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Celiac Disease

From the Bench to the Clinic

Edited by Luis Rodrigo and Carlos Hernández-Lahoz

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Meet the editors

Dr. Luis Rodrigo MD is Emeritus Professor of Medicine at the University of Oviedo (Spain). He has been the Chief of the Gastroenterology Service at HUCA Hospital in Oviedo for more than 40 years. He obtained his PhD in 1975 and has developed a long teaching and research career. He has published a total of 575 scientific papers, 297 written in English and the rest in Spanish. Dr. Rodrigo has participated as main investigator in a total of 45 clinical trials and has directed 40 doctoral theses. He has contributed actively to the formation of around 100 specialists in gastroenterology working in his hospital and other hospitals in Spain and abroad. He has written around 35 chapters in books on several subjects and has been the editor of 22 books in his specialty and related diseases.

Carlos Hernández-Lahoz, MD, PhD, studied High School Studies at the Institute of Logroño (Spain) from 1955 to 1961. He studied at the Medical School at Zaragoza (Spain) from 1962 to 1968 obtaining his Licensee Degree in Medicine. He was also a medical resident at the Hospital General de Asturias obtaining the title of neurologist in Oviedo (Spain) in 1972, a neurology consultant at the Hospital Central de Asturias (HUCA), Oviedo (Spain), from 1990 to 2014, and an associate professor of neurology at the Medical School, University of Oviedo (Spain), in 1990, giving theoretical and practical teaching to medical students for over 24 years, finishing in 2014. He has published a total of 125 articles and 20 chapters in books on neurology, some of them on the subject of neurogluten.

Contents

Preface

It is our great pleasure and honor to present this new book *Celiac Disease - From the Bench to the Clinic*, which refers to the application of experiments to clinical practice to achieve a better and quicker diagnosis of the treatment of this interesting and chameleonic disease. It is the transfer of knowledge from the bench to the bed, as happens in many human diseases, or, in other words, to translate new knowledge from basic investigations to their applications in the clinic.

Celiac disease (CD) can be defined as an autoimmune disease, which may affect several organs but particularly the small intestine, related to gluten toxicity that appears in genetically susceptible persons. It is diffusely distributed worldwide and its incidence is placed between 1 and 2% of the human population. In the last few decades, knowledge about this disease has grown substantially in different areas, and this book is a small sample of these advances.

The editors have written an introductory chapter, which reviews CD from a clinic point of view and describes the associated conditions and their relative frequencies.

Related to the diagnosis of CD, there is an interesting chapter related to serological diagnosis using a new type of test called the point-of-care test, which is an easy and speedy application of several serologic tests in ambulatory clinical consultation. This chapter is written by Prof. Shimoni and clearly describes the different models designed for its application. This has several advantages compared to traditional methods and the most important is the possibility of early detection of CD by general practitioners.

Another chapter refers to the genetics of the disease. Classically, there are two genotypes placed in the HLA system class II associated with the presence of CD, and they are recognized as the most common HLA-DQ2 (90% of cases) and HLA-DQ8 (5% of cases). Prof. Nóbrega et al. describe that the genotype DQ2.5/DQ2.2 is predisposed to this disease, mainly in children.

A very important basic contribution to the pathogenesis of the disease are the autophagic mechanisms, and their role in the production of intestinal lesions in affected children has been described and analyzed by Prof. Bozzola.

The extra-intestinal complications of CD are described by Prof. Jabeen, including ferropenic anemia, osteopenic processes, and some cancer types especially in difficult cases of long evolution and with poor response to gluten-free diet.

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Section 1

Introduction

Chapter 1

Introductory Chapter: Celiac Disease - An Overview

Luis Rodrigo and Carlos Hernandez-Lahoz

1. Introduction

Celiac disease (CD) is a systemic disorder of an autoimmune nature that occurs in genetically susceptible individuals. It is caused by gluten and related prolamins and is characterised by the presence of a variable combination of gluten-dependent clinical manifestations, the presence of CD-specific antibodies, along with genetics compatible with HLA-DQ2 or HLA-DQ8 haplotypes and the presence of varying degrees of enteropathy [1].

It is triggered by the consumption of foods that contain or are made with gluten, mainly through wheat proteins (gliadins), and also rye (secalins), barley (hordeins) and certain varieties of oats (avenins).

It is a chronic disease with a genetic basis that affects, or may affect, various organs and systems in which inflammation of the small intestine may lead to various symptoms and eventually to malabsorption of nutrients. Treatment of CD consists of permanently following a gluten-free diet (GFD), which was developed in 1951 by the Dutch paediatrician Dr. Willem Karel Dicke, in the course of treating children suffering from chronic diarrhoea with malnutrition who had been admitted to the Children's Hospital of Utrecht after the Second World War. He found that they improved when foods containing wheat flour were removed from the diet. This was the starting point for the introduction of the GFD, which is the only treatment for CD that is effective throughout the world, and it has been applied ever since [2].

Genetics, immunology and aspects of the environment are important factors in the development of CD. Its principle determinants are the class II genes of the HLA system, which are largely related to the presence of HLA-DQ2 and HLA-DQ8 [3]. It is primarily an immune disorder, mediated by T cells, that affects the intestinal mucosa of genetically predisposed individuals. CD4+ T cells recognise gluten peptides, which are selectively present in the context of the molecules HLA-DQ2(+) and DQ8(+) [4].

The enzyme transglutaminase 2 (TG2) deaminates positively charged gluten peptides. Gluten-specific CD4+ T cells, such as the cytotoxic intraepithelial CD8 T lymphocytes, play an important role in the development of intestinal lesions. Gluten is the most important environmental factor involved in its development, but other environmental factors have been implicated, such as infections, dysbiosis and exposure to drugs [5, 6].

The Consensus Conference of Experts Meeting in 2012 and 2013, celebrated in Oslo and London respectively have accurately described the terms related to CD and also the sensitivity to non-celiac gluten sensitivity (NCGS) and wheat allergy, to unify criteria and accurately define the differences between such disorders [7, 8].

2. Clinical presentations of CD in adults

In adults, the average age of presentation of CD is 44 years (with a wide age range between 14 and 81 years). It has a clear female predominance (3:1), which has been confirmed in young children. Strikingly, approximately 15–25% of cases are diagnosed in people over 60 years of age [9].

In some cases, there is a recorded history of growth retardation or other symptoms that suggest that CD was present in childhood. The classic presentation of the disease with malabsorption, diarrhoea, weight loss and abdominal distension is less common in adults than in children [10].

Diarrhoea is the main way in which CD presents itself, although it occurs in fewer than 50% of patients; constipation is not uncommon in celiac patients and is accompanied by non-specific gastrointestinal symptoms that overlap considerably with those of functional dyspepsia (FD), irritable bowel syndrome (IBS) and functional diarrhoea [11, 12].

Patients with CD may frequently have symptoms that are also characteristic of IBS, including abdominal pain (77%), abdominal distension (73%), chronic diarrhoea (52%), constipation (17%) and/or the presence of a pattern of alternating bowel movement in an intermediate percentage (24%). This means that IBS is often the initial diagnosis for many patients, before the discovery of CD several years later [13, 14].

The presence of symptoms related to gastroesophageal reflux disease (GERD) that do not respond well to treatment with anti-secretory drugs should make the doctor think that the patient may be celiac. For example, in an Argentine study, Nachman et al. evaluated the presence and intensity of GERD symptoms at the time of diagnosis of CD in adult patients and found a significantly higher mean score of reflux symptoms than in healthy controls. In baseline terms, 30.1% of patients with CD presented moderate or severe GERD symptoms, compared with 5.7% of controls [15]. A study conducted by Usai et al. of cases and controls in patients with CD and associated GERD confirmed that the GFD improved symptoms and was a useful strategy for preventing recurrences [16].

The prevalence of extra-intestinal manifestations is very high among adult patients, especially if a specific search for them is carried out. Anaemia, caused mainly by iron deficiency, osteoporosis, dermatitis herpetiformis, recurrent aphthous stomatitis, hypertransaminasaemia and a series of neuropsychiatric disorders may be a common form of presentation of CD in adults [17, 18].

Serological screening in high-risk groups, especially in families of patients with CD, have increased the frequency of detection of the disease in children and adults, some of whom are asymptomatic or have mild, nonspecific symptoms [19].

3. Diagnostic criteria of CD in adults

The diagnostic strategy for an adult patient with suspected CD is complex, given the diversity of possible clinical scenarios in which it can occur. Determining the specific serology for CD by measuring titres of tissue transglutaminase (tTG), endomysial (EMA) and deamidated gliadin (PGD) antibodies should be the initial diagnostic test, because of its simplicity and low cost. It should be carried out in patients presenting signs, symptoms and/or associated diseases.

When the tTG titres are 10 times higher than their normal values (>100 U/ml), they are considered to be diagnostic of CD by themselves, thereby avoiding taking duodenal biopsies, since the probability of finding associated villous atrophy is very high. Hills et al. confirmed that the finding in adults of tTG values >30 U/ml

1. Clinical symptoms suggestive or compatible with CD
2. Positive serology with high titres
3. Positive genotyping for HLA DQ2/DQ8
4. Enteropathy compatible with CD in duodenal biopsies
5. Positive response to gluten-free diet

Table 1.
Criteria of Catassi and Fassano for the diagnosis of CD [22] (five-point rule).

(N < 10) with the Celikey test has a positive reductive value of 100%. Before deciding against taking biopsies during the endoscopy, the positivity of the EMAs should be confirmed and the presence of the genetic markers (DQ2 and DQ8) determined, since their presence reinforces the diagnosis of CD [20].

Conversely, when the levels of the tTG antibodies are less than three times the normal values, a gastroscopy should be done with multiple duodenal biopsies (usually six, of which two must be from the bulb). If the histological results reveal enteropathy, a GFD should be initiated and followed strictly and permanently. In patients with CD-compatible enteropathy and negative serology, genotyping of the HLA-DQ2/DQ8 system may be useful, because if both markers are negative, they make the diagnosis unlikely [21].

Nevertheless, it should be considered that, for any case, neither serological and genetic tests, nor the results of duodenal biopsies are pathognomonic. This means that, in certain cases, it is very difficult to confirm or rule out the presence of CD, given the great variability of possible findings, and it is impossible to simplify the wide range of diagnostic possibilities available for use in the clinical setting.

However, some easily applicable algorithms are available that can be of use. In this regard, in 2010, Catassi and Fassano suggested using a simple five-point rule that is very easy to include and evaluate, assigning a unit value to responses to questions about such items as: clinical symptoms, positivity of serology, the presence of compatible genetic markers, the positivity of histological findings, and the serological and histological responses to the GFD. The presence of four of these five criteria (or three of four if genetic markers are not available) is indicative of a probable diagnosis of CD, if the patient can be or not diagnosed. This system has not yet been prospectively validated, so it is not in general use (**Table 1**) [22].

This system is only of indicative value and is little used in clinical practice because, despite its simplicity, it is difficult to interpret and the weight of each of the included items is not taken into account. Under no account should it be applied with rigid criteria, to conclude whether a particular patient is celiac or not.

4. Non-celiac gluten sensitivity

Non-celiac gluten sensitivity (NCGS) is an emerging disorder, characterised by the presence of intestinal and extra-intestinal symptoms, related to the consumption of foods containing gluten. It appears in individuals who are not affected by CD or by wheat allergy. Despite the lack of reliable epidemiological data, it is estimated that its prevalence worldwide is between 5 and 10 times that of CD (5–10% in the general population). This has contributed to the great increase (a tripling in the US, for example) in the worldwide consumption of gluten-free food in recent years.

NCGS was originally described in 1976 and 1978 [23, 24] and the first series of studies on the subject was published in 1980 [25]. However, it was not until 2010, with the sharp increase in the number of publications, that this apparently novel

A. Relationship of their presence with consumption of food containing gluten
B. Exclusion of wheat allergy
C. Serology of negative CD
D. Absence of villous atrophy

Table 2.
Diagnostic criteria compatible with the presence of an NCGS [26–28].

entity was brought to the attention of physicians and researchers, presenting a challenge to those working in the field of gluten consumption-related disorders.

It is characterised from the clinical point of view by the presence of digestive and extra-digestive symptoms in relation to the consumption of food containing gluten. No precise diagnostic criteria are available, which is why it is fundamentally diagnosed by the exclusion of CD in patients with similar clinical characteristics. The diagnostic criteria of the NCGS are based on additionally ruling out the presence of symptoms related to wheat allergy. Antibodies to CD are negative or have low titres, and duodenal biopsies may show inflammatory changes, but always without any intestinal villous atrophy being present (**Table 2**) [26–28].

The physiopathology of NCGS is still not fully understood. Several pioneering studies have suggested an important role for innate intestinal immunity in the pathogenesis of NCGS, in contrast to CD, in which an adaptive immune response is activated [29, 30].

No cases of family aggregation, presence of associated diseases or long-term complications have been described in NCGS, unlike what occurs in the case of CD. A diagnosis of NCGS in patients with gluten-dependent symptoms, a family history of CD or associated autoimmune diseases, can call into question whether a case of NCGS is, instead, really a case of mild CD, because their very similar clinical characteristics make them very difficult to distinguish.

5. Diseases associated with CD

The extra-intestinal diseases most frequently associated with CD are iron deficiency anaemia, type 1 diabetes mellitus, osteoporosis, thyroid disorders and dermatitis herpetiformis [31]. Autoimmune diseases are generally between 3 and 10 times more frequent in patients with CD than in the general population.

Some hypotheses have been proposed to explain the increase in the prevalence of autoimmune diseases in patients with celiac disease. One of these is that a longer exposure to gluten prior to diagnosis could be a risk factor for the development and appearance of related diseases [32, 33]. However, other researchers determined that the presence of autoimmune diseases in patients with a late diagnosis of CD is not associated with the duration of gluten consumption [34].

From the immunological perspective, CD is characterised by over-expression of interleukin-15 (IL15) at the level of the mucosal surface of the small intestine. There is some evidence about its importance in the association with autoimmune diseases, because, due to the presence of these increased levels of cytokine, the effector T cells in the intestinal epithelium are not suppressed by the regulatory T cells, which would generate a loss of gluten tolerance and a greater presence of antibodies such as auto-antigens [35].

Vitamin D deficiency is another factor that has been implicated in the pathogenesis of autoimmunity in CD due to it frequently being detected at low levels in the blood of patients with CD and other autoimmune disorders. Vitamin D is an

important biological inhibitor of inflammatory hyperactivity, even in the presence of several malignant tumours. Its real role and the details of the mechanisms by which it acts have not yet been fully elucidated [36].

6. Ferropenic anaemia and associated CD

Anaemia without other clinical signs of intestinal malabsorption is one of the most common extra-intestinal manifestations of CD [37]. CD is frequently diagnosed in patients referred for evaluation for iron deficiency anaemia, which is found in 1.8–14.6% of patients [38].

A prospective study conducted in patients with iron deficiency anaemia published in 2005 [39] reported a 5% prevalence of celiac disease. Subsequent studies have confirmed that between 4 and 6% of patients with refractory iron deficiency anaemia of unknown origin have CD. Associated autoimmune gastritis is found in 20–27% of patients, 50% of whom also have an associated active *H. pylori* infection that responds effectively to the eradicating treatment.

The most obvious cause of this anaemia is a decrease in intestinal absorption of iron and other nutrients, including folate and cyanocobalamin. Villous atrophy of the intestinal mucosa is a significant cause of the decrease in iron absorption, as confirmed by the microcytic and hypochromic anaemia revealed in the haemograms of the majority of anaemic patients with CD [40].

The decreased absorption of iron in CD is also revealed by the failure of the serum iron levels to increase following oral administration of iron supplements, whereas the problem is resolved rapidly when iron is administered parenterally.

7. Diabetes mellitus type 1 (DMT1) and associated CD

In children, the first cases with CD and associated DMT1 were reported in 1969 [41]. Thus, a 10-year controlled longitudinal monitoring study of 335 adult celiac patients, diagnosed between 1980 and 1990, compared with a broad group of age- and sex-matched control subjects with various gastrointestinal symptoms, confirmed the high prevalence of endocrine diseases in patients with CD (11.9% in patients versus 4.3% in the control group; $p < 0.003$). More recently, other researchers found a higher prevalence of type 1 diabetes mellitus (5.4–7.4%) in patients with CD compared with controls [42, 43].

DMT1 is diagnosed in more than 90% of cases before CD is confirmed. Patients with diabetes mellitus and symptoms associated with CD who are following a GFD show a clear overall clinical improvement. In children, an increase in the growth rate and a rise in haemoglobin levels are often observed. Better metabolic control of diabetes mellitus is observed, as clearly confirmed by the reduction in the number of hypoglycaemic episodes, and the reduced need for daily insulin if the patient is following a GFD [44].

Juvenile diabetic patients present an average prevalence of CD of about 5% of cases. This strong association is largely due to the fact that celiac patients with and without diabetes share the same genetic base represented by the presence of the HLA-II haplotype, DR3-DQ2, demonstrating that it is appropriate to systematically screen for CD in patients with T1DM. Strategies for follow-up include periodic serological determinations for specific antibodies, first at the time of diagnosis, then, repeated every 6 months for the first year and at least annually for 5 years or more. Patients with positive responses to specific serological tests and in whom genetic susceptibility markers (HLA-DQ2 and/or HLA-DQ8) are present require duodenal biopsies to be taken to confirm the diagnosis of CD. Although many clinical

guidelines recommend carrying out systematic screening for CD, their application in clinical practice, particularly in children, adolescents and young adults, has not yet reached the desired level of performance in many countries of the world [45, 46].

8. Altered bone metabolism: osteopenia, osteoporosis and CD

The association of celiac disease with metabolic bone disorders was known even before the origin and treatment of celiac disease was understood. Osteomalacia, a condition characterised by low bone mineral density (BMD), marked deformities and rickets, has frequently been described in the medical literature. This disease preferentially affects children with CD [47], but is rarely part of their routine clinical presentation of CD [48]. The development and availability of the means to measure bone mineral density by non-invasive techniques has confirmed the clear relationship between low BMD and the presence of CD. BMD determination has been routinely used for adult celiac patients since 2005 [49]. Metabolic bone disease remains a significant and very frequent complication of CD determined at the time of diagnosis in children and adults.

The presence of low BMD leads to a deterioration in the quality of life [50], aggravated by its frequent complications, such as the presence of various repeated bone fractures, spontaneously, or after minor trauma. Currently, the finding of a low BMD is the first diagnostic criterion for confirming the presence of osteoporosis, metabolic and skeletal disease defined by lesions at the level of the bone microarchitecture, increased bone fragility and susceptibility to an increased risk of breaks. The WHO has established diagnoses of osteoporosis when bone mass values are less than −2.5 times the standard deviation (SD) of the maximum bone mass (the maximum value of BMD in an adult), and of osteopenia when these values are between −1 and −2.5 SD less than the maximum.

It is estimated that, at the time of diagnosis, one-third of paediatric patients present osteoporosis, and one-third have osteopenia. Only the remaining third of patients with CD have normal BMD [51]. Although more than half the children with CD have low BMD at the time of diagnosis [52], once a GFD has been initiated, most children with CD achieve a normal height and weight for their age, and their rate of bone mineralisation accelerates, in such a way that most of them attain their maximum bone mass when their bones finish growing [48]. The most serious problem arises when CD is diagnosed during adulthood, by which time bone growth is complete and maximum bone mass has been reached. The prevalence of osteoporosis in adult patients with CD is twice that of adults of the same age in the unaffected population [53]. The average prevalence of low BMD in adult CD patients is 40%, compared with 15% in the general adult population. In one series of adult patients with CD, the prevalence of low BMD reached 75% [54]. This low BMD is also characteristic of patients with dermatitis herpetiformis [55].

The first-line treatment for osteoporosis in CD is to establish a permanent GFD. Several studies, of children and adults, have demonstrated its effect on bone density and calcium absorption [56–58]. The greatest gain in bone mass described in these studies has been shown to occur during the first year on the GFD. It leads to an increase of at least 5% in bone mass after 1 year, although this is not enough for the bone mass to become normalised. In clinical practice, the degree of adherence to the GFD also determines the extent of recovery of bone mass, which is generally estimated at around 30% [59]. In addition, the recovery rate is higher in young patients with CD than in adults. This is explained by the fact that 97% of the bone mass accumulates during the first two decades of life, and because complete recovery is difficult for people older than 20 years of age [60].

In addition to the GFD, an adequate supply of calcium and vitamin D should be ensured, since they are critical factors for the acquisition and maintenance of good bone mass. Adult patients with untreated CD typically experience a decrease of 45% in the level of intestinal absorption of calcium that is followed by a 52% improvement 6 months after beginning the GFD. Serum vitamin D levels at diagnosis are very low in most adult patients. The intake of 1200-1500/day (suppress daily and long-term) calcium and 400 U of vitamin D is recommended, administered in exactly the same way as it is in cases of osteoporosis that are not associated with CD [61].

9. Thyroid diseases and CD

It is well known that CD is present in a higher proportion of patients with autoimmune-based thyroid diseases (e.g., Graves' disease and Hashimoto's thyroiditis), with a prevalence of 2–7% [62–65]. Similar observations have been made in celiac patients, whereby their serological signs of autoimmune thyroid disease were present in up to 26% of cases. Thyroid dysfunction was detected in up to 10% of the cases of CD and it was estimated that the risk of disease was at least three times higher than in healthy controls [66–69].

It has been reported that patients with CD who follow a GFD could develop thyroid problems of an autoimmune nature. In contrast, other studies have described declining anti-thyroid antibody titres after a period of 2–3 years on the GFD [70, 71]. These different results could have arisen because patients had been on their GFD for different lengths of time. The authors prospectively evaluated the presence of thyroid autoimmunity in children and adolescents with CD who had adopted a GFD. After 2 years on the diet, a 7% increase in thyroid autoimmunity was observed, based on levels of L-thyroxine in the CD patients. Thyroid autoimmunity did not appear to be more frequent in paediatric patients and adolescents with CD who followed a GFD than in control groups. Since their clinical development does not seem to affect growth, the authors concluded that a long-term programme screening for thyroid disease might not be necessary for all patients with CD who follow a GFD, but may be advisable for those for whom there is a suspicion of thyroid disease [72].

The coexistence of CD and autoimmune thyroid disease has been explained in terms of several mechanisms, such as the genetic predisposition and the association of both diseases with the gene that codes for antigen 4, which is associated with cytotoxic T lymphocytes and which confers susceptibility to thyroid autoimmunity. It has also been shown that the tTG-2 IgA reacts with thyroid tissue and that this association could contribute to the onset and development of thyroid disease in patients with CD [73].

10. Dermatitis herpetiformis and EC

Dermatitis herpetiformis (DH) was first described in 1885 by the French dermatologist Louis Duhring, and, indeed, is still known as Duhring's disease in some countries. In 1966, Marks et al. identified the presence of histological alterations in the small intestine of these patients that were identical to those observed in individuals with CD [74, 75].

The primary cutaneous lesions appear as erythematous papules, associated with liquid-containing vesicles in different areas of the body, especially those where there is rubbing, where they are distributed symmetrically on the extensor surfaces of the extremities. The vesicles produce a great deal of itching, causing patients to scratch themselves frequently, bursting their blisters, which releases their liquid content,

and causing erosions and abrasions. Subsequently, the papules become scabs that later detach, leaving a slightly hyperpigmented area. Generally, they predominate in young adults, but they can also affect children and older adults, especially atopic children. The vast majority of patients note the onset of symptoms during the warm months from the beginning of spring to the end of summer [76, 77].

The majority of patients with DH have no intestinal manifestations, or else only very mild ones. Sometimes patients only have iron deficiency anaemia. Males are more likely to be affected than are women (1.5–2:1) in contrast to CD, which clearly predominates in the female sex (2–4:1) [78].

The most characteristic histological finding is the presence of IgA-type granular deposits located in the papillae of the dermis and throughout the basement membrane, as can be demonstrated by direct immunofluorescence in skin biopsies. These accumulations promote an inflammatory response with infiltration of neutrophils around the vesicles of the affected areas [79]. The immunological basis of its development is linked to the pathogenesis of gluten intolerance in CD. The tTG-3 antibody is the main responsible auto-antigen, which is located in the skin of these patients, leading to the appearance and maintenance of the inflammatory response [80].

The main treatment for DH is the adoption of a GFD, which must be adhered to strictly and constantly throughout life. The skin lesions disappear several weeks after starting the GFD. Some cases may require a brief complementary treatment with Dapsone. This drug targets rashes by inhibiting neutrophil migration, and is used until the skin lesions have completely disappeared [81]. A Finnish study, carried out between 1971 and 2010, on the death rate and causes of death of 476 consecutive patients with DH, found significant reductions in all-cause mortality and cerebrovascular disease. The standardised mortality rate for all-cause mortality was significantly reduced to 0.70 (95% CI: 0.55–0.87). This value was similar in both sexes and was almost identical to that in dermatitis herpetiformis patients (0.73) without villous atrophy of the small intestine [82].

11. Cerebellar ataxia and EC

This disease encompasses cases previously known as "idiopathic sporadic ataxia," which are accompanied by positive antibodies against gluten. It is a type of cerebellar ataxia that appears in patients with associated gluten intolerance.

The most common form of clinical presentation consists of balance and gait disorders with associated dysarthria. Less frequently, it manifests as diffuse or focal myoclonic contractions. It is accompanied by nystagmus and other ocular signs in more than 70% of cases. In general, it begins gradually, appearing in individuals aged over 50 years, and with equal prevalence in men and women. It usually has a slow evolution, with a stationary clinical course and intermittent episodes of aggravation. Most patients have a prior history of recurrent digestive symptoms, and many patients have not been previously diagnosed with CD or with associated NCGS.

The Sheffield group led by Dr. Hadjivassiliou was the first to describe this type of association, and has made significant contributions to the field since 1970 [83]. The diagnosis of gluten ataxia is confirmed by the presence of anti-gliadin antibodies (AGAs) [84] as well as anti-tTG- and, when available, anti-tTG-2–6.

Fewer than 40% of patients with gluten ataxia present anti-tTG-2-positive IgA. When combined with anti-tTG-6, it can attain a positivity of 85% [85]. Autoantibodies to tTG-6 have been identified in immune-mediated ataxia patients with gluten sensitivity, suggesting an important role for transglutaminase 6 in cortical and cerebellar neurons [86, 87]. Gluten ataxia occasionally presents a familial character, affecting several first-degree relatives [88].

Gluten ataxia is considered an autoimmune disease characterised by the presence of cerebellar damage, mainly localised at the level of Purkinje cells, and the presence of circulating antibodies against gluten or related enzymes. Affected patients must follow a strict and sustained GFD. The degree of response to the withdrawal of gluten from the diet depends on the time elapsed between the appearance of the first signs of ataxia and the commencement of the GFD: the sooner it is established, the greater the chances of remission or recovery from the signs of ataxia.

It is important to remember that coexisting nutritional deficiency and autoimmunity can also cause neurological dysfunction in CD. A wide variety of neurological phenotypes with different aetiologies was found in 68 gliadin-positive patients with CD or non-CD over a period of 10 years (2002–2012): cerebellar ataxia, neuropathy, dementia, myeloneuropathy, autoimmune disease, deficiencies of vitamin E, folate and copper, genetic disorders and metabolic or toxic syndrome, among others. The authors concluded that exposure to gluten can cause neurological dysfunction even in those patients without established CD [89].

12. Neurogluten

Non-celiac gluten sensitivity (NCGS) is a clinical entity related to the ingestion of gluten-containing food, but the patients are not affected by celiac disease (CD). NCGS does display the typical histology of CD, although it may share a low level of duodenal intraepithelial lymphocytosis, but not crypt hyperplasia or villous atrophy. Neither does NCGS have the typical serology of CD, in which anti-transglutaminase type 2 IgA antibodies are present in the serum. However, they have a low level of them, and sometimes they are positive for antigliadin IgG antibodies. The sensitivity and specificity of both processes are limited. NCGS may affect individual or familial cases, but unlike in CD, the HLA-DQ2/DQ8 haplotype is not required in order for it to develop [90–93].

Like CD, NCGS is a common, chronic process, and often responds well to a GFD, even in severe cases. To date, in the absence of other biomarkers, the most specific method for confirming the diagnosis is a positive, self-reported response by the patient to a strict GFD, adhered to for a period of 6–12 months, and the verified improvement of the clinical signs when examined. To sustain the benefits, long-term or lifelong adherence, according to the severity of the syndrome, to a GFD will be the most widely recommended therapy [94, 95].

NCGS gives rise to important neurological and neuropsychiatric disorders. The most frequent of these are gluten ataxia and peripheral neuropathy, which have both frequently been associated with depression and anxiety. All patients can improve with early adoption of a GFD. However, without that therapy, progressive neurological dysfunction and cerebellar atrophy, and axonal nerve injury appear in MRI and neurophysiological patterns, respectively. Cerebrospinal fluid alterations are less frequent [96–99].

Other cases have been described that are associated with CD, but these probably correspond more closely to NCGS. They are all grouped together as neurogluten, a term that evokes different disorders in the nervous system that have the same cause, as is the case in neurosyphilis [100, 101].

Controversy arises when neurological entities without a determined diagnosis are included and related to that cause by a functional improvement that has not been objectively verified over a sufficiently long period. Given the current convenience and availability of GFD, safer diagnostic criteria, based on expert consensus, are needed to make more accurate diagnoses until such times as reliable biomarkers become established.

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Section 2

Diagnosis

Chapter 2

Challenges with Point-Of-Care Tests (POCT) for Celiac Disease

Huan Wu, Michael Wallach and Olga Shimoni

Abstract

Current screening test for celiac disease involves blood test in centralized pathology laboratories, typically performing enzyme-linked immune-sorbent assays (ELISA) to detect specific celiac disease antibodies. Most of the current available celiac disease antibody tests detect anti-gliadin (AGA), anti-endomysial (EMA), anti-transglutaminase (tTG), or deamidated gluten peptide (DGP) antibodies from serum or whole blood samples. It requires blood collection from untreated celiac patients, which is often invasive and inconvenient. There is a rapid growth in demand for noninvasive celiac tests for the early and fast diagnosis of celiac disease to help potential celiac patients obtain results and take corresponding actions. Over the last decade, several point-of-care tests (POCT) have been introduced to the market, but these tests have not been widely accepted by clinicians. Moreover, the 2009 NICE guideline CG 86 recommended that self-tests and/or POCT for celiac disease should not be used as a substitute for laboratory-based tests. Here, we provide a background on the evolution of POCT for celiac disease. We discuss general principle of operation for the known commercial kits as well as the use of various antigens and antibodies in different tests developed over the years. Finally, we discuss challenges for future research directions in celiac disease POCTs.

Keywords: celiac disease, point-of-care tests, lateral flow test, immunoassays

1. Introduction

Celiac disease is defined as a lifelong condition as a result of ingestion of gluten among genetically susceptible people that can be relieved by the introduction of gluten-free diet; the condition relapses with gluten intake [1]. Recent studies show that it is prevalent around the world, covering from the western world, such as Europe and America, to Oceania, Africa, and Asia. The number of celiac sufferers increases, doubling its number every two decades [2]. The symptoms vary at a wide range, from flatulence, constipation, anorexia, irregular bowel habits, and irritability to numbness in limbs, foggy mind, diarrhea, and depression [3]. Therefore, it is hard to recognize the condition and deliver the diagnosis. In fact, almost 90% of celiac patients remain undiagnosed, due to the nonspecific or absent symptoms over a long period [4]. Thereby, it is of paramount significance to achieve the early-stage diagnosis of celiac disease that can lead to improving the patients' quality of life.

The current gold standard of celiac disease diagnosis, which has been also developed in a much earlier period, is to observe the small intestine atrophy obtained

through biopsy [1]. This process is highly invasive, time-consuming, and inconvenient to both patients and clinicians.

Over the last several decades, researchers have found that the concentration of some certain antibodies circulating in celiac patients' body increased, and the detection of celiac disease can be achieved with the detection of these antibodies. In fact, the surface of mucosa represents the major targeted sites when foreign antigens attack the body [5]. Plausibly, 80% of all cells producing immunoglobulin (IgG) in the human body are in small bowel mucosa, which also produces the dimers of IgA [6]. Therefore, the antibodies of celiac disease among untreated patients are located in the mucosal surface [7], as extracellular deposit, some present in jejunal juice [8], and most in the intestine [9, 10]. For untreated celiac patients, the additional generation of antibodies leads to an increase of antibodies specific to celiac disease (IgA− class), most of which can be found in the circulating blood and some other bodily fluids. Among all the celiac-specific antibodies, anti-reticulin (ARA), anti-jejunal (JEA), endomysial (EMA), and tissue transglutaminase antibodies (tTG) are among the patients' own endogenous biomolecules that form as a result of immune response to antigen in the intestine, whereas anti-gliadin antibody (AGA) and deamidated gliadin peptide antibody (DGP-Ab) are formed directly against dietary gliadin [11].

Over the years, the detection of the celiac-related antibodies has shown promising results, and whole blood- or serum-based pathology tests are regularly used for screening for celiac disease. Typically, screening for celiac disease includes tests for identification of titers for AGA and/or anti-tTG antibodies, and most of these tests are based on enzyme-linked immunoassays (ELISA). Even though the method of ELISA can reach a high sensitivity and specificity, these tests cannot be used on their own in the process of celiac disease diagnosis. Furthermore, pathology tests are typically confined to centralized laboratories, where expert personnel is required, leading to slow- and high-cost detections. Thereby, it is not suitable for the use outside hospitals, such as clinical offices or home settings, leaving a high number of undiagnosed celiac cases, especially when their symptoms are not obvious or do not affect their normal life. Thus, simple and rapid detection methods are in high demand to be developed.

Fast, accurate, and noninvasive early diagnosis methods and/or devices are needed to achieve the detection of celiac disease with high sensitivity and specificity, especially facing the rapid increasing number of celiac patients. Over the last decade, several point-of-care blood tests have been developed and applied for celiac diseases screening. The most prominent tests are Simtomax® Blood Drop system (Augurix SA, Switzerland) and Biocard™ celiac test (AniBiotech®, Finland), lateral flow immunoassays that detect anti-tTG and/or anti-deamidated gliadin peptide antibodies.

Lateral flow test, also called lateral flow immunoassay or test strip, has been widely and commercially used in the rapid detection of many diseases and conditions, such as HIV, illicit drugs, and early pregnancy [12]. In the following sections, we will discuss and compare several commercial kits available as POCT devices for celiac disease. The principle of lateral flow test is outlined in the next section.

2. Lateral flow immunoassays

Lateral flow immunoassay is a simple immunochromatography technology that has successfully applied for rapid diagnostic testing [12, 13]. It typically made of nitrocellulose or paper-based porous membrane that makes it ideal to fabricate low-cost devices with little maintenance requirements. Porous membrane enables the separation, capture, and recognition of the target analytes. In addition, porous

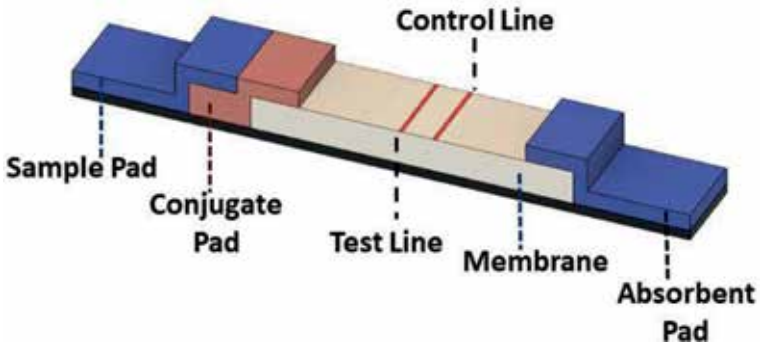

Figure 1.
Schematic representation of lateral flow immunoassay in a capture format. Various important parts of the test are identified as sample, conjugate, and absorbent pads, nitrocellulose membrane with test and control lines.

nature of the material facilitates movement of fluids, such as a whole blood, serum, or urine, by means of capillary action with no external force required.

There are several types of lateral flow assays available, but the most popular is the capture format. There are two types of capture: "sandwich" and competitive capture [13].

Overall, lateral flow immunoassay consists of four parts: sample pad, conjugate pad, nitrocellulose membrane (test line and control line labeled on it), and absorbent pad (also called wicking; **Figure 1**). Typically, fluid sample (blood, serum, urine, saliva) is added onto sample pad. Through the capillary action, the liquid moves to the conjugate pad, where preloaded recognition element (conjugated nanoparticles or colored reagent) is imbedded. The sample and the recognition reagent react commonly through antigen-antibody interaction. The sample together with the recognition element continues flowing within nitrocellulose membrane toward test and control lines. Depending on the type of a capture, sandwich or competitive, the test line would show a colorful line or disappear, respectively. Control line always shows colorful signal to indicate the correct functionality of the test. Absorbent pad function is to collect all the unreacted reagents as well as excess of liquids.

Lateral flow immunoassay for celiac disease is typically used to detect antibodies, such as anti-tTG, anti-DGP, and AGA antibodies from the whole blood or serum in a sandwich type of detection [14–18]. Preloaded reagent can be gold nanoparticles or dye conjugated to antigens, such as transglutaminase, deamidated gliadin peptides, and gliadin protein fragments. The detection of celiac disease-related antibodies will present as color test line that can be usually seen by the naked eye.

Lateral flow test or strip test is often used as a point-of-care test (POCT) due to its high specificity, visual color confirmation, and, especially, because of no additional instrumentation is required. In fact, most of the current commercial POCTs for celiac disease are based on lateral flow assay to detect antibodies. In the following section, we will discuss and make a comparison among commercial kits available together with the outlining principles of POCT device for celiac disease.

3. Point-of-care tests for celiac disease

3.1 Current commercial point-of-care tests

One of the most widely used commercial kits for celiac disease detection is the new generation of Biocard™ celiac test (AniBiotech®, Vantaa, Finland). In

this commercial kit, lateral flow method was utilized to detect human anti-tTG IgA antibodies from a whole blood. Gold-labeled anti-human IgA antibodies are prefixed on the conjugate pad, protein that binds to tTG antigen on the test line and anti-mouse IgG antibody on the control line [19].

The procedure is as follows: first, a drop of whole blood is taken from a finger prick, and then, the assumption is if the blood sample contains anti-tTG antibody, it will complex with liberated self-tTG found on hemolyzed red blood cells. Then the complex will flow to the conjugated pad due to the capillary force, forming a larger complex with anti-human IgA labeled on gold colloids. The larger aggregate is then recognized and captured by the tTG binding protein on the test line, and the red color will appear. The excess gold-labeled anti-IgA antibodies migrate further to control line, combining with anti-mouse IgG antibodies, which is prefixed on the control line, producing another red line (**Figure 2**). Both red lines represent positive result; only one red line in the control line means negative. If neither of the lines turns red, it means the subject is IgA deficient, or the test did not function properly. Usually the result can be viewed within 5–10 min; positive result can even appear after 2 min.

Since the Biocard™ celiac test has been made available on the market, a considerable number of papers have evaluated its sensitivity and specificity. Though the reported sensitivity and specificity vary in a wide range, most of them are around 90%, where some can reach as high as 93 and 94% for sensitivity and specificity, respectively [19–24]. However, this result is slightly lower than laboratory-based celiac disease test (more than 95%); therefore, this commercial kit can be only used for screening for celiac disease, but not for diagnosis. Nevertheless, this test can be also used to detect patients who have IgA antibody deficiency.

Another widely available POCT is Simtomax® Blood Drop system (Augurix SA, Switzerland). It is also a lateral flow assay device but slightly more sophisticated as it presents with two test line, A and B, in addition to a control line [25]. Test line A is used to detect both anti-DGP IgA and IgG antibodies, while test line B is for the detection of the whole IgA antibodies. Control line detects the presence of antibodies by capturing with anti-mouse antibodies. On the test line A, synthetic DGP is embedded to capture and detect anti-DGP IgA and IgG. For the test line B, mouse anti-human IgA detects total IgA. For the conjugate pad, it is secondary antibodies combined with gold colloid. If anti-DGP present in the patient's serum, anti-DGP will be captured by the secondary antibodies in the conjugate pad, and then the

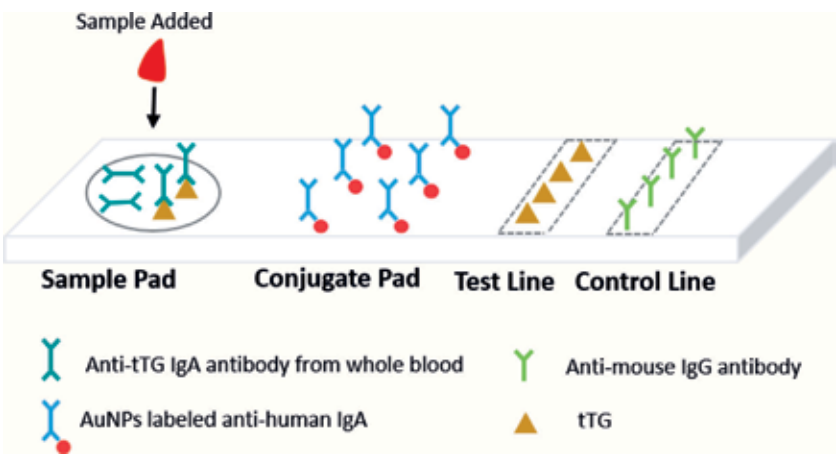

Figure 2.
Scheme of Biocard™ celiac test.

complex will flow further to the test line A and B, captured by A and B, and line A and B will become red. For the control line, goat anti-mouse antibodies are pre-attached; the excess conjugate of gold colloid with secondary antibodies can interact with it forming red line. All these three lines can be formed after 10 minutes, but not later than 15 min. Three red lines mean that the patient is celiac disease positive and has no IgA deficiency. If only two lines become red, in test line A and control line, then it represents that the subject is positive for celiac disease and IgA deficient, while two red lines, line B and control line, indicate that the subject is healthy, negative for celiac disease, and has IgA deficiency. Only one red line on the control means that the subject is negative for celiac disease but positive for IgA deficiency. If all of these three lines do not turn red demonstrating a non-valid result, meaning the patient needs a further test with a new device.

Various assessments have been done for this commercial kit, and it has been proved to have high sensitivity (95–100%) and specificity (93.1–95.7%) [26–28]. However, specificity of this test drastically reduced when used for patients on a gluten-free diet [26, 29]. This is not surprising as the amount of celiac-related antibodies will decrease with a strict following of gluten-free diet but still can be present due to unaware consumption of gluten.

Except the above two simple and popular POCT kits, additional lateral flow test has made commercially available, Stick CD 1 and 2 [30]. Stick CD 1 can detect IgA, IgG, and IgM antibodies against human tTG, while Stick CD 2 also detects AGA antibodies. It was demonstrated that the sensitivity of Stick CD 1 was 97% and as to CD 2, 95% for anti-tTG antibodies and 63% for AGA antibodies. The specificity of CD1 has been shown to reach 99%; as for CD 2, it was 99% for anti-tTG antibodies [30].

There are multiple ELISA-based tests that are widely available to identify celiac disease, such as Celikey® or QUANTA®. Typically, these kits use ELISA assay to detect IgA anti-tTG and/or anti-DPG antibodies. The reported sensitivity and specificity have been reported to be higher than 90% [31–33]. QUANTA® products have a various series of commercial kits and can detect different biomarkers with high sensitivity and specificity for celiac disease detection [34] (**Table 1**), including IgA anti-DGP, IgG anti-DGP, IgA human anti-tTG, IgA, and anti-tTG/DGP screening. It can be seen from **Table 1** that the performance of traditional ELISA-based test is still slightly higher than that of any lateral flow tests. This is one of the reasons that most of the gastroenterologists have not accepted the use of POCT as an alternative to the lab-based tests.

3.2 Research development for point-of-care tests

Although there are some commercial products for the assay of celiac disease in the market, the effort has not been stopped to devise a low-cost kit with high

Test	Sensitivity (95% CI) (%)	Specificity (95% CI) (%)
IgA DGP	98.4 (91.4–99.7)	92.7 (85.5–97.1)
IgG DGP	95.2 (86.7–99.0)	100.0 (96.2–100.0)
IgA human tTG	95.2 (86.7–99.0)	97.9 (92.8–99.7)
IgA and IgG DGP screen	96.8 (89.0–99.5)	99.0 (94.4–99.8)
tTG/DGP screen	100.0 (94.3–100.0)	92.8 (85.8–97.1)

Table 1.
Performance of QUANTA lite celiac disease tests in a high-risk population.

accuracy. In 2005, Korponay-Szabó et al. developed a POCT that could rapidly detect the autoantibodies of tTG from blood sample [35]. The test is based on a Nunc-Immunostick (Denmark) principle with a four-wing stick. The two wings of the stick are pre-covered with gelatine to recognize and capture self-tTG/anti-tTG antibody complexes from the hemolyzed patient blood sample. The third wing is fixed with anti-human IgA antibodies, to combine with plasma IgA, which is used as a positive control. The fourth wing has no coating leading to the absence of antibody capture, and it serves as a negative control. In the test process, one drop of blood is inserted into the hemolyzing solution and incubated with the stick for 15 min. Then, the stick is washed with water and immersed for another 15 min in the solution of peroxidase-labeled anti-human IgA. To obtain a visible signal, the stick is washed again and then inserted into tetramethyl benzidine solution, which is used as a color reagent, to observe the color change. If these three wings become blue within 5 min, it means that the result is positive for celiac disease. Negative result can be confirmed when only the IgA-sensitive part turns blue. If no blue color appears, it indicates that the sample was IgA deficient, and the test is invalid. It was demonstrated that the sensitivity and specificity were 97.0 and 96.9%, respectively, after testing 164 human blood samples of untreated celiac patients [35].

In recent years, another design for POCT device to detect celiac disease has been based on electrochemical biosensor. The desirable features of POCT, such as low cost and ease of operation, match well with the utilization of electrochemical biosensor [36]. It makes biosensors suitable for the commercial applications. Moreover, commercial device to detect blood glucose has been widely used in clinics and households, boosting motivation of researchers to devise electrochemical sensors for the rapid test of celiac disease.

In 2012, Adornetto et al. developed a novel fast immunosensor to achieve anti-tTG antibody detection based on magneto-electrochemistry from serum samples [37]. In this system, tTG antigen-coated magnetic beads are used to capture and detect antibodies against tTG from positive serum samples. Alkaline phosphatase-labeled anti-human IgA antibodies are used as a control. Magnetized screen-printed electrodes coupled with a portable instrument serving to read out electrochemical signal, which is produced after the addition of α-naphthyl phosphate that is enzymatically converted into the electrochemically active α-naphthol product. The device was used to analyze 107 blood serum samples (46 positive vs. 61 negative samples), and it was able to identify with a clinical sensitivity of 100% and a specificity of 98.36%, while the cutoff was 1.0 AU/ml. This is comparable to spectrophotometric ELISA kits (98.57%, 100%, and 7.00 AU/ml, respectively).

Interestingly, in 2015, Adornetto et al. have engineered another electrochemical immunoassay system to detect the IgA anti-tTG antibodies [38]. The authors described a similar system, but the biggest change was that this device is used for the detection of celiac disease in saliva sample with high sensitivity. This is the first report that overcomes the problem associated with saliva samples, such as low levels of IgA anti-tTG antibodies and high liquid viscosity. In this device, magnetic beads were covered with the tTG antigen to react with antibodies against IgA anti-tTG, which would typically be present in saliva samples of positive celiac disease patients. The marker in this case was the conjugate of anti-human IgA and alkaline phosphate enzyme. The electrochemical transducer was created with a strip of eight magnetized screen-printed electrodes. This device showed the clinical sensitivity of 95% and specificity of 96% when analyzed in 66 saliva samples. The results show the suitability for this POCT as noninvasive screening for celiac disease.

In another study, a modular electrochemical peptide-based sensor was developed to detect anti-DGP antibody [39]. In this approach, firstly a short helical support peptide (SP) was immobilized on the surface of a gold electrode, followed

by functionalization of SP with DGP and methylene blue (MB), which are used as the antigen and electrochemical tag, respectively. When the added anti-DGP IgG monoclonal antibody was recognized and then bound with the DGP, the transfer electrons efficiency between DGP and gold surface was reduced, leading to a signal decrease in a potentiometer. This unique modular style could guarantee the background of high currents when DGP antibody is absent, even at low surface densities of DGP. This means that this system could achieve a low-limit detection of anti-DGP. Although this system presents a great potential, it was not properly assessed on a real human serum or saliva sample from celiac patients. Nevertheless, it represents an alternative direction for the future development of POCT device.

A creative electrochemiluminescence immunosensor for the test of tTG was designed based on the detection platform of a membrane-templated gold nano-electrode ensemble [40, 41]. In this platform, tTG antigen was first immobilized on the surface of polycarbonate to capture the target anti-tTG antibody present in the sample. Then it could react with the biotinylated secondary antibody, which was labeled with ruthenium-based electrochemiluminescence reagent modified with streptavidin. The application of an oxidizing potential could induce the generation of intense and sharp electrochemiluminescence signal, which was used to analyze different concentrations of anti-tTG. The result showed that its linear range was between 1.5 ng/mL and 10 μg/mL, with a detection limit of 0.5 ng/mL. This system was applied to detect human sera samples from five celiac patients and two healthy controls as a proof of concept for screening test of celiac disease with great outcomes. Nevertheless, this test still requires more human samples and more vigorous validation for the potential in POCT application.

Recently, our group has developed a novel one-step test for screening celiac disease [41]. The test is based on a precipitation principle of gliadin peptide-coated gold nanoparticles. In this test, diluted serum is added to the prepared peptide-coated gold colloids in a small tube. If AGA antibodies are present in serum, it causes agglutination of gold colloids and essentially leads to a colloid precipitation. The test was used on 30 human serum samples (26 positive celiac samples and 4 controls) in a blinded assessment. The test demonstrated an overall sensitivity and specificity of over 85%, indicating that this assay has potential to be adopted as screening tool for celiac disease. Furthermore, this test could be a part of an exclusion-based diagnostic strategy in testing high risk of celiac disease populations.

4. Outlook for the future POCT development

Over the last decade, several POCTs have been introduced to the market, but these tests have not been widely accepted by clinicians [42]. Moreover, the 2009 National Institute for Clinical Excellence (NICE) guideline CG 86 recommended that self-tests and/or POCT for celiac disease should not be used as a substitute for laboratory-based tests [43]. Therefore, even though the current POCT devices can be used to detect celiac disease with a relatively high sensitivity, their specificity somewhat lags behind lab-based tests. However, one of the biggest drawbacks of the current available POCTs is their lack of usability by a non-trained person.

Most of the lab-based tests for celiac disease are performed by trained clinical technicians, but POCTs are generally aimed to be performed by a non-trained person. Multiple steps and components, such as blood drawn from a finger prick, accurate amount of blood requirement, addition of dilatants or other solutions and visual interpretation, would typically introduce user errors leading to a decrease in accuracy of the tests. The usability of POCTs has been assessed on the example of HIV self-testing kits [44]. In this particular study, authors found that almost 50%

of the untrained participants performing POCT HIV test had made multiple errors during testing. It is clear that for a successful adaptation of POCTs across the globe, ideal self-test kits must include easy-to-understand instructions, preferably one-step operation and easy-to-interpret results.

As discussed above, most of the POCT systems utilize whole blood or serum samples for celiac disease detection. Drawing blood from vein or from finger prick can be viewed by some people as invasive and painful leading to a withdrawal from voluntary testing. In addition, all the mentioned laboratory or POCTs target autoantibodies, such as antibody against tTG, DGP, or even EMA. It means that celiac disease can only be detected after antibodies are circulated in the human body. However, antibody-based tests generally fail at the latent or silent cases of celiac disease or people on voluntary gluten-free diet. Can we achieve celiac disease detection when there is a low amount or no antibodies found? Maybe it is another challenge that researchers have to face when devising the future screening methods and tools for celiac disease.

In other fields of analyte detection, including the detection of blood glucose, on-site alcohol and illicit drug tests, and confirmation of pregnancy, there are already various well-developed and commercialized products from different brands. These examples comprise of the use of nanotechnology, microfluidics, or the combination of these two methods together that provide some inspiration for future methods of POCT for celiac disease. In particular, highly accurate and sensitive fast test of HIV [45, 46], tuberculosis [47–49], and malaria [50–52] combine the utilization of lateral flow microfluidics with visual colorimetric observation detection. Indeed, these POCT diagnostic devices can probably provide the most effective and useful tool for mass diagnosis. Additionally, these tests have been proven to be cost-effective, simple, and portable, as well as with capacity for multiplexing.

