Ministry of Higher Education and Scientific Research

College of Medicine

University of Karbala

Department of Medical Microbiology

Study of Serum Level of Zonulin Protein and Interleukin-15 in Celiac Disease Patients

A Thesis

Submitted to the College of Medicine and the Committee of Post Graduate Studies \ University of Karbala in Partial Fulfillment of the Requirements of Master Degree of Science in Medical Microbiology

By

Israa Hussein Hajjoj AL-Jarrah

M.B.Ch.B.

Supervised by

Assistant Prof.

Dr. Alaa Saad Alattabi

M.B.CH.B. - FIMCS path/immunology

1440 A.H **2019 A.D**

وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ

﴿ المجادلة / ١١ ﴾

صدق الله العلي العظيم

DEDICATION

To our time Imam, the guardian of the matter, and the commander of the nation, **Al-Hujjah Ibn Al- Hassan** "God accelerated his appearance", who has always been my patron to almighty gracious Allah in the completion of my project and passing the difficult stages of it.

To the soul of my father and my mother ....Who I hope they are happy for my graduation.

To my faithful husband Assistant Prof. Abdul-Ridha.....Who was always standing next to me in every moment of this journey? His limitless support and encouragement made this work come into existence.

To my brothers and sisters for their extraordinary assistance, in particular Ikhlas, who helped me in more ways than anyone else?

To my kids Mohammed, Mo'ammal, Salih, Fwatim, and Ghadeer....Whom their love contributed to my emotional well-being throughout the completion of the project at hand.

To my precious friends and relatives .....For their everlasting question and support.

I present this humble work.....

ISRA'A

FEBRUARY/2019

ACKNOWLEDGMENT

First of all....."Praise to almighty merciful Allah for blessing me much more than I deserve".

Really, it is the time to present my sincere thanks and express my deep gratitude to all people who had a direct or indirect role in achieving this project.

I would like to present my deep and sincere appreciation to the smart supervisor, Assistant Prof. Dr. Ala'a Sa'ad Al-Ataabi..... For her continued supervision and boundless help, manuscript revision, and providing expert advice, suggestions and support along the project time.

I express my thanks and gratitude to Assistant Prof. Dr. Ali Mansour and the academic staff of Microbiology department, College of Medicine/ University of Karbala for everything they provide.

My thanks and appreciation to Internal Medicine Consultant Dr. Ali Nasir in Al-Hindiya General hospital, to Pediatric Specialists Dr. Mohammed Firas and Dr. Waleed Khalid in Pediatric-Teaching Hospital...For their help, advice, and guidance, which played integral roles in the project.

I present my thanks and gratefulness to Immunity Consultant Dr. Mohammed Salih for his professionalism and advice and I am indebted to my best friends Dr. Fatima Abdullah and Dr. Rasha Khahtan in Al-Hussein Medical City hospital for their brilliance and friendship in the lab. Their endless support enabled me to accomplish my project.

Special thanks to Dr. Ban Jabir and M.A. Ali AL-Saadawi for their help in statistical analysis.

I also would like to express my gratitude to all bacteriologist and lab assistants in Al-Hussein Medical City hospital, Pediatric-Teaching Hospital, Al-Hindiya General hospital, and Al-Kafeel Surgical hospital.....as their cooperation and help were crucial in achieving my lab work.

I wish to express my respect and appreciation to the celiac patients and their families.

This thesis would have been impossible without the extraordinary support of my family.

Lastly "Thanks to anyone I've forgotten who was instrumental in this project.

ISRA'A

FEBRUARY/2019

Summary:

Celiac disease is an immune mediated chronic enteropathy with a broad range and variable severity of presenting symptoms, triggered by ingested gluten found in wheat, barley, and rye in genetically predisposed people of all ages. Celiac disease is unrivaled disease pattern to study the pathogenic processes induce autoimmune disorders in humans. In addition to individual genetic construction and triggering (environmental) elements, another key factor, in which an increased intestinal permeability was intended in the disease pathogenesis. The aim of this study is to look at the zonulin role as a biomarker for impaired intestinal permeability and IL15 as predictive marker for inflammatory process of small intestine in Iraqi people with celiac disease since both markers are upregulated in various stages of the disaese. Method: Case control study was carried out to test 108 celiac patients of different age groups and different phases of the disease by measuring serum level of the two markers using a sandwich ELISA technique and to be compared with 118 healthy controls matched for age and gender. Results: High serum zonulin concentrations were noticed in the majority of the celiac disease patients with different phases of the disease, which probably attributed to a non-adequate restriction of gluten in the diet of most of the candidates, while no detectable level for circulating IL15 revealed in patient's sera except for small proportion (16 patients), and this may reflect the local effect of this inflammatory mediator. However this study demonstrates a significant statistical difference in zonulin and IL15 serum levels among celiac patients and their matched for age and gender controls ($p< 0.0001$for zonulin and $p< 0.001$ for IL15).Conclusion: The high serum zonulin levels in the majority of patients illustrates how much GFD is a public burden needs to be solved and may provide some rationale to seek

a non-food alternatives. This review also calls for expanding our actually limited knowledge about IL15 to get more autoimmune disorders at the level of our population.

CONTENTS:

List of tables

List of figures

List of abbreviations

Symbols	Descriptions
ACG	American College of Gastroenterology
AGA	Anti-gliadin antibody
AJs	Adherens Junctions
Anti-GDP	anti-deamidated gliadin derived peptides
Anti-tTG	anti-tissue transglutaminase antibodies
BSG	British Society of Gastroenterology
CD	Celiac Disease
CXCR3	Chemokine receptor 3
DCs	Dendritic cells
DH	Dermatitis herpetiformis
DM-1	diabetes mellitus- Type 1
ELISA	Enzyme-Linked Immuno Sorbent Assay
ESPGHAN	European Society of Pediatric Gastroenterology, Hepatology and Nutrition
EGFR	Epidermal growth factor receptor
FAS	First apoptosis signal
FOXP3	fork-head box transcription factor P3
GALT	Gut-associated lymphoid tissue
GFD	gluten-free diet
HLA	human leukocyte antigen
HP	Pre-haptoglobin
IB	Intestinal barrier
IELs	Intra-epithelial lymphocytes
IFA	indirect immunoflourescent assay
Ig	Immunoglobuline
IL-15	InterLeukin-15
IP	Intestinal Permeability
LP	lamina propria
MHC	Major histocompatibility
MICA molecules	major histocompatibility complex class I chain-related molecules
MLNs	Mesenteric lymph nodes

ncRNAs	non-coding RNAs
NK	Natural killer
NKGD2D	Non-MHC class I natural killer receptor
NKTs	Natural killer T cells
PAR2	proteinase-activating receptor 2
PPs	Peyer's patches
RCD	Refractory celiac disease
TCR	T-cell receptor
TGF-β	Transforming growth factor-β
Th1	T-helper 1 cells
Th2	T-helper 2 cells
TJs	Tight Junctions
TLR	Toll-Like Receptors
Treg	T-regulatory cells
tTG	tissue Transglutaminase
WGO	World Gastroenterology Organization
ZOs	(ZO)-proteins
Zot	zonula occludens toxins

Chapter One

Introduction

&

Literature Review

Chapter One

1.1-Introduction:

Coeliac disease (CD) is a chronic small intestinal immune-mediated enteropathy induced by ingestion of gluten in genetically susceptible people (Ludvigsson *et al*., 2013). The significant role of the genetic vulnerability is definite as most (>95%) of patients express HLA-DQ2 and nearly 5% express HLA-DQ8 (Sollid and Thorsby, 1990; Sollid *et al*., 1998; Wolters and Wijmenga 2008). CD is ultimately common genetic disease worldwide and affects about 1 in 100 of the people in the world (Biagi *et al*., 2010; Lionetti and Catassi, 2011; Gujral *et al*., 2012). The disease has a higher prevalence in females than males with a ratio of 3: 1(Ciacci *et al*., 1995). In spite of the obvious increase in the disease prevalence, however a frequent delay in diagnosis is often attributed to the variation in the manifested feature from one patient to another (Volta and Villanacci, 2011).

Clinically CD presented with highly variable manifestation ranging from the typical (gastrointestinal) features to atypical (extra intestinal) symptoms (Caproni *et al*., 2009). The diagnosis depends on the clinical presentation, disease-specific antibodies (Ludvigsson *et al*., 2013; Kaswala *et al*., 2015). A duodenal mucosal biopsies are the golden standard to confirm diagnosis. The serology and biopsy should both be accomplished before adherence to gluten-free diet (GFD) (Husby *et al*., 2012; Rubio-Tapia *et al*., 2013). HLADQ2/DQ8 genotyping can be carried out in certain situations (Rostom *et al*., 2006; Husby *et al*., 2012; Rubio-Tapia *et al*., 2013; Ludvigsson *et al*., 2014).The only safe and efficiently curable treatment, is a life-long restriction of glutin from diet (Greetje *et al*., 2010; Ludvigsson *et al*., 2013; Rubio-Tapia *et al*., 2013).

The small intestine normally optimize the digestion and absorption of nutrients, in addition to control macromolecules(Antigen) trafficking between the host and external environment through a barrier mechanism, which is physiologically orchestrated by intestinal tight junctions (TJs) competency (Fasano, 2008).Yet the TJs are modulated or held in assembly state by, among others, the well-recognized protein, zonulin (Wang *et al*., 2000; Fasano, 2008) A protein that documented as pre-haptoglobin (HP)2 (Tripathi *et al*., 2009) with a main biological function is modulation of intercellular TJs and reversible regulation of intestinal permeability(IP) ((Wang *et al*., 2000; Fasano, 2000; Fasano, 2011).

The Zonulin upregulation has a potential effect, in one way or other, by increasing gut permeability (Fasano, 2011),with consequent increase entry of stressors(like gliadin) into submucosal layer via either paracellular pathway (Fasano *et al*., 2000; Cummins *et al*., 2001; Clemente *et al*., 2003; Thomas *et al*., 2008) or retrrotranscytosis by which secretory immunoglobulin A (IgA)ensure passage of gliadin peptide by binding to CD71 (transferrin) receptors (Matysiak-Budnik *et al*., 2008).

Moreover gliadin peptides induce innate and adaptive immune responses (Maiuri *et al*., 2003; Gianfrani *et al*., 2005; Meresse *et al*., 2009). The toxic peptide (19-mer) plays on innate response and cumulates the production of interleukin (IL)-15, a pro-inflammatory cytokine(Fasano 2012), from enterocytes, macrophages, and dendritic cells, mediating intra-epithelial lymphocytes (IELs) expansion and enterocytes apoptosis by upregulation of the non-MHC class I NK receptor (NKG2D and CD94) on IELs and directs the killing of stressed enterocytes express MICA ligand perpetuating the cytotoxic damage of the epithelium (Kagnoff 2007; Schuppan *et al*., 2009; Fasano 2012; Han *et al*., 2013; De Re *et al*., 2013; Kim *et al*., 2015).

On the other hand, the deamidation of 33-mer gliadin (immunogenic peptide) by tissue transglutaminase 2 (TG2) enzyme yields a deaminated gliadin peptides with high affinity to HLA-DQ2 or -DQ8 contexts on antigen presenting cell (APC), presented to activated T cells (CD4+) and unleash the adaptive immune response. Notably, both of the deamidated gliadin and auto-antigenTG2 are the goal of B-cell responses specific for celiac (Di Sabatino and Corazza, 2009; Stamnaes and Sollid, 2015).

This improper adaptive immune response with peculiar picture results in mucosal damage which healed upon refraining of dietary gluten (Stamnaes and Sollid, 2015).

The studied theory suggests that since the immunopathogenic signal is evoked, it is not self-perpetuating. Instead, the continuous interplay between genes and the environment can be modulated or even reversed (Fasano 2011; Fasano 2012). Since zonulin-dependent TJ desintegrity permits such interplay, the aim of modern therapeutic designs is to rehabilitate the intestinal barrier competency by down regulating zonulin signaling cascade which provide a novel methods to be explored for managing of these chronic disastrous diseases (Fasano 2011; Fasano 2012).

1.2- Study objectives:

To compare the serum level of Zonulin protein and Interleukin-15 (IL15) in celiac disease's patients in Karbala province as they contribute to the pathogenesis of celiac disease. Also to prove that zonulin-dependent TJ disintegration is a responsible factor for a continuous interplay between genes and the environment.

1.3-Literatures Review:

1.3.1-Celiac disease definition:

CD is a chronic systemic immune-mediated disorder associated with variable small-intestinal mucosal lesion caused by gluten consumption in genetically susceptible individuals (Green and Cellier, 2007; Ludvigsson *et al*., 2012; Husby *et al*., 2012). It is also called gluten intolerance, gluten-sensitive enteropathy, and coeliac sprue (AGA Institute, 2006; Crowe, 2011), and celiac as term (‘‘hollow’’ in Greek) refers to the intestinal origin of the disease (Van de Kamer *et al*., 1953). CD is outlined by a wide range gluten-related clinical features, specific antibodies, HLA-DQ2 or HLA-DQ8 positivity; a distinct model of intestinal damage (inflammatory cell infiltration, crypt hyperplasia, ending with villous atrophy); with clinical and serologic resolution on adhering to GFD (Fasano and Catassi, 2001; Husby *et al*. 2012; Schuppan and Zimmer, 2013). Coeliac disease could be considered as a systemic condition rather solely a gastrointestinal disorder in view of the various extraintestinal features of the disease (Reilly *et al*. 2012).

CD varies from food allergies, where the pathogenesis is mounted by an immediate hypersensitivity reaction in which IgE-antibodies are the master-key mechanism. Instead, in CD an adaptive immune response orchestrating release of cytokines causing mucosal inflammation manifested by variable clinical features(Constantin *et al*., 2005; Tanabe, 2008). Between the common clinical scenarios, the frequency of CD ranges from moderately increased, like irritable bowel syndrome, to substantially increased, like iron-deficiency anemia (Ford *et al*., 2009; van der Windt *et al*., 2010; Aziz and Sanders, 2012). Adherence to a life-long GFD, leads to interruption of the gluten-induced intestinal immune response, permitting the recovery of villous architecture to normal state and alleviation of clinical symptoms (Campanella *et al*., 2008; Malamut *et al*., 2009). So CD is a site where the effect of MHC

class II alleles in individual immunopathology is decoded, the processes that link tolerance to food proteins and autoimmunity to be analyzed, and T cell lymphoma agenesis due to chronic activation of IELs can be investigated (Meresse *et al*., 2012).

1.3.2- History of celiac disease:

*CD first described by Greek physician Aretaeus from Cappadocia about 100 AD, and later, by other clinicians from Europe and America (Adams, 1856; Simoons, 1981; Losowsky, 2008).

* In 1888 Samuel Gee linked CD to food ingestion (Gee, 1888)

* In 1950s, Willem Dicke, a Dutch pediatrician and colleagues (Dicke *et al*., 1953) demonstrated that consumption of wheat and rye causing CD (van Berge-Henegouwen and Mulder, 1993; Losowsky, 2008; Yan and Holt, 2009; Elli *et al*., 2015), and restriction of these grains from the diet improves symptoms (Van de Kamer *et al*., 1953).

*In 1954, Paulley (Paulley, 1954) was the first who linked the CD clinical manifestations with small intestinal mucosal destruction, which followed by a histological classification of celiac lesions presented by Marsh (Marsh, 1992).
* In 1969, establishment of a ‘diagnostic criteria’ or protocol for CD by the European Society for Paediatric Gastroenterology and Nutrition (ESPGHAN) which also known Interlaken criteria (Meeuwisse, 1970)

*Later linkage of CD to genetic elements, where studies proposed that HLA class II loci predisposed to celiac (Houlston and Ford, 1996).
* In the 1980’s and 1990’s, specific, sensitive, and non-invasive screening tests where introduced with marked improvement in the diagnosis of CD by the identification of enzyme tissue transglutaminase 2 (TG2) (Dieterich *et al*., 1997).

*Beyond that, every aspect of CD is virtually studied, including innovative forms of treatment have emerged (Freeman, 2013).

1.3.3- Epidemiology of celiac disease:

The availability and accuracy of noninvasive diagnostic tools motivated a potent research on CD epidemiology (Reilly *et al*., 2012; Ianiro *et al*., 2015; Ianiro *et al*., 2016). CD prevalence is about 1% of the general population, with evidence suggests that only small percent (about 10% to 15%) of them (both children and adults) have been diagnosed and treated (Rubio-Tapia *et al*., 2013). Therefore epidemiology of CD has an iceberg model or configuration, in which the tip or visible portion represent typical cases of CD being diagnosed due to indicative symptoms, while the invisible portion of the iceberg that submerged below the waterline denotes to all the under diagnosed cases that often show either extra-intestinal or minimal or even no manifestations (Fasano and Catassi, 2001; Bai *et al*., 2005; Lionetti and Catassi, 2011). Hence CD is a common food-dependent, genetically-linked disorder in the world with prolonged duration (Lohi *et al*., 2007). Choung et al have shown that undiagnosed celiac is more in children and younger adults (Choung *et al*., 2017).

Mustalahti et al mentioned that active serological screening would uncover many undiagnosed CD cases (Mustalahti *et al*., 2010).The World Gastroenterology Organization (WGO) ensured that considering the local resources important to meet the ‘epidemic’ of CD: hence it recommended adjusting a diagnostic protocol relying on available resources (Bai *et al*., 2013). Originally CD was considered almost exclusively a white Europeans disorder, but now it is widely distributed (Fasano and Catassi, 2001), and extends to people beyond the European origin, with recognized prevalence seen in diverse populations such as the Asia, Middle East, North Africa and

South America (Shahbazkhani *et al*., 2003; Sood *et al*., 2003; Green and Cellier, 2007).

