Chapter 1
Everything That Must Be Known About the Relationship of Gluten to Human Health

Francesca Di Sario, Chiara Monachesi, Anil K. Verma, and Carlo Catassi

Abbreviations

Anti-tTG	anti-tissue transglutaminase antibodies
ATIs	Amylase-trypsin inhibitors
CD	celiac disease
DGP	anti-deamidated gliadin peptides antibodies
EATL	enteropathy-associated T cell lymphoma
EFSA	European Food Safety Authority (EFSA)
ELISA	enzyme-linked immune sorbent assay
EMA-IgA	IgA class anti-endomysial antibodies
ESPGHAN	European Society for Pediatric Gastroenterology, Hepatology, and Nutrition
FDA	Food and Drug Administration
FODMAPs	fermentable oligosaccharides, disaccharides, monosaccharides and polyols
GFD	gluten-free diet
GIP	gluten immunogenic peptides
GRD	gluten-related disorders
NCGS	non-celiac gluten sensitivity
RCD	Refractory celiac disease
T1DM	type 1 diabetes mellitus

F. Di Sario
Division of Pediatrics, DISCO Department, Polytechnic University of Marche, Ancona, Italy

C. Monachesi · A. K. Verma
Celiac Disease Research Laboratory, Polytechnic University of Marche, Ancona, Italy
e-mail: c.monachesi@univpm.it

C. Catassi (✉)
Division of Pediatrics, DISCO Department, Polytechnic University of Marche, Ancona, Italy

Mucosal Immunology and Biology Research Center, Division of Pediatric Gastroenterology and Nutrition, Massachusetts General Hospital, Boston, MA, USA
e-mail: c.catassi@univpm.it; ccatassi@mgh.harvard.edu

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WA	wheat allergy
WDEIA	Wheat-dependent exercise induced anaphylaxis

1.1 Introduction

Gluten-related disorders (GRD) include celiac disease (CD) and other medical conditions like non-celiac gluten sensitivity (NCGS) and wheat allergy (WA) that are triggered by the ingestion of gluten-containing cereals, particularly wheat, and cured by the elimination of gluten-containing food from the diet. In this chapter we will briefly describe the clinical features of these disorders and point out how they can be differentiated and diagnosed in clinical practice. Finally, aspects related to the gluten-free diet (GFD), its nutritional quality, the gluten contamination issue and the impact of this treatment on daily life will be discussed.

1.2 Celiac Disease and Other Gluten-Related Disorders

1.2.1 Celiac Disease (CD)

CD is a chronic, autoimmune enteropathy triggered by the ingestion of gluten in genetically predisposed individuals. Gluten is the main protein component of wheat, rye and barley and is responsible for the baking properties of these cereals giving them cohesivity, viscosity and dough elasticity. Gluten includes two major protein fractions, i.e. gliadins and glutenins. Both gliadins and glutenins are rich in proline and glutamine that increase resistance to gastrointestinal proteolysis, since gastric, pancreatic, and brush border enzymes are deficient in proline endopeptidase activity. The final product of this incomplete digestion is a mix of peptides that may activate immune response (both innate and adaptive) leading to intestinal damage (Catassi et al. 2022).

Pathophysiology of CD

The etiology of CD is multifactorial and results from a complex interplay between genetic, environmental factors and immunological mechanisms. CD is strongly associated with the expression of specific human leucocyte antigen (HLA) class II molecules, i.e. HLA-DQ2 (HLA-DQA1*05:01-DQB1*02:01) and HLA-DQ8 (DQA1*03:01-DQB1*03:02). DQ molecules are expressed on the surface of antigen presenting cells (APC) and interact with selected gluten peptides generating an abnormal immune response (Megiorni et al. 2012). Although these predisposing HLA genotypes are present in most cases of CD (at least 95% of patients), their expression is necessary but not sufficient for the development of the condition. HLA alone does not fully explain genetic susceptibility and additional genetic factors

may be involved. Gluten exposure is essential for the development of CD. In the intestinal lumen, gluten-derived peptides cross the epithelial barrier and reach the lamina propria where they are deamidated by tissue transglutaminase and presented by antigen presenting cells (APC) to CD4+ T-cells in the context of HLA-DQ2 or HLA-DQ8 molecules. The activation of CD4-lymphocytes produces high levels of pro-inflammatory cytokines leading to the typical mucosal damage of CD (infiltration of inflammatory cells, crypt hyperplasia and villous atrophy). Moreover, the T-cell mediated immune response activates B cells which secrete anti-gliadin, anti-endomysium and anti-tissue-transglutaminase antibodies (Kagnoff 2007).

