

## Original Article

# Risk factors for neural tube defects in Riyadh City, Saudi Arabia: Case-control study

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## ABSTRACT

Both genetic and non-genetic environmental factors are involved in the etiology of neural tube defects (NTD) which affect 0.5-2/1000 pregnancies worldwide. This study aimed to explore the risk factors for the development of NTD in Saudi population, and highlight identifiable and preventable causes. Similar studies are scarce in similar populations of the Arabian Peninsula and North Africa. This is an unmatched concurrent case-control study including NTD cases born at King Khalid University Hospital, Riyadh during a 4-year period (2002-2006). The case-control study included 25 cases and 125 controls (case: control ratio of 1:5). Years of formal education, employment, household environment (including availability of air conditioning) and rate of parental consanguinity did not differ between mothers of cases and controls. Significantly higher proportion of mothers of cases

had history of stillbirth compared to control mothers (16% vs 4.1%,  $P=0.02$ ). Also family history of hydrocephalus and congenital anomalies were more prevalent in cases than controls ( $P$  values=0.0000 and 0.003, respectively). There was significant protective effect of periconceptional folic acid consumption both prior to conception (OR 0.02, 95% CI 0.00-0.07) and during the first 6 weeks of conception (OR 0.13, 95% CI 0.04-0.39). Further research, including a larger cohort, is required to enable ascertainment of gene-nutrient and gene environment interactions associated with NTD in Saudi Arabia.

### Key words:

Neural tube defects; Risk factors; Genetics; Folic acid supplementation; Saudi Arabia.

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## INTRODUCTION

Neural tube defects (NTD) are a common cause of central nervous system anomalies affecting 0.5-2 per 1000 pregnancies worldwide, with marked regional and racial variations [1,2]. Globally, more than 10% of infant mortalities secondary to congenital anomalies are caused by nervous system defects [3]. They arise following failure of the neural tube, which is the embryonic precursor of the brain and spinal cord, to close between the third and fourth week of embryonic development. Although, the incidence of NTD has fallen over recent years in industrialized countries [4-8], it still remains high in the less-developed countries in Latin America, Africa, the Middle East, Asia and the Far East, with estimated incidence ranging between >1 to 11/1000 births [9-12].

The majority of NTD are sporadic and both genetic and non-genetic environmental factors are involved in its etiology [2,13]. Recognized risk factors associated with NTD include deficient folic acid [14], maternal diabetes [15], maternal exposure to certain teratogens such as valproic acid taken by mothers who have epilepsy [16], lead in drinking water [17], in utero exposure to arsenic [18], and mycotoxins and fungus contaminants of maize [19]. Other associated risk factors include hyperthermia following episodes of maternal fever or heat exposure [20,21], maternal "flu" in the first trimester [22], certain parental occupations [23-25], and lower socioeconomic status [26].

Several clinical trials concluded that periconceptional folate supplementation substantially reduces the incidence of NTD [3,27] and a declining population prevalence of NTD by 30-50% was observed following folic acid fortification of food in many countries [28-32].

Few studies, addressing the epidemiology of NTD, have been reported from different parts of Saudi Arabia [33-38]. All of these have been directed

towards estimating the incidence of the disease which ranged between 0.78-1.83/1000 live births. Following fortification of flour in Saudi Arabia in 2001, a decline in NTD from 1.9/1000 live birth during the period 1997-2000 to 0.76/1000 afterwards (2001-2005) was reported in a study [39] from the Western Region of Saudi Arabia. Studies to evaluate risk factors for NTD are scarce in Saudi Arabia, as well as in other countries of the Arabian Peninsula and North Africa with similar demographic characteristics. Consanguinity was found to be a significant risk factor ( $p < 0.0005$ ) in a study from Al-Madinah Al-Munawarah, in Saudi Arabia [38]. Another study [40] identified low maternal serum vitamin B12 as a risk factor for NTD in Egyptians.

The aim of this case-control study was to explore the risk factors for the development of NTD in Saudi population, and highlight identifiable and preventable causes.

## MATERIALS AND METHODS

This is an unmatched concurrent case-control study. A case of neural tube defect (NTD) is defined as defects in neural tube formation, including anencephaly and myelomeningocele. A control is defined as apparently normal infant born immediately after delivery of a NTD case in the same hospital where cases were recruited.

All NTD cases born at King Khalid University Hospital (KKUH) in Riyadh City during a 4-year-period (4.6.1423 – 3.5.1427 [Hijri Calendar], [13.8.2002 – 31.5.2006 [Gregorian Calendar]), regardless of their gestational age, were included in the study. Cases were identified at delivery rooms soon after their birth. Five controls were taken for each NTD case recruited for the study. Cases of NTD referred to the hospital were not recruited for this study. The medical records of the mothers of the participating cases and controls were carefully reviewed for abstraction of

demographic variables, data on antenatal history, and medical care during antenatal, natal and postnatal periods. Mothers were asked about underlying medical illnesses and exposure to some potential risk factors during pregnancy. Two Neonatal Intensive Care Unit doctors (L C I and J E de J) interviewed mothers of cases and controls using a structured questionnaire. The interviewers participating in the study have thoroughly been trained and were supervised. The data collection in the structured questionnaire had been tested before conducting the present definitive study to reveal unforeseen logistic problems. Occurrence of NTD within the family and consanguinity between parents were also ascertained. Cases and controls were scrutinized for the state of the infant at birth, in case it was not possible to examine the child immediately after birth. Severity of the NTD condition and results of laboratory investigations pertinent to NTD and its etiology were also recorded.

#### Sample size and statistical analysis

The minimum sample size required on assumption of 30% exposure among controls, odds ratio of 4 with 80% study power at 95% level of significance was calculated to be 23 cases and 115 controls i.e. to get valid results, the number of recruited cases should be at least 23.

All collected data was recorded before data entry,

including data on consanguinity and open-ended answers. Epi Info (Version 6.06b) was used for data management and preliminary analyses: univariate and bivariate analyses. Proportions were compared using standard approaches for categorical data. Differences between means of cases and controls were calculated using the 2-tailed t-test as indicated. Level of significance ( $\alpha$ ) was set at  $p=0.05$ . Odds ratios (OR) and the corresponding 95% confidence intervals (95% CI) were calculated for comparison between cases and controls in relation to underlying potential risk factors and for estimating the strength of the associations.

## RESULTS

**Case Series:** There were 25 cases included in the study, 11 (44%) had anencephaly, 11 (44%) spina bifida/myelomeningocele and 3 (12%) occipital encephalocele. Nineteen (76%) cases were diagnosed antenatally, 6 (24%) at birth and 7 cases (28%) received treatment for their condition during hospitalization (Table 1). Brain ultrasound was antenatally performed for 24 cases (96%). In addition, 8 (32%) had ultrasound after birth too. Only one case had MRI in utero. Twelve cases (48%) had CNS and other systems abnormalities, 4 (16%) had arthrogryposis and 5 (20%) had associated dysmorphic features.

**Table 1 - Characteristics of 25 cases of neural tube defects born in King Khalid University Hospital 2002-2006**

Variable	No	%
<b>Time at diagnosis</b>		
Antenatal	19	76
At birth	6	24
<b>Type of NTD</b>		
Anencephaly	11	44
Occipital encephalocele	3	12
Myelomeningocele	7	28
Spina bifida	4	16
<b>Treatment during hospital stay</b>		
Yes	7	28
No	18	72

NTD - Neural tube defect

**Case Control Study:** The study included the 25 cases of NTD detected at KKHU, as well as 125 controls who were recruited from the same hospital (case: control ratio of 1:5). The mean age of mothers of cases was 28.76 + 6.64 years compared to 29.9 + 6.16 years for controls. Father's mean age of cases (34.55 + 6.74) was younger than that of controls' (36.32 + 9.44). The differences in mother's and father's age were not statistically significant between cases and

controls ( $p > 0.05$ ).

Years of mother's formal education were slightly higher for cases than controls (12.7 + 3.39 and 11.91 + 4.74, respectively). The majority of mothers of cases were not working (80%) compared to 70% of control's mothers. The difference was not statistically significant ( $p > 0.05$ ). Likewise, the majority of cases (90.9%) and controls (90.2%) were Saudis (Table 2).