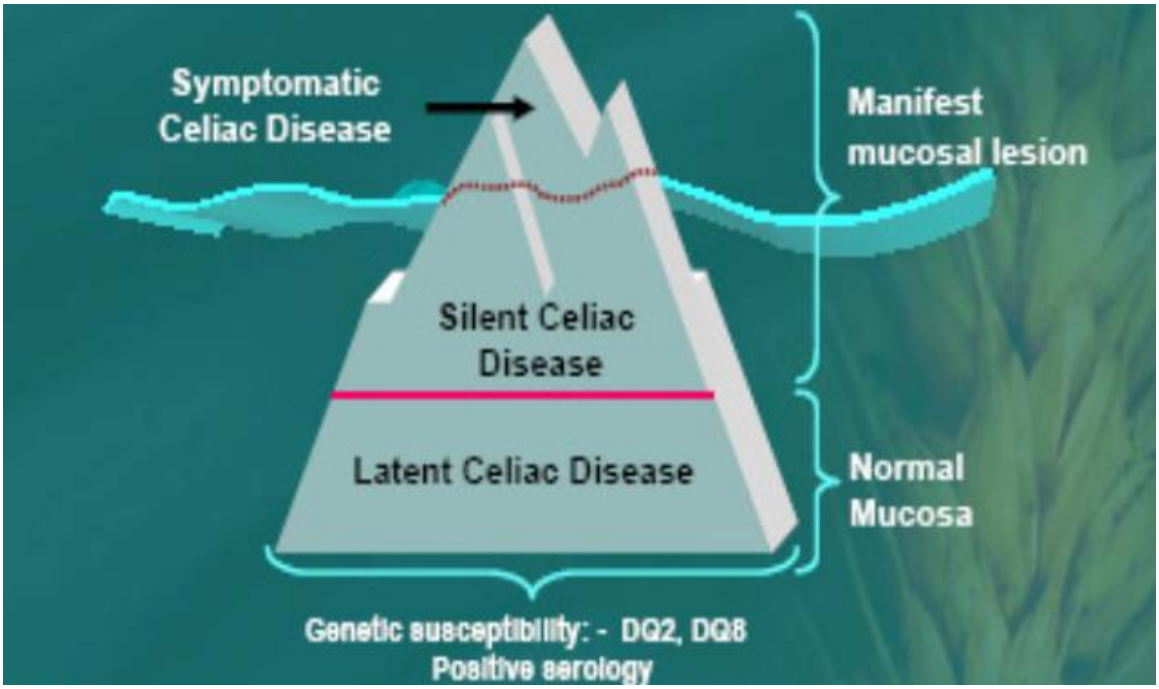

Figure (1.1): The iceberg model of CD (Fasano, 2006)

Many developing countries are also common with CD in particular the Middle East (Dalgic *et al*., 2011) and North Africa (Alarida *et al*., 2011), this may be due to the continuous 'Westernization' of the diet (Fasano and Catassi, 2012; Sapone *et al*., 2012). Relatively, a common cause of chronic diarrhea in Iran, Iraq and Kuwait is celiac, while 2%-8% of type 1 diabetic patients in Iran, Israel and Saudi Arabia diagnosed to have CD (Rostami *et al*., 1999).

1.3.3.1-Gender differences: Interestingly, the prevalence of CD among women is higher than men and the female: male ratio is 2.8:1 (Polanco *et al*., 1981; Thomas *et al*., 2009). Children are in contrast to adults (twice in females) have equal sex ratio 1:1, and same ratio also have interestingly recognized in the elderly (Freeman, 1995). Here in Iraq, CD also occurs more in female than in male (Hameed *et al*., 2016).

1.3.3.2-The age of onset of CD: CD was traditionally considered a paediatric disorder (Ivarsson *et al*., 2000; Marine *et al*., 2011), but recently it

can affect any age group, however the presentation mostly occurs either in young children (from 9 to 24 mo) or in adulthood (third or fourth decade) (Ciclitira *et al*., 2001; Fasano and Catassi, 2001). However, the frequency of CD is also recognized to be more in elderly (Freeman, 1995), with a greater chance (1:10) of developing lymphoma was seen in people diagnosed over 50 years (Ruoff, 1996). Again in Iraq, CD occurs in children more than other ages (Hameed *et al*., 2016).

1.3.4-Etiology:

1.3.4.1-Environmental factors:

CD is known to be a diverse disorder where the interactions between environmental and genetic factors are well recognized (Sanz *et al*., 2011). Many environmental factors had been suggested to trigger and participate in the disease pathogenesis (Hameed *et al*., 2016) including: 1- The delivery mode (normal vaginal VS cesarean section) shows a significant influence on molding the initial composition of gut microbiota (Dominguez-Bello *et al*., 2010). 2- The type of milk-feeding and the breast-feeding duration, can also participate by influencing the gut micro environment (Silano *et al*., 2010). 3- High wheat consumption and early-life infections (Myléus *et al*., 2012). Recurrent rotavirus infection had reported to carry high risk of early childhood disease (Stene *et al*., 2006). 4- Substantial increase in intestinal Gram-negative with reduction in Bifidobacteria,(change in microbiota) has been recognized in patients with CD (Sanz *et al*., 2011). 5- Drugs like antibiotics and others that frequently used are also familiar triggers exert significant influence on the gut microbiota (Zeissig and Blumberg, 2014). 6- Subjects lifestyle and dietetic elements like alcohol and energy-filled Western mode of diet can alter IP (Massey and Arteel, 2012; Pendyala *et al*., 2012). These factors can cause mucus layer alterations, gut microbiota modulations, and mucosal damage, with consequent alteration in IP and translocation of

luminal antigens to the submucosal layer (Massey and Arteel, 2012; Pendyala *et al*., 2012), precipitating the disease in individuals with genetic susceptibility (Ménard *et al*., 2010).

1.3.4.2-Genetic susceptibility:

A recently published meta-analysis has shown that the first-degree relatives have a risk to develop CD of 7.5% (Singh *et al*., 2015), with more importance for the high concordance found in monozygotic twins reaching 75% for CD development, a number that significantly more than respective figures in other autoimmune disorders (Nistico *et al*., 2006). A lower rate is probably applied for the second-degree relatives (Fasano *et al*., 2003).

CD as a disorder has a complex mode of inheritance far of Mendelian mode, falling within both MHC and non-MHC loci. The MHC region lies on 6p21 and represent the main genetic element for CD. A plenty of genes within MHC region have immunological functions, thus setting the highest association signals recognized in various immune-mediated disorders (Palma *et al*., 2012; Kurppa *et al*., 2012). About 90-95% of patients harbor HLA-DQ2 (variant DQ2.5) encoded in one of two 1. A cis- presentation by the DQB1*0201 and DQA1*0501 genes on DR3-DQ2 allele. 2. A trans-presentation by DQA1*05 and DQB1*02 chains located on disparate haplotypes (DR5-DQ7 and DR7-DQ2) (Sollid *et al*., 1989; Lundin *et al*., 1990; Kurppa *et al*., 2012). The residual 5-10% of patients carries DR4-DQ8 alleles (Sollid *et al*., 1989; Lundin *et al*., 1990; Spurkland et al., 1992; Kurppa *et al*., 2012; Sollid *et al*., 2012). At the time in which the HLA-DQ2.5 variant has particularly higher risk for CD, the gene dosage effect of MHC has also an impact in CD with more risk in HLA homozygous individuals (Ploski *et al*., 1993; Karell *et al*., 2003). The risk of HLA genotypes for developing is about 30%, but only 1%-3% encounters the disease (Sollid *et al*., 1989; Silano *et al*., 2010). However, individuals who harbor HLA-DQ2 or HLA-DQ8

alleles are generally risk factors for disease development (Ludvigsson *et al*., 2013).

Among Iraqi celiac patients, DQ2 genotype is the most prevalent of HLA genes. DRB1*04 alleles shown to have a role in celiac disease development, thus negative result of DQ2 and /or DQ8 don't exclude celiac disease (Hameed *et al*., 2016).

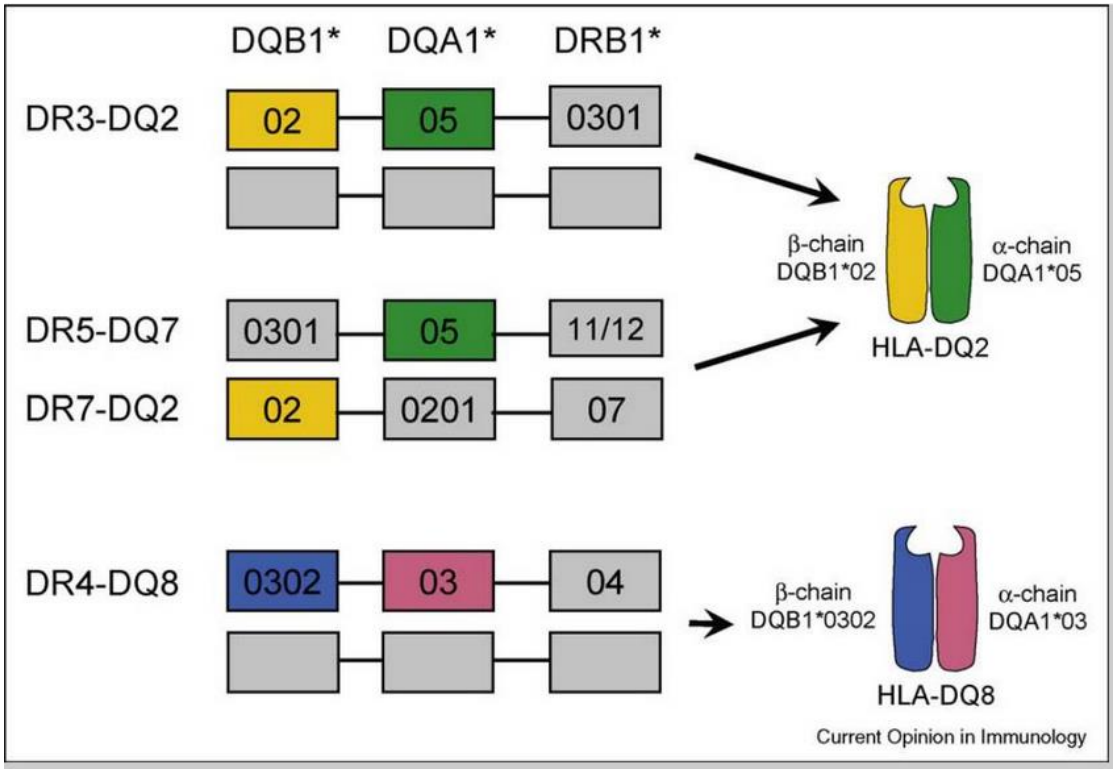

Figure (1.2): DQ2 and DQ8 (Qiao *et al*., 2009)

On the other hand, a fair number of non-HLA genes linked to CD have been determined by genome-wide association studies (Abadie *et al*., 2011).They collectively share to genetic background of the disease more than HLA. Even so, each one adds little part to disease development depending on the gene multitude (Hunt *et al*., 2008; Dubois *et al*., 2010; Trynka *et al*., 2011; Kumar *et al*., 2011).

For genes expressed in MHC I of APC, HLA B8 had been linked to CD in Algeria (López-Vázquez *et al*., 2004), Iraq (Dawood *et al*., 1981; Jabbar, 1993) and Turkey (Erkan *et al*., 1999). Another is MICA allele was reported

to be over-expressed in atypical CD Saharawi patients(López-Vázquez *et al*., 2004), and have also been found in Western countries (Martín-Pagola *et al*., 2004), while HLA-A25 reported only in Turkish children(Erkan *et al*., 1999). Regarding the CD-associated single nucleotide polymorphisms, which mostly located on a non-exon intergenic regions where they seem to exert their effect by regulation of gene expression, in particular of T and B cells (Trynka *et al*., 2011; Kumar *et al*., 2011).

A new emerging field was evolved few years ago, which explains that the changes in cellular phenotype or gene expression can be induced by epigenetic modifications, like methylation or ncRNAs (transcribed non-translated RNA molecules). The two participate in the regulation of gene expression next to transcription (Remely *et al*., 2014). Another study has elucidated an impact of microbiota on the level of methylation of the free fatty acid receptor 3 gene enrolled in metabolic process in addition to inflammatory response (Remely *et al*., 2014).

1.3.5-Association of CD with other disorders:

There are a series of conditions shown to accompany CD. These associated conditions are more frequent in CD patients than others (Barker and Liu, 2008), among these conditions, the autoimmune diseases which have frequency of about 3–10 times more in CD patients comparing to general population (Rubio-Tapia *et al*., 2008; Frost *et al*., 2009; Wouters *et al*., 2009; Sattar *et al*., 2011; Volta *et al*., 2011).This association is simply due to the sharing of a common genetic regions (HLA loci like HLA-DQ2, HLA-DR3, and other else genes involved in the development of ADs (Houlston *et al*., 1997; Smyth *et al*., 2008) .

Down syndrome, Turner syndrome, Williams's syndrome, and other genetic diseases had been seen more in CD patients (Barker and Liu, 2008). Type-

1DM is one of the most recognized and widely investigated diseases linked to CD (Akirov and Pinhas-Hamiel, 2015). Ludvigsson et al reported that type 1-DM poses 5-10 double risk for developing CD in a considerable study group of children (Ludvigsson *et al*., 2006).

Additionally, autoimmune thyroid disease such as Hashimoto's thyroiditis and Graves' disease, Sjo¨gren's syndrome, and Addison's disease are also associated with either frank or silent clinical types of CD (Collin *et al*., 2002). CD is also more likely in psoriasis patients, declaring a shared immune-linked pathogenesis (Lundin and Wijmenga, 2015). Other study reveals that many of immune or non-immune diseases of the gastro-intestinal tract can be associated with CD such as microscopic colitis, Crohn's disease, ulcerative colitis, and autoimmune liver diseases (Collin *et al*., 2002). At the time in which IgA deficiency affects 1 in every 400- 800 person of the general population, it is more frequent (2–3 %) in CD patients and 1 % in those undergoing tests for CD (McGowan *et al*., 2008; Conrad *et al*., 2012). Moreover, a number of neurological and psychiatric manifestations have frequently been found to be related to the gluten-mediated immune response, including ataxia, epilepsy and neuropathy (Jackson *et al*., 2012), and a small proportion of celiac patients, developed health-related anxiety following their diagnosis (Ukkola *et al*., 2011).

1.3.6-The gluten protein:

The term gluten is usually used to describe complex proteins in wheat (gliadin), barley (hordein), rye (secalin) that are intolerable by CD patients (Ludvigsson *et al*., 2013; Diaz-Amigo and Popping, 2013). Gluten has two major components, alcohol-insoluble glutenin polymers and soluble prolamines (gliadin monomers) (Dieterich *et al*., 1997; Nikulina *et al*., 2004), and the latter is the major protein being involved in the immunogenic reaction in CD (Shan *et al*., 2002). Glutenins include high and low molecular weight

proteins, while the gliadin family contains α-,β-,γ- and ω- proteins (Nikulina *et al*., 2004), according to their electrophoretic mobilities (Mamone *et al*., 2011). Among these, α- gliadin have been found to be the most harmful in coeliac disease (Stoven *et al*., 2012). Gluten is one of the widely used and most plenteous dietary components for majority of populations (Sapone *et al*., 2012)**.**As the wheat flour is easily obtained and because of the functional properties (visco-elastic properties) of its gluten proteins, this may interpret their wide use in food processing and food industries (Day *et al*., 2006; García-Manzanares and Lucendo, 2011). Maize and oat do not trigger immune response in majority of CD patients (Koskinen *et al*., 2009).

Characteristically gliadin has a specific sequence of peptides (proline) rendering it poorly digested in the small intestine of people with or without CD (Shan *et al*., 2002). The proline content and incomplete degradation by prolylendopeptidase in the small intestine yield long gluten-derived peptides, with two characteristic subsets, the short toxic (Barone *et al*., 2011; Camarca *et al*., 2012) and long immunogenic (Shan *et al*., 2002; Qiao *et al*., 2004). Cytotoxic α -gliadin peptides (p31-49) have no affinity to HLA-DQ2 and -DQ8 molecules, instead they cause IL-15 upregulation (Kagnoff *et al*., 2007; Schuppan *et al*., 2009; De Re *et al*., 2013).

In contrast, the long 57-89 peptide α-gliadin fraction has a variety of characteristics (Shan *et al*., 2002). This immunogenic peptide contains 6 partly engaged amino acid sequences for HLA-DQ2-binding and is also considered as a coeliac superantigen, a protein that circumvents normal processing and directly links the TCR of T-cells with the MHC class II molecule , produces a powerful immune response(Schuppan *et al*., 2009; Fasano, 2011).

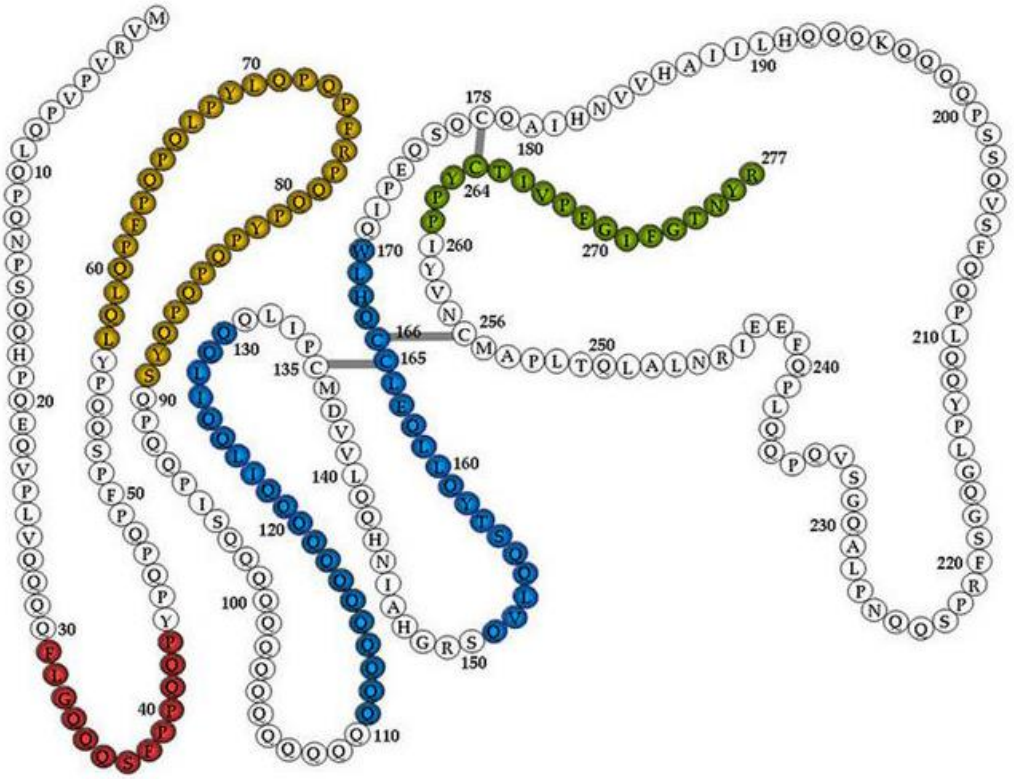

Figure (1.3): charting of α-gliadin ingredients: Peptides with cytotoxic activity31–43 and 31–49 are shown in red, immune-modulatory activity 57–89 (33-mer) in yellow, zonulin releasing and gut permeating activity 151–170 in blue, CXCR3-binding peptide111–130, and IL-8 releasing peptide 261–277 in dark green (Fasano, 2011).

