DOI 10.1515/pterid-2013-0028 — Pteridines 2013; 24(3-4): 257–263

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Demographic factors and associated anomalies in fetuses with neural tube defects

Abstract: The aim of this study was to identify the types of abnormalities associated with neural tube defects (NTDs) and the magnitude of the risk for their expression under the influence of the following factors: maternal age >35 years, consanguinity and season of conception. One hundred and fifty fetuses were autopsied during the period 2006–2009 at the Center for Maternity and Neonatology, Tunisia. A mother's age of >35 years increases the probability of intrauterine growth retardation by two-fold [odds ratio (OR) 2.043, confidence interval (CI) 0.880–4.741]. Consanguinity increases the relative risk for abnormalities in the facial shape (OR 3.031, CI 1.279–7.183) and adrenal hypoplasia (OR=2.787, CI 1.140–6.814). The autumn-winter period of conception increases the relative risk for the expression of cleft palate by more than nine times (OR 9.035, CI 1.161–70.258) and by about three times for abnormalities of the excretory tract (OR 2.935, CI 0.954–9.141). The prenatal ultrasound diagnosis of NTDs with risk factors such as maternal age >35 years, consanguinity and conception during the autumn-winter period should be targeted to the search for lower-than-normal fetal weight and abnormalities of the excretory tract and the adrenal glands. The head should be examined with special care for deviations in the cranial perimeter and cleft palate.

Keywords: consanguinity; mother's age; neural tube defects (NTDs); seasonal variations; ultrasound.

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Introduction

Numerous advances have been made in the search for neural tube defects (NTDs). Some of the more significant are the widely introduced triple test (α-fetoprotein, estradiol and β-horion gonadotropin) and ultrasound examinations during the 12th, 16th and 22nd weeks of gestation. Nonetheless, a large percentage of NTDs still remain undiagnosed. In Europe, the frequency of NTDs remains at a rate of 2.3 per 1000 births [1]. The reasons for some NTDs not being diagnosed on time may lie in their multifactorial genesis and in the incompletely understood role of many risk factors, such as maternal age >35 years.

The relationship between incidence of NTDs and maternal age is a U-shaped curve according to Vieira and Castillo [2]. The largest values of this curve are <20 and >35 years of age, and the smallest values are between 20 and 29 years of age. Knowledge of this epidemiological dependence, however, is not sufficient for the effective prevention of NTDs. With the introduction of advanced *in vitro* fertilization techniques in older women, it is imperative to study in depth the influence of maternal age on the incidence of NTDs.

Another similar factor is consanguinity. The frequency and degree of consanguinity in a certain population depend on the religion, culture, educational level and other factors [3]. The main impact of consanguinity is the increased probability of homozygosity and autosomal recessive genetic diseases [4]. According to Al-Gazali et al. [5], an increased risk for NTDs was observed in mothers homozygous for the mutant gene *C677*. A recent study shows that many mothers who have given birth to a child with an NTD may be carriers of a mutation on the level of the gene *methylenetetrahydrofolate* reductase [6]. Another study shows that consanguinity has an effect on postnatal mortality and on the appearance of congenital malformations [7]. Consanguineous union is still prevalent in some societies, and, therefore, the degree and ways in which it influences the genesis of NTDs continue to be an interesting area of research.

Research on the seasons of conception and their influence on NTDs shows that the months May and June represent the peak for the incidence of NTDs in the Northern Hemisphere. These peaks can be explained by the intensity of solar radiation and the oxidative stress in the body that affect the closure of the neural tube as well as the process of lateralization [8]. Probable risk factors for NTDs are overheating during the warmer months and febrile conditions, according to Botto et al. [9].

The susceptibility of fetuses to NTDs during the colder months of the year could be attributed to reasons such as inadequate and less nutritious diet. One author has confirmed the teratologic influence of consumption of unsuitable and improperly stored food during the autumn-winter period on fetuses with NTDs [10]. Numerous studies have found that deficiency in folic acid and vitamin B_{12}, intake of anticonvulsant and antipyretic drugs, maternal diabetes, hypertension and other factors could lead to defects in the closure of the neural tube [11–13].

Information about the mother's age, the type of marriage and the date of conception is collected during the first obstetric history. It is a simple and routine practice; therefore if the impact of these factors is known, not only in an epidemiological aspect, but also in the way they affect the morphogenesis, the diagnosis of NTDs during early ultrasound examination will be more effective. In this context, the aim of this study was to identify the types of abnormalities associated with NTDs and to define the magnitude of the risk for their expression under the influence of the following risk factors: maternal age >35 years, consanguinity and season of conception. Proven associations between the different indicators may be used to develop specific markers for preventive and educational programs for professionals in the field of fetal medicine.

