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Clinical Paper
Cleft Lip and Palate

Screening for maternal coeliac disease as a potential risk factor for orofacial clefts—a pilot study

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Abstract. There is increasing evidence that dietary folic acid deficiency in utero may increase the risk of developing the 'cleft lip with or without cleft palate' (CL ± P) variant of orofacial cleft. Coeliac disease is a common cause of folic acid malabsorption, and in the majority of cases remains undiagnosed. This pilot study assessed the seroprevalence of undiagnosed coeliac disease in a cohort of mothers of infants with CL ± P in the Hyderabad area of India. The seroprevalence of coeliac disease of 1.15% (95% confidence interval 0.37–2.66%) was little different from the expected figure based on published population studies, making a clinically significant association unlikely.

Key words: cleft lip; cleft palate; coeliac disease.

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Non-syndromic orofacial cleft (OFC) is one of the most common congenital malformations, found in up to 0.2% of live births in Europe[1–3] and India.[4] It is characterized by incomplete separation of the nasal and oral cavities without any associated anomalies, and is composed of two distinct but related entities: cleft lip with or without cleft palate (CL ± P) and cleft palate isolated (CPI). The aetiology of non-syndromic OFC is complex and incompletely understood, but there appear to be genetic and environmental factors, with distinct differences between CL ± P and CPI. The genetic influence is proportionately greater for CPI than for CL ± P.[5] Predisposing environmental factors include folic acid deficiency – this is known to cause OFC in rodents,[6] and there is evidence for a similar consequence in humans – both as a consequence of dietary insufficiency and maternal use of dihydrofolate reductase inhibitors.[2,7–9] Furthermore, a recent large case–control study in Norway showed that folic acid supplementation during early pregnancy reduced the risk of CL ± P by 39%, whilst there was no effect on the risk of CPI.[3]

Coeliac disease (CD) is a chronic inflammatory disorder of the small bowel, with characteristic mucosal histology.[10] Whilst the pathogenesis is not fully understood, there is overwhelming evidence that CD results from mucosal exposure in genetically predisposed individuals to dietary gluten, a family of related proteins found in the cereals wheat, barley, and rye. The immune system is strongly implicated in mediation of the inflammatory response in CD,[10] and one manifestation of this is that most patients with untreated CD acquire circulating autoantibodies, in particular endomysial antibody (EMA) – the dominant component of which is directed against the enzyme, tissue transglutaminase 2.[10] EMA has a relatively high sensitivity and specificity for untreated CD, and has therefore been used widely to determine the seroprevalence of CD in population studies.[10,11]

CD has been increasingly recognized in Asian populations over recent years. Recent seroprevalence studies would suggest that CD is as common in Indian populations as it is elsewhere in the world, with figures in the range 0.5–1.3%.[12–15] The majority of individuals with CD are

undiagnosed (and therefore untreated) even in Western countries, leading to the concept of the 'coeliac iceberg'.[16] Undiagnosed CD commonly results in silent malabsorption of vitamins and nutrients, in particular folic acid.[10] The question arises, therefore, as to whether undiagnosed maternal CD with consequent folic acid deficiency might predispose to development of the CL ± P variant of OFC.

Materials and methods

The overall study plan involved two stages: firstly a pilot study to assess the seroprevalence of CD in a cohort of mothers of infants with CL ± P, and secondly, if the results of the pilot study suggested that an increased prevalence was likely, a large case–control study to define the risk more precisely. The current report concerns stage one, the pilot study, for which formal ethics committee approval was obtained.

The mothers of infants with non-syndromic CL ± P seen at a high volume cleft centre in Hyderabad, South India between November 2009 and November 2011 were identified. Subjects were excluded if they (1) were aged under 18 or over 30 years, (2) had a previous child with OFC, or (3) had an established diagnosis of CD. Following informed consent, a small blood sample was taken and serum stored for batch testing.

