifida, 24 patients with anencephaly and 17 patients with encephalocele. The most anomalies resulted from NTDs was hydrocephaly and pes equinovarus. The most detected chromosomal abnormality was trisomy 13 (3 out of 7), and the most additional anomaly was the cleft lip and palate and detected in 4 (5.88%) patients. Pregestational DM was seen in 12/68 of pregnancies with fetal NTD.
Conclusion: The rate of additional anomalies and chromosomal abnormalities in cases diagnosed with fetal NTDs is high. Thus, a detailed ultrasonographic examination and genetic tests of fetuses with NTD is essential. The pregnancies complicated with pregestational DM have an increased risk for fetal NTD.

Keywords: anencephaly; congenital anomalies; inencephaly; nervous system malformations; neural tube defect

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Introduction

Neural tube defects (NTDs) result from incomplete or aberrant neural tube closure during embryogenesis. The neural tube closure process starts from the cervical area and continues toward cranial and caudal directions [1]. If this process interrupted in any way could result in NTDs. The NTDs types vary according to the affected region. The affected area is in the skull results in anencephaly, and the vertebra results in spina bifida [2, 3]. The most associated anomalies with NTDs are facial cleft, cardiac defects, limb reduction defects, abdominal wall defects and renal abnormalities. Less common anomalies include polydactyly and holoprosencephaly [3]. A series of anomalies contain clubfoot, Chiari 2 and hydrocephaly generally accepted as secondary structural anomalies resulted from NTDs. The rate of underlying chromosomal abnormalities with NTDs is high and varied according to its types. Among NTDs types, the cephalocele has the highest chromosomal abnormality rate (14%), and lethal group such as anencephaly or iniencephaly have the lowest chromosomal abnormality rate (2%). Besides underlying genetic abnormalities, environmental and nutritional factors have an essential role in developing the NTDs. The well-known ones are low folic acid intake, using several drugs such as valproic acid, diabetes mellitus and exposure to high temperature [4- 8].

Because the NTDs have increased mortality and severe morbidity rates and, high prevalence, screening the fetus for these abnormalities is fundamental to routine ultrasound examinations [9]. Detecting a pregnancy with foetal NTD has a crucial role for counselling parents to assist for further management. The prognosis of foetuses with NTD mostly depends on the type of NTD and co-existing malformations [2, 10]. Screening a fetus concerning NTD via alpha-fetoprotein (AFP) and prenatal ultrasound are both accepted methods. However, due to AFP's low sensitivity and specificity, the ultrasound is the more advisable one [1].
In this study, we aimed to demonstrate the risk factors, chromosomal abnormalities, associated abnormalities in pregnancies diagnosed with fetal NTD.

Material and methods

For this study, after the local institutional ethics committee approved, the data of patients diagnosed with fetal NTD at our hospital from January 2016 to August 2020 were retrieved from the hospital database. The family and antenatal history of patients included maternal age, maternal education level, diabetes mellitus, exposure to teratogenic drugs, smoking, and folic acid intake before or during the first trimester, siblings with a congenital abnormality, consanguinity was obtained.

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The maternal serum AFP MOMs (multiple of median) value was noted. Multifetal pregnancies and pregnancies who did not terminated were excluded. According to our clinical protocol, all pregnant women who referred to our centre diagnosed or suspected for a fetal NTD are screened again on detailed ultrasound for exact diagnosis and possible additional anomalies by perinatologist who eight years were experienced in fetal anomaly screening via Voluson E6 equipped with 5–9-MHz volumetric transvaginal transducers and a 4–8-MHz volumetric convex transducer, (GE Medical Systems, Horten, Norway). Pregnancies before 14 gestational weeks, have low imaging quality on abdominal USG due to maternal fat, suspected fetal brain abnormality with cephalic presentation or patients who were thought to provide additional information, the vaginal probe used in adjunction to abdominal probe for diagnosis. Once a case diagnosed with fetal NTD, further evaluation was performed for the additional central nervous system and other system anomalies. Prenatal invasive testing was offered to all patients diagnosed with fetal NTD. The selection type of prenatal invasive testing determined according to patients' gestational weeks. If patients' gestational weeks had been between 11 and 14 the chorion villus sampling, between 16 and 22 the amniocentesis and after 22 cordocentesis was performed. For the fetuses who could not be tested genetically in the antenatal period, the cord blood samples were taken at pregnancy termination for a genetic test. When a fetus diagnosed with NTD, the family were counselled by an obstetrician, a pediatric surgeon, a neurologist for the prognosis and course of the fetal NTD. The choice of pregnancy termination was routinely offered to these cases. The final diagnosis was revealed from the fetal autopsy, physical examination and/or radiograph after pregnancy termination.

