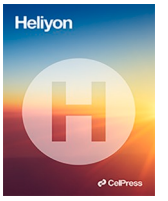
Heliyon 10 (2024) e40685

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Prevalence of celiac disease-specific antibodies and their association with clinical status and environmental factors

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ARTICLE INFO

Keywords:
Celiac disease
Anti-transglutaminase
Anti-gliadin
Anti-DGP
Infertility
Environmental health

ABSTRACT

Background and aims: Celiac disease (CeD) affects 1–2% of the world's population. The aim of this study was to relate the incidence of CeD-related serological markers to symptoms, pathologies, and environmental exposure to wheat flour, given the number of flour mills present in the region.
Materials & methods: Serum samples were collected from 537 inhabitants from a rural city. Levels of anti-transglutaminase (a-tTg), anti-gliadin, anti-DGP antibodies and total IgA levels were measured. Volunteers completed a questionnaire covering environmental factors, demographics, pregnancies, other diseases, symptoms, and CeD diagnosis. Geo-referencing of volunteers' homes and mills in the city was performed, and correlations between the different parameters assessed were analysed.
Results: A CeD incidence of 1.76 % was found. However, a-tTg and a-gliadin levels were elevated in the population without CeD diagnosis (9.6 % and 30.1 %). Subjects with CD diagnosis showed diarrhoea and colic pain. Women with CeD had fewer pregnancies. Positive a-tTg and number of CeD-associated symptoms appear to correlate with proximity to flour mills.

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https://doi.org/10.1016/j.heliyon.2024.e40685
Received 17 April 2024; Received in revised form 28 October 2024; Accepted 22 November 2024
Available online 23 November 2024

Conclusion: A high prevalence of CeD-related specific antibody positivity in a rural population was found, possibly due to environmental factors related to flour mills. Further research is needed to better understand CeD's pathogenesis and its health implications.

1. Introduction

CeD is currently considered to be the most common chronic intestinal disease. An approximate global prevalence of 1–2% has been estimated. However, it is very difficult to determine the true incidence and prevalence in the population, given the variety of forms in which it can manifest itself [1]. Epidemiological studies carried out in Argentina [2] have shown that the paediatric population has a prevalence of 1.26 %, while in the adult population it is 0.6 %, being higher in women (0.8 %) than in men (0.4 %) [3]. It is estimated that more than 400,000 people suffer from the disease in Argentina [4].

The inflammatory response in the intestinal mucosa leads to the appearance of specific antibodies (Abs), including anti-gliadin (a-Gli), anti-transglutaminase (a-tTg) and anti-deamidated gliadin antibodies (a-DGP) which serve as powerful tools for the diagnosis and monitoring of CeD patients. In addition, IgA a-tTg antibodies are markers of active CeD. Early diagnosis and treatment of CeD are essential to reduce the risk of associated diseases. In adults, CeD often manifests with digestive symptoms like abdominal distension, diarrhoea, gas, and colic pain. However, when the disease remains undiagnosed and develops silently, it can lead to severe outcomes. Also, CeD is associated with several autoimmune diseases, such as autoimmune thyroid disease, type 1 diabetes mellitus, autoimmune liver diseases, and inflammatory bowel disease although the reason remains unclear.

Chivilcoy is a rural city in the province of Buenos Aires, Argentina, where wheat production and bulking is one of the main economic activities. In this sense, and to analyse the current prevalence situation, the aim of this work was to study the incidence of serological markers related to CeD in the inhabitants of this city and relate them with symptoms, history of pathologies, family relationship with CeD patients and environmental exposure to wheat flour, given the number of flour mills and storage silos present in the region.

2. Methods

2.1. Patients

This study was approved by the Ethics committees of Universidad Nacional de Luján (DISPSEACAD-LUJ:0000125–20). All participants provided written informed consent prior to enrolment in the study. This research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki (2008). The selection of patients for this study was carried out on the basis of two major campaigns. The first of them, in the exhibition of the rural association of Chivilcoy, where the general population attends every year. Random people were invited to take part in the study (no matter their clinical status related to CeD). In order to have a larger number of volunteers in the group of people diagnosed with CeD, targeted campaigns were conducted at events of the Argentine Celiac Association (ACA). Serum samples were collected from adults and stored at −80 °C until use. All volunteers were geo-referenced, as were the mills and flour collection points in the sampled city. For each subject, the distance between the household and the nearest mill was calculated.

2.2. Clinical and biochemical data

A software was designed by us in order tocreate a database with the clinical and biochemical data of the volunteers. Data from environmental factors, sex, age, number of pregnancies and abortions, other diseases, CeD related symptoms, and if they had a medical diagnosis of CeD were also included. The data provided for this study was anonymized and shuffled.

2.3. Determination of antibodies associated with CeD

Serum samples obtained were analysed by an ELISA developed in our laboratory (CELISAR) to determine the Abs IgA a-tTg (a-tTgA) and a-Gli (a-GliA). RecombinanttTg*E. coli*producer bacteria werekindlyprovidedby Laboratorio de Inmunoendocrinología, Cátedra de Inmunología, Facultad de Farmacia y Bioquímica, UBA. Briefly, we immobilized tTg or gliadin (Sigma Chemical Co, St Louis, MO, USA) to a 96-well plate and incubated with serum samples and anti-Human IgA Peroxidase-conjugated was added (Jackson Immuno Research), stopped with sulfuric acid and read at 450 nm. All values were relativized to the cutoff value calculated as mean of normal serum plus 2 standard deviations. Those samples with relative values greater than 1.0 arbitrary units were considered positive. The control sera used were previously characterized with commercial kits (OrgentecDiagnostika GmbH-ORG540A and ORG534A).

