Risk Factors of Neural Tube Defects in Northern Iran

Mohammad Jafar Golalipour¹; Mostafa Qorbani²,³; Arezo Mirfazeli⁴; Elham Mobasherī⁵

¹Gorgan Congenital Malformations Research Center, Golestan University of Medical Sciences, Gorgan, IR Iran
²Department of Public Health, Alborz University of Medical Sciences, Karaj, IR Iran
³Non-Communicable Diseases Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, IR Iran
⁴Department of Pediatrics, Gorgan Congenital Malformations Research Center, Golestan University of Medical Sciences, Gorgan, IR Iran
⁵Department of Obstetrics and Gynecology, Gorgan Congenital Malformations Research Center, Golestan University of Medical Sciences, Gorgan, IR Iran

*Corresponding Author: Mohammad Jafar Golalipour, Gorgan Congenital Malformations Research Center, Golestan University of Medical Sciences, P. O. Box: 49175-1141, Gorgan, IR Iran. Tel/Fax: +98-1714425165, E-mail: mjgolalipour@yahoo.com

Received: August 27, 2012; Revised: March 24, 2014; Accepted: April 5, 2014

Background: Neural tube defects (NTDs) including spina bifida and anencephaly are the second most common birth defects with 2.8 per 1000 births in northern Iran.

Objectives: This study was conducted to determine the risk factors of neural tube defects in Gorgan, north of Iran.

Patients and Methods: This hospital-based, case-control study was carried out on all NTD-affected pregnancies (n = 59) during February 2007 - August 2010, and 160 healthy pregnancies were selected via convenient sampling method in three hospitals in Gorgan, north of Iran. Risk factors including maternal body mass index (BMI), season of birth, gender of the newborn, mother’s age, ethnicity, consanguineous marriage, folic acid consumption, nutrition, habit, and education, were assessed through interviews with mothers. Uni- and multivariate logistic regression analyses were used to estimate the risks by odds ratios (ORs) and 95% confidence intervals.

Results: The multivariate analysis showed that maternal BMI (normal/underweight OR: 0.23, overweight/underweight OR: 0.15, obese/underweight OR: 0.013) and maternal ethnicity (Fars/Sistani OR: 3.49) and maternal nutrition (good/poor OR: 0.46) were significantly correlated with NTDs in the newborns.

Conclusions: This study showed that maternal ethnicity, insufficient nutrition, and BMI, were the main risk factors of NTDs in northern Iran.

Keywords: Neural Tube Defects; Ethnicity; Body Mass Index; Nutritional Sciences

1. Background

Neural tube defects (NTDs) are a group of congenital malformations including spina bifida, anencephaly, and encephalocele, which arise during the process of neurulation between the third and fourth weeks of human pregnancy (1, 2). NTDs contribute to miscarriage, infant mortality, and serious disability (3). NTDs are caused by partial or complete failure of fusion in the cranial and spinal regions of the neural tube during early embryogenesis (4). NTDs are the second most common birth defects with incidence rate of 0.97 per 1000 child births in European contraries (4). Multifactorial disturbances in embryonic neurulation have been identified as causes of NTDs (5). Incidence of different NTDs varies according to geographic conditions, race/ethnicity (5, 6), sex of the newborn (6, 7), as well as high caffeine intake, low calorie diet, alcohol consumption (4), lack of folate supplementation at any time of pregnancy (4, 6, 7), oral contraceptive usage, and passive smoking (8). In addition, other factors associated with one or both NTDs include maternal birthplace, parity, timing of prenatal care initiation, socio-economic status (9), as well as maternal age (7, 9), education, nutritional status, drug usage, presence of maternal chronic diseases, and acute infections during pregnancy (10). A previous study showed that incidence rate of NTDs is 2.88 per 1000 in northern Iran (3).

2. Objectives

There was no documented study regarding the risk factors of NTDs in this area; therefore, this study was conducted to determine the risk factors of NTDs in Gorgan, north of Iran.

3. Patients and Methods

This hospital-based, case-control study was carried out on all NTD-affected pregnancies (n: 59) during February 2007 - August 2010, and 160 healthy pregnancies were selected via convenient sampling method from three hospitals of Gorgan, north of Iran.