The data were evaluated using version 23.0 (SPSS Inc., Chicago, IL, USA). Frequencies were calculated using independent t-test and descriptive statistics and presented as number and percentage.

Results

During the study period, 68 patients who fulfil the study criteria were included in the study. All included patients 'pregnancies were terminated due to fetal NTDs.

The mean age of patients was 28.98±6.69 years and the mean weeks of gestation were 16.71±3.94 weeks at the time of diagnosis. Most of the cases aged between 18-30 years (55.58%). Only 4 of 68 patients included in the study were illiterate, and high school graduates constituted the majority of literate patients (47.06%). Accordingly, most patients (73.53%) used folic acid before or during the first trimester of pregnancy recommended by a family physician or an obstetrician. The most used prenatal invasive testing was amniocentesis (44.12%), followed by CVS (39.71%). A total of 33 patients screened with AFP, and 10 out of them have AFP level above 2.5 MOMs. As an etiologic cause of NTDs, two patients were exposed to teratogenic drugs: one used lamotrigine and another used carbamazepine for epilepsy. Twelve patients (17.65%) have pregestational DM (Table 1.).

Table 2 demonstrates the numbers and percentages of patients according to NTDs types. Spina bifida was the most prevalent type of NTDs. The majority of spina bifida were localized in the lumbosacral area (48.15%). Because the acrania, exencephaly and iniencephaly are precursor lesion of anencephaly, we noted these anomalies as anencephaly. The anencephaly and encephalocele were detected in 24 (35.29%), and in 17 (25%) patients, respectively.

Table 3. depicts additional anomalies revealed from fetuses diagnosed with NTD. At least one anomaly was seen in 34 of all fetuses having NTDs (50%). There are two main types of anomalies can be detected in fetuses with NTDs; the anomalies resulted from the direct or indirect effect of NTD, such as hydrocephalus or clubfoot, and the anomalies unrelated to the effect of NTDs [3, 8, 10, 11]. As expected, the most related additional anomaly was hydrocephalus, followed by pes equinovarus (36.25% and 8.82%, respectively). The cleft lip and palate were the most detected anomaly among those irrelevant to the mechanical effect of NTD (5.88%).

Table 1. Demographic characteristics of the patients.

Variables		Number	Percentage (%)
Maternal age	<18	1	1.47
	18-30	38	55.88
	30-35	14	20.59
	>35	15	22.06
Mother's education level	İlliterate	4	5.88
	Primary school	6	8.82
	Secondary school	13	19.12
	High School	32	47.06
	University	13	19.12
Type of invasive testing	CVS	27	39.71
	AS	30	44.12
	Cord sampling after TOP	11	16.18
Previous miscarriages	Yes	23	33.82
	No	45	66.18
AFP(MOM)	≥2.5	10	14.70
	<2.5	23	33.82
Smoking	Yes	5	7.35
	No	63	92.65
Consanguinity	Yes	13	19.12
	No	55	80.88
Maternal DM	Yes	12	17.65
	No	56	82.65
Folic acid intake	Yes	50	73.53
	No	18	26.47
Teratogenic drugs	Yes	2	2.94
	No	66	97.05
Gender	Male	32	47.05
	Female	36	52.94
Chromosomal anomaly	Yes	7	10.29
	No	61	89.70
Additional anomaly	Yes	27	39.70
	No	41	60.29
		Mean±SD	
Gestational age(week)		16.71±3.94	
Maternal age(year)		28.97±6.69	

TOP: Termination of pregnancy, DM: Diabetes mellitus, AFP: Alpha fetoprotein

Prenatal invasive testing was performed on all patients, and abnormal chromosomal results revealed from 7 (10.29%) patients. The chromosomal abnormalities detected were-trisomy 13(3), trisomy 18 (1), tetraploid (1), and chromosomal structural abnormalities included inversion of 9th chromosome long arm [inv (9) q12q13] (1) and translocation on 17th, 4th and 9th chromosomes [q21; q 21q32; q34] (1) (Table 4.).

