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Original article

Risk factors of neural tube defects: A reality of Batna region in Algeria

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ABSTRACT

Background: Neural tube defects (NTDs) are severe birth defects, with genetic and/or environmental risk factors.
Aim: The objective of this study was to analyze data on NTDs cases at the Batna Maternity Hospital and to investigate some environmental and two genetic risk factors suspected in the etiology of NTDs.
Subjects and methods: This study was conducted on 82 healthy participants and 48 mothers with an NTD child. Peripheral blood samples were collected, in EDTA tubes and frozen at −20 °C until DNA extraction by conventional method. Genetic analysis of methylene tetrahydrofolate reductase C677T polymorphism was determined by real time PCR, while cystathionine-beta-synthase 844 insertion was investigated by traditional PCR. Chi-square analyses were used to evaluate differences in the distribution of data. The odds-ratio was also calculated. A P-value less than 0.05 were significant.
Results: The incidence of NTD in Batna region was 1.58 per 1000 births. The rate of NTD was significantly higher in females than males, highest affected NTD newborn's was observed in mothers aged between 25 and 29 years and the consanguinity among all NTD cases was 30%. Data showed no significant association of NTDs with personal education, obesity, diabetes, but regarding folic acid consumption, about 86% of NTD's mothers in our region didn't take pre-conceptional supplementation with this vitamin .Genetic factors results didn't show a significant association of NTDs with specific mutations of the variant C677T MTHFR, and no gene-gene interaction of CBS insertion and C677T polymorphism was found, despite a significant difference in heterozygote frequency of CBS 844ins68 genotype between NTD's mothers and controls, OR: 2.85(1.18–6.88).
Conclusion: NTD represents a real public health problem in Batna, Algeria. Various genetic and/or nutritional factors are implicated, although the mechanism is not clear. We suggest that further research should continue planning for preventive measures and effective treatment to reduce the incidence of NTDs in Algeria.

1. Introduction

Neural tube defect (NTD) is a general term for a congenital malformation of the central nervous system occurring secondary to lack of closure of the neural tube [1] with a worldwide incidence ranging from 1.0 to 10.0 per 1000 births [2]. Classically, there are two main groups: (a) anencephaly and encephalocele, and (b) spina bifida [3] .Anencephaly is fatal in all cases, whereas children with spina bifida frequently suffer from severe disability and require continued medical treatment [4].

NTD's varies by race, geographical location, socioeconomic class, nutritional status and multiple factors of predisposition with a very high prevalence among the Irish, and low in the black ethnic groups [5] .NTDs are multifactorial disorders, arising from a complex combination of genetic and environmental interactions involving nutritional deficiencies, genetic predisposition, in addition to some trace elements and vitamins that could partially explain these anomalies [6] .Many studies have investigated the risk of NTD' pregnancy for contributions of socioeconomic status (SES), parental education, maternal and paternal ages and occupations, smoking, alcoholism, maternal reproductive history, including maternal country of birth and country of conception, hyperthermia during early pregnancy, hyperglycemia, diabetes or obesity, and maternal use of caffeine and medications during early pregnancy [2]. Even so, these risk factors are thought to account for only a small proportion of NTDs, suggesting the presence of other risk factors [7].

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Due to the known role of folate in NTD prevention [8], considerable research has been focused on genes involved in this vitamin metabolic pathway; however, few of the corresponding genes have been associated with an increased risk of NTDs [9]. The MTHFR gene is the most extensively studied of all the folate metabolism candidate with 32 published papers, including a wide spectrum of populations [10]. The MTHFR gene is the main focus of so many studies, because MTHFR enzyme converts 5, 10-methylene-THF to 5-methyl-THF (5-MTHF), the intracellular form of folate utilized by both the folate and methionine cycles. The association between NTDs and the MTHFR C677T polymorphism showed ambiguous results; the risk was increased for Dutch and Irish populations [11], in contrast to other populations where no association have been established [12]. The most studied common mutations in different ethnic populations of the CBS gene in association with NTD is the 844ins68 insertion [11]. The majority of studies didn't find a significant association between NTDs and the CBS gene [13,14]. It is well established that maternal folic acid supplementation may reduce the rate of occurrence of anencephaly or Spina bifida in some populations, but after more than two decades of research, mechanism (s) by which folic acid may intervene to prevent NTDs is not fully elucidated [12].