2.4. Determination of total IgA and a-DGP antibodies

Sera from subjects who were a-tTg negative but reported symptoms associated with CeD were measured by radial immunodiffusion plates to determine total IgA (Diffu-Plate®, Biocientífica, Buenos Aires, Argentina). Subjects with normal total IgA levels were assayed for a-DGP IgA and subjects with total IgA deficiency (<90 mg/dl, according to kit manufacturer's specifications) were assayed for a-

DGP IgG (OrgentecDiagnostika GmbH-ORG551A and ORG551G).

2.5. Statistical analysis

Statistical analysis was performed using graph Pad Prism 8.0 software using simple analysis of variance (ANOVA). Multivariate regressions were performed to associate the analyses, adjusting for demographic characteristics. Significant differences between means were calculated with Tukey's or Chi square test. Values of $p \leq 0.05$ were considered as significant.

3. Results

3.1. Volunteers recruitment and characteristic

After carrying out the campaign at the rural association of Chivilcoy, 337 samples from volunteers were collected, 6 of whom (1.78 %) had a diagnosis of CeD. In order to have a larger number of volunteers in the group of people diagnosed with CeD additional campaigns were conducted at events of the ACA (14 % of diagnosed CeD), reaching 537 volunteers, with a total of 34 CeD diagnosed cases (6.33 %). The participants were predominantly female (76.3 %), with an average age of 48.9 ± 13.5 years. The percentage of participants with a medical diagnosis of CeD were similar for both sexes (6.58 % for women and 5.51 % for men). However, comorbidities related to CeD (anaemia, diabetes, thyroidism, osteoporosis), comorbidities less related to CeD (allergy, irritable colon, diverticulitis, fibromyalgia, glaucoma, thyroid gland nodules, poliomyelitis, psoriasis, rosacea, tuberculosis, vitiligo), symptoms widely described as related to CeD (abdominal distention, diarrhoea, gas, and colic pain), and symptoms less specifically related to CeD (weight loss, loss of appetite, tiredness, muscle pain, and cramps) were more frequent in females (44.9 % vs 14,3 %, $p \leq 0.0001$; 49,5 % vs 8,73 %, $p \leq 0.0001$; 78.8 % vs 55.5 %, $p \leq 0.05$, and 79.7 % vs 54.8 %, $p \leq 0.05$, respectively) (Table 1).

3.2. A-GliA and a-tTgA antibodies determination

The presence of a-tTgA and a-GliA were determined by ELISA in the 537 volunteers. The results showed high positivity rates for a-tTgA (9.7 %) and a-GliA (32.2 %). When comparing groups with and without CeD diagnosis, the first group had higher positivity of a-GliA (55.9 %) compared to the group without CeD diagnosis (30.1 %), while a-tTgA rates remained similar (8.8 % and 9.7 %, respectively) (Table 2).

To carry out a more detailed analysis of the data, the a-tTgA negative group was divided into two groups: "negative" (cut off minus one SD: <0.8 arbitrary units) and "uncertain negative" (0.8–1.0 arbitrary units). Similarly, the group of positives was divided into "positive" (cut off plus one SD: >1.2 units) and "uncertain positive" (1.0–1.2 arbitrary units). The proportions of these 4 groups are shown in Fig. 1A and B.

Then, the a-GliA levels within each of the a-tTgA groups were analysed. Volunteers with negative a-tTgA showed a higher proportion of negative a-GliA. On the other hand, volunteers without a CeD diagnosis but with positive or uncertain a-tTgA had high proportions of positive a-GliA. There was also a correlation between a-tTgA and a-GliA in volunteers diagnosed with CeD (Fig. 1C and D). Among the uncertain positive a-tTgA volunteers with CeD (2 volunteers total), one of them was a-GliA negative, and the other one was a-GliA positive (Fig. 1 D panel III). Finally, only one volunteer with CeD had both positive a-tTgA and a-GliA markers (Fig. 1 D panel IV).

3.3. Analysis of symptoms and comorbidities

Fig. 2 (A and B) shows the proportions of volunteers with CeD-related symptoms (abdominal distention, diarrhoea, gas, and colic pain), discriminating according to CeD diagnosis and a-tTgA level. Both groups, with CeD and without CeD, presented similar percentages of volunteers with abdominal pain and gas (58.8 % vs. 59.6 % and 52.9 % vs. 53.3 %, with CeD vs. without CeD, respectively), however volunteers diagnosed with CeD had a higher proportion of diarrhoea (38.2 % vs. 23.1 %) and colic pain (50.0 % vs. 38.8 %), but differences are not significant (Chi square $p > 0.05$). In addition, within the group diagnosed with CeD, there is a trend towards a higher proportion of individuals with each related symptom and higher a-tTgA levels (Fig. 2 B).

To further analyse our groups of study profile, we observed the presence of comorbidities declared. As shown in Fig. 2(C and D), no

Table 1
Characteristics of the volunteers.

	Age (average)	With CeD (%)	Comorbidities associated (%)	Comorbidities not associated (%)	Widely related symptoms (%)	Symptoms less related (%)
Total (n = 537)	48.9	6.33	37.6	39.8	73.2	73.7
Female (n = 410)	47.3	6.58	44.9**	49.5**	78.8*	79.7*
Male (n = 127)	49.6	5.51	14.3**	8.73**	55.5*	54.8*

Asterisk mean significant differences between women and men (*$p < 0.05$; **$p < 0.0001$).