Ethical approval for the study was obtained from the Ethics Committee of Golestan University of Medical Sci-
ences. The mothers’ consents were obtained for the study, along with a clearance from the institutional ethical committee. The subjects were chosen from three hospitals (Masoud, Falsafi, and Deziani) of Gorgan, north of Iran.

Newborns with NTDs after confirmation by a pediatrician (neonatologist) as case group, normal newborns as control group, and their mothers, were evaluated. For every case, we selected the subsequent two or three healthy newborns as control group. NTDs were defined according to the International Classification of Diseases, tenth revision (ICD-10).

Deziani Hospital is the largest specialized obstetrics and gynecology referral hospital (120 beds) in the city, with annual rate of more than 6000 deliveries. Patients are usually from moderate-to-low socioeconomic class families of various ethnic backgrounds. Masoud (60 beds) and Falsafi (100 beds) are two private general hospitals with annual rate of more than 1500 deliveries. In Gorgan, capital of Golestan province in north of Iran, three main ethnic groups are Fars, Turkman, and Sistani. The region has a population of about 350,000. Native Fars is the predominant inhabitant with the most members, Turkman is the ethnic group emigrated from central Asia more than three centuries ago, and the Sistani group emigrated from southeastern Iran half a century ago (3, 5).

Risk factors including maternal BMI, season of birth, gender of the newborn, mother’s age, ethnicity, consanguinity marriage, folic acid consumption, nutritional habitat, and education level, were assessed through interviews with mothers and recorded in a questionnaire for each mother in the case and control groups. BMIs < 18, 18-24.99, 25-30, and > 30, were considered as underweight, normal, overweight and obesity, respectively (3, 5, 10).

3.1. Statistical Analysis

Data analysis was performed using SPSS version 16. To investigate the NTD-affecting factors, logistic regression model was used to measure the crude odds ratio (OR) of NTD occurrence for each of the independent variables. Multiple Logistic Regression (MLR) by backwards method was used to control the confounders; the independent variables with P value < 0.2 in the univariate analysis were entered in the MLR model. The results are expressed as OR with 95% confidence interval (CI). Significance level was adjusted as < 0.05.

4. Results

In the case group, 32 (54.2%) affected newborns were male and 27 (45.8%) were female; parents of 33 (55.9%) and 26 (44.1%) affected newborns lived in rural and urban areas, respectively. In the control group, 77 (48.5%) newborns were male and 93 (51.9%) were female; parents of 96 (60.4%) and 63 (39.6%) control newborns lived in rural and urban areas, respectively (Table 1).

The results of univariate analysis of maternal character-

istics and NTD risk factors are depicted in Table 1. Based on our results, maternal age ≥ 35 (OR = 1.73, CI 95%: 0.51-5.81), folate supplementation (OR = 1.13, CI 95%: 0.62-2.06), gender of newborn (OR = 1.19, CI 95%: 0.65-2.17), consanguineous marriage (OR = 1.1, CI 95%: 0.56-2.13), residency (OR = 1.2, CI 95%: 0.65-2.19), birth in summer (OR = 0.9, CI 95%: 0.4-2.03), diploma/illiterate mother (OR = 1.89, CI 95%: 0.54-6.51), gravidity > 3/prime gravid (OR = 0.71, CI 95%: 0.21-2.34), and history of abortion (OR = 1.52, CI 95%: 0.7-3.29) were not significantly associated with NTDs as risk factors.

Multivariate analysis showed that maternal BMI (normal/underweight OR: 0.23, overweight/underweight OR: 0.15, obese/underweight OR: 0.13), maternal ethnicity (Fars/Sistani OR: 3.49) and maternal nutrition (good/poor OR: 0.46) were significantly correlated with NTDs (Table 2).

5. Discussion

The rate of NTDs in different ethnic groups showed that NTDs risk in native Fars was 3.49 times more than Sistani ethnic group. Other researchers such as Canfield et al. (9) showed that anencephaly and spina bifida rates were higher among Hispanics than non-Hispanic whites and blacks. In addition, Velle et al. (11) suggested that race and ethnicity might be affecting factors in the rate of NTDs.

