

Maternal factors and associated anomalies in NTD fetuses from Tunisia

Research Article

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Abstract: Aim of the study: To determine the impact of maternal age, consanguinity, season of conception and variation in the amount of amniotic fluid for the appearance of anomalies associated with neural tube defects (NTDs). Materials and methods: 150 NTD fetuses, a result from autopsies (macroscopic autopsy; microscopic study of biopsy fragments; macro and microscopic brain examination), have been examined during a period of three years (01.2006, 01.2009), in the Clinic of Fetopathology, at the Center of Maternity and Neonatology – Tunisia. Results: Anomalies associated with NTDs occur three times more often in pregnancies with an abnormal amount of amniotic fluid. Also, their likelihood of cardiovascular and reproductive system anomalies is increased four times. Nearly 80% of the NTD fetuses conceived during the autumn-winter period have acrania. Women older than 35, are twice more likely to have underweight children and children with defects of the digestive system and hand. They are also three times more likely to have fetuses with endocrine system abnormalities. Anomalies incompatible with life occur twice as often in consanguineous unions. Conclusion: The mother's age, consanguinity, season of conception, and variation in the amount of amniotic fluid have considerable impact on the emergence of associated anomalies in fetuses with NTDs.

Keywords: Neural tube defects (NTD) • Consanguinity • Amniotic fluid • Seasons • Mother's age

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1. Introduction

World statistics show that neural tube defects (NTDs) rank foremost among congenital malformations. According to EUROCAT (European Surveillance of Congenital Anomalies), following an analysis of births in 22 European countries, neural tube defects are at a rate of 2.3 per 1,000 births for the period 2003-2007 [1]. Numerous studies on the subject have found that deficiency of folic acid and vitamin B12, intake of anti-convulsant and antipyretic drugs, maternal diabetes, hypertension and other factors could lead to defects in the closure of the neural tube [2-4]. Some of the more recent genetic studies show that many mothers

who have given birth to a child suffering from a neural tube defect may be carriers of a mutation on the level of gene MTHFR [5-8]. In another study, Martinez and Al Gazali emphasize the risk for birth to a child suffering from NTDs in mothers homozygous for the mutated gene C677T [9,10]. Nonetheless, the precise role of the exogenous and endogenous maternal factors in the etiopathogenesis of neural tube defects (NTDs) and accompanying additional malformations remains unclear.

An overview of the NTD occurrence rates with accompanying information in the nations outside the European Union hint at some of the factors that could affect the occurrence of defects. Overall, the incidence

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of neural tube defects outside the European Union is much higher. To the east, in Turkey, according to Dogan H. (2005) and Onrat ST. (2009) the frequency of NTDs is close to four per one thousand births [11,12]. The different regions of Iran show a varying incidence of NTDs (1.27 to 4.2 per 1,000 births), and consanguinity is found in about one third of the cases (29.5%). Neural tube defects in the country have been found to be more common in fetuses of multi-gravida women; especially ones that are over 35 years of age [13-15]. In northern Iran, the rate of NTDs is 4.89/1000 in newborns of mothers aged >35 years, where 28% of cases have been the result of consanguineous marriages. 12.8%, 9%, 3.8%, and 2.5% of these marriages had a degree of relatedness of 3, 4, 5 and 6, respectively. Demographically, the rate of anencephaly in three different ethnic groups was respectively 1.2, 1.6, and 0.7 in 1000 births with incest as a factor [14]. The incidence of NTDs in south-western Iran is 4.2 per 1000 births; NTDs in first pregnancy cases are at a frequency of 42.6% and 57.4% in cases of following pregnancies. NTDs detected in fetuses of mothers between 21 and 30 years of age represent 72.2%, where 31% are married couples with a consanguineous connection [16].

In Al-Ramadi Maternity and Children's Hospital, western Iraq, neural tube defects have a 3.3/1000 rate among newborns, where in two-thirds of cases, inter-marriage was reported as a factor [17].

