Development of Celiac Disease; Pathogenesis and Strategies to Control: A Molecular Approach

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Abstract

Celiac disease (CD) is an intestinal chronic disorder with multifactorial etiology resulting in small intestinal mucosal injuries and malabsorption. Trigger from gluten and related cereal proteins, HLA-DQ2/DQ8 molecules and autoantibodies to tissue transglutaminase, are essential to precipitate the disease. Genetic, dietary and immunological factors explain geographically regional differences in CD. Specific anchoring sites in DQ2/DQ8 peptide binding motifs show affinity to tTG deamidated peptides of gluten and present to gluten restricted T cells to form celiac lesions. The present article reviews the causes, molecular details of CD development, strategies to control and significance of probiotics in reducing the gluten burden in CD.

Keywords: Celiac disease; Gluten; Transglutaminase; Villous atrophy; HLA DQ2/DQ8; Gluten free diet

Abbreviations: CD: Celiac disease, tTG: tissue Transglutaminase; HLA: Human Leukocytic Antigen,

Introduction

Celiac Disease (CD) is a form of chronic enteropathy affecting the small intestine in genetically predisposed individuals and is precipitated by the ingestion of gluten containing foods. It is also referred as gluten sensitive enteropathy, celiac sprue and non tropical sprue. Many a times CD was mistaken with tropical sprue for overlapping symptoms. But CD can be clearly differentiated from this on the basis of serological tests for the detection of auto antibodies generated in response to gluten ingestion and small bowel biopsy. Moreover, CD is mainly associated with type I diabetes mellitus, auto immune thyroiditis, and chronic liver disease compared to tropical sprue [1]. The disease is characterized by severe immune mediated damage to the small bowel, typically involving chronic diarrhea, abdominal bloating and distention, weight loss, iron deficient anemia, malnutrition and metabolic bone disease [2,3]. Assessment of Indian CD children for clinical, nutritional and pathological characteristics showed the symptoms of vitamin deficiency, abnormal fat excretion, delayed puberty and also altered histology in the small bowel such as partial and subtotal villous atrophy with hyperplasic crypts, increased intra epithelial lymphocytes and mononuclear infiltration in the lamina propria [4]. Not only typical symptoms but some atypical symptoms like enamel defects in adult celiacs have also been reported and was obvious since gliadin and gluten proteins shared common antigenic motifs [5]. Recently several studies agree that, atypical presentation of CD has become more common compared to classical presentation. This may be attributed to the factors such as, onset of symptoms with age, early and late diagnosis which correlates with the dietary practices, geographical distribution and socio-economic aspects [3,5,6].

Earlier, it was thought that prevalence of CD is high in western population. But recent studies showed that CD has become worldwide in its distribution. Though, accurate statistics of CD has not yet been determined since its diagnosis follow iceberg model. However, World Gastroenterology Organisation (WGO) data states that the prevalence of CD in a healthy adult population varies between roughly 1 in 100 and 1 in 300 in most parts of the world and female to male ratio was 2:1. Serological diagnosis (anti-gliadin, anti-endomysium and anti-transglutaminase antibody assays) of CD in Middle East, North Africa, and India showed that the prevalence of CD in these regions is almost same as western countries. In these regions, prevalence of CD at-risk population ranges between 3% and 20% and in people with type I diabetes, the prevalence was nearly 3-5%. In USA, the prevalence of CD in first and second degree relatives of CD patients was found to be 4.5% and 2.5% respectively. Whereas in Europe, prevalence of CD is between 0.5% and 1% [7-9]. Present review aims to understand the mechanism of development of CD, causes and existing strategies of treatment. This would perhaps help to take possible course of action in surmounting the lacuna in existing therapies of CD treatment in the near future.

