REVIEW OF CURRENT EVIDENCE FOR FOLATE IN THE PREVENTION OF NEURAL TUBE DEFECTS

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The incidence of neural tube defects (NTD) among black South Africans living in urban areas is low compared with reports of NTD incidence in rural areas.[1,2] A NTD incidence of 0.95 per 1 000 live births was reported in Cape Town,[1] while an incidence of 0.99 per 1 000 live births was reported in a study performed at Kalafong Academic Hospital, Pretoria.[2] In contrast, the prevalence of NTD in the black population in rural Transkei was 6.13 per 1 000 live births,[3] and in rural Northern Province it was 3.55 per 1 000 live births.[4] In view of the association between folic acid status and NTD, we performed a study in rural and urban communities to determine whether folate or vitamin B_{12} status and/or abnormal homocyst(e)ine metabolism could explain why the incidence of NTD in rural areas is so high. (Homocyst(e)ine (tHcy) refers to the sum of concentrations of free homocysteine, protein-bound homocysteine, the disulphide homocystine, and the mixed disulphide homocysteine-cysteine.)

FOLATE, ABNORMAL HOMOCYST(E)INE METABOLISM AND NTD

Elevated maternal plasma homocysteine as a causal agent in NTD

The mechanism by which folate deficiency may cause NTD is by impairing homocysteine metabolism. Elevated plasma homocyst(e)ine[1] concentrations and elevated homocyst(e)ine concentrations in amniotic fluid aspirates have been reported previously in mothers with NTD births.[5-8] Further evidence for an association between abnormal homocysteine metabolism in some NTD cases is given in a report of increased post-methionine load tHcy concentrations in 22% of women with a history of NTD.[9] Abnormal homocysteine metabolism may be due to homozygosity for the 677 C to T mutation in the gene coding for methylene tetrahydrofolate reductase (MTHFR). The mutated enzyme is thermolabile.[10] It has a lower level of activity,[9-11] which results in ineffective homocysteine remethylation. Homozygosity is associated with higher plasma total homocyst(e)ine (tHcy) levels,[11-14] especially when dietary folate intake is low.[12,14] Furthermore, a 2 - 3-fold increase in the prevalence of homozygosity for the 677 C→T mutation in the

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gene coding for the enzyme MTHFR was observed in parents of neonates with NTD,[10] and fibroblast cultures from fetuses with NTD showed that homozygosity for the 677 C→T mutation was associated with a 7.2-fold increased risk of NTD.[15]

Folate supplementation and NTD

Folate supplementation in the peri-conceptual period appears to reduce the frequency of NTD.[16,17] Although the findings are not entirely consistent, a recent review concluded that NTD were associated with decreased folate concentrations during the first trimester of pregnancy.[18] Early in pregnancy serum and red-cell folate concentrations were reported to be significantly lower in women who delivered babies with NTD compared with controls,[19] and a continuous dose-response relation between a risk of NTD and red-cell folate levels was established.[20]

Rural black South African mothers with a history of NTD

As folate and/or vitamin B_{12} deficiencies are considered to be causally related to NTD,[21] we hypothesised that differences in folate/vitamin B_{12} nutritional status could explain the differences in NTD incidence in rural and urban black populations. Folate deficiency is common in pregnant rural black women[22] and in rural black children.[23] It is not known whether impaired homocysteine metabolism exists in black women with a history of NTD pregnancies, or whether homozygosity for the thermolabile variant of MTHFR increases the risk of NTD. We therefore investigated whether vitamin B_{12} and/or folate status and elevated homocysteine concentrations could explain the high incidence of NTD in rural black populations by determining plasma tHcy, vitamin B_{12} and folate concentrations in apparently healthy rural black women and urbanised black women. We also studied folate and vitamin B_{12} status, homocyst(e)ine metabolism and MTHFR genotypes (677 C→T mutation) in 54 rural black women with histories of NTD.

Population survey of rural and urban women

Apparently healthy, urbanised black women (mean (SD) age 25.4 (3.9) years, $N = 101$) from the Pretoria area had lower plasma folate concentrations ($P < 0.001$) compared with rural black women (mean (SD) age 31.3 (7.5) years, $N = 107$) who lived in Northern Province. Lower plasma tHcy concentrations were observed in the rural population but the difference was not statistically significant ($P = 0.09$).

Homocysteine metabolism and MTHFR genotype in women with a history of NTD

Since 1990, the Department of Human Genetics, University of Pretoria, has provided a clinical genetic service to seven rural hospitals in Northern Province. Fifty-four black women with a recorded history of NTD between 1990 and 1995 were approached to participate in the study. Of the 54 NTD cases, 30 were documented as spina bifida, 10 as anencephaly, 7 as cranioracheischisis, and 7 as encephalocoele. Age and body mass paired controls were selected from the rural population sample described above. None of the cases or controls were receiving vitamin supplementation. Methionine load tests were performed on these subjects and no significant differences were observed in plasma vitamin B_{12}, folate, fasting homocyst(e)ine, methionine, and the post-methionine load increase in plasma homocyst(e)ine concentrations between NTD cases and controls. More than 50% of both NTD cases and controls had a post-methionine load increase in plasma tHcy concentrations below the 5th percentile calculated in a healthy white control group (64 apparently healthy white women with no history of NTD or previous miscarriages (mean (SD) age 35.9 (8.1) years) recruited from major employers in Pretoria).

The finding that black rural women with a history of NTD do not differ significantly from controls supports the concept that a frank folate deficiency is not a prerequisite for NTD occurrence.[24] Abnormal homocysteine metabolism in NTD has been reported for Caucasian populations,[5-8] but our results question the assumption that homocyst(e)ine metabolism is causally related to NTD in black subjects. Black subjects have a high incidence of NTD, while they appear to metabolise methionine much more effectively than white subjects, as was observed in the present study as well as in previous studies.[25]

The presence of 677 C→T mutation in the MTHFR gene was investigated in women with a history of NTD and in controls by polymerase chain reaction (PCR) of genomic DNA and *Hinfl* digestion of the PCR product. Of the controls 9/54 (16.6%) were heterozygous and of the NTD cases 12/58 (20.7%) were heterozygous, while no homozygotes for the 677 C→T mutation in the MTHFR gene were found in either group. A population genetic test showed that this black population sample was in Hardy-Weinberg equilibrium. Homozygosity for the 667 C→T mutation in the gene coding for MTHFR may not be a genetic risk factor for NTD in black subjects, as was reported for Caucasians.[10,15]

Conclusion

No explanation can be derived from this study for the high incidence of NTD in rural black women since neither folate or vitamin B_{12} deficiency, nor aberrant homocysteine metabolism were evident in NTD subjects compared with controls. The prevalence of folate deficiency (plasma folate levels < 6.75 nmol/l) was high in rural non-pregnant black women (21%) and even higher in the urban population group (46%). In a previous study performed in Northern Province 48% of black women in the second or third trimester of pregnancy were

reported to be folate-deficient.[22] A significant trend to lower folate intake with urbanisation[26] may explain why the urban group in our study had significantly lower folate concentrations compared with the rural group. Although the urban group had a higher prevalence of folate deficiency than the rural group, the NTD prevalence was only 0.99/1 000 live births,[2] compared with the NTD prevalence in rural areas ranging from 3.55 - 6.13 per 1 000 live births.[3,4]

Folate deficiency does not appear to increase the risk of NTD in black subjects. As our data question the role of folate deficiencies and abnormal homocysteine metabolism in the aetiology of NTD in black subjects, the relevance of folate supplementation in this population group to prevent NTD needs further investigation.

References

1. Buccimazza SS, Molteno CD, Dunne TT, Viljoen DL. Prevalence of neural tube defects in Cape Town, South Africa. *Teratology* 1994; **50:** 194-199.
2. Delport SD, Christianson AL, Van den Berg HJS, Wolmarans L, Gericke GS. Congenital anomalies in black South African liveborn neonates born in an urban academic hospital. *S Afr Med J* 1995; **85:** 11-15.
3. Ncayiyana DJ. Neural tube defects among rural blacks in a Transkei district. *S Afr Med J* 1986; **69:** 618-620.
4. Venter PA, Christianson Al, Hutamo CM, Makhura MP, Gericke GS. Congenital anomalies in rural black South African neonates — a silent epidemic? *S Afr Med J* 1995; **85:** 15-20.
5. Steegers-Theunissen RPM, Boers GHJ, Trijbels FJM, *et al*. Maternal hyperhomocysteinemia: A risk factor for neural-tube defects? *Metabolism* 1994; **43:** 1475-1480.
6. Mills JL, McPartlin JM, Kirke PN, *et al*. Homocysteine metabolism in pregnancies complicated by neural-tube defects. *Lancet* 1995; **345:** 149-151.
7. Lucock MD, Wild J, Lumb CH, *et al*. Risk of neural tube defect-affected pregnancy is associated with a block in maternal one-carbon metabolism at the level of N-5-methyltetrahydrofolate: homocysteine methyltransferase. *Biochem Mol Med* 1997; **61**: 28-40.
8. Steegers-Theunissen RP, Boers GH, Blom HJ, *et al*. Neural tube defects and elevated homocysteine levels in amniotic fluid. *Am J Obstet Gynecol* 1995; **172:** 1436-1441.
9. Frosst P, Blom HJ, Milos R, *et al*. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995; **10:** 111-113.
10. Van der Put NMJ, Steegers-Theunissen RPM, Frosst P, *et al*. Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. *Lancet* 1995; **346:** 1070-1071.
11. Nishio H, Lee MJ, Fujii M, *et al*. A common mutation in methylenetetrahydrofolate reductase gene among the Japanese population. *Jpn J Hum Genetics* 1996; **41:** 247-251.
12. Jacques PF, Bostom AG, Williams RR, *et al*. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase and plasma homocysteine concentrations. *Circulation* 1996; **93:** 7-9.
13. Harmon DL, Woodside JV, Yarnell JW, *et al*. The common "thermolabile" variant of methylenetetrahydrofolate reductase is a major determinant of mild hyperhomocysteinaemia. *QJM* 1996; **89:** 571-577.
14. Malinow MR, Nieto FJ, Kruger WD, *et al*. The effects of folic acid supplementation on plasma total homocysteine are modulated by multivitamin use and methylenetetrahydrofolate reductase genotypes. *Arterioscler Thromb Vasc Biol* 1997; **17:** 1157-1162.
15. Ou CY, Stevenson RE, Brown VK, *et al*. 5,10 Methylenetetrahydrofolate reductase genetic polymorphism as a risk factor for neural tube defects. *Am J Med Genet* 1996; **63:** 610-614.
16. Medical Research Council Research Group. Prevention of neural tube defects: Results of the Medical Research Council vitamin study. *Lancet* 1991; **338:** 131-137.
17. Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* 1992; **327:** 1832-1835.
18. Wald NJ, Hackshaw AK, Stone R, Sourial NA. Blood folic acid and vitamin B_{12} in relation to neural tube defects. *Br J Obstet Gynaecol* 1996; **103:** 319-324.
19. Kirke PN, Molloy AM, Daly LE, Burke H, Weir DG, Scott JM. Maternal plasma folate and vitamin B_{12} are independent risk factors for neural tube defects. *QJM* 1993; **86:** 703-708.
20. Daly LE, Kirke PN, Molloy A, Weir DG, Scott JM. Folate levels and neural tube defects. Implications for prevention. *JAMA* 1995; **274:** 1698-1702.
21. Kirke PN, Molloy AM, Daly LE, Burke H, Weir DG, Scott JM. Maternal plasma folate and vitamin B12 are independent risk factors for neural tube defects. *QJM* 1993; **86:** 703-708.
22. Baynes RD, Meriwether WD, Bothwell TH, Costa FJF, Bezwoda WR, MacPhail AP. Iron and folate status of pregnant black women in Gazankulu. *S Afr Med J* 1986; **70:** 148-151.
23. Van der Westhuyzen J, van Tonder S, Gilbertson I, Metz J. Iron, folate and vitamin B_{12} nutrition and anaemia in black preschool children in the northern Transvaal. *S Afr Med J* 1986; **70:** 143-145.
24. Allen WP. Folic acid in the prevention of birth defects. *Curr Opin Pediatr* 1996; **8:** 630-634.
25. Ubbink JB, Vermaak WJH, van der Merwe A, Becker PJ, Potgieter H. Effective homocysteine metabolism may protect South African Blacks against coronary heart disease. *Am J Clin Nutr* 1995; **62:** 802-808.
26. Bourne LT, Bourne DE, Sayed AR, Wolfaardt D. Folate levels in an urban population — implications for supplementation for the prevention of neural tube defects. *S Afr Med J* 1997; **87**: 932-933.