5. Conclusion

In conclusion, this chapter has reviewed the current commercially and laboratory-based developed POCT devices and the challenges to be faced with for a rapid and simple test of celiac disease. It is expected that more efforts of multidisciplinary research involved in immunology, lateral flow technology, microfluidics, nanotechnology, and genetics could provide a great opportunity for the fast, accurate, and early diagnosis of celiac disease, dramatically improving the quality of human life.

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Conflict of interest

Authors declare no conflict of interest.

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Section 3

Genetic

Chapter 3

Genotype DQ2.5/DQ2.2 (β2/β2) and High Celiac Disease Risk Development

Yanna Karla de Medeiros Nóbrega

Abstract

Celiac disease (CD) is a genetically determined immune-mediated disorder in which gluten immunogenic peptides are presented to CD4 T cells by HLA-DQ2.5, DQ8, DQ2.2, and their combinations. CD is considered one of the most well-characterized autoimmune diseases, having a described environmental factor, a well-established pathogenesis, associated genetic factors, and a well-established laboratory diagnosis, although it is still considered a difficult-to-classify disease. In the last decades, advances in laboratory diagnosis with the emergence of molecular biology techniques have allowed a specific characterization of the CD-associated genotypes and, although clinically the disease management was not modified by this factor, the follow-up of patients at risk of CD development has greatly benefited from the possibility of specifically finding the inherited genotype, and whether it represents a greater or lesser risk for developing the disease. In some populations, it is already possible to calculate the exact risk associated to the inherited genome by each individual, but the genotypes available in several countries sometimes disregard the relevance of searching beyond the genotypes DQ2.5/DQ2.5, DQ2.5/DQ8, and DQ2.5/DQ2.2, which also present a high risk for developing the disease.

Keywords: celiac disease, HLA, disease risk, HLA-DQ2.2, HLA-DQ2, HLA-DQ8

1. Introduction

1.1 Definition, prevalence, and classification

Celiac disease (CD) is a genetically determined immune-mediated disease, and individuals with CD have specific HLA haplotypes (DQ2 and/or DQ8) that trigger an immune response to gluten intake, leading to intestinal and clinical signs and symptoms [1, 2], besides other autoimmune-associated CD diseases, such as dermatitis herpetiformis [3], type 1 diabetes mellitus, Hashimoto's thyroiditis, and Sjögren syndrome [4]. Also, there are some genetic syndromes that may be CD associated such as Down syndrome [5, 6], Turner syndrome [7], and Williams syndrome [8].

As CD is one of the most well-elicited autoimmune diseases and one of the most common permanent food intolerances among humans [9], its prevalence in the general population from Europe, USA, and countries where the population is

predominantly of European origin is approximately 1% [2]. Prevalence is lower, ranging from 0.15 to 0.84%, in Latin American countries such as Brazil [10–14]. When Brasília city (Brazilian capital and population representation) is considered, since it is a city formed by people from all regions of the country, the prevalence found in the general population is 0.34%, considering 0.21% in adults and 0.54% in children [11].

The CD prevalence can still be related to the cereal consumption that contains gluten (mainly wheat) and to the distribution of predisposing HLA alleles in the population [15, 16]. Besides, the existence of genetic and environmental factors may influence the CD prevalence rate in a region [15]. Last but not least, we highlight the microbiota variability, the existence of intestinal infections, and socioeconomic conditions, which are also factors that may influence the CD development and prevalence [17, 63].

According to clinical signs and symptoms, laboratory and histopathological findings, which together have been called "clinical forms," CD can be classified into five distinct forms (**Table 1**).

1.2 CD pathogenesis and immune response (IR), the HLA importance

CD pathogenesis, which is an inflammatory enteropathy with autoimmune characteristics, is triggered by gluten ingestion [17]. In nonceliac individuals, gluten is cleaved by digestive enzymes into small fragments for eliciting an immunogenic response and is digested by gastrointestinal system without causing damages. In CD patients, the gluten digestion induces gliadin fragments initiating an innate and adaptive immune response resulting in tissue damage of the intestinal mucosa and clinical CD manifestations [24].

Classification	Clinical forms
Classic or typical	Frequently in children, characterized by gastrointestinal manifestations that can arise after the introduction of gluten foods (weeks, months, or even years). Patients have positive serology for CD and HLA compatible; and, in intestinal biopsy, there are usually lesions of variable severity, but they are frequently characterized by hypotrophy or atrophy of intestinal villi and varying degree of intestinal cryptic hyperplasia.
Atypical or nonclassical	Minimal or no gastrointestinal manifestations and recurrent extraintestinal manifestations. It can appear at any age (but commonly in teenagers and adults). It presents positive serology, HLA, and CD-compatible biopsy.
Silent or asymptomatic (subclinical)	Signs and manifestations commonly associated to CD are nonexistent. Patients are diagnosed occasionally in screening programs or because they are in risk CD groups (carriers of autoimmune diseases or celiac relatives), by positive serology, HLA, and CD compatible biopsy.
Potential	Patient may or may not present manifestations, as well as may not develop mucosal lesions in the future. CD-positive serology, CD-compatible HLA typing, normal intestinal mucosa, or with subtle abnormalities (increase of intraepithelial lymphocytes) and absence of significant enteropathy.
Latent (controversy)	Characterized by the presence or absence of antibodies in the normal intestinal mucosa, HLA typing is CD compatible, but for being defined as having CD, there must be a prior diagnosis of at least the presence of an enteropathy associated to gluten consumption.

Adapted from Fasano and Catassi, [18]; Lionetti and Catassi [17]; Admou et al. [19]; Bao, Green and Bhagat [20]; Husby [8]; Kaneepkens and von Blomberg [21]; Ludvigsson et al. [22]; Sapone et al. [23].

Table 1.
Celiac disease classification and clinical forms.

Gluten is the energy storage protein found in wheat, rye, barley, and oat grains, which has a large amount of prolamins (glutamine and proline) in its primary structure. Proline-rich peptides are resistant to gastrointestinal digestion [25].

Specifically in wheat, gluten proteins are divided into gliadins and glutenins [25], according to the solubility of the prolamins present. Gliadin is soluble in alcohol, while glutenin is soluble in acidic and basic dilutions [26]. Gliadin is an alcohol-soluble, 30 kDa protein, particularly rich in glutamine and proline residues, which are contained in polyglutamine sequences, represented as a single chain of polypeptides, which can be divided in four different groups: α-, β-, γ-, and Ω-gliadin [26]. The N-terminal domain of α-gliadin contains the most immunogenic fragment, which has the peptide 31–43 and the 33-mer fragment, which contains six significant epitopes for CD pathogenesis [27, 28].

The presence of gliadin and its peptides is the external factor triggering the immune response in CD, which implies the need for its entry through the mucosa and presence in the lamina propria with consequent obligatory passage through the cells of the intestinal epithelium. This epithelium entry occurs by three mechanisms: (1) through the transcellular route, where gluten is endocytosed in lysosomes, which degrade it in small nonimmunogenic peptides [29]; (2) via the paracellular route, by regulating the TJ junctions responsible for the union of the epithelial cells, promoting a change in cellular permeability and, consequently, the entry of gliadin peptides into the mucosa, such as regulation through zonulin produced by epithelial cells of celiac patients that alter the permeability between epithelial cells [29, 30]; (3) by transepithelial transport in cells of celiac patients, where there is an increase in CD71 (transferrin receptor) expression. This receptor recognizes IgA complexed with gliadin through the Fc portion of the immunoglobulin, and releases this association without processing in the lamina propria [31].

Although these mechanisms of entry and processing by digestive enzymes are described in the literature, it is believed that these peptides interact with the intestinal epithelial cells and produce an inflammatory response before presenting themselves in the lamina propria, promoting gene alterations in this cell by mechanisms not yet fully elucidated [24].

In an *in vitro* cellular CD model by using $CaCo_2$ cells, intact gliadin and its immunogenic peptides from the 33-mer fragment (nondeaminated—P56-88, P57-68, P69-82, P31-43, and deaminated P57-68 E65 and P69-82 E72) were used for understanding this interaction mechanism in the first 24 and 48 h. Results showed that following interaction with $CaCo_2$ cells, these peptides modulated receptor gene transcripts such as TLR-4, cell permeability altering protein genes such as zonulin and occludin, as well as inflammatory cytokines (IL-1, IL-6, IL-8, and IL-15) very important for CD pathogenesis, besides increasing the production mediators of oxidative stress such as nitric oxide. Afterward, IL-6 and TNF-α levels revealed the secretion of these cytokines in culture supernatant, confirming the inflammatory process initiated in the first 24 and 48 h after interaction with these human epithelial cells when culture (unpublished data) [32]. Earlier data had previously confirmed that the peptide p31-43 activates the innate immune response through the activation of proinflammatory cytokines, while the p57-68 peptide has been identified as immunodominant and capable of activating the adaptive immune response through recognition by T cells-CD4 [33].

When gliadin peptides reach the lamina propria, they are modified by the action of the tissue transglutaminase 2 (tTG2) enzyme, which in the presence of calcium, converts glutamine residues to glutamic acid, the negative charge of glutamic acid increases the affinity of tTG2 for gliadin and the gliadin-tTG2 complex also increases the affinity of the gliadin-tTG2 complex and the gliadin peptides with the MHC class II molecules HLA-DQ2/DQ8 [15, 34–36].

These gliadin peptides are recognized and processed by the HLA-DQ2/DQ8 MHC class II antigen presenting cells (APCs) and are presented to CD4 T cells, which become active and begin to produce IFN-γ and IL-15. T-CD4 lymphocytes, activated by APCs on the lamina propria, differentiate into intraepithelial lymphocytes (IELTs) and infiltrate epithelial cells in response to IL-15 stimuli produced by enterocytes. Also, in response to IL-15, IELTs display cell membrane receptors for natural killers (NK), which promotes the cascade recruitment of new NK cells, which promote destruction of the epithelial barrier, cryptic hyperplasia, and atrophy of the intestinal villi [25, 37, 38].

APCs migrate to the mesenteric lymph node and display the gliadin peptides complexed with tTG2 to immature CD4 T cells. In mesenteric nodules, T-CD4 cells differentiate into effector T-CD4 cells (T-CD4$^+$), which increases the proliferation of reactive B cells to the gliadin-tTG2 complex. Reactive B cells differentiate into plasma cells and produce IgA and IgG antibodies, not only against glutamine residues modified to glutamic acid, but also against tTG2, which may still be complexed with these peptides [39, 40].

The continuous recognition by APCs of the gliadin-tTG2 complex as an immunogenic stimulus accentuates the immunological and proinflammatory response, triggering the autoimmune response found on CD [8]. However, in healthy individuals, the recognition of these peptides when presented by MHC of class II originated of HLA DQ2/DQ8 [41] also occurs.

The entire inflammatory process induced by gliadin and its peptides on CD is a result from the synergism between the innate and adaptive immune response that occurs in two distinct sites in the small intestine, that is, in the epithelium and in the intestinal lamina propria [31, 42].

Studies suggest that CD is primarily mediated by adaptive immunity, where CD4 T cells recognize gliadin peptides through MHC II molecules, which are encoded by the HLA (*Human Leukocyte Antigen*) DQ2 and DQ8 genes present in celiac patients, which confirms the strong genetic basis [15, 31, 42].

For all these reasons, CD is an excellent model for studying the genetic factors that contribute to the development of immune-mediated disorders. Among these reasons, we can highlight the fact that it has a well-known environmental triggering factor—gluten, an autoimmune disease with a well-described genetic predisposition associated to the MHC HLA DQ2/DQ8 alleles, the involvement existence of other non-MHC genes, and the high incidence of other immunological diseases reported in both celiac and familial patients, in which the innate and adaptive response plays a key role [43]. CD is also considered a multifactorial disease caused by the interaction of different genetic factors that act in consonance with nongenetic effects, since nonceliac individuals also have such alleles, suggesting that additional complementary mechanisms are necessary for the disease development [39].

Similar to other autoimmune diseases, CD is a polygenic disorder and the MHC gene is the most important genetic factor. Most celiac patients carry a specific genetic variance of HLA-DQ2 (DQA1 * 05: 01, DQB1 * 02: 01, known as DQ2.5), and those who are not HLA-DQ2.5 almost always carry the HLA variance -DQ8 (DQA1 * 03, DQB1 * 03: 02) or another variant of HLA-DQ2 (DQA1 * 02: 01, DQB1 * 02: 02), known as DQ2.2 [9]. Since all celiac patients carry specific HLA variations, this factor may be considered necessary for CD diagnosis, but alone it is not sufficient for CD development [25].

Recently, studies based on Genome Wide Association Studies (GWAS) has been allowing the identification of single-nucleotide polymorphisms (SNPs) in each gene in the human genome associated to a cell metabolic pathway or a specific phenotype such as CD. In general, GWAS tests hundreds of thousands of SNPs throughout the patient genome and matched the control ethnic group [44]. These SNPs often

affecting the recognition of transcription factors, resulting in differences in the expression of regulatory genes shared with other autoimmune diseases. After GWAS studies, it was possible to verify by immunochip analysis that several non-MHC genes have been related as CD susceptibility factors. Until then, 39 loci with 57 independent association signals were described, contributing 14% of the genetic variance for CD [45].

Many of these genetic variances are shared with other autoimmune diseases such as type 1 diabetes mellitus and rheumatoid arthritis [46]. After GWAS studies, once evidenced correlations with metabolic pathways and shared inflammatory response between CD and other autoimmune diseases, new strategies that make use of different cellular models can be applied to CD [47].

1.3 Laboratory diagnosis and HLA determination relevance

Clinical CD manifestations are very heterogeneous and often subtle, which may confuse the clinician and delaying the definitive diagnosis. According to the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN), CD diagnosis depends on clinical manifestations, significant level of the presence of specific antibodies (positive serology), presence of predisposing HLA-DQ2 and/or HLA-DQ8 genes, and presence of histopathological abnormalities from the intestinal mucosa evidenced by the biopsy [8].

ESPGHAN advises that CD diagnosis should be considered in children and adolescents who present gastrointestinal (diarrhea, abdominal pain, nausea, vomiting, etc.) and extraintestinal manifestations (anemia, dermatitis herpetiformis, chronic fatigue, etc.). It is also recommended that CD diagnosis be evaluated in asymptomatic children and adolescents (but belong to a risk group for CD development). Risk CD groups are composed of individuals with type 1 diabetes, Down's syndrome, Turner's syndrome, Williams's syndrome, autoimmune thyroid disease, autoimmune liver disease, selective IgA deficiency, and first-degree relatives of celiac [8]. American Gastroenterology Association recommends that CD diagnosis be considered in any individual with a clinical condition indicative of CD or belonging to at-risk groups [48].

The production of anti-endomysium (EMA), anti-gliadin, antitransglutaminase (anti-tTG) and gliadin-tTG complexes is part of the CD pathogenesis process. Serological tests used in the laboratory CD diagnosis are intended to detect levels of these antibodies in the serum (CD-suspected individuals). The available CD diagnostic tests include anti-gliadin IgA and IgG antibodies, anti-EMA IgA and IgG, IgA and IgG anti-tTG [48, 49].

Anti-gliadin antibodies are not currently considered sufficiently sensitive or specific to be used in the CD diagnosis [20] and have been replaced by anti-gliadin deaminated (anti-DGP) antibodies of both IgA and IgG because they have greater sensitivity and specificity. Anti-DGP IgG test is used in IgA deficiency cases where anti-DGP IgG antibodies are detected [49]. Both IgA and IgG anti-DGP assays are commonly used as additional tests in patients who are negative for other serological tests but presenting characteristic clinical CD symptoms, especially in patients younger than 2 years old [8, 49].

Anti-tTG antibodies are commonly detected by ELISA method usually by human recombinant tTG as antigen [8]. Anti-tTG IgA serological test is considered the most sensitive method for diagnosing CD, with sensitivity close to 97% [20]. This test has high specificity, close to 99% [48]. Although anti-tTG IgA assay has high sensitivity and specificity, it is possible to find false-positive results in patients with liver disease, congestive heart failure, arthritis, and inflammatory bowel disease [48]. Anti-tTG IgA test is generally used as the first test in the initial

approach for diagnosing CD because it is a quantitative test that can be automated and does not depend on the observer interpretation such as the anti-endomysium test [8, 49].

Although the main methodology used for dosing tTG and gliadin is performed by ELISA, in the last two decades, new methodology is available; it is worth mentioning the indirect chemiluminescence immunoassay (CLIA) [50, 51] and the fluorescent enzyme immunoassay (FEIA or EliA) [52].

IgA-EMA antibodies are detected by indirect immunofluorescence, which requires microscopic evaluation. This method is of subjective evaluation, being subject to variations depending on different observer interpretations. However, when well interpreted by the experienced observer, the specificity of the IgA-EMA serological test is close to 100% [48], being considered a reference test for detecting specific CD antibodies [8].

For the anti-endomysium (EMA), although new methods have not been developed, it is now possible to carry out the technical procedure in a fully automated way, and reading by integrated software. Even with this great advance and agility in sample processing time, and in the technical standardization of the employed method, the existence of characteristic fluorescence patterns still requires that the analyzes be interpreted according to the knowledge and subjective observation of the microscopist or the observed, which causing high intra- and interlaboratory variability, which in laboratory practice is considered the major problem for diagnosing autoimmune diseases in general.

Also, the use of molecular methodologies in the laboratory diagnosis currently allows the detection of HLA genotypes associated to CD, in a highly specific way, mainly using the RT-PCR methodology.