Materials and methods

One hundred and fifty fetuses were examined during the period 2006–2009 at the Center for Maternity and Neonatology, Tunis, Tunisia. The fetuses were the result of pregnancy terminations for medical reasons (95.4%), intrauterine fetal death (3.3%) and spontaneous abortions (1.3%).

The data for the mother and the fetus were collected in a file of autopsy findings, from the fetus and the placenta. Written informed consent was obtained from both parents of each fetus. The additionally collected information was about the age of the mother, the number of previous pregnancies and births, types of medications taken, the pathology of pregnancy, blood group data, consanguinity, season of conception, place, method and term of pregnancy. The birth weight, gender, gestation week and origin (single or multiple pregnancies) were recorded for each fetus. The file also contains data from the karyotype study, fetal biometry, autopsy and diagnosis, as well as photographic documentation. The procedures followed were in accord with the Declaration of Helsinki and its revisions.

The structural distribution of the examined cases according to the type of NTDs is as follows: spina bifida (myelomeningocele), 39.3% (59); encephalocele and exencephaly, 16.0% (24); craniorachischisis, rachischisis and iniencephaly, 22.0% (33); and anencephaly, 22.7% (34). About 80% of the NTDs were diagnosed before the 28th week of gestation. The percentage of NTDs diagnosed between 29 and 34 weeks of gestation and after the 34th week of gestation was 18.9% and 3.4%, respectively. The karyotypic study was conducted for 14.67% of fetuses with NTDs.

The data had been statistically processed by a univariate nonparametric test (Pearson's χ^2 criterion of agreement) and by the Fisher's exact test using the statistical program SPSS v. 17 (IBM Corporation, Armonk, NY, USA).

Results

The average age of the mothers in this study was 30.93±0.673. The youngest mother was 21 years old, and the oldest was 43 years old. Anomalies affected both fetal genders, but were more prevalent in females; sex ratio was 1.15%. The average weight for the fetuses was 620.76 g (standard error ±58.52), the lightest being 16 g and the heaviest was 4250 g.

Karyotypic study

A total of 8.67% of the cases have a normal karyotype and 6% have chromosomal aberrations. There were two numerical chromosomal aberrations: trisomy 21 and trisomy 13. Structural chromosomal aberrations were discovered in one fetus with exencephaly, one with craniorachischisis and three with spina bifida. Structural aberrations were not discovered in cases of anencephaly.

Age of mother

Malformations incompatible with life such as anencephaly, craniorachischisis, rachischisis, iniencephaly and encephalocele were twice more common (29.7%) in mothers aged >35 years than spina bifida (16.9%) [odds ratio (OR) 2.152, confidence interval (CI) 1.001–4.625] (Table 1). With increasing maternal age in women aged >35 years, cases of NTDs associated with hypoplasia of the adrenal gland (42.3%) were two times more common than isolated cases (20.8%) (OR 2.787, CI 1.140–6.814) (Figure 1). Also, when the factor "maternal age >35 years" is present, there were almost twice more cases of NTDs associated with abnormalities of the digestive system (gastrointestinal, mesenteric and parenchymal) and of the hand and fingers (syndactyly, clinodactyly of the thumb and the fingers, polydactyly, reduction anomalies) (32.2% and 34.7%, respectively) (OR 1.926, CI 0.909–4.083), as compared to isolated cases of NTDs (19.8% and 19.8%, respectively) (OR 2152, CI 1.001–4.625). With a maternal age >35 years, there were almost twice more cases of NTDs associated with a less-than-normal fetal weight (29.5%)

Table 1 Associated abnormalities in fetuses with NTDs, as determined by the age of the mother.

Indicators	Groups	Under 35 years of age		>35 years of age		Total		χ^2	p-Value	OR (CI)
		n	%	n	%	n	%			
NTDs	Anomalies incompatible with life[a]	64	70.3	27	29.7	91	100.0	3.938	0.047	2.152 (1.001–4.625)
	Spina bifida	49	83.1	10	16.9	59	100.0			
	Total	113	75.3	37	24.7	150	100.0			

[a]Anencephaly, craniorachischisis, rachischisis, iniencephaly and encephalocele.

compared to those with a normal fetal weight (17%) (OR 2.043, CI 0.880–4.741). A lower-than-normal fetal weight was found in 64.2% of the examined fetuses according to the reference values of Guilhard-Costa and Larroche [14]. A less-than-normal weight is an indicator of intrauterine growth retardation.

