

Duration of gluten exposure in adult coeliac disease does not correlate with the risk for autoimmune disorders

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Abstract

Background and aims—Duration of gluten exposure seems to predispose adolescents with coeliac disease to autoimmune diseases. In a retrospective cohort study, we assessed the relationship between autoimmune disorders and actual gluten exposure in patients in whom coeliac disease was diagnosed in adult life (≥ 16 years).

Methods—We screened for the presence of autoimmunity in 605 controls (16–84 years) and 422 patients (16–84 years), all of whom had been on gluten withdrawal for at least one year (median follow up 9.5 years). A logistic regression analysis, setting the prevalence of autoimmunity as the dependent variable, was employed to control for independent covariates as predictors of the risk of autoimmunity.

Results—The prevalence of autoimmunity was threefold higher ($p < 0.00001$) in patients than in controls. Mean duration of gluten exposure was 31.2 and 32.6 years for patients with or without autoimmunity. Logistic regression showed that increased age at diagnosis of coeliac disease was related to the prevalence of autoimmune disease while “actual gluten exposure” which takes into account diet compliance, follow up, and age at diagnosis of autoimmune disorders were not predictive for the risk of developing autoimmune diseases (odds ratio 0.82 per year).

Conclusion—The prevalence of autoimmune diseases in patients with a late coeliac disease diagnosis does not correlate with duration of gluten intake. Early exposure to gluten may modify the immunological response. Gluten withdrawal does not protect patients with a late diagnosis from autoimmune diseases. (*Gut* 2001;49:502–505)

Keywords: coeliac disease; autoimmune disorders; prevalence

Current knowledge considers coeliac disease (CD) an autoimmune (AI) disorder triggered by ingestion of gluten in genetically predisposed individuals.¹ Indeed, gluten induces the production of autoantibodies directed against fibroblast derived extracellular matrix proteins: the target autoantigen is tissue transglutaminase (tTG), a ubiquitous intracellular enzyme

which may be released during cellular wounding.² Gliadin is the preferred substrate for this enzyme, giving rise to novel antigenic epitopes by cross linking molecules of the extracellular matrix with gliadin or with tTG-gliadin complexes.³

Given the essential role of gliadin in inflammation and autoimmunity drive, and the ubiquitous diffusion of tTG in the body, recently Ventura and colleagues,⁴ by screening 929 adolescents with CD, hypothesized that the duration of gluten exposure may predispose to autoimmunity in other organs. The results of a prospective multicentre study on the prevalence of thyroid disorders in adults with CD did not support this hypothesis, at least for thyroid involvement.⁵

The present study was undertaken with the aims of screening for the prevalence of AI disorders among patients in whom CD was diagnosed in adult life (≥ 16 years) and followed up in a single centre, and of verifying whether the onset of AI diseases correlates with duration of gluten exposure.

Patients and methods

In this retrospective cohort study we reviewed the hospital medical records of 713 adult patients in whom CD was diagnosed at our centre, from January 1968 to May 1999.

PATIENTS

In all cases the diagnosis of CD was made by demonstrating the typical histological appearance of the intestinal mucosa, which improved after gluten withdrawal.

We enrolled only patients in whom a diagnosis of CD had been made at age ≥ 16 years and who were in clinical remission and on a gluten free diet (GFD) for at least one year; compliance with the diet was ascertained by direct enquiry by an expert or by healing of intestinal lesions at repeat biopsy and/or negativity of serum antiendomysium antibodies which, presently, are considered the most reliable serological markers of CD.⁶

A total of 422 CD patients (128 males, 294 females; mean age 39.3 years, range 17–84) met the established criteria and entered the study. Median follow up was 9.5 years (range 1–32).

Abbreviations used in this paper: AI, autoimmune; CD, coeliac disease; DH, dermatitis herpetiformis; GFD, gluten free diet; IDDM, insulin dependent diabetes mellitus; tTG, tissue transglutaminase.

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Table 1 Prevalence of each autoimmune (AI) disease among coeliac disease (CD) patients and controls: 151 AI diseases in 127 of 422 patients (30%) (multiple AI diseases occurred in single patients); 70 AI diseases in 605 controls (11.57%) ($p < 0.00001$). Excluding dermatitis herpetiformis, the prevalence of AI diseases resulted in 111 AI diseases in 91 of 422 patients (21.5%) ($p = 0.00001$ compared with controls)

AI diseases	CD patients (n (%))	Controls (n (%))	χ^2 test (p value)
IDDM	16 (3.79)	2 (0.33)	15.341 (<0.0005)
Dermatitis herpetiformis	42 (9.95)	0 (0)	60.269 (<0.0001)
AI thyroid diseases	57 (13.5)	12 (2)	50.852 (<0.0001)
AI hepatitis	2 (0.5)	1 (0.16)	0.952 (NS)
AI atrophic gastritis	3 (0.71)	0 (0)	2.218 (NS)
AI anaemia, neutropenia, thrombocytopenia	7 (1.65)	1 (0.16)	5.371 (=0.0218)
Connective tissue diseases	7 (1.65)	15 (3.19)	0.455 (NS)
Psoriasis	13 (3.08)	36 (5.95)	3.897 (=0.0488)
Alopecia	1 (0.24)	3 (0.5)	0.021 (NS)
Epilepsy with occipital calcifications	3 (0.7)	0 (0)	2.218 (NS)

IDDM, insulin dependent diabetes mellitus.

CONTROLS

The control group included 605 volunteers (180 males, 425 females; mean age 39.5 years, range 16–84) recruited among medical students, medical and nursing staff, and their relatives: CD was excluded in all either by clinical findings or by negativity of IgA-antiendomysium antibodies.

METHODS

The presence of AI disorders was assessed using a structured questionnaire completed by patients with the assistance of ad hoc trained personnel and from interviews with patients and careful examination of medical records, verifying if all diagnostic criteria had been met.

In both groups we searched for the following AI disorders, according to the study of Ventura and colleagues⁴: endocrine diseases (insulin dependent diabetes mellitus (IDDM), AI thyroid diseases, Addison's disease), AI hepatitis, primary biliary cirrhosis, AI atrophic gastritis, AI anaemia, AI neutropenia, AI thrombocytopenia, connective tissue diseases (systemic lupus erythematosus, Sjogren's syndrome, rheumatoid arthritis, dermatomyositis), alopecia aerata, psoriasis, and epilepsy with occipital calcifications. Dermatitis herpetiformis (DH) was also included, as in Ventura's study, although this skin disorder is one manifestation of gluten intolerance and not an associated disease.⁷

Age at diagnosis of AI disorders was recorded for the two groups.

For each patient, the following clinical-epidemiological features were recorded:

- age at CD diagnosis (as an indirect mirror of duration of gluten exposure);
- diet compliance (defined as: 1, strict; 2, partial; 3, poor);

- histological outcome, as detected by repeated intestinal biopsy (1, persistent total or partial villous atrophy; 2, mucosal recovery or appreciable improvement);
- years of follow up at our centre;
- actual duration of gluten exposure: this information provides a better indication of the effective duration of exposure to gluten, by taking into account diet compliance, follow up, and age at diagnosis of AI disorders. The beginning of a strict GFD, in patients in whom recovery or appreciable improvement of intestinal mucosa was ascertained, was considered as the end time of gluten exposure. Otherwise, the period of gluten exposure matched the time of AI disorder onset, except for patients in whom diet compliance interrupted gluten exposure;
- age at last survey (or present age): this parameter may be regarded as a confounding variable, considering that age itself is a risk factor in AI disease onset;
- sex, which is considered another possible confounding variable, due to the higher prevalence of AI diseases in females.

The study was performed according to the principles of the Declaration of Helsinki, and informed consent was obtained from all patients and controls.

STATISTICAL ANALYSIS

Statistical data were generated using SAS software, version 6.12 (SAS Institute Inc., North Carolina, USA).

Descriptive statistics are presented as mean (SD). For comparison of means, the unpaired Student's *t* test was applied. When data were regarded as not normally distributed, the non-parametric Mann-Whitney U test and Wilcoxon signed rank test were used. Differences in frequencies were tested using the χ^2 test or Fisher's exact test, where indicated.

Logistic regression analysis, with the prevalence of AI diseases as the dependent variable, was employed to control for independent covariates as predictors of the risk of autoimmunity.

For all statistical analyses, a two tailed *p* value <0.05 was considered significant.

