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Oral pathology in untreated coelic disease

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SUMMARY

Background
Many coeliac disease patients with atypical symptoms remain undiagnosed.

Aim
To examine the frequency of oral lesions in coeliac disease patients and to assess their usefulness in making coeliac disease diagnosis.

Patients and methods
One hundred and ninety-seven coeliac disease patients and 413 controls were recruited and the oral examination was performed.

Results
Forty-six out of 197 coeliac disease patients (23%) were found to have enamel defects vs. 9% in controls ($P < 0.0001$). Clinical delayed eruption was observed in 26% of the pediatric coeliac disease patients vs. 7% of the controls ($P < 0.0001$). The prevalence of oral soft tissues lesions was 42% in the coeliac disease patients and 2% in controls ($P < 0.0001$). Recurrent aphthous stomatitis disappeared in 89% of the patients after 1 year of gluten-free diet. Multi-logistic analysis selected the following variables as the most meaningful in coeliac disease patients: dental enamel defects (OR = 2.652 CI = 1.427–4.926) and soft tissue lesions (OR = 41.667, CI = 18.868–90.909). Artificial Neural Networks methodology showed that oral soft tissue lesions have sensitivity = 42%, specificity = 98% and test accuracy = 83% in coeliac disease diagnosis.

Conclusions
The overall prevalence of oral soft tissue lesions was higher in coeliac disease patients (42%) than in controls. However, the positive-predictive value of these lesions for coeliac disease diagnosis was low.

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INTRODUCTION

Coeliac disease (CD), one of the most common chronic diseases among Caucasians, is an autoimmune enteropathy triggered by the ingestion of gluten-containing grains in genetically predisposed subjects. During recent decades, the use of serological assays with a good diagnostic accuracy has shown that its frequency is much higher than previously considered, ranging between 1:85 and 1:300 both in Europe and in the USA.[1–7] At the same time, it has become evident that the 'typical' clinical presentation of CD, i.e. the malabsorption syndrome, is nowadays less frequent and many CD patients show 'atypical' – non-gastroenterological – symptoms or are asymptomatic[8–12]. This different clinical presentation makes diagnosis difficult; in fact, a serological screening study revealed that the ratio of known CD cases to undiagnosed CD cases was 1–7.[13] Lack of diagnosis may have important consequences as dietary avoidance of gluten determines remission of the disease and avoids the onset of malignancy and thus decreases mortality in CD patients.[14–16] Consequently, paediatricians, gastroenterologists and internists must look beyond the intestine to suspect a CD diagnosis. In fact, haematological, dermatological, neurological, obstetric, gynecological and the most proteiform clinical presentations of CD should be considered.[17, 18] In this respect, the oral cavity, an apparatus which is very easy to examine, can be very interesting. In fact, mainly recurrent aphthous stomatitis (RAS) and dental enamel defects have been reported to be associated with CD.[19–24]

The primary aim of this study was to assess the frequency of oral diseases, including soft and hard tissue lesions in CD patients, in comparison with otherwise healthy controls; the secondary objective was to consider what contribution an oral clinical examination could give to help suspect a CD diagnosis.

PATIENTS AND METHODS

The total study population of this prospective study consisted of 610 subjects; of these, 197 were CD patients, recruited at the time of diagnosis. They were consecutively enrolled, between January 2004 and June 2005: the adult patients (90 cases: 65 F, 25 M, age range 18–75 years, median 31) in two centres – gastroenterology and internal medicine – of the University Hospital of Palermo and the children (107 cases: 59 F, 48 M, age range 2–17 years, median 9 years) in the Pediatric Gastroenterology Unit of the 'Di Cristina Hospital' in Palermo.

Coeliac disease patients aged under 2 years were excluded.

Four hundred and thirteen healthy subjects who were age/sex-matched (180 adults: 130 F, 50 M age range 19–77 years, median 32; 233 children: 120 F, 113 M, age range 2–17 years, median 8.5) and living in the same geographical area as that of the CD group were enrolled as controls. Paediatric controls were recruited (by simple randomization) at a day nursery, and at Primary and Secondary Schools during a health prevention programme for oral diseases; these subjects did not refer any diseases, had no family history of CD and showed normal growth (weight/height ratio between 25th and 75th centiles). Adult controls were recruited among otherwise healthy patients consecutively referred to the Dental Unit of the University of Palermo for third molar surgery; they were tested for serum anti-transglutaminase (anti-tTG) antibodies and were negative.