There is no documented study regarding the risk factors of NTDs in this region of Algeria. Therefore, this study was conducted to investigate the possible association of the MTHFR C677T polymorphism and the CBS 844ins68 in mothers of Batna region, as well as some environmental factors suspected in the etiology of NTDs.

2. Subjects and methods

2.1. Study population and data collection

A retrospective study of children born with a neural tube defect identified from the Maternity Hospital of Batna, Algeria was conducted within a period of 2 years (2012, 2013), during which more information was collected for each case, including the age of the mother, the date of birth, sex; birth single or twins; the residence and consanguinity. Incidence (birth) rates per 1000 births were examined for each year of the study period. Furthermore, other laboratory tests were performed on a group of 82 apparently healthy participants (control group) and 48 mothers who had conceived a previous NTD child aged between 24 and 48 years, from Batna Hospital, Algeria. An informed consent for genetic analysis was obtained from the parents and/or from the individual controls. Additional data were obtained from NTD's mothers concerning medical pre-pregnancy history, personal education, body mass index (BMI), and folic acid consumption. BMIs 18–25, 25–30, and >30, were considered as normal, overweight and obesity, respectively.

2.2. DNA samples and genotyping analysis

Peripheral blood samples were collected, in EDTA tubes and frozen at −20 °C until their transfer to Ankara/Turkey for DNA extraction by conventional phenol-chloroform method. After hemolysis of the blood in hypotonic solution, DNA was isolated by using a simple proteinase K treatment at 65 °C in the presence of SDS, followed by ammonium acetate precipitation of debris and ethanol precipitation of the DNA. DNA amount and purity were quantified for each sample by spectrophotometry (Nanodrop ND-100).

Genetic analysis of the MTHFR C677T polymorphism was determined according to an analysis of melting curves performed on the Light Cycler (Roche 1.5 light Cycler, Mannheim, Germany) in capillary tubes with a detection Kit of this polymorphism (Roche Molecular Biochemicals, Mannheim, Germany). Genotyping of the 644ins68 in the CBS gene was analyzed by PCR method in a thermal cycler (Biometra), for the study populations using 5 μl of 10× PCR Buffer, 25 mM $MgCl_2$, 10 mM of dNTP's mix, 10 pmol of each primer forward: 5′-GCA GTT GTT AAC GGC GGT AT-3′ (Fermentas) and reverse 5′-GTT GTC TGC TCC GTC TGG TT-3′, (Fermentas) and 5 U of Taq polymerase (Fermentas) in a total reaction volume of 50 μl. PCR conditions were as follows: denaturation at 94 °C for 1 min, annealing at 63 °C for 1 min, and extension at 72 °C for 1 min by 34 cycles, followed by a final extension to 72 °C (12 min). A 10 μl aliquot of PCR product was electrophoresed in a 2% agarose gel stained with ethidium bromide and visualized under UV light.

2.3. Statistical analysis

Chi-square analyses were used to evaluate significant differences in the distribution of data. A *P*-value less than 0.05 were considered as significant. In addition, genotype and allele frequencies of cases and control subjects were determined and the odds ratios (OR) as well as their 95% confidence intervals (CI) were calculated to evaluate the possible association between different genotypes and NTDs.

3. Results and discussion

Neural tube defects (NTDs) are among the most common, costly, and deadly of all human congenital anomalies whose etiologies remain largely unknown. The annual incidence of all NTD_S types during the period of study (2012–2013) in the region of Batna was calculated from Maternity-hospital birth files and the results from our investigation demonstrated 0.58 and 2.58 NTD cases per 1000 live births and foetal deaths with an average rate of 1.58 cases per 1000 births.

Despite widely reported advances, NTDs continue to present a major public health burden afflicting 1 in 2000 births in the US, and significantly more births in developing areas such as China and Latin America [15]. We previously found that the incidence of NTDs in Setif, Algeria was 7.5 per 1000 births [16]. This high rate can be attributed to the low dietary folate intake by our female population [17].

The relatively low prevalence of NTDs in this region of country, Batna (Algeria) may be due to the awareness by our population of NTD's impact or to giving birth in private clinics or early pregnancy's loss of child affected by these anomalies.