Another important factor involved in CD pathogenesis is represented by increased gut permeability. In normal circumstances, intestinal epithelial barrier is poorly permeable to macromolecules. In CD the up-regulation of zonulin (a protein involved in the integrity of the Tight Junction system) causes an upregulation of the intestinal barrier function facilitating the translocation of gluten peptides into the subepithelial compartment (Fasano et al. 2000).

Epidemiology

CD is one of the most common life-long disorders affecting approximately 1% of general population worldwide with female and children predominance (Fasano et al. 2012; Singh et al. 2018). The incidence of CD has increased during the last decades due to the availability of sensitive diagnostic tools, awareness of the wide clinical polymorphism, and increased serological screening of individuals considered to be at high risk for the disorder. CD tends to be more common in people who have family history of CD, as well as in individuals with some genetic disorders (e.g. Down and Turner syndrome), IgA deficiency, type 1 diabetes mellitus (T1DM), thyroiditis and other autoimmune diseases, and inflammatory bowel diseases. From an epidemiological point of view, CD may be represented by an iceberg with the majority of cases, i.e. the submerged part of the iceberg, remaining undetected (Catassi et al. 1994).

Clinical Presentation

The clinical spectrum of CD is wide including symptomatic cases with intestinal and/or extraintestinal symptoms, subclinical forms which are occasionally diagnosed because of serological screening and potential cases (positivity of serum celiac autoantibodies and HLA-DQ2/DQ8 haplotypes, and a normal small intestinal mucosa at the small intestinal biopsy). Gastrointestinal symptoms include chronic diarrhea, steatorrhea, weight loss, bloating, flatulence, abdominal pain, abdominal distension and constipation. However, the majority of people with CD have signs and symptoms unrelated to the digestive system, including iron deficiency and microcytic anemia (because of iron malabsorption, chronic inflammation and resistance to oral iron therapy), bone disorders (osteopenia or osteoporosis as a result of impaired calcium absorption and vitamin D deficiency), hair loss, unexplained hypertransaminasemia, cutaneous manifestations such as dermatitis herpetiformis, mouth ulcers (aphtous stomatitis), tooth enamel defects, chronic fatigue, neurological disorders such as ataxia, peripheral neuropathy, epilepsy (mainly with occipital

calcifications), endocrinologic dysfunctions, growth failure, gynecologic and fertility problems (Fasano et al. 2001). Loss of gluten tolerance can present at any age after the introduction of gluten, but symptoms differ in children and adults. Young children tend to have the more classical intestinal signs of CD, including growth problems, while older children and adults may present with symptoms that are non-specific or atypical.

Complications

CD complications are rare and affect approximately 1% of patients (Singh et al. 2018). Several studies demonstrated that a late diagnosis of CD (after the age of 50) and/or poor GFD compliance is associated with higher mortality compared to that of the general population (Rubio-Tapia et al. 2016). Complications of CD include refractory CD, ulcerative jejunoileitis, enteropathy-associated T cell lymphoma (EATL) and splenic hypofunction.

Refractory celiac disease (RCD) is characterized by the persistence of clinical symptoms and histological lesions after at least 12 months on a strict GFD. In most cases the absence of response is caused by intentional or unintentional gluten intake. RCD includes type 1 (IEL population has a normal CD3+CD8+ phenotype) and type 2 (presence of a monoclonal rearrangement of T cell receptor). The distinction between the two forms is imperative because of different therapeutic management and prognosis. Type II RCD is more aggressive and can lead to the development of intestinal lymphoma.

Ulcerative jejunoileitis is characterized by multiple ulcerations of the small bowel that can evolve in strictures and cause obstruction, bleeding and perforation with peritonitis. It is clinically characterized by abdominal pain, diarrhea, weight loss and low-grade fever. The recognition of this condition is important since mortality is very high.

The EATL is a rare and aggressive form of non-Hodgkin lymphoma. It is more common in men over 60 years and is often preceded by type 2 RCD. Unfortunately, these patients have a very poor outcome with a reported 5-year survival rate lower than 20%.