Regarding gender, our results indicated that the NTDs rate was higher in males than females, but our results were in contrast with other studies in southwest Iran (12) and China (8). Yin’s study in china showed that the overall sex ratio (SR) of NTD was M/F = 0.59; anencephaly, spina bifida, and encephalocoele were more common in females than males, with SRs of 0.40, 0.72, and 0.82, respectively (8).

Our study showed that mother’s nutrition is significantly associated with NTD. This result was similar to Mandracioglu’s study (10), reporting that mothers poorly nourished during pregnancy were more prevalent among the case group (OR = 4.89, CI 95%: 2.84-8.42, P = 0.000). In De Marco’s study in Italy (4), high caffeine intake (OR = 10.82, 95% CI: 3.78-31), low-calorie diet (OR = 5.15, 95% CI: 1.79-14), and occasional consumption of fruits and vegetables (OR = 3.38, 95% CI: 1.67-6.82) were the main spina bifida risk factors in the multivariate analysis.

This study showed that there was no significant difference between mothers’ ages and NTDs, whereas maternal age of over 40 years in Texas (9) and maternal age of over 30 years in Russia (13) were associated with NTDs. In Turkey (14) and Italy (4), significant association between mothers’ ages and NTDs was found.

In this study, a nonsignificant association was observed between seasonal variation and NTDs. The incidence rate of NTDs was higher in the summer, but in Nil’s study in Tehran, birth in spring and summer was significantly associated with NTDs (7). Furthermore, Obeidat and Amarín’s study in Jordan showed that more NTD-affected babies
| Risk Factors               | Case         | Control      | OR  | CI (95%)         | P Value |
|---------------------------|--------------|--------------|-----|------------------|---------|
| **BMI**                   |              |              |     |                  |         |
| Underweight               | 8 (15.4)     | 6 (4.1)      | 1   | -                | -       |
| Normal                    | 23 (44.2)    | 66 (45.5)    | 0.26| 0.08-0.83        | 0.02    |
| Overweight                | 11 (21.2)    | 42 (29)      | 0.19| 0.05-0.68        | 0.01    |
| Obese                     | 10 (19.2)    | 31 (21.4)    | 0.24| 0.06-0.86        | 0.02    |
| **Season of birth**       |              |              |     |                  |         |
| Spring                    | 13 (22)      | 29 (18.1)    | 1   | -                | -       |
| Sumer                     | 23 (39)      | 57 (35.6)    | 0.9 | 0.4-2.03         | 0.8     |
| Autumn                    | 7 (11.9)     | 32 (20)      | 0.48| 0.27-1.39        | 0.17    |
| Winter                    | 16 (27.1)    | 42 (26.2)    | 0.85| 0.35-2.03        | 0.71    |
| **Consanguineous marriage**|             |              |     |                  |         |
| Yes                       | 17 (28.8)    | 43 (26.9)    | 1.1 | 0.56-2.13        | 0.77    |
| No                        | 42 (71.2)    | 117 (73.1)   | 1   | -                | -       |
| **Maternal age**          |              |              |     |                  |         |
| ≤ 20                      | 16 (27.1)    | 37 (23.1)    | 1   | -                | -       |
| 20-34                     | 37 (62.7)    | 115 (71.9)   | 0.74| 0.37-1.48        | 0.4     |
| ≥ 35                      | 6 (10.2)     | 8 (5)        | 1.73| 0.51-5.81        | 0.37    |
| **Folic acid consumption**|              |              |     |                  |         |
| Yes                       | 28 (47.5)    | 71 (44.4)    | 1.13| 0.62-2.06        | 0.68    |
| No                        | 31 (52.5)    | 89 (55.6)    |    | -                | -       |
| **Nutrition**             |              |              |     |                  |         |
| Poor                      | 33 (55.9)    | 67 (42.9)    | 1   | -                | -       |
| Good                      | 26 (44.1)    | 89 (57.1)    | 0.59| 0.32-1.08        | 0.09    |
| **Habitat**               |              |              |     |                  |         |
| Rural                     | 33 (55.9)    | 96 (60.4)    | 1.2 | 0.65-2.19        | 0.55    |
| Urban                     | 26 (44.1)    | 63 (39.6)    | 1.2 | 0.65-2.19        | 0.55    |
| **Sex**                   |              |              |     |                  |         |
| Male                      | 31 (52.5)    | 77 (48.1)    | 1.19| 0.55-2.17        | 0.56    |
| Female                    | 28 (47.5)    | 83 (51.9)    | 1   | -                | -       |
| **Ethnicity**             |              |              |     |                  |         |
| Sistani                   | 15 (25.4)    | 59 (36.9)    | 1   | -                | -       |
| Turkman                   | 6 (10.2)     | 31 (19.4)    | 0.76| 0.26-2.15        | 0.6     |
| Fars                      | 38 (64.4)    | 70 (40.6)    | 2.13| 1.07-4.26        | 0.03    |
| **Education**             |              |              |     |                  |         |
| Illiterate                | 4 (7.1)      | 20 (13)      | 1   | -                | -       |
| Under diploma             | 32 (57.1)    | 90 (58.4)    | 1.77| 0.56-5.59        | 0.32    |
| Diploma                   | 14 (25)      | 37 (24)      | 1.89| 0.54-6.51        | 0.31    |
| Higher education          | 6 (10.7)     | 7 (4.5)      | 4.28| 0.92-39.79       | 0.06    |
| **Gravidity**             |              |              |     |                  |         |
| 1                         | 30 (50.8)    | 75 (46.9)    | 1   | -                | -       |
| 2                         | 15 (25.4)    | 42 (26.2)    | 0.89| 0.43-1.84        | 0.76    |
| 3                         | 10 (16.9)    | 29 (18.1)    | 0.86| 0.37-1.98        | 0.72    |
| > 3                       | 4 (6.8)      | 14 (8.8)     | 0.71| 0.21-2.34        | 0.57    |
| **Abortion history**      |              |              |     |                  |         |
| No                        | 47 (79.7)    | 137 (85.6)   | 1   | -                | -       |
| Yes                       | 12 (20.3)    | 23 (14.4)    | 1.52| 0.7-3.29         | 0.28    |