Bluewell enzyme-linked immunosorbent assay (ELISA) kits (D-tek, Mons, Belgium) were used for the quantification of IgA antibodies to gliadin-activated transglutaminase in human serum. An antibody concentration greater than 25 U/ml was considered positive, as recommended by the manufacturer. Mothers testing positive were informed of the result and encouraged to take appropriate medical advice. Exact confidence intervals (CI) for CD seroprevalence were calculated from the binomial distribution using Bland's Biconf software.[17]

Results

The mothers of 436 infants with CL ± P seen during the study period met the inclusion criteria and agreed to serological testing. The maternal age range was 18–30 years (median 23 years). The malformation pattern was cleft lip and palate in 379 of the infants and isolated cleft lip in the remaining 57. Of the 436 mothers, five (1.15%) proved to be transglutaminase antibody-positive (95% CI 0.37–2.66%).

Discussion

The increasing evidence implicating dietary folate deficiency in the pathogenesis of CL ± P[2,3,6–9] raises a realistic possibility that folate malabsorption might have a similar influence. CD is the most common cause of folate malabsorption in many populations of the world – and as malabsorption is generally partial rather than complete, the consequences of this are likely to be most striking in parts of the world where dietary nutrient intake is already limited. The pilot study reported here was designed to explore the possibility of an association between CL ± P and undiagnosed maternal CD in the Hyderabad area of India. This lies on the Deccan plateau, where gluten-containing cereals form a major part of the staple diet.

It is important to establish whether a link between OFC and maternal CD exists, because CD can be screened for by widely available serological testing.[10,11] Once diagnosed, treatment by exclusion of dietary gluten might then be expected to reduce the subsequent risk of intra-uterine complications. The possibility of an association between OFC and maternal CD has previously been raised in a small study, but the finding of one subject with positive coeliac serology amongst 37 mothers of infants with CL ± P neither confirms nor refutes this.[18]

Studies of the seroprevalence of CD in many populations around the world including India have almost invariably yielded figures in the range 0.5–1.5%.[10–15] A seroprevalence in our study group substantially greater than this would imply that CD might indeed be a significant factor underlying the development of CL ± P, and would justify a more detailed study with an appropriate control group.

In the event, the seroprevalence of CD in the study participants of 1.15% (95% CI 0.37–2.66%) was little different from the expected figure based on published population studies. This does not entirely exclude the possibility of an association, but it does make it unlikely that there is a clinically significant link. Furthermore, it implies that an extremely – and probably unrealistically – large case–control study would be required to have sufficient power to detect a statistically significant association.

Given evidence relating dietary folic acid deficiency to the risk of CL ± P, one can only speculate as to the reason(s) for the negative result of our study. There are several potential reconciling explanations to consider. Firstly, it might be that the sensitivity of serology for CD in the study group is unexpectedly poor, as it is in some subgroups of patients with CD such as smokers and the elderly.[19] Secondly, the assumption made about the population prevalence of CD in the Hyderabad area might be invalid. Thirdly, the result might be the equivalent of a type II statistical error.[17] Finally, it might perhaps be that in maternal malabsorptive states, dietary folic acid is in some poorly defined way preferentially channelled to the foetus. With regard to this possibility, our findings are in accord with studies showing no excess of CD in the mothers of children with neural tube defects, for which there is also evidence of a link with dietary folic acid deficiency.[20,21]

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Competing interests

None declared.

Ethical approval

Ethical approval was given by the Independent Ethics Committee of the GSR Institute of Craniofacial and Facial Plastic Surgery on 11 April 2009, with reference number GSR-EC-02-2009.