Coeliac disease diagnosis was based on the positivity of serum anti-tTG and/or anti-endomysium (EmA) antibodies, presence of clinical symptoms and positive histological evidence of villous atrophy with crypt hyperplasia and increase in intraepithelial lymphocytes on a gluten-containing diet, and the disappearance of the symptoms and normalization of serum anti-tTG and/or EmA on a gluten-free diet (GFD).[25]

The clinical manifestations of CD were classified as 'typical' when they included: chronic diarrhoea, failure to thrive, anorexia, abdominal distension and muscle wasting; other clinical manifestations were considered 'atypical'.[17] When CD diagnosis was made in subjects who were apparently asymptomatic, it was classified as 'silent'.

Immediately after CD diagnosis, the patients underwent an intra-oral examination of the soft and hard tissues. All evaluations were performed independently by two of the authors (C. D. and D. L. C., who were trained in oral health survey) and then tested for concordance.

We focused on hard tissue lesions (i.e. dental enamel defects), soft tissue lesions (e.g. presence of RAS, aspecific atrophic glossitis and geographic tongue) and clinical delay of dental eruption.

The enamel defects affecting deciduous and permanent teeth were graded 0–IV according to Aine's classification.[26] As regard RAS, we included recurrent ulcerative lesions clinically observed by two of the authors (C. D. and D. L. C.) during the intra-oral

examination. However, as clinical evidence of the lesions is not always found because healing occurred before the oral investigation, but patients could have had a past history of aphthous ulcers, we also considered ulcerative events, noted by parents or patients, or reported in hospital clinical records, with clinical features pathognomonic of RAS.

To evaluate delayed eruption in the paediatric patients, we used the conventional eruption tables for the Caucasian population[27] and we considered delayed eruption as when the teeth were not in arch after their normal age of eruption, with a range of ±6 months.

In all individuals, dental hygiene was categorized into nominal variables using three values: 0 (poor), 1 (sufficient) and 2 (good).[28]

All CD patients with oral soft tissue lesions were re-evaluated 1 year after the beginning of GFD.

Finally, all the paediatric controls positive for oral hard and/or soft tissue lesions potentially associated with CD were tested for anti-tTG antibodies to exclude the disorder.

Informed consent was obtained for all participants in the study and the study was approved by the Ethics Committee of the University Hospital of Palermo.

Statistical analysis

Data were analysed by means of SAS for Windows ver. 9.0, (SAS Institute Inc., Cary, NC, USA) and by means of STATISTICA 6.0 (Statsoft Inc., Tulsa, USA). To measure the association level, crude odds ratio (OR) and the 95% corresponding test-based CI were calculated. Student's *t*-test was used to calculate significant differences between cases and controls at baseline for normally distributed variables. The Mann–Whitney *U*-test was used to calculate differences between not-normally distributed variables (e.g. Aine's scores).

The concordance rate of the oral evaluation between the observers was evaluated using Cohen's kappa statistic, measuring agreement beyond that expected by chance (expressed as a coefficient ranging from 0 to 1.00).[29]

The relationship between CD and other variables was analysed within conditional multivariate frameworks.

A conditional logistic regression model was constructed stratified by age and dental hygiene; the same model permitted a stepwise selection procedure to obtain the most parsimonious model. The maximum likelihood ratio and adjusted OR were obtained using the iterative weighted least squares procedure. In all of the evaluations, *P*-values ≤0.05 were considered statistically significant.

Furthermore, in a blind manner, three Artificial Neural Network (ANNs) models were applied to the same data, as previously described.[30] Fully-connected multilayer feedforward networks were used. The learning rule employed was the well-known back-error propagation, which adjusts the internal parameters of the networks over the repeated training cycles to reduce the overall error.[31] The networks were validated with a new set of data, different from the training ones. The performance measured by mean squared error (MSE), accuracy, sensitivity and specificity values as well as the receiver operating characteristic (ROC) area, which can ascertain the degree of meaningful prediction,[32, 33] was calculated for the significant associations. In this study, all variables were selected as input ones, except for CD (the output variable) for ANNs system analysis.

RESULTS

There was a substantial agreement (kappa = 0.85) between the observers for the main dental and oral lesions.

Table 1 summarizes the main findings of the oral examination in CD patients and controls.

Forty-six out of 197 CD patients (23%) were found to have systematic and symmetrical enamel defects vs. a lower rate of 9% (37/413) in controls [$P < 0.0001$; OR = 3.510 (95% CI = 2.135:5.770)]. The frequency of the enamel defects was very similar in the adult and paediatric CD patients. The severity of enamel defects in CD patients, evaluated according to Aine (17), was: grade I in 87%, grade II in 11% and grade IV in 2%. Figure 1a,b shows grades 1 and 4 lesions, respectively, observed in CD patients who were included in the study.

Clinical delayed eruption was observed in 28 out of 107 paediatric CD patients (27%) vs. 16 out of 233 (7%) controls [$P < 0.0001$; OR = 5 .932 (95% CI = 3.407:10.330)].

The overall prevalence of oral soft tissue lesions was 42% (82/197) in CD patients and 9/413 in controls (2%) [$P < 0.0001$; OR = 22.257 (95% CI = 13.828:35.824)]; frequency was similar in adult and paediatric CD. RAS was found in 37/197 (19%) CD patients vs. 3/413 (1%) controls [$P < 0.0001$; OR = 18.9505 (95% CI = 9.552:37.595)]. In CD

Table 1. Oral examination findings in coeliac disease (CD) patients and controls, grouped by adult and paediatric age

	Adult individuals				Paediatric individuals			
	CD patients (n = 90)	Controls (n = 180)	P-value	Unadjusted OR (95% CI)	CD patients (n = 107)	Controls (n = 233)	P-value	Unadjusted OR (95% CI)
Dental enamel defects (%)								
No	69 (77)	164 (92)	0.001	3.12 (1.53–6.33)	82 (77)	212 (91)	0.0003	3.07 (1.63–5.80)
Yes	21 (23)	16 (8)			25 (23)	21 (9)		
Soft tissue lesions (%)								
No	56 (62)	177(97)	<0.0001	21.49 (8.02–57.59)	59 (55)	229 (98)	<0.0001	46.57 (16.14–134)
Yes	34 (38)	5 (3)			48 (45)	4 (2)		
Recurrent aphthous stomatitis (%)								
No	71 (79)	179 (99.5)	<0.0001	47.36 (6.22–360.53)	89 (83)	231 (99)	<0.0001	23.35 (5.31–102)
Yes	19 (21)	1 (0.5)			18 (17)	2 (1)		

patients, RAS was directly observed during the medical visit in 34 cases and simply recalled by the parents in three cases. Figure 1c,d shows an image of RAS observed in CD patients who were included in the study. The other soft tissue lesions detected were aspecific atrophic glossitis and geographic tongue. The first was found in 31/197 (16%) CD patients vs. 1/413 (0.2%) controls [$P < 0.0001$; OR = 22.464 (95% CI = 10.500:48.063)], while geographic tongue was noted in 14/197 (7%) CD patients vs. 5 out of 413 (1%) controls [$P < 0.0001$; OR = 7.0326 (95% CI = 2.650:18.666)]. CD patients showed a better dental hygiene status than controls [$P < 0.0001$; OR = 4.848 (95% CI = 2.7027:8.695)].

None of paediatric controls with oral hard and/or soft tissue lesions presented positive serological markers for CD.

As regards the clinical manifestations of CD, 'typical' symptoms were more often observed in children (60/107 cases, 56%) than in adults (40/90 cases, 44%), whereas the frequency of silent cases was similar (7% in adults vs. 7% in children). However, oral hard or soft tissue lesions were observed with an almost identical frequency in patients with 'typical' and 'atypical' CD symptoms. Furthermore, as among patients without any signs and symptoms potentially related to CD, as diagnosed during familial CD screening, we found cases of oral soft tissue lesions, they should be