Hyposplenism (functional or anatomic) is often associated with CD affecting more than one-third of adult celiac patients. Due to splenic hypofunction CD patients are at risk of developing severe complications, such as thromboembolic events and encapsulated bacterial infections.

Diagnosis

The diagnosis of CD relies on a combination of clinical, serological and histopathological findings. In current clinical practice, IgA anti-tissue transglutaminase antibodies (Anti-tTG) (or IgG class in subjects with IgA deficiency) is the most useful marker for diagnosing CD with a 95% sensitivity and 97% specificity (Rostom et al. 2005). Anti-endomysial antibodies (EMA-IgA) are highly specific for CD (about 100% of specificity in active CD) but their determination is expensive and operator-dependent. Therefore, they are usually used as a confirmatory test. IgG anti-deamidated gliadin peptides antibodies (DGP), are useful in subjects with IgA

deficiency and are the first marker that become positive in young children. HLA-DQ2 and HLA-DQ8 testing should not be used routinely but only in selected clinical situations, such as a screening test in high-risk individuals, due to their high negative predictive value.

In adult patients, an antibody positive test requires an endoscopy with small bowel biopsy for the definitive diagnosis of CD. The histological hallmark of CD is an increased intraepithelial lymphocyte count and a variable degree of villous atrophy. In children, according to the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) guidelines for diagnosis of CD, small intestinal biopsy may be omitted in children showing high titer of anti-TTG antibodies (higher than tenfold the upper normal limit) and EMA positivity (Husby et al. 2012).

Dietary Management

At present, the only accepted treatment for CD is the GFD (Kupper 2005; Ciacci et al. 2015). Strict adherence to the GFD normally leads to the resolution of symptoms (either intestinal and extraintestinal), normalization of serological tests and of intestinal villi architecture. Full normalization of both anti-tTG levels and histopathology may take over 2 years (severe small bowel lesions and high anti-tTG levels at diagnosis) (Monachesi et al. 2020). Additionally, a correct GFD is a key factor for decreasing risk of complications (particularly bone disorders, infertility and malignancy). Adhering to a GFD may have a negative impact on the quality of life, especially in adolescents, and may induce subtle vitamin and mineral deficiencies.

1.2.2 Non Celiac Gluten Sensitivity (NCGS) and Wheat Allergy (WA)

Non Celiac Gluten Sensitivity

NCGS is characterized by intestinal and extra-intestinal symptoms related to the ingestion of gluten (and potentially other wheat components) in subjects that are not affected by either CD or WA (Catassi et al. 2015). This entity has been described recently and many aspects of its epidemiology, pathophysiology, clinical spectrum and treatment are still unclear. Current evidence suggests that NCGS is more common in adults, particularly in females, but the overall prevalence in the general population is not clearly defined. As regards to disease mechanisms, experimental data suggest the role of an abnormal wheat-induced innate immune response. Clinical presentation of NCGS includes both intestinal (Irritable Bowel Syndrome like symptoms) and not intestinal symptoms. The most common intestinal symptoms are abdominal pain, bloating and alternating bowel habits (either diarrhea or constipation). Extraintestinal manifestations include headache, foggy mind (difficulties in concentrating), fatigue, joint and muscle pain, leg or arm numbness, dermatitis (similar to eczema), recurrent oral ulceration, anxiety and depression.

The latency between gluten ingestion and the appearance of symptoms is usually short, within hours or days. Unlike CD, patients with NCGS do not seem at risk for long-term complications.

Unfortunately, no sensitive and/or specific diagnostic biomarkers of NCGS have been identified so far. In contrast with CD, TTG, EMA and DGP antibodies are generally negative (except for an isolated positivity of first-generation antigliadin antibodies particularly of IgG class) and duodenal biopsies are either normal or show isolated duodenal lymphocitosis. Moreover, the CD-predisposing HLA-DQ2 and DQ8 genotypes are found in only 50% of patients with NCGS, and have no relevance for diagnosing this condition.

The diagnosis of NCGS is based on: (a) exclusion of CD and WA (b) defining patient's clinical response to the GFD (at least 6 weeks of GFD) and evaluation of the effect of reintroducing gluten by a double-blind, placebo-controlled challenge (Catassi et al. 2015). However, this approach is difficult to apply in daily clinical practice because of frequent self-diagnosis and treatment with GFD among patients. Moreover, since NCGS is not a chronic condition, gluten tolerance should be re-evaluated over time.