In retrospect, the birth rates of children with neural tube defects in northern Africa are much lower. In Algeria, the established NTD rate is 0.75 per 1000 births where, in 13% of the cases, incest was found [18]. The influence of the seasons of conception for the presence of NTDs has been the subject of numerous studies with varying results [19,20].

The purpose of this study was to investigate the impact of the factors: maternal age, consanguinity, season of conception and variation in the amount of amniotic fluid for the appearance of anomalies associated with neural tube defects (NTDs).

According to the severity of the malformation, NTDs have been separated in two groups – compatible and not compatible with life. The only compatible with life NTD is spina bifida.

2. Methods

Monitored in this study are the anomalies associated with neural tube defects in 150 autopsies of fetuses from the whole county of Tunisia, carried out during a period of three years (01.2006-01.2009) in the clinic of Fetopathology at the Center for Maternity and Neo-

natology - Tunis (Tunisia). The fetuses have been the result of interrupted pregnancies for medical reasons, intrauterine foetal death, spontaneous abortions and neonatal death. All examination has been performed after obtaining informed consent from the parents for autopsy, genetic testing and biopsy specimen collection.

The full range of data available for each case (fetus) has been combined in a personal file which includes: photographic documentation, X-rays, karyotypic study, foetal biometry, documentary information from the autopsy of fetuses obtained from visual macroscopic inspection (situs thoracis, situs abdominis, pelvic cavity and retroperitoneal compartment examination) and internal microscopic examination of the internal organs. The supporting documents for each case are documents of ultrasound, which have been taken from the medical records of the Clinic of Neonatology and Clinic of Obstetrics and Gynecology at the Center for Maternity and Neonatology - Tunis.

The cranial cavity and the brain were examined after fixation with formalin (an aqueous solution of 40% formaldehyde) for a period of one to six months for cases with malformations spina bifida, encephalocele and rachischisis. Each fetus's organs were weighed and fragments were taken for histological examination of the system from different parts of the brain and spinal cord, meninges, eyes, nerves, skin, thymus, heart, etc., as well as from any abnormalities found. For defects of the neural tube, fragments were taken from the places of transition to a healthy zone. The results of the autopsy were added to the files in three stages: the first stage - from macroscopic autopsy, the second - from microscopic results of biopsy fragments and the third stage - from the macro and microscopic brain examination.

The data has been statistically processed by variational, parametric (Pearson's χ^2 -chi-squared test), Fisher's Exact Test, and correlational analysis using the statistical program SPSS-V17.

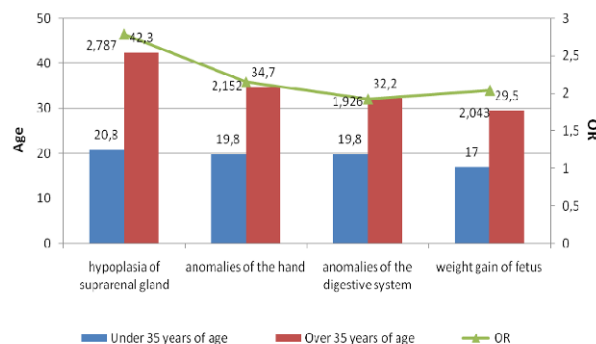
3. Results

The structural distribution of the cases by type of NTD is shown in Table 1. A karyotypic study was done on 21 of the fetuses (14.66%). Of these fetuses, 14 (63.3%) had a normal karyotype and 8 (36.37%) had pathologies: one karyotype of trisomy 13, two karyotypes of trisomy 21 and 5 karyotypes with a chromosomal aberration.

The average age of mothers in the study was 30.93 ± 0.67 , with a minimum age of 21 years and a maximum age 43 years. A mother's age of over 35 years has significant impact on the emergence of endocrine abnormalities in fetuses with neural tube defects (Figure 1).

Table 1. Distribution of fetuses according to the type of NTD.

Type of anomaly	Number of cases	%
Spina bifida aperta (meningocele, myelomeningocele, dyastematomyelia)	59	39.3
Encephalocele, exencephalia	24	16.0
Craniorachischisis, rachischisis, encephalocele	33	22.0
Anencephalia	34	22.7
Total	150	100.0

**Figure 1.** Associated abnormalities in fetuses with NTDs, determined by age of mother.

The share of hypoplasia of the adrenal gland is 22 points higher in fetuses of mothers aged over 35 years. A higher maternal age poses a higher risk for digestive system anomalies and abnormalities of the hand - single transverse palmar crease, clinodactyly of the thumb and the fingers associated with NTDs. In addition, women over 35 have nearly twice as often underweight fetuses and fetuses with a decrease in intrauterine development.

One quarter of those surveyed reported consanguineous unions which is similar but a little less than the results of other authors [21]. From the research conducted in Algeria for a period of three years (2004-2006), it has been concluded that consanguinity is a factor in fetuses with NTDs in 13% of the cases (28/215 cases), which is twice less than our results [18].

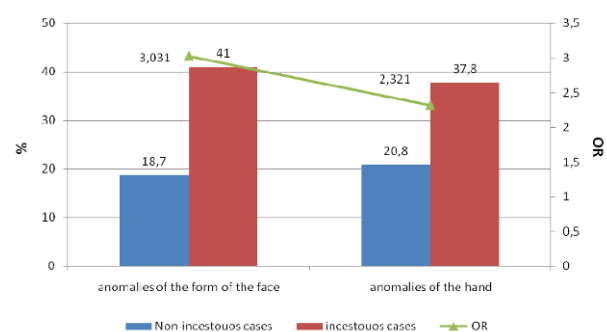
Consanguinity was a significant factor for the expression of facial dysmorphia, where, the presence is 23 points higher in consanguineous cases (Figure 2).

Table 2. Consanguinity and associated anomalies in fetuses with NTDs.

Indicators	Groups	Non-cosanguineous cases		Consanguineous cases		total		χ^2	P	OR (CI)
		qty.	%	qty.	%	qty.	%			
spina bifida and anomalies*	anomalies*	48	67.6	23	32.4	71	100.0	3.587	0.058	2.464 (0.953-6.372)
	spina bifida	36	83.7	7	16.3	43	100.0			
	total	84	73.7	30	26.3	114	100.0			
telorism	normal	50	78.1	14	21.9	64	100.0	3.447	0.063	2.551 (0.934-6.969)
	hypotelorism	14	38.3	10	41.7	24	100.0			
	total	64	72.7	24	27.3	88	100.0			

Abbreviations: CI, confidence intervals; OR, odds ratio; χ^2 , chi-square; P, sig; qty, quantity.

* anomalies incompatible with life

**Figure 2.** Consanguinity and associated anomalies in fetuses with NTDs.

Around 40% of fetuses with associated anomalies of the hand - monkey groove, clino-, polydactyly, and syndactyly emanate from consanguineous unions, which show a trend of dependence between the parameters. In addition, the consanguinity factor demonstrates significant influence on the type of NTD, where abnormalities incompatible with living - craniorachischisis, anencephaly, exencephaly and encephalocele were twice as often manifested in consanguineous unions (Table 2). The same dependence is found in the indicator - hypotelorism.

Changes in the amount of amniotic fluid demonstrated a significant influence on deviations of the distance between the eyes, where hypertelorism is three times more common in cases when there was a presence of oligoamnios or polyhydramnios (Table 3).

The same dependence is found in fetuses with the defect - macrocrania. Variations in the quantity of amniotic fluid has an impact on the occurrence of associated anomalies of the cardiovascular, digestive and reproductive systems in fetuses with NTDs (Figure 3).

Abnormalities of the respiratory system are 10 points more frequent as a result of the impact of the factor - abnormal amount of amniotic fluid. Almost all of the identified anomalies associated NTDs occur approximately three times more often in pregnancies with an abnormal amount of amniotic fluid, except in respiratory anomalies (Table 4).

Table 3. Amniotic fluid - quantitative changes and associated anomalies in fetuses with NTDs.