**Table 2 - Demographic characteristics of cases with neural tube defects (n=25) and their controls (n=125)**

Characteristics	Cases (n=25)	Controls (n=125)	P
<b>1. Mother's age (years)</b>			
Mean	28.76 ± 6.64	29.90 ± 6.16	0.41
Median	27.00	30.00	
<b>2. Father's age (years)</b>			
Mean	34.55 ± 6.74	36.32 ± 9.44	0.42
Median	35.00	35.00	
<b>3. Years of formal education</b>			
Mean	12.7 ± 3.39	11.91 ± 4.74	0.45
Median	12.0	12.00	
<b>4. Mother's employment</b>			
Working	4 (20.0)	33 (30.6)	0.34
Housewife	16 (80.0)	75 (69.4)	
<b>5. Country of origin</b>			
Saudi Arabia	20 (90.9)	101 (90.2)	
Other countries	2 (9.1)	11 (9.8)	0.91
<b>6. Tribal ascertainment</b>			
Yes	13 (65)	86 (76.8)	0.26
No	7 (35)	26 (23.2)	

Mothers' employment was not known for 5 cases and 17 controls. Also country of origin was unknown for 3 cases and 13 controls. Tribal ascertainment not reported for 5 cases and 13 controls.

Around half (52%) of cases and 51.2% of controls live in villas whereas 36% of cases and 44.8% of controls live in apartments. There was no significant difference in the type of housing between cases and controls ( $p>0.05$ ). The crowding index for cases was  $1.77 + 0.69$  and  $1.91 + 0.83$  persons/room for controls, with no significant difference between the 2 groups ( $p>0.05$ ).

Regarding reproductive history of mothers of cases and controls (Table 3), both the number of pregnancies and parity were slightly lower in cases than control

( $4.12 + 2.45$  vs  $4.51 + 3.06$  and  $2.56 + 2.06$  vs  $2.99 + 2.68$ , respectively). However, the differences were not statistically significant ( $p>0.05$ ). About one third of control mothers gave history of abortion (35.5%) compared to 40% of mothers of cases. Significantly higher number of cases' mothers than controls' reported still births (respectively, 16% and 4.1%,  $p=0.02$ ). Neonatal losses were more reported among mothers of cases (4.2%) than those of controls (1.7%), with no significant difference between the two groups ( $P=0.42$ ).

**Table 3 - Reproductive history of mothers of cases of neural tube defects and their controls.**

Characteristics	Cases (n=25)	Controls (n=125)	P
<b>1. Number of pregnancies</b>			
Mean $\pm$ SD	4.12 $\pm$ 2.45	4.51 $\pm$ 3.06	
Median	4.00	4.00	0.55
<b>2. Parity</b>			
Mean	2.56 $\pm$ 2.06	2.99 $\pm$ 2.68	
Median	2.00	2.50	0.45
<b>3. History of abortion</b>			
Yes	10 (40)	44 (35.5)	
No	15 (60)	80 (64.5)	0.67
<b>4. History of still birth</b>			
Yes	4(16.0)	5 (4.1)	
No	21 (84.0)	117 (95.9)	0.02
<b>5. History of neonatal loss</b>			
Yes	1 (4.2)	2 (1.7)	
No	23 (95.8)	118 (98.3)	0.42

Two fifths (39.1%) of cases and 36.6% of controls reported history of consanguinity. Only 2.5% of controls compared to 29.2% of cases had family history of hydrocephalus with statistically significant difference between the groups ( $p=0.0000$ ). Congenital anomalies were, significantly, more common among .(cases (21.7%) than controls (4.4%,  $p=0.003$

The risk of NTD (Table 4) was less among infants with mothers who received multivitamins or folic acid irrespective of the timing during pregnancy, however the decreased risk was not statistically significant

(OR=0.39, CI=0.02 – 23.8). There was a protective effect of periconceptional folic acid consumption. This applied for intake of folic acid prior to conception (OR 0.02, 95% CI 0.00 – 0.07) and during the first 6 weeks of conception (OR 0.13, 95% CI 0.04 – 0.39). However, the risk of NTD in children of mothers who received folic acid after 6 weeks of gestation was 65.2% in cases compared to 83.6% in controls. This difference in risk was not statistically significant (OR .(0.37, 95% CI 0.12-1.16

**Table 4 - Folic acid and vitamins supplementation among cases of neural tube defects (n=25) and their controls (n=125)**