The only available treatment for NCGS is the GFD, although it is not already established if patients have to follow it on a life-long basis (Khan et al. 2020). Recently data suggest that other wheat components, particularly amylase-trypsin inhibitors (ATIs) and fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) may be involved in triggering NCGS-like symptoms.

Wheat Allergy

WA is one of the most common food allergies. It is defined as a hypersensitivity reaction to wheat proteins triggered by an IgE-dependent mechanism. It shows greater prevalence in children while it is quite uncommon in adult population.

Symptoms typically occur within minutes to hours after the ingestion of wheat proteins and may be gastrointestinal (abdominal pain, bloating, nausea and vomiting, diarrhea), respiratory (bronchial obstruction, rhinitis), cutaneous (hives, atopic dermatitis). Rarely angioedema or anaphylaxis may occur (Inomata 2009). Wheat-dependent exercise induced anaphylaxis (WDEIA) is a particular type of WA which occurs when ingestion of wheat is followed within a short period of time by physical activity. Diagnosis of WA is classically based on skin tests (prick or patch technique and intradermal injections), measurement of total IgE and specific IgE antibodies (for example anti- wheat – barley and rye). However, since these tests are inadequately sensitive and specific, an open food challenge can be considered to confirm WA. WA can be transient, especially in children with predominantly gastrointestinal manifestations, or can persist as a lifelong disorder. Dietary allergen avoidance is the primary treatment for wheat allergy. Table 1.1 summarizes the main features and differential characteristics of gluten-related disorders.

Table 1.1 The main features of gluten-related disorders

	Celiac disease	Non Celiac Gluten Sensitivity	Wheat allergy
Time interval between gluten exposure and onset of symptoms	Weeks-years	Hours-days	Minutes-hours
Pathogenesis	Autoimmunity (Innate + Adaptive Immunity)	Immunity? (Iinnate Immunity?)	Allergic Immune Response
HLA	HLA DQ2/8 restricted (∼97% positive cases)	Not-HLA DQ2/8 restricted (50% DQ2/8 positive cases)	Not-HLA DQ2/8 restricted (35-40% positive cases as in the general population)
Auto-antibodies	Almost always present	Always absent	Always absent
Enteropathy	Almost always present	Always absent (slight increase in IEL)	Always absent (eosinophils in the lamina propria)
Symptoms	Both intestinal and extra-intestinal (not distinguishable from GS and WA with GI symptoms)	Both intestinal and extra-intestinal (not distinguishable from CD and WA with GI symptoms)	Both intestinal and extra-intestinal (not distinguishable from CD and GS when presenting with GI symptoms)
Complications	Co-morbidities Long term complications	Absence of co-morbidities and long term complications (long follow up studies needed to confirm it)	Absence of co-morbidities. Short-term complications (including anaphylaxis)

1.3 Nutritional Considerations of the GFD

1.3.1 Gluten Exclusion in the Management of CD and Other GRD

Due to its nutritional properties, affordability, adaptability, and accessibility, over the last thousand years wheat has become a worldwide staple food. More than 25,000 different cultivars have been produced by wheat breeding, mostly consumed after processing into bread, pasta, noodles, bulgur and couscous. Wheat grain include three major components: starch, proteins, and cell wall polysaccharides, accounting for about 90% of the dry weight. Gluten is the main structural protein complex in wheat with equivalent toxic proteins in rye, barley, spelt, einkorn, khorasan wheat (usually marketed as Kamut), and triticale. The wide availability and functional properties of gluten proteins contribute to its wide use as an ingredient in food processing. Gluten is not a single protein but a mixture of proteins named prolamins, representing about 80% of the total protein content in grain. Gluten proteins

are divided into two major fractions: (i) gliadins (monomeric, subdivided into ω-, γ-, and α/β-gliadin fractions), and (ii) glutenins (large polymers). The most immunogenic gluten fragment is the 33-mer peptide, which consists of 33 amino acids of the α-gliadin fraction (Lionetti et al. 2011; Sapone et al. 2012; Catassi et al. 2013). All the gluten-containing cereals are excluded in the GFD.

