TERATOLOGY 51:71–78 (1995)

Reduced Recurrence of Orofacial Clefts After Periconceptional Supplementation With High-Dose Folic Acid and Multivitamins

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ABSTRACT To assess effects of periconceptional multivitamin and folic-acid supplementation on recurrence of cleft lip with or without cleft palate (CL±P), we prospectively evaluated 221 pregnancies in women at risk of a child with CL±P. The 10-step protocol included multivitamin supplementation with SPOFAVIT (A, B1, B2, B6, C, D3, E, nicotinamide, calcium pantothenicum) and folic acid (10 mg/day), beginning ≥2 months before planned conception and continuing for ≥3 months thereafter. A comparison group comprised 1,901 women at risk of a child with CL±P who received no supplementation and gave birth within the same period as the study group. In the supplemented group, 3 of 214 informative pregnancies ended with infants with CL±P, a 65.4% decrease (observed versus expected value −5.67; $P = 0.031$, Fisher's exact test); the expected value of 8.7 was calculated based on the incidence of cleft among first-degree relatives among the comparison group. Subset analysis by proband's sex, severity of CL±P, and both variables showed highest supplementation efficacy in probands with unilateral cleft (82.6% decrease, $P = 0.024$, Fisher's exact test). No efficacy was observed for female probands with bilateral CL±P. Generally, efficacy was greater for subgroups with unilateral than with bilateral cleft and for male than female probands. These findings confirm the need for a randomized, double-blind, controlled multicenter trial to establish whether periconceptional vitamin supplementation prevents CL±P and, if it does, whether the effective agent is folic acid or multivitamins, or both combined.

More than 20 years after the first studies in experimental animals indicated that vitamin deficiency in a mother could cause congenital malformations in the offspring (Hale, '33; Warkany and Nelson, '40; Warkany and Schraffenberger, '43), it was shown that the formiminoglutamic acid excretion test for defective folate metabolism was more often positive in women pregnant with a child with a neural tube defect (NTD) or other congenital abnormality than in control subjects (Hibbard and Smithells, '65), and that periconceptional supplementation with multivitamins (Smithells et al., '81) or folic acid (Laurence et al., '81) had a role in the prevention of NTDs. Nonetheless, prevention of congenital anomalies seemed impossible to realize as the ultimate goal of teratology (Warkany, '81) until a randomized, controlled, double-blind, multicenter trial sponsored by the British Medical Research Council (MRC) showed a 72% decrease in the recurrence of NTDs when women ingested 4 mg/day of folic acid from the day of randomization before conception and during 12 weeks thereafter (Medical Research Council Vitamin Study Research Group, '91; Wald, '93). All but one (Mills et al., '89) of the interventional (Czeizel and Dudas, '92; Vergel et al., '90; Kirke et al., '92) and observational (Yates et al., '87; Bower and Stanley, '89; Milunski et al., '89; Mulinare et al., '88; Mills et al., '89; Werler and Mitchell, '92) studies in the field suggest that periconceptional dietary folate and either multivitamin or folic acid supplementation reduce a woman's risk of having a child with NTD.

It was for cleft lip and palate anomalies, however, that the first attempts were made to use prophylactic multivitamin therapy, including folic acid, to prevent recurrence in humans (Douglas, '58; Conway, '58; Peer et al., '58). Based on the results of those studies, Burian ('64), of the Czechoslovak Academy of Sciences in Prague, initiated a study in which women who had given birth to a child with orofacial cleft began taking the multivitamin supplement preparation SPOFAVIT either immediately after a subsequent pregnancy was

Received April 18, 1994; accepted November 6, 1994.

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Presented in part at the 50th Anniversary Meeting of the American Cleft Palate-Craniofacial Association in Pittsburgh, Pennsylvania (April 1993) and the 7th International Congress on Cleft Palate and Related Craniofacial Anomalies in Broadbeach, Australia, November 1993.

confirmed or periconceptionally when pregnancy had been planned. Although Burian's observations were mainly empirical, a prospective trial of periconceptional multivitamin and high folic acid supplementation was conducted in women at risk of a child with cleft lip with or without cleft palate (CL±P) from 1976 through 1980 (Tolarova, '82a,b). The positive results of that work were later confirmed (Tolarova, '87, '89, '90a,b, '92). We report the final detailed findings of this study with the complete sample, which was performed in the former Department of Medical Genetics of the Czechoslovak Academy of Sciences in Prague to assess whether a folate-containing multivitamin supplement administered during the periconceptional period would prevent the recurrence of CL±P.

