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Gluten-Free Diet: An Approach to Reconcile Nutritionally and Technologically Adequate and Safe Food for Celiac Patients

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Abstract: ***The rise in incidence of celiac disease (CD), a chronic multisystem autoimmune disorder associated with intestinal mucosa inflammation, intestinal villous atrophy and other indicators has increased in recent years. Study for pathogenesis and molecular mechanisms are the key aspect of nutritionally balanced gluten-free diet. Currently, gluten-free diet is the sole efficient treatment for celiac subjects. This may lead to ramifications corresponding to nutritional imbalances and deficiencies. Role of oats as source of minerals, proteins, vitamins and fiber and their use in gluten-free diet to compensate for the nutritional deficiencies is highlighted while their consumption with respect to safety for celiac patients is debated upon. Nutritional quality of pseudo-cereals and minor cereals is focused in the light of dietary advices and nutritional properties as well as the effort being made to formulate new generation of cereals. Finally, few strategies which make use of biotechnological and enzymatic methods to detoxify the dietary gluten which can be used in developing cereals having nutritional and baking qualities are described.***

Keywords: Celiac disease, gluten-free diet, autoimmune disorder, pathogenesis, molecular mechanisms, safe food.

1. Introduction

Celiac disease is a food intolerance disorder related disorder that, regardless of being under-analyzed, is a standout amongst the most successive ceaseless gastrointestinal issue. It creates in hereditarily inclined people in whom unidentified natural elements (diseases, changes in microbial vegetation, and so forth) can trigger bias to gluten contained in wheat, grain, rye and oats [1], [2]. Gluten is an unpredictable mixture of proteins which are called as prolamins. The prolamins in wheat are named as gliadins and glutenins. An average typical for the following proteins is the closeness of various proline and glutamine stores, which makes them impenetrable to gastrointestinal assimilation and are introduced to deamination by transglutaminase.

Celiac illness retards when the patients are exposed to gluten-rejection diet regimen, and the patients backslide when gluten is reinculcated in eating routine [1], [2]. Gluten has numerous unique attributes that support its utilization in different nourishment items. Since a lot of gluten is created amid starch, it has a moderately less cost. This can end up being tricky for individuals on a gluten-free diet, as the gluten proteins can be developed in unforeseen origin, for example, meat, fish or etc. Due to these elective ways to deal with the GFD are effectively looked for, which incorporate the scan for and improvement of new grains or gluten with low immunogenic substance [3].

1.1 Macronutrients

Many attempts demonstrate that gluten free products regularly have more carbohydrate and lipid content as compare to their gluten accommodating counterparts [4], [5]. The analysis of the wholesome structure of GF breads showed that they are rich in starch and glycemic and low in protein while high fat content [6]. With respect to composition of lipid, it was demonstrated that financially accessible GF bread rolls have rich saturated fat contrasted with the gluten containing counterparts [7].

1.2 Micronutrients and minerals

It is observed that GFPs have low amount of folates, vitamin B and the folates and they are not strengthened when compared to their gluten containing parts. Two reviews were directed on US monetarily accessible GFPs. The creator broke down the folate, vitamin B and B iron organization of these items and contrasted them and the arrangement of their gluten containing parts, knowing that GFPs were fundamentally lower in vitamin B, iron and folates.

1.3 Dietary fiber

A few reviews have detailed that GFD is related with a lower intake of dietary fiber than a standard gluten containing diet. A review led to the understanding that the eating regimen of CD patients which were on GFD had low fiber consumption [8].

2. Celiac Disease

Celiac disease is termed as a chronic genetic autoimmune disorder caused due to enduring intolerance to a protein called gluten in hereditarily prone characters. It is not only a common enteropathy induced by food in humans but is also similar to multisystem immunological syndrome [9], [10]. Patients of pediatric age are sometimes symptomless, though a number of symptoms are presented by gluten intolerance with gastrointestinal manifestations being the major one along with weight loss, chronic diarrhea and in some cases, anemia. Symptoms of CD vary and greatly depend upon factors like patient's age, extent and the duration of extra intestinal symptoms, however, a major

percentage of patients however exhibit symptoms like bone fractures, skin lesions and infertility [11]. In the intestinal mucosa of subjects, damaged broad villi and chronic inflammatory lymphoepithelial cell infiltrate was supported by accurate clinical report of CD [12]. Later the association of gluten response and abnormalities of the intestinal mucosa of celiac patients was described [13]. CD prevalence (5.66%) is highest in Sahrawi as compared to much lower prevalence in other regions [14].

2.1 Pathogenesis and molecular mechanism

Pathogenesis of CD involves a number of factors including genetic, immunologic and environmental factors as shown in Figure 1, along with some molecular mechanisms including interactions between intestinal epithelium, gluten peptides and autoantigens [15].

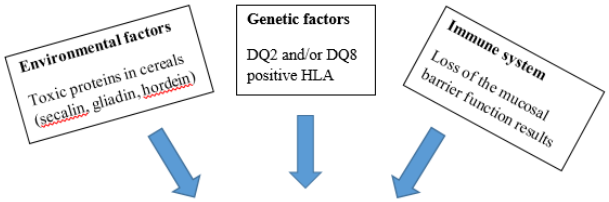

Figure 1: Factors involved in pathogenesis of celiac disease.

Gluten and its domains are resilient to pancreatic and gastric degradation as well as are resistant to proteases present in the intestinal mucosa which results in larger sized peptide fragments accumulation (33mer sequence). Molecular mechanisms and detailed study of degree of toxic effects of different protein fragments revealed that they have the ability to induce damage to the mucous membrane both in vitro and in and display immunogenic characteristics including the stimulation of intestinal T cells and immunitary cells [16]. One of the hypotheses was that the preliminary stages of the CD pathogenesis on the intestinal mucosal epithelium might be due to wheat peptides which were different from those which T cells recognized resulting in villous atrophy of intestines and subsequently into malabsorption and anemia [17].

3. Gluten-Free Diet

The dietary foods in the gluten-free diet (GFD) are made from ingredients free from wheat, rye, barley or their variations while making sure that the level of gluten is not above 20 mg/kg in the food. Though it is nearly impossible to avoid contamination of gluten in GF products that are processed, yet it is a key aspect in processed and natural foods for ensuring food safety. Therefore a safe threshold has been defined by some authors according to whom gluten contamination can be regulated. Food and Drug Administration (FDA) has projected gluten-free as a food which claims of not containing any prohibited ingredient or an ingredient which is from an unprocessed grain for removing gluten and that use of the that specific ingredient should not exceed the safe limit (20 ppm) of the gluten (Standard, 2007). In celiac patients folate, iron and calcium malabsorption is much common, however, some studies stated that following the GFD can overcome these deficiencies, though, GFD consumers show higher levels of sugars, lipids and salt which can compensate for limitations in GFD [18].

3.1 Dietary advices and nutritional properties

Different regions display varied lifestyles and dietary habits, thus making it difficult to specify nutritional recommendations applicable to celiac patients of different regions. Following are described few nutritional properties and dietary recommendations of foods included in GFD to avert malnutrition.

3.1.1 Carbohydrates

Although the grains as carbohydrate source are not common, legumes and seeds are allowed. Pseudo-cereals and minor cereals can be represented as a suitable carbohydrate, minerals, vitamins and dietary fiber source [19]. Table 1 shows the chemical composition of pseudo-cereals [17].

Table 1: Chemical composition (% dry mass) of pseudo-cereals compared to wheat

Component	Amaranth	Quinoa	Buckwheat	Oat	Wheat
Starch	67.3	69.0	67.2	nd	61.0
Protein	15.2	13.3	10.9	13	11.7
Fat	8.0	7.5	2.7	7.5	2.0
Minerals	3.2	2.6	1.59	3.1	1.8

3.1.2 Protein

Intake of protein should be about 15% of the total calories. Animal foods are the major source of protein in GDF. Protein quality and content is much higher in minor cereals and pseudo-cereals as compared to wheat [20].

3.1.3 Lipids

As compared to unprocessed foods, processed GF products have higher levels of *trans* and saturated fatty acids. But pseudo-cereals have the advantage of having higher levels of linolenic acid which is important for coronary heart disease prevention [21].

3.1.4 Dietary fiber

It is a combination of plant constituents and breakdown-resistant molecules. Diets rich in fiber avert many ailments including diabetes, colon cancer and coronary heart disease. In CD patients, (20-35g/d) of fiber intake is advised [22]. Quantities of fiber in different foods derived from plants are mentioned in Table 2 [17].

Table 2: Quantity of fiber in a variety of plant-derived foods

	Fibre (g/100g)
Cereals	
Oat	10.3
Wheat	9.5
Barley	9.2
Teff	8.0
Corn	7.3
Spelt	6.8
Rice	2.8
Pseudo-cereals	
Buckwheat	10.0
Quinoa	7.0
Amaranth	6.7
Fruit and vegetable	0.5-5.0
Nuts	4.0-12.0
Pulses	5.0-18.0

3.1.5 Micronutrients

Deficiency of micronutrients can be compensated by increased intake of vegetables and fruits rich in minerals and vitamins to lower the heart disease and cancer risk and antioxidant compounds protects against oxidative damage [23].