Table 2
Percentages of positivity for the different Abs studied in volunteers with and without CeD diagnosis and separated by sex.

		a-tTgA positive (%)	a-GliA positive (%)
With CeD diagnosis	Female (n = 27)	11.1	59.3
	Male (n = 7)	0.0	42.9
	Total (n = 34)	8.8	55.9*
Without CeD diagnosis	Female (n = 383)	8.2	28.8
	Male (n = 120)	14.4	35.6
	Total (n = 503)	9.7	30.1*

Asterisk mean significant differences between volunteers with and without CeD diagnosis (*$p < 0.05$).

significant statistical associations were found between the comorbidities analysed (anaemia, diabetes, thyroidism, and osteoporosis) and a-tTgA levels or CeD diagnosis (Chi square $p > 0.05$).

3.4. Total IgA determination

In subjects negative for a-tTg but showing CeD-associated symptoms (colic pain and diarrhoea) (69 subjects), 8.7 % were found to be deficient in total IgA (<90 mg/dl) (Fig. 3A). Among subjects with normal total IgA levels, a-DGP IgA was determined, and 9.5 % tested positive for this antibody. For the total IgA deficient volunteers, a-DGP IgG was tested, and all results were negative. Additionally, 60.9 % of the subjects tested for total IgA had values lower than the population average (220 mg/dl) reported by the manufacturer (Fig. 3B).

3.5. Pregnancies and abortions

In relation to the number of pregnancies, a lower number of pregnancies was observed in those women diagnosed with CeD (2.04 $\pm$ 0.26 mean and mean error, respectively) compared to those without a diagnosis of CeD (2.85 $\pm$ 0.10) ($p \leq 0.05$) (Fig. 3C). On the other hand, the number of abortions did not differ significantly between the groups, (0.42 $\pm$ 0.05 and 0.22 $\pm$ 0.09) ($p > 0.05$) (Fig. 3D).

3.6. Geographical distribution

Georeferencing the volunteers home's addresses in Chivilcoy showed a distribution mirroring the city's population density, with more volunteers in the city centre and a gradual decrease outward. However, among subjects with a-tTg>1.0, a different pattern emerged, indicating a higher proportion of individuals with a-tTg>1.0 near the main flour mills and collection centres (Fig. 4A). Interestingly, the distance to the nearest mill showed an inverse correlation with a-tTg levels and CeD-related symptoms. The stronger inverse correlation becomes more evident while moving the analysis from the "positive" group to the "negative", where no correlation was observed in subjects with antibodies a-tTgA negative or uncertain negative, but a trend was seen in those with a positive or uncertain positive biomarker, suggesting higher levels of specific antibodies as the distance to the nearest mill decreased. Additionally, the number of symptoms (diarrhoea, colic pain, gas, and abdominal distention) shows an increasing trend of subjects living closer to the mills (Fig. 4B).

4. Discussion

A systematic review conducted by Singh et al. (2018) reported a global seroprevalence of CeD at 1.4 %, with regional variations ranging from 1.3 % in South America to 1.8 % in Asia [5]. Although the incidence of CeD is well studied in Europe, North America, and Oceania, there is a lack of population-based studies on CeD incidence in Africa, Asia, and Latin America [6]. One challenge in epidemiological studies is the presence of numerous undiagnosed individuals due to various factors, such as some patients having minimal or no symptoms [7]. Additionally, some patients who eliminate gluten from their diet for other purposes may have normal blood test results, further complicating the accurate diagnosis of CeD. This can lead to late diagnosis, which may not only worsen the condition, but also promote the development of complications [8,9]'

We conducted sampling campaigns to analyse CeD-related Abs in Chivilcoy, Buenos Aires (70.765 habitants). In the first of them, carried out in the exhibition of the rural association of Chivilcoy, we identified 1.76 % of volunteers diagnosed with CeD (n = 337). Previous studies reported a prevalence of 0.6 % in the Argentinean adult population in 2001 and 1.26 % in the Argentinean paediatric population in 2012 [9,10]. This increasing trend in prevalence aligns with literature suggesting that environmental factors, alongside diagnostic improvements, might contribute to the rise in CeD [10] [-12]. For instance, studies in Finland indicated a near-doubling of CeD prevalence over 20 years, possibly influenced by environmental factors [11]. The "Swedish epidemic" in children born between 1984 and 1996 revealed insights into potential causal mechanisms, including high gluten intake after breastfeeding [13]. Additionally, research in Norway and Denmark associated antibiotic use in infancy with an increased CeD risk [14]. The hygiene hypothesis has also been proposed as an explanation, with reduced early-life microbial exposure potentially leading to an overactive immune response later in life [15]. Other factors like season of birth, early childhood infections, latitude, and mode of delivery have also been suggested