Among the cases of NTDs (66.66%) had open spina bifida, 19.5% had anencephaly, and (5.5%) was affected by encephalocele, and/or associated – hydrocephalus; however 8.33% had spina bifida associated with anencephaly. It has been reported that open or closed spina bifida is the more frequent NTD malformation with an incidence of 2.6/10,000 [18].

The sex distribution of NTD cases was significantly ($p < .05$) higher in females (70%) compared to males (28%). This is in agreement with other studies in southwest Iran [19], and China [20]. On the other hand, there were non-significant male dominance cases of NTDs in north of Iran [21], however, in the province of Shanxi in China, there was no significant difference in the distribution according to gender [22].

Our study also showed that 30% of children with neural tube defects are from consanguineous marriage and taking into account the age of the mothers who had an affected child, the highest rate (30.6%) were observed in the group aged between 25 and 29 years without a significant difference ($p = 0.41$). This is in disagreement with some researches which showed a linear relationship between the rate of NTDs and maternal age [23], but also with others where NTDs were unrelated to age [24]. The observed frequencies of the

various genotypes and alleles of 677 C → T polymorphisms in the MTHFR gene are shown in Table 1. In the control group, 35 (43%) were heterozygous (CT) and 14 (17%) were homozygous (TT). These frequencies were not significantly different (OR = 1.01 (0.42–2.41) from those observed in the NTDs mothers group where 6 (12.5%) were homozygous (TT). In addition, there was no statistically significant difference between allele frequencies for the MTHFR 677C → T polymorphism in NTDs mothers and control mothers (OR = 0.7 (0.4–1.19).

Some epidemiological studies have investigated the correlation of C677T polymorphism in MTHFR gene with several pathological processes, dependent on the metabolism of folate; like NTDs [10]. The homozygous C677T MTHFR gene polymorphism shows an incidence ranging from 10 to 20% in some populations [25]. Our data disagree with another research Carter et al. [26] who reported a high rate of homozygosity (TT) in mothers with an NTD child compared with controls. The difference in consumption of folate and riboflavin in various countries, can explain the association with NTDs in some populations and not others [27].

The distribution of different genotypes of CBS 844ins68 polymorphism shows that there is a significant difference between the genotypic frequencies of heterozygous between NTDs mothers and controls (31 and 13%) for this study population (OR = 2. 85 (1.18–6.88)) Table 2.

These results agree with others [9,12]. In contrast, Grandone et al. [28], showed an increased risk of NTDs significantly associated with paternal genotype mutation 844ins68 in the gene of the CBS, but according to other studies, no significant association was found between NTD and the CBS gene [13,14].

Single-gene analyses indicate that maternal genes associated with metabolic conditions (obesity, diabetes) may influence the risk of neural tube defects (NTDs). However, to our knowledge, there have been no assessments of maternal-fetal metabolic gene-gene interactions and NTDs [29].

Our results described in Table 3, show no evidence for a significant association between the MTHFR polymorphism and CBS insertion in NTDs mothers compared to controls (OR = 0.59 (0.06–5.83)). In contrast to the study of De Franchis et al. [30] suggesting that the CBS gene variant may occur more frequently in combination with the MTHFR variant in mothers with an NTD child, indicating the presence of gene-gene interactions in the etiology of NTDs in some population [31].

As illustrated in Table 4, maternal age, education, obesity, and consanguineous marriage were not significantly associated with NTDs in our population. This study showed that there was no significant difference between mothers' age and NTDs, as reported in the north of Iran [21], whereas in other studies, significant association between mothers' ages and NTDs was found in Russia [32] and in Turkey [33]. In a meta-analysis study of maternal age as a risk factor for NTDs, the authors found an increased risk associated with mothers of 40 years and younger than 19 years [34].

According to our results, the rate of consanguinity among NTD group is 30% but there is no significant relation between consanguineous marriage of parents and NTDs. This agrees with the research of Golalipour et al. [21], but others have reported higher incidence (63.6%) of NTDs with consanguinity in Iraq [35]. We previously found that the rate of consanguinity among all NTD cases was 13% in Setif region of Algeria [16]. The possible role that consanguinity could play as a risk factor for NTDs in Algeria requires further investigations.

The key role that environment play in NTD etiology is highlighted by the important impact of maternal nutrition, specifically folate intake [15]. Similar to our result, several studies reported significant relation between low folic acid consumption and NTDs in western Iraq [35], and Austria [36]. However, others demonstrated a non significant association between folic acid consumption and NTD cases, and this can be due to mandatory flour fortification with folic acid in this area [21].