METHODS

Protocol for a multivitamin intervention regimen

A 10-step protocol for the primary prevention of CL±P was followed:

1. A genetic evaluation was made, which entailed a physical examination of all first-degree relatives, including all siblings of the proband, both prospective parents, and, for adult patients, the proband's children. Its purpose was to diagnose all micromanifestations (microforms) of cleft anomaly and to exclude syndromic cases.
2. Risk of recurrence was calculated in nonsyndromic cases. This risk figure differs significantly relative to the sex of the proband and the severity of the CL±P anomaly (Tolarova, '87, '89) and was stated individually for each case; the precise value is closely linked to the correct diagnosis.
3. All prospective mothers had a medical examination with a routine biochemistry panel, all hematologic evaluations including tests of liver and pancreatic function, X-ray examinations of the lungs and heart, and an electrocardiogram (ECG). All had serologic tests for toxoplasmosis and most had an endocrinologic evaluation of iodine accumulation in the thyroid gland (Kremenova et al., '81) to screen for diminished thyroid glandular function that may affect the health of mother, and possibly the development of the child.
4. A gynecologic examination was performed, which included a vaginal examination with cytology samples and a vaginal smear for cytologic examination. Other examinations were done by appropriate specialists, if necessary as indicated by the medical history.
5. All pathologic conditions revealed by the examinations were treated before periconceptional vitamin supplementation was begun.
6. Based on the prospective mother's history and health status and on the families' schedules and habits, an optimal time for conception was chosen that took into account seasonal individual differences in risk. Except for women who suffered from such problems as seasonal exacerbation of a disorder (e.g., hay fever or duodenal ulcer), the late spring and summer months were generally suggested for conception because of the better availability of fresh fruit, green vegetables, and other natural sources of vitamins, including provitamin D from more intense sunshine, and because of the lesser chances of influenza and other respiratory infections that can cause an increased body temperature and poorer appetite with consequent changes in the nutrient supply.
7. Living conditions of the prospective parents were discussed and, if necessary, changes were suggested to make them optimal for conception and pregnancy.
8. Periconceptional administration of multivitamins was begun at least 2 months before conception and continued during at least the first 3 months of pregnancy. The SPOFAVIT multivitamin preparation was given as 3 tablets daily (1 tablet containing vitamin A, 2,000 IU; vitamin B_1, 1 mg; vitamin B_2, 1 mg; vitamin B_6, 1 mg; vitamin C, 50 mg; vitamin D_3, 100 IU; vitamin E, 2 mg; vitamin PP [nicotinamide], 10 mg; calcium pantothenicum, 1 mg), and 10 mg of folic acid was given once daily. Most of the women also received supplemental iron (75 mg/day of Ferrum II fumaricum) and additional vitamin B_6 (1 mg/day in 1 tablet). If iodine metabolism testing showed diminished thyroid glandular function, an iodine supplement was given. A few women received vitamin E supplementation at the gynecologist's suggestion. No other medication was taken.
9. All women in the supplemented group were monitored continually and were evaluated in our department once each month from the time they entered the study until their child was born or their pregnancy ended.
10. The newborn child was first examined in our department between 6 weeks and 3 months after birth, again at 6 months of age, and then again at 1, 3, and 5 years of age, in order to diagnose possible dysmorphic signs and to follow the course of development.

Sample selection

Probands were 1,603 children born with nonsyndromic CL±P in the Czech Republic from 1970 through 1982, and 1,930 adult women or their husbands born with nonsyndromic CL±P from 1930 through 1962. In all cases, CL±P was "isolated;" that is, no other major anomaly was present (Fig. 1). The individuals with cleft anomaly were selected from the registry of cleft lip and palate and craniofacial anomalies of the Czechoslovak Academy of Sciences. In order to evaluate the effect of multivitamin and folic acid supplementation in a group that was as homogenous as possible, the study included only cases with no family history of clefting (i.e., sporadic cases).

A personal letter was sent to offer the multivitamin intervention to all individuals with sporadic cleft

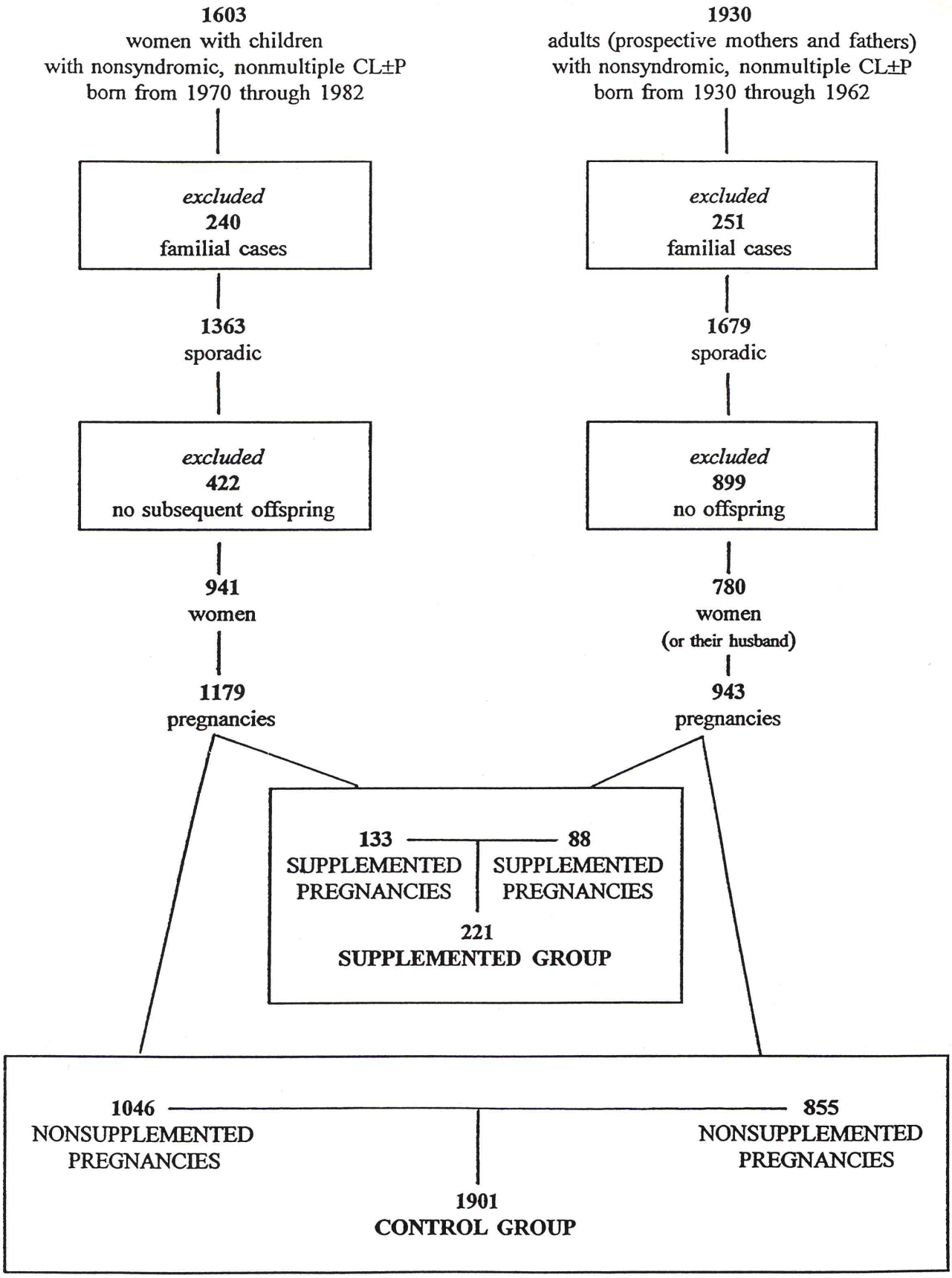

Fig. 1. Strategy of sample selection.

TABLE 1. Structure of supplemented and nonsupplemented group of probands affected with cleft lip with or without cleft palate

Probands with cleft				Number (supplemented/nonsupplemented)			
Children	CL	Uni	M	16/201	33/362		
			F	17/161		34/392	
		Bi	M	1/17	1/30		
			F	0/13			133/1046
	CLP	Uni	M	58/328	72/497		
			F	14/169		99/654	
		Bi	M	17/108	27/157		
			F	10/49			221/1901
Parents	CL	Uni	M	6/199	24/338		
			F	18/139		26/379	
		Bi	M	2/21	2/41		
			F	0/20			88/855
	CLP	Uni	M	19/245	41/369		
			F	22/124		62/476	
		Bi	M	15/72	21/107		
			F	6/35			

CL, cleft lip; CLP, cleft lip and palate; Uni, unilateral; Bi, bilateral; M, male; F, female.

anomalies identified in the registry: 1,363 mothers of children with CL±P and 1,679 adults identified with CL±P (Fig. 1). Excluded were 1,321 cases from both groups in which no pregnancy occurred during the study period (Fig. 1). All of the 221 women who agreed to participate in the study and complied with the 10 steps of the multivitamin intervention regimen were included in the supplemented group. The comparison group consisted of 1,901 women who either did not respond to the letter, refused to participate in the study, started multivitamin supplementation after the embryonic period, or discontinued supplementation before or during the embryonic period. Thus, subjects were not randomized into supplemented and comparison groups. The strategy of sample selection is shown in Figure 1. For probands affected with CL±P, the structure of supplemented and nonsupplemented pregnancies is shown in Table 1.

Medical outcome assessment

All probands and all their first-degree relatives who lived in the Czech Republic and were born between 1964 and 1982 were examined and followed in our department (Tolarova, '87, '90a,b). The bases for determining risk in the comparison group were the same as those for the supplemented group. Mothers in the comparison group had a genetic evaluation and received counseling in regard to an optimal time for conception like that given in the study group; risk of recurrence was calculated in nonsyndromic cases. All were monitored routinely at the regional prenatal clinic until their child was born or their pregnancy ended.

Statistical evaluation

The proportions of affected and unaffected newborns and other informative outcomes of pregnancies were calculated for both the supplemented and the nonsupplemented groups. Differences in informative outcomes of pregnancies were evaluated using the chi-square test and Fisher's exact test. The difference between the observed number of infants affected with cleft in the supplemented group and the expected number was used as a measure of effectiveness of the supplementation in preventing cleft recurrence.

RESULTS

In all, 221 pregnancies of mothers at risk of having a child with CL±P were supplemented.

TABLE 2. Prevention of cleft lip with or without cleft palate by periconceptional vitamin (particularly high folic acid) supplementation[1]

Proband	Nonsupplemented (without/with cleft)	Supplemented (without/with cleft)	Efficacy	
			Expected occurrence	Decreased by (%)
Cleft lip with or without cleft palate (Cl±P)*	1,824/77	211/3	8.67	65.4
Male with CL±P**	1,149/42	129/1	4.58	78.2
Female with CL±P**	675/35	82/2	4.14	51.7
Unilateral CL±P†	1,511/55	163/1	5.76	82.6
Bilateral CLP±P†	313/22	48/2	3.29	39.2

[1]Fisher's exact test was used for all results.
*Results for the whole group, $P = 0.030579$.
**Results for subgroups of males and females, respectively, $P = 0.063169$; $P = 0.227924$.
†Results for subgroups of unilateral and bilateral cases, respectively, $P = 0.02433612$; $P = 0.3734264$.

Group at risk of CL±P

Of the 221 supplemented pregnancies, 7 pregnancies ended in a spontaneous abortion that obviated an examination for the presence or absence of cleft. Of the other 214 pregnancies, 211 culminated in a liveborn child, 2 in a prematurely born fetus, and 1 in termination in the second trimester. One pregnancy was terminated because a fetus with karyotype 46XY + 21 was diagnosed prenatally during an examination prompted by the mother's advanced age; the fetus had no cleft. One ended prematurely at week 31; the male child, who had no cleft, died. Another pregnancy ended unsuccessfully in a premature birth at 19 weeks, 4 weeks after visualization fetoscopy; this fetus also had no cleft. One pregnancy ended with twins at term; neither had cleft.

Altogether, 211 term babies (209 singletons, 1 pair of twins) and 3 fetuses (2 premature, 1 terminated) were examined for orofacial cleft. Of those 214 infants, 3 were born with a CL±P anomaly, 1 boy with hypospadia, and 1 girl with syndactyly; the syndactyly was the expression of an autosomal dominant gene inherited from the mother. Visualization fetoscopy was done in 1 case because of a high risk of recurrence (Tolarova and Zwinger, '81). In the 3 cases of recurrence, 1 girl had bilateral complete cleft lip and palate (CLP). She was the mother's second daughter to have bilateral complete CLP; there was no other case of cleft in the pedigree. The mother had had a fever and had taken an antihistaminic and antiallergic drug together with periconceptional supplementation, but she had no other illness during pregnancy. Another girl, who had unilateral CLP, was her mother's second child. Her older brother had no cleft. Her father had bilateral CLP. Both mothers' pregnancies had been periconceptionally supplemented with vitamins and folic acid; no history of illness was registered during either pregnancy. In the third case, a unilateral microform of cleft lip occurred in a male child born to a mother with unilateral CLP; later in the study, the mother's second supplemented pregnancy produced a girl with no cleft.

The frequency of cleft in the infants born was generally lower among the mothers receiving supplementation than in the nonsupplemented group (Table 2). In contrast to 8.67 cases of cleft expected in the supplemented group, only 3 infants were born with cleft ($P = 0.031$, Fisher's exact test). In the nonsupplemented group, 4.05% of pregnancies ended with an infant with cleft lip or CLP. By contrast, only 1.4% were affected in the supplemented group, representing a 65.4% decrease. When the supplemented group was divided according to sex, the male subgroup showed a greater difference between the observed and expected values (Table 2). When that group was divided according to severity by unilateral or bilateral cleft, the subgroup with unilateral cleft showed the bigger difference between the observed and expected values. When the supplemented group was divided according to sex and severity combined, the biggest difference between the observed and expected values was found in the subgroup of male probands with unilateral cleft (no recurrence in 96 supplemented pregnancies versus 28 recurrences in 973 nonsupplemented pregnancies), whereas there was no difference between those values in the subgroup of female probands with bilateral cleft. As predicted by our hypothetical four-threshold model of liability for CL±P (Tolarova, '87), the best results of supplementation were obtained in the subgroup of male probands with unilateral cleft. This finding is consistent with our observation that, in general, the effectiveness of supplementation increases in proportion to the increasing incidence of the subgroup in the general population and the decreasing values of heritability and risk of recurrence.

In general, then, a 65.4% decrease of cleft recurrence was found for the supplemented group. The best efficacy of supplementation was found in the subset with unilateral cleft ($P = 0.024$, Fisher's exact test), in which the difference between observed and expected cases was −4.76 (3.51% versus 0.61%) (Table 2), for a decrease by 82.6%. No difference was found in the subgroup of female probands with bilateral cleft.

DISCUSSION

We are aware that we could not randomize study subjects into supplemented and comparison groups. Furthermore, during the study we suggested that the supplemented group conceive in the spring, while no such recommendation was made for the comparison group. It is therefore possible that selection bias occurred and that an another unknown factor, other than multivitamins/high-dose folic acid supplements, could explain the observed results.

Although based on relatively small numbers of recurrent clefts, our findings suggest that a periconceptional multivitamin and high-dose folic-acid regimen may be associated with a reduced recurrence of CL±P in risk pregnancies, and they support the potential efficacy of a daily multivitamin and folic-acid supplementation beginning at least 2 months before conception and continuing during at least the first 3 months of pregnancy.