The only effective treatment for CD is a lifelong and strict GFD (Lionetti et al. 2011; Fasano et al. 2012). Unlike food allergies, CD does not always cause immediate and severe symptoms after gluten ingestion. This is why many patients following the GFD, mostly young and asymptomatic or with mild symptoms when diagnosed, tend to occasionally ingest amounts of gluten. The protracted ingestion of gluten traces (>10–50 mg/day) is sufficient to cause significant intestinal mucosa damage (Catassi et al. 2007), possible reappearance of CD specific autoantibodies in blood, increased risk of long-term complications such as osteoporosis and lymphoma (Lionetti et al. 2011). Based on the above daily threshold, a maximum tolerable amount of gluten of <20 parts per million (ppm) in gluten-free food has been calculated (Catassi et al. 2007), a threshold that has been endorsed by the major international regulatory agencies, e.g., the Codex Alimentarius, the US Food and Drug Administration (FDA), and the European Food Safety Authority (EFSA) (2008, 2013).

Nutritional problems may be found at both diagnosis and after starting the GFD. At diagnosis, deficiencies are the consequence of nutrient malabsorption caused by the intestinal mucosal damage with frequently low levels of iron, copper, folate, B12 vitamin and zinc. In the course of the GFD, the dietary problems are likely to be related to the reduced nutritional quality of the gluten-free products and inadequate alimentary choices.

1.3.2 Dietary Guidelines for a Balanced GFD

Following a strict GFD requires critical attention in the selection of gluten-free food, ingredients, and nutritional content. To avoid unintentional gluten exposures, it is extremely important for CD patients to look for the gluten-free label before buying processed gluten-free foods (2013). There are multiple organizations certifying gluten-free foods, complying with differing criteria for certification, from a level of gluten <5 ppm up to <20 ppm. Allowed fresh foods, that can be consumed confidently without extra screening, are fruits and vegetables, many kind of seeds, legumes and nuts in their unprocessed forms, eggs, lean, unprocessed meats, fish and poultry, amaranth, buckwheat, flax, rice, soy, corn, potatoes, millet, quinoa, teff, and alcoholic beverages produced from naturally gluten-free ingredients. Alcoholic beverages made from gluten-containing grains processed to remove gluten must carry a label stating that gluten content cannot be precisely determined and may contain some gluten traces. Foods labeled “gluten-free” may include naturally gluten-free food, processed food without gluten-containing ingredients, or food with a gluten-containing ingredient that has been processed to remove gluten.

Newly diagnosed CD patients are advised to receive dietary counseling on food labeling, and the most appropriate gluten-free foods (Penagini et al. 2013). The consumption of naturally gluten-free cereals and pseudo-cereals should be encouraged in CD patients. Amaranth, quinoa and buckwheat are an excellent option, since they are good sources of carbohydrates, protein, dietary fiber, vitamins (folate, riboflavin, vitamin C and vitamin E) and polyunsaturated fatty acids. Recent evidence showed that long-term introduction of selected uncontaminated and nonreactive varieties of oats can be safely included in the diet of patients with CD, with no deleterious effects at the clinical, serologic, or mucosal levels (Lionetti et al. 2018). Noteworthy, the inclusion of oats in the range of safe gluten-free options has many potential benefits for CD patients, being a good source of fiber (especially beta-glucans), iron, thiamine, B complex vitamins, providing a higher satiety value than other gluten-free cereals, and increasing the palatability and dietary variety.

A GFD is a nutritionally safe intervention that allows remission of the disease without any major risks. Apart from maintaining the above-mentioned safe limit of gluten intake (below 10–50 mg/day), a suitable GFD must also be nutritionally adequate to prevent micronutrients and fiber deficiencies, allow appropriate growth in children and pubertal development, and avoid long-term potential harms like hyperlipidemia, and hyperglycemia. Studies have shown sub-optimal intake of some nutrients over the long run, particularly fiber, calcium, iron, folate and other vitamins, with the GFD (Mariani et al. 1998; Lionetti et al. 2020).

GFD imbalance may occur because of the need of excluding several cereals or the different nutritional composition of gluten-free products as compared to their unrefined counterparts. In this regard, national/international food pyramids for food groups' consumption references and recommended energy and nutrient intake levels should be followed. Gluten-free raw materials result in gluten-free food which is less palatable than regular foods. The manufacturing of gluten-free products often requires the addition of additives such as hydrocolloids, and some macronutrients such as fats to mitigate the lack of gluten. Commercially available gluten-free products are quite expensive, and eating out can be socially challenging (Mariani et al. 1998; Valletta et al. 2010; Kabbani et al. 2012).