1.4 HLA and the importance of risk genotypes in laboratory genotyping

CD is an example of a multifactorial disorder in which the genetic test is of great clinical relevance, since the disease rarely develops in the absence of HLA-specific genes (HLA-DQ2 and HLA-DQ8) [15, 53]. The HLA-DQ2 and HLA-DQ8 genes are required for developing CD but are not sufficient [54]. If an individual carries these genetic markers, it does not necessarily mean that the subject will develop CD, but having a risk for developing the disease. Therefore, the absence of the HLA-DQ2 and HLA-DQ8 genes has a high negative predictive value for the diagnosis of CD, ie, the chance of an individual who does not have these genes develop CD is extremely low, whereas the presence of these genes markers has a relevant positive predictive value [55].

HLA typing can be used to rule out the diagnostic hypothesis of CD in patients with doubtful diagnosis, excluding the disease possibility in individuals who do not have these genes. Chang and Green [53] suggested that HLA typing be performed prior to serological testing to reducing the number of false-positive results and thereby decreasing the number of biopsies required. However, ESPGHAN recommends that the HLA test be performed prior to the serological tests only in the case of asymptomatic patients belonging to risk groups (first-degree relatives of celiac, type 1 diabetic patients, and Down syndrome, for example) [8].

In CD, the heterodimers are called human leukocyte antigen (HLA) and belong to HLA-DQ loci present on chromosome 6. Genotypes that are strongly associated to the onset of the immune response triggered by gluten are HLADQ2.5, HLA-DQ2.2, and HLA-DQ8 [56–58]. It is known that genotypes HLA-DQ2.5 (DQA1 * 05: 01, DQB1 * 02: 01), HLADQ2.2 (DQA1 * 02: 01, DQB1 * 02: 02), and HLA-DQB1 * 03: 02) are necessary but not sufficient for developing CD, since more than 30% of the general population in the world have these genotypes and only 3–5% will develop CD [9, 59–61].

Virtually, all CD patients carry the alleles encoding the HLA-DQ2 and/or DQ8 molecules or at least one DQ2 heterodimer chain, usually the DQB1 * 02 allele-encoded strand. The CD occurrence in the absence of these risk factors in DQ is extremely rare, but the presence of these molecules also fails to predict with precision when and if the CD will develop, since they are present in 25–50% of the general population, although the vast majority of these individuals never develop the disease throughout life [58].

Even with this knowledge, performing HLA-DQ typing for determining future CD risk has been widely discussed, although its practical use is mostly associated to risk groups where genetic testing of individuals could eliminate the need for future antibody tests in more than 60% of the population considered to be at low CD risk (DQ2 or DQ8 negative). On the other hand, the identification of high-risk individuals would allow a safer prospective screening, allowing an early therapeutic intervention [5], and a more precise monitoring, since the risk of developing the disease is more likely in these individuals. In the scientific literature, the first study that calls attention to the determination of CD risk development associated to the presence of HLA-DQ genotypes was performed by Megiorne et al. [62] in the Italian population, the best characterized thesis in the world. Results showed that considering the prevalence of 1: 100 established in this population, which corresponds to 1% that is the prevalence in populations of Caucasian origin in the world population, the risks for developing CD were higher when associated to the presence of genotypes DQ2.5/DQ2.5 and DQ2.5/DQ2.2, in addition to DQ2.5/DQ8, where the risk found was 1: 7, 1:10, and 1:24 (**Figure 1**).

After this publication, rare study has appeared, is the case of Almeida et al. [2] and Murad et al. [64], that given the existence of the estimated prevalence of these populations in other studies in the same region, were able to calculate the CD risk development in the populations of Brazil and Syria, respectively.

Results found by Almeida et al. [2] showed that the risks associated to DQ2.5/DQ2.5, DQ2.5/DQ2.2 and DQ2.5/DQ8 genotypes in the Brazilian population were 1:7, 1:10, and 1:19, respectively, such as described by Megiorni et al. [62].

Murad et al. [64] found in the Syrian population, a slightly different risk for DQ2.5/DQ2.5, DQ2.5/DQ2.2, and DQ2.5/DQ8 genotypes and the associated risks were, respectively, 1:12.5, 1:20, and 1:10, emphasizing that in this population of origin other than Caucasians, the risk associated to these genotypes are somewhat different in terms of prevalence, but they continue to confer the greatest risks for developing CD (**Figures 2** and **3**).

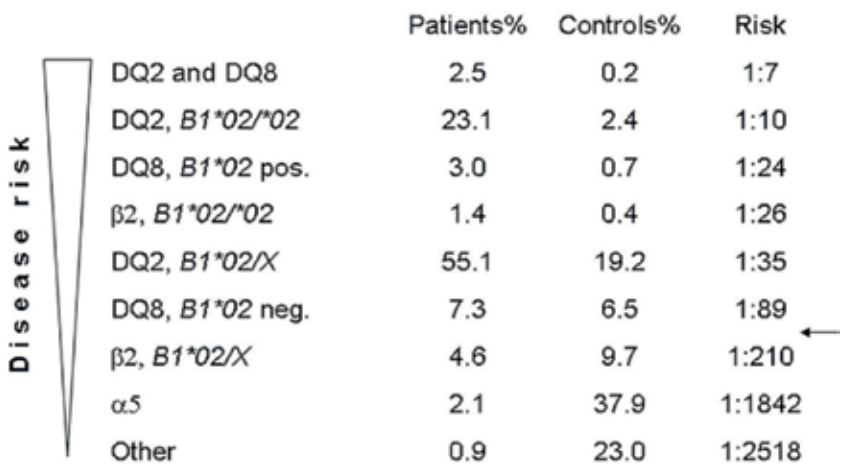

Figure 1.
Risk calculated by Megiorni et al. [62] in the Italian population. Adapted from Megiorni et al. [62].

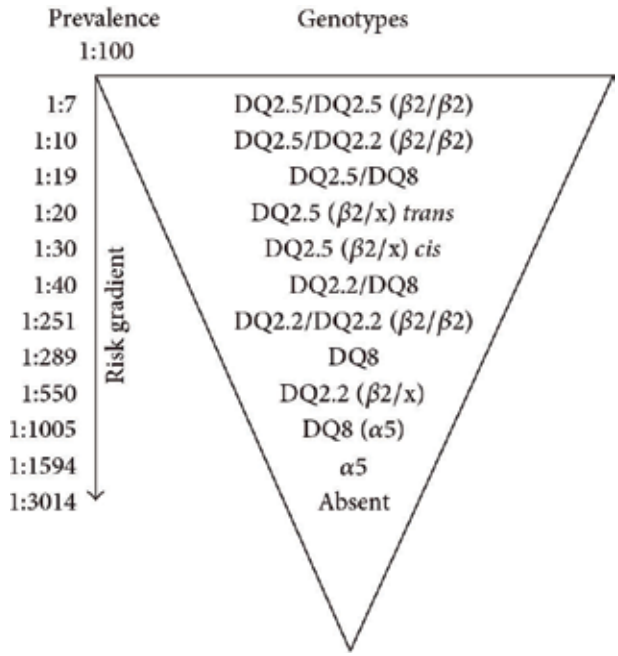

Figure 2.
Calculated Risk (Brazilian population)—Almeida et al. [2].

HLA Genotype	Patient ($n = 49$)		Control ($n = 58$)		Odds ratio	95% CL	Relative risk	*P*-value
	n	Fr (%)	*n*	Fr (%)				
DQ2.5/DQ8	5	10.2	1	1.7	6.48	0.73–57.46	1/10	0.09
DQ2.5/DQ2.5	24	49	6	10.3	8.32	3.02–22.93	1/12.5	< 0.01
DQ2.5/DQ2.2	5	10.2	2	3.4	3.18	0.59–17.19	1/20	0.18
DQ8/DQX	4	8.2	2	3.4	2.49	0.44–14.21	1/25	0.31
DQ2.5/DQ7	8	16.3	4	6.9	2.63	0.74–9.35	1/25	0.13
DQ2.2/DQ8	1	2	1	1.7	1.19	0.07–19.92	1/50	0.90
DQ2.2/DQ2.2	2	4.1	3	5.2	0.78	0.13–4.87	1/100	0.79
DQ2.x	0	0	4	6.9	0.12	0.01–2.33	0	0.16
DQX/DQX	0	0	35	60.3	0.01	0.00–0.11	0	< 0.01

Figure 3.
Calculated Risk—Murad et al. [64].

Because it is not possible to calculate the risk for the various world populations, there are many where the absence of disease prevalence data does not allow this calculation to be carried out, an estimate for populations of Caucasian origin seems to produce very close results suggesting that calculations are accurate in these populations. Considering the relevance of risk demonstrated by the most prevalent and important genotypes for the development of CD, DQ2.5/DQ2.5, DQ2.5/DQ2.2, and DQ2.5/DQ8, it can be recommend that population studies, especially those for clinical diagnosis, which until now considering the risk for development CD associated only to the presence of the DQ2.5/DQ2.5 and DQ2.5/DQ8 genotypes, which consider the inclusion of the DQ2.5/DQ2.2 genotype in their research, because this genotype does indeed pose a high risk for CD development and should not be neglected.

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Section 4

Pathogenesis and Autophagy

Chapter 4

The Emerging Role of the Autophagy Process in Children with Celiac Disease: Current Status and Research Perspectives

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Abstract

Celiac disease (CD) affects approximately 1% of the population in Europe and North America, but the number of patients currently undiagnosed is estimated to be far higher than that of diagnosed cases owing to the presence of prevalent forms with nonspecific symptoms. The toxicity of gliadin in children with CD is not destroyed through digestion with gastropancreatic enzymes. An innate immunity to gliadin plays a key role in the development of CD. Autophagy, a physiological catabolic process, plays also a crucial role in the pathogenesis of several inflammatory diseases. Recent studies have described functional involvement of the regulation of autophagy within a pediatric CD cohort. Furthermore, the contribution of autophagy has been highlighted in the degradation and in the reduction of extracellular release of gliadin peptides, thus suggesting novel molecular targets to counteract gliadin-induced toxicity in CD.

Keywords: gluten, autophagy, celiac disease, gluten-free foods, gluten-free diet

1. State of the art

Celiac disease (CD) is an immune-mediated disorder triggered by gluten ingestion in genetically susceptible subjects. About 1% of the European and North American population are affected, but the number of CD cases currently undiagnosed is suspected to be far superior to known cases due to the prevalence of forms with nonspecific symptoms. An increasing incidence of CD has been observed in developing countries, possibly due to westernization of the local diet, changes in wheat production/preparation, and increasing simplicity of diagnostic techniques. The principal determinant of genetic susceptibility to CD is the major histocompatibility class II HLA molecules. The HLA-DQ2 haplotype is expressed in the majority of patients with CD, whereas the HLA-DQ8 haplotype is expressed only in a minority of patients. However, although the presence of the DQ2 and DQ8 haplotypes is a necessary condition, it is not sufficient for the development of CD. In point of fact, only 10% of people with a genetic predisposition goes on to develop CD.

Gluten is a protein complex rich in proline and glutamine and is found in wheat, rye, and barley. The term gluten refers to a group of prolamins of wheat (gliadin and glutenin). Other prolamins are found in rye (secalin) and barley (hordein) and are genetically similar to each other.

It is particularly interesting that maize, while containing prolamins, causes no mucosal damage in celiac patients, most likely because a different phylogenetic evolution of maize prolamins makes its consumption safe for celiac subjects and not toxic.

Gluten is poorly digested in the human intestine regardless of the presence of celiac disease. Its oligopeptides cross the intestinal mucosa and reach the submucosa where they are deamidated by transglutaminase type 2 (tTG2). Deamidation promotes high affinity binding with HLA DQ2 and DQ8 expressed on the surface of T lymphocytes. In celiac patients, this process triggers an inflammatory and immune-mediated response, typical of the disease. T lymphocytes recognize the HLA complex and release various cytokines including IL-15 and IFN-γ. These molecules induce the activation and clonal expansion of B cells which produce antibodies against gluten as well as autoantibodies against tTG2. Other cytokines stimulate fibroblasts and inflammatory cells to secrete matrix metalloproteinases with consequent tissue remodeling and further release of tTG2 in the extracellular compartment. At the same time, there is an increase in intraepithelial lymphocytes with cytolytic activity which determines epithelial damage.

The typical histological lesions of celiac disease are villous atrophy, intraepithelial lymphocytosis, and crypt hyperplasia. Villous atrophy consists of decreased villous height and alteration of normal crypt/villous ratio (3:1) until total disappearance of villi. Intraepithelial lymphocytosis is defined as a number of intraepithelial lymphocyte (IEL) greater than 30 per 100 enterocytes. Crypt hyperplasia is the extension of the regenerative epithelial crypts associated with changes in the presence of more than one mitosis per crypt.

These elementary lesions associated with celiac disease are identified through duodenal biopsies from endoscopic evaluation. Multiple biopsies of the duodenum (at least one biopsy of the bulb and four of the distal part of the duodenum) are required to make a diagnosis as elementary lesions are not exclusive and frequently may be patchy [1].

In children, adding biopsies of the bulb increases diagnostic reliability, owing to the fact that in 10% of pediatric patients, villous atrophy is exclusively located in the duodenal bulb [2].

Histological changes can be classified according to the Marsh classification which identifies three entities: (1) type 1 or infiltrative lesions (normal villous and crypt architecture, normal villous/crypt ratio, and an increased number of intraepithelial lymphocytes); (2) type 2 or hyperplastic lesion (normal villous architecture, hyperplasia of the glandular element with an increased number of mitoses, and increased intraepithelial lymphocytes); and (3) type 3 or destructive lesion (varying degrees of villous atrophy associated with hyperplasia of the glandular crypt and increased intraepithelial lymphocytes). Oberhuber et al. [3] proposed a different classification dividing the Marsh type 3 lesion into three subgroups according to the severity of villous atrophy: (3a) mild villous atrophy and pathological increase of IELs; (3b) moderate villous atrophy and pathological increase of IELs; and (3c) total villous atrophy and pathological increase of IELs.

Diagnosis of CD is based not only on histology but also on the presence of specific serological markers which should be performed in patients on a gluten-containing diet.

Anti-tTG2 is circulating, gluten-dependent, autoantibodies that target transglutaminase 2, the principal self-antigen involved in pathogenesis of CD. IgA anti-tTG2 has high sensitivity (97%) and specificity (91%) and is deemed the single most

reliable test for detection of CD in primary care in cases of clinical suspicion or as a screening test in relatives of celiac patients or in people with an increased risk of developing CD. In comparison with the anti-endomysial antibody, the anti-tTG2 IgA assay has greater sensitivity and reproducibility. It is important to remember that IgA deficiency is more common among celiac patients than in the general population; therefore, in cases of low serum IgA levels, IgG class anti-tTG2 should be evaluated.

Anti-endomysial antibodies (EMA) are directed against the intermyofibril substance of smooth muscle, which may correspond to either a reticulin-like structure or a surface component of smooth muscle fibrils. These are detected by indirect immunofluorescence on monkey esophagus cells and on human umbilical cord cells as a substrate. The EMA assay specificity is high (100%), but it is also IgA-based and the EMA IgG assay is not widely available. Anti-endomysial antibodies are considered a confirmatory assay and should be used only in cases of borderline positive or possibly false positive results for anti-tTG2.

Testing for antibodies directed against native gliadin is no longer recommended. However, antibodies against synthetic deamidated IgG class gliadin peptides (anti-DPG) have a diagnostic role in cases of IgA deficiency.

Histological evaluation and serological markers are important for diagnosis but also for testing efficacy of alternative therapies for celiac disease, as mentioned below.

Currently, the only effective treatment for celiac disease is a strict gluten-free diet (GFD). The aim of dietary regimen is recovery of intestinal damage (usually within 24 months), disappearance of serological markers, and resolution of symptoms, when present. Moreover, a GFD improves nutritional parameters including body mass index and bone mineral density.

Celiac patients should avoid all products containing wheat, barley, and rye for life. Complete elimination of gluten is very difficult, especially due to contamination of other foods with traces of gluten.

Strict avoidance of gluten is demanding especially in Mediterranean countries where gluten ingestion in normal diet is high as well as in adolescence and in asymptomatic children diagnosed by screening. Therefore, CD subjects should be monitored annually for adherence to the GFD. Verification of the disappearance of the specific antibodies is important during follow-up.

The definition "gluten-free" is reserved for foods having less than 20 parts per million of gluten. The lowest quantity of gluten known to be responsible for mucosal damage ranges from 10 to 50 mg per day. A gluten intake of less than 10 mg per day is unlikely to cause mucosal damage.

Nevertheless, some patients may be more sensitive than others are to gluten exposure. Therefore, compliance to GFD should be strict and lifelong.

The gluten-free diet has numerous difficulties; a significant lifestyle change is required by patients, and it may be challenging especially in Western countries where gluten is contained in a lot of foods.

Furthermore, gluten-free products are more expensive [4] and are known to have poor palatability and high fat content.

Hypothetical gluten exposure in restaurants may also be a source of anxiety for celiac patients [5].

It is extremely important that patients know potential hidden sources of gluten and obtain precise information about gluten-free substitute and their fiber and nutrient content. Celiac patients should have high-fiber diets and frequently need supplementation of iron, folic acid, vitamin B12, and vitamin D. It may be very useful to refer patients to a dietitian at the time of diagnosis.

Serological markers are used to assess adhesion to gluten-free diet and its efficacy. First of all, in a patient with persistent symptoms, despite a gluten-free diet, it is mandatory to verify strictly compliance to GFD and in particular to investigate

the possibility of inadvertent gluten exposure. Second, it is important to exclude other causes of persistent symptoms. It is necessary to underline the hypothetic presence of alternative diagnosis (i.e., lactose or fructose intolerance, irritable bowel syndrome, microscopic colitis, pancreatic insufficiency, and small intestinal bacterial overgrowth). Another reason for the recurrence of clinical manifestations is refractory celiac disease, whereby CD patients present symptoms of malabsorption and villous atrophy despite a GFD for more than 12 months. In the primary form, there is no initial response to a diet, while in the secondary one, a relapse occurs after an initial response to a GFD. Patients with refractory CD are at risk of developing enteropathy-associated T cell lymphomas.