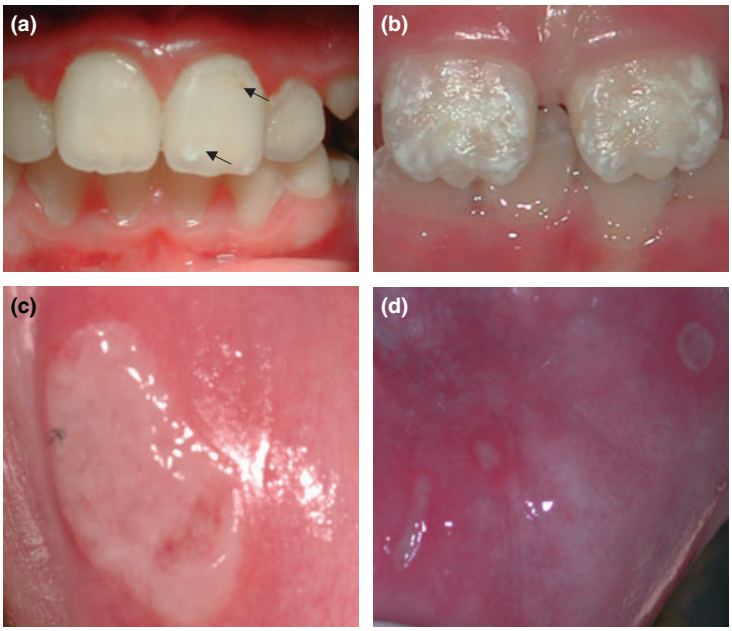

Figure 1. (a) Enamel defects of upper incisors (arrows indicate the color changes); (b) Enamel defects of upper incisors (structural changes); (c) major type of recurrent aphthous stomatitis and (d) numerous minor-type lesions of RAS.

considered as patients with 'atypical' and not with 'silent' CD. In CD patients, the frequency of the oral hard and/or soft tissue lesions did not differ according to the severity of intestinal mucosa damage or to the ideal body weight.

After a 1-year follow-up, 33 out of 37 CD patients (89%) with RAS at diagnosis referred that they had no longer suffered from RAS since beginning the GFD. The other four patients (11%) did not strictly adhere to GFD, as confirmed by persistently elevated serum anti-tTG antibodies, and did not report any improvement in RAS recurrence and number of ulcers per episode. Also, atrophic glossitis disappeared in all the patients who adhered to GFD. As regards geographic tongue, no cases were present in the sample followed longitudinally.

Conditional multi-logistic analysis in the stepwise procedure selected Oral Mucosa Lesions and Dental Enamel Hypoplasia as the most meaningful variables in CD patients (Table 2).

Artificial Neural Networks methodology consistently proved that CD was the most meaningful variable related to soft tissue lesions in the present dataset, with MSE equal to 0.321. Performance indexes showed the following values: accuracy = 83%; sensitivity = 42%, specificity = 98%. The ROC area was equal to 0.83.

On the basis of the 3% prevalence of CD recorded in our centres during the study period, and on the basis of the sensitivity and specificity shown by the ANN methodology, the positive and negative predictive values of the oral lesions were 39% and 99%, respectively. On the basis of the 1% CD prevalence in the general population, the positive and negative predictive values of the oral lesions in CD diagnosis were 17% and 99%, respectively.

DISCUSSION

Recent epidemiology data have shown a prevalence of CD approaching 1% in the general population.[34–36] However, there has been a noticeable change in the clinical presentation of CD, as almost 50% of the patients with newly diagnosed CD do not present with gastrointestinal (GI) symptoms[37, 38] thus making diagnosis difficult. This is a paramount aspect as a GFD in CD patients is known to prevent many of the extra-intestinal symptoms, such as osteoporosis,[39] recurrent abortions,[40] and above all, it protects against the development of cancer.[41] Thus, to identify the greatest number of 'atypical' or 'silent' CD patients and prevent complications, clinicians must investigate 'at-risk subjects', e.g. those with chronic anaemia,[42, 43] hypertransaminasemia or hyperamylasemia of unknown origin,[44, 45] osteoporosis,[39] autoimmune thyroid disorders.[46] Furthermore, it is known that CD is an auto-immune disease resulting from an inappropriate T cell-mediate immune response against ingested gluten.[47] Although the proximal part of the intestinal mucosa represents the main site of the gut involved in CD, it has been demonstrated that gluten-driven T-cell activation is not restricted to the small intestine, but is present in the whole GI tract. The mouth, the first part of the GI system, represents a site very easy to detect and an oral examination could give a useful diagnostic contribution as lesions of the hard[48, 49] and soft tissues[50, 51] have been reported in CD.

Ours is the largest uni-centre study to have investigated the risk for CD patients of suffering from dental or oral mucosa lesions and shown the sensitivity and specificity of oral soft tissue lesions in suggesting a CD diagnosis.