---

\[a\] Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.

\[b\] Data are presented as No. (%).
Table 2. Multivariate Models of the Association Between Maternal Risk Factors and Neural Tube Defects Occurrence a

| Risk Factor       | OR   | CI (95%)       | P Value |
|-------------------|------|----------------|---------|
| **BMI**           |      |                |         |
| Underweight       | 1    |                |         |
| Normal            | 0.22 | 0.06-0.81      | 0.02    |
| Overweight        | 0.15 | 0.04-0.6       | 0.007   |
| Obesity           | 0.13 | 0.03-0.55      | 0.006   |
| **Mother's ethnicity** | | | |
| Sistani           | 1    |                |         |
| Turkman           | 1.36 | 0.4-4.62       | 0.61    |
| Fars              | 3.49 | 1.48-8.19      | 0.004   |
| **Nutrition**     |      |                |         |
| Poor              | 1    |                |         |
| Good              | 0.46 | 0.22-0.94      | 0.03    |

a Abbreviations: CI, confidence interval; OR, odds ratio.

were conceived in the late summer and early autumn and concluded that seasonality affected the incidence of NTDs in the north of Jordan (15). Regarding our results, there was no significant relation between consanguineous marriage of parents and NTDs. Various reports have attributed higher incidence of NTDs to consanguinity (16-19). Murshid in Saudi Arabia (16) reported that consanguinity of parents was found in 89% of the spina bifida parents and only 67% of the controls (P < 0.0005). A study in India (17) reported that NTD was significantly higher among babies born to parents of consanguineous marriages (P < 0.01). Furthermore, a study in Iran has shown that consanguineous marriages of all types were related with increased congenital malformations (with ratio of 43/1000 for consanguineous marriages and 28/1000 for nonconsanguineous ones) (18). A study in Iraq reported that 63.6% of NTD cases were results of consanguineous marriages (19). The possibility that consanguinity could be a risk factor for NTDs in Iranian population requires further investigations.

In this study, 55.9% and 44.1% of parents with affected newborns lived in rural and urban areas, respectively. This result was similar to a report from Texas (20). According to Luben’s study in Texas, there was no evidence that urban or rural residency was associated with changes in the rate of anencephaly or spina bifida without anencephaly in unadjusted or adjusted analyses.