The innate and adaptive immune responses triggered by gliadin peptides (van de Water *et al*., 2010; Roshan *et al*., 2011) mounts duodenal and to a less degree of the proximal jejunal inflammation,.(Van Bergen *et al*., 2015).

Gluten is also responsible for a set of immune-based disorders other than CD such as dietary wheat allergy and gluten sensitivity (Sapone *et al*., 2012, Sicherer and Sampson, 2014; Czaja-Bulsa, 2015), in addition to some neurological disorders have been shown to join CD, like ataxia, epilepsy, and neuropathy (Jackson *et al*., 2012; Gerace *et al*., 2017).

Worldwide regulations of gluten-free labeling had been fulfilled to protect consumers at risk where most of these are depending on the recommendations of Codex Alimentarius Standard 118–1979, which defines foods with gluten level not more than 20 mg/1kg in total as "gluten-free" and those with 100 mg/1kg labeled as 'low-gluten' (Codex Alimentarius, 1979 rev. 2008). By the

current Codex, foodstuffs convening these criteria can be labeled as a ‘gluten-free food’ or ‘gluten-low food’ respectively (Codex Alimentarius, 1979 rev. 2008; FDA., 2013).

1.3.7- Physiology of small intestine:

1.3.7.1-The intestinal epithelial barrier:

Anatomically the wall of small intestine contains four layers arranged as, mucosa, submucosa, muscularis and serosa (larke, 2009; Hering *et al*., 2012).

The human intestinal mucosa or so-called intestinal barrier (IB) is composed of a surface mucus layer (simple columnar epithelium), and a submucosal immune cell containing lamina propria (LP) (Helander and Fandriks, 2014). The epithelial layer coats a large area of about 400 m^2 (Brandtzaeg, 2011), which is folded into up evaginations(villi) and down invaginations(crypts). It separates the host, sterile sub-mucosal side from the external environment, luminal side (Madara, 1990; Cummings *et al*., 2004; Bischoff *et al*., 2014),where the interaction of the external "physical" barrier and the inner immunological one maintains equilibrated permeability of the gut (Scaldaferri *et al*., 2012)**.**

The intestinal epithelium serves two essential biological functions: 1. Barrier function occluding the passage of enteric pathogens and their toxins and other foreign particles into submucosa. 2. Selective filtration of food particles, and by that permeates the essential dietary nutrients, electrolytes and water from the intestinal lumen into the blood stream via either transcellular or paracellular pathway (Mandel *et al*., 1993; Groschwitz and Hogan 2009; van der Flier and Clevers, 2009; Suzuki, 2013).

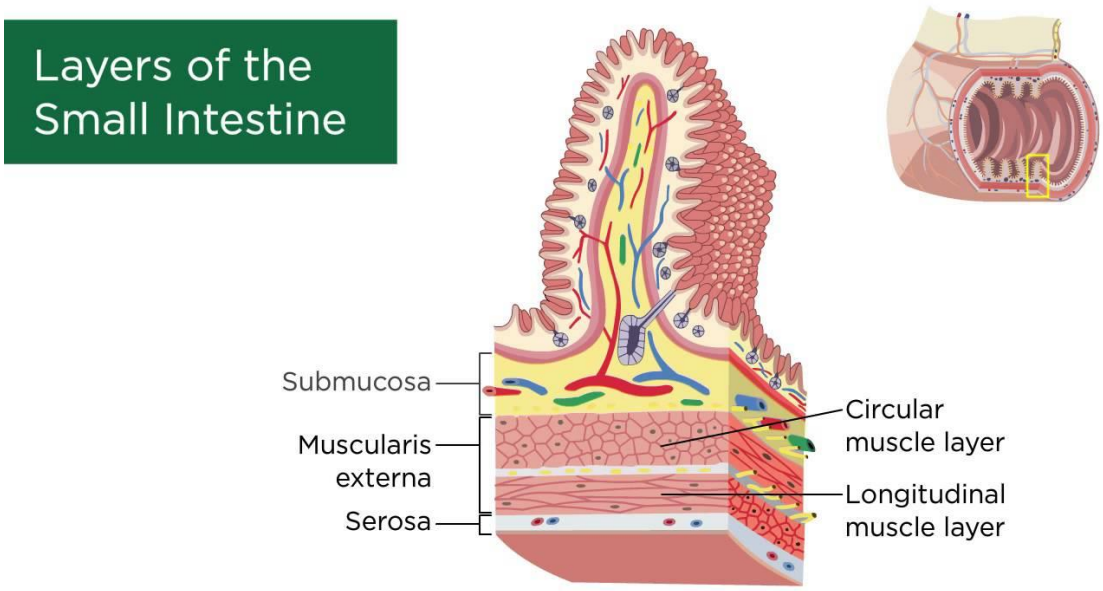

Figure (1.4): Small intestinal wall structure.

Other structures including blood vessels, enteric nervous system, and smooth muscle cell layers, all play a role in IB function by their ability to induce especial defense mechanisms in case of danger (Groschwitz and Hogan 2009; Bischoff, 2011; Wada *et al*., 2013).Generally, the role of IB rely on the structural organization of three components: the specialized epithelium composing of mucus- and antimicrobial peptides-producing cells; the intestinal microbiota; and the immune cells of gut-associated lymphoid tissue, (Moens and Veldhoen, 2012; Antoni *et al*., 2014; Bischoff *et al*., 2014). Plasma cells producing sIgA which has an important protective function in intestinal immunity by separating a potentially harmful, luminal antigens, preventing pathogens from adhering to the intestinal epithelium, and blocking their toxins (Menard *et al*. 2010; Horton and Vidarsson 2013). Hence the mucosal epithelium can be considered as a translator between the immune system and the gut microbiota (Martini *et al*., 2017).

1.3.7.2. -Composition of intercellular tight junction:

A collection of intercellular junction proteins accomplish the epithelial barrier function including the apical tight junctions, underneath adherens junctions,

and desmosomes (Farquhar and Palade, 1963; Laukoetter *et al*., 2006; Vanheel *et al*. 2013).). Beside the important role in controlling epithelial adhesion and in turn barrier function, the intercellular junctional proteins also play a substantial role to regulate epithelial homeostasis such as cell generation, immigration, and differentiation (Schlegel *et al*., 2010; Nava *et al*., 2013; Kamekura *et al*., 2015).

Tight Junctions (TJs): TJs inhabit the interface between the apical and basolateral plasma membrane zones. They contribute in cell polarity setting in addition to controlling the transport of micronutrients and fluids in the paracellular pathway (Cereijido *et al*., 1998; Tsukita *et al*., 2001).

Four protein components constitute the TJs and act to seal adjacent epithelial cells producing a physical barrier between endothelial and epithelial cells (Anderson and Van Itallie, 1995) 1- Occludin (Furuse *et al*., 1993), 2-Claudin family which comprises of 24 proteins with tow subsets, one reduces permeability, the sealing claudins and other increasing permeability, the pore-forming claudins (Kotler *et al*., 2013), 3-Tricellulin, which makes tricellular connections (Ikenouchi *et al*., 2005), and 4-Junction adhesion molecule A(JAM) (Mandell *et al*., 2005). A direct interaction of these transmembrane junction proteins with membrane-associated zonula occludens (ZO)-proteins (ZO-1, ZO-2, and ZO-3) gives the shape of the peri-junctional actinomyosin ring or F-actin ring (Nusrat *et al*., 2000; Lal-Nag and Morin, 2009; Bauer *et al*., 2010; Marchiando *et al*., 2011).

ZOs hold the TJ flexibility and permeability (Bauer *et al*., 2010) and coordinate the transport of ions and small particles (Halpern and Denning, 2015).The intercellular TJs are dynamic structures that can constantly reconstruct their building blocks with a relatively rapid turnover to react with external stimuli and optimize intestinal integrity (Tsukita *et al*., 2001; Forster, 2008; Halpern and Denning, 2015; Zihni *et al*., 2016).

b- Adherens Junction(AJ): This junctional complex had a role in initiating and maintaining epithelial cell–cell contact, regulating the actin cytoskeleton, and mediating intracellular signals and transcription regulation(Ivanov and Naydenov, 2013).

c- Desmosomes have visualized in ultra-structural studies as spot fusions among epithelial cells. These junctions are lying below the TJs and AJs within the lateral membrane of cells (Farquhar and Palade, 1963; Nekrasova and Green, 2013).

Intestinal barrier integrity and maintaining of the assembly state is not confined to TJs, but relied on crosstalk among TJs, AJs, and desmosomes and additive effects of each component (Holthofer *et al.*, 2007; Hartsock and Nelson 2008).

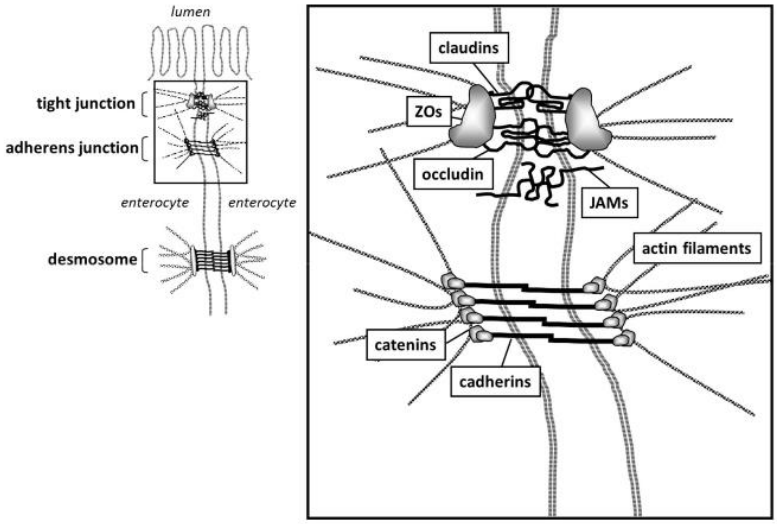

Figure (1.5): Intercellular tight junction between epithelial cells (Suzuki *et al*.,2013).

1.3.7.3-The mechanism of transport via the intestinal barrier:

Intestinal barrier and intestinal permeability are two different sides related to a single anatomical structure (Clarke, 2009; Hering *et al.*, 2012) IP in common sense is the measurable trait of the IB(Brandtzaeg, 2011; Bischoff, 2011).

The transportation of molecules from the intestinal luminal side into the lamina propria is carried out through two essential mechanisms:

1-The paracellular diffusion: TJs in between intestinal epithelial cells are the rate-limiting agent for permeation of molecules via paracellular route (Adson *et al*., 1994). They pose small pores in the epithelial villi and larger pores in the crypts (Powell, 1981; Fihn *et al*., 2000). Several physiological stimuli and signaling pathways can enhance TJs to adjust their tightness there by modulate the para-cellular macromolecules trafficking into mucosal cells (Harhaj and Antonetti, 2004). The paracellular space dimensions are 10–15 Å proposing that solutes more than 15 Å which nearly 3.5 kDa molecular radius (including proteins) are usually not filtered through this pathway (Fasano and Shea-Donohue, 2005). Moreover, claudins by ion-selective pores formation within the TJs designate paracellular permeability to small molecules. A recent study has been revealed claudin-2 channels to be gated with opening and closure on a sub millisecond timescaling (Weber and Turner, 2017).

2-The transcellular transport pathways are accomplished by:

A. Transcytosis (endo- and exocytosis) mediated or not by membrane receptors (Menard *et al*., 2010) permit the access of macromolecules from intestinal lumen into the sub mucosa by vesicular transport inside the enterocytes (Menard *et al*., 2010), where they undergo endolysosomal degradation; and basolateral release in the lamina propria. Microfold cells present over the mucosal lymphoid aggregates and enhance transcytosis of large particles across the epithelium (Stadnyk, 2002; Lavelle *et al*., 2010). During physiological conditions, most of antigens (~90%) transported through the transcellular pathway, as it is a well-organized pathway that achieves antigens lysosomal degradation into small non-immunogenic peptides (Fasano, 2011).
B. Formation of an immune-complex with immunoglobulin(Ig), including isotypes IgA, IgM and IgG (Horton and Vidarsson, 2013), however IgA is the most commonly present Ig in the mucosal layer

and the mechanism by which IgA polymers are transported in the intestine a involves receptor-mediated basal-to-apical secretion (Menard *et al*., 2010; Horton and Vidarsson, 2013).

An ex vivo analyses of intestinal biopsies in Ussing chambers demonstrated that transcellular passage of intact 19-mer and 33-mer gliadin peptides was much more in patients with active CD than patients on GFD (Heyman *et al*., 2011) However, another recent study reveals that exposure to gliadin causes a raise in IP in all individuals, even those not affected with CD (Hollon *et al*., 2015).

1.3.7.4-Structure and function of Zonulin:

Fasano and his team had discoverd zonula occludens toxins (Zot), an enterotoxin which is able to reversibly open intracellular tight junctions (Fasano *et al*., 1991). Subsequently robust studies had been conducted for the many years ago to identify the TJ physiologic modulators, and these studies end successfully with the identification and description of zonulin (Tripathi *et al*., 2009). The combination of Ussing chamber experiments and anti-Zot antibodies gave rise to the identification a ~47 kDa Zot-analog in human, named zonulin (Fasano *et al*., 2000) and ex vivo studies demonstrate that human zonulin is only protein known to date capable of increasing permeability in both jejunum and ileum (Wang *et al*., 2000).

Later on, zonulin was described as the precursor of HP2; plasma glycoproteins that encompassed both alpha- and beta-polypeptide chains (heterodimeric) with disulfide bonds covalently bind them. Only the beta-chain is glycosylated (Haugen *et al*., 1981), and has one form of about 36 kDa, while the alpha-chain has two forms (alpha 1about 9 kDa, and alpha 2 about 18 kDa), so the presence of alpha-1 or alpha -2 or both chains in the pre haptoglobin give rise to three forms HP(1–1), HP(2–1), and HP(2–2) (Tripathi et al., 2009), however this particular type of polymorphism has been seen only in humans (Levy *et al*., 2010). The genes responsible for

Haptoglobin (HP) coding lie on chromosome 16q22, and HP2 produced by a non-homologous intragenic duplication of exons 3 and 4 of HP1(Maeda *et al*., 1984) resulting in these phenotypes . Serum HP can be detected and measured in all mammals (Levy *et al*., 2010). The human Zot analog intestinal protein was named zonulin owing to its action on the zonula occludens proeins (Wang *et al*., 2000). However, the actions of both Zot and zonulin are reversible (Fasano *et al*., 2000; Fasano, 2011). The intact single-chain precursor of zonulin protein plays a role in regulating IP by trans activating the epidermal growth factor receptor (EGFR) through proteinase-activating receptor 2 (PAR2) activation (Tripathi *et al*., 2009), while the cleaved 2-chain format it is transformed into haptogloblulin, a protein with antioxidant, heme (iron)-binding, and antimicrobial properties. Haptoglobin has no effect on TJs (Levy *et al*., 2002; Fasano, 2012).

Zonulin can be utilized as a biomarker of impaired barrier function for multiple neurodegenerative, autoimmune disease, and tumors and can be a robust therapeutic target to treat these debilitating disorders (Fasano, 2012).

1.3.7.4.1- The mechanism of action of zonulin:

Under the steady state, TJ proteins are linked in both hetero- and homophilic protein-protein interactions which safeguard integrally closed TJs (Fasano and Shea-Donohue, 2005; Fasano, 2012). In genetically susceptible individuals, this powerful regulation of macromolecules trafficking is abolished awing to prolonged uregulation of zonulin, with consequent increase in the IP (Fasano, 2008). An enteric pathogen or a specific gliadin peptide would stimulate chemokine receptors (CXCR-3) causing zonulin release in MyD88-dependent pattern (MyD88 is a master adapter molecule in the signaling cascade of Toll-Like Receptors) (Lammers *et al*., 2008). The activated zonulin pathway in turn transactivates EGFR through PAR2 (Tripathi *et al*., 2009). Then the protein completes the activation pathway that in combination with other intracellular signaling events affecting TJ resulting in occludin displacement,

actin polymerization, and myosin-1C phosphorylation (Papp *et al*., 2008; Tripathi *et al*., 2009; Jorge *et al*., 2010; Vanuytsel *et al*., 2013). These events as a whole contribute to a thorough junctional complex rearrangement that eventually lead to transient disassembly of TJs. However this effect is reversible and as the zonulin signaling is over, the TJ steady state spontaneously resumed (Tripathi *et al*., 2009; Fasano, 2012).

1.3.7.4.2-Environmental stimuli causing intestinal zonulin release:

There are variable intestinal stimuli that can induce zonulin release, among which dietary gluten and exposure to bacteria are the most potential triggers that have been so far known (Lammers *et al*., 2008; Fasano, 2011), bringing a zonulin release from intact intestinal epithelial cell (El Asmar *et al*., 2002; Clemente *et al*., 2003). Enteric pathogens cause IB dysfunction, thus play a substantial role in the pathogenesis of wide range of diseases, including autoimmune, allergic, and inflammatory diseases (Fasano, 2011). This might be due to their ability to produce Enterotoxins (El Asmar *et al*., 2002), which enhance zonulin-forced unlock of the paracellular route that per se may be part of host defense program, by which can flush out microbial particles and thus participating in the innate body immunity toward bacterial settlement in the gut (Fasano, 2012).

On the other hand, gliadin is also has been proposed to induce zonulin release (Clemente *et al*., 2003; Thomas *et al*., 2006) only when be in contact with the epithelial apical surface (Clemente *et al*., 2004; Lammers *et al*., 2008), where engaging of the chemokine receptor CXCR3 occurs. CXCR3 receptors had demonstrated to be expressed at the luminal side of the intestinal epithelium and over-expression of these receptors was revealed in CD patients (Lammers *et al*., 2008).

At the time gluten is restricted from the diet, circulating zonulin levels decrease, the intestine restores the baseline barrier function, with normalization of the autoantibody titers, arresting of the autoimmune process, and consequently complete healing of the intestinal damage (that represents the biological outcome of the autoimmune process) (Fasano, 2012).

1.3.7.5-Gut-Associated Lymphoid Tissues (GALT):

The GALT is the major immune tissue in the body and is the primary route by which antigens exposure take place (Moog, 1981; Mestecky *et al*., 2015).The mucosal epithelium is the main barrier separating gut luminal antigens from the largest lymphocytes population of the GALT. However, the GALT is primarily described to be a tolerogenic climate (Mowat, 2003; Commins, 2015) or a containment system that motivates systemic tolerance toward luminal antigens by a mechanism involves the regulatory T cells induction and secretion of polymeric IgA (Bjorkman et al., 1987; Cuvelier et al., 1987; Mirenda et al., 2005; Song et al., 2006).

Oral tolerance is the active process by which orally ingested antigen does not induce an immune response (Commins, 2015), thus administration of the dietary proteins may bring about modulation of the host immune system, a mechanism designated oral tolerance by bystander suppression (Bayrak and Mitchison, 1998; Weiner *et al*., 2011; Pabst and Mowat, 2012).

1.3.7.5.1- The GALT organization:

The GALT is organized within two compartments:

1-The diffuse lymphoid tissue: diffusely dispersed within submucosa (lamina propria) below the epithelial layer, and also through the same mucosal layer. This compartment is predominated by plasma cells which mostly IgA-producing cells and CD4+T lymphocytes which more common in this compartment comparing to CD8+ T cells. The CD4+ T cells might

functionally be divided into effector T helper (Th) cells and T regulatory (Treg) cells (Hill and Artis, 2010; Russell and Ogra, 2010). In addition, the submucosa contains a plenty of macrophages and dendritic cells (DCs), which have locally adapted to the antigen-enriched environment (Mestecky et al., 2005; Garrett *et al*., 2010; Hill and Artis, 2010; Russell and Ogra, 2010). As DCs are one of professional antigen presenting cells, they play key roles during the induction phase of immune responses, giving the outcome directed toward either protective immunity or tolerance. (Kalinski *et al*., 1999; Matteoli *et al*., 2010; Pletinckx *et al*., 2011)

The innate immune T-cells located between epithelial cells of intestinal mucosa are called intestinal epithelial lymphocytes (IELs) (Dalton *et al*., 2006). These cell populations are infrequent elsewhere in the immune system, and most (about three quarters) of IELs are CD8+ "cytotoxic T cells" (Ke *et al*., 1997; Mayer, 2005; Perrier and Corthesy, 2011). Of which, some specialized IEL carrying the TCR gamma delta (γδ IEL) have been reported to support IB function by occluding bacterial transport (Dalton *et al*., 2006), production of antimicrobial factors (Ismail *et al*., 2011), and through interactions with occludin, a TJ protein (Edelblum *et al*., 2012). TCR γδ IEL can also promote epithelial repair and regulate inflammatory processes (Chen *et al*., 2002; Yang *et al*., 2004). It has been noted that a depletion of γδT cells has been reported to weaken oral tolerance induction (Ke et al., 1997). On the other hand a rise in IELs cytotoxic T cell receptor (TCR) αβ+ subset shown to be commonly correlated to the existing atrophy of intestinal villi (Kutlu *et al*., 1993), and the malignant switching of IELs is characteristic for CD (Stein *et al*., 1988; Spencer *et al*., 1989), which proposes the involvement and un usual activation of these cells in CD (Abadei *et al*, 2011).

A considerable number of resident natural killer T cells (NKTs) and natural killer (NK) cells also present in this part. They are dependent on enterocyte expression of non-classical MHC molecules to participate in distinguished mechanisms of antigen presentation. NK cells act together with T cells to monitor the enterocytes and may precipitate enterocytes apoptosis in occasions of infection or IL-15upregulation (Jabri *et al*., 2000; Srivastava, 2006).

2-**The organized lymphoid follicles:** Like Peyer's patches (PPs) are aggregates of lymphoid tissue with overlying epithelial dome containing specialized epithelial cells called microfold (M) cells which provide effective delivery of luminal antigens to the underlying immune cells (Menard *et al*., 2010), and they are more abundant in the terminal ileum, where they collect to produce apparent macroscopic clusters. No afferent lymphatics for these follicles they have the ability to sample and respond to several microbial and dietary luminal antigens. Draining of the PPs via efferent lymphatics pass to the mesenteric lymph nodes (MLNs), where further amplification of immunological signals occurs (Menard *et al*., 2010; Abadei *et al*, 2011).

1.3.7.5.2- Interleukin 15(IL15) cytokine:

IL-15 has been discovered in 1994 (Burton *et al*., 1994; Grabstein *et al*., 1994), with enormous expansion of the IL-15 action from a mere growth factor of T-cell to pleiotropic cytokine that substantially plays in all innate and adaptive immune cells. IL-15 is one of type I cytokines family involving IL-2, IL-4, IL-7, IL-9, IL-21, and IL-15 (Fehniger and Caligiuri; Rochman *et al*., 2009). Both IL-2 and IL-15 have structural relativeness as they share two receptor subunits, CD122 (β chain) and CD132 (γ(c) chain), nevertheless the expression manner and physiological actions of IL-2 and IL-15 individual receptor certainly different (Votavova *et al*., 2014).

1.3.7.5.2.1- IL15 receptors, presentation and expression:

IL-15 is unrivaled cytokine, because it is not excreted and might be up regulated on the surface of every body cell in response to inflammatory and stressful conditions (trans- presentation delivery mechanism). The well-studied source of IL15 are the DCs (Colpitts *et al*., 2013), and the 'private' IL-15Rα chain is required for IL15 expression on the cell surface (Bergamaschi *et al*., 2008; Bergamaschi *et al*., 2009). Given the intracellular expression and association are mandatory for the cytokine activity hence, the same cell would express both IL-15 and the IL-15Rα (Sandau *et al*., 2004; Burkett *et al*., 2004). In the IL-15 producing cells, IL-15Rα is expressed in the endoplasmic reticulum and links to IL-15 with high affinity (Duitman *et al*., 2008; Votavova *et al*., 2014), then transported to the surface, and to be trans-presented to responding cells like CD8 memory T cells, NK cells, and IELs (Duitman *et al*., 2008) expressing IL-15Rβ and γc (CD122/CD132 dimeric receptor); So IL-15 signaling pathway shows a cell contact-dependent pattern (Dubois *et al*., 2002; Bergamaschi *et al*., 2008; Bergamaschi *et al*., 2009).

At the time IL2 works as a free molecule after its secretion by exclusively stimulated T cells, the IL-15 expression is primarily by myeloid cells and acts in a cell surface-liked manner "trans-presentation mechanism" (Bergamaschi *et al*., 2009; Abadie and Jabri, 2014; Votavova *et al*., 2014).

However in human and murine IL-15 with IL-15Rα may exist in soluble form (cis-presentation) in addition to membrane bound (trans- presentation). Thus, a multitude of IL-15Rα/IL-15 soluble complexes could detach from the cells surfaces (Mortier *et al*., 2008; Bouchaud *et al*., 2010; Bergamaschi *et al*., 2012).

IL-15 expression must be tightly regulated as the overt lymphoproliferative disorders observed when IL-15 production is deregulated and excess of cytokine to act (Fehniger *et al*., 2001).

1.3.7.5.2.2-The biological functions of IL15:

IL-15 has extended biological functions which are fundamental for the function and maintenance of multiple cell types (Abadie and Jabri, 2014). A fine-tuned IL-15 expression at multiple levels is importantto guarantee that the plentiful cytokine functions can undertake, as an imbalanced high IL-15 expression is seen in multiple autoimmune inflammatory diseases (Waldmann, 2004). The IL-15 exerts a significant actions in the gut of celiac patients, where excess of cytokine produced, including: 1. Anti-apoptotic and a pro-in flammatory cytokine. 2. The ability to stimulate the expansion and activation of IELs (Hue *et al*., 2004; Malamut *et al*., 2010). 3. Endanger local immunoregulation (Ben Ahmed *et al*., 2009; Depaolo *et al*., 2011). 4. IL-15 promotes IFN-γ production which in turn results in activation of intraepithelial type 1 innate lymphoid cells (Fuchs *et al*.,2013), giving the perception about important defensing action exerted by IL-15 to protect gut against invading pathogens (Di Sabatino *et al*., 2011; Perera *et al*., 2012). 5. In vitro studies also proposed that IL-15 can enhance the proliferation and differentiation of active B cells, thereby can modulate B-cell activities as well as immunoglobulin production (Armitage *et al*., 1995; Park *et al*., 2004). 6. It was also demonstrated that IL-15 evokes expression of both CD25 and FOXP3 "key transcriptional regulators of Treg cells" in peripheral CD4+CD25 T cells in state lacking antigenic activation (Imamichi *et al*., 2008).

7. Besides multiple effects of IL 15 on lymphocytes, it also exerts an effect on neutrophils, dendritic, and mast cells through frustrating their death (apoptosis) (Hoontrakoon *et al*., 2002; Dubois *et al*., 2005). However other study reported an implication role of DCs in IL-15 trans-presentation to a plenty immune cell subsets, and also suggested the action of other cellular sources of IL-15 in conjunction with DCs to preserve gross peripheral homeostasis (Castillo *et al*., 2010; Colpitts *et al*., 2013).

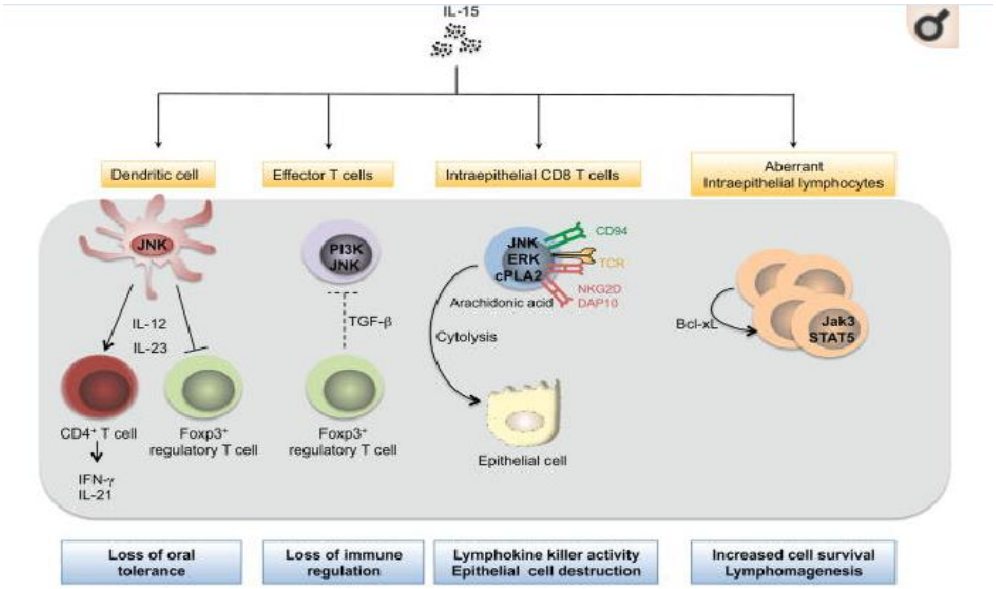

Figure (1.6): Action of IL15 in celiac disease pathogenesis (Abadie and Jabri, 2014).

1.3.7.5.2.3-The interaction between gliadin fractions and IL15 in CD:

IL-15 is the master key in CD pathogenesis and several data had shown its approved action in different disease stages and the extended effect on various cell lineages and immune responses (Jabri *et al*., 2000; Roberts *et al*., 2001; Green and Jabri, 2003). Patients with active-phase disease demonsrate IL15 up-regulation in both mucosa and submucosal layers (Di Sabatino *et al*., 2006; Harris *et al*., 2010) by the action of the toxic gliadin peptide 31-43 (mer 31–43). Gliadin considered as a stressful molecule which strongly enhance MICA molecules expression on epithelial cell surface upon gliadin challenge (Rossi *et al*., 2005).

This gliadin- induced production of IL15 by DCs, macrophages, and epithelial cells in turn activates cytotoxic cells (CD8+) IELs independent of TCR specificity (Han *et al*., 213; Kim *et al*., 215). Enhancement of IELs cytolytic activity by IL15 *via* the increasing their expression of the NKG2D and CD94/NKG2C and directing IELs to damage intestinal epithelial cells express stress-ligands including MICA and MICB. Moreover IL-15 acts significantly to raise IELs resistance to the inhibitory actions of counter-regulatory mechanisms of T-reg cells (Han *et al*., 213; Kim *et al*., 215). The end result of

the up-regulated IL-15 is apoptosis of epithelial cells (villi damage), disintegrated IP and passage of various antigens, including 33-mer (immunogenic) gliadin and perpetuating the adaptive immune response in the lamina properia (Abadie and Jabri, 2014). Hence the toxic gliadin peptide is capable of initiating both "stress" and "innate immune" responses plus IL-15 key players in the gut of celiac patients mediating these processes (Maiuri *et al*., 2003).

1.3.7.6- Gut microbiota:

The intestines inhabit a great number of bacterial colonies in the body, which by far immensely more than the host body cells (Eckburg *et al*., 2005; Sender *et al*., 2016). Normally intestinal colonization with microbes occurs within 24 hours after birth from both the birth canal and the environment (Eckburg *et al*., 2005). This colonization of microbiota is usually confined to the external "loose" mucus, leaving the internal "adherent" mucus is vastly freed from bacteria (Johansson *et al*., 2009). Another point is the composition and function of intestinal microbiota which have a crucial balancing effect on the host's conditions (health and disease). One study on CD patients who have persistent symptoms in spite of a long gluten restriction, have analyzed the duodenal microbiota and revealed a low number of Bacteroidetes in that patients (Wacklin *et al*., 2014). Yet the interactions between the host and microflora are important for a proper development of intestinal barrier structure and function (Kim *et al*., 2010), as the intestinal epithelium may be considered as a translator between the immune system and the microbiota (Martini *et al*., 2017). Although gluten is essential in the activation cascades causing CD, it is evident that disturbed equilibrium between the intestinal epithelium and microbiota could precede the specific gliadin- induced immune response. Especial genetic elements such as "HLA-DQ2/-DQ8" alleles or other non-MHC genes are likely to determine the CD-associated microbial fingerprint (Cukrowska *et al*., 2017).

Dysbiosis has been proposed to play a role in autoimmune disease by modifying the normally balanced inflammatory \tolerogenic ingredients of the microbial colonies, and in turn, the local immunological response (Kamada *et al*., 2013), thus intestinal dysbiosis can be regarded as sign of multiple immunological diseases (including celiac disease) , as raised pathobionts number might induce the pro inflammatory cascades, evoke "gluten tolerance" breakdown, and declare CD (Kurashima *et al*., 2013; Cukrowska *et al*., 2017).

1.3.7.7- The enzyme tissue Transglutaminase:

Transglutaminases (TGs) are enzymes with calcium-dependent activity related to (protein-glutamine γ-glutamyltransferases family) and catalyze the binding of a "glutaminyl residue" with a "lysyl residue". To date eight of human TGs are at least known, as recent studies have mentioned their actions in multiple disorders (Martin *et al*., 2012). "Tissue" transglutaminase (TG2) is the most copious member and widely expressed of the transglutaminase proteins (Di Sabatino *et al*., 2012), and its localization in different cellular parts is influenced by alterations in the cellar response to different stimuli or stress (Park *et al*., 2010; Piacentini *et al*., 2014). TG as multifunctional protein with ubiquitous expression is involved in a various biological processes, either as a Ca^{2+}-dependent enzyme or by forming complexes with other proteins (Lorand and Graham, 2003). TGs produced in the cytosol of host cells, and much of the enzyme, however, is shuttled to the extracellular compartment via an unconventional pathway (perhaps recycling endosomes) (Zemskov *et al*., 2011). In the extracellular compartment, TG2 is shown to 1. Participate in matrix proteins cross-linkage 2. Interact with fibronectin (Upchurch *et al*., 1987; Hoffmann *et al*., 2011) 3. Affect the cell adhesion, differentiation, and survival (Iismaa *et al*., 2009).

1.3.7.7.1- TG2 enzymetic activity:

A plenty of enzymatic activities have been reported, however well-known catalyzing activity of TG2 is the Ca2+-dependent selective modifications of glutamines either by transamidation, in which covalent linkage of the target glutamine to an amine of lysine residue, or by deamidation, where conversion (hydrolysis) of the glutamine into glutamic acid(negatively charged) occurs (Lorand and Graham, 2003). The enzyme TG2 targets the peptide glutamine in sequence-dependent mode (glutamine residues in the sequence QXP is the preferred one) (Vader *et al*., 2002; Fleckenstein *et al*., 2002). Interestingly, such component is usually found in gliadin, therefore are excellent substrates for this enzyme. A plenty of peptides found in a gluten digest, but the best TG2 substrates are those peptides recognized by activated T cells proposing that TG2 has a role in "epitope selection" of these cells (Dorum *et al*., 2010).

1.3.7.7.2-The role of TG2 in celiac disease:

A variety of human disorders reflect TG2 implication in their pathogenesis, of which the role of TG2 in CD is well studied and recognized. By gluten peptides deamidation, TG2 give the generation of immunogenic epitopes, the best identified by activated T cells (celiac $CD4^+$ T cells) within grooves of HLA-DQ molecules (Dieterich *et al*., 1997; van de Wal *et al*., 1998). It is seldom coincident where TG2 is implicated in the formation of $CD4^+$ T-cell epitope and at the moment being a target for autoantibodies. Stamnaes and his colleagues significantly explain how this self-antigen being a target of the robust B-cell response noted in CD. They demonstrated that the enzyme show self- cross-link, creating a covalently bound self-multimers even in presence of multiple competing substrates (amine- or glutamine-containing substrates). Gluten peptides which ready for incorporation into TG2-multimers, sufficiently activate TG2-specific B cells. Hence, the enzyme multimers and gliadin peptides shape the design that can be effective antigenic motif

involved in breakdown of B-cell tolerance in CD (Stamnaes *et al*., 2015). Appreciation of this interaction is one of the main defying for getting a complete comprehension of how gluten induces tissue damage and remodeling of the intestinal mucosa (Lionetti *et al*., 2014).

1.3.7.7.3- Autoantibodies to tissue Transglutaminase 2 enzyme:

Previous studies had identified that the core domain of TG2 has two adjacent Ca^{2+} binding sites (S4 "aa 151–158" and S5 "aa 433–438"), among the two, (S4) robustly marks antigenic properties for antibodies in CD (Király *et al*., 2009). The finding of these conformational epitopes on the major auto-antigens and their implication in celiac presentation favor that the autoantibody response affect the pathogenic process of the disease. These identifications might be of interest to design more studies that may bring the light to the therapeutic potential interfering with the effects of celiac antibodies (Simon-Vecsei *et al*., 2012). Another point deserves viewing is the TG2-specified B cells are activated by gliadin -specific ($CD4^{+}$) T cells next to uptake and display of TG2-gliadin complexes on the surface of Bcells (Sollid *et al*., 1997; Sollid and Jabri, 2013; Sollid *et al*.,2015). The action of TG2-specified B cells as APCs for the anti-gliadin T cell response might also be a novel strategy for CD treatment by clipping this amplification loop (Di Niro *et al*., 2012). Auto-antibodies toward TG2 are a key mark of CD, and the anti-TG2 IgA are first-line diagnostic tool, with sensitivity and specificity more than 95% (Rostom *et al*., 2005). Even in rare seronegative cases, anti-TG2 antibodies shown to be generated, which confirmed in small intestinal biopsies containing antibodies deposits (Salmi *et al*., 2006). IgA differs from IgM, as the latter can stimulate the classical pathway of complement thereby plays a role in CD inflammatory process (Halstensen *et al*., 1992) in particular IgA-deficient patients (Borrelli *et al*., 2010).

On average, the lesion of untreated celiac patients may contain a 10% of IgA-producing cells with TG2 reactivity (Di Niro *et al*., 2012). Nevertheless, other

studies reveal no clear effect described for these antibodies as their role in celiac might be due to the action of antibodies as antigenic receptors on the surface of B cells instead of being free (soluble) Igs (Stamnaes and Sollid. 2015).

1.3.8. -The pathogenesis of celiac disease:

It is a multifaceted, including genetic, environmental (gluten, intestinal infections), and immunological factors as demonstrated in figure (1.7) (Stein and Schuppan, 2014). Abnormal processing of gluten peptides by mucosal cells might cause excessive activation of the local immune system and precipitate the disease (Heyman *et al*., 2011).

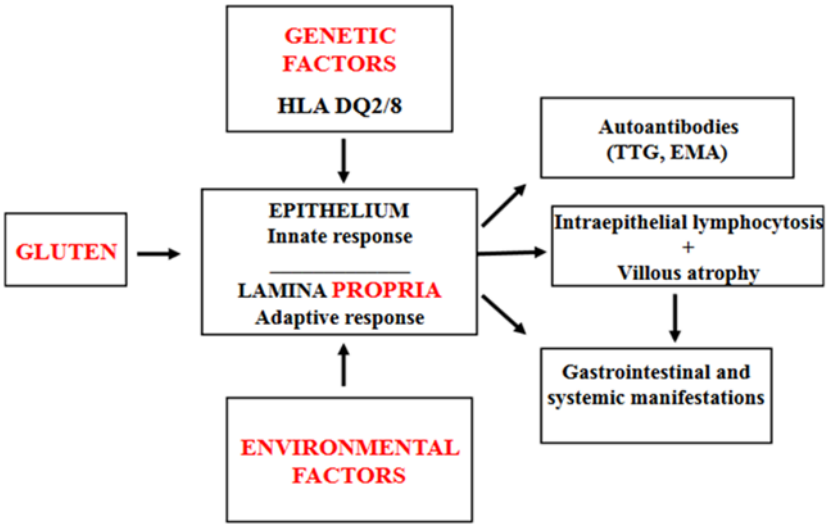

Figure (1.7): Pathogenesis of celiac disease (Peter *et al.*, 2015).

This is a new model that modifies the previous theories (molecular mimicry and/or the bystander effect) regarding autoimmunity, but instead, a continuous stimulation by environmental triggers (non-self-antigens) looks important to perpetuate the pathgenesis (Fasano, 2008; Fasano, 2012). Early in the disease, the main events probably to outrun the activation of the adaptive immune response are: 1. Alteration in digestive enzymes processing, 2. Increasing IP due to zonulin release, 3. Activation of innate immunity

(Fasano, 2011; Sapone *et al*., 2012). The innate immunity has a substantial effect in evolving CD as the uncontrolled gliadin transport via intestinal routes might result from early stimulation of non-specific (innate) immune response, relying on the functional properties of the epithelial cells and IELs distributed throughout the mucosa (Jabri and Abadie, 2015). A previous data revealed that the innate immunity has a complementary role coordinated by the pro inflammatory cytokine IL-15 brings the IELs expansion, reinforces the later lymphocytes to guide an autoimmune attack against the mucosal epithelium, and fosters the beginning of T lymphomas. IL-15 also enhances expression of NKG2D/ CD94 on IELs, so induce apoptosis of MICA-expressing mucosal cells. More IL-15 frustrates the Treg cells counter-regulatory actions and prohibit TGF-β regulatory cascade (Meresse *et al*., 2008; Han *et al*., 213; Kim *et al*., 215). Sjöberg and coworkers hypothesized that the defeat in promoting and/or preserving tolerance to dietary gluten that leading to CD might be due to activation of pro-inflammatory cytokine producing lymphocytes and down-regulation of the T-reg cells (Sjöberg *et al*., 2013).

Following translocation of intact gliadin peptide into submucosa through paracellular or retrotranscytosis, thereby increasing IP to immunogenic peptide and promoting immune-mediated responses (Coulon *et al*., 2011).

In the lamina properia, TG2 enzyme present in the extracellular part of the sub-epithelial area or at the brush border (Matthias *et al*., 2011) drives deamidation of gluten peptides ,which then strongly bind to HLA-DQ2\HLA-DQ8 molecules expressed on DCs (P4, P6, and P7 pockets in HLA-DQ2 while P1 and P9 pockets in HLA-DQ8) (Schuppan *et al*., 2009; Abadie *et al*., 2011), introduced to activated T-cells (Hell and West, 2006), with consequent pro-inflammatory cytokines release (IFN-γ, IL2, IL-17, and IL-21) (Björck *et al*., 2015). Th1 cytokines enhance IELs and NK T cells cytotoxicity, where

enterocytes killing to be induced via: 1. Fas/Fas ligand order. 2. IL-15-induced perforin/granzyme cytotoxicity. 3. Homodimeric NKG2D–MICA killing signals (Di Sabatino *et al*., 2006; Di Sabatino and Corazza, 2009). The epithelial inflammation and the direct cytotoxic action on the mucosa, both eventually result in small intestinal villous atrophy. On the other hand, the Th2 cytokines production induce and activate a B-lymphocytes clonal expansion with subsequent differentiation into plasma cells (Di Sabatino and Corazza, 2009; Björck *et al*., 2015), that secrete both anti-gliadin and anti-tissue-transglutaminase antibodies (Björck et al., 2015; van Bergen *et al*., 2015). Moreover, Sjöberg *et al* in 2013 reported that the bacterial structure (dysbiosis in the intestinal microbiota), considered a triggering component for CD in two possible ways, first by direct influence in the mucosal immune reactions, and the second by promoting inflammatory process against gliadin (Sjöberg et al., 2013). The resident microbiota has also a direct action on the gluten breakdown, there by formation of immunogenic peptides (Cukrowska *et al*., 2017).

The immunopathological processes ends with small intestinal inflammation, causing a disorganized mucosal architecture (flattened/ blunt villi and crypts hyperplasia)in addition to lymphocytes infiltration which clinically revealed by the malabsorption symptoms. These abnormalities occur in steps and in a slow progression from normal to complete villous atrophy, as qualified by Marsh in 1992 and later a modification done by Oberhuber for grading the intensity of intestinal damage (Oberhuber, 2000).

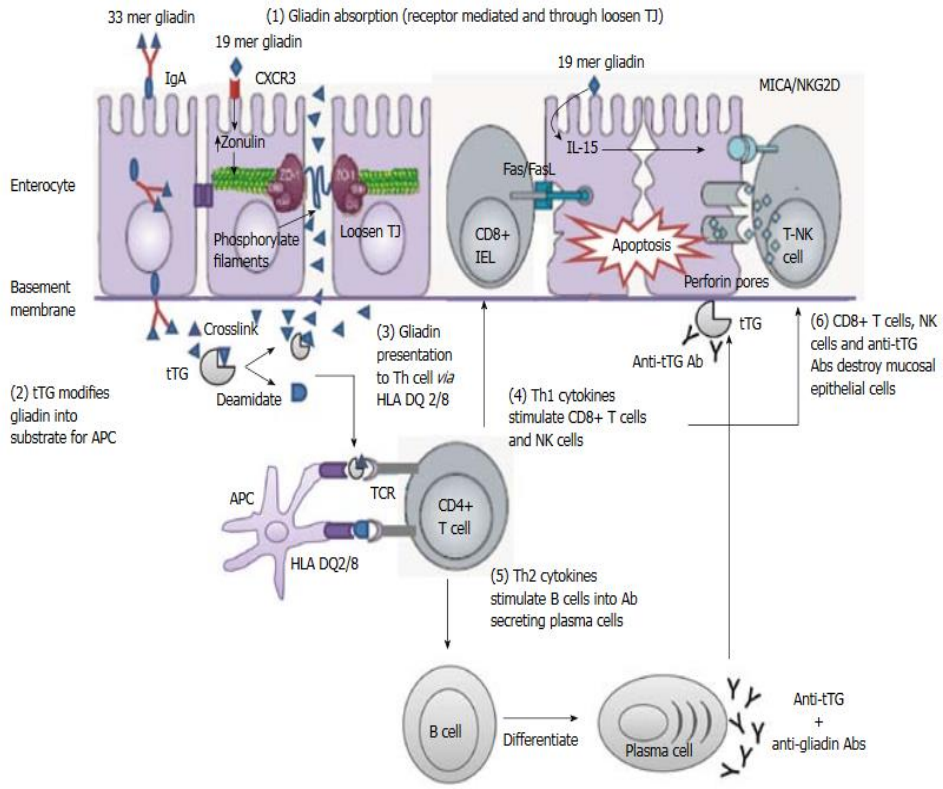

Figure (1.8): Mechanisms of mucosal damage in celiac disease (Di Sabatino and Corazza, 2009).

1.3.9- The clinical presentation of CD:

Nowadays, in particular resource-rich countries, more heterogeneous clinical manifestations of CD may be reported, including typical gastroenterological symptoms as well as a myriad of extra-intestinal disorders (Jeon *et al*., 2013). The GI symptoms (diarrhea and malabsorption syndrome) are predominating in young children, while failure to thrive, short stature, and other growth problems occur in all peadiatric age groups(Reilly *et al*., 2011).Generally extra-intestinal (atypical) clinical features occur in children more than 5-year-old and so in adults (Akirov *et al*., 2015).

Several factors may cause this protean clinical picture of CD including patient immune and genetic background, with gender, presentation age, dietary habits, and degree of mucosal trauma (Bai *et al*., 2005), all can affect the disease clinical manifestations. The natural history of CD is widely variable between patients and some studies propose number of consequent events:

positive serology, the evolvement of intestinal abnormalities, presenting complaints, and later on the complications. However, it is not the norm that all these events to occur and every disease-stage might have a prolonged duration (ranging weeks to decades) (Tosco *et al*., 2011; Fasano and Catassi, 2012). Several CD subtypes have been described (Ludvigsson *et al*., 2-13) as shown below.

Table (1.1): Clinical Presentation of CD (Husby *et al*., 2013).

Clinical Presentation of CD
1. Symptomatic: Includes -Gastrointestinal -Extraintestinal
2. Asymptomatic: Includes
- Silent
-Potential (Latent)

1.3.9.1. Typical (Gastrointestinal) form:

This characteristically presented by clinical features developed due to disorganized absorption of digested food like diarrhea, steatorrhea and malabsorption causing weight loss (Ludvigsson *et al*., 2-13). Generally occurs following the introduction of weaning foods containing glutens between 6 and 24 mo of age (Tully, 2008). Histological picture (villous atrophy and crypt hyperplasia) confirm the diagnosis.

1.3.9.2. Atypical (Extraintestinal) form:

This form is distinguished due to predominance of extra-intestinal symptoms with or without the common features of abnormal absorption, and it usually encountered in older children and adults (Ludvigsson *et al*., 2013). A number of studies revealed that approximately half of CD patients present with this form and having one or more of various manifestations (anemia, osteoporosis, hypo plastic dental enamel, and neurological problems) (Rampertab *et al*., 2006; Sapone *et al*., 2012; Husby *et al*., 2012).

1.3.9.3-Silent or asymptomatic form:

This form points to the several individuals who show positive serology and characteristic intestinal histology without evidence of clinical symptoms (Ludvigsson *et al*., 2013) Here the risk for developing complications, notably autoimmune (Cosnes *et al*., 2008) or seldom a malignant disease is high (Elfstrom *et al*., 2011).On average, patients with associated autoimmune (like DM-type 1), those with a positive family history for CD , and individuals with genetic disorders (like Down, Turner, or Williams syndrome) are often more liable to this form of CD (Ludvigsson *et al*., 2013).

1.3.9.4-Latent (potential) form:

A minimal number of asymptomatic individuals have positive serumTG2 antibodies but normal histology of small intestine (Ludvigsson *et al*., 2013). A previous study assumed that the high level of endomysial antibodies in such patients might be a substantial predictor for disease progression (Troncone *et al*., 1996). In a subgroup of these patients, an overt mucosal damage develops with time (Tosco *et al*., 2011). In potential CD, TG2 antibodies and considerable IELs milieu (TCRγδ+ lymphocytes in particular) could often be seen locally in intestinal biopsy (Koskinen *et al*., 2008; Ludvigsson *et al*., 2013).

1.3.9.5-Refractory form (RCD):

A subtype that affects small proportion (5%) of CD patients and is often described as recurrent or persistent malabsorptive complaints beside the biopsy findings (villous atrophy) in spite of a strict GFD (Rubio-Tapia and Murray, 2010; Ludvigsson *et al*., 2013; Malamut an Cillier, 2014) for at least 1 year (Ho-Yen *et al*., 2009). RCD has two subtypes, type 1(RCD I) and and type 2(RCD II) (Green, 2005; Green, 2007). Type 1 (where IELs are normal), and type 2 (abnormality of IELs; clonal IELs without surface markers known for T-cells like CD3, CD8, and TCR) (Daum *et al*., 2005; Freeman *et al*., 2010).

1.3.9.6-CD and skin manifestation:

Dermatitis herpetiformis (DH) is a dermatologic aspect of small intestinal immune-based enteropathy evolved by gluten consumption (Fry *et al*., 1967; Zone, 2005; Bolotin and Petronic-Rosic, 2011). It is rarely seen in children, and usually manifested in the third decade of life (Parzanese *et al*., 2017). Characteristically the skin shows diffuse symmetrical herpetiform clusters of urticaria, vesicles, and pruritic papules particularly on the buttocks, knees, and elbows which due to the presence of IgA precipitates or deposits within the dermal papillae (van der Meer, 1969; Zone *et al*., 1996) which resolve on keeping a GFD (Fry *et al*., 1973; Garioch *et al*., 1994). Both DH and CD have the same HLA association with HLA-DQ2 in almost 90% of patients and HLA-DQ8 for the remainders 5% (Collin and Reunala, 2003; Holmes *et al*., 2009).

DH Patients have serum antibodies reactive against tTG2 and tTG3, a cutaneous form of TG2, which owing either to cross-reaction or spreading of epitope (Sa'rdy *et al*., 2002). Of note, the T cells are absent in DH skin lesions, and the blistering areas often contain deposits of IgA and TG3 plus infiltrating neutrophils (Zone *et al*., 2011).

1.3.10-Complications associated with CD:

1. About four times more risk of death or excess mortality seen in under diagnosed CD (Rubio-Tapia *et al*., 2009)
2. Severe consequences might occur in undiagnosed subjects, both adult and children (Rubio-Tapia *et al*., 2009; Holmes, 2010; Norström *et al*., 2011) since a gluten-rich food might increase the risk for lymphoproliferative disorders, Non-Hodgkin lymphoma (Smedby *et al*., 2005), gastrointestinal cancer, and a plenty of micronutrient deficiencies and metabolic abnormalities (Richir *et al*., 2010; van der Windt *et al*., 2010; Lacy *et al*., 2012; Sharaiha *et al*., 2012), in addition to osteoporosis, ulcerative jejunoileitis.

3. The males and females reproductive system could be a target of CD and infertility cases reported in the two (Ozgör and Selimoğlu, 2010). In addition to miscarriage, pregnancy complications, and more seen in affected females (van der Windt *et al*., 2010; Lacy *et al*., 2012).

4. Moreover in CD, cerebral calcifications, behavioral disturbances, and even malignancy due to the continued gluten ingestion regardless of apparent symptoms or signs. Because of all these disease outcomes, promoting accurate diagnosis and correct information to patients are mandatory. (Amil Dias, 2017).

1.3.11-The differential diagnosis of CD:

With regard to other disorders exclusion, multiple causes of an inflammatory cell infiltration in duodenal mucosa with or without villous atrophy; these include cow milk allergy, post viral enteritis, Crohn's disease, common variable immunodeficiency, drugs like non-steroidal anti-inflammatory, autoimmune enteropathy, Giardia, tropical sprue, intestinal TB, gluten sensitivity, and others (Hill *et al*., 2005; Green and Cellier, 2007).

1.3.12- Diagnosis of celiac disease:

Since 2012, gastrointestinal organizations have published four guidelines on CD diagnosis. The coincide usage of serologic tools and biopsy for disease diagnosis is recommended by these guidelines. European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) consensus guideline \2012 , American College of Gastroenterology (ACG) guideline \2013, the British Society of Gastroenterology (BSG), and recently the World Gastroenterological Association(WGO) guideline for adult CD patients (Kaswala *et al*., 2015) . A substantial overlapping among these guidelines with the main variation is that ACG and BSG entail histological findings for diagnostic confirmation, while ESPGHAN and WGO permit disease diagnosis regardless histological findings in definite cases. (Kaswala *et al*., 2015) The guidelines of the ESPGHAN authorize for CD diagnosis without

biopsies in children present a distinctive clinical features and levels of TG2-IgA antibodies 10-fold or more the upper normal level (UNL), detection of anti-endomysial antibody in another test plus genetic study (HLA-DQ2/DQ8 typing) for confirmation [triple test " ESPGHAN criteria] (Husby *et al*., 2012). Recent guidelines from the WGO for diagnosis of adult CD patients recommend serological tests including the known antibodies "anti-tTG and/or anti-EMA, or anti-DGP" and suggest histology which is considered not obligatory. This is convenient in areas where healthcare facilities are restricted (Bai *et al*., 2013). However, celiac disease diagnosis cannot be accomplished via a single test and the table below shows the diagnostic tools for the diagnosis of celiac disease.

Table (1.2): Diagnostic Tools in Celiac Disease (Husby *et al*., 2013).

Diagnostic Approach in CD
Clinical manifestations
Serological tests
Genetic study (HLA-DQ2/DQ8 genotyping)
Small intestinal biopsy\ histological findings

1.3.12.1-Serologic tests in CD:

None of serological tests is absolutely specified for CD and presence of antibodies often favor the diagnosis despite the dramatic variation in the diagnostic accuracy between laboratories (Leffler and Schuppan, 2010).

Serological tests (anti-tissue transglutaminase, anti-endomysial, and anti-deamidated gliadin peptides antibodies) are celiac-specific are often the first step while screening for CD (Rostom *et al*., 2005; Lewis and Scott, 2010). All serologic tests are automated except for the EMA test, since it is observer-dependent and needs more time comparing to other tests. Although both IgA and IgG antibodies can be measured for each serologic test, however, the

standard antibody to be measured in CD is IgA measurement. Generally, CD diagnosis should be accomplished before gluten being restricted from diet since both circulating antibody levels\ small intestine pathology might improve and even resolve when restrict gluten (Green and Cellier, 2007; Rubio-Tapia *et al*., 2013).

1.3.12.1.1-Anti-gliadin antibody (AGAs):

It had been found that the native gliadin is target of antibodies (IgA and IgG) and hence AGAs were the initial serologic tools mark the necessity of small intestinal biopsy in celiac patients. AGA tests are of limited accuracy (Leffler and Schuppan, 2010), and they broadly fell out of priority in the 1990s after a wide availability of more accurate tests (Gasbarrini *et al*., 2010)comparing to the inferior accuracy and low sensitivity \ specificity of this test , except in younger children (Rubio-Tapia *et al*., 2013).

1.3.12.1.2-Anti-tissue transglutaminase antibody (Anti-tTG):

In 1997, Dieterich and coworkers identification of the tissue transglutaminase enzyme to be a self-antigen reacting with EMA had led to the evolution of another technique (ELISAs) for detecting auto antibodies towards this enzyme (Dieterich *et al*., 1997; Husby *et al*., 2012). Serum tTG (IgA) measurement is the premier testing recommended for individuals where associated IgA deficiency is not expected due to its excellent standardization and being a high sensitive and specific test (Zintzaras and Germenis, 2006; Lewis and Scott, 2010), while tTG (IgG) antibodies are the serologic tool in IgA-deficient individuals(Rubio-Tapia *et al*., 2013). The greater the antibody titer, the higher the probability of a valid positive result (van der Windt *et al*., 2010) The testing is most commonly based on an ELISA technique and less commonly on radioimmunoassay (Li *et al*., 2006).

1.3.12.1.3- Anti-endomysial antibodies (Anti-EMA):

Antibodies to endomysium (a smooth muscle connective tissue) can be detected by indirect immunoflourescent assay (IFA) using either human

umbilical cord or monkey esophageal tissue, with results being reported in titers (Leffler and Schuppan, 2010). EMA is considered a significant indicator for CD with high specificity and sensitivity (Piacentini *et al*., 1999; Salmi *et al*., 2006; Leffler and Schuppan, 2010). However a less sensitive EMA has been proposed, in particular young children < 2 years (Maglio *et al*., 2010) and in elderly patients (Salmi *et al*., 2006) and a false negative result in children aged < 2 years and in case of IgA deficiency is expected (Ludvigsson and Green, 2011).

While EMA still is the most sensitive CD test in a reference lab, the test technical disadvantages yielding a considerable inter-observer and inter-site variability have directed the effort toward the substitution of EMA by a recent ELISA based assays (Dieterich *et al*., 1997; Piacentini *et al*., 1999)

1.3.12.1.4- Anti-deamidated gliadin derived peptides (Anti-DGP):

Recently, antibody testing against DGP has become clinically obtainable. The action of intestinal tTG to convert peptides in gluten into deamidated one provides the bases for DGP test (Dahlbom *et al*., 2005; Prince, 2006), and these antibodies offers a highly specific and sensitive test for CD than previous AGAs (Prince, 2006). They are especially useful in young children < 2years of age (Rashtak *et al*., 2008).

Moreover it has been reported that measurement of DGP (IgG) class is superior to tTG (IgG) antibodies on screening of CD cases with IgA-deficiency (Tonutti *et al*., 2009). In addition anti-DGP assays demonstrated to be of value in monitoring dietary adherence to GFD in children (Basso *et al*., 2009; Monzani *et al*., 2011), and more important part in monitoring celiac autoimmunity. In addition, a retrospective study demonstrated that IgG class of anti-DGP antibodies is specifically preceding the tTG (IgA) antibodies mostly in young children. This finding refers to a possibility of early CD

detection by regular follow-up using DGP test in some children with a genetic risk for CD (Lammi *et al*., 2015).

1.3.12.1.5-The role of combined serological tests:

The clinicians few years ago tend toward prescribing a serial testing to increase the diagnostic accuracy (Parzanese *et al*., 2017). Since no single test is completely sensitive \ specific for CD, hence, combination of individual tests in a commercial panel (Rashtak *et al*., 2008) is more useful as they have additive role in detecting celiac patients with IgA deficiency. However DGP (IgG) even detect some of patients with adequate IgA who are sero-negative for tTG IgA atibodies (Rashtak *et al*., 2008)

Other data have shown that concomitant use of tTG (IgA) and DGP (IgG) as screening tests having a better specificity in different age groups (adults and children) (Liu *et al*., 2007; Villalta *et al*., 2010).

1.3.12.1.6-The role of total IgA level in serological tests:

At the time in which measuring IgA antibodies is the standard for the CD immunoassays, false negative result may occur in IgA-deficient cases (Rashtak *et al*., 2008)

Individuals in whom CD is predominantly suggestive even before screening, so measuring IgA level is proposed in such category, in particular when serological tests of IgA-class are negative. A clinical approach is to start with total IgA measurement to determine IgA level sufficiency and, in case of deficiency, so to incorporate testing of IgG-class into the serology testing. In such circumstance, the preferred tests would be DGPs (IgG) and / or tTG (IgG) (Villalta *et al*., 2007; Villalta *et al*., 2010).

1.3.12.2- Genetic testing:

HLA (DQ) alleles genotyping has additional role in CD diagnosis (Hadithi *et al*., 2007), and the remarkable point in HLA determination is for excluding the presence of or susceptibility to CD (Hadithi *et al*., 2007;

Husby *et al*., 2012). According to the 2012 ESPGHAN guidelines, the value of genetic testing in CD is due to the fact that the disease is improbable when HLA-DQ2 and HLA-DQ8 haplotypes are disproved (Husby *et al*., 2012), and HLA –typing was considered the first tool in screening individuals at risk of CD.

HLA genetic testing at birth have been debated as an approach of mass population screening to identify those with a genetic predisposition for CD before appearance of clinical manifestations and consequent health issues (Anderson, 2006).

1.3.12.3-Intestinal endoscopy and biopsy:

In reference to CD guidelines, histologic picture of small intestine is needed to confirm diagnosis. Endoscopy and sampling of small intestinal biopsies is the golden norm to diagnose CD in adulthood (Fasano *et al*., 2003; Bonamico *et al*., 2004; Villanacc *et al*., 2011; Bao *et al*., 2012; Mills and Murray, 2016). Different entities to be integrally assessed in the histology of small bowel, including: low-height enterocytes, crypt hyperplasia, blunt\ atrophied villi, and infiltration of inflammatory cells. Marsh classified these intestinal histological lesions into four distinct diagnostic grades (scoring system) (Marsh, 1992; Villanacc *et al*., 2011), later Oberhuber proposed modifications of Marsh grading where M III grade subdivided into a, b, and c scores (modified Marsh-Oberhuber) (Oberhuber, 2000; Mills and Murray, 2016) or the recently introduced and simplified Corazza classification. The histolopathlogical features are also important in verifying the causes of malabsorption (differential diagnosis) (Owens and Greenson, 2007; Yantiss and Odze, 2009). The grading of these histological changes according to the Marsh Modified Classification as follow: Marsh 0 is a merely preinfiltrative stage; Marsh I is identified by IEL infiltration in a normal villous architecture; Marsh II is defined when the crypt hyperplasia accompanies the IEL

infiltration; and Marsh III is characterized by villous atrophy (partial, subtotal, or total) in addition to crypt hyperplasia and IEL infiltration (Marsh, 1992).

Recent studies have reported the introduction of methods for quantifying the pathologic lesions present in the intestinal biopsy (Ciaccio *et al*., 2015)

1.3.12.4-Gluten challenge test:

Glutin challenge is the mode by which a clinically suspected patient, but uncertain CD where formerly managed by gluten restriction returns to a usual, gluten-containing diet, along medical monitoring, enabling the disease diagnosis (Lahdeaho *et al*., 2011; Leffler *et al*., 2012). In the past this test was a routinely used to diagnose CD, however a less frequent use nowadays due to the high accuracy of serological tests.

Gluten challenge is still the best test to diagnose CD in those patients with positive HLA-genotyping, but normal findings for both serology and biopsy as they done on gluten restriction. Although the norm for this test being a diet containing as low as 10 g of gluten per day for1-2 months, only a few data indicating the diagnostic adequacy of the test, the optimum duration, and the dose of gluten (Ansaldi *et al*., 1988; Rujneret *et al*., 2002). A recent study reported that diagnostic changes are found in majority of patients within 2 weeks of gluten ingestion even if a patient could not tolerate > 3 g of gluten per day (Leffler *et al*., 2012). Some studies found that normal histology preserved when <10 mg gluten intake per day, whereas 50 mg daily gluten show a raised ratio of villous height-to-crypt depth and considerable abnormalities (increased IEL and villous atrophy) with 100 to 500 mg daily ingestions /day (Akobeng and Thomas, 2008). In addition, the gluten challenge advent has enabled experts in designing a diagnostic algorithms and monitoring clinical responses in clinical trials. Also the test presents a beneficial role in ameliorating the incoming therapies as an adjuvant therapy to GFD (Castillo *et al*., 2015).

* **In vitro-gluten challenge test:**

Nowadays, many data suggested that in celiac disease, the immune reaction against gluten could be stimulated *in vitro* where culture (mucosal) cells taken from intestinal biopsies can be used (Khalesi *et al*, 2016).

1.3.13-Treatment of celiac disease:

At present time, the solitary adequate approach to treat CD is by a lifelong restriction of gluten from diet (Dick *et al*., 1953; Green and Cellier, 2007; Hassan and Kader, 2014; Makharia, 2014; Plugis and Khosla, 2015) Since as low as 50 mg/day of gluten could induce immune response, so complete withdrawal of whole dietary items and other gluten- containing products like drugs, make-up, and other else is very important (Catassi *et al*., 2007). To accomplish good adherence to a GFD, considerable patient education, motivation, and follow-up are necessary (Sverker et al., 2005; Rubio-Tapia *et al*., 2013).

Generally a few weeks are enough to achieve clinical improvement while recovery of the mucosal damage needs 1-2 years (Lionetti and Catassi, 2011). An accompanying brush border lactase insufficiency may be associated with CD due to destruction of surface epithelial cells, so avoidance of dairy products including milk is a must during the initial treatment time. Also multivitamins exempt from gluten should be taken by all patients to compensate deficient vitamin B which often occurs during the long restriction time (Ludvigsson and Green, 2011). Precocious diagnosis and treatment are essential in pediatric celiac patients to overcome a number of irreversible outcomes like growth default, osteoporosis, and retarded dentition (Freeman *et al*., 2011). In the general population the daily gluten intake has approximately been calculated to be 15–20 g (van Overbeek *et al*., 1997), however several studies in CD patients have shown that a less than 1 g daily doses of gluten are enough to bring epithelial changes, so gluten amount of

10–50 mg per day has been estimated to be a safe threshold for those patients (Collin *et al*., 2004; Hischenhuber *et al*., 2006; Catassi *et al*., 2007).

Other aspect in treating CD patients is by monitoring response to or compliance with a GFD, thereby observing the late outcomes that can manifest in the scope of disease activity (Green *et al*., 2003; Cosnes *et al*., 2008).

Table (1.3): The overall target of treatment of CD (Makharia, 2014).

***Inducing remission including:**
1-Clinical resolution by symptoms relief and improved patient quality of life
2-Serologic settlement of antibodies to base line level
3-Histologic reversibility of mucosal abnormalities
4-Avoiding late outcomes
*Maintaining remission by continuous restriction of gluten from diet
*Screening family members for CD
*Secondary prevention: By precociously detecting and treating of the disease

Some studies had established that more than a year is needed for complete recovery of the villus architecture in the small-bowel mucosa, and furthermore, intraepithelial lymphocytosis may continue for years (Lanzini *et al*., 2009), whereas around half of patients have persistent active disease in spite of gluten restriction (Lee *et al*., 2003). Moreover another study had observed that a varying grade of villous atrophy present in most of celiac patients despite adherence to GFD from 1 to 4 years (Morón *et al*., 2013).

Nevertheless, a small proportion of fully adherent individuals may manifest opposition to gluten restriction with no clinical or histological improvement despite of GFD. Therefore, it is not surprising to find CD patients being more concerned in therapeutic substitutes away of gluten restriction. Recent

advance to understand the pathogenesis of CD and the fact of GFD being a social burden has unlocked the gate for a numerous novel therapeutic agents (Stein and Schuppan, 2014; Makharia, 2014).

-Some clinical trials have carried out using drug called Larazotide acetate (AT-1001, developed by Alba Therapeutics, USA). This octapeptide derivative of cholera toxin, ZOT has been shown to be effective antagonist for zonulin via receptor blockade (Fasano *et al*., 1997; Paterson *et al*., 2007), thereby reducing the paracellular transport of gluten and hence suppress the activation of the pathological immune response.

-Bifidobacteria has shown a high therapeutic potency and may have improving-role in disease prognosis (Sanz *et al*., 2011; Golfetto *et al*., 2014). A potentially used probiotics (useful bacteria) in CD management signify the general association of intestinal dysbiosis with the disease and the effect referred to these probiotics is to preserve IB function and regulate the immune responses (Olivares *et al*., 2014; Klemenak *et al*., 2015).

-Malamut and coworkers in 2010 reported that IL-15 has a significant role in the immnopathogenic process of refractory CD, especially type 2 (RCDII), they proposed that the disease controlling and preventing the consequent evolvement to T lymphomas may be achieved by IL-15 blocking or its signaling pathway (Malamut *et al*., 2010) or by prohibiting of the JAK3-kinase downstream (Baslund *et al*., 2005)

The use of antibodies to block IL-15have demonstrated the ability to cause IELs death (apoptosis) in the intestinal mucosa of human IL-15 transgenic mouse models (Rashtak and Murray, 2012).

Furthermore, blockage of this cytokine and its receptor regulation (IL-15/IL-15system) assumed to be used in treating inflammatory autoimmune diseases, since IL-15 enhances other pro-inflammatory cytokines (like TNF-a, IL-1b) (Waldmann, 2004).

The treatment lines behind GFD could be categorized according to the site of action in various pathogenic stages of the disease for which they proposed and include: Immunodominant gluten peptides, impaired mucosal barrier, adaptive immunity, inflammatory response, lymphocyte recruitment (homing), and immunomodulation (Stien and Scuppan, 2014).

Novel therapeutic approaches:

a. Therapeutic approaches act at the luminal level of the gut, either to degrade or bind the ingested gluten peptides in the small intestine (glutenases, gluten binders, neutralizing antibodies).

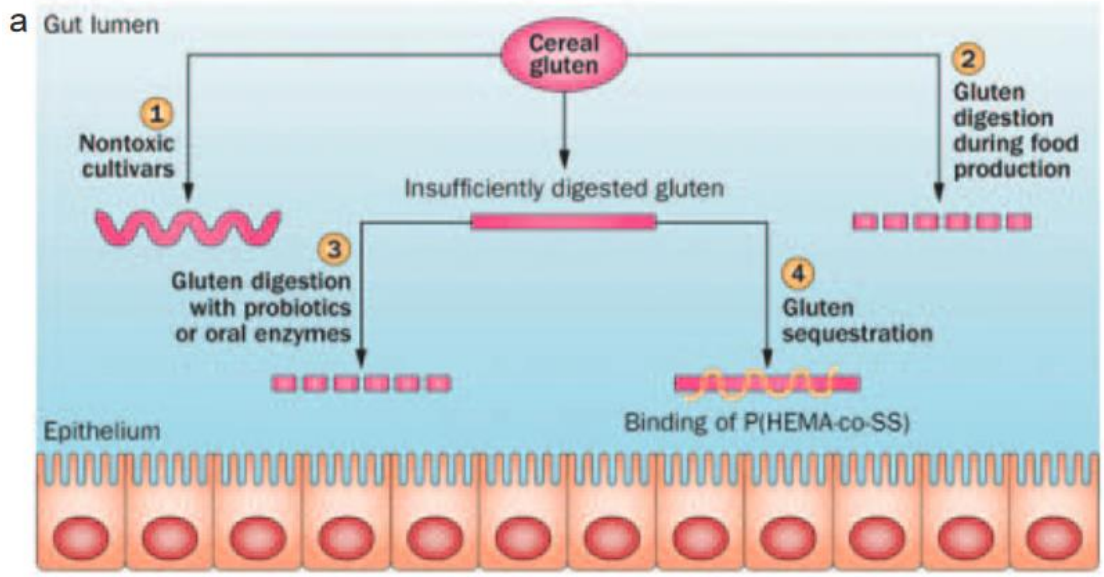

Figure (1.9): Therapeutic lines play in the small intestinal lumen (Kaukinen *et al.*, 2014).

b. Proposed treatment lines that impede gluten effects in the intestinal mucosa: The use of octapeptide AT-1001 for blocking the ZOT receptor to reduce intestinal permeability is another option. Moreover, as gliadin peptides deamidation by the enzyme tTG2 with consequent presentation by HLA-DQ2/8 commences the adaptive immune responses, so inhibiting this enzyme and using peptides to block DQ2 are likely to be a possible way to ameliorate inflammation.

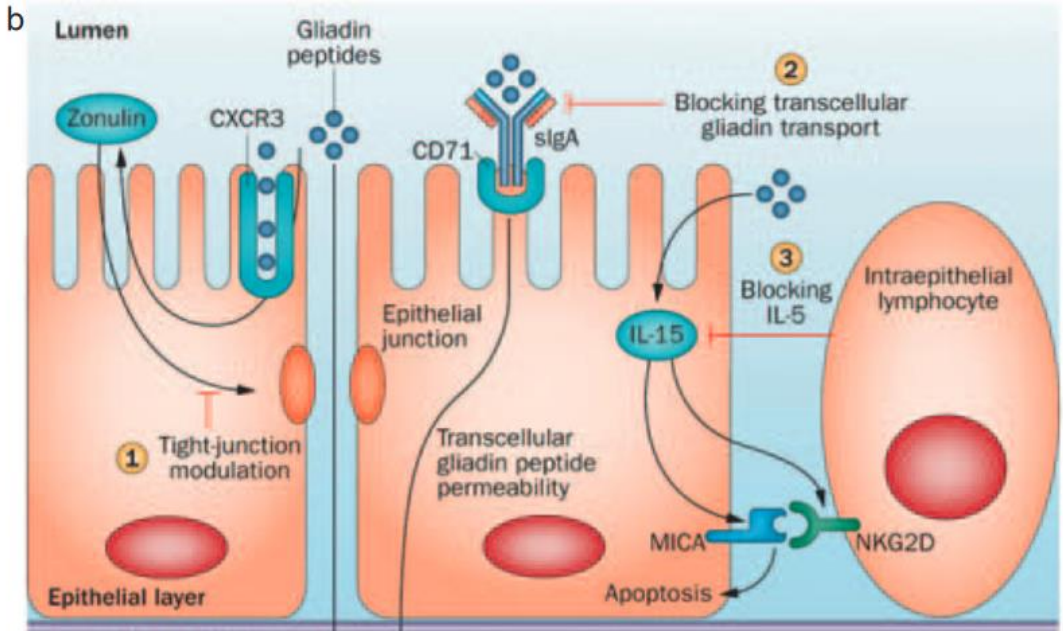

Figure (1.10): Proposed treatment lines that restrain the action of gluten in the intestinal mucosa (Kaukinen *et al*., 2014).

c. Treatment strategies mandate the prohibition of immunological signals in submucosal cells (in particular patients with RCD). This accomplished either by blocking of lymphocyte (anti-IL-15, anti-CCR9, and others) or by inducing local tolerance.

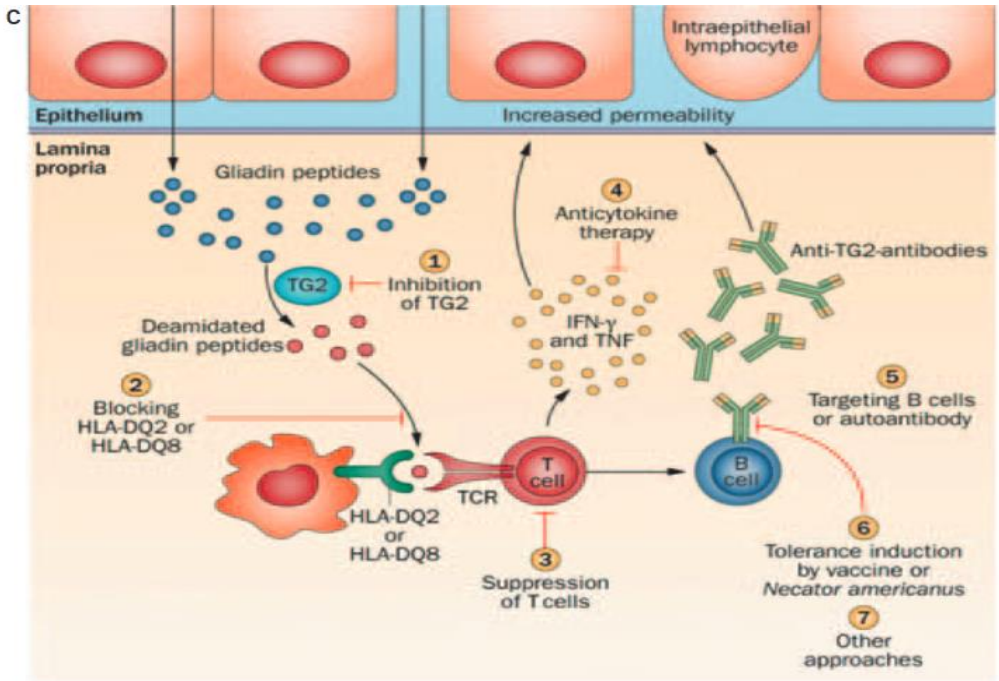

Figure (1.11): Therapeutic options based on prevention of immunological cascades in the submucosal cells (Kaukinen *et al*., 2014).

Chapter Two

Materials &Method

Chapter Tow

2. Material and Method:

2.1. The study design:

This is a retrospective multi-center case–control study. Consecutive patients who are already or newly diagnosed to have CD (cases) to be compared with controls matched for age and gender.

2.2. Setting:

Al-Hussein-medical city hospital and the pediatric teaching hospital are teaching hospitals (university of Karbala), in addition to Al-Hindiya general hospital and their endocrine centers provide diagnostic and follow-up care for in- and outpatients.

2.3. Study group selection:

In this study, a random selection of all enrolled candidates (cases and controls of pediatric and adult age groups) was carried out for the period from August 2017 to February 2018 in Karbala province hospitals.

2.3.1. Patients sampling:

A total of 108 patients who were attending endocrine centers in Al-Hussein-medical city hospital, the pediatric teaching hospital and Al-Hindiya general hospital, were enrolled in the study. The age groups ranging between 1 to 58 years with 68 of them are below 18 years. The patient's sex was 75 female VS 33 male.

2.3.2. Controls sampling:

The total number of controls is 118 with 48 of them are adults where enrolled in the study while they were attending prenuptial examination unit in Al-Hindiya general hospital, while the 70 pediatric controls, those who attended primary care unit for vaccinations or routine checking in pediatric

teaching hospital. Age group range 1.5 -57 years, with 75 of them are female VS 43 male of the total.

2.3.3. Clinical data:

Information including age, sex, residence, complaints, duration of disease(old cases), family history, skin manifestations, associated autoimmune disorders, serum antibody titer, indications for endoscopy and biopsy result, challenge test, treatment, complications, and more others collected directly from patients or their family members via a questionnaire sheets.

2.3.4. Descriptive variables of the study group:

2.3.4.1. Inclusion criteria:

a-For patients: The rule 'four out of five ' was a quantitative approach and was suggested for the last few years where disease diagnosis in a patient is definite as he fulfills at minimum 4of 5criteria (Catassi and Fasano, 2010):

1. Manifestations typically suggesting for CD.
2. High positive titer of serum tTG (IgA) class autoantibodies.
3. Positive HLA-(DQ2 and/or DQ8) genotyping.
4. Celiac histologic picture on duodenal biopsy.
5. Improvement on restricting dietary gluten (Catassi and Fasano, 2010)

Thus for the study group and according to current international guidelines:

*****All age groups of patients were included.

*Patients diagnosed to have CD by specialists where their clinical manifestations were highly suggestive for CD.

*Positive serological markers for CD (tTG IgA> 10 folds upper limit of normal according to revised criteria of ESPGHAN 2012).

*Confirmation by histopathological picture of duodenal biopsy in adults and some children who their parent accepted undergoing biopsy, while challenge test done for all children who refused biopsy.

*Response to GFD for old patients.

b-The controls who enrolled in the study were healthy persons from all age groups and they were sero-negative for tTG and DGP antibodies.

2.3.4.2. Exclusion criteria:

a-For patients: Two categories excluded.

1-Those with intestinal manifestations for CD, but have border-line serological tests with a biopsy either negative or not carried out.

2- Those with clinical presentation suggestive for CD, positive serology, M1 mucosal changes, and later had negative serology even on gluten-containing diet.

b-For controls: At time of study, we excluded those with:

1. Food allergies.
2. Acute or chronic gastroenteritis.
3. Infectious disease.
4. Cancerous disease.
5. Family history of CD or they have border line serological tests.
6. Irritable bowel disease or inflammatory bowel disease.

2.4. Ethical issues:

Ethically, the selection of the study groups and data collection were accomplished after we have been taken the approval of Karbala Health Directorate to work in its hospitals and a practical supervisor has been set in each hospital to follow the workflow. In addition, the permission and agreement of laboratory department of each hospital to work in the corresponding immunity unit of each lab also have been taken.

Regarding blood samples, we obtained the permission from patients themselves or the parents of participating children to aspirate or use the collected blood from patients to perform serological or chemical tests, either as part of their follow-up regimen (old cases) or to screen or confirm diagnosis of CD after challenge test (pediatric new cases). For adult controls,

the permission taken only to use the already present blood samples in the corresponding unit mentioned above, while for children control, their parents agree to aspirate blood.

*Both candidates (cases and controls) were informed that these tests are for research and are not diagnostic, and interestingly, all participants were cooperative and good respondents.

*Permission of patients and controls to publish the study.

2.5. Blood samples processing:

The 5ml freshly aspirated venous blood samples using disposable syringes were collected sequentially from children and adults patients, in addition to controls at the aspiration unit in the lab of the hospitals were the patients attended. The blood aspirated in a gel tubes and submitted to a barcode to be labeled and sent to immunity unit in the lab, where they centrifuged at 3000 revolutions per minute (rpm) for 10 minutes to separate serum. Later, from each sample, we took 1/2 to 1 ml of serum in a plane tube, labeled, and kept in the deep freeze of the unit (for zonulin and IL15 measurement), while the original tubes kept in the fridge to pass in the weekly run of celiac serology (TG [IgA, IgG], DGP [IgA, IgG] antibodies) that done by immunity unit staff of the mentioned labs.

2.6. Laboratory instruments used in the study:

Down, a list of all laboratory instruments and equipment used during the study:

Table (2.1): Laboratory instruments and equipment

Instrument	Manufacturer origin
Disposable syringe (5ml)	Medi, China
Gloves	Malaysia
Gel and plane test tubes	Jordan
Cylinder: 1000ml MBL, Boro 100ml Hlalab	Germany
Multi-channel 100 M pipette Micropipettes 100 M, 1000M	Slamed, Germany Slamed, Germany
Plastic rack	China
Centrifuge	Kokusan, Japan
Distillator	Nuve, Turkey
Cold incubator	Memmert, Germany
Deep freezer	ARCTIKO, Japan
Electronic sensitive balance	RADWAG, Poland (UK)
ELISA reader and printer ELISA automated washer	BioTek, USA BioTek, USA
Vortex	England

2.7-The laboratory technique:

The serological markers which currently used as a first step in the screening for CD, and the study marker (zonulin), both are measured by indirect ELISA (Enzyme-Linked ImmunoSorbant Assay) technique. While the other maeker (IL15) measured by antibody sandwich ELISA.

2.8- Materials (kits) used in the study: Two different kits had been used in this study:

2.8.1- The IL -15 DuoSet ELISA kit:

DuoSet ELISA kit from R&D systems (ENGLAND) was used for serum IL-15 level measurement. The kits contain the basic components required for the development of sandwich ELISAs to measure natural and recombinant human interleukin 15 (IL-15). DuoSets are designed for the analysis of cell culture supernatants, serum, and plasma samples (DuoSet ELISA, DEVELOPMENT SYSTEM).All the kit contents are illustrated in the table (2.2) below.

Table (2.2): The reagents of IL15 ELISA kit (DuoSet ELISA, DEVELOPMENT SYSTEM)

Reagents	Quantity
Assay plate(12×8 coated Microwells)	1(96 wells)
Human IL-15 Capture Antibody	1 vial
Human IL-15 Detection Antibody	1 vial
Human IL-15 Standard	2 vials
Streptavidin-HRP	1 vial
PBS Bovine Serum Albumin (BSA)	12 tablet 1 gm
Tween 20	1ampule(6 ml)
Wash Buffer: 0.05% Tween 20 in PBS, PH7.2-7.4	Manually prepared
Reagent Diluent:1%BSA in PBS, PH7.2-7.4	Manually prepared
Substrate Solution: 1:1mixture of color reagent A (H2O2) and color reagent B (Tetra-methyl benzidine)	
Plate Sealers	2

2.8.2- The zonulin COSABIO ELISA kit:

Serum zonulin protein level measurement, where ELISA kit from CUSABIO (U.S.A.) was used for quantitative determination of human zonulin concentration in serum, plasma, and tissue homogenates (User Manual,Catalog Number, CSB EQ027649HU; CUSABIO).

Table (2.3): The reagents of zonulin ELISA kit (CUSABIO User Manual)

Reagents	Quantity
Assay plate(12×8 coated Microwells)	1(96 wells)
Standard(freeze dried)	2
Biotin-antibody(100×concentrate)	1*120ml
HRP-avidin(100×concentrate)	1*120ml
Biotin- antibody Diluent	1*15ml
HRP-avidin Diluent	1*15ml
Sample Diluent	1*50ml
Wash Buffer(25×concentrate)	1*20ml
TMB Substrate	1*10ml
Stop Solution	1*10ml
Adhesive strip(for 96 wells)	4
Instruction Manual	1

The detection range of each of the markers is demonstrated in the table (3.4) below.

Table (2.4): The detection range of serum zonulin and IL15 levels

The marker	Minimum detectable dose	Detection range
Zonulin(in reference to COSABIO User Manual)	Less than 0.156ng/ml	0.625ng/ml-40ng/ml
IL15	Undetectable as it part of innate immune response.	

2.9-The method (procedure):

The serum zonulin and IL15 level measurements of the total patients and controls sera carried out concomitantly in tow separated runs, were tested at the Immunity unit of Al-Hussain medical city lab. The first run in 23/11/2017, while the second run accomplished at the same lab in 22/2/2018.The same instructions of an individual marker were referred for both runs.

2.9.1-The procedure for the study markers measurement:

* General instructions for the laboratory work:

1-Both kits should be stored at 2-8C and to be used within the expiration date of each kit.

2-It is important to bring all reagents to room temperature (18-25C) before use for 30 minutes.

3-The steps and reagents of each ELISA assay may vary, and it is better to use the test specific information for the assay being worked with.

4-Distilled water (DW) from Distillates in the biochemistry unit of the lab, put in a graduated glass container (cylinder/1000ml), to be used for preparation of reagents.

5-By using PH indicator, we measured the PH of the DW and was acidic (5.7).

6-Be sure that pipettes used are calibrated.

7-Use graduated glass containers to prepare the reagents.

8- A list of patients and controls that their samples passed in the run.

9-The required information from both kits admitted into the ELISA reader, to facilitate the OD measurement of each individual marker.

10- Early in the morning of the work day, all samples to be tested were brought out the deep freeze and allowed to thaw, also the kit brought out the refrigerator to reach room temperature before use.

11-Labeling of test tubes and containers of each marker separately was carried out.

2.9.1.1- Serum zonulin level measurement:

We strictly refer to the Assay Layout Sheet (User Manual; COSABIO) instruction:

1-Principle of the assay: This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for zonulin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any zonulin present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for zonulin is added to the wells. After washing, avidin-conjugated horseradish peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of zonulin bound in the initial step. The color development is stopped and the intensity of the color is measured.

2-Reagents preparation for zonulin COSABIO kit: According to instructions of COSABIO User Manual**,** we prepared the reagents**:**

Biotin-antibody (1×): Centrifuge the vial before opening. It requires a 100-fold dilution. A suggested 100-fold dilution is 10 Ml of Biotin-antibody + 990 Ml of Biotin-antibody Dliuent.

HRP-avidin (1×): Centrifuge the vial before opening. It requires a 100-fold dilution. A suggested 100-fold dilution is 10 Ml of HRP-avidin + 990 Ml of HRP-avidin Dliuent.

Wash Buffer (1×): Mix gently to dissolve any crystals if present**.** Dilute 20 ml of Wash Buffer concentrate (25×) into 480ml of DW to prepare 500 ml

of Wash Buffer (1×). Again, PH indicator used and the WB was neutral (7.2) and kept in the refrigerator.

Standard: Centrifuge the standard vial at 6000-10000pm for 30s. Reconstitute the standard with 1.0 ml of ready to use Sample Diluent (SD) (don't use other diluent). in a labeled plastic plane tube, to get a stock solution of 40ng/ml(S7) which serves as the high standard and allow it to sit for a minimum of 15 minutes with gentle agitation prior to making dilution.

3-Assay procedure:

a-The working standards by taking seven plane tubes labeled sequentially (S6-S0), as shown in the table below.

Table (2-5): Zonulin standards arrangement in the plate

Tube	S7	S6	S5	S4	S3	S2	S1	S0
ng/ml	40	20	10	5	2.5	1.25	0.625	0

A 250Ml of SD were pipetted in the tubes from S6 to S0, and the stock Solution (S7) used to produce a 2-fold dilution series by pipetting a 250Ml of S7 into S6, then a 250Ml of S6 into S5 and repeated sequentially to S1.The S0 contain only SD and serve as Standard zero (0 ng/ml).

b- The patient's samples sequentially arranged in a plastic rack according to their name's list, and the same for control's samples in another separate rack.

c-The plastic microtiter plate (96 wells) was unsealed from the pouch, placed on the working bench to start addition using a multi-channel pipette graduated to 100Ml.

d- A100Ml of standard started from S0 to S7 in double wells, and 100Ml of undiluted samples were added to the remaining 80 wells, covered with adhesive strip, and incubated for 2 hours at 37C.

*Note: Changing of pipette tips between additions of each standard and samples

* During incubation period, all other reagents of the kit prepared as mentioned above.

*After incubation, only removing of the liquid of each well was carried out without washing.

e-A100Ml of Biotin-antibody (1x) added to each well, covered with a new adhesive strip, and incubated for 1 hour at 37C.

* During incubation period, priming of the ELISA automated washer to zonulin WB done, and a three-wash program chosen.

*Aspiration of liquid and washing for 3-times carried out, filling each well with 200Ml of WB per wash cycle.

*Complete removal of any remaining WB ensured by inverting the plate and blotting against clean paper towels.

f-A100Ml of HRP-avidin (1x) added to each well, covered with a new adhesive strip, and incubated for 1 hour at 37C. Aspiration of liquid and washing for 5-times carried out, and complete removal of any remaining WB ensured by inverting the plate and blotting against clean paper towels.

g-Addition of 90Ml of TMB Substrate to each well, incubated for 15-30 minutes at 37C, and protected from light, with observation once every 10 minutes for change of color.

h- Addition of 50Ml of Stop Solution to each well and gentle tapping to ensure thorough mixing done as shown in plate (3.1).

i- Determination of the optical density (OD) of each well within 5 minutes, using a microplate reader set to 450 nm.

4- Calculation of results: Average the duplicate readings for each standard and sample and subtract the average zero standard OD. Construct a standard curve by plotting the mean absorbance of each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The samples used were undiluted, so no need to multiply the concentration from the standard curve by dilution factor.

2.9.1.2- Serum IL15 level measurement:

The DouSet ELISA kit for serum IL15 level measurement recommends many extra steps.

1. Reagents preparation for IL15: Reagents preparation was accomplished according to instructions of DouSet User Manual**:**

Phosphate buffer saline (PBS): One tablet of PBS (sigma Chemical Company, Poole Dorset, UK) to be dissolved in 200ml of distilled water and stored at 0-5C.

Washing Buffer: PBS solution contains 0.05% of Tween 20 was prepared to be used for washing the plates.

Reagent Diluents: PBS solution containing 1% bovine serum albumin was prepared. Reagent diluent was used in blocking step and as a diluent's solution for the standards, detectable and streptavidIn conjugates.

Biotinylated Mouse anti-human IL15 detection antibody: A vial contain more than 200 Ml, also divided in tow eppendorf tubes (100Ml/tube), and kept in the freeze to be used later. Each 100Ml of anti-human IL15 detection antibody dissolved in 11ml of reagent diluent.

Streptavidin-HRP: Each vial contains 2.0 ml of streptavidin conjugated to horseradish-peroxidase to be diluted to the working concentration with dilution ratio of 1:40 (for example, 100Ml Streptavidin-HRP in 4 ml of reagent diluent**).**

Substrate Solution: The substrate solution is not ready to use and it is comprised of a substrate buffer solution and a color reagent (OPD). One capsule of phosphate citrate buffer with Sodium perborate (sigma) was dissolved in 100 ml of DW. The substrate solution was prepared by adding one tablet of OPD into 25mls of substrate buffer.

Stopping solution: H_2SO_4 solution to stop the ELISA reaction was not supplied, and we used a stop solution of EUROIMMUN kits.

3- The procedure of the kit:

a-The standard vial, on receiving the kits, was dissolved in 0.5ml of PBS and distributed in 5 eppendorf tubes(100 µ /tube) and kept in the freeze to the time of work, where prepared using 2-fold serial dilution in reagent diluent as shown in the table (2-6). Later 100 µ of the blank and standards were added in double run in the first two strips of a 96-well microplate, while a 100 per well of labeled samples were added to the remaining strips, covered with adhesive strip and incubated 1.5 hour at RT.

Table (2-6): IL15 standards and blank arrangement in the plate

Tube	S7	S6	S5	S4	S3	S2	S1	Blank
Conc. Pg/ml	1000	500	250	125	62.5	31.3	15.6	**zero**

*Aspiration\wash was repeated as mentioned above.

b. A 100Ml of diluted detection antibody were added to each well, and the plate covered with new adhesive strip, and incubated for 1.5 hour at RT.

*Aspiration\wash was repeated as mentioned above.

c. A 100Ml of the working dilution of Streptavidin-HRP were added to each well, the plate covered, and incubated for20 minutes at RT away of direct light.

*Aspiration\wash was repeated as mentioned above.

d. Later on 100Ml of substrate was added to each well and the microplate incubated away of direct light for 20 minutes.

e-To stop reaction, a 50Ml of EUROIMMUN kits stop solution was to added each well as shown in plate (3.2), and determination of OD of each well carried out immediately using a microplate reader set to 490 nm.

4- Calculation of results: Average the duplicate readings for each standard and sample and subtract the average zero standard OD. Construct a standard

curve by plotting the mean absorbance of each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The samples used were undiluted, so no need to multiply the concentration from the standard curve by dilution factor.

2.9.2-The procedure for the serum antibody measurement: The serological markers which currently used in screening of suspected patients for CD are: Anti-tissue transglutaminase antibody (tTG IgA, IgG), and anti-deaminated gliadin peptide (DGP IgA, IgG). All these tests are available in Al-Hussein- medical city, while only tTG IgA, IgG were performed as screening and follow-up tests for children with symptoms suggestive for CD in Peadiatric teaching hospital.

2.9.2.1-Serum anti- tissue Transglutaminase antibody measurement:

1-Anti-tissue Transglutaminase ELISA (IgA) test kit principle: This kit provides a semiquantitative or quantitative in vitro assay for human autoantibodies of the IgA class against tissue Transglutaminase in serum or plasma. The test kit contains microtiter strips, each with 8 break-off reagent wells coated with human tissue Transglutaminase. In the first reaction step, diluted patient samples are incubated in the wells, and in case of positive samples, specific IgA antibodies (also IgG and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labeled anti-human IgA (enzyme conjugate) catalyzing a color reaction. (EUROIMMUN Medizinische Labordiagnostika AG).

Indications: Glutin-sensitive enteropathy (CD), Duhring's Dermatitis herpitiformis.

Calculation of results: Results can be evaluated quantitatively using "point-to-point" plotting of the extinction values measured for the 3 calibrators

against the corresponding units (linear/linear) for calculation of the standard curve by computer.

*The upper limit of the normal range (**cut-off**) recommended by EUROLMMUN is 20 relative units (RU) /ml. EUROLMMUN recommends interpreting results as follows:

<20RU/ml: negative , ≥20RU/ml: positive

2- Anti-tissue Transglutaminase ELISA (IgG) test kit principle: This kit provides a semiquantitative or quantitative in vitro assay for human autoantibodies of the IgG class against tissue Transglutaminase in serum or plasma. The test kit contains microtiter strips, each with 8 break-off reagent wells coated with human tissueTransglutaminase. In the first reaction step, diluted patient samples are incubated in the wells, and in case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labeled anti-human IgG (enzyme conjugate) catalyzing a color reaction. (EUROIMMUN Medizinische Labordiagnostika AG).

Calculation of results: 1-Quantitative interpretation of results. The extinction value of the calibrator defines the upper limit of the reference range of healthy subjects (**cut-off**) recommended by EUROIMMUN. Values above the indicated cut-off are to be considered as positive, those below as negative. 2-A semiquantitative evaluation of results is possible by calculating a ratio according to the following formula:

$Ratio =$ (Extinction of control or patient sample)/Extinction of the calibrator (**cut-off**)

*EUROLMMUN recommends interpreting results as follows:

Table (2.7): Result interpretation

Ratio	Finding
<1.0	Negative
≥1.0 to 2.0	Weak positive
≥2.0 to 5.0	Positive
≥5.0	High positive

2.9.2.2- Serum anti- DGP antibody measurement:

Indications: Glutin-sensitive enteropathy (CD), Duhring's dermatitis herpitiformis.

1-Anti-gliadin (GAF-3X) ELISA (IgA) test kit principle: This assay provides a semi-quantitative or quantitative in vitro assay for human antibodies of the IgA class against gliadin (GAF-3X) in serum or plasma. The test kit contains microtiter strips, each with 8 break-off reagent wells coated with gliadin(GAF-3X). In the first reaction step, diluted patient samples are incubated in the wells, and in case of positive samples, specific IgA antibodies (also IgG and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labeled anti-human IgA (enzyme conjugate) catalyzing a color reaction (EUROIMMUN Medizinische LabordiagnostikaAG).

Calculation of results: Results can be evaluated quantitatively using "point-to-point" plotting of the extinction values measured for the 3 calibrators against the corresponding units (linear/linear) for calculation of the standard curve by computer.

*The upper limit of the normal range (cut-off) recommended by EUROLMMUN is 25 relative unit (RU) /ml.

EUROLMMUN recommends interpreting results as follows:

<25RU/ml: negative , ≥25RU/ml: positive

2- Anti-gliadin (GAF-3X) ELISA (IgG) test kit principle: This kit provides a semi-quantitative or quantitative in vitro assay for human antibodies of the IgG class against gliadin (GAF-3X) in serum or plasma. The test kit contains microtiter strips, each with 8 break-off reagent wells coated with gliadin(GAF-3X). In the first reaction step, diluted patient samples are incubated in the wells, and in case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labeled anti-human IgG (enzyme conjugate) catalyzing a color reaction. (EUROIMMUN Medizinische Labordiagnostika AG).

Calculation of results: Results can be evaluated quantitatively using "point-to-point" plotting of the extinction values measured for the 3 calibrators against the corresponding units (linear/linear) for calculation of the standard curve by computer.

*The upper limit of the normal range (cut-off) recommended by EUROLMMUN is 25 relative unit (RU) /ml. EUROLMMUN recommends interpreting results as follows:

<25RU/ml: negative , ≥25RU/ml: positive

The procedure for measuring serum antibodies titer:

As the method of measurement is the same for the all four kits, we mentioned it once to overcome repeating.

***The coated wells, calibrators and controls, enzyme conjugate, sample buffer, chromogen/substrate, and stop solution all are ready to use.

* The wash buffer is a 10x concentrate, diluted as 1 part reagent plus 9 part deionized or distilled water (for example, 50 ml of wash buffer in 450 ml DW).

* Patient samples are diluted 1:201in sample buffer (for example, we diluted 5 Ml serum in1.0 ml sample buffer and mixed well by a vortex [mixer]).

*__Step one__: Transfer 100 Ml of the calibrators, positive or negative control or diluted samples into the individual microplate wells according to pipetting protocol. Incubate for 30 minutes at room temperature (+18 C to +25C).

*Automatic emptying and washing of wells (program setting: Three times washing) using the prepared washing buffer specific for each kit, thoroughly dispose of all liquid from microplate by tapping it on absorbent paper.

*__Step two:__ Pipette 100 Ml of enzyme conjugate (peroxidase-labeled anti-human IgA) into each well. Incubate for 30 minutes at room temperature (+18 C to +25C).

*Wash as described above.

***Step three:** Pipette 100 Ml of chromogen/substrate solution. Incubate for 15 minutes at room temperature (+18 C bis 25C), protect from direct sunlight.

***Step four:** Pipette 100 Ml of stop solution into each well in the same order and at the same speed as the chromogen/substrate solution was introduced.

***Step five:** Photometric measurement of the color intensity should be made using a microplate reader set at a wavelength of 450 nm and a reference wavelength between 629 nm and 650 nm within 30 minutes of adding the stop solution. Prior to measuring, a slight shaking of the microplate is required to ensure a homogenous distribution of the solution.

2.10-Waste disposal:

The patient's and control's samples, the microplates and the remainders of all kits content had been handled as infectious waste and disposed according to Al-Hussein medical city lab-disposal approach.

2.11-Statistical analysis:

Data analysis was performed using the SPSS version 18 soft-ware package (Statistical Package for Social Science; Inc., Chicago, IL).

Quantitative data were expressed as Mean ± standard deviation (SD), and the quantitative variables were analyzed using non-parametric t-test. On the other hand, qualitative data were expressed as numbers (N) and percentages, and qualitative variables were analyzed using Chi-square test. Pearson correlation analysis was used to reveal the association between two or more of the related quantitative variables.

*A p values <0.05 were considered statistically significant**.**

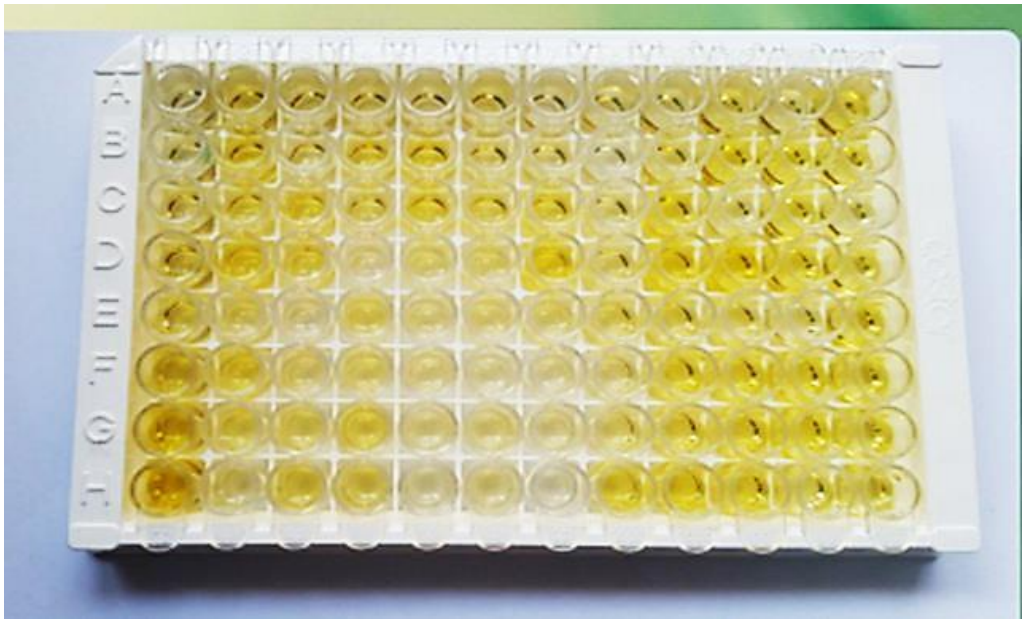

Plate 2.1: Zonulin microplate result

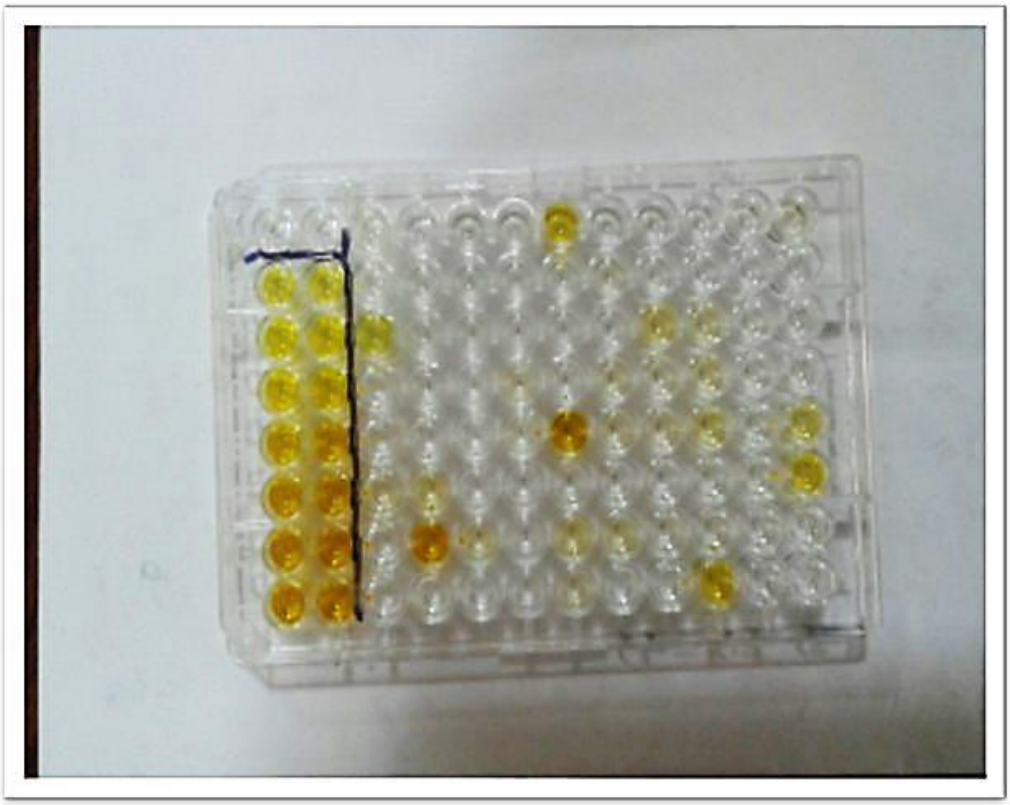

Plate 2.2: IL15 microplate result

Chapter Three

Study Results

Chapter Three

3-Results:

3.1-Socio-demographic characteristics of celiac patients and controls (age, gender and residence):

Age: A total of 108 celiac patients (N =108), with age ranging from 1 year to 58 years and their mean age ± (SD) is 18.39±14.08 year , where children account to about two third (N=68 ; 63%) of the celiac patients. **Gender:** Females in this study group are more than males (69.4**%** VS 30.6**%**) with the female to male ratio being 2.27: 1. **Residence:** About a three quarters of patients of urban residence (74.1**%** VS 25.9**%**), and most of them are literate. Patients and control were matched for age, gender and residence with a p-value more than 0.05 for each demonstrated in table (3.1) below.

Table-(3.1): Socio-demographic characteristics (age, gender, and residence) of celiac patients comparing to controls.

Variable		Patients No. (%)	Controls No. (%)	P value
Age in years	Less than 18	68(63)	70(59.3)	0.07
	18 and above	40(37)	48(40.7)	
	Total	108(100)	118(100)	
	Mean ±SD	18.39±14.08	21.76±13.78	
	Range	1- 58	1.5- 57	
Gