Celiac disease (CD) is a common autoimmune disorder of the small intestine, followed by intestinal inflammation that causes destruction of intestinal villi (villous atrophy), elongation of crypts (crypt hyperplasia) (1) and alters intestinal barrier (2). For the first time, CD reported with a prevalence 1: 184 in an Italian scholar population (3). Prevalence of CD varies from 0.006-5.6% through different populations of world. As per recent reports prevalence of CD in United States is 0.79% (4). In European countries, its prevalence is 4% in Spain (5), 2.4% in Finland (6), 0.9% in Germany (7), 0.6% in Northern Sweden and Hungary (8, 9), lesser in Scotland 0.01% (10) and least in Netherlands 0.006% (9) whereas in Asian countries the prevalence is 0.3% in Iran, 0.5% in Turkey, 0.7% in Israel (11) and an average of 0.73% from different regions of India (12). Saharawi population of Africa accounts 5.6% as highest prevalence worldwide (13). The presence of variant forms of human leukocyte antigen (HLA) genes are reported to be associated with the intestinal inflammation in CD but all types of genetic variations could define 48% of the disease risk, suggesting that there are some other factors associated with pathogenesis (14). The extent of gluten intake is strongly associated with prevalence of CD rather than HLA genetics (12), but as gluten itself does not explain the reason for disease incidence and disease progression, so environmental factors other than gluten need to be addressed (15). In context to the current scenario, intestinal dysbiosis is well reported in CD and certain environmental factors are associated with disease markers and microbiota composition in infants at risk of CD (16). Intestinal microbial ecology of commensals, symbiotic and pathogenic microorganisms play an important role in pathogenesis of many gastrointestinal diseases. Intestinal bacteria interfere with the mammalian immune system and regulate differentiation of pro-inflammatory and anti-inflammatory T-cells through many pathways among which Toll-like Receptor (TLR) pathway (17, 18) is a key factor for integrity and functionality of the tight junction barrier of the intestinal epithelial layer (19).

Even for several decades, CD has been known as a culprit among autoimmune diseases. Despite of knowledge about antigenic trigger, there is no successful therapy available to alter the option of gluten free diet (GFD). Strict lifelong adherence to GFD always remains a challenge for patients and unexpectedly ingestion of gluten contaminated food facilitates reoccurrence of gluten induced inflammation because
intestinal microbial balance is not restored even after being on GFD (20-22), indicating persistent disease susceptible environment.

Microbiome dysbiosis in CD is reported by several studies (23). In a pursuit to explore the role of HLA genes in pathogenesis of CD, a recent report of 22 individuals genetically at risk of CD concluded that HLA-DQ2 genotype selects early intestinal microbiota composition when compared to individuals at lower genetic risk/carriers. The individuals at high risk showed the different intestinal microbiota i.e. high-risk infants had significantly less *Bifidobacterium* and unclassified *Bifidobacteriaceae* proportions and more *Corynebacterium, Gemella, Clostridium sensu stricto, unclassified Clostridiaceae, unclassified Enterobacteriaceae* and *Raoultella* proportions (24). On the other hand, a recent Indian study on 23,331 adults, supported the importance of other factors rather than genetics because HLA genes were not associated with prevalence of CD (12) but the extent of gluten intake was. An association of microbiota with CD was first established in GFD Treated-CD (T-CD) and Untreated-CD (U-CD) subjects (20-22) and thus a concept of dysbiosis was put forward. An Italian study reported that intestinal infections were strongly associated with disease onset that were further significantly associated with antibiotic use (25). Moreover, early age infections and infants’ antibiotic intake is also reported as the cause of dysbiosis and alterations in lymphocyte subpopulations (26) that can be correlated to disease activity i.e. increased cellularity (increase in number of intraepithelial lymphocytes) and atrophy of small intestinal mucosa, a characteristic feature of CD (27). Such antibiotic induced dysbiosis was characterized by decreased counts of *Bifidobacterium longum* and increased counts of *Bacteroides fragilis* (26). Moreover, dose-response relationship of antibiotics is significantly associated with CD onset and risk of CD is strongly increased by cephalosporin intake (25). In contrast, several previous studies reported that dysbiosis in CD is characterized by decrease in *Bifidobacterium* counts (28-31), pointing on antibiotics and infections as the key players of dysbiosis that may be a reason of disease susceptibility. In such a situation, a very likely question arises that “Environmental trigger is only gluten or there is something else too i.e. microbiome dysbiosis, infections and antibiotics, or antibiotic induced dysbiosis” (Figure 1). Intestinal microbial overgrowth is characteristic of CD and specific pathobionts namely *Klebsiella oxytoca, Staphylococcus epidermidis, and Staphylococcus pasteuri* were reported to outnumber...
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commensals and they have potential to exclude commensals from intestine (32).

Figure 1. This figure represents the interaction of gluten, microbes and intestinal immune regulation.

Section (A) of the image represents healthy microbial ecology where there is a balance between beneficial bacteria (green) and harmful bacteria (red yellow) and beneficial microbes preventing the adhesion of harmful ones to intestinal mucosa. Section (B) represents exposure to environmental infectious agents (red) that compete the beneficial microbes to adhere the intestinal mucosa and after adherence they disturbed intestinal barrier function (tight junctions) by activating different inflammatory pathways at intestinal mucosal surface. Disturbed intestinal barrier leads to exposure of intestinal immune cells to the dietary antigens (i.e. gluten). On the other hand infections impose elastase activity to the peptides that are more
potent to translocate through intestinal barrier. These gluten peptides (blue coloured) are presented by Antigen Presenting Cells (APCs) to the T lymphocytes leading to cascades of immune processes leading to gluten specific immune responses and tissue remodelling to develop gluten intolerance. Section (C) highlights that the catalyst role of antibiotics that are given to patients to treat infection. Antibiotics eradicate infections along with beneficial intestinal microbes thereby leading to dysbiosis. In this condition, the opportunistic harmful microbes may prove to be dangerous because beneficial microbes can no longer protect intestinal mucosa leading to adherence of the former to the intestinal epithelial cells and creating disease susceptible microenvironment (25). Colour of beneficial microbes, Green; Gluten peptides, blue coloured; Colour of Harmful/ Opportunist microbes, Grey coloured; Colour of Infections, Red coloured;