Reported studies of multivitamin and folic acid supplementation in CL±P

In a study of prevention of cleft anomaly in a series of several thousand women, Douglas ('58) used a 1-a-day multivitamin and mineral capsule and a supplementary capsule of vitamins A and B complex, folic acid, and pantothenic acid; supplementation began soon after conception and lasted for at least the first trimester. In Conway's ('58) nonrandomized controlled trial, mothers receiving a supplement took a multivitamin with 0.5 mg of folic acid; no cleft occurred among 59 children in the supplemented group of mothers who had previously had a child with cleft; however, 4 of 78 infants were born with cleft anomaly (2 cases of CL±P and 2 of cleft palate (CP) in the nonsupplemented group.

In an extensive, nonrandomized, controlled trial (Peer et al., '58, '63, '64; Briggs, '76), pregnant women with a prior pregnancy affected with CL±P or CP took a multivitamin supplement with 5 mg of folic acid daily from the first suspicion of pregnancy until at least the fifth month. A 72% decrease in CL±P recurrences was observed in the supplemented group; 4 CL±P recurrences occurred among 261 children in the supplemented group as compared to 15 among the 275 children in the nonsupplemented group. No such effect was seen in regard to CP. Whereas vitamin supplementation started at the beginning of pregnancy in each of those three studies, supplementation in our study was taken periconceptionally.

Like previous studies of CL±P and CP prevention, ours is an interventional study focused on recurrences in the family in which cleft had already occurred in a first-degree relative. In some attempts to decrease recurrences (von Kreybig et al., '78; Gabka, '81; Schubert et al., '90), either the samples were small or the available data were insufficiently detailed to permit comparisons with the other studies discussed here.

Two recent studies have focused on occurrences. One was an observational, population-based case-control study of dietary folate (Bower and Stanley, '92). Based on information from 59 mothers of infants with a midline defect, of whom 13 infants had facial cleft, the investigators concluded that there was no evidence of an association between nonneural midline birth defects and either dietary folate ingestion or folic acid supplementation. In the other, a randomized, prospective, blind, interventional study (Czeizel and Dudas, '92; Czeizel, '93), multivitamins including 0.8 mg of folic acid reduced the first occurrence of NTDs, but not nonneural midline defects. There were 4 cases of CL±P among 2,104 children in the group receiving the multivitamin supplement, and 3 cases of CL±P and 2 of CP among 2,052 children in the group receiving trace elements.

Preventive effect in subsets of CL±P

As far as we know, no previous study has distinguished different subgroups of CL±P. We found that the effectiveness of vitamin supplementation varied in subsets of CL±P formed according to the sex of the proband, severity of the malformation, and the combination of those two variables. Although further subsets of these subgroups were not sufficiently large to permit detailed analyses, differences in the effectiveness of supplementation apparently exist in relation to the proband's sex and the severity of the anomaly.

The greatest effectiveness of the supplemental regimen, resulting in an 82.6% decrease in cleft recurrence, was found in the largest subgroup, the male probands with unilateral cleft, which also has the lowest heritability value and the lowest risk of recurrence. According to our hypothesis of a four-threshold model of liability (Tolarova, '87, '90b) and the multifactorial threshold (MF/T) model (Carter, '65), this is also the subgroup in which liability for CL±P is determined in large part by environmental factors. In both this and our previous study (Tolarova, '82a,b), the best results of periconceptional supplementation were obtained in the subgroup of probands with unilateral clefts. In contrast, periconceptional supplementation apparently did not reduce recurrences in the subgroups in which the proband was female with bilateral cleft or in cases of bilateral cleft generally; that is, in the subgroups in which a liability to CL±P was determined in large part by genetic factors.

Dose of folic acid

The therapeutic dose of folic acid used in our study is higher than doses used in the interventional studies of NTD and in previous studies of cleft anomalies. The folic acid dose level of 10 mg was chosen because, at the time this study began in Czechoslovakia, the only folic acid preparation available was in the form of a pill that contained a 10-mg dose and was difficult to split. We consulted hematologists about safety considerations re-

garding use of this dose level and were assured that, if the blood count was monitored before and during the folic acid supplementation, pregnant women would not be at risk for adverse events.