As regards the hard tissues, we found systematic and symmetrical enamel defects in 23% of CD patients, with an OR >3 in comparison with the controls. The enamel defects resulted in a dental deformity, which can be easily recognized (see Figure 1), although low-grade lesions must also be accurately investigated. Other studies have reported a wide range of frequencies of enamel defects in CD patients,[26, 48, 52–59], but our data are in agreement with other studies performed in Italy, and the differences in frequency probably depend on environmental, dietetic and, above all, genetic factors.[54] The same hypothesis could be made for the severity of enamel defects which appeared less severe in our study (87% of the patients had a grade I lesion) than in other studies.[48]

The aetiopathogenesis of these defects in CD patients still remains unclear. As the crowns of deciduous and permanent teeth develop from 4 to 5 months of

Table 2. Conditional multi-logistic analysis in the stepwise procedure. Characteristics and risk factors were stratified by age and oral care

	Odds ratio	95% CI
Gender (female vs. male)	1.980	1.253–3.130
Dental enamel hypoplasia	2.652	1.427–4.926
Oral soft tissue lesions	41.667	18.868–90.909

intra-uterine life to the 7th year of life except for the third molar, nutritional, immunological or genetic factors (association with the HLA DR3 allele) have been hypothesized as causing developmental defects in the enamel.[53, 60] Hypocalcaemia caused by malabsorption during dental development has been considered to be implicated in enamel hypoplasia. Regarding the nutritional dynamics, despite some doubts raised by Maki *et al.*,[60] it has been hypothesized that a gluten-induced immunological process could occur between 6 months and 7 years in the enamel-producing organ, resulting in defective enamel formation. Finally, these dental anomalies have been found to be significantly related to HLA antigen DR3.[53, 56, 60] Furthermore, an Italian study,[53] reporting a frequency of 29% in coeliac patients vs. 15% in controls, did not report any statistically significant differences in calcium concentrations, but a coincidence in 77% of CD and enamel defects in DR3-positive subjects. Another finding on dental hard tissues was the significantly higher frequency of delayed eruption, observed in 27% of CD children. Only few papers in the literature have dealt with this issue,[49, 61–63] and they are in agreement with our data. The delayed dental eruption could be seen as a possible sign of malnutrition (such as is delayed puberty) and advises for serological CD screening.

However, the most important finding of the present study is related to the oral soft tissue investigation: in fact, mucosa lesions were found in 42% of CD patients with an OR of 22 vs. controls. Within this group of lesions, RAS was found in 37/197 (19%) CD patients with an OR of 19 vs. controls. RAS frequency in CD observed in our study is in agreement with that reported by other authors.[64, 65] It is also very interesting that almost all (89%) the CD patients with RAS no longer suffered after beginning GFD and the lack of healing in the remaining patients was probably linked to the lack of adherence to GFD. Consequently, RAS persistence in CD patients could cautiously be considered a marker of lack of GFD adherence. This hypothesis is supported by a study which reported that RAS and intestinal histological alterations relapsed after gluten challenge.[66]

Although several studies have reported the presence of oral mucosa lesions in CD, our study reports the first evaluation of the risk of such lesions in CD patients using univariate, multi-logistic regression, ANN sensitivity and specificity testing.

However, despite a good test accuracy, the presence of oral lesions showed a low positive-predictive value of 16%, giving in the general population a 1% prevalence of CD.

In conclusion, our study showed a higher frequency of oral alterations in CD patients in comparison with healthy controls. However, the presence of these lesions had a low positive-predictive value in CD diagnosis.

AUTHORS' CONTRIBUTIONS

All the authors participated in drafting the article and/or revising it critically and gave the final approval of the version to be published. Furthermore, in particular, they worked as follows: Giuseppina Campisi, Chief Investigator of the Dental Care Unit, did the conception and design of the study and interpretation of the data. Chiara Di Liberto, Investigator of the Dental care Unit, was in charge of the acquisition of data. Giuseppe Iacono, Chief investigator of the Paediatric Gastroenterology Unit, was in charge of the acquisition of data. Domenico Compilato, Investigator of the Dental care Unit, was in charge of the acquisition and interpretation of data. Lidia Di Prima and Carmela Sferrazza, Investigators of the Internal Medicine Unit, were in charge of the acquisition of data. Francesco Calvino was responsible for the statistical analysis, analysis and interpretation of the data. Vito Di Marco, Investigator of the Gastroenterology Unit, was in charge of the acquisition of data. Lorenzo Lo Muzio did the analysis and interpretation of data. Calogero Scalici, Investigator of the Paediatric Gastroenterology Unit, was in charge of the acquisition of data. Craxì Antonio, Chief Investigator of the Gastroenterology Unit, did the conception and design of the study. Carroccio Antonio, Chief Investigator of the Internal Medicine Unit, did the conception and design of the study and interpretation of data.