Causes

Presence of Human Leukocytic Antigens mainly HLA-DQ2 or HLA DQ-8 molecules, trigger from gluten protein of wheat and related cereal, and the generation of circulating autoantibodies to tissue transglutaminase (tTG) are essential to precipitate the disease [10,11]. Apart from this, several environmental and immunological factors such as ingestion of gluten rich diet to infants, occurrence of certain gastrointestinal infections also increases the risk of CD. However, prolonged breast feeding and delayed ingestion of gluten delays the onset of CD in early childhood [4]. It was corroborated by a study of Swedish children, where the risk of CD in early childhood has shown to be reduced when gluten is ingested to infants while they are still being breast fed [12]. However, not always the late introduction of gluten in diet reduces the risk of CD in children. Children not exposed to gluten and related proteins containing diet till seven months were exhibited slightly increased hazard of celiac disease autoimmunity compared to those children who were exposed to such diet earlier to four months [13].
Mechanism

Human Leucocytic Antigen (HLA) - DQ2 and DQ8 in celiac disease: CD is one of the HLA-linked disorders. Two gluten/gliadin peptide presenting HLA-DQ molecules namely DQ (α1*0501, β1*02)/DQ2 or to a lesser extent DQ (α1*03, β1*0302)/DQ8 which are present on antigen presenting cells are considered as principal genetic factors responsible for CD. HLA DQ2 heterodimer is prevalent and present in 90% of patients with CD. HLA- DQ2 molecules can be expressed in cis form or in trans form [14]. DR3 haplotype exists in cis form; where HLA-DQ alleles HLA-DQA1*0501 encode α chain and HLA-DQB1*0201 encode β chain of the dimer. A1-B8- DR3 is a classical Caucasian haplotype whereas as A26-B8-DR3 and Ax-B21-DR3 are characteristic haplotypes of CD in Asian Indians [15]. In case of trans form, DR7 haplotype carry closely related DQB1*0202 allele that encodes β chains. DR5 haplotype (HLA DR5 is a broad antigen serotype that is further split into HLA DR11 and DR12 antigens) with HLA-DQA1*0501 allele on other chromosome encodes α chain, then both encoded α and β chains unite to form CD associated dimer (Figure 1). In case of HLA-DQ8, α and β chains are encoded by HLA-DQA1*03 and HLA-DQB1*0302 respectively [16,17].

Gluten and tissue transglutaminase in celiac disease: Wheat gluten majorly contains glutenin and gliadin protein fractions. Based on the sequence, gliadin protein fractions are subdivided into α, γ, ω gliadins. Compared to gliadin, gliadin fractions are more immunogenic because they are rich in glutamine and proline residues and possess epitopes which exhibit affinity towards DQ2/DQ8 molecules on antigen presenting cells and are accepted solely by gliadin reactive T cells which are found only in the intestinal mucosa of persons positive for CD [18]. However, a gliadin peptide epitope capable of activating DQ8 restricted T cell proliferation with residues GGYYPSPQ楚 has also been identified [19]. Gut T cells recognized three gliadin derived DQ2 restricted epitopes: DQ2-a-I-gliadin, DQ2-a-II-gliadin, DQ2-γ-I-gliadin and two DQ8 restricted DQ8-a-I-gliadin and DQ8-I-glutenin epitopes one each derived from gliadin and glutenin respectively [18,20-22].

High proline content enables gluten peptides resistant to gastric, pancreatic and intestinal protease activity and enhances their survivability in the small intestine. Either by epithelial transcytosis or by increasing the epithelial tight junction permeability, gluten peptides reach lamina propria and undergo tissue transglutaminase (tTG) mediated deamidation [11,23] (Figure 2). tTG catalyzes selective

### Figure 1: Illustration of the expression of HLA DQ 2 in cis and trans forms:
In cis form, α and β chains of DQ2 are expressed by HLA DQA1*0501 and HLA DQB1*0201 alleles of DR3 haplotype respectively. Whereas in case of trans form, HLA DQA1*0501 allele of DR5 haplotype encodes α chain and DR7 haplotype with DQB1*0202 allele on other chromosome encodes β chain. Then these two chains unite to form HLA DQ2 molecule on Antigen Presenting Cells.