Folic acid and vitamins supplementation	Cases n (%)	Controls n (%)	OR and (CI) 95%
<b>Folic Acid at any time (1)*</b>			
Yes	23 (95.8)	119 (98.3)	
No	1 (4.2)	2 (1.7)	0.39 (0.02 – 23.8)
<b>Prior to conception (2)*</b>			
Yes	4 (20.0)	94 (94.0)	0.02 (0.00 - 0.07)
No	16 (80.0)	6 (6.0)	
<b>First six weeks of conception (3)*</b>			
Yes	8 (33.3)	79 (81.4)	0.13 (0.04 – 0.39)
No	14 (66.7)	18 (18.6)	
<b>After 6 weeks of pregnancy (4)*</b>			
Yes	15 (65.2)	97 (83.6)	0.37 (0.12 – 1.16)
No	8 (34.8)	19 (16.4)	

CI - confidence interval, n – number, OR - odds ratio

\*Unknown history has been reported for: (1)\* one case and 4 controls, (2)\* four cases and 25 controls, (3)\* three cases and 28 controls, and (4)\* two cases and 9 controls

The effect of diseases and exposure to recognized risk factor for NTD, as well as other significant gestational events are shown in Table 5. None of the mothers of cases or controls had history of exposure to drugs or toxins during pregnancy. Exposure to radiation, hypertension and febrile illnesses were slightly more among cases than controls (respectively, 4%, 8% and 20% vs 3.2%, 2.4% and 18.4%); whereas history of diabetes and urinary tract infections were more common among controls than cases (21.6% vs 16% and 13.6% vs 8%, respectively). Yet, none of these

differences attained the level of significance.

Duration of pregnancy was significantly longer in controls than cases (38.6±2.9 vs 34.7±6.8 weeks; P=0.0000). Normal spontaneous vaginal delivery was common among controls than cases (67.2% vs 60%, respectively) whereas the reverse was true regarding delivery by lower segment cesarean section (23.2% vs 32%, respectively). However, the differences between the groups in these two parameters were not significant.



**Table 5 - Gestational events in cases of neural tube defects (n=25) and their controls (n=125)**

Event	Status		Odds ratio	95% confidence interval
	Cases n (%)	Controls n (%)		
- Exposure to radiation	1 (4)	4 (3.2)	1.26	0.02 – 13.47
- Diabetes	4 (16)	27 (21.6)	0.69	0.16 – 2.31
- Hypertension	2 (8)	3 (2.4)	3.54	0.28 – 32.3
- Febrile illness	5 (20)	23 (18.4)	1.11	0.29 – 3.49
- Diminished fetal movement	2 (8.0)	0 (0.0)		Undefined
- Urinary tract infection	2 (8)	17 (13.6)	0.55	0.06 – 2.61
- Bleeding (per vaginum)	1 (4)	0(0.0)		Undefined
- Pre-eclampsia	0 (0.0)	1 (0.1)	0.00	(0.00 – 195.0)
- Polyhydramios	1 (4)	0 (0.0)		Undefined

Table number 6 shows the neonatal characteristics of cases and controls. More males were included as controls than cases (44.8% vs 32%), and head circumference was slightly larger in controls than cases (20.8+15.1 and 20.2+14.8, respectively). However, the differences between the groups were not significant in these respects. The birth weight was significantly heavier in controls than cases (3036.1 + 792.6 g and 2266.3 + 1333.5 g, respectively; P=0.001). Also the height was more among controls than cases (73.95 + 102.6 cm and 61.21 + 74.1 cm, respectively;

P=0.02). More cases were born cyanosed and pale (20% vs 13% and 4% vs 0%, respectively). On the other hand, more cases had poor sucking and feeding (18.2% vs 2.4%; P=0.002) and the majority of cases (68%) required nasogastric tube feeding compared to a minority (4%) of controls (P=0.00011). Jaundice was only observed in controls (1.6%) whereas bladder and fecal incontinence, manifesting as continuous dribbling of urine, patulous anus and staining of pampers, was present only in cases (38.1%; P=0.0000

**Table 6 - Neonatal characteristics of neural tube defects cases (n=25) and their controls (n=125)**

Characteristics	Cases n (%)	Control n (%)	P
<b>1. Infant sex</b>			
Male	8 (32)	56 (44.8)	
Female	17 (68)	69 (55.2)	0.24
<b>2. Head circumference (cm)</b>			
Mean	20.2 ± 14.8	20.8 ± 15.1	
Median	22	33	0.86
<b>3. Weight (g)</b>			
Mean	2266.3± 1333.5	3036.1± 792.6	
Median	2550	3095	0.001