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REFERENCES

1 Korponay-Szabo IR, Kovacs JB, Czinner A, Goracz G, Vamos A, Szabo T. High prevalence of silent celiac disease in preschool children screened with IgA/IgG antiendomysium antibodies. *J Pediatr Gastroenterol Nutr* 1999; **28**: 26–30.

2 Hill ID, Bhatnagar S, Cameron DJ, *et al.* Celiac disease: Working Group Report of the First World Congress of pediatric gastroenterology, hepatology, and nutrition. *J Pediatr Gastroenterol Nutr* 2002; **35** (Suppl. 2): S78–88.

3 Catassi C, Ratsch IM, Fabiani E, *et al.* High prevalence of undiagnosed coeliac disease in 5280 Italian students screened by antigliadin antibodies. *Acta Paediatr* 1995; **84**: 672–6.

4 Kolho KL, Farkkila MA, Savilahti E. Undiagnosed coeliac disease is common in Finnish adults. *Scand J Gastroenterol* 1998; **33**: 1280–3.

5 Carlsson AK, Axelsson IE, Borulf SK, Bredberg AC, Ivarsson SA. Serological screening for celiac disease in healthy 2.5-year-old children in Sweden. *Pediatrics* 2001; **107**: 42–5.

6 Not T, Horvath K, Hill ID, *et al.* Celiac disease risk in the USA: high prevalence of antiendomysium antibodies in healthy blood donors. *Scand J Gastroenterol* 1998; **33**: 494–8.

7 Cataldo F, Montalto G. Celiac disease in the developing countries: a new and challenging public health problem. *World J Gastroenterol* 2007; **13**: 2153–9.

8 Lo W, Sano K, Lebwohl B, Diamond B, Green PH. Changing presentation of adult celiac disease. *Dig Dis Sci* 2003; **48**: 395–8.

9 Steens RF, Csizmadia CG, George EK, Ninaber MK, Hira Sing RA, Mearin ML. A national prospective study on childhood celiac disease in the Netherlands 1993–2000: an increasing recognition and a changing clinical picture. *J Pediatr* 2005; **147**: 239–43.

10 Ravikumara M, Tuthill DP, Jenkins HR. The changing clinical presentation of coeliac disease. *Arch Dis Child* 2006; **91**: 969–71.

11 Torres MI, Lopez Casado MA, Rios A. New aspects in celiac disease. *World J Gastroenterol* 2007; **13**: 1156–61.

12 West J, Logan RF, Hill PG, Khaw KT. The iceberg of celiac disease: what is below the waterline? *Clin Gastroenterol Hepatol* 2007; **5**: 59–62.

13 Catassi C, Ratsch IM, Fabiani E, *et al.* Coeliac disease in the year 2000: exploring the iceberg. *Lancet* 1994; **343**: 200–3.

14 Swinson CM, Slavin G, Coles EC, Booth CC. Coeliac disease and malignancy. *Lancet* 1983; **1**: 111–5.

15 Corrao G, Corazza GR, Bagnardi V, *et al.* Mortality in patients with coeliac disease and their relatives: a cohort study. *Lancet* 2001; **358**: 356–61.

16 Anderson LA, McMillan SA, Watson RG, *et al.* Malignancy and mortality in a population-based cohort of patients with coeliac disease or "gluten sensitivity". *World J Gastroenterol* 2007; **13**: 146–51.

17 Fasano A, Catassi C. Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. *Gastroenterology* 2001; **120**: 636–51.

18 Abdulkarim AS, Murray JA. Review article: the diagnosis of coeliac disease. *Aliment Pharmacol Ther* 2003; **17**: 987–95.

19 Sedghizadeh PP, Shuler CF, Allen CM, Beck FM, Kalmar JR. Celiac disease and recurrent aphthous stomatitis: a report and review of the literature. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002; **94**: 474–8.

20 Bucci P, Carile F, Sangianantoni A, D'Angio F, Santarelli A, Lo Muzio L. Oral aphthous ulcers and dental enamel defects in children with coeliac disease. *Acta Paediatr* 2006; **95**: 203–7.