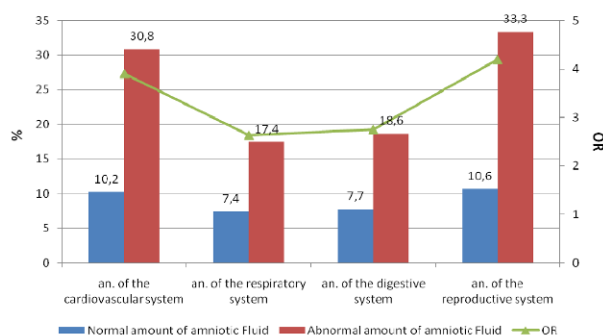
Indicators	Groups	Normal amount of amniotic Fluid		Abnormal amount of amniotic Fluid		total		χ^2	P	OR (CI)
		qty.	%	qty.	%	qty.	%			
telorism	other types	63	92.6	5	7.4	68	100.0	3.990	0.046	3.150 (0.980-10.123)
	hypertelorism	36	80.0	9	20.0	45	100.0			
	total	99	87.6	14	12.4	113	100.0			
macrocrania and other anomalies	other anomalies	91	92.9	7	7.1	98	100.0	6.316	0.012	3.488 (1.262-9.642)
	macrocrania	41	78.8	11	21.2	52	100.0			
	total	132	88.0	18	12.0	150	100.0			

Abbreviations: CI, confidence intervals; OR, odds ratio; χ^2 , chi-square; P, sig; qty, quantity.

Table 4. Relationships between the seasons of conception and associated abnormalities in fetuses with NTDs.

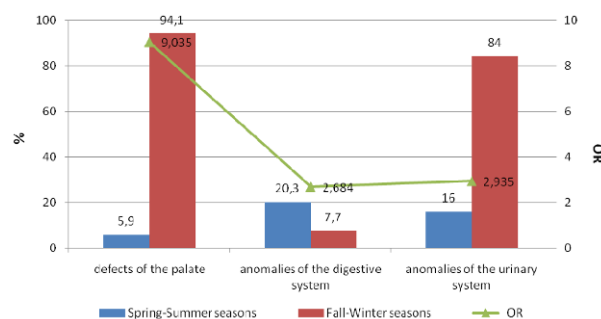
Indicators	Groups	Spring-Summer seasons		Autumn -Winter seasons		Total		χ^2	P
		qty.	%	qty.	%	qty.	%		
anomalies of the head's circumference	acrania	13	22.0	46	78.0	59	100.0	11.885	0.008
	microcrania	10	66.7	5	33.3	15	100.0		
	normocrania	10	41.7	14	58.3	24	100.0		
	macrocrania	16	30.8	36	69.2	52	100.0		
	total	49	32.7	101	67.3	150	100.0		
telorism	Hypertelorism	11	24.4	34	75.6	45	100.0	6.217	0.045
	hypotelorism	10	30.3	23	69.7	33	100.0		
	normal	12	54.5	10	45.5	22	100.0		
	total	33	33.0	67	67.0	100	100.0		

Abbreviations: CI, confidence intervals; OR, odds ratio; χ^2 , chi-square; P, sig; qty, quantity.

**Figure 3.** Amniotic fluid: Quantitative changes and associated anomalies in fetuses with NTDs.

The season of conception is crucial for the emergence of abnormal head circumference (Table 4). The presence of acrania is 56 points higher when conception was during the autumn-winter seasons, compared to conception during the warmer seasons. For the presence of macrocrania, this seasonal difference is nearly 40 points. It is worth noting, however, that the presence of microcrania is 33 points higher when conception was in the spring and summer, compared to the autumn and winter months. Telorism and urinary excretory pathways are significantly influenced by conception during the autumn-winter period (Table 4) (Figure 4).

The seasons significantly influence the occurrence of anomalies of the palate (Figure 4). Nearly 95% of the proven palate anomalies derived from pregnancies

**Figure 4.** Relationships between the seasons of conception and associated abnormalities in fetuses with NTDs.

that were conceived during the autumn-winter period (Figure 4).

Anomalies of the digestive system are about 13 points more common when conception was during the spring-summer period.