<b>4. Height (cm)</b>			
Mean	61.21± 74.1	73.95± 102.6	
Median	46.0	49.0	0.02
<b>5. Medical status</b>			
Cyanosed	5 (20)	16 (13.0)	
Jaundiced	0 (0.0)	2 (1.6)	0.0000
Pale	1 (4.0)	0 (0.00)	
Other	12 (48.0)	2 (1.6)	
Normal	7 (28.0)	103 (83.7)	
<b>6. Poor sucking</b>			
Yes	4 (18.2)	3 (2.4)	
No	18 (81.8)	120 (97.6)	0.002
<b>7. Poor feeding</b>			
Yes	4 (18.2)	3 (2.4)	
No	18 (81.8)	120 (97.6)	0.002
<b>8. Apneic episodes</b>			
Yes	2 (9.1)	4 (3.3)	
No	20 (90.9)	119 (96.7)	0.21
<b>9. Jaundice</b>			
Yes	2 (9.1)	9 (7.3)	
No	20 (90.9)	114 (92.7)	0.77
<b>10. Reflux</b>			
Yes	0 (0)	1 (0.08)	
No	22 (100)	122 (99.2)	0.67
<b>11. Nasogastric tube feeding</b>			
Yes	7 (68.2)	5 (4.1)	
No	15 (31.8)	118 (95.9)	0.00011
<b>12. Bladder incontinence</b>			
Yes	8 (38.1)	0 (0)	
No	13 (61.9)	106 (100)	0.0000
<b>13. Fecal incontinence</b>			
Yes	8 (38.1)	0 (0.0)	
No	13 (61.9)	106 (100)	0.000
<b>14. Other complications</b>			
Yes	1 (4.8)	6 (5.3)	
No	20 (95.2)	108 (94.7)	0.92

cm – centimeter, g – gram, n – number, SD - standard deviation



## DISCUSSION

This prospective unmatched concurrent case control study consisted of 25 cases of NTD and 115 of their control who were born at KKHU. The disease was twice as common in females (female: male ratio of 2.1:1), similar to what has been reported before [41]. The majority of these (76%) cases were diagnosed antenatally, in contrast with a study [39] from the Western Region of Saudi Arabia where only 29% were reported to have been diagnosed antenatally. In the present cohort, myelomeningocele/spina bifida equaled anencephaly in proportion (44%). This also contrasts with studies from the Southern [37] and Western [39] Regions where the majority of NTD cases presented as myelomeningocele (70% and 83%, respectively).

In the present study, none of the demographic characteristics including mother age, father age, years of formal education, mother employment and country of origin was statistically different between cases and controls. Likewise, the household environment of cases with NTD did not differ between the two groups. Of special note was the availability of air conditioning which was similar in cases (84%) and controls (80.6%). It's noteworthy that maternal hyperthermic exposure in the first trimester of pregnancy has been documented to increase the risk for the development of NTD [20,21]. A combination of hot-tub use, febrile illness, or sauna use was associated with a six fold increase in risk [1].

Comparing the reproductive and genetic factors of mothers of cases and controls (Tables 4 and 5), there was no statistically significant difference between the two groups with regards to the number of pregnancies, parity, abortions, neonatal loss and parental consanguinity. A significantly higher proportion of mothers of cases had history of stillbirth compared to control mothers (16% vs 4.1%,  $P=0.02$ ). Also, family history of hydrocephalus and congenital anomalies were more prevalent in cases than controls

( $P$  values = 0.0000 and 0.003, respectively). Taken together, these might suggest genetic factors as important contributors to the occurrence of NTD in this cohort. It has been known that the recurrence risk for a second affected child is increased by 3-5 folds for couples with one affected infant and by 10 fold for siblings of affected individuals, as compared with the general population [13,42]. This recurrence fits a multifactorial polygenic or oligogenic pattern, rather than single dominant or recessive gene mode of inheritance; with reduced penetrance [43]. On the other hand, syndromes, often associated with chromosomal anomalies, account for <10% of all NTD cases [44-47]. Also, simple nucleotide polymorphisms (SNPs) and polymorphisms in genes of folate metabolism, which are known to be associated with NTD [48], might have been contributing to these observed differences between the cases and control groups.