21 Smith DM, Miller J. Gastro-enteritis, coeliac disease and enamel hypoplasia. *Br Dent J* 1979; **147**: 91–5.

22 Wierink CD, van Diermen DE, Aartman IH, Heymans HS. Dental enamel defects in children with coeliac disease. *Int J Paediatr Dent* 2007; **17**: 163–8.

23 Bossu M, Bartoli A, Orsini G, Luppino E, Polimeni A. Enamel hypoplasia in coeliac children: a potential clinical marker of early diagnosis. *Eur J Paediatr Dent* 2007; **8**: 31–7.

24 Procaccini M, Campisi G, Bufo P, *et al.* Lack of association between celiac disease and dental enamel hypoplasia in a case-control study from an Italian central region. *Head Face Med* 2007; **3**: 25.

25 Revised criteria for diagnosis of coeliac disease. Report of Working Group of European Society of Paediatric Gastroenterology and Nutrition. *Arch Dis Child* 1990; **65**: 909–11.

26 Aine L. Dental enamel defects and dental maturity in children and adolescents with celiac disease. *Proc Finn Dent Soc* 1986; **82**: 227–9.

27 Nowak AJ. *Oral Management of Pediatric Patients for Non-Dental Professionals, A Study Guide*. Iowa: University of Iowa, 2001.

28 Campisi G, Margiotta V. Oral mucosal lesions and risk habits among men in an Italian study population. *J Oral Pathol Med* 2001; **30**: 22–8.

29 Cohen J. Weighted kappa: nominal scale agreement with provision for scaled disagreement or partial credit. *Psychol Bull* 1968; **70**: 213–20.

30 Lo Muzio L, D'Angelo M, Procaccini M, *et al.* Expression of cell cycle markers and human papillomavirus infection in oral squamous cell carcinoma: use of fuzzy neural networks. *Int J Cancer* 2005; **115**: 717–23.

31 Mehrotra K, Mohan CK, Ranka S. *Elements of Artificial Neural Networks*. Cambridge, MA: MIT Press, 1997.

32 Azuaje F. A computational neural approach to support the discovery of gene function and classes of cancer. *IEEE Trans Biomed Eng* 2001; **48**: 332–9.

33 Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* 1982; **143**: 29–36.

34 Fasano A, Berti I, Gerarduzzi T, *et al.* Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. *Arch Intern Med* 2003; **163**: 286–92.

35 Tommasini A, Not T, Kiren V, *et al.* Mass screening for coeliac disease using antihuman transglutaminase antibody assay. *Arch Dis Child* 2004; **89**: 512–5.

36 Maki M, Mustalahti K, Kokkonen J, *et al.* Prevalence of celiac disease among children in Finland. *N Engl J Med* 2003; **348**: 2517–24.

37 Maki M, Kallonen K, Lahdeaho ML, Visakorpi JK. Changing pattern of childhood coeliac disease in Finland. *Acta Paediatr Scand* 1988; **77**: 408–12.

38 Pare P, Douville P, Caron D, Lagace R. Adult celiac sprue: changes in the pattern of clinical recognition. *J Clin Gastroenterol* 1988; **10**: 395–400.

39 Kemppainen T, Kroger H, Janatuinen E, *et al*. Osteoporosis in adult patients with celiac disease. *Bone* 1999; **24**: 249–55.

40 Rostami K, Steegers EA, Wong WY, Braat DD, Steegers-Theunissen RP. Coeliac disease and reproductive disorders: a neglected association. *Eur J Obstet Gynecol Reprod Biol* 2001; **96**: 146–9.

41 Askling J, Linet M, Gridley G, Halstensen TS, Ekstrom K, Ekbom A. Cancer incidence in a population-based cohort of individuals hospitalized with celiac disease or dermatitis herpetiformis. *Gastroenterology* 2002; **123**: 1428–35.

42 Carroccio A, Iannitto E, Cavataio F, *et al*. Sideropenic anemia and celiac disease: one study, two points of view. *Dig Dis Sci* 1998; **43**: 673–8.

43 Harper JW, Holleran SF, Ramakrishnan R, Bhagat G, Green PH. Anemia in celiac disease is multifactorial in etiology. *Am J Hematol* 2007; **82**: 996–1000.