1.3.3 Adherence to the GFD

Frequent monitoring is crucial to promote adherence to GFD and early identification of nutritional deficiencies and/or metabolic imbalances. Several procedures are available to evaluate the compliance with the GFD, such as clinical symptoms monitoring, interview by an experienced dietitian, structured dietary questionnaires, serological tests (i.e. anti-tTG antibodies and DGP antibodies), intestinal permeability test, and small intestinal histology. Among the gluten-dependent biomarkers, serological tests are highly sensitive and specific for diagnosis, but not enough to detect occasional transgressions (Ludvigsson et al. 2018; Myléus et al. 2020). The use of serial biopsies is invasive and impractical for close monitoring. Normalization

of serology and histopathology may take over 2 years, particularly in those with severe small bowel lesions and high anti-tTG antibodies levels at diagnosis (Sansotta et al. 2020; Monachesi et al. 2020). In recent years gluten immunogenic peptides (GIP) determination has gained scientific attention (Moreno et al. 2017). GIP are fragments of gluten proteins excreted in urine and in stools, detectable using specific monoclonal antibodies, particularly A1 or G12. However, there are some open questions about their clinical performance (i.e. latency between gluten exposure and appearance in stool/urine, and the relationship between the quantity of ingested vs eliminated gluten) (Monachesi et al. 2021a). So far, a reliable biomarker to test the adherence to the GFD in the long-term follow-up of CD patients is still lacking.

1.4 Gluten Contamination: Current Scenario and Advances

1.4.1 What Is Gluten Cross-Contamination?

Gluten cross-contamination occurs when a gluten-free grain/food item is directly or indirectly cross-contacted with a gluten-containing grain/food item. Cross-contamination can occur at various stages, for example when cereals are grown, harvested, processed, and/or bulk traded (Lee et al. 2014). Crop rotation is a beneficial farming practice, but this allows mixing of grains. Lentils grow in cooler climates similar to wheat and barley. Hence crop rotation between wheat and lentils increases the possibility of mixing the grains. Apart from this, transportation of grains in the same vehicle, use of shared harvesting equipment, and shared storage facilities cause cross-contamination (Lee et al. 2014).

1.4.2 Worldwide Status of Gluten Contamination of the GFD

In an early study, Gibert et al. (2013) showed a safe threshold level of gluten (i.e. <20 mg/kg gluten) in a wide range of commercially available European gluten-free food products (n = 205) (Gibert et al. 2013). However in recent years, studies from USA, Brazil, and Canada have shown a variable level of gluten contamination (i.e. 20.5%, 21.5%, and 9.5% respectively) in the locally available commercial gluten-free products (Koerner et al. 2013; Lee et al. 2014; Farage et al. 2017). In an interesting study, Farage et al. showed that 21.5% of commercially available Brazilian bakery products were cross-contaminated with gluten and not safe for CD patients (Farage et al. 2017). However, in another study the authors investigated gluten contamination in naturally gluten-free meals collected from food services in Brazil, and found a low occurrence of gluten contamination in naturally gluten-free preparations (Farage et al. 2019). A study conducted by Atasoy et al. reported a significant amount of gluten contamination (17.5%) in manufactured gluten-free products available on the Turkish market (Atasoy et al. 2020). On the other hand, recent

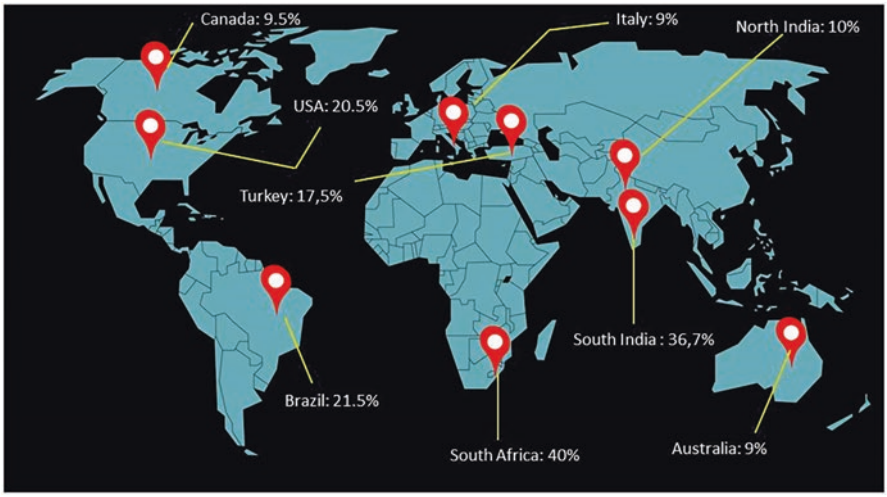

Fig. 1.1 Percentage of labelled gluten-free products containing gluten above the threshold limit (20 ppm) in different countries