Table 2. NTD types and localization

NTD type	N	Percentage (%)
Anencephaly	24	35.29
Encephalocele	17	25.00
Spina bifida	27	39.27
Localization		
Thoracal	2	7.41
Thoracolumbar	6	22.22
Lumbar	4	14.81
Lumbosacral	13	48.15
Sacral	2	7.41

Discussion

The primer prevention of fetal NTDs is a folic acid fortification which accepted as a national program in many countries and similarly in Turkey [5]. The second prevention of fetal NTDs is screening the fetuses on prenatal USG. With advanced

technologies of USG devices and sonographers' experience, the detection ratio of the NTDs is increased and most fetuses detected in the first or early second trimester. Compared to USG, AFP's sensitivity and specificity remain low for screening NTDs and losing their first popularity.

Table 3. Anomalies related to other systems in patients with NTD.

		Number	Percentage (%)
Neurological anomalies	Alobar holoprosencephaly	1	1.47
	Agenesis of CC	1	1.47
	Hydrocephaly	25	36.76
	Microcephaly	1	1.47
	Ventriculomegaly	1	1.47
Orthopedic anomalies	Thoraco-lumbar scoliosis	2	2.94
	Pes equinovarus	6	8.82
	Kyphosis+scoliosis	2	2.94
Congenital heart disease	Inlet VSD	1	1.47
	Large VSD	1	1.47
Urinary system anomalies	Multicystic dysplastic kidney	1	1.47
	Polycystic kidney	1	1.47
Other systems anomalies	Cleft lip and palate	4	5.88
	Single UMA	2	2.94
	Diaphragm hernia	1	1.47
	Omphalocele	3	4.41
	Frontal bossing	1	1.47
	Placentae circumvallate	1	1.47
	Cystic hygroma	1	1.47
	Hydrops fetalis	1	1.47
	Cervical jugular cyst	1	1.47

CC: Corpus callosum, UMA: Umbilical artery

The prevalence of NTDs is affected by nutritional factors, genetics, and folic acid supplementation. The reported NTDs prevalence, in Eastern Mediterranean (2.1-124.1; 21.9 per 10,000 births) European (1.3-35.9; 9.0 per 10,000 births), Americas (3.3-27.9; 11.5 per 10,000 births), South-East Asian (1.9-66.2; 15.8 per 10,000 births) [12]. A study evaluated the prevalence of NTDs in the Turkish population found 11.1 per 10.000 birth [13]. In our study, the prevalence of NTDs was 14 per 10.000 pregnancies.

Studies evaluated the relation between maternal age and the prevalence of NTDs are conflicting. A study found a higher incidence of NTDs with younger maternal age connected to this result with that the younger maternal age had been related to a lower folic acid uptake and higher substance abuse ratio [9]. Contrary to this study, a study that evaluated 205 pregnancies with fetal NTD found a strong association between maternal age and the rate of fetal NTD [14].

Table 4. Ultrasound findings in chromosomally abnormal fetuses with neural tube defects

Case	MA (years)	NTD types	AFP (MOM)	GA (weeks)	Additional ultrasound findings	Karyotype
1	29	Cephalocele	3.44	13	Vertebral scoliosis	46,X*,İNV(9)(q12q13)
2	28	Spina bifida (thoracal)	1.04	17	CHD (Inlet type VSD), cleft lip and palate	Trisomy 13
3	25	Spina bifida (lumbosacral)	1.32	16	Bilateral polycystic kidney	Trisomy 13
4	37	Cephalocele	2,13	11	Alobar holoprosencephaly	Trisomy 18
5	27	Cephalocele		13	CHD(VSD), abnormal facial features (small jaw, low-set ears, hypertelorism)	Tetraploid
6	24	Spina bifida (lumbosacral		14	Cervical jugular cyst	46,X*,t(17;4;9) (q21;q21q32;q34)[3]
7	23	Cephalocele	4.54	12	Single umbilical artery, thoraco-lumbar scoliosis, abdominal wall defect	Trisomy 13

CHD, congenital heart defect; VSD, ventricular septal defect; GA, gestational age; AFP, alpha fetoprotein; IUGR, intrauterine growth restriction; MA, maternal age; NTD, neural tube defect; SUA, single umbilical artery

There are several factors affecting the development of NTDs are affected by maternal age. First, increasing with maternal age, the literacy ratio heightens using maternal folic acid rate and receiving prenatal health care ratio.

Second, advanced maternal age increases maternal chronic disease such as diabetes mellitus and fetal chromosomal ratio that potentially could possess a risk for fetal NTDs. In the current study, most of the pregnant women were aged, between 18 and 35 years there was not relationship between maternal age and NTDs frequencies.

The maternal literacy rate in patients included in the study was high, and only four women were illiterate. Similarly, the rate of using folic acid in the periconceptional period or during the first trimester of pregnancy was high, and 73.53% of patients used folic acid. Using folic acid before and during the first trimester has proven to prevent fetal NTDs as high as 2 out of 3 cases [5,6, 9,14, 15]. However, in this study, we cannot determine the effect of using folic acid on preventing NTDs because of the lack of a control group and a relatively small study group.

Causing a higher maternal glucose level and oxidative reactions, which both disrupt neural tube closure, the DM has shown to increases the rate of fetal NTD. Because the neural tube closure completes during early pregnancy weeks, the gestational DM is not a factor of NTDs. A metaanalysis evaluating 41 studies investigating the prevalence of DM in Turkish pregnant women found the DM prevalence as 7.7% [16].

Comparing to these results, the prevalence of DM in this study was high, and 17.65% of patients included in the study have DM. An interesting relationship was reported between smoking and passive smoking and the development of fetal NTDs, which found that passive smoking was a causing factor, but smoking was not [7]. İn this study, the smoking rate was 7.35% and did not differ from the results of studies that questioned the rate of smoking among pregnancies without fetal NTDs [17]. However, we had not questioned the status of passive smoking in this study.

Patients with epilepsy have an increased risk of fetal NTD. İt is unknown whether this risk altogether results from a drug used for epilepsy or being with epilepsy solely increases this risk. However, it is clear that the drugs used for epilepsy increase the risk of fetal NTDs. Unfortunately, this risk cannot be prevented by using folic acid completely. A study that evaluated the risk of drugs used in epilepsy found that valproic acid has the highest, carbamazepine has the modest, and lamotrigine has the lowest risk of developing fetal NTDs. Also, monotherapy is better than multidrug therapy [18]. İn this study, there were two cases with epilepsy which one used lamotrigine, and another used carbamazepine was, and both had used folic acid.

Several studies showed a link between previous spontaneous abortus and the risk of fetal NTDs [19, 20]. The NTDs constitute a high proportion of congenital fetal anomalies, and congenital fetal malformations are the most etiologic cause of early spontaneous abortion. A similar underlying mechanism could explain this link. Like previous studies, 33.82% of patients had been at least one last spontaneous abortus.

The reported rate of consanguineous marriages in our country is high (18.5%) and varies according to the region and socio-cultural levels [21]. The consanguinity potentially can increase the risk of fetal NTDs by boosting the risk of autosomal recessive chromosomal dependent syndromes that are responsible for NTDs and central nervous system anomalies [22]. The rate of consanguinity in this study was 19.12% and similar to the rate of pregnancies without fetal NTDs.

Studies on NTDs reported a predominance of female over male fetus [23]. Similarly, there was a slight predominance of the female fetus in this study with a ratio of 53%. İnterestingly, as showed by several studies, this ratio is on the way towards equality after fortification of folic acid [15].