### Figure 2: Illustrating mechanism of CD pathogenesis:
Ingestion of proline rich gluten by genetically susceptible individual results in the generation of gluten peptides. Since, these peptides are resistant to brush border proteases, they enter lamina propria and undergo crosslinking and deamidation by ITG. Deamidated peptides possess more immunostimulatory epitopes and are present to gluten reactive population of CD4 T cell by HLA DQ2/DQ8 heterodimer expressing APCs. Further, activated T-cells produce auto antibodies and other immuno components that lead to tissue damage which includes increased permeability, dysfunction of intestinal tight junction, infiltration of IELs, flattening of villi, inflammation and malabsorption as in late phase of pathogenesis of CD. (This figure is created by authors and team based on the data collected from references cited in the present review article).
crosslinking or deamidation of protein-bound specific glutamine residues whereas acidic pH in the stomach results in random deamidation of numerous peptides [10]; However, microorganisms are also known to produce transglutaminases (mTG) and are used in food industry to improve the quality and texture of the food and they also can generate T cell reactive gluten epitopes by deamidation [24]. Hence, it is advisable that not to use mTG in the food formulations for CD patients.

Deamidated peptides and HLA DQ2/DQ8 binding motifs: The conversion of glutamine to glutamic acid residues by deamidation would result in relatively large numbers of negatively charged residues in gliadin peptides that bind to HLA-DQ2 or HLA-DQ8 molecules with great affinity. Moreover, most of the DQ2 specific gliadin epitopes recognized by T cell are tTG targeted residues [23]. Since glutenins and gliadins of wheat contain high amount of glutamine and proline, they serve as preferred substrates for TG2. For instance, celiac super antigen, a 33mer peptide from α2-gliadin which has been considered as model peptide in several preclinical studies, contains amino acid sequences that can be deamidated by TG2 [25]. In addition to deamidation, affinity between gliadin epitopes and peptide binding motif of HLA DQ2/ DQ8 has also been important. Pool sequencing analysis has helped to understand the requirements for a peptide to bind HLA DQ2/8 motifs. Interim, it has also been shown that though α and β chains of peptide binding motifs of both HLA DQ2 and HLA DQ8 have 91% sequence identity, they differ in their preference for deamidated gluten peptides. The subtle changes in the sequence of α and β chains of binding motifs can be ascribed to the difference in their preference for binding peptides. DQ2 peptide binding pocket prefers bulky hydrophobic polar residues at p1 anchor site, aliphatic residues at p4, whereas β71 Lys residue influentially allows negatively charged amino acid residue to p6/p7, while bulky hydrophobic negatively charged residues are preferred at p9 anchoring sites. In case of DQ8 binding pocket, negatively charged amino acids are preferred at p1 due to the close-knit of α 53 Arg, while aliphatic amino acids at p4, p6/p7 and negative or polar at p9 anchor sites in the groove. This preference may be due to the side chains of the residues in the binding grooves of the DQ2/DQ8 motifs (Figure 3a and 3b) [18,26-29]. Peptide binding groove has been occupied by a HLA Class II associated invariant chain peptide (CLIP) with conventional sequence MR-MA-PLLM. Mass spectrometry study revealed that 22 variants of invariant chain derived peptides are associated with HLA-DQ2 and majority of them do not possess the conventional sequence. Instead, they have an alternative partly coinciding sequence TPLLMQALPM and the nine amino acid binding core sequence of DQ peptide binding motif with PLLM-QALPM sequence. The alternative CLIP peptide has better HLA-DQ2 binding properties compared to conventional CLIP and it had also been found to be related with HLA-DQ8 [30].

Activation of gluten specific CD4 T cells: tTG deamidated gluten residues presented by HLA-DQ2 and DQ8 molecules turn on the gliadin reactive CD4 T cells which is crucial for CD pathogenesis. Although, several studies tried to explore the mechanism of T cell activation in CD, further research at molecular level has to be undertaken to elucidate the biochemical mechanisms. Abundant CD163+CD1C+ dendritic cells with less CD103+ and CD1C+ cells have been found in the celiac lesion sections of duodenal biopsies [31]. Then, the conception was drawn from this study that a distinct group of subpopulations of antigen presenting cells were present in the duodenal mucosa and they activate gliadin restricted T cells. This was also supported by another study, where deamidated peptide specific T cell proliferation was observed when T cells were mixed with deamidated 33mer peptide incubated with monocyte derived dendritic cells [25]. Moreover, HLA class I and killer cell immunoglobulin like receptors (KIRs) interactions