**Figure 2. Diagrammatic representation of microenvironment proposed for CD development.**

Infections and antibiotic intake produce CD prone environment that affects commensals communities. Decreased number and diversity of commensals results in immune dysregulation because lesser numbers of commensals (microbes secreting glutenases) may lead to inefficient gluten digestion followed by decreased intestinal barrier function and leaving behind significant amount of intact immunotoxic peptides for immune activation.

Thus, CD is interplay of three important factors that are gut microbiota, genetics and immunotoxic gluten peptides. The probable model for disease
Microbiota and gluten peptides are the major pathogenic factor responsible for CD. Literature on this part is scarce to define the types of microbes that may cause CD. Thus the study was designed to explore microbial diversity in disease versus non-celiac control subjects. On the other hand, beneficial microbes (Lactobacillus species) were isolated from healthy subjects so as to evaluate their immunomodulatory effects against gliadin in-vitro cell line model (Caco-2 cell line). Another objective was focused on the identifying therapeutic peptides from gluten that can be used as early diagnostic marker. After analyzing the immunotoxic nature of gluten peptides, a consensus peptide was developed to be evaluated for T-cell proliferation in type 1 diabetes mellitus (T1DM) subjects so as to look for an early diagnostic marker for diseased subjects. Since T1DM and CD has strong associations and gluten peptides are known to have immunotoxic actions that is reflected in T-cell proliferation. Thus, objectives for the work plan are described below that may help us to understand better the pathophysiology of disease and developing some new therapeutic approaches:

I. To investigate gut microbiota changes associated with CD.

II. To screen Lactobacillus bacteria having protective effects against gliadin.

III. To establish the mechanism (s) of action of probiotics against gliadin induced immunotoxicity.

IV. To predict the autoimmune basis of β islet cell autoimmunity by using in-silico (immunoinformatics) and in-vitro approaches.

**OBJECTIVE I. To investigate gut microbiota alterations associated with CD.**

In this objective, we have characterized the microbial diversity of duodenal microbiome in celiac disease patients by using culture dependent approaches and metagenomics. Duodenal biopsies were collected from 13 CD patients and 6 non-celiac controls. The biopsy samples were homogenised and serially diluted onto different media plates in aerobic and anaerobic conditions. We reported prevalence of some new/ rare microbes in CD patients, thus their genomes were sequenced to predict the possible role in pathogenesis of CD. All those genomes were annotated by using Rapid Annotations using Subsystems Technology (RAST) server and were...
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compared with a literature searched experimentally proven invasive strain of CD i.e. 
*Neisseria flavescens*. A comparison between the gene categories; Virulence disease 
and defense, Membrane transport, Iron acquisition system, Phages and prophages of 
all isolates was evaluated.

**OBJECTIVE II and III. To screen lactobacillus bacteria having protective 
effects against gliadin.**

While *Lactobacilli* are the deficient microbes in intestine of CD patients as 
compared to non-CD controls and they are known to present the protective 
immunomodulatory effects. In search of a good probiotic against CD, the 
lactobacillus bacteria were isolated from stool samples of healthy individuals. The 
strain *L. pentosus* HY10 was evaluated for its immunomodulatory actions against 
immunotoxicity induced by gliadins *in vitro* Caco-2 cell line model. Instead of using 
live microbial cultures, cell free supernatant (CFS) of the cultured microbe (*L. 
pentosus* HY10) was used to evaluate the immunomodulations exerted by it against 
gliadin induced immunotoxicity. Further, the possible mechanism of action of 
probiotics against gliadin induced immunotoxicity was established.

**OBJECTIVE IV. To predict the autoimmune basis of β islet cell autoimmunity 
by using in-silico (immunoinformatics) and in-vitro approaches.**

CD is very prevalent in type 1 diabetic subjects. Interestingly, type 1 diabetes 
mellitus (T1DM) does not strike all people who carry the risk genes. In addition, 
incidence of T1DM in monozygotic twins is approximately 50%. These facts indicate 
that environmental factors play a significant role in T1DM aetiology and 
pathogenesis, out of which dietary protein exerts the greatest influence on the 
incidence of T1DM, and wheat gluten is the most diabetogenic of common food 
proteins. However, only a few studies, to date, have attempted to explore possible 
relationships between wheat proteins and β cell autoimmunity in humans. Therefore, 
to study the role of immunootoxic gluten peptides in T1DM may provide hope for new 
therapeutic. The objective was based on designing a consensus peptide that should 
represent most of the immunogenic peptides till date available in literature. To 
accomplish this objective, by using immunoinformatics tools all the proteins of wheat
were evaluated for their immunogenic potential. The aim was accomplished by using prediction tools for B-cell and T-cell activation. The selected potential peptides were aligned by using multiple sequence alignment tools and a consensus sequence was generated that was representing the common peptide pattern. To evaluate the immunogenic potential of this celiac peptide, T-cell proliferation was evaluated by using fluorescence-activated cell sorting (FACS) in the PBMCs derived from T1DM patients. The PBMCs isolated from blood samples of subjects (T1DM subjects and Healthy controls) were subjected to carboxyfluorescein succinimidyl ester (CFSE) staining followed by stimulation with synthetic peptide.