3.1.6 Mineral salts

Minerals salts are essential and include calcium, sodium, chloride, phosphorus, magnesium and potassium along with trace elements (zinc, iron). Vegetables and animal products are important source of minerals. Pseudo-cereals have high mineral content. In minor grains, calcium and iron contents are much higher as compared to those in rice, wheat and sorghum [24].

3.1.7 Vitamins

In order to prevent vitamin deficiencies and its subsequent disorders, sufficient vitamins intake is vital for celiac subjects. Green vegetables and cereals are rich in folic acid (vitamin B9), though it has been found that folic acid is present in GF cereals in significant amounts. B2 and B6 vitamins are also present in pseudo-cereals [25].

3.1.8 Phytochemicals

Polyphenols are a type of phytochemicals, contained in plant-derived foods and gives them distinct nutritional and organoleptic properties and is beneficial to humans. Cereals are demonstrated as a suitable polyphenols source [22].

3.2 Formulation and nutritional value of gluten-free products and recent advances

With respect to nutritional aspect, excluding gluten from diet does not cause much repercussions, however, GFD being restricted to low biological and nutritional value, pose many curbs. Commonly it has low content of essential components and poses a risk of developing obesity and metabolic syndromes [26]. These challenges along with GF products' consumers being discontented due to poor flavor and aroma and low nutritional quality has given way to greater research concerns in recent years. Today, corn and rice flour are being used for gelatinization of starch particles as an alternative to gluten, however, the food prepared would have a high glycemic index which can cause in celiac subjects metabolic syndromes [27]. Uncertainty still prevails that whether non- fortified GF therapy is a proper alternative to the nutritionally well balanced diet. Studies for new generation of gluten-free cereals are still in rounds.

4. Nutritionally balanced diet for celiac patients

4.1 Wheat and barley

One type of wheat, *Triticum aestivum*, is highly utilized in the industrialized world, because of its expanded protein creation and also its strength in cold atmospheres. It has likewise been resolved that the proteins that are harmful for celiac infection reside on gliadin part of wheat [28]. With such an expansive wheat species accessible, a lot of research is centered around the investigation of various wheat species and cultivars as an alternative for strict GFD [29].

4.2 Other cereals and pseudo-cereals

It is outstanding that the high nourishing estimation of gluten containing grains and the viscoelastic system created by the gluten that empowers a phenomenal circulated air through structure in sustenance items. Interestingly, grain based without gluten items are rich in starches and fats, but they lack macronutrients and micronutrients. In outcome, long exposure to GFD can instigate supplements insufficiencies. The consolidation of different fixings/supplements like 3-omega lipids, particular proteins, and so forth is a contrasting option to enhance the nourishing arrangement of without gluten items. It is critical that grains are firmly identified with wheat; rye and grain are viewed as dangerous in light of scientific classification.

4.3 Rice, maize and sorghum

There is prominent increment in the utilization of rice flour for gluten free foods for their hypoallergenic qualities despite its important as a hydrocolloid [30], [31]. Maize is utilized as another option to expound without gluten food items. Celiac patients which are considered stubborn to the GFD treatment enhanced when corn eating routine was recommended [32]. Nonetheless, a few reviews have demonstrated the specific maize prolamins contain amino corrosive successions that look like gluten peptides and cause proteolysis in gastrointestinal tract [33]. Sorghum, dry season and warmth tolerant oat grain that develops in semiarid conditions. About 40% of the sorghum generation is utilized for human sustenance in. Immunological reviews upheld that sorghum may give a decent premise to without gluten sustenance [34].

4.4 Minor cereals

Minor oats, supposed on the grounds that they are less normal and are just developed in a couple of little areas include fonio, teff, millet, teosinte and Job's tears. Fonio can get by in poor soil, for example, sandy and acidic soils and its organization is like that of different millets: constrained

in lysine, however rich in methionine [35]. Teff is an oat customarily developed to make level bread. Millet are little grained, annual cereal grasses. Pearl millet id considered as sustenance utilization as it is comparable with rice flour. Job's tears is normally gluten free, however like different grains it can be polluted during preparation by blending with gluten grains [36]. Figure 2 summarizes, by means of a schematic representation, a proposed approach towards a nutritionally adequate and balanced gluten-free diet [37].