Figure 3a and 3b: Peptide binding grooves of HLA- DQ2 and DQ8: Schematic illustration the peptide binding groove of HLA-DQ2 and HLA-DQ8 molecules and requirements of the peptides to bind respectively. (This representation is created by authors and team based on a compiled data from references Johansen et al. [26]; Godkin et al. [27]; van de Wal et al., [19]; Mazzarella et al., [28]; Costantini et al., [29]).
modulated T cell function. Over expression of KIR3DL1 which serves as a marker for the augmentation of natural killer cells reprogrammed T cells which are observed in the biopsy samples from the untreated CD patients compared to those with GFD and non CD individuals [32]. The T cells after accepting the gluten residues by HLA DQ2/8 further trigger humoral mediated immune pathway and activates B cells to produce antibodies against gluten peptides, tTG and also produces array of cytokines: interferon-γ, interleukin (IL)–1β, tumor necrosis factor-α, IL-6 and IL-8 at higher levels [33]. These cytokines make the enteric lymphocytes as cytotoxic cells and affects the enteric cells resulting in inflammation (Figure 2). However, gluten induced T cell triggered immune pathogenesis also affects the synthesis of useful immune components. For instance, T cell secreted IL17 and IL22 are essential for the regulation of intestinal mucosa but in a study of gluten sensitive rhesus macaques, their level decreased markedly and resulted in adverse mucosal structure. However, withdrawal of gluten from diet increased the secretion of cytokines IL17 & IL22 with improved mucosal structure [34].

Thus, understanding pathogenesis at molecular level allows various strategies to treat CD at different levels more effectively.

### Possible Therapies for CD

Several non-dietary strategies have been suggested to treat CD. These novel strategies provide promise of alternative, adjunctive treatment options but also raise important questions regarding safety, efficacy and monitoring of long term treatment effect. Moreover, the responses of CD individuals to these strategies are found to be not so satisfactory. In contrast to non dietary strategies, gluten free dietary therapies are found to be safe without much side effects and remains mainstay of CD treatment. This review focuses on the gluten free diet and importance of intense expert dietary counseling for all patients with CD.

#### Permeability inhibition

To induce toxicity, the gluten peptides must cross intestinal barrier. Proper functioning of intestinal tight junction (ITJ) decreases the permeability of intestine barriers to gluten fractions and prevents the initiation of gluten induced pathogenesis. Binding of gliadin to CXCR3, a chemokine receptor leads to MyD88 dependent zonulin release that accounts for intestinal tight junction dysfunction with increased permeability, exposing the submucosal cells to immunogenic peptide induced toxic effects (Figure 4) [11]. Zonulin is an analogue to zonula occludens toxin (Zot), an enterotoxin expressed by Vibrio cholerae that reversibly opens TJ’s. The amino termini of zonula is similar to Zot and share a octapeptide (GGVLVQPG) motif essential for intestinal receptor binding [35]. Expression of CXCR3 was high in intestinal tissues of CD individuals compared to non-CD controls and returned to baseline levels during the remission of disease after implementing gluten free diet [36]. These findings suggest that maintenance of ITJ is essential in preventing the initial onset of CD pathogenesis and in recent years researchers are focusing on drugs that target the functioning of ITJ. In an in vitro study, Larazotide acetate (LA), a tight-junction regulator peptide prevented the opening of intestinal epithelial TJ. The safety, tolerance and pharmacokinetic effect of LA was carried out in celiac disease subjects challenged with gluten in a randomized, double blinded, placebo- controlled study. The patients were given either 12 mg doses of LA or placebo. After an acute exposure to gluten, patients treated with LA did not show any increase in the intestinal permeability while placebo patients showed 70% increase in intestinal permeability. In addition, frequent gastrointestinal symptoms and increased interferon-γ concentrations were observed in placebo (57%) compared to LA (29%) treated subjects [37]. Oral administration of this drug before each gluten ingestion would help the patients to include gluten in their diet which makes their life better but still there is a need of further improvement in the efficacy of the drug in reducing gastrointestinal symptoms.

#### tTG blockage

Once the gliadin and glutenin peptides cross the intestinal barrier, they encounter an endomysial auto antigen in CD namely tTG. As discussed earlier, tTG deamidates the gluten peptides and enhance their affinity towards HLA DQ 2/8 binding motifs. Inhibiting the tTG activity may hamper the development of CD pathogenesis by preventing the activation of gluten reactive T cells. Therefore, tTG blockage is one of the important strategies to be considered for the effective management.