Among the most notable environmental risk factors for NTDs are maternal pre-gestational insulin-dependent diabetes [37] and maternal pre-pregnancy obesity [38]. Our results showed that obesity is not a risk factor for NTDs (OR = 0.40; 0.42).

Several published studies showed that being overweight in women was significantly associated with an increase of NTD's rate [21]. It is believed that glucose homeostasis plays an important role in NTD; at the time of neural tube closure, mothers with poorly regulated glucose levels are likely to have an altered intrauterine environment leading to abnormal organogenesis [29]. Maternal variants in the obesity-related genes *FTO*, *LEP*, and *TCF7L2* are also associated with NTDs, consistent with maternal obesity as a risk factor [39].

As reported in our study, mother's education level was not related to NTDs, in contrast with other studies in Italy [40] and Turkey [33], reporting significant associations between education level and NTDs. This difference may be due to our small sample size.

Table 1
Genotype and allele frequency of the MTHFR (677C → T) polymorphism.

Genotype	Control mothers n (%)	NTD's mothers n (%)	Odds ratio (95% CI)
CC	33 (0.40)	25 (0.52)	1
CT	35 (0.43)	17 (0.35)	0.64 (0.3–1.4)
TT	14 (0.17)	6 (0.125)	1.01 (0.42–2.41)
C	101 (0.62)	67 (0.70)	1
T	63 (0.38)	29 (0.30)	0.7 (0.4–1.19)

* Odds ratio (95% CI) vs. controls.

Table 2
Genotype distribution of the 844ins68 at cystathionine β-synthase (CBS).

Variable	Control mothers n (%)	NTD mothers n (%)	Odds ratio (95% CI)
Negative insertion	69 (0.81)	33 (0.68)	1
Heterozygous	11 (0.13)	15 (0.31)	2.85 (1.18–6.88)
Homozygous	5 (0.06)	0 (0.01)	–

Table 3
Frequency of control and NTD's mothers for 844ins68 at CBS gene, and homozygous genotype for MTHFR (TT).

Group	844ins68/TT	other genotype's combinations	Odds ratio (95%CI)
Controls	3	85	1
NTD's mothers	2	48	0.59 (0.06–5.83)

4. Conclusion

Most NTDs are sporadic, and their occurrence translates to a great cost in terms of physical, emotional, and financial burden of the affected children and their families. Our study confirms that NTD still represents a real public health problem in Algeria. Moreover, various genetic and/or environmental factors as well as their complex interactions, nutritional factors like folate deficiency and seasonal changes in diet, are implicated in the pathogenesis of NTD in Algeria, although the mechanism is not clear. Etiologic study of NTDs requires a large base population.

We suggest increased attention to this problem for the development of alternative therapies to prevent these defects. This can be achieved by food fortification in our country to increase a woman's

Table 4
Association of maternal risk factors and NTD occurrence in Batna, Algeria.

RISK FACTORS	Control n(%)	NTD's mothers n(%)	OR	CI (95%)
Maternal Age				
20–24	17 (20)	10 (20 0.8)	1	
25–35	52 (61.17)	27 (56.25)	0.88	0.355–2.19
>35	16 (18.82)	11 (23)	1.17	0.39–3.49
Consanguineous marriage				
Yes	25 (30)	15 (31.2)	1	
No	60 (70)	33 (68.7)	0.92	0.42–1.97
Body mass index				
Normal	35 (41.17)	30(62.5)	1	
Overweight	20 (23.52)	7(14.58)	0.40	0.15–1.098
Obese	30 (0.35)	11(23)	0.42	0.18–0.99
Folic acid consumption				
Yes	13(15.29)	2 (4.16)	1	
No	72 (84.7)	46 (95.83)	4.15	0.89–19.25
Education				
Illiterate	6(7)	10(20.83)		
Under diplôma	57 (67)	33 (68.75)	0.35	0.11–1.04
Diplôma	22 (26)	5 (30.42)	0.14	0.03–0.55

* OR: odds ratio; CI: confidence interval. Data are presented as No. (%).

daily average consumption of folate. In addition, a national birth defects registry is essential to the determination of the burden of these defects in Algeria.

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