With advanced USG devices quality and experiences in fetal screening, most cases with fetal NTD diagnosed in the first trimester. The detection rate of defects located in the fetal skull, such as anencephaly or encephalocele, is above ninety per cent. İn this study, the mean diagnosis weeks are 16±71 week, and most patients were in the first trimester. Because anencephaly is not compatible with the survival of life, the pregnancies with this anomaly are terminated. As for spina bifida, in most countries like ours, after detailed counselling to family, the route of pregnancy is left to a family decision. A study evaluated 116 pregnancies with fetal NTDs, found that spina bifida had an incidence of 0.342 (85 cases, 68%), and anencephaly had an incidence of 0.113 (28 patients, 22.4%) [23].

The rate of NTDs type varies according to diagnosis time. Most fetuses with anencephaly died during the prenatal period, and a few of them who could survive until delivery will eventually die in the first weeks of life. However, fetuses with spina bifida have a high survival rate in utero and after delivery. Accordingly, two studies evaluated cases in the prenatal period and reported the anencephaly ratio of 42.8% and 54.1%, occipital encephalocele 17.9% and 5.4%, spina bifida 39.3% and 40.5%, respectively [24, 25]. A study evaluated cases at the delivery time found anencephaly 2.1%, encephalocele 7.0%, and spina bifida 91.9% [26]. İn this study, we found that the ratio of anencephaly 35.29%, encephalocele 25%, spina bifida 39.27% and similar to studies that evaluated prenatal cases. The lumbosacral region is the most affected area of fetuses diagnosed with spina bifida [24-26]. Similarly, in this study, spina bifida mostly on the lumbosacral region (48.15%) followed by the thoracolumbar region (22.22%).

The reported frequency of associated anomalies with NTDs was 15-32% [10, 11]. The abnormalities result from NTDs is much higher; however, these anomalies should not be classified as associated anomalies because they result from the direct or indirect effect of NTDs. The most abnormalities that result from NTDs is hydrocephaly Arnold Chiari 2 and club foot. İn our research, 25 out of 68 fetuses had hydrocephaly, and six fetuses had a club foot. The variability and detecting additional abnormalities mostly depend on gestational weeks at the screening. For example, detecting anomalies involved the middle cranial structures (i. e agenesis of the corpus callosum or vermian agenesia) and the most cardiac defects are not possible in the first trimester, generally. The reported associated anomaly with the greatest frequency is facial clefting, cardiac defects, anotia, microtia, limb reduction defects, abdominal wall defects, and renal abnormalities [3, 8]. İn this study, the most associated anomalies were cleft lip and palate (n=6), omphalocele (n=3), ventricular septal defect (n=2), single uterine artery (n=2).

Trisomy 13 and 18 were the most chromosomal anomalies with NTDs reported in studies [27, 28]. Similarly, in our study, the rate of chromosomal abnormality was 10.29, and the most chromosomal anomaly was trisomy 13, which detected in 3 out of 7 patients with chromosomal abnormalities. The rate of chromosomal abnormality was 10.29. The reported rate of chromosomal abnormalities varies by 1.8% and 16% [28, 29]. However, the reported rate of chromosomal abnormality with fetal NTDs in our country was lower than the current study and previous studies [11, 13, 18]. The rate of chromosomal abnormality in fetuses with NTD varies and depends on the study's populations, folic acid intake, type of NTD and the presence of additional abnormality. Our institution is a tertiary referral centre, and most complicated cases are referred, which may explain the higher chromosomal abnormality than previous studies conducted in our country.

There is some weakness of this study that should be mentioned. First, as being a tertiary referral center, there was a possibility that more complicated cases had been referred to our hospital, which could change the ratio of the additional abnormality and chromosomal abnormality with fetal NTDs. Second, due to relatively small cases, the comparison of types of NTDs could not be performed.

The study’s strength is that the research is single centred, cases well defined, diagnosis of all fetuses were confirmed after the termination of the pregnancy.

Conclusions

The rate of additional anomalies and chromosomal abnormalities in cases diagnosed with fetal NTDs is high. Thus, a detailed ultrasonographic examination and genetic tests of fetuses with NTD is essential. The pregnancies complicated with pregestational DM have an increased risk for fetal NTD.

Disclosure

Authors have no potential conflicts of interest to disclose.

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