In the present study, the rate of consanguinity between parents was slightly higher in the cases (39.1%) than controls (36.6%). However, the difference between the groups was not significant. Consanguinity has been reported as risk factor in previous studies in Egyptians [49], Palestinians [50] and Saudis [37, 38], yet none of these was a cases control study designed to examine critically the effect of consanguinity on NTD. Also of note was that the consanguinity rate among the cases in the present study (39.1%) was below the range (50-80%) observed in previous studies on NTD from Saudi Arabia [37-39].

Studying events during gestation and labor pertaining to cases and controls was only significant for a longer duration of pregnancy in controls compared to cases (38.6+2.9 vs. 34.7+6.8 weeks, respectively). The difference between the groups was highly significant in this respect ( $p=0.0000$ ). Other gestational events did not differ significantly between mothers of cases and their controls. These included exposure to radiation, the occurrence of diabetes, hypertension, febrile illnesses, diminished fetal movements,

urinary tract infection, vaginal bleeding, pre-eclampsia, polyhydramnios, and method of labor. It's noteworthy that infants of diabetic mothers were reported to be at risk for the development of NTD, including anencephaly and other central nervous system anomalies [51,52]. Oxidative stress in the embryo, caused by maternal hyperglycemia, inhibits expression of Pax-3 leading to increased neuroepithelial apoptosis in the embryo and abortion of the neural tube closure process; and antioxidants including vitamin E, vitamin C, a combination of antioxidants and lipids, or N-acetylcystein, might have a protective impact on the outcome of pregnancy [53]. . On the other hand, a febrile illness episode in the first trimester was found to be associated with an increased risk for having a NTD-affected pregnancy [54]. A combination of hot-tub use, febrile illness, or sauna use was associated with a six fold increase in risk [1]. The use of sulphonamides during early pregnancy was found to be associated with anencephaly [55], and the use of trimethoprim, which disturbs folate-related metabolism, was also linked to the causation of NTD [56].

The neonatal characteristics among cases and controls generally reflected the sequence and complications of NTD. Compared to controls, cases had significantly higher birth weight ( $P=0.001$ ), shorter length ( $P=0.02$ ), and had poorer medical status (e.g. cyanosis) at birth ( $P=0.0000$ ). Subsequently in hospital, a higher proportion of cases compared to controls manifested poor sucking, poor feeding and required nasogastric tube feeding ( $P$  values = 0.002, 0.002 and 0.00011, respectively). Bladder and fecal incontinences, which were seen only in cases, affected 8 babies (38.1%;  $P=0.0000$ ).

One of the major objectives of the present study was to examine whether periconceptional folic acid supplementation was protective against the development of NTD. Comparing mothers of cases and controls, there was an overall trend for a protective

effect of periconceptional folic acid consumption. This applied for intake of folic acid prior to conception and during the first 6 weeks of conception. However, the small sample size (25 cases) in the present study did not allow for controlling confounders, such as the impact of genetic factors. It has been observed that case control studies with small sample size and low prevalence of exposure, coupled with small to moderate effect sizes, can result in biased estimates of association between exposure and disease status [57]. Another confounding factor was the fact that the present study was conducted in the era following fortification of flour in Saudi Arabia [58]. This meant a low prevalence of exposure (i.e. absence of periconceptional folic supplementation) in the present case-control study. A decline in the incidence of neural tube defects has been reported from the Western Region of Saudi Arabia from 1.9/1000 to 0.76/1000 live births in the post-fortification period [56]. The authors attributed the relatively high incidence after fortification, compared to other countries, to the fact that fortification in Saudi Arabia was carried out only in flour, while it included all cereals and grains in the US [39,58]. Similar observations were recorded from Brazil, where fortification of wheat and corn flour with 150 micrograms of folic acid /100 grams became mandatory in June 2004 [58]. A study in the city of Recife [59] found no significant difference between global prevalence of NTD in the pre- and post-fortification periods (0.72 and 0.51/1000 live births). This was partly explained by the inadequate intake of sufficient amounts of fortified food by this population due to local diet habits characterized by low consumption of wheat and corn flour. This Brazilian study also highlighted the importance of considering the staple food in future programs of food fortification with folic acid.

Further research, through conducting the study with larger cohort (which necessitates a longer period of time than the present study) is required. This will

enable ascertainment of gene-nutrient and gene environment interactions, as well as specific risk factors associated with NTD in Saudi Arabia.

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