4. Discussion

An age of over 35 should be considered as a possible risk factor for the occurrence of NTDs incompatible with life and requires increased attention during pregnancy (Table 2). The risk for mothers older than 35 to have a fetus with NTDs and abnormalities in the endocrine system is almost three times higher, compared to

younger women. While the probability of abnormalities in the digestive system and a weight gain retardation, in fetuses of women older than 35, is increased approximately two-fold, respectively. The share of presence of the combined anomalies of the hand - single transverse palmar crease, clinodactyly of the thumb and clinodactyly of fingers with NTDs is also almost two times higher in fetuses of older women. In consanguineous unions, the chance of defects of the hand is about twice as high as well. In these cases, the likelihood of anomalies in the shape of the face and hypotelorism increases about three times. Similar are the results for associated limb anomalies in children with myelomeningocele that are from consanguineous marriages [22].

Hypertelorism, macrocrania, anomalies of the cardiovascular, reproductive, digestive and respiratory system are three times more often manifested in case of the factor - *abnormal quantity of amniotic fluid*. Also, an abnormal quantity of amniotic fluid increases the risk of manifestation of cardiovascular pathologies and defects of the reproductive system around four times. The results, of the dependencies between variation in the amount of amniotic fluid and the associated malformations found, most likely have pathophysiological and pathoanatomical genesis which is related to impaired microcirculation and reabsorption of cerebrospinal fluids [23,24]. Some authors have found that variations in the circumference of the head are usually the result ventriculomegaly due to an obstruction of the flow of cerebrospinal fluid.

References

- [1] Dolk H., Loane M., Garne E. The prevalence of congenital anomalies in Europe. *Adv Exp Med Biol*, 2010, 686, 349-364
- [2] Henk J. Folic acid, methylation and neural tube closure in humans. *Birth Defects Res.*, 2009, 85, 295-302
- [3] Lindenbaum J., Savage D.G., Stabler S.P., Allen R.H. Diagnosis of cobalamin deficiency: relative sensitivities of serum cobalamin, methylmalonic acid and total homocysteine concentrations. *Am J Hem.*, 1990, 34, 99-107
- [4] Seyit C.T., Beyan C., Atay V., Yaman H., Alanbay I., Kaptan A. Serum vitamin B12 and homocysteine levels in pregnant women with neural tube defect. *Gynecological Endocrinology*, 2010, 26, 578-581
- [5] Stonek F., Hafner E., Philipp K., Heffler L.A., Bentz E.K., Tempfer C.B. Methylenetetrahydrofolate reductase C677T polymorphism and pregnancy complications. *Obstet Gynecol.*, 2007, 110, 363-368
- [6] Koch M.C., Stegmann K., Ziegler A., Schröter B., Ermer A. Evaluation of the MTHFR C677T allele and the MTHFR gene locus in a German spina bifida population. *Eur J Pediatr.*, 1998, 157, 487-492
- [7] Mornet E., Muller F., Lenois-Furet A., Delezoide A.L., Col J.Y., Simon-Bouy B. Screening of the C677T mutation on the methylenetetrahydrofolate reductase gene in French patients with neural tube defects. *Hum Genet*, 1997, 100, 512-514
- [8] Weitkamp L.R., Tackels D.C., Hunter A.G., Holmes L.B., Schwartz C.E. Heterozygote advantage of the MTHFR gene in patients with neural-tube defect and their relatives. *Lancet*, 1998, 1554-1555
- [9] Martínez-Villarreal L.E., Delgado-Enciso I., Valdéz-Leal R., Ortiz-López R., Rojas-Martínez A., Limón-Benavides C. Folate levels and N(5), N(10) - methylenetetrahydrofolate reductase genotype (MTHFR) in mothers of offspring with neural tube defects: a case-control study. *Arch Med Res.*, 2001, 32, 277-282
- [10] Al-Gazali L.I., Padmanabhan R., Melnyk S., Yi P., Pogribny I.P., Pogribna M. Abnormal folate metabolism and genetic polymorphism of the folate path-