In our findings, although 47.5% of women with affected newborns did not intake folic acid periconceptionally, this difference was not significant. Similar to our result, Yin’s study in China (8) reported that intake of folic acid was nonsignificantly associated with risk for anencephaly (OR = 0.46, CI 95%: [0.25, 0.84]). On the other hand, several studies reported significant relations between low folic acid consumption and NTDs in Italy (4), Iran (7), western Iraq (19), and Austria (21).

Lack of relation between intake of folic acid and NTD can be due to mandatory flour fortification with folic acid in this area which started from June 2006. Our results indicated that rate of NTDs was significantly higher in overweight women compared with normal-weight ones. Several studies showed that being overweight in women was significantly associated with increase of NTDs rate (22-27).

In this study, the mother’s education level was not related with NTDs, which was in contrast with other studies in Italia (4) and Turkey (14), reporting significant associations between education level and NTDs. This difference may be due to small sample size in our study. Based on our findings, maternal abortion history was not associated with NTDs. This result was similar to De Marco’s study (4) in Italy, reporting that previous history of spontaneous abortions did not prove to be a spina bifida risk factor. Our study showed that gravidity was not associated with NTD. In contrast with our study, De Marco et al. (4) in Italy showed that birth order was a significant risk with two to three folds higher risk, if the index case was second-born (OR = 2.15, 95% CI: 1.25-3.69) or third-born (OR = 3.93, 95% CI: 1.69-9.17), respectively.

Regarding various populations in this area, survey of nutritional factors was the strong point, and lack of all types of hospitals in our province was the limitation of this study. Concerning the high rate of NTDs in this region and due to multifactorial causes of NTDs, this study indicated that maternal overweight, ethnicity and nutrition, may be effective in predisposition to NTDs.

Acknowledgements

We specially thank the Neonatal Ward staff for their considerable help, the Deputy of Golestan University of Medical Sciences, director and personnel of Dezyani Hospital,
and Maliheh Sedehi and Nafiseh Kaviani, personnel of Gorgan Congenital Malformations Research Center, for their cooperation in this project.

**Authors’ Contribution**

All authors cooperated in design, conduction, data gathering, data analysis, and writing the manuscript.

**Financial Disclosure**

The authors declared no conflict of interest.

**Funding/Support**

Gorgan Congenital Malformations Research Center financially supported this research (grant number 35/6991).