Two complications are associated with high-dose folic acid administration. The first is a masking or precipitation of neurologic signs of pernicious anemia. Although the incidence of pernicious anemia is extremely low among women of child-bearing age, this potential risk was excluded in our study by detailed medical examinations, including blood tests, and the evaluations every woman received before taking supplementation. The second complication is interference with anticonvulsant control in patients with grand mal epilepsy, a risk also excluded by the thorough medical evaluations. No woman in our study was being treated for grand mal epilepsy. In two women with less severe manifestations of epilepsy, the folic acid and multivitamins were administered under the close supervision of a neurologist.

From prevention studies on the NTDs, it has been concluded that folic acid supplementation with 4 mg/day is effective and acceptably safe under medical supervision, and that a folic acid dose of 0.4–0.8 mg/day is accepted as safe for general use without medical supervision (Wald, '93). While no such guidelines existed for multivitamin supplementation during the years of our study, no women participating in the study showed or reported signs of adverse effects, either while taking the folic acid regimen of 10-mg/day or during subsequent follow-up review. Whether the lower dosage apparently effective against NTDs may be effective against CL±P remains to be tested.

Homogeneity of the analyzed sample

The degree of homogeneity of the population in our study is unusual in that, in both study groups, all first-degree relatives who were alive and living in the former Czechoslovakia were examined and followed by the same research team. Also, in the Czech Republic at that time under a socialist regime, there were not large differences in socioeconomic status among our supplemented and comparison groups. All medical examinations and follow-up evaluations in this study were completely paid for by government, including paid leave from work and travel expenses. It seems unlikely that a 65.4% reduction in CL±P recurrence in the mothers taking the supplement could be due purely to chance. As was true before the MRC study (the Medical Research Council Vitamin Study Research Group, '91; Wald, '93) established an effect of folic acid in NTDs, however, it remains in question whether multivitamin supplementation or folic acid, or both in combination, may prevent some cases of CL±P, or whether women who choose to take the vitamin supplements represent a select group with a lower risk of having another affected child.

Conclusion and outlook

Unlike preceding studies of prevention of cleft anomalies with a regimen of multivitamin and folic acid supplementation during pregnancy, the features characterizing this study are (1) the initiation of vitamin supplementation before conception; (2) higher genetic homogeneity of the analyzed sample, in that syndromes and multiple malformations were excluded and there was homogeneity of a family history of cleft; and (3) a large enough sample accrued to permit evaluation of subsets of CL±P. Our results in this study confirm our earlier findings (Tolarova, '82a,b, '87, '90a,b, '92) and suggest that high-dose folic acid and multivitamin supplementation is associated with a substantial 65.4% decrease in recurrence of CL±P in risk families that had one occurrence of CL±P among the first-degree relatives of the awaited child. The results of this study, when considered in combination with those of previous studies of recurrences and recent interventional and observational studies of occurrences, establish the need for a double-blinded, randomized, multicenter trial to determine (1) whether occurrences as well as recurrences of CL±P anomalies can be prevented by periconceptional supplementation of the diet with folic acid and multivitamin supplements; and (2) if so, whether the effective agent is the folate, the vitamins, or both the vitamins and folic acid in combination. In such a study other risk factors can be measured and included as part of the analysis. Involvement of multiple centers in such a study could enlarge the sample of probands to a number providing greater statistical power.

ACKNOWLEDGMENTS

This study was carried out in Prague, Czech Republic, in the former Czechoslovak Academy of Sciences, starting at the Department of Birth Defects of the Laboratory of Plastic Surgery and continuing at the Laboratory of Clinical Genetics of the Institute of Experimental Medicine.

This work would not have been possible without professional contributions of local geneticists, gynecologists, obstetricians, internal medicine specialists, and other physicians in the Czech Republic. Especially valuable help in the follow-up of our patients was contributed by Jarmila Prochazkova, M.D., Ph.D., Alena Rohackova, Ivana Vodochodska, and Rosa Kubatova. We are also grateful to Susan Eastwood, E.L.S.(D.), for help in editing the paper.

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