Consanguinity

Consanguinity was present in 26.3% of the cases in this study. The values for the first, second and third degree of consanguinity were 12.8%, 9% and 2.5%, respectively. In 2% of the cases, the degree of consanguinity was not specified.

In the analysis of the factor "facial dysmorphia", the "frog face" in fetuses with NTDs was twice more often manifested in cases with consanguinity (41%) than in non-consanguineous cases (18.7%) (OR 3.031, CI 1.279–7.183) (Figure 2).

About 40% of the anomalies of the hand were found in fetuses with NTDs that originated from consanguineous unions. Anomalies of the hand were twice more common (37.8%) in cases with consanguinity as compared to fetuses without consanguinity (20.8%) (OR 2.321, CI 0.979–5.500) (Figure 2).

Hypotelorism is a discrete deviation from the indicator expressing the ratio "length of the eyelid/distance between the eyes" and is twice more frequent (41.7%) when compared to the normal distance between the eyes (21.9%) in fetuses with NTDs who were from consanguineous unions (OR 2.551, CI 0.934–6.969) (Figure 3).

In addition, the consanguinity factor demonstrates a significant influence on the type of NTD. In cases with the consanguinity factor, anomalies incompatible with life – craniorachischisis, rachischisis, anencephaly, exencephaly and encephalocele – were twice more common (32.4%) than spina bifida (16.3%) (OR 2.464, CI 0.953–6.372) (Figure 3).

Seasonal risk factors

Almost 95% of the defects of the palate (cleft palate) were diagnosed in fetuses with NTDs who were conceived during the autumn-winter period (OR 9.035, CI 1.161–70.258) (Figure 4). Conception during the autumn-winter period presents a three times larger relative risk for

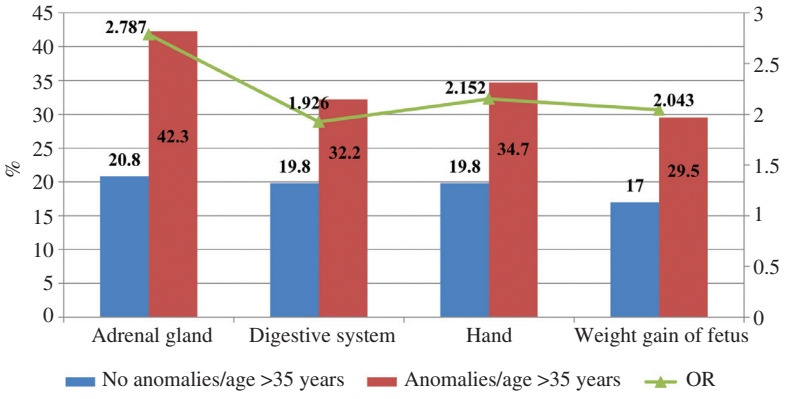

Figure 1 Associated abnormalities in fetuses with NTDs, as determined by the age of the mother.

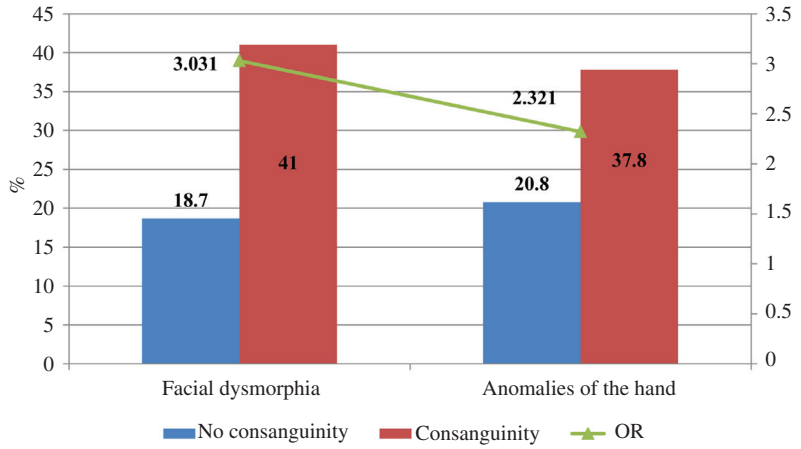

Figure 2 Consanguinity and associated anomalies in fetuses with NTDs.

NTDs being associated with abnormalities of the excretory system (84%) when compared to the spring-summer period (16%) (OR 2.935, CI 0.954–9.141). The spring-summer period increases by three-fold the probability of association of digestive system abnormalities with NTDs (20.3%) in comparison to the autumn-winter period (7.7%) (OR 2.684, CI 1.256–5.735). Although hyper- and hypotelorism are discrete indicators, they have two- to three-fold greater potential to become markers for the presence of NTDs, especially for the autumn-winter period fetuses (75.6% and 69.7%, respectively) (Table 2).

Acrania is over 3½ times more frequent when the conception was during the autumn-winter period (78%), and over two times more often the fetuses have NTDs associated with macrocrania (69.2%) as compared to the spring-summer period (22% and 30.8%, respectively) (Table 2). The opposite, microcrania, is doubled in frequency (66.7%), when conception was during the spring-summer period in comparison to the autumn-winter period (33.3%).

Discussion

Age of mother

According to Golalipour et al. [15, 16], NTDs are more common in fetuses of multi-gravida women, especially in those who are >35 years of age. Our study shows that a maternal age >35 years should be considered as a possible risk factor for the occurrence of NTDs incompatible with life – craniorachischisis, anencephaly, exencephaly, encephalocele and rachischisis – and requires increased attention during prenatal examinations (Table 1).

The probability of intrauterine growth retardation in the fetuses of women aged >35 years is increased by approximately two-fold (Figure 1). The growth of the fetus depends on its overall metabolic development. Intrauterine growth retardation is a multihormone syndrome. The growth hormone and insulin-like growth factors have direct effects on fetal growth. The release of insulin to

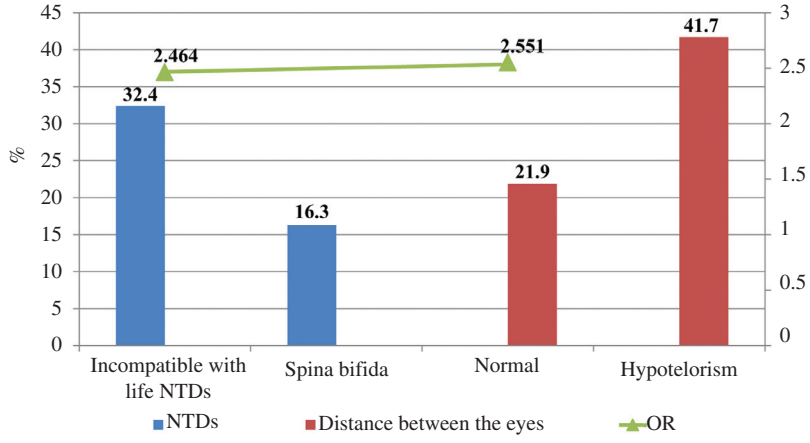

Figure 3 Anomalies associated with NTDs in fetuses with consanguinity.

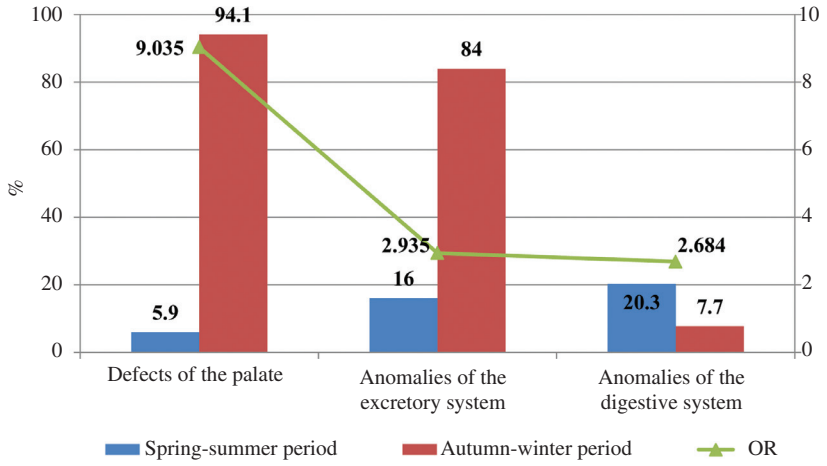

Figure 4 Relationships between the seasons of conception and associated abnormalities in fetuses with Ntds.

the fetus depends on the transfer of glucose through the placenta. The growth of the fetus during pregnancy is coordinated by growth factors that are limited by maternal factors and placental function (Figure 1) [17].

The risk for mothers aged >35 years to have a fetus with NTDs and adrenal hypoplasia is almost three times higher in comparison to younger mothers (Figure 1). The embryonic origin of the adrenal gland has two embryonic primordia. The adrenal cortex originates from the mesoblast, and the adrenal medulla originates from the neural crests. The adrenal glands are larger than a kidney during the first trimester, and by the end of the third trimester their size decreases to one third of their original size [18]. The study of the adrenal glands in the antenatal period is difficult. The anomalies in the volume of the glands are related mainly to the cortex. Our research reaffirms the importance of the study of the adrenal glands during ultrasonographic examinations, especially when the age of the mother is >35 years.