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References

1. Mossey PA, Little J, Munger RG, Dixon MJ, Shaw WC. Cleft lip and palate. *Lancet* 2009;**374**:1773–86.
2. Kelly D, O'Dowd T, Reulbach U. Use of folic acid supplements and risk of cleft lip and palate in infants: a population-based cohort study. *Br J Clin Pract* 2012;**62**:466–72.
3. Wilcox AJ, Lie RT, Solvoll K, Taylor J, McConnaughey DR, Vindenes H, et al. Folic acid supplements and risk of facial clefts: national population-based case–control study. *Br Med J* 2007;**334**:464–7.
4. Reddy SG, Reddy RR, Bronkhorst EM, Prasad R, Ettema AM, Sailer HF, et al. Incidence of cleft lip and palate in the state of Andhra Pradesh. *South India Indian J Plast Surg* 2010;**43**:184–9.
5. Sivertsen A, Wilcox AJ, Skjaerven R, Vindenes HA, Abyholm F, Harville E, et al. Familial risk of oral clefts by morphological type and severity: population-based cohort

study of first-degree relatives. *BMJ* 2008;**336**:432–4.

[6] Munger RG. Maternal nutrition and oral clefts. In: Wyszynski DF, editor. *Cleft lip and palate: from origin to treatment*. Oxford: Oxford University Press; 2002. p. 170–92.

7. Shaw GM, Lammer EJ, Wasserman CR, O'Malley CD, Tolarova MM. Risks of orofacial clefts in children born to women using multivitamins containing folic acid periconceptionally. *Lancet* 1995;**346**:393–6.
8. van Rooij IA, Ocke MC, Straatman H, Zielhuis GA, Merkus HM, Steegers-Theunissen RP. Periconceptional folate intake by supplement and food reduces the risk of nonsyndromic cleft lip with or without cleft palate. *Prev Med* 2004;**39**:689–94.
9. Hernandez-Diaz S, Werler MM, Walker AM, Mitchell AA. Folic acid antagonists during pregnancy and the risk of birth defects. *N Engl J Med* 2000;**343**:1608–14.
10. Green PH, Cellier C. Coeliac disease. *N Engl J Med* 2007;**357**:1731–43.
11. Rostom A, Dubé C, Cranney A, Saloojee N, Sy R, Garritty C, et al. The diagnostic accuracy of serological tests for coeliac disease: a systematic review. *Gastroenterology* 2005; **128**(Suppl. 1):S38–46.
12. Sood A, Midha V, Sood N, Avasthi G, Sehgal A. Prevalence of coeliac disease among school children in Punjab. *North India J Gastroenterol Hepatol* 2006;**21**:1622–5.
13. Yachha SK, Poddar U. Celiac disease in India. *Indian J Gastroenterol* 2007;**26**:230–7.
14. Kumar A, Meena M, Begum N, Kumar N, Gupta RK, Aggarwal S, et al. Latent coeliac disease in reproductive performance of women. *Fertil Steril* 2011;**95**:922–7.
15. Makharia GK, Verma AK, Amarchand R, Bhatnagar S, Das P, Goswami A, et al. Prevalence of celiac disease in the northern part of India: a community based study. *J Gastroenterol Hepatol* 2011;**26**:894–900.
16. Catassi C, Ratsch IM, Fabiani E, Rossini M, Bordicchia F, Candela F, et al. Coeliac disease in the year 2000: exploring the iceberg. *Lancet* 1994;**343**:200–3.
17. Bland M. *An introduction to medical statistics*. 3rd ed. Oxford: Oxford University Press; 2009.
18. Hozyasz KK. Coeliac disease and birth defects in offspring. *Gut* 2001;**49**:738–42.
19. Boger P, Thomas PW, Nicholas DS, Surgenor SL, Snook JA. Determinants of endomysial antibody status in untreated coeliac disease. *Eur J Gastroenterol Hepatol* 2007;**19**:890–5.
20. Dickey W, Stewart F, Nelson J, McBreen G, McMillan SA, Porter KG. Screening for coeliac disease as a possible maternal risk factor for neural tube defect. *Clin Genet* 1996;**49**:107–8.
21. De Almeida RC, Lima BO, Castro LC, Gandolfi L, Riccardo P. Maternal celiac disease: improbable risk factor for neural tube defect. *Eur J Gastroenterol Hepatol* 2009;**21**:805–8.

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