2. The autophagy process

Eukaryotic cells digest their cytoplasmic content through different processes that come under the general term autophagy (from the Greek words *auto* meaning "self" and *paghein* meaning "to eat"). Autophagy includes different forms of digestive pathways such as macroautophagy, microautophagy, chaperone-mediated autophagy, and noncanonical autophagy. Generally, the term autophagy refers to macroautophagy, and this process depends on specialized autophagy-related proteins (ATGs) to digest different targets, such as organelles, large aggregates of proteins, and microorganisms. Autophagy also plays a key role in direct microorganisms and virus clearance, in the control of inflammation through the inhibition of inflammasome, in antigen presentation, in regulating T cell homeostasis, and the secretion of immune mediators [6]. It is worth nothing that autophagy impairment plays a crucial role in several diseases, in particular proteopathies, such as Parkinson's [7] and Huntington's disease [8].

As analytically described by Codogno et al. [9], there are four types of autophagy:

- Macroautophagy: organelles or other cargos (proteins, lipids, or nucleic acids) are sequestered in the autophagosome, a double-membrane vesicle, and delivered to the lysosome for degradation.

- Microautophagy: small cytosolic materials are degraded after their engulfment in lysosomes through membrane invagination processes.

- Chaperone-mediated autophagy: proteins with the specific KFERQ target sequence are recognized by chaperone Hsc70 protein and then degraded by lysosomes action.

- Noncanonical autophagy: under specific circumstances autophagosome formation in macroautophagy can bypass the canonical steps. To date two noncanonical pathways have been described: Beclin-1-independent autophagy and a pathway that bypasses the action of specific autophagy-related proteins such as ATG5, ATG7, and LC3.

To date, several autophagy-related genes (*ATG*s) have been described and exert a finely coordinated function at different stages of the pathway. It is widely accepted that autophagy consists of six sequential steps: (1) initiation; (2) nucleation or phagophore formation; (3) ATG5-ATG12 conjugation, interaction with ATG16L, and multimerization at the phagophore; (4) LC3 processing and insertion into the nascent autophagosome; (5) capture of random/selective targets for degradation; and (6) fusion of the autophagosome with the lysosome.

3. Autophagy and autoimmune diseases

Autophagy plays a crucial role in the pathogenesis of several autoimmune diseases. In particular, in Crohn's disease, an inflammatory bowel disease is caused by a combination of environmental, immune, and bacterial factors in genetically susceptible individuals. It has been demonstrated that genetic polymorphisms in the *ATG16L* and *IRGM* autophagy-related genes lead to a strong predisposition for the development of Crohn's disease [10]. Despite this significant association, the role played by the ATG16L protein in the disease pathogenesis is still under debate. In particular, the ATG16L protein is known to suppress the inflammatory process, as demonstrated in Atg16L-deficient mice, which were find to be highly susceptible to colitis induced by dextran sulfate sodium. Subsequently the symptoms developed by these mice were effectively treated with injections of anti-IL-1β and IL-18 antibodies [11]. Recently, nucleotide polymorphisms within the key regulatory autophagy gene *ULK1* have been shown to increase susceptibility to Crohn's disease, thus demonstrating that autophagy might contribute to the pathogenesis of this disease [12]. Similarly, the ATG5 protein, another essential component of the autophagic machinery, is implicated in the development of systemic lupus erythematosus (SLE), multiple sclerosis (MS), and rheumatoid arthritis (RA). SLE is an autoimmune disease in which the patient's immune system attacks healthy tissues. Different single nucleotide polymorphisms (SNPs), identified near and within the *ATG5* locus, are associated with SLE initiation and/or development, although the pathogenetic mechanisms involved are still unknown. In another study, it was demonstrated that T cells in SLE patients are autoreactive and autophagy promotes their survival and contributes to their persistence in autoimmune conditions [13]. MS is a demyelinating disease that affects the brain and the spinal cord. Changes in the expression of the ATG5 protein correlates also with immune-mediated myelin injury in MS-derived mice and in affected patients. Specifically, ATG5 is overexpressed in circulating T cells of relapsing–remitting MS patients compared with healthy controls. ATG5 altered expression seems to extend T cell survival and proliferation during active disease; moreover, ATG5 expression profiles correlate with the severity of the disease in mice models [14]. In RA, anti-citrullinated protein antibodies are the most powerful biomarkers in the diagnosis of this disease. During inflammation, the arginine residues of self-proteins are converted to citrulline (a nonessential alpha amino acid) by the peptidylarginine deiminase enzyme, in a process known as citrullination, thus leading to an altered immune response. Presentation of these peptides is blocked after treatment with 3-methyladenine, an autophagy modulator drug, or by reducing ATG5 protein expression, confirming a key role of autophagy in RA pathogenesis [15].

The clinical spectrum of celiac disease is broad, and often it may be not so easy to discern between poor compliance, difficult acceptance of therapy, or presence of disease complications.

It is important to emphasize experimental therapies, in terms of alternative treatment (versus gluten-free diet) or GFD adjuvant.

Recent advances in the "non-dietary" treatment of CD include engineering gluten-free grains, degrading immunodominant gliadin peptides, decreasing intestinal permeability, and inducing oral tolerance to gluten with a therapeutic vaccine.

4. Non-dietary therapies

As outlined above, there are many reasons behind the need to identify new therapeutic options for celiac disease, especially non-dietary therapies. The purpose is to offer a better quality of life to celiac patients.

Over the years several studies regarding alternative therapies have been conducted.

The aim of experimental research is to find a drug that reduces bowel inflammation despite gluten exposure. Evaluation of mucosal damage is the best way to verify the efficacy of alternative drugs, but it is an invasive procedure and especially distressing for children. Noninvasive markers of efficacy could be serological normalization (using tTG-IgA title) or the improvement of clinical symptoms.

In celiac patients exposed to gluten, increased intestinal permeability has been observed and is due to defects in tight junctions, which are structures involved in regulating the passage through the paracellular space. Increased permeability determines the passage of gluten peptides, which reach lamina propria and stimulate inflammatory response [16].

Larazotide acetate is an oral peptide derived from the zonula occludens toxin secreted by *Vibrio cholerae*. It is believed to be involved in the modulation of tight junctions, consequently preventing gliadin passage through the epithelial barrier. Numerous trials have been conducted to verify the efficacy of larazotide. It has been shown to reduce symptoms in patients on a gluten-free diet [17].

Significant effects of larazotide on serological markers have not been demonstrated, and mucosal damage healing does not appear to be the focus of the evaluation of this drug's efficacy.

Another target of alternative drug is the degradation of toxic gluten peptides making them non-immunogenic. Several *endopeptidases* have been studied for this purpose.

AN-PEP (*Aspergillus niger* prolyl endoprotease) is an *Aspergillus niger*-derived endopeptidase which was studied in a randomized trial where celiac patients were divided into two groups: one of which received AN-PEP and the other a placebo. All patients followed a diet with gluten exposure (7 g of gluten daily) for 2 weeks. AN-PEP appeared to be well-tolerated, but no difference as regards mucosal damage was observed between two groups [18].

ALV003 is a combination of two types of glutamine-specific endoprotease, EP-B2 from barley and a prolyl endopeptidase (SC PEP from *Sphingomonas capsulata*); it is active at gastric pH and able to detoxify gluten [19].

It has been observed that ALV003 can prevent mucosal damage, secondary to gluten exposure, in celiac patients with moderate gluten consumption [20].

Over the years, the emerging role of gut *microbiota* in different diseases has been noted. Variations in the composition of microbiota could play a causative role in the pathogenesis of inflammatory and autoimmune diseases.

The microbial community is composed of more than 1000 species of microbes which exert various functions on the immune system, including protecting the body against pathogens, harvesting nutrients and energy from diet, and fermenting nondigestible carbohydrates.

A specific role of intestinal microbiota in the development of celiac disease has been suggested.

Frequent infectious diseases and consequent antibiotic treatments (with secondary effects on the intestinal microbiota) have also been associated with the onset of celiac disease in genetically susceptible infants [21].

In celiac patients, a prevalence of *Bacteroides* spp. has been found [22] along with a reduction in numbers of *Bifidobacterium* species [23].

Furthermore, regardless of GFD, celiac patients presented less variability of *Bacteroides* species in biopsy samples of the duodenal microbiota in comparison with controls [24].

Several studies regarding the association of *Bifidobacterium* and celiac disease have been conducted. *Bifidobacterium infantis* and *Bifidobacterium lactis* were found to contrast the increase of permeability, secondary to gluten ingestion, in the celiac bowel. They are also thought to play a role as downregulators of immune response in celiac patients [25–27].

Therefore, a therapeutic function of *Bifidobacteria* may be considered and it could be investigated through further studies.

BL-7010 is an orally available polymer with a high affinity for gliadins. Polymeric binders are used to reduce the intestinal absorption of endogenous or exogenous molecules.

BL-7010 sequesters gliadin and masks it from enzymatic degradation. Through this mechanism, it is thought to prevent the formation of immunogenic peptides that trigger the immune system.

It is chemically stable at a wide pH range, is non-biodegradable, and is water-soluble. It exerts its function locally in the gastrointestinal tract and is not absorbed systemically; therefore, it has a very good safety profile as was demonstrated in preclinical studies. In vitro and in mouse models have shown that BL-7010 is able to bind gliadin and to prevent barrier dysfunction, changes in IELs, and villous/crypt ratios induced by gliadin [28].

Another therapeutic target identified in recent years is a vaccine called *Nexvax2*. The aim of this drug is to restore immune tolerance to gluten using peptide-based immunotherapy to induce T cells to make a regulatory response. Murine models were used for the study, and three immunogenic peptides have been identified. These peptides elicit an immune response in patients with celiac disease who carry the HLA-DQ2 immune recognition gene. Tolerance is based on reduction of CD4 T cell proliferation, decreasing IL-2 and IFN-γ levels and promoting expression of T cells with regulatory functions. It was reported that patients treated with the vaccine (especially with high doses) presented gastrointestinal side effects similar to celiac symptoms. This finding corroborates the hypothesis that the vaccine has a similar action to gluten ingestion as regards intestinal immunity [29].

Another attempt at immuno-modulation was made using *CCX282-B* an antagonist of the CCR9 chemokine receptor. Increased blood levels of T cells expressing CCR9 have been found in celiac patients. It is thought that CCX282-B may prevent T cell migration from the blood to the intestinal mucosa. Unfortunately the results of this study are not yet available.

A further consideration is the hygiene hypothesis, whereby excess hygiene is thought to trigger the inflammatory process through immune imbalance resulting in autoimmune disorders. The effect of helminth infections on immunity has also been studied, in particular their role in celiac disease and in inflammatory bowel disease.

Interestingly, it has been reported that the *Necator americanus* species of hookworm enabled individuals with celiac disease to tolerate escalating challenges with dietary gluten [30]. The exact mechanism responsible for tolerance is not known, and further studies are expected.

As previously described, reasons justifying the search for an alternative therapies for celiac disease are numerous, and several studies have been conducted to assess their efficacy. The aim is to identify a new drug and, at the same time, to define new hypothetical therapeutic targets to improve celiac patients' quality of life.

Over the years we have studied the autophagy process. Our results have highlighted the possible contribution of this process to the degradation and the reduction of extracellular release of gliadin peptides and suggest novel molecular targets to counteract gliadin-induced toxicity in CD.

5. Autophagy and celiac disease

The primary link between autophagy and celiac disease is that autophagy is conventionally described as a catabolic pathway where the cytoplasmic material sequestered by autophagosomes is degraded. Therefore, the exogenous gliadin

peptides content might be a potential target of the autophagy clearance process. The autophagy escape, on the other hand, might specifically lead to MHC antigen presentation by dendritic cells or to other unspecific exocytic/endocytic processes between different cells.

Autophagy is known to modulate two crucial aspects of the adaptive immune response involved in the pathological context of the celiac condition: it can enhance priming of CD4+ T cell responses, but at the same time, by allowing the presentation of self-peptides, it may also regulate the establishment of peripheral T cell tolerance [31]. Importantly, antigen presentation by MHC class I or II proteins is dependent on the activity of the proteasome or the endocytic/phagocytic system, respectively, and therefore associated with the functionality of the autophagy process.

To date, however, only a few scientific publications have attempted to investigate the functional role of the autophagy process in celiac disease.

Weersma and colleagues [32] first reported the absence of association of *IL23R* and *ATG16L1* mutations with celiac disease susceptibility, in contrast to that of Crohn's disease. This result was subsequently confirmed independently by genetic analysis in a different cohort of celiac disease patients [33].

More recently, Rajaguru and colleagues [34] evaluated the expression of the LC3 autophagy marker in duodenal biopsies of celiac patients at initial presentation and after 6 months of gluten-free diet, reporting a time-related reduction of LC3 expression in dendritic cells through immunohistochemical analysis. The authors concluded that the observed typical histological pathological hallmarks in duodenal biopsies were associated with a reduction in activated dendritic cells expressing autophagic proteins. This alteration within the autophagy executor organelles may well play an important role in the pathogenesis of autoimmune disorders like celiac disease.

Comincini and collaborators [35] have recently tackled a different question regarding celiac disease, i.e., the possibility to identify novel molecular markers in order to increase the sensitivity and specificity in the diagnosis of pediatric celiac disease patients. To this end, the expression levels of two key autophagy executor genes (*ATG7* and *BECN1*) and their regulatory validated miRNAs (miR-17 and miR-30a, respectively) were analyzed by relative quantitative real-time PCR on a cohort of confirmed celiac patients compared to age-related controls, analyzing peripheral blood, and corresponding duodenal specimens. Among the investigated gene/miRNA targets, statistical analysis indicated the highest significant association as that of *BECN1* expression profiles with the pathological status in the blood, while in intestinal biopsies, all the investigated sequences were positively associated with the celiac disease condition. The authors were also able to identify specific celiac/control molecular subtypes based on specific genes and miRNA expression signatures. Overall, the authors described novel molecular markers that might be useful in increasing the accuracy in CD diagnosis and in molecular-based stratification of the patients, further reinforcing the functional involvement of the regulation of the autophagy process in digestive and autoimmune-related disorders such as CD.

In the latest PubMed contribution, Manai and collaborators [36] reported that in Caco-2 cells, a widely used in vitro model for celiac disease studies, the administration of enzymatically digested gliadin (PT-gliadin) peptides significantly reduced the expression of the LC3-II autophagy-related marker. Furthermore, electron and fluorescent microscope analysis suggested a compromised function of the autophagosome apparatus. The improvement of the dysregulated autophagy process, along with a reduction of PT-gliadin toxicity, was achieved by means of a starvation induction protocol and by 3-methyladenine administration, while rapamycin, a well-known autophagy inducer, did not produce significant improvement in the clearance of extra- and intracellular fluorescent PT-gliadin amounts. Importantly, these results highlighted the possible

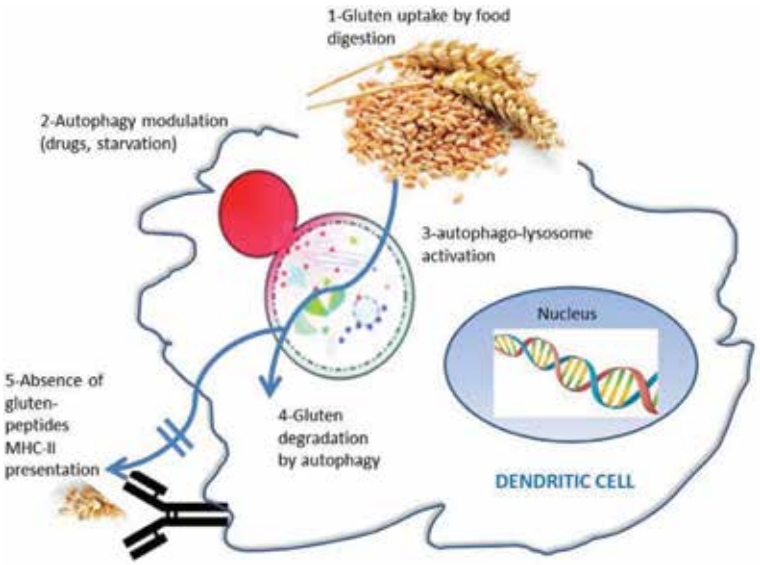

Figure 1.
Gluten degradation by autophagy process. Gluten digested peptides (1) can be internalized into different cell types, including human dendritic cells. These peptides form relatively large intracellular aggregates, partially resistant to intracellular catabolic process, and they can also be released to other surrounding cells by exocytic processes. The discussed approach, (2) based on the autophagy activation using drugs (rapamycyn, chloroquine, etc.) or by means of starvation induction, (3) leads to the fusion of autophagosomes and lysosomes to produce autophago-lysosomes, able to digest the gluten digested peptides. (4) this intracellular digestion produces a reduction of the exocytosis of the gluten peptides, and, mostly, (5) it might reduce the gluten-peptide presentation by MHC-II molecules (HLA-DQ2.5) and therefore the pathological activation of T cell in the celiac disease.

contribution of the autophagy process to the degradation and the reduction of extracellular release of gliadin peptides and suggest novel molecular targets to counteract gliadin-induced toxicity in celiac disease. A schematized summary of the autophagy-modulation strategy to counteract the gluten-derived cellular toxicity is illustrated in **Figure 1**.