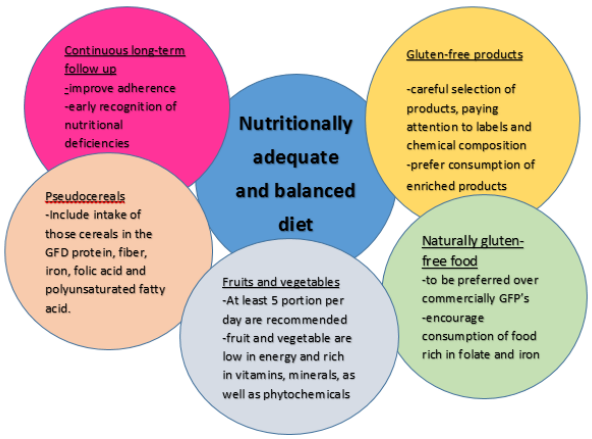

Figure 2: Schematic representation of proposed approach to a nutritionally adequate and balanced gluten-free diet.

4.5 Role of oats in celiac disease

Oat grains are a good source of proteins, have high nutritional content. They have good taste, dietetic properties and stimulate metabolic changes in animals and humans. They contain dietary fiber, antioxidants, vitamins, minerals, phenolic compounds and unsaturated fatty acids. The nutritional components of oats are given below:

Table 3: Nutritionally important components in the oats

	Nutritional components	*Properties*
Proteins	Albumins, globulins, prolamins and glutenins	Oats have high and distinct protein concentration. Globulins are major storage proteins [38].
Carbohydrates	B-glucan, glucose, fructose, pentose, saccharose, kestose, neokestose, bifrucose, neobifrucose, acid galactoarabinoxylan, etc.	The main dietary component obtained from oats is B-glucan. It exhibits high viscosity at lower concentrations [39].
Lipids	Unsaturated fatty acids	Oats lipids have high antioxidant properites [40].
Antioxidants	Vitamin E, phytic acids, phenolic compounds, flavonoids and sterols	Antioxidants inhibit the growth of cancerous cell and reduce the cholesterol concentration in the serum [41].

Table 4: Health benefits of the oats

Effects	*Findings*
Hypocholesterolemic	This effect is derived from the B-glucan content. The significance of cholesterol reduction by it is variable and controversial [42], [43].
Hypoglycaemia	By consuming oats insulin response is decreased, fasting blood glucose level and chances of post-prandiahypoglycaemia is reduced [44].
Prevention of cancer	In oats selenium is present which repairs DNA and lowers the risk of cancer particularly colon cancer. Moreover, it is also present in high fiber food [45].
Reduction of hypertension	To prevent hypertension, soluble and fiber rich oats are beneficial and act as dietary therapy [46].
Immunomodulatory	Oats stimulate the immune response through B-glucan and inhibit the growth of various bacteria, fungi, parasites and viruses [47].
Antioxidant	Certain chemicals are present in oats that act as antioxidants. For example, phenolic compounds, vitamin E and phytic acid. (41).
Antiatherogenic	Studies have shown that by consuming oats bran level of plasma cholesterol is reduced in artherosclerosis. But it is not clear that whether this antiatherogenic effect is due to reduced plasma cholesterol level or due to other components of the oats [48].
Obesity control	Oats are effective in reducing obesity [39].

4.5.1 Relationship between oats and celiac disease

The avenin components present in oats seeds exist either as monomores or in disulfide linkages [49] as prolamins and are rick in glutamins and proline residues. The proteins which are enriched in these two amino acids evoke celiac disease. However, prolamins of oats are different from other cereal grains i.e. wheat, rice and maize as they account for only 10-20% total protein content, they
contain lowest proline and glutamine content i.e. 35-50%, the domains of oat avenins are two in number with high content of proline and glutamine and the pattern of disulfide linkage is also different and have low molecular weight

glutenins [50]. However, all oats are not pure and studies revealed that oats cannot be tolerated by persons who are sensitive to gluten due to contamination with the wheat, rye and barley and are not tolerated by persons suffering from celiac disease.

5. Methods to convert cereal varieties into harmless gluten free diet

Two methods are generally used to convert gluten containing cereal varieties into harmless gluten free cereal varieties these are as follows:

5.1 Biotechnological method

Gluten detoxification is carried out by down regulating the gene expression mainly by RNA interference (RNAi). The technique is useful for reducing immunotoxicity of gluten and is also used for some wheat varieties and crops [51]. By using RNAi, we silence the multigene family and also able to down regulate the gliadin and glutenin proteins. Mainly the expression of different gliadins is down regulated i.e. α-gliadins, γ-gliadins and ω-gliadins [52]. These specific genes commonly have immunogenic epitopes and are silenced by RNAi. For use by celiac patients and other pathologies related to gluten i.e. gluten allergies transgenic lines are used in foodstuffs which are deficient in all three gliadins. Humanes and Pistonperformed an experiment in which they construct hpRNA to silence the γ-gliadins in the bread wheat genotypes [53]. They reported that in transgenic strains γ-gliadins was reduced to 65-97%. But by reducing γ-gliadins, other storage proteins i.e. ω-gliadins, α-gliadins also reduced. Moreover, there was no significant decrease in total amount of gliadin in transgenic lines as compared to wild type. Later on, they construct hpRNA containing chimeric sequences having conserved genes in the α-, ω- and γ-gliadins [54]. These chimeric sequences are able to silence the expressions of all three types of gliadin genes ranging from 70-88%. In these lines, the overall content of gluten proteins reduced to 56% while albumins and globulins the non-glutein proteins increased and these are good to be used in foodstuff. We can quantify the amount of gluten in the food by doing monoclonal antibody assay based competitive ELISA. By monoclonal antibody testing, it was seen that in transgenic lines content of gliaidin was reduced by 92%. From this we conclude that transgenic lines relative to wild type containing 10% α-gliadins, 30% ω-gliadins and 1% γ-glaidin were unable to stimulate T-cells lesion derived celiac disease [54].

These is one important question that by silencing the gliadin or gluten proteins either quality if food is affected or not? The answer to this question is by silencing the α-gliadins, the baking quality is not affected but the bread made from them had lower volumes i.e. 11% as compared to normal wheat breads. Mixing properties were determined by using SDS sedimentation test and mixograph these test showed that reduction of γ-gliadin have not any effect on the baking and mixing quality of dough. The absence of gliadins is compensated by adding non-toxic flours of pseudocereals, gluten free cereals and non-toxic oat cereals and enhances the variety of wheat [55].

5.2 Enzymatic method

To cleave food proteins normally gastric and pancreatic enzymes are used but gluten proteins are resistant to digestion of the proteins due to their high proline and the glutamine contents. Some gastrointestinal enzymes could hydrolyze gluten proteins and convert them into non-toxic fragments. Moreover, toxicity could also be diminished by complete acidic hydrolysis [56]. Three common approaches are used to detoxify gluten; use of Propyl Endopeptidase (PEP) to cleave gliadin fragment, use of germinating cereals for degradation of toxic peptides and transamidation of the cereal flours.

5.2.1 Propyl Endopeptidase (PEP)

PEP was extracted from different organism and was used to cleave the gluten peptides and reduce its toxicity [15]. This information is obtained on the basis that the large amount and site of the proline residues is an important factor for gastrointestinal resistance. From this further researches have shown that the fermentation of wheat, rye and the barley flours with peptidases cause the remarkable decrease in the toxicity of gluten. These propyl endopeptidases are present in many organisms such as bacteria, fungi, plants and animals but particularly, Lactic acid bacteria (LAB) have greater peptidase system [57]. These lactobacilli species have an abundance of peptidase that are able to degrade gluten specially fragments of α-gliadins. Moreover during fermentations lactobacilli species are also able to hydrolyze wheat prolamins. Recently, lactobacilli are combines with the fungal peptidases to diminish the wheat toxicity by doing long term fermentation. Thus, by using specific proteases we are able to eliminate gluten toxicity during food processing.

5.2.2 Germinating cereals

During the germination of wheat seeds, the endogenous cereal proteases produce would be able to hydrolyze the proteins extensively [58]. These proteases from germinating seed varieties could be used to produce safe cereal varieties that are harmless for celiac patients. The study of the protein content by reversed phase- HPLC shows efficient degradation of prolamins during wheat, rye and barley germination. Further studies shows that protease from these germinating cereals catalyze rapid cleavage of peptides into harmless fragments and nine amino acids [58]. These enzymes obtained from germinating cereals are excellent alternatives to recombinant proteases and have several advantages over bacterial and fungal peptidases. The extraction of germinating cereals is simple process.

5.2.3 Transamidation

Two types of reaction are catalyzed by a class of enzyme transglutaminase: transamidation and deamidation. In the former one primary of γ-carboxamide group is transferred to later one and in the second reaction, conversion of glutaminyl residue to glutamyl residue occur [59]. The immuopathogenesis of celiac disease is due to tissue transglutaminase because due to deamidation immunogenicity of gluten peptides increases.

6. Conclusion

Due to high incidence of the celiac disease, nutritionally adequate gluten free food is produced which is consumed by the gluten sensitive patients. But due to contamination of gluten it is not suitable and best alternatives are altered cereals and pseudo-cereals. By enzymatic and biotechnological methods we can detoxify gluten and produce high quality food for patients suffering from celiac disease. The sequence of gluten storage proteins can also be modified to decrease their toxicity.

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