The season of conception has a considerable impact on the occurrence of NTDs. Nearly 80% of the NTD fetuses conceived in the cold part of the year have acrania, while 70% have macrocrania. The risk for palate defects in NTD fetuses conceived during the autumn and winter seasons increased nine-fold. In addition, hypertelorism is three times more common and hypotelorism more than twice as common in these cases. The presence of abnormalities of the digestive system is almost four times lower and abnormalities in the urinary excretory tract are almost three times more common, in cases when conception occurred during the autumn-winter period, as compared to NTD fetuses conceived during the warmer months. The studied dependencies show that the autumn-winter period of conception is more unfavourable as it poses a greater risk for the emergence of a number of anomalies. A possible reason for this vulnerability is less agreeable weather and unhealthy nutrition during this segment of the year.

Several authors have confirmed the teratologic influence of consumption of unsuitable and improperly stored food during the autumn-winter on fetuses with NTD. Additional studies are needed on the influence of these factors in order to find a relationship between them and the defects in the morphogenesis.

From the study results, we conclude that the mother's age, consanguinity, season of conception and variation in the amount of amniotic fluid are etiopathogenetic factors determining the emergence of several anomalies in the surveyed fetuses with NTDs.

- way in a child with Down syndrome and neural tube defect. *Am J Med Genet*, 2001, 103, 128-132
- [11] Doğan H., Sahinoglu S. Fetuses with neural tube defects: ethical approaches and the role of health care professionals in Turkish health care institutions. *Nurs Ethics.*, 2005, 12, 59-78
- [12] Onrat S.T., Seyman H., Konuk M. Incidence of neural tube defects in Afyonkarahisar, Western Turkey. *Genet Mol Res.*, 2009, 8, 154-156
- [13] Gholipour M.J., Mobasher E., Vakili M.A., Keshtkar A.A. Epidemiology of neural tube defects in northern Iran, 1998-2003. *East Mediterr Health J.*, 2007, 13, 560-566
- [14] Gholipour M.J., Najafi L., Keshtkar A.A. Neural Tube Defects in Native Fars Ethnicity in Northern Iran. *Iranian Journal Of Public Health*, 2010, 3, 116-123
- [15] Afshar M., Gholipour M.J., Farhud D. Epidemiologic aspects of neural tube defects in South East Iran *Neurosciences*, 2006, 11, 289-292
- [16] Behrooz A. Prevalence of neural tube defect and its relative factors in south-west of Iran. *Pakistan Journal Of Medical Sciences*, 2007, 23, 654-656
- [17] Al-Ani Z.R., Al-Hiali S.J., Al-Mehimdi S.M. Neural tube defects among neonates delivered in Al-Ramadi Maternity and Children's Hospital, western Iraq. *Saudi Med J.*, 2010, 31, 163-169
- [18] Houcher B., Bourouba R., Djabi F., Houcher Z. The prevalence of neural tube defects in Setif University Maternity Hospital, Algeria-3 years review (2004-2006). *Pteridines*, 2008, 19, 12-18
- [19] De la Vega A., López-Cepero R. Seasonal variations in the incidence of some congenital anomalies in Puerto Rico based on the timing of conception. *P R Health Sci J.*, 2009, 28, 121-125
- [20] Siffel C., Alverson C.J., Correa A. Analysis of seasonal variation of birth defects in Atlanta. *Birth Defects Res A Clin Mol Teratol.*, 2005, 73, 655-662
- [21] Perveen F., Tyyab S. Frequency and pattern of distribution of congenital anomalies in the newborn and associated maternal risk factors. *J Coll Physicians Surg Pak.*, 2007, 17, 340-343
- [22] Sreenivas T., Nataraj A.R. Parental consanguinity and associated factors in congenital talipes equinovarus. *Foot*, 2012, 1, 2-5
- [23] Abd E., Ghani A., El Ansarry K. Neural Tube Defects. *Asjog.*, 2006, 3. Available from: URL: <http://www.asjog.org> Accessed November 12, 2010
- [24] Dias M.S., Partington M. Embryology of myelomeningocele and anencephaly. *Neurosurg Focus*, 2004, 16, E1