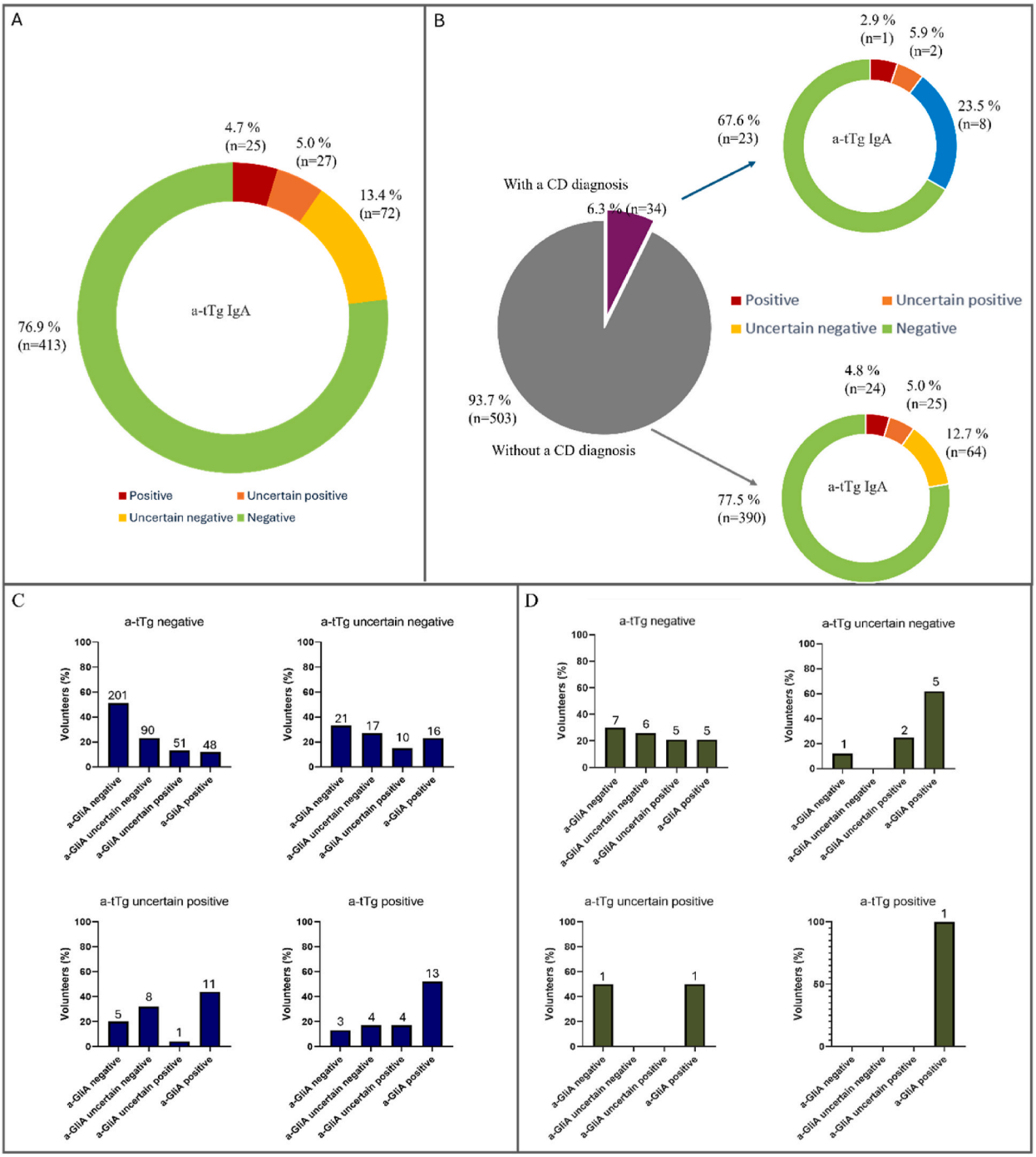

Fig. 1. **A.*Volunteers grouped according to the values of arbitrary units of a-tTg IgA***: positive (>1.2 arbitrary units), uncertain positive (between 1.0 and 1.2), uncertain negative (between 0.8 and 1.0), and negative (<0.8). **B.** Proportion of volunteers with and without a diagnosis of CeD and proportions of volunteers grouped according to the values of arbitrary units of a-tTg IgA in each case. **C.Volunteers without a diagnosis of CeD** and grouped according to the values of arbitrary units of a-gli IgA: (I) negative (<0.8 arbitrary units); (II) uncertain negative (between 0.8 and 1.0); (III) uncertain positive (between 1.0 and 1.2); and (IV) positive (>1.2 arbitrary units). Each bar represents the proportion of volunteers with a-Gli IgA corresponding to each group. a-tTg negative (n = 390), a-gli uncertain negative (n = 64), a-gli uncertain positive (n = 25), a-tTg positive (n = 24). **D.*Volunteers diagnosed with CeD*** are shown (I) a-tTg negative (n = 23), (II) a-tTg uncertain negative (n = 8), (III) a-tTg uncertain positive (n = 2), (IV) a-tTg positive (n = 1). The numbers on the bars indicate the total number (n) of volunteers in each group.