The probability of abnormalities in the digestive system in the fetuses of women aged >35 years is increased by approximately two-fold. According to Forrester and Merz [19], NTDs are often complicated with abnormalities of the digestive system. NTDs can be associated with gastroschisis, omphalocele, stenosis, atresia, intestinal malabsorption, obstructive genitourinary defects, reduction defects and deformities of the limbs [19].

The probability for anomalies of the hand – syndactyly, clinodactyly of the thumb and fingers, polydactyly and reduction anomalies – being associated with NTDs is almost two times higher in the fetuses of women aged >35 years (Figure 1).

A maternal age >35 years increases the relative risk for the association of NTDs with retardation in the weight gain of the fetus, hypoplasia of the adrenal gland, and abnormalities of the digestive system and hand.

Consanguinity

In cases of consanguinity, there is a statistically significant increase in the incidence of anomalies incompatible

Table 2 Relationships between the seasons of conception and associated abnormalities in fetuses with NTDs.

Indicators	Groups	Spring-Summer period		Autumn-Winter period		Total		χ^2	p-Value
		n	%	n	%	n	%		
Circumference of the head	Acrania	13	22.0	46	78.0	59	100.0	11.885	0.008
	Macrocrania	16	30.8	36	69.2	52	100.0		
	Microcrania	10	66.7	5	33.3	15	100.0		
	Normal	10	41.7	14	58.3	24	100.0		
	Total	49	32.7	101	67.3	150	100.0		
Distance between the eyes	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		

with life: craniorachischisis, anencephaly, exencephaly, encephalocele and rachischisis (Figure 3). In consanguineous unions, the probability for the presence of defects of the hand is about twice higher as well. In these cases, the likelihood of anomalies in the shape of the face and hypotelorism increases by about three times (Figures 2 and 3). The results are similar for associated limb anomalies in children with myelomeningocele who are from consanguineous unions [20].

Abnormalities in the shape of the face ("frog face"), the hand (syndactyly, clinodactyly of the thumb and fingers, polydactyly, reduction anomalies), as well as deviation in the distance between the eyes have an increased relative risk for occurrence as abnormalities associated with NTDs.

Season of conception

The season of conception has a considerable impact on the occurrence of NTDs. According to De la Vega and López-Cepero [21], the appearance of spinal dysraphisms such as anencephaly and spina bifida, cleft palate, congenital anomalies of the aorto-pulmonal truncus of the heart and congenital luxation of the hip is affected by seasonal variations. Roughly eighty percent of the fetuses with acrania and about 70% of those with macrocrania were born during the autumn-winter period (Table 2). There were over 3½ times increase in the relative risk for acrania and more than two-fold increase in the relative risk of NTDs in association with macrocania when the period of conception was autumn-winter as compared to spring-summer (Table 2).

There was a more than nine-fold increase in the probability for the association of cleft palate with NTDs when the fetus was conceived during the autumn-winter period (Figure 4). The existence of this association between cleft palate and NTDs could be due to effects of identical etiologic factors. The discovery of such factors can be targeted to genetic testing [22].

The probability of abnormalities of the excretory system in association with NTDs when the fetus was conceived during the autumn-winter period increases almost three times (Figure 4). Deviations of the cranial perimeter (acrania, macrocrania), cleft palate and abnormalities of the urinary tract are the most commonly associated anomalies in fetuses with NTDs conceived during the autumn-winter period. Our research shows that the autumn-winter period of conception is more unfavorable as it poses a greater risk for the emergence of associated anomalies. Possible reasons for this vulnerability of the fetuses are the less agreeable weather and the less-nutritious diet during this time of the year.

A maternal age >35 years, consanguinity and the season of conception are not only important epidemiological markers, but also risk factors affecting the organogenesis and morphogenesis during the intrauterine development. When either of the factors – maternal age >35 years, consanguinity or conception during the autumn-winter period – is present, the attention of clinicians during the process of prenatal ultrasound diagnosis should be focused on the following parameters: abnormally low weight of the fetus; abnormalities of the hand; and, particularly, deviation in the cranial perimeter, cleft palate and the distance between the eyes, for the detection of NTDs. The ultrasound study of the internal organs should be targeted to anomalies of the urinary tract and adrenal hypoplasia.

Conflict of interest statement: The authors declare no conflict of interest.

Received September 21, 2013; accepted October 28, 2013

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