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**Figure 4:** Elucidating the mechanism of action of Larazotide acetate in preventing intestinal permeability. Larazotide acetate is a intestinal tight junction regulator peptide that prevents the opening of intestinal epithelial tight junctions and prevents the crossing of immunogenic gluten peptides across the intestinal barrier and prohibits the onset of disease pathogenesis at initial stages. This could be considered as an efficient strategy in managing Celiac Disease.
of CD. Autoantibodies against tTG can block its catalytic activity and reduce the deamidation of glutamine residues. In an in vitro and in situ study serum purified IgG and IgA antibodies from celiac patients showed dose dependent inhibition of tTG activity [38]. However, monoclonal antibody CUB7402 inhibited tTG activity expressed in Madin–Darby Canine Kidney (MDCK) tTG cells in vitro and in situ study. The addition of anti tTG CUB7402 and single chain monoclonal antibodies from intestinal mucosa of celiac patients decreased tTG activity in umbilical cord sections. Whereas anti tTG antibodies from non CD patients failed to inhibit tTG activity indicating that they do not specifically bind the epitopes of tTG present in substrate binding site. The results showed that anti tTG antibodies from CD patients could inhibit tTG activity partially. Although, anti tTG antibodies inhibit tTG activity when targeted to active site at higher concentrations, residual enzyme activity is sufficient for pathogenesis [39]. In this regard, further research is required to screen for the therapeutic agents that completely block tTG.

**Corticosteroids**

At present, there is no single drug or treatment available to mitigate the symptoms of CD. A combination of several strategies has been implemented in treating the complications of CD. Administration of budesonide is one among such strategy to alleviate the burden of CD. Budesonide is one of the corticosteroid drugs which reduce diarrhea, inflammation, tissue damage in intestine. Effectiveness of budesonide in treating adult CD was assessed. Where, one group of patients with malabsorption was given only gluten free diet (GFD) and the other was given gluten free diet with 6 mg of budesonide daily. After 4 weeks, patients treated with both GFD and budesonide showed better health compared to receiving gluten-free diet alone. The following discussion would help to outline the possible explanation for the mechanism of action of budesonide in CD individuals. Gliadin peptides are responsible for the expression of HLA-DR in crypt enterocytes. In in vitro studies, budesonide reduced the expression of HLA-DR elicited by gliadin-derived peptides. The expression of cyclo-oxygenase (COX)-2 and intercellular adhesion molecule (ICAM)-1 in the lamina propria was also reduced in patients treated with budesonide. Reduced expression of COX-2 (pharmacological inhibition of which reduces inflammation and pain) and ICAM-1 (ligation of which produces pro inflammatory effect), in the lamina propria of biopsy specimen of celiac patients elucidated the effects of budesonide in reducing symptoms [40].

Moreover, in an open labeled non controlled study, immunomodulatory medications like systemic steroids and azathioprine with budesonide are used to treat refractory CD. The patients were given 9 mg of budesonide per day for a range of 1 to 36 months. Based on the severity of the symptoms, prednisone and azathioprine were administered along with budesonide in some patients. 51% of patients were administered with budesonide only. Among which 80% showed complete response and 20% showed very poor response. After budesonide therapy, numbers of bowel movements in the subjects were decreased drastically and 86% of the patients under study showed improvement in the disease recovery [41]. Azathioprine is an immunosuppressant and used with corticosteroids to treat many auto immune diseases. However, studies revealed that it is carcinogenic to human [42]. Azathioprine and prednisone are used to treat type I and type II refractory celiac disease (RCD). The combination of these drugs found to be effective in treating RCD type I but in case of type II RCD, high risk of development of enterocyte associated T-cell lymphoma is alarming the use of azathioprine as immunosuppressive drug in humans [43]. Although, many studies suggested the use of budesonide in effective management of refractory CD, experience of its use in CD is however limited. Since budesonide has its efficacy in terminal ileum and right colon, available oral budesonide is predominantly used to treat illness of the lower intestine which is unsuitable to treat CD since it primarily involves inflammation at proximal small intestine and rarely entire small intestine. Formulation of new generic budesonide drug that targets the upper intestine is of interest in treating CD.