Results

PREVALENCE OF AI DISORDERS

As shown in table 1, the overall prevalence of AI diseases was significantly higher ($p < 0.00001$) among patients (151 AI diseases in 127 of 422 patients—30%) than controls (70

Table 2 Epidemiological features of coeliac disease patients related to the presence (group 1) or absence (group 2) of associated autoimmune (AI) diseases. Column A: dermatitis herpetiformis included as AI disease; column B: dermatitis herpetiformis regarded as not an AI condition (in six of 42 patients with dermatitis herpetiformis, other AI diseases occurred)

Coeliac disease patients	Group 1		Group 2		p Value	
	A	B	A	B	A	B
Mean age (y)	43.2	43.9	37.6	38.05	< 0.0005*	<0.001*
Mean age at diagnosis (y)	35.7	37	28.3	29	< 0.00004*	<0.00001*
Mean "actual" gluten exposure (y)	31.2	32.2	32.6	32.8	NS*	NS**
Males	45/127	20/91	83/295	108/331	NS**	NS**
Females	82/127	71/91	212/295	223/331	NS**	NS**

*Unpaired Student's *t* test; ** χ^2 test.

AI diseases in 605 subjects—11.6%). IDDM (3.79%), DH (9.95%), and AI thyroid diseases (13.5%) showed the highest prevalence in CD patients. Even excluding DH, the prevalence of AI diseases was significantly higher among patients (111 AI diseases in 91 of 422 patients—21.5%) than controls ($p=0.00001$).

In 82 of 127 patients (64.5%), AI disorders were diagnosed before CD while in 45 patients (35.5%) with associated AI disorders, CD was diagnosed before AI disease onset. AI disease distribution according to sex among both patients and controls showed that females were more frequently affected than males.

CD patients were further grouped according to the presence (group 1) or absence (group 2) of associated AI diseases. As shown in table 2 (column A: DH patients included; column B: DH patients excluded), present age and age at CD diagnosis were statistically higher in group 1 while neither effective duration of gluten exposure nor sex was found to be a significant predictor of the risk of developing an AI disease.

After excluding from the statistical analysis patients with DH, as this skin disease may be considered part of the spectrum of CD, overall results did not change.

LOGISTIC REGRESSION ANALYSIS

The logistic regression model, using an univariate approach, confirmed the finding of a significantly increased prevalence of AI diseases in older patients and in those with a longer diagnostic delay.

As shown in table 3 (column A: DH patients included; column B: DH patients excluded), older age and delayed diagnosis were predictors of an increased risk for developing AI diseases (relative risk 1.073 and 1.15 per year, respectively) while unexpectedly, actual duration of gluten exposure appeared to reduce this hazard (relative risk 0.82 per year). None of the other clinical-epidemiological features reached statistical significance. Again, after excluding DH patients, the odds ratio for actual gluten exposure did not change (relative risk 0.82 per year).

For each subject included in the study, the accuracy of the model as a predictor of the odds for the development of AI diseases was tested by further comparing expected versus observed probabilities: good agreement was found between expected and observed results (79.2% of cases).

Table 3 Logistic regression model in which the prevalence of autoimmune (AI) disorders was set as the dependent variable: older age and delayed diagnosis of coeliac disease yielded an increased risk for developing AI diseases while "actual duration of gluten exposure" (see text) appeared to reduce this risk. None of the other clinical-epidemiological features reached statistical significance. Column A: dermatitis herpetiformis included as AI disease; column B: dermatitis herpetiformis regarded as not an AI condition

Variable	p Value		Odds ratio	
	A	B	A	B
Present age	0.0023	0.07	1.073	1.05
Age at diagnosis of coeliac disease	0.001	0.0004	1.15	1.18
Actual duration of exposure to gluten	0.0001	0.001	0.82	0.82

Discussion

There is a large body of literature describing both the association of AI disorders with CD and, conversely, a higher than expected prevalence of CD, mainly clinically silent, in patients with recognised AI diseases.⁸⁻¹⁴ The association of CD with AI disorders is attributed both to a common genetic background, in particular HLA haplotypes, and to a similar immune mediated disease mechanism.¹⁵ CD has many features in common with AI diseases although it does not fit the current concept of autoimmunity, where: (1) a triggering agent is not known; (2) the disease is thought to be self perpetuating; and (3) irreversible destruction of the target organ is the postulate to the onset of symptoms. In contrast, in CD, a known extrinsic agent (gluten) is of primary importance for the pathogenesis and its removal leads to recovery of intestinal lesions.

The intestinal tract is the major immunological organ of the human body and it plays an essential role in induction of oral tolerance. Taking into account the fact that immunologically mediated reactions to foods occur outside the gut environment and can affect almost all organ systems,¹⁶ involvement of some extrinsic factor (such as gluten) which perturbs immune regulation may also be possible in AI disorders.¹⁷

The beneficial effect of gluten withdrawal on DH skin lesions is well recognised¹⁸; the disappearance, in some cases, of microscopic haematuria in patients with IgA mesangial nephropathy¹⁹ and reversal of cerebellar ataxia after institution of a GFD have been described.²⁰ A role for gluten ingestion in longstanding untreated CD, even if clinically silent, in predisposing for other AI diseases was recently hypothesized in children and adolescents.⁴

Our study confirms the elevated prevalence of AI disorders in CD (30% of our adult patients with CD had at least one AI disease), with an overall 2–3-fold higher frequency than in controls. We do not believe that subjects in the control group were healthier than the general population: there were almost 50% more controls than CD patients and age was comparable in the two groups. Furthermore, unsatisfactory health conditions were not considered exclusion criteria for controls. To confirm our hypothesis, the prevalence of AI disorders among controls was even higher than the overall estimate in a large epidemiological study from the USA.²¹

Most of the AI disorders included in our study have been reported to be associated with CD^{10,22} and are the same as those considered by Ventura and colleagues.⁴ Among them, although DH has always been at the top of the list of associated disorders, there is unequivocal evidence that it is one of the manifestations of gluten intolerance and that it may be considered as CD of the skin.⁷ We included this skin disorder to compare our results with those of Ventura and colleagues.⁴ However, exclusion of these data did not change the overall results.

Epilepsy with occipital calcifications, alopecia aerata, and psoriasis are currently considered AI disorders.²³⁻²⁵ Surprisingly, psoriasis was even less common in CD patients than in controls: the beneficial effect of a GFD on this lesion was recently suggested.²⁶

In our study, when adult patients with and without AI associated disorders were compared, age at CD diagnosis, considered as an indirect mirror of duration of gluten exposure, was significantly higher in patients affected by both diseases than in patients with CD alone, thus supporting Ventura's hypothesis.⁴

However, some data were not convincing: firstly, if the presence of AI disorders was related to duration of gluten exposure (in Ventura's paper it was 23% in patients aged 10-25 years), their prevalence in adults should have been much higher than the observed 30%. Furthermore, although in 64.5% of patients AI disorders became manifest before the diagnosis of CD (that is, during a gluten containing diet), in 35.5% of patients an AI disease appeared after diagnosis of CD (while on gluten withdrawal), even in subjects strictly compliant with the diet.

As a single variable may influence the outcome when assessed by univariate regression (because it is a covariate with another variable related to the outcome), a multivariate analysis, which eliminates confounding variables, was carried out.

"Actual gluten exposure" (by taking into account not only age at CD diagnosis but also age at diagnosis of AI disorders, follow up at our centre, diet compliance, and histological outcome of intestinal mucosa) was considered a more reliable marker than age at CD diagnosis alone. The beginning of a strict GFD, in patients in whom recovery of intestinal lesions could be ascertained, was considered as the end time of gluten exposure. In contrast, in patients with associated AI disorders, the period of gluten exposure matched the time of AI disease onset, except for patients in whom diet compliance interrupted gluten exposure.

However, many AI diseases considered in this study could remain undetected for many years thus misleadingly prolonging the estimated "gluten exposure". Notwithstanding these potentially confounding variables, results regarding present age and age at CD diagnosis did not change while "actual gluten exposure" was similar in both patients with and without associated AI disorders, with a relative risk for AI diseases of 0.82 per year. Thus it can be hypothesized that gluten ingestion plays a central role in modifying the immunological response earlier in life: as observed by Ventura and colleagues,⁴ the prevalence of AI disorders in children in whom CD was diagnosed before the age of two years and who later did not undergo gluten challenge, was comparable with that of controls.

Conversely, this study showed that in patients with a late CD diagnosis, gluten withdrawal did not protect from AI diseases thus implying that onset of AI disorders does not correlate with duration of gluten exposure.

Misleading complaints, which were the cause of earlier recognition of AI diseases, are probably responsible in many patients for the diagnostic delay in CD.

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