6. Concluding remarks

Celiac disease is an increasingly complex disease, with a well-established genetic background but with a plethora of molecular/cellular actors involved. Despite this emerging complexity, the cellular uptake of the digested gliadin components and their ultimate fate is the key determinant for this disease. Once within a cell, gliadin peptides, as with any exogenous components, undergo different catabolic processes, including the relatively low-energy consumption processes such as exocytosis. In this scenario, autophagy protein turnover might represent a pro-survival process to counteract a surge in potentially toxic gliadin. However, for reasons still unknown, the autophagy process seems to be impaired in the celiac condition: as a result, gliadin is easily internalized in different types of cells, but no marked signs of a prominent degradation are reported. On the other hand, more and more is being learned about the process of autophagy and its molecular players, and, consequently, a relatively large number of molecular and pharmacological modulators are being put on the market and assayed in clinical trials for different pathologies. Therefore, once the alterations of the steady-state status of the autophagy process are clarified by comparing physiological to celiac pathological conditions, one could realistically hope to counteract gliadin toxicity by improving its catabolism within the cells, bearing in mind however

that the exacerbation of the fine autophagy intracellular balance might also lead to other, even more complex pathological conditions such as cancers.

This article focuses on the results of researches carried out by authors in the field of celiac disease.

It is of the upmost importance to investigate new therapeutic options for celiac patients, especially non-dietary therapies, in order to improve their quality of life.

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The authors declare no conflict of interest.

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This article is the focus of the researches carried out by authors in the field of celiac disease.

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Section 5

Complications

Chapter 5

Complications of Celiac Disease

Rakhshinda Jabeen

Abstract

Celiac disease is a small bowel disorder, due to defect in gluten diet, leading to mucosal inflammation, villous atrophy and crypt hyperplasia. For the diagnosis of celiac disease, one has to be on gluten free diet. Due to commonly available various serologic tests and histopathology, celiac disease, can be categorized as asymptomatic, silent or potential. Between 80 and 90% of all patients with celiac disease remained undiagnosed. Because of this late diagnosis, patients may develop various complications including anemia, bone loss, depression and cancers. Patients may have different types of anemia including iron deficiency, folic acid or B12 deficiency. Any of these may occurred separately or may be manifested together. The same variation is seen in bone loss, starting from osteopenia, osteomalacia to osteoporosis and even dysplasias. Patient may develop lymphoma, gastric or oesophageal carcinomas as well. Celiac disease is also associated with other autoimmune illnesses as it is an autoimmune process by itself. The complications of celiac disease, is either due to direct consequence of celiac, or due to significant damage to the small intestine. With the early detection and diagnosis, the symptomatology and complications of celiac disease can be spared.

Keywords: complications, anemia, bone loss, cancers

1. Introduction

Celiac disease (CD) is one of the commonest malabsorptive syndromes, of either one or more nutrients. It was historically known as a disease of whites, but in recent era, it is as commonly seen in other parts of the world, including Asian and African countries [1, 2]. Because of its various categories starting from full blown CD, to completely asymptomatic variety, the clinical presentation dispersed among individuals. Although the commonest presentation is the gastrointestinal manifestation but many individuals may present with malignancies associated with CD. The logical answer for this diverse and late manifestation may be due to prolonged breast feeding and late commencement of gluten diet among infants, which was the usual presenting age among infants [3]. The usual age of presentation of CD now is 10–40 years [4].

In recent days, young patients still presenting with classical symptoms of CD, i.e. gastrointestinal, although may have complications at the time of initial presentation. The older individuals usually present with complications of different varieties, which make CD to diagnose late [5] (**Table 1**).

Among different categories of CD, including classical CD, atypical CD, asymptomatic and latent CD the presentation are different and thus complications.

Classical celiac disease: The classical one, including three features: villous atrophy, symptoms of malabsorption and resolution of symptoms with gluten free diet.

Gastrointestinal complications	Non-gastrointestinal complications
Malabsorption	Nutrient and mineral deficiencies
Intestinal lymphomas	Osteoporosis/osteomalacia
Collagenous sprue	Dental defects
Other GI malignancies	Idiopathic pulmonary hemosiderosis
	Glomerular IgA nephropathy
	Infertility
	Cardiomyopathy/myocarditis

Table 1.
Complications of celiac disease.

This variant presents with malabsorption with vitamin and nutrient deficiencies. The malabsorptive symptoms include steatorrhea, weight loss, abdominal pain and diarrhea [6]. These patients also developed muscle weakness, muscle and bone pain, tooth enamel defect and lactase malabsorption [7]. These patients have various kind of nutrient and mineral deficiency including iron, folic acid, vitamin B12, vitamin D and zinc mostly, although they may have deficiency of other minerals as well.

Non-classical and atypical CD: The non-classical CD patients may have both gastrointestinal symptoms as in classical one, or they present with other associated manifestations of CD, including dermatitis herpetiformis, IgA deficiency, type 1 diabetes mellitus, autoimmune thyroid illness, enamel defects and infertility. These patients also have severe mucosal damage and having specific celiac antibody pattern.

The asymptomatic or silent CD: This variant remained undiagnosed, or incidentally diagnosed upon screening. They usually do not have any symptomatology except a little fatigue, which occurs after introduction of gluten free diet. Although these patients have classical architectural remodeling of intestinal mucosa as in classical CD, i.e. crypt hyperplasia and villous atrophy, but do not developed gastric symptoms; neither had they developed complications related to CD.

Latent CD: There are some patients among CD who have minor or no symptoms with normal jejunal mucosa, and remained asymptomatic if on gluten free diet. In this variant only 20% remained asymptomatic till adulthood and do not develop any complication of CD and having normal architecture throughout. The rest of the patients developed various degree of villous atrophy [8] and thus may develop complications related to CD. These patients may develop malabsorption with multiple nutrient deficiencies and lymphoma.

Refractory CD: It is defined as persistent or recurrent symptoms typically diarrhea and weight loss with signs of malabsorption .despite on strict gluten free diet. It is accompanied by villous atrophy. Both the variants of refractory CD is associated with increased risk of lymphoma [9].

2. Gastrointestinal complications of CD

The classical CD may present with complications related to gastrointestinal symptomatology including recurrent foul smelling, bulky loose stools, and due to steatorrhea and flatulence. The mechanism of developing steatorrhea is primarily due to changes in jejunal mucosal function. Due to changes in jejunal brush border enzymatic function; these patients may develop secondary lactase deficiency, leading to diarrhea even after ingestion of milk and milk products, which further

increases the development of complications [10]. In cases of extensive involvement of ileum, patients with CD may complicate with bile acid malabsorption resulting in bile acid induced fluid secretin in the colon. Crypt hyperplasia may lead to endogenous fluid secretion (**Figures 1** and **2**).

CD presenting in early age may complicate with short stature or stunted growth due to deficiency of multiple nutrients and minerals. These patients may also have severe weight loss, anemia of different types, and osteopenia. Due to shifting of classical symptoms to asymptomatic variety, patients with CD, may presents with irritable bowel syndrome type symptoms.

An association of CD is seen with gastroesophageal reflux disease, and it is much higher in CD than controls. These patients usually responded on gluten free diet within 3 months' time [11]. Eosinophilic esophagitis is another common complication among patients with CD. Patients with persistent reflux or dysphagia should be evaluated for CD [12].

Because same pro-inflammatory polymorphism of the IL-23, receptor in both ulcerative colitis and CD, they may co-exist. The incidence of ulcerative colitis is

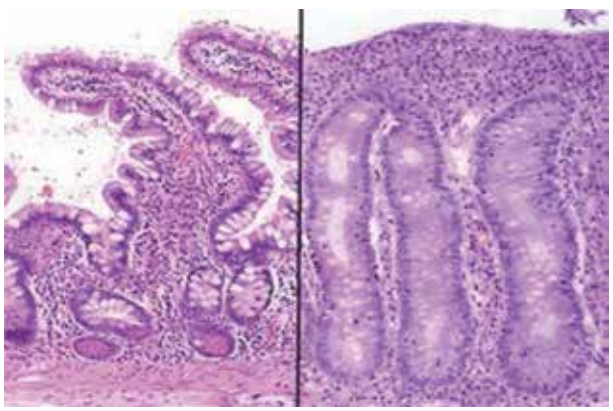

Figure 1.
Comparison of blunting and flattening of villi with normal mucosa (left) in CD.

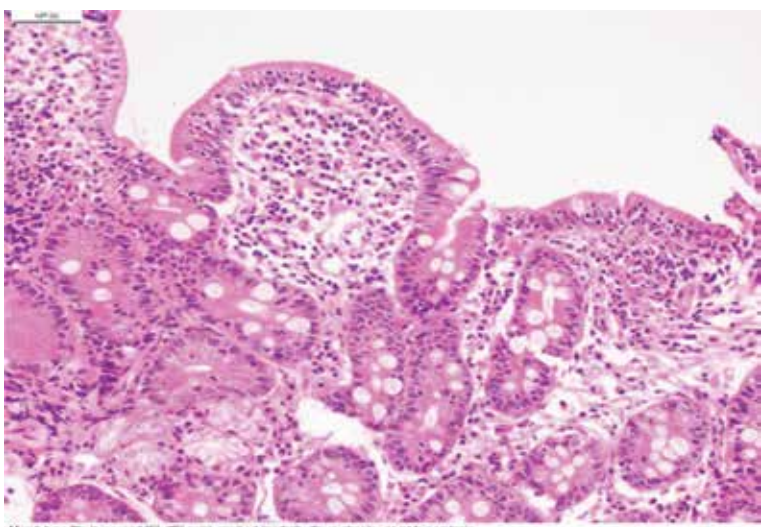

Figure 2.
Villous atrophy in CD.

much higher than Crohn's disease in patients with CD [13]. Patients having both CD and ulcerative colitis have more chances to have pancolitis as compared to patients having ulcerative colitis alone [14].

Oral lesions including erythema or atrophy, and soreness or burning sensation of the tongue have been associated with CD and can be resolved with gluten free diet.

Adult patients may rarely develop celiac crisis, presenting as profuse diarrhea and severe metabolic disturbances, if remained undiagnosed [15].

3. Nutrient deficiencies in CD

Now a days, many patients with CD, may presents with no or minor gastrointestinal symptoms. The anemia including microcytic due to iron deficiency, macrocytic either due to vitamin B12 or folic acid deficiencies or even normocytic due to combined deficiencies is the initial manifestation of CD [16]. The possible explanation for high prevalence of nutritional deficiencies among CD patients include insufficient nutritional intake.

One of the commonest complications of CD is iron deficiency anemia; these patients may remain undiagnosed till their adult life [17]. The major cause of iron deficiency anemia includes decreased dietary intake, reduced absorption and blood loss. Among these causes, blood loss remained the most important one and it can be due to various sites including abdomen or urogenital. Reduced absorption of iron is an uncommon cause of iron deficiency, especially in healthy individuals and in resource rich countries. Iron is absorbed in the upper GI tract, and the duodenum is the site of maximum absorption [18]. There are multiple medical conditions which may lead to reduced absorption, and among them celiac is not an uncommon cause. The other conditions are atrophic gastritis, helicobacter pylori infection and bariatric surgery. Normal heme iron and normal gastric environment without acid reducing medications, facilitates absorption [19]. CD can contribute to anemia by several mechanisms, including iron deficiency by reduced absorption of supplemental iron and malabsorption of the other nutrients required for RBC production including B12, folic acid and copper [20]. There may also be a component of anemia of chronic disease and blood loss. Although the exact cause of blood loss in CD is unclear [21]. The causative pathology among these patients either includes mucosal abnormalities leading to oesophagitis and gastritis, or occult gastrointestinal bleeding [22]. The occult bleeding is due to excessive loss of intestinal cells and/or malabsorption of peroxidase-containing foods rather than loss of red blood cells [23]. Although the occult bleeding is not a common manifestation among patients with CD, thus occult bleeding is not a major contributing factor to iron deficiency anemia [24]. The classical presentation among these patients is recurrent iron deficiency with no recovery on supplemental therapy. The presentation of iron deficiency among these patients may include mild iron deficiency to severe heart failure.

The water soluble vitamins are B6 and folic acid which absorbed proximally and B12 which absorbed distally are the other commonly deficient vitamins [25]. The fat soluble vitamin A and D are also deficient in CD. Among these water and fat soluble vitamins, folic acid is the deficient most varying from 18 to 90% [26], followed by B12, vitamin A and D respectively. Overall vitamin deficiencies are barely seen in healthy individuals except for vitamin B12 which is commonly seen in healthy individuals [27]. The clinical presentation of folic acid includes mild cheilitis and sore mouth. Vitamin B12 and B6 deficient patients although presents with more severe manifestation including neurological symptoms.

There is deficiency of other minerals including magnesium, copper, and zinc. Minerals form only 5% of the typical human diet but are essential for normal health and function. Although magnesium deficiency in humans is very unusual, it has been seen in individuals on a highly restricted diet or with celiac disease [28]. Magnesium is absorbed throughout the small intestine but the co-efficient is very low, possibly as low as 5% [29]. Its absorption decreases following high dietary intake or when total body magnesium is sufficient [30]. Due to deficiency patients may develop scaly dermatitis and dyslipidemia. Copper is the other mineral which may reduce in CD. It is absorbed in the proximal small intestine and stomach [31]. Though rare, acquired copper deficiency has been seen in humans. The common causes include fore gut surgery, premature infants, chronic malabsorptive conditions including CD and hemodialysis [32–34]. Copper deficiency may presents with fragile, abnormally-formed hairs, depigmentation of skin, muscle weakness and neurological abnormalities [35]. The neurological abnormalities are same as B12 deficiency. It may also causes anemia mimicking iron deficiency and neutropenia [36]. The other commonest deficient mineral is zinc. It is mainly absorbed in the duodenum and jejunum, and to a lesser extent in the ileum and large intestine [37]. In CD, the zinc deficiency may be due to increased endogenous losses of zinc, rather than abnormal zinc absorption. The clinical presentation of zinc deficiency includes wide array of skin lesions, growth retardation and hypogonadism [38]. The cell mediated immunity and antioxidants buffer capacity may be compromised as well [39].

4. Neuropsychiatric complications of CD

There is an established association of CD with different neuropsychiatric symptoms including headache, peripheral neuropathy, ataxia, depression, dysthymia, anxiety and epilepsy [40]. Peripheral neuropathies, characterized by burning, tingling, and numbness in hands and feet is quiet common among CD patients and sometimes the initial presentation as well. These neuropathies are associated with deficiencies of different vitamins including B1(thiamine), B2(riboflavin), B3(niacin), B6(pyridoxine), B12(cobalamin) and E, and mineral including copper. However, these deficiencies occurred when there is severe and extensive bowel involvement. Neuropathies may also be associated with lymphoma as well. Patients presenting with neurological manifestation has significant structural and functional brain deficits on MRI as compared to controls. The exact mechanism in relation to depression and epilepsy is not clear yet [41]. Patients with peripheral neuropathies do not responded on gluten free diet as compared to other neurological manifestations.

5. Metabolic bone disease and complications related to joints in CD

The relationship of bone derangement and CD has been recognized since a long time, and can occur with or without gastrointestinal symptoms (**Table 2**). Low mineral density, reduced bone mass and increased fragility leading to increased risk of fracture is commonly seen in CD. These bone alteration are the consequence of impaired calcium and vitamin D absorption and secondary hyperparathyroidism, resulting primarily from the loss of villous cells in the proximal intestine, where calcium is mostly absorbed accounting for 90% of overall calcium absorption [42]. Minor amount of calcium is absorbed from stomach and intestine, the colon accounts for <10% of calcium absorption. Calcium absorption from intestine,

Factors	Mechanism of action
Hypocalcaemia	Vitamin D deficiency Intestinal mucosal damage Alterations in calcium-transport mechanism Inadequate calcium intake Reduced consumption of dairy products Steatorrhea
Hypovitaminosis D	Alterations in vitamin D metabolism Decreased level of vitamin D-binding proteins Decreased intake of vitamin D Steatorrhea
Bowel inflammation hormones	Chronic release of proinflammatory cytokines PTH Estrogens and androgens
Corticosteroids	Reduction of intestinal calcium absorption Increase of renal calcium excretion Impairment of osteoblast function Alteration of osteoclast resorption cycle
Additional risk factors	Autoimmune alternations Diagnosis in adult life Lapses from GFD Active disease Low BMI Lifestyle factors

Table 2.
Factors contributing to bone alteration in celiac disease.

reabsorption from the kidneys and excretion from bones is tightly controlled. Calcium balance is regulated through the calcitropic hormone, parathyroid hormone, 1,25(OH)2 D3, exert complex coordinated activities to maintain normal serum calcium levels [43]. When the extracellular calcium concentration decreases, there is rapid increase in parathyroid hormone release that promotes bone turnover and calcium bone loss. Hyperparathyroidism (**Figure 3**) is common in patients with newly diagnosed CD, 27% in adults and 12–54% in children [44, 45]. It is more common in refractory C, rather than in those who responded on gluten free diet [46]. Low BMD in adult CD patients is related to secondary hyperparathyroidism and osteomalacia due to calcium and vitamin D malabsorption [47]. Vitamin deficiency is present in both females and males accounting for 71 and 64% respectively. Dietary vitamin D is absorbed as a fat soluble vitamin in small intestine, and because of intestinal involvement in CD its absorption decreases thus leading to deficiency of particular vitamin [48]. The other factor of hypovitaminosis is intestinal mucosal lesion [49]. Steatorrhea may also impair the absorption of 25(OH)D undergoing enterohepatic circulation, especially in acute exacerbation of CD. In patients with CD, the regulation of 1,25(OH)2D3 is through genomic action involving the classical vitamin D receptor (VDR), although non genomic regulator is also involved in its absorption [50]. VDR is normally expressed in duodenal mucosa of patients with CD, notwithstanding mucosal damage and atrophy of villi [51]. Although there is no difference in frequency of VDR gene is seen in patients with CD and healthy subjects, therefore VDR gene is unrelated to low BMD [52]. The main factor is reduced calbindin and calcium binding protein due to damaged intestinal mucosa which leads to calcium loss and secondary hyperparathyroidism [53]. In atypical patients of CD, many presented with back pain, diffuse musculoskeletal pain and proximal muscle weakness, due to osteomalacia, osteopenia and osteoporosis. All these patients have low BMD.