**Sequestering polymers**

Another possible strategy to manage celiac disease is the use of sequestering polymers such as Polyhydroxyethylmethacrylate-co-styrene sodium sulfonate (P(HEMA-co-SS)) is a non absorbable drug that targets the upper intestine is of interest in treating CD. At present, there is no single drug or treatment available to mitigate the symptoms of CD. A combination of several strategies has been implemented in treating the complications of CD. Administration of budesonide is one among such strategy to alleviate the burden of CD. Budesonide is one of the corticosteroid drugs which reduce diarrhea, inflammation, tissue damage in intestine. Effectiveness of budesonide in treating adult CD was assessed. Where, one group of patients with malabsorption was given only gluten free diet (GFD) and the other was given gluten free diet with 6 mg of budesonide daily. After 4 weeks, patients treated with both GFD and budesonide showed better health compared to receiving gluten-free diet alone. The following discussion would help to outline the possible explanation for the mechanism of action of budesonide in CD individuals. Gliadin peptides are responsible for the expression of HLA-DR in crypt enterocytes. In in vitro studies, budesonide reduced the expression of HLA-DR elicited by gliadin-derived peptides. The expression of cyclo-oxygenase (COX)-2 and intercellular adhesion molecule (ICAM)-1 in the lamina propria was also reduced in patients treated with budesonide. Reduced expression of COX-2 (pharmacological inhibition of which reduces inflammation and pain) and ICAM-1 (ligation of which produces pro inflammatory effect), in the lamina propria of biopsy specimen of celiac patients elucidated the effects of budesonide in reducing symptoms [40].

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**Sequestering polymers**

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efficiently to gliadin compared to other proteins and found safe at high dose of 15 mg/day in Balb/c mice. It also reduced gliadin induced cytotoxicity on primary cultures of human differentiated enterocytes and hypersecretion in HLA-DQ8/HCD4 transgenic mice [45]. The ability of (P(HEMA-co-SS)) in preventing formation of immunogenic peptides of Wheat peptide and barley hordein in HLA HCD4/DQ8 mice was studied. Predominant excretion of P (HEMA-co-SS) in feces was observed after single administration of 150mg/kg co polymers of P (HEMA-co-SS) to Sprague-Dawley rats. Recovery of 98% of the total radioactivity in feces indicated that the polymer did not get absorbed into the blood. In the presence of P (HEMA-co-SS), the formation of 33-mer immunogenic peptide of gliadin was reduced to 50%-70%, HLA-DQ2 restricted T cell epitopes of hordein were reduced to 45%-65% and also reduced the formation of glutenin epitopes by 60%. Because the copolymer induced changes in the conformation of the secondary structure of gluten, gliadin and hordein proteins and entraps to form larger aggregating complexes without allowing the peptides to interact with the enzymes to form immunogenic peptides and excrete out of the body while no signs of toxicity of the polymer were found during the study. When P(HEMA-co-SS) incubated with intestinal biopsy samples from celiac patients, reduction in the secretion of cytokines responsible for inflammation cascade was observed [46]. Further work aimed to assess safety and efficacy of this polymer in human may reduce the burden of celiac disease.

Gluten reduced diet

Although several strategies are in practice to treat CD, nutritional therapy is considered to be more safe, effective and relevant in treating celiac disease without side effects. Nutritional therapy involves life time elimination of wheat, rye and barley from the diet (Table 1). Several studies revealed that follow-up of strict gluten free diet ameliorated the pathogenicity of CD [4]. Gluten content of food can be reduced by breeding less immunogenic varieties of wheat and also by biotechnological approaches using microbial proteases to hydrolyze immunogenic gluten peptides in the diet (Figure 6). Research in probiotics proved the efficacy of several strains of Lactic Acid Bacteria (LAB) in treating metabolic disorders and also colon cancer [47-49].

Since microbial proteases are known for efficient proteolytic activity they are extensively used to reduce gluten in the food for celiac patients. But major problems associated with the use of these proteases include the in vivo efficacy of the microbial or enzyme preparations, efficient delivery against gastric conditions in the stomach, formulation and dosage.