**References**

1. Boyles AL, Billups AV, Deak KL, Siegel DG, Mehrttretter I, Slifer SH, et al. Neural tube defects and folate pathway genes: family-based association tests of gene-gene and gene-environment interactions. *Environ Health Perspect*. 2006;114(10):1547–52.
2. Ray JG, Wyatt PR, Thompson MD, Vermeulen MJ, Meier C, Wong PY, et al. Vitamin B12 and the risk of neural tube defects in a folic-acid-fortified population. *Epidemiology*. 2007;18(3):562–6.
3. Golalipour MJ, Mohabehri E, Vakili MA, Keshkhar AA. Epidemiology of neural tube defects in northern Iran, 1998-2003. *East Mediterr Health J*. 2007;13(3):560–6.
4. De Marco P, Merello E, Calevo MG, Mascelli S, Pastorino D, Crocetti L, et al. Maternal periconceptional factors affect the risk of spina bifida-affected pregnancies: an Italian case-control study. *Childs Nerv Syst*. 2011;27(7):1073–81.
5. Golalipour M, Najafi L, Keshkhar A. Neural tube defects in native fars ethnicity in northern Iran. *Iran J Public Health*. 2010;39(3):116–23.
6. Khattak ST, Naheed T, Akhtar S, Jamal T. Incidence and risk factors for neural tube defects in Peshawar. *Gomal J Med Sci*. 2008;6(3):1–4.
7. Nili F, Jahangiri M. Risk factors for neural tube defects: a study at university-affiliated hospitals in Tehran. *Arch Iran Med*. 2006;9(10):20–5.
8. Yin Z, Xu W, Xu C, Zhang S, Zheng Y, Wang W, et al. A population-based case-control study of risk factors for neural tube defects in Shenyang, China. *Childs Nerv Syst*. 2011;27(1):549–54.
9. Canfield MA, Marengo L, Ramadhanii TA, Suarez L, Brendler JD, Scheurer A. The prevalence and predictors of anencephaly and spina bifida in Texas. *Paediatr Perinat Epidemiol*. 2009;23(1):41–50.
10. Mandiracuglou A, Ulman I, Iuleci E, Ulman C. The incidence and risk factors of neural tube defects in Izmir, Turkey: a nested case-control study. *Turk J Pediatr*. 2004;46(3):214–20.
11. Velie EM, Shaw GM, Malcoe LH, Schaffer DM, Samuels SJ, Todoroff K, et al. Understanding the increased risk of neural tube defect-affected pregnancies among Mexico-born women in California: immigration and anthropometric factors. *Paediatr Perinat Epidemiol*. 2006;20(3):219–30.
12. Behroz A, Gorjizadeh MH. Prevalence and Correlates of Neural Tube Defect in South West Iran: Retrospective analysis. *Sultan Qaboos Univ Med J*. 2007;7(1):31–4.
13. Petrova JG, Yaktshloyd A. The incidence of neural tube defects in Norway and the Arkhangelskaja Oblast in Russia and the association with maternal age. *Acta Obstet Gynecol Scand*. 2009;88(6):667–72.
14. Onrat ST, Seyman H, Konuk M. Incidence of neural tube defects in Afyonkarahisar, Western Turkey. *Genet Mol Res*. 2009;8(6):154-61.
15. Obeidat AZ, Amarir Z. Neural tube defects in the north of Jordan: is there a seasonal variation? *J Child Neurol*. 2010;25(7):564–6.
16. Murshid WR. Spina bifida in Saudi Arabia: is consanguinity among the parents a risk factor? *Pediatr Neurosurg*. 2000;32(4):10-2.
17. Mahadevan B, Bhat BV. Neural tube defects in Pondicherry. *Indian J Pediatr*. 2005;72(7):557–9.
18. Rahmani SA, Aboualsoltani F, Pourbarghi M, Dolatkhah H, Aghazade AM. The frequency of consanguineous marriages and their effects on offspring in Tabriz City. *Shiraz E Med J*. 2010;11(1):238–7.
19. Al-Ani ZR, Al-Hulali SJ, Al-Mehimid SI. Neural tube defects among neonates delivered in Al-Ramadi Maternity and Children’s Hospital, western Iraq. *Saud Med J*. 2010;31(2):161–9.
20. Luben TJ, Messer LC, Mendola P, Carozza SE, Horel SA, Langlois PH. Urban-rural residence and the occurrence of neural tube defects in Texas, 1999-2003. *Health Place*. 2009;15(3):384–54.
21. Schiller-Fruhwirth I, Mittermayr T, Wild C. [Neural tube defects in Austria: Assumption and calculations on the prevention potential through folic acid enrichment and supplementation]. *Gesundheitswesen*. 2010;72(12):380–5.
22. Werler MM, Louik C, Shapiro S, Mitchell AA. Prepregnancy weight in relation to risk of neural tube defects. *JAMA*. 1996;275(14):1089–92.
23. Shaw GM, Velie EM, Schaffer D. Risk of neural tube defect-affected pregnancies among obese women. *JAMA*. 1996;275(14):1093-6.
24. Stothard KJ, Tennant PW, Bell R, Rankin J. Maternal overweight and obesity and the risk of congenital anomalies: a systematic review and meta-analysis. *JAMA*. 2009;301(6):596–600.
25. Waller DK, Mills JL, Simpson JL, Cunningham GC, Conley MR, Lassman MR, et al. Are obese women at higher risk for producing malformed offspring? *Am J Obstet Gynecol*. 1994;170(2):541–8.
26. Weller DK, Shaw GM, Rasmussen SA, Hobbs CA, Canfield MA, Siega-Riz AM, et al. Prepregnancy obesity as a risk factor for structural birth defects. *Arch Pediatr Adolesc Med*. 2007;161(7):745–50.
27. Blomberg MJ, Kallen B. Maternal obesity and morbid obesity: the risk for birth defects in the offspring. *Birth Defects Res A Clin Mol Teratol*. 2010;88(1):35–40.