studies from India found 10% contamination in labeled and 36.7% contamination in naturally gluten-free products (Raju et al. 2020; Mehtab et al. 2021). In a landmark large survey, our group showed a low level (9%) of gluten contamination in the gluten-free food products available in Italian supermarkets. This study also confirmed that naturally gluten-free products (lentil, oats) are at a significantly higher risk of gluten contamination as compared to certified gluten-free food products (Verma et al. 2017).

In a meta-analysis-based review article, Guennouni et al. reported that 15% of gluten-free food products contain gluten levels higher than the accepted safe threshold (>20 mg/kg). The study also confirmed that naturally gluten-free foods are significantly more often contaminated than labelled gluten-free products (Guennouni et al. 2021).

In summary, all these studies report the possibility of gluten contamination in products that are labelled as gluten-free (Fig. 1.1). These data show that there is an unmet need for a strict and universal gluten-free labeling regulations all over the world. A rule should also be imposed on the food manufacturers to declare the product ingredients on the package.

Is it possible to quantify the amount of contaminating gluten into the diet of the "average" celiac patient? Syage et al. estimated that treated CD patients on average consume up to 400 mg/day of gluten accidentally (Syage et al. 2018). However, this is likely to be an overestimation, at least talking about patients who do their best to comply with the requirements of the GFD. In another study from our group (Monachesi et al. 2021b) we quantified the accidental gluten contamination in the diet of Italian children. The results of this study found that gluten contamination in the daily diet is unusual and is usually below the safety threshold of 10 mg/day in the Italian pediatric population. This safe situation was achieved thanks to the high

awareness of CD in this country (Monachesi et al. 2021b). However, unintentional gluten exposure is likely to be more common and needs further investigation in countries where CD awareness is not as high as in Italy.

1.4.3 Gluten Contamination in the Kitchen

See et al. in 2015 described four main ways of gluten cross-contamination, i.e. in the field, the factory or retail premises, restaurants, and home (See et al. 2015). During food preparation, gluten-free items may be contaminated in several ways. In a recent study conducted by Thompson et al. shared fryer, i.e. a fryer that uses gluten-free fries and oil but is used for cooking also gluten-containing food, has a high risk of gluten cross-contamination (Thompson et al. 2021). Cross-contamination at home can occur through shared kitchenware, the same cooking platform, and the use of gluten-containing food ingredients with gluten-free food ingredients. Miller et al. in 2016 reported that gluten-free foods should be kept at a minimum distance of 2 meters in a shared kitchen to avoid cross-contamination (Miller et al. 2016). A study conducted by Studerus et al. in 2018 confirmed that gluten cross-contamination in the kitchen environment may occur but using the same utensils for cooking gluten-containing food and gluten-free food generally does not cause cross-contamination in a domestic environment. However, the kitchen ladle used to cook gluten-containing pasta should be avoided for cooking gluten-free pasta.

Recently Weisbrod et al. (2020) have shown that the preparation of gluten-free food in close contact with gluten-containing food may not always cause gluten cross-contamination. In this study the authors investigated the gluten transfer and efficacy of washing methods during food preparation: (1) cooking pasta, (2) toasting bread, and (3) slicing cupcakes. They found that cooking gluten-free pasta with the water used to boil gluten-containing pasta is not safe but rinsing the pasta with fresh running water makes it safe to eat for CD patients. They also found that using the same toaster for gluten-containing and gluten-free bread does not cause cross-contamination and the use of the same knife to slice the gluten-containing and gluten-free cupcakes does not always cause cross-contamination (Weisbrod et al. 2020).

1.4.4 Methods to Detect Gluten Traces in Food Products

Several methods are available to measure traces of gluten in food items. However, no test is fully reliable so far. Nonetheless, the enzyme-linked immune sorbent assay (ELISA) test is considered a trusted method for this purpose (Verma et al. 2017).