However, VSL#3 probiotic preparation effectively reduced the gluten toxicity during long term wheat flour fermentation. When jejuna biopsies of celiacs were exposed to peptic- trypic digest from VSL#3 fermented dough, there was no increase in the infiltration of CD3+ intraepithelial lymphocytes and zonulin release reduced when biopsy samples were exposed to VSL#3 treated gliadins [49]. Moreover, gluten detoxifying prolyl endopeptidase (PEP) from Myxococcus xanthus was characterized and an enteric coated capsule containing 100 mg of MX PEP with other blended materials was developed. Gelatin encapsulated capsules of MX PEP were enteric coated to protect from gastric environment and to ensure its safe release into duodenal region. In in vitro assessment of enteric capsules, no change in the activity of PEP compared to dry powder before encapsulation [50]. Whereas,

<table>
<thead>
<tr>
<th>List of gluten rich and gluten free foods</th>
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<tbody>
<tr>
<td><strong>Grains with gluten</strong></td>
</tr>
<tr>
<td>Wheat (includes spelt, kamut, semolina, triticale),</td>
</tr>
<tr>
<td>Rye, Barley (including malt)</td>
</tr>
<tr>
<td><strong>Gluten free grains</strong></td>
</tr>
<tr>
<td>Rice (white, brown, wild, basmati, jasmine)</td>
</tr>
<tr>
<td>amaranth, buckwheat, corn, millet, quinoa, sorghum, tef (an Ethiopian cereal grain), montina (Indian rice grass), oats, ragi, job’s tears</td>
</tr>
<tr>
<td><strong>Sources of gluten-free starches</strong></td>
</tr>
<tr>
<td>Tubers: arrowroot, jicama, taro, potato, tapioca (cassava, manioc, yucca)</td>
</tr>
<tr>
<td>Legumes: chickpeas, lentils, kidney beans, navy beans, pea beans, peanuts, Soybeans</td>
</tr>
<tr>
<td>Nuts: almonds, walnuts, chestnuts, hazelnuts, cashews</td>
</tr>
<tr>
<td>Seeds: sunflower, flax, pumpkin</td>
</tr>
</tbody>
</table>

| Table 1: List of gluten rich and gluten free foods. (Source: celiac disease foundations, Gluten Intolerance Group of North America and Whole Grains Council). |

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**Figure 6**: An overview of possible approaches to reduce gluten in diet for celiac patients.

The gluten in the diet for celiac patients can be reduced mainly by three approaches. 1) Fermentation of gluten foods with probiotics or sourdough bacteria helps to reduce the formation of immunogenic peptides and also improves the nutritional quality of the food. 2) Administration of either capsules containing gluten degrading proteases (i.e. oral supplementation) or the potent probiotic formulation that proven to degrade gluten can be consider as a strategy to reduce gluten burden in diet for celiac patients. 3) By breeding less immunogenic wheat, rye and barley also gluten content of the foodstuffs for celiac patients can be reduced.
prolyl endo pro tease from Aspergillus niger, was found to be active at
stomach pH 2 and resistant to pepsin degradation [51]. As it resists
gastric conditions without any encapsulations and adjuvants, further
evaluation of the enzyme for its direct use as oral supplementation may
serve as reliable, affordable potential therapy for celiacs. However, in
an another in vivo study after ingestion of gluten pretreated with ALV003
(preparation of mixture of highly specific glutanases) to CD patients,
gluten specific T cell response was reduced compared to placebo
 treated gluten [52]. These studies gave an idea of using microbial
proteases as a potential tool for the treatment of CD either by oral
pro tease supplementation or by development of gluten reduced food
stuffs. Mean while it is well documented that the patients treated with
 gluten free diet (GFD) for long time showed vitamin deficiency [53].
Therefore, for patients under GFD, vitamin supplementation at regular
intervals has been recommended. Alternatively, fruits, vegetables and
other food stuffs which are gluten free may also be consumed as a
source of vitamins and other micro nutrients (Table 1).