Osteopenia is found in in almost all patients with CD, either treated or untreated. It is even more common in patients who remained unrecognized. The prevalence of osteopenia and osteoporosis in adult is around 14–35% [54]. Osteoporosis (**Figures 4** and **5**) is characterized by low bone mass, micro architectural disruption and skeletal fragility resulting in decreased bone strength and increase risk fracture. It is not only dependent on BMD, but also related to rate of bone formation and resorption, bone size and shape and micro architecture. It has no clinical manifestation until one developed pain due to fracture. The commonest site of involvement of osteoporosis is either lumbar spine or neck of femur or radius. The commonest site of involvement is also lumbar spine accounting almost 26% [48]. The risk is almost doubled as compared to general population. It is more common in peripheral skeleton and common in males with classical presentation than females. Loss of bone density is much increased in older patients with late diagnosis as compared to younger patients and usually not resolved completely with gluten free diet. There is increased fracture risk, almost doubled in CD patients as compared to general population [55].

Children with CD are at risk of reduced BMD, hyperparathyroidism, decreased calcium especially in those with untreated CD [56]. They may or may not have

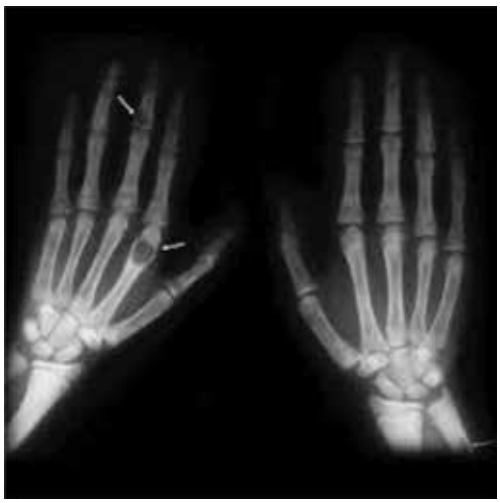

Figure 3.
Hyperparathyroidism.

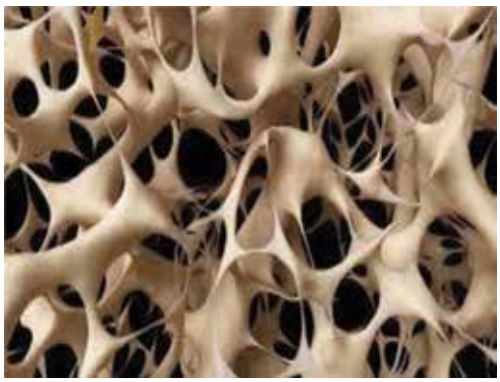

Figure 4.
Cross-section of bone tissue-osteoporosis.

associated gastrointestinal symptoms. There is also risk less-than optimal peak bone mass leading to growth retardation as well. Bone density increases until the end of puberty. If there is lack of achievement of proper peak mass, there is more chance of development of osteoporosis in adulthood [57]. The rate of bone metabolism is also altered in children with CD, which is another factor for osteopathy [58]. In children who are unable to catch up growth need to be evaluated for concomitant growth hormone deficiency, as growth hormone exert its effect on bone mineral density.

Patients with CD commonly have osteomalacia (**Figures 6** and **7**) as well and presenting as aches and pain with bone tenderness unlike to osteoporosis. These patients also presented with proximal myopathy and spontaneous fracture [59]. The exact prevalence of osteomalacia though is not clear in CD [60]. The major factor for development of osteomalacia is decreased absorption of calcium and vitamin D. Diet plays a major and important role in proper bone mineralization. A gluten free diet is low in nutrient, vitamins and minerals, including calcium [61].

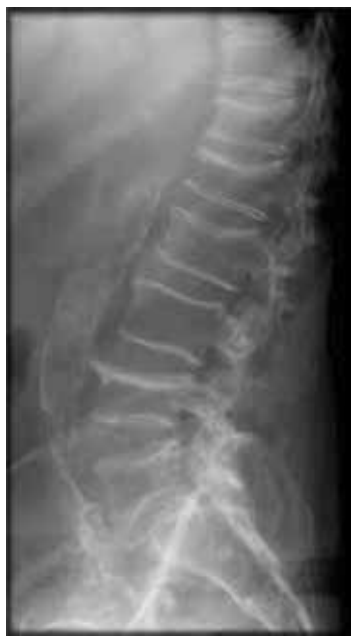

Figure 5.
Radiology-osteoporosis.

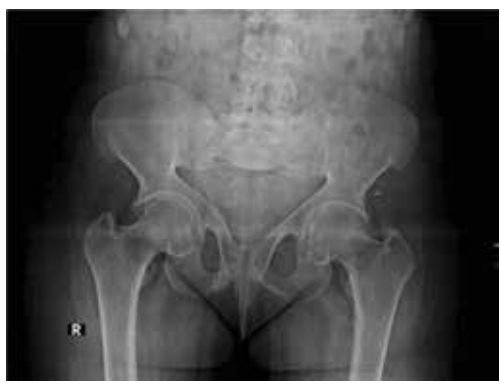

Figure 6.
Tri-radiate pelvis-osteomalacia.

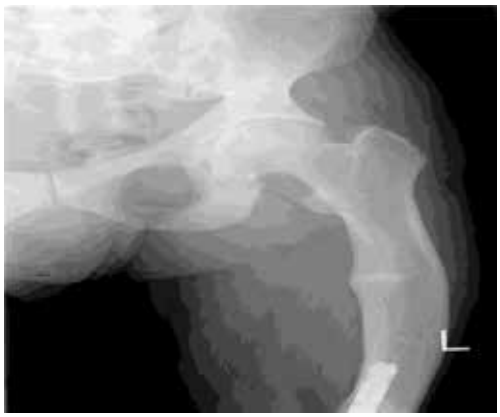

Figure 7.
Looser's zone-osteomalacia.

Thus they consume less vitamin D and calcium, as compared to normal diet [62]. It further decreases due to lactose intolerance resulting from decreased lactate production by the damaged villi. This factor further decreases BMD, and aggravates osteoporosis and osteopenia.

Osteoarthritis is the most common type of arthritis world over. It is inflammatory condition due to damage of cartilage involving any joint of the body. It is commonly presents in patients with CD, but the exact causal relationship is not known [63].

6. Malignancies associated with CD

The most important complication of CD is development of cancer. The incidence of gastrointestinal and non-gastrointestinal both is common among CD patients. The commonest among the malignancies is the gastrointestinal lymphomas accounting for 18% of all cancers. The gastrointestinal tract is the predominant site of extranodal lymphoma involvement. Primary lymphomas are less common than secondary lymphomas. Primary lymphomas typically involve any section of gastrointestinal tract from oropharynx to rectum [64]. It can involve single or multiple sites. These are usually non-Hodgkin's lymphomas although Hodgkin's lymphoma has been reported as well. The commonest site of involvement of non-Hodgkin's lymphoma is stomach, present in 68–75%, followed by small bowel than ileocecal junction. Diffuse colonic involvement is present only in 1% [65]. Distribution of primary lymphomas varies among population; gastric lymphoma is more common in United States, while intestinal lymphoma is seen in Middle East and Mediterranean areas. GI Lymphomas are commonly seen in patients with helicobacter pylori infection, autoimmune disease, immunodeficiency and immunosuppressive states, inflammatory bowel disease and CD. Both the T and B cell are commonly seen in patients with CD. The exact etiology of development of these malignancies is under controversy, both the autoimmune and inflammatory factors contribute to the risk. The T cell variant enteropathy-associated T-cell lymphoma (EATL) (**Figure 8**) involving the small intestine mostly, is the commonest malignancy seen in celiac disease, although uncommonly seen [66, 67]. It is most commonly found in adult males with a median age of 60 years. EATL patients mostly presented with acute bleeding, perforation or obstruction, or it should always be suspected if there is clinical deterioration of CD, despite on a strict gluten free diet. It is highly suggested to screen a patient for CD, even if not diagnosed before, if

presented with EATL. Patients with enteropathy associated T cell lymphoma of the small intestine, involving jejunum demonstrates large circumferential ulcer without overt mass. The involved area typically shows lymphoma, while the non-involved sites usually show villous atrophy.

The other variant of EATL, ulcerative enteritis, is another complication of long standing and refractory sprue. It presents with abdominal pain, nausea, vomiting and diarrhea. The other complication of CD includes intestinal ulceration independent of lymphoma and so called refractory sprue and collagenous sprue (**Figure 9**). It is a clinicopathological entity characterized by diarrhea and malabsorption accompanied by the histological findings of subepithelial collagen deposition and severe villous atrophy of small bowel mucosa [68]. The occurrence of collagenous sprue has been seen in patients with celiac disease, tropical sprue, milk intolerance and common variable immunodeficiency states [69]. Regardless of etiology it has a poor prognosis. Collagenous sprue associated with CD usually does not respond to gluten free diet and has a poor prognosis [70].

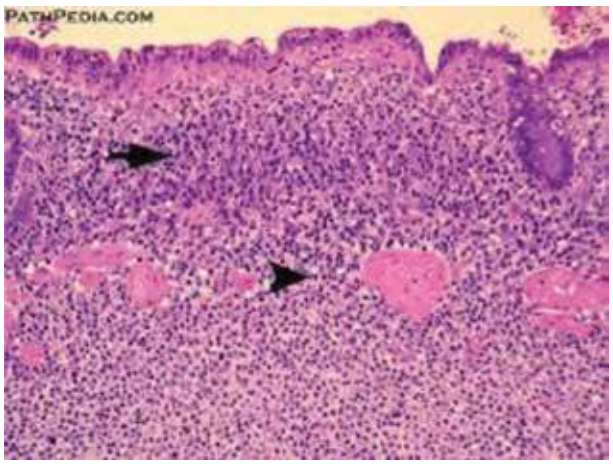

Figure 8.
Lymphoma.

Figure 9.
Collagenous colitis.

The risk of other digestive tract malignancies is also commonly seen in CD patients, including oropharyngeal, colorectal, small intestinal adenocarcinoma and hepatocellular carcinomas. In oropharyngeal carcinomas, the commonest is the squamous cell carcinoma. In contrast to gastrointestinal carcinomas, the non-gastrointestinal malignancies, including breast carcinomas are not seen commonly in CD patients. There is no evidence that gluten free diet may decrease the development of malignancies in CD.

7. Hyposplenism in CD

One of the earliest manifestations of CD is hyposplenism, though the exact mechanism of development of hyposplenism is not known. Due to hyposplenism there is increased susceptibility of infection due to encapsulated bacteria especially. Vaccination against prophylaxis for pneumococcus is not highly recommended [71, 72].

8. Venous thromboembolism in CD

Hypercoagulability with elevated homocysteine level and low vitamin-K-dependent anti-coagulant proteins (protein C & S) are relatively common in CD patients. Due to these factors, i.e. increased homocysteine level and decreased protein C &S, along with autoimmunity of CD, there might be increased susceptibility to venous thromboembolism. Although not commonly observed in CD patients [73].

9. Kidney disease in CD

Glomerular IgA deposition is common, occurring in as many as one-third of patients. Although the clinical manifestation is not evident, due to no associated complement deficiency. This indicates that a high circulating load of polyclonal IgA is not adequate to cause nephritis, but other abnormalities of IgA are necessary to translate into mesangial activation and glomerular injury. This mesangial IgA deposition is also seen in healthy individuals from 3 to 16% [74]. Thus its association with other disease is relatively high.

10. Idiopathic pulmonary hemosiderosis in CD

Lane-Hamilton syndrome, the co-existence of CD and idiopathic pulmonary hemosiderosis, is not an uncommon entity in CD patients, although the exact prevalence is not known [75]. It is a rare lung disease characterized by alveolar capillary bleeding and accumulation of hemosiderin in the lungs. Diffuse alveolar hemorrhage is characterized by hemoptysis, dyspnea, alveolar opacities on chest X-ray and anemia. It may lead to iron loss through swallowing of iron-laden alveolar or bronchial epithelial cells. This may lead to functional iron deficiency [76].

11. Dental defects in CD

Oral manifestations are overlooked in CD patients; the long list of clinical signs and symptoms associated with CD includes dental enamel hypoplasia, aphthous

ulcers and delayed eruption of teeth [77]. The dental enamel defects are more common in deciduous dentition. It can involve all four quadrants, but more commonly involved maxillary and mandibular incisors and molars [78]. The exact cause is not understood but it may be due to increased level of HLA DR3 in their blood. This antigen has an association with celiac disease. Dental enamel hypoplasia, a nutritionally related defect of the enamel, presented as pits, lines or grooves on the teeth. Its prevalence ranges from 10 to 97%, and appears to more common in children with CD, as compared to adults [79]. Its prevalence in CD, is much higher than general population, and it is contributed both to nutritional and immunological factors. Another enamel defect, either partial or complete, can sometimes be the only symptoms in children with CD. It is thus advisable to screen children with enamel defects for CD. The other oral manifestation aphthous ulcer or canker sores are also seen in CD, though not specific for CD. Aphthous ulcers, though regresses on gluten free diet [80].

Delayed tooth eruption, another manifestation of CD, has been reported in 27% of patients with CD. The possible cause is probably malnutrition. A high prevalence of enamel hypoplasia is around 66% in CD patients. Formation of plaque is less frequent in patients who are on gluten free diet, probably because of multiple meals in between and use of fluoride toothpaste.

There are other oral problem related to celiac disease, which include recurrent aphthous stomatitis, atrophic glossitis, dry mouth syndrome and squamous cell carcinoma of the oropharynx.

12. Reproductive complications in CD

Females with untreated CD may have multiple complications in relation to reproductive problems. They may have late menarche, recurrent miscarriages, infertility, preterm delivery and low birth weight. These patients may directly presented with these problems and do not have any gastrointestinal issues. All these issues can be resolved with gluten free diet [81].

Males with CD also have infertility, characterized by sperm dysmotility and morphological changes. They may also have androgen resistance leading to infertility. All these can be resolved with gluten free diet [82].

13. Cardiac complications in CD

Autoimmune myocarditis and idiopathic dilated cardiomyopathy are associated with CD, though the prevalence is 5%. Not all patients have gastrointestinal symptoms but almost all of them iron deficiency anemia. These patients responded on gluten free diet with or without immunosuppressive therapy [83].

These patients also have strong association with ischemic heart disease as well.

14. Autoimmune diseases in CD

CD is closely associated with other autoimmune illnesses, like type 1 diabetes mellitus and autoimmune thyroiditis. Type 1 diabetes mellitus and CD has strong genetic association with HLA-DR3, HLA-DQ2, and HLA-DQ8 [84]. Because of the same genetic association, they share same pathogenesis of tissue damage from autoimmunity or intolerance to dietary antigen. The patients with HLA-DQ2 also have raised IgA autoantibodies to tissue transglutaminase and thus likely to have CD with

type 1 diabetes mellitus. Although age of onset of diabetes mellitus is not dependent of CD, neither it triggers the autoimmunity leading to it. Whether a gluten free diet helps in improvement of diabetes mellitus is not clear yet.

There is increased incidence of autoimmune thyroiditis among patients with CD, and hypothyroidism is more common. Association of CD, autoimmune thyroiditis and type 1 diabetes mellitus is part of polyglandular autoimmune syndrome type 111.

15. Liver disease in CD

CD may be associated with nonspecific mild chronic elevation in serum aminotransferase levels. AST ranges from 29 to 80 while ALT from 60 to 130. These increased transaminases may get normalize with gluten free diet. Patients with CD may also have severe liver disease including congenital liver fibrosis, massive steatosis, and progressive hepatitis of unknown origin [85]. There is also an association of primary biliary cirrhosis and primary sclerosing cholangitis with CD.

16. Skin manifestation in CD

Dermatitis herpetiformis in CD is the commonest and pathognomonic skin lesion. It presents usually on external surface in grouped in the form intensely pruritic papules and vesicles. The diagnosis is confirmed on histology by the demonstration of granular IgA deposits along the non-affected subepidermal basement membrane [86]. Similar to CD they have increased antibodies against tissue transglutaminase IgG. Dermatitis herpetiformis and CD, are associated with HLA-DQ alpha beta heterodimers, and may have association with other autoimmune illness as well. It responds well on gluten free diet though requires longer duration.

Patients with CD, also have increased incidence of atopic dermatitis as compared to general population [87].

17. Mortality in CD

There is an increased mortality in patients with CD, due to severe clinical course but as a whole the data is inconclusive. There is twofold rise in death especially in severe disease [88]. The increased mortality is mostly associated with malignant and cardiovascular diseases [89].

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Celiac disease (CD) occurs in about 1% of people worldwide. Diagnosis rates are increasing due to a true rise in incidence, rather than increased awareness and detection. CD affects genetically susceptible individuals who are triggered by the ingestion of gluten. The disease has many clinical manifestations, ranging from severe to minimally symptomatic or non-symptomatic presentations. Diagnosis requires the presence of duodenal chronic inflammation, and most patients have circulating antibodies against tissue transglutaminase. Our understanding of the basic and clinical aspects of CD increases, which is as a major health problem of almost global occurrence. Case finding, distinguishing CD from other gluten-sensitive conditions, better care, and balanced use of resources are the current challenges.

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