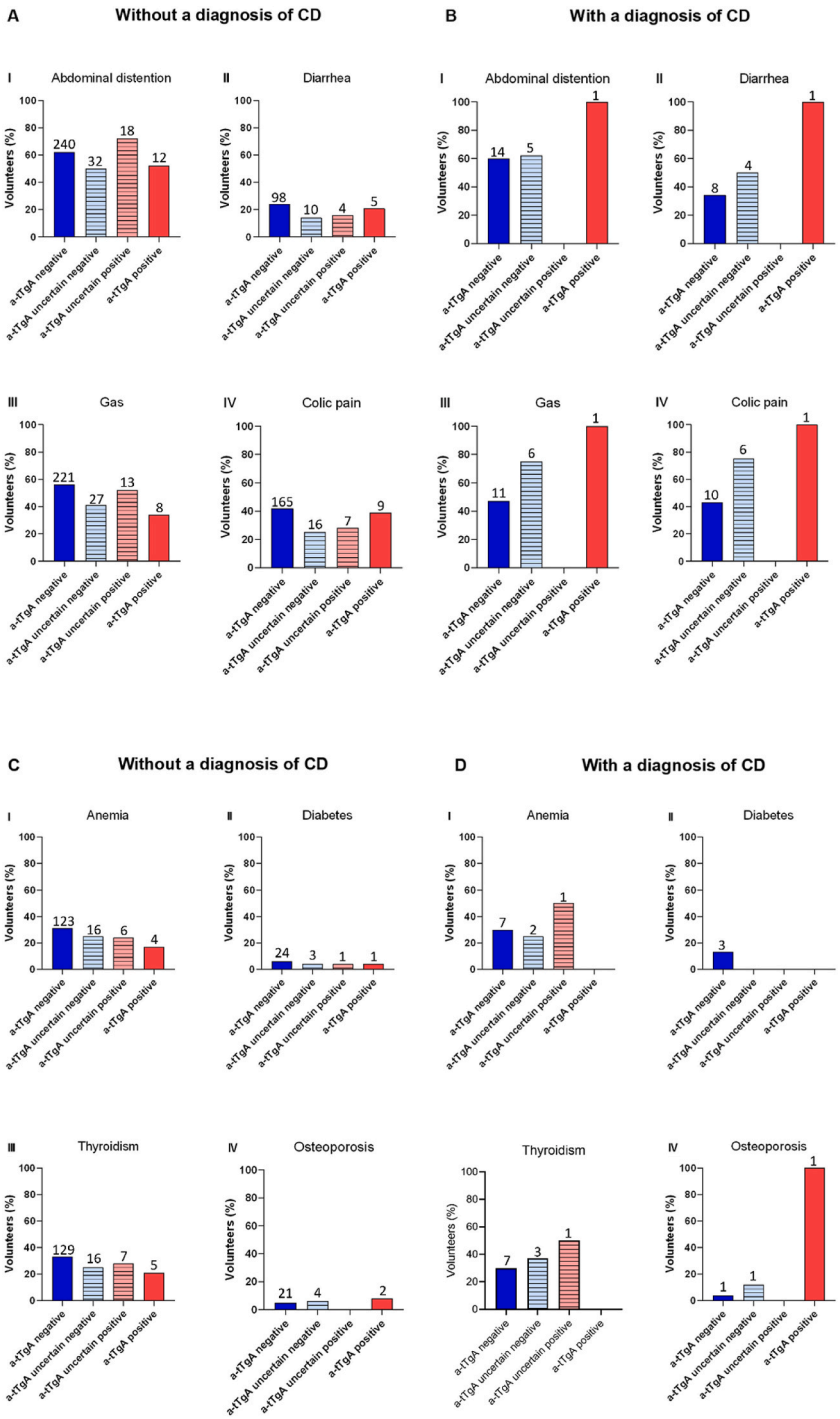

Fig. 2. A. *CeD-related symptoms in volunteers without a diagnosis of CeD*, according to the values of arbitrary units of a-tTg IgA: positive (>1.2 arbitrary units), uncertain positive (between 1.0 and 1.2), uncertain negative (between 0.8 and 1.0) and negative (<0.8). (I) Abdominal distention (n = 302); (II) Diarrhoea (n = 113); (III) Gas (n = 267); (IV) Colic pain (n = 197). **B.** Similarly, the results for volunteers diagnosed with CeD are shown. (I) Abdominal distention (n = 20), (II) Diarrhoea (n = 13), (III) Gas (n = 18), (IV) Colic pain (n = 17). **C.*Comorbidities in volunteers diagnosed with CeD***, according to the values of arbitrary units of a-tTg IgA: positive (>1.2 arbitrary units), uncertain positive (between 1.0 and 1.2), uncertain negative (between 0.8 and 1.0) and negative (<0.8). (I) Anemia (n = 149); (II) Diabetes (n = 29); (III) Thyroidism (n = 157); (IV) Osteoporosis (n = 27). **D.** Similarly, the results for volunteers diagnosed with CeD are shown. (I) Anemia (n = 10); (II) Diabetes (n = 3); (III) Thyroidism (n = 11); (IV) Osteoporosis (n = 3). The numbers on the bars indicate the total number (n) of volunteers in each group. In those cases where the chi square analysis could be performed, $p > 0.05$.

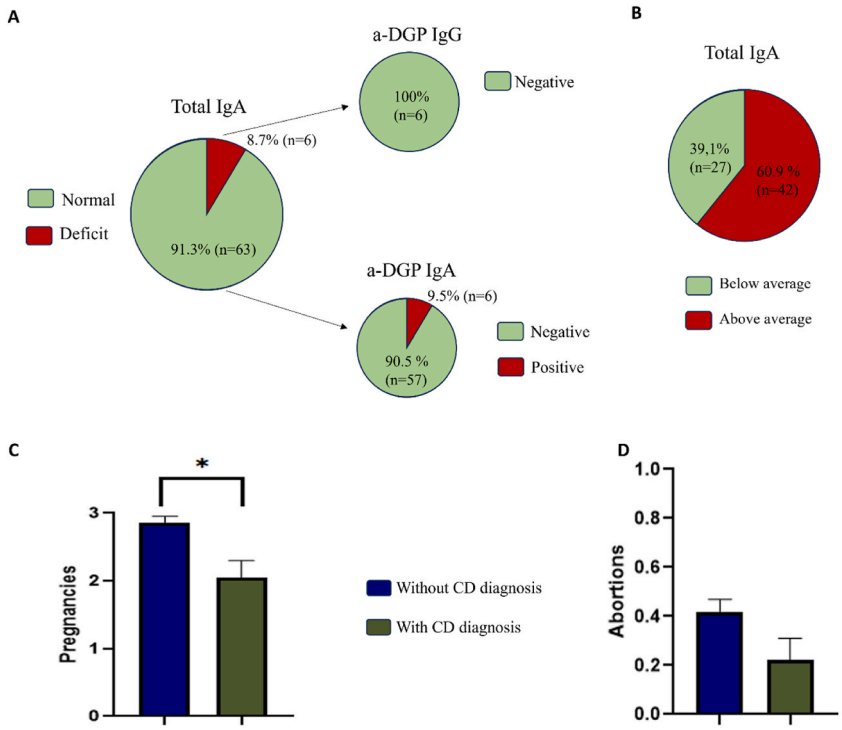

Fig. 3. **A.** Proportion of volunteers with total IgA deficit (<90 mg/dl) and a-DGP levels. **B.** percentage of volunteers below and above the median value of total IgA (220 mg/dl). **C.** Number of pregnancies and **D.** Number of abortions in women with and without a diagnosis of CeD. The means and the mean error are shown. Significance * = $p < 0.05$.

as potential CeD risk factors [16–19]. Future research is necessary to better comprehend CeD's aetiology.

The targeted campaigns increased the number of volunteers to 537 (0.76 % of the population of Chivilcoy), of whom 6.33 % had been diagnosed with CeD (34 volunteers). Although no significant difference in CeD incidence was observed between sexes, the rate was slightly higher in women (6.58 % in women and 5.51 % in men). Screening studies in adults have previously shown similar CeD incidence in men and women [10,20]. The disparity in CeD diagnosis may be linked to gender differences in health care utilisation patterns, with certain comorbid conditions more commonly diagnosed in women (e.g., hypothyroidism), potentially leading to a higher detection rate of CeD [21].

The remarkable high percentage of positive antibodies, both a-tTgA (9.7 %) and a-gliA (32.5 %), is noteworthy. These percentages remain high in both the group without CeD diagnosis (9.7 % and 30.1 %, respectively) and the group with diagnosis (8.8 % and 55.9 %, respectively). While this might partially be due to underdiagnosed cases, the persistently high percentages could be associated with non-compliance with the diet, the presence of cross-contamination of foods with gluten or environmental factors, leading to continuous gluten exposure, as will be discussed below. Particularly, only 3 of the volunteers with CeD declared not complying with the gluten-free diet. These 3 volunteers presented a-tTgA and a-GliA (they are the 3 cases that constitute the 8.8 % a-tTgA positive of the CeD group). However, other 16 volunteers with CeD were a-GliA positive too, reinforcing the hypothesis of cross-contamination of foods or the presence of environmental factors. The serological tests used for CeD diagnosis are primarily ELISAs, utilising strict cut-off values to distinguish negative from positive results. For a more detailed analysis of clinical associations with antibody levels, four subject groups were identified based on ELISA values: "negative" (<0.8), "uncertain negative" (0.8–1.0), "uncertain positive" (1.0–1.2), and "positive" (>1.2).

As previously mentioned, there is strong evidence of a significant association between CeD and other immune-mediated diseases [22]. In our study, the prevalence of thyroid disease did not exhibit a significant difference between the groups with and without a CeD diagnosis. Previous studies have shown a positive association between thyroidism and CeD [23] [24]. However, in our sample, both the percentage of subjects without a CeD diagnosis with thyroidism (31.2 %) and the percentage of subjects with CeD and thyroidism (32.5 %) were similar to each other, and higher than the population average. Elevated levels of arsenic in water have been suggested as a risk factor for thyroid disease, and the samples for this study were collected in a city in the Pampas region of Argentina where high arsenic levels in water have been detected [25,26].

Type 1 diabetes mellitus (T1DM) has been identified as a disease associated with CeD. Gluten exposure in untreated CeD patients may promote the development of CeD-associated immune-mediated diseases [27], suggesting a connection between gluten consumption in infancy and the development of CeD and T1DM [28]. Our study found a higher percentage of subjects with diabetes in the group with CeD diagnosis (8.82 %) compared to the group without a diagnosis (5.76 %), consistent with previous literature findings [22].

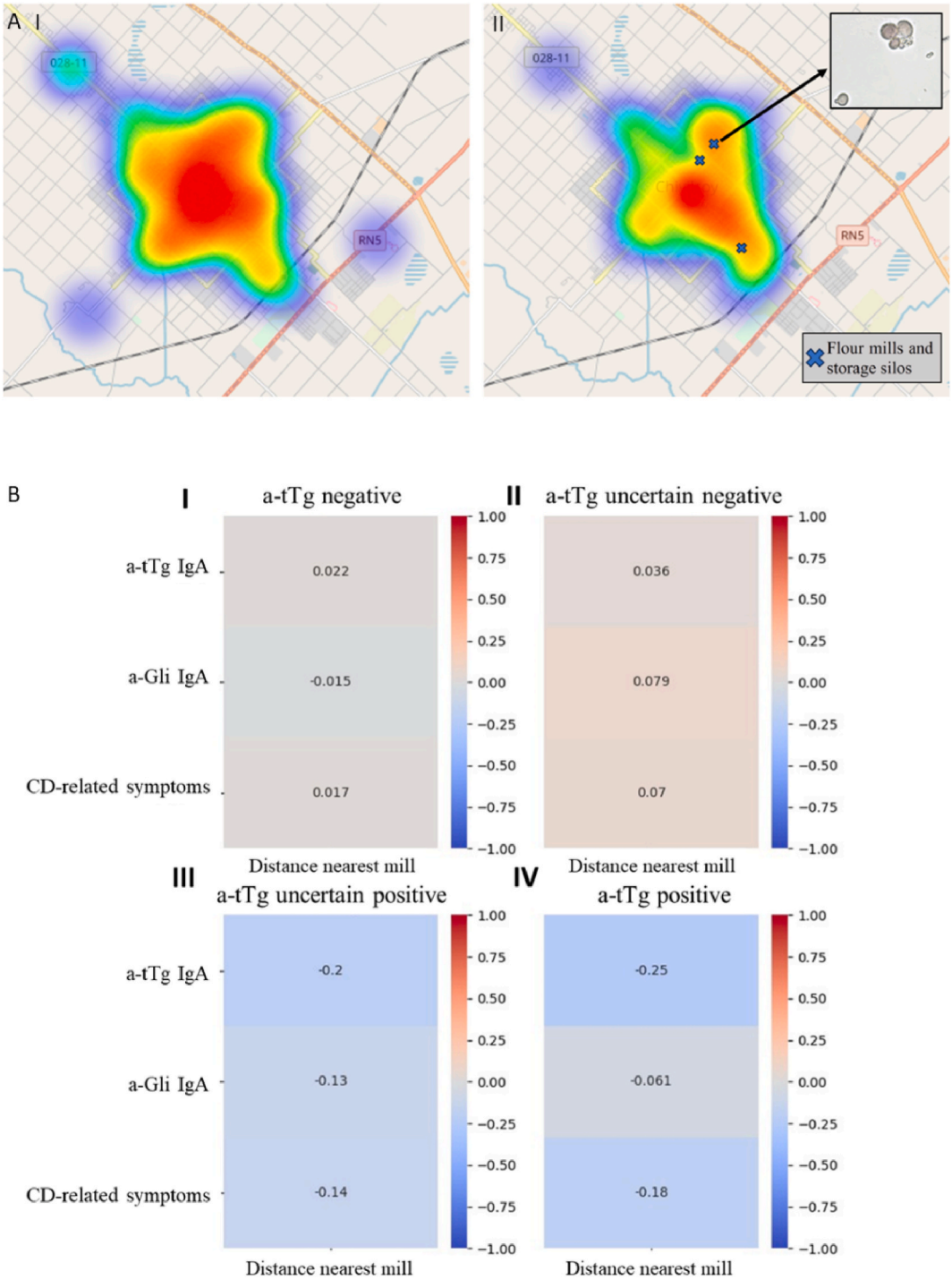

Fig. 4. A. Heatmap showing the density of people belonging to **I-** the negative and uncertain negative (a-tTg<1 arbitrary units) **II-** Positive and uncertain positive group (a-tTg>1 arbitrary units). A warmer colour (orange/red) means a higher density of people in the area. In addition, an optical microscope photograph (40X) of flour particles successfully captured in the environment suspension near a flour mill is shown. This was confirming by polarized light that allows detect starch through the formation of a mark known as a "malte cross" and alternatively by staining with Lugol.**B**. Correlation plots between antibodies (Tg-IgA and Gli-IgA) and the number of symptoms related to celiac disease regarding the distance to the nearest mill considering the four groups of studied people, according to the values of arbitrary units of a-tTg IgA: positive, uncertain positive, uncertain negative and negative. The dark blue scale shows the nearest the mill with a higher value of the variable and red colour indicates a greater distance from the mill.

Among adults with CeD, nutritional deficiencies such as folate, B12 or zinc are common. A previous study reported that 32 % of individuals had iron deficiency anaemia [29]. Our study found similar results, with 29.4 % of subjects diagnosed with CeD reporting anaemia, but no significant difference observed compared to the group without a CeD diagnosis (29.6 %). In adults, osteoporosis may be present in 10 % of patients at the time of CeD diagnosis [30]. In our analysis, 8.82 % of subjects with CeD reported having osteoporosis, while the percentage was lower in the group without a CeD diagnosis at 5.37 %.

In our study, four symptoms associated with CeD were examined: abdominal distention, diarrhoea, gas, and colic pain. Abdominal distention and gas showed no significant difference between the undiagnosed CeD group and the diagnosed CeD group. However, colic pain and diarrhoea were more prevalent in the diagnosed CeD group, consistent with existing literature, where diarrhoea is recognized as a primary symptom in classical CeD cases [30–32]. Factors contributing to diarrhoea in CeD patients include malabsorption leading to increased faecal volume, bacterial metabolism of stool volume producing fatty acids, disrupted intercellular junctions increasing water and solute transport, and bile acid malabsorption [32,33].

While there is less literature on colic pain as a symptom associated with CeD, abdominal pain is associated with the condition. Episodes of colic pain can occur due to bowel distension by large amounts of gas produced by bacterial fermentation [32]. In our study, 38.7 % of control subjects reported this type of pain, compared to 50.0 % of subjects with CeD, suggesting it may be an important symptom indicative of CeD.

This study also examined the rate of pregnancies and abortions. It was found that women diagnosed with CeD had a lower number of pregnancies compared to those without a CeD diagnosis ($p < 0.05$). The association between female infertility and CeD was first observed by Morris et al., in 1970 [34] when they reported three cases of infertility in women with CeD, which was reversed upon adopting a gluten-free diet. Subsequent studies have shown an increased prevalence of CeD in women with infertility [35,36]. Singh et al. (2018) conducted a meta-analysis and found that women with "all-cause" infertility had approximately 3.5 times higher odds of having CeD, while those with unexplained infertility had approximately 6 times higher odds [5]. However, it has been documented that infertility in CeD patients can be reversed by adhering to a gluten-free diet [37,38].

There is an association between CeD and total IgA deficiency [39,40]. Previously, the incidence of IgA deficiency in patients with CeD has been found to be 2–3%, indicating a 10 to 15-fold increase compared to subjects with normal IgA levels [42]. We analysed the total IgA levels in the group of volunteers who tested negative for a-tTgA but reported colic pain and diarrhoea. Of this group, 8.7 % were deficient, but none were a-DGP IgG positive. Of the 63 subjects without total IgA deficiency, 6 were a-DGP IgA positive, so it would be advisable to follow up these subjects who may have CeD even if they do not yet express the other markers.

Finally, the results of our study shed light on the possible association between residential proximity to flour mills and elevated levels of a-tTg antibodies. Our research, conducted in a rural region with varying distances to flour mills, revealed a notable trend of increased antibody levels in individuals living closer to these mills. These results suggest that exposure to mill emissions or by-products may be a contributing factor to the observed immunological response. However, it is important to consider the possibility of other confounding variables that could influence this association. Further investigations, including controlled experimental studies and detailed exposure assessments, are warranted to establish the causal relationship between proximity to flour mills and a-tTg antibody levels. Furthermore, research into the possible mechanisms of exposure and the role of genetic predisposition in this context will be of vital importance to improve our understanding of this association and to be able to design policies that safeguard the health and quality of life of citizens.

Our hypothesis is that exposure to wheat flour in the air is an environmental factor capable of inducing the activation of antigen-presenting cells in both the lungs and the intestine, with the consequent response of the adaptive immune system (B and T cells, among others) and the production of a-tTgA and a-GliA. This would be because the flour present in the air could be both inhaled and swallowed, as well. This increase in specific antibodies would cause, over time, the appearance of symptoms associated with CeD, the development of CeD or some other pathology such as gluten intolerance. To the best of our knowledge, this is the first work that raises this hypothesis, so it is still necessary to conduct more studies to confirm these alternatives. For example, analyzing the relationship of these results with the presence of HLA alleles associated with celiac disease (DQ2/8). However, some previous works demonstrated interesting results that may be related to our hypothesis. Some authors have linked allergies (rhinitis, asthma and dermatitis) to the presence of gluten in the air[41,42,43]. Other work showed that components of flour present in aerosols can reach an intracellular location, or that gluten can trigger symptoms in patients without celiac disease (so-called 'non-celiac gluten sensitivity' or NCGS) [44]. In addition, a case of chronic cough has been reported that improved markedly with a gluten-free diet [45].

In conclusion, our findings show high CeD-specific antibodies in a rural population. This may be due to environmental factors, as an association was observed between household proximity to flour mills and wheat collection sites with higher levels of specific antibodies and CeD-associated symptomatology. In this regard, our group is studying environmental pollutants in the air of the city of Chivilcoy that could influence antibody production. For this purpose, an environmental particle capturing device has been set up, and the captured particles are currently being analysed through optical microscopy. Fig. 4A depicts flour particles successfully captured in the ambient suspension near a flour mill. On the other hand, a higher incidence of diarrhoea and colic pain as well as anaemia was observed in subjects with positive antibodies. High incidence of positive a-tTg antibodies volunteers suggests that this population requires to be tested with a genetic test or small-intestine biopsy. Our results also show that women with CeD have fewer pregnancies, suggesting an association between CeD and infertility. Our results support the hypothesis of underdiagnosis of this disease. Moreover, further research is needed to understand the pathogenesis of CeD and its consequences on patients' health.

CRediT authorship contribution statement

Gabriel Alejandro de Diego: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Natacha**

Cerny: Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gabriel Tolosa:** Software, Formal analysis, Data curation. **Maximiliano Lulic:** Software, Formal analysis, Data curation. **Mariel Fusco:** Investigation. **Fiorella Sabrina Belforte:** Methodology. **Brian Martínez Ruiz:** Investigation. **María Inés Tamborenea:** Writing – review & editing, Methodology, Investigation. **Ana Cánepa:** Methodology, Investigation. **Margarita Cimarelli:** Methodology, Investigation. **Rosana Ghiglieri:** Methodology, Investigation. **Eugenia Díaz:** Investigation. **Exequiel Giorgi:** Investigation. **Claudio Pérez:** Writing – review & editing, Methodology. **Marisa Gassmann:** Methodology, Investigation. **Emilio Malchiodi:** Writing – review & editing, Funding acquisition, Conceptualization. **Rubén Iácono:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Mauricio César De Marzi ,:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Data availability statement

Data will be made available on request.

Funding

Gabriel de Diego and Natacha Cerny are grateful for their doctoral and postdoctoral fellowships granted by CONICET. This work was supported by the under grant from UNLu (PCDD-CB N° 287–18 and DISPPCDD-CB N°27/2023) and PICT-2021-GRF-TI-00507 (to MCDM). Declarations of interest: none.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e40685.

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