However, there is a possibility of cross contamination of gluten
free food with gluten at various levels from harvesting fields to
transportation, processing and marketing. And it is also difficult for
patients to eliminate gluten rich food from their diet for life time. To
overcome these FAO/WHO regulations helped CD patients by defining
some standards and specifying quality factors for gluten free foods.
FAO/WHO defines gluten-free food products as that the total content of
 gluten in foods which are made from non gluten ingredients shall
not exceed 20 mg/kg and foods consist of one or more ingredients from
wheat (i.e., all Triticum species, such as durum wheat, spelt and kamut),
rye, barley, oats or their crossbred varieties, which have been specially
processed to reduce the gluten content to a level above 20 up to 100 mg/
kg in total, based on the food as sold or distributed to the consumer.
It also specifies that “Gluten-free” foodstuffs, substituting important
basic foodstuffs should supply approximately the same amount of
vitamins and minerals as the original foodstuffs they replace and the
product shall be prepared with special care under Good Manufacturing
Practices (GMP) to avoid contamination with prolamin.

In India, CD population has been raising and is found more
common in northern states where wheat is the staple food. Increased
prevalence of CD in recent years parallels with the increased intake of
 gluten rich food [54]. Due to diabetes, even for south Indians, wheat
has become a staple food in recent times. This may also contribute to
a rise in the number of celiacs in India in near future. Accordingly the
meetings of Indian Task Force for Celiac Disease aimed to focus on
research on the prevalence of CD, diagnosis, legislation for labeling
of gluten free products and subsidies on such foods to make them
affordable to celiacs. Moreover there is also an elevated risk of CD in
first degree and second degree relatives of celiac [55] and hence they
need to be taken care of their diet. Even though class I and class II
HLA haplotypes are necessary factors, but they are insufficient for the
development of CD in the absence of gluten load. As a precaution it is
advisable to the first and second degree relatives of risk group to follow
strict gluten free diet to escape from celiac disease.

In developed countries like United Kingdom, New Zealand and
United States and also in other provinces like Island of Jersey, where
prevalence of CD is comparatively high, their government subsidizes
the gluten free food products. But in developing countries like Middle
East, some parts of Europe, Asia, there is no such provision for CD
patients. Moreover, in these countries, rate of diagnosis is below the
water level in an iceberg model for CD diagnosis [56] and there is an
 elevated risk of CD in first degree and second degree relatives. Hence
it is difficult for patients in these regions to get suitable diet guidelines
and also hard to get gluten free food products. If it is available, it costs
high and common people cannot afford to have it.

Summary

Celiac disease, a chronic enteropathy is worldwide in its
distribution. HLA DQ binding motifs, gluten and other environmental
factors are involved in disease development. Deamidation of gluten
peptides by tTG, antigen presenting cells with HLA DQ2/DQ8 binding
motifs, gluten restricted T cells and generation of auto antibodies
are central components in mechanism of pathogenesis. Research on
molecular aspects of disease development suggested many strategies
like use of corticosteroids to alleviate the symptoms of CD, sequestering
polymers that prevent the enzymatic action on gliadins responsible for
the formation of immunogenic peptides, permeability inhibition by
Larazotide acetate, autoantibodies against tTG to block its catalytic
activity. However, these methods failed to give complete solution to
CD. Only available efficient treatment is nutritional therapy which
involves the strict exclusion of gluten for life time. Since CD accounts
for permanent gluten intolerance, early diagnosis is critical for better
disease management. However, CD diagnosis follows iceberg model
because of its overlapping symptoms with other gastrointestinal
disorders, its awareness at the level of primary health centers by
pediatricians must be encouraged. Celiac disease occurs as the result
of interplay between the genetic tendency of certain individuals to
develop disease and excessive ingestion of gluten. Predisposition can
be controlled by breeding less immunogenic varieties of wheat and by
biotechnological approaches. Proteolysis by probiotic isolates has
been suggested as a new tool for food processing for celiac persons.
Therefore more emphasis must be given to improve gluten hydrolyzing
probiotic isolates. Such that pre digestion by glutanases to produce
 gluten reduced wheat and other related cereal foods and oral enzyme
supplementation of glutanases would be a promising strategy for celiac
prone individuals, perhaps a safe and cost effective method.

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Declaration of Conflict of Interest

Authors declare that ‘There is no conflict of interest among the authors or
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