44 Bardella MT, Fraquelli M, Quatrini M, Molteni N, Bianchi P, Conte D. Prevalence of hypertransaminasemia in adult celiac patients and effect of gluten-free diet. *Hepatology* 1995; **22**: 833–6.

45 Carroccio A, Di Prima L, Scalici C, *et al*. Unexplained elevated serum pancreatic enzymes: a reason to suspect celiac disease. *Clin Gastroenterol Hepatol* 2006; **4**: 455–9.

46 Ventura A, Neri E, Ughi C, Leopaldi A, Citta A, Not T. Gluten-dependent diabetes-related and thyroid-related autoantibodies in patients with celiac disease. *J Pediatr* 2000; **137**: 263–5.

47 Schuppan D. Current concepts of celiac disease pathogenesis. *Gastroenterology* 2000; **119**: 234–42.

48 Aine L, Maki M, Collin P, Keyrilainen O. Dental enamel defects in celiac disease. *J Oral Pathol Med* 1990; **19**: 241–5.

49 Balli MP, Balli ME, Mengoli M, Balli C, Balli F. Growth, skeletal and dental age in chronic diarrhea in childhood. *Pediatr Med Chir* 1988; **10**: 277–82.

50 Andersson-Wenckert I, Blomquist HK, Fredrikzon B. Oral health in coeliac disease and cow's milk protein intolerance. *Swed Dent J* 1984; **8**: 9–14.

51 Lahteenoja H, Toivanen A, Viander M, *et al*. Oral mucosal changes in coeliac patients on a gluten-free diet. *Eur J Oral Sci* 1998; **106**: 899–906.

52 Ventura A, Martelossi S. Dental enamel defects and coeliac disease. *Arch Dis Child* 1997; **77**: 91.

53 Mariani P, Mazzilli MC, Margutti G, *et al*. Coeliac disease, enamel defects and HLA typing. *Acta Paediatr* 1994; **83**: 1272–5.

54 Rea F, Serpico R, Pluvio R, *et al*. Dental enamel hypoplasia in a group of celiac disease patients. Clinico-epidemiologic correlations. *Minerva Stomatol* 1997; **46**: 517–24.

55 Rasmusson CG, Eriksson MA. Celiac disease and mineralisation disturbances of permanent teeth. *Int J Paediatr Dent* 2001; **11**: 179–83.

56 Aguirre JM, Rodriguez R, Oribe D, Vitoria JC. Dental enamel defects in celiac patients. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1997; **84**: 646–50.

57 Ballinger A, Hughes C, Kumar P, Hutchinson I, Clark M. Dental enamel defects in coeliac disease. *Lancet* 1994; **343**: 230–1.

58 Ciccarelli NPDDF, Greco L. Lipoplasia dello smalto dentario dei denti permanenti di soggetti celiaci in challenge con glutine. *Congr Naz SIP* 1993; **19/S-2**: 195.

59 Mariani P, Ferrante E, Margutti G. Difetti dello smalto dentario in un gruppo di bambini e adolescenti celiaci italiani. *Congr Naz SIP* 1993; **19/S-2**:196.

60 Maki M, Aine L, Lipsanen V, Koskimies S. Dental enamel defects in first-degree relatives of coeliac disease patients. *Lancet* 1991; **337**: 763–4.

61 Marzec-Koronczewska Z. The condition of the stomatognathic system in children with gluten-dependent coeliac disease. *Czas Stomatol* 1990; **43**: 207–12.

62 Prati C, Santopadre A, Baroni C. Delayed eruption, enamel hypoplasia and caries in childhood celiac disease. *Minerva Stomatol* 1987; **36**: 749–52.

63 Ansaldi N, Morabito A, Balocco P, Galleano E. Dental changes in children with malabsorption. *Minerva Pediatr* 1989; **41**: 581–5.

64 Petrecca S, Giammaria G, Giammaria AF. Oral cavity changes in the child with celiac disease. *Minerva Stomatol* 1994; **43**: 137–40.

65 Meini A, Pillan MN, Plebani A, Ugazio AG, Majorana A, Sapelli PL. High prevalence of DRW10 and DQW1 antigens in celiac disease associated with recurrent aphthous stomatitis. *Am J Gastroenterol* 1993; **88**: 972.

66 Majorana A, Sapelli PL, Malagoli A, *et al*. Celiac disease and recurrent aphthous stomatitis. The clinical and immunogenetic aspects. *Minerva Stomatol* 1992; **41**: 33–40.