Several antibodies (R5, A1/G12, α-20, MIoBS, DQ2.5-glia-α3, and Skerritt) have been developed to detect the gluten traces in food products (Verma et al. 2017;

Panda et al. 2019). All these antibodies use different gluten extraction methods and different gluten standards. That is why they show discrepancies in their gluten quantification results. Apart from these, matrix interference and antibody-binding affinities also cause variability in the ELISA results. There are no official reference materials approved, and there is no universal unit of measurement of gluten (Diaz-Amigo et al. 2013; Sharma et al. 2013; Hochegger et al. 2015; Osorio et al. 2019). However, these antibodies are highly specific for gluten traces. Gluten ELISA provides a satisfactory result in short time and it is an easy method to perform. Despite some limitations, the ELISA method is considered adequately reliable and the method-of-choice to detect gluten contamination in food products.

Among the developed gluten detection antibodies, R5 and G12/A1 (commonly known as G12 antibody) antibody ELISAs are the most frequently used gluten ELISA method. R5 antibody-based ELISA method is the most prevalent and is also a reference method for gluten estimation (Verma et al. 2017; Scherf et al. 2021). This method has been endorsed by the Codex Alimentarius as a Type 1 method to determine gluten in food (Scherf et al. 2021).

The R5 monoclonal antibody is raised against the omega-Secalin from rye, and strongly recognizes the most toxic fragments of gliadin i.e. QQPFP, QQQFP, LQPFP, and QLPFP sequences (Osman et al. 2001). G12/A1 antibody-based sandwich ELISA is another frequently used ELISA method to detect gluten in foodstuff (Escarnot et al. 2018; Silvester et al. 2018). A1/G12 are highly sensitive monoclonal antibodies developed against the α2-gliadin 33-mer toxic peptide of the gliadin (Morón et al. 2008b). G12 antibody is claimed to have a broader specificity for the prolamins that are more toxic for CD patients (Morón et al. 2008a, b). It is restricted to the detection of the QPQLPY sequence of 33-mer toxic gliadin fragments, while A1 has a broader range of epitope recognition, and apart from the QLPYPQP sequence, it also recognizes two more sequences (QQPFPQP and QLPFPQP) (Morón et al. 2008b).

Some studies have assessed the performance of the R5 and G12/A1 antibodies-based ELISA methods and found that both antibodies work well and provide similar results (Bruins Slot et al. 2015; Hochegger et al. 2015; Yu et al. 2021).

1.4.5 Future Methods to Detect Gluten Traces in Food Products

Apart from these immunological methods (ELISA), in the last decades, several non-immunological methods have been developed to allow precise gluten quantification in food products (Osorio et al. 2019). Among these methods, DNA-specific genomics methods (i.e., PCR, QC-PCR, RT-PCR, real-time immune-PCR) are more sensitive than ELISA, but sometimes they show false-positive results and cannot be applied to hydrolysed food products (Osorio et al. 2019). Proteomics-based methods (i.e., column chromatography, gel permeation chromatography, MALDI-TOF

MS, LC-MS/MS, NIR spectroscopy) have also been established as a method to accurately quantify gluten (Mejías et al. 2014; Scherf et al. 2016). LC-MS/MS method is an extremely sensitive analytical technique that can effectively characterize, and quantify gluten fragments (Osorio et al. 2019). However, due to their respective drawbacks, such as complexity of the technique and difficulty in differentiating gluten and non-gluten proteins, these approaches are not regularly in use. In recent years, biosensors (gold nanoparticle-based immune sensors) were found to be a highly specific test for gluten quantification, but these tests require further validation (Khot et al. 2012; Vinci et al. 2019).

Despite these various tools, analysis of gluten contamination remains a significant issue. There is certainly a need to develop a reliable gluten tracking method with high accuracy and precision. This topic will be deepened in Chap. 10, because of its relevance in this book.

1.5 Conclusions

CD and other gluten-related disorders are common and affect humans worldwide with a wide array of clinical manifestations, both intestinal and extra-intestinal. Specific investigations and algorithms have been developed to correctly diagnose and treat these conditions, even though a biomarker of NCGS is still lacking. The GFD is the treatment of choice of gluten-related disorders. This is a safe nutritional intervention that is however difficult to maintain since gluten is a pervasive ingredient that may contaminate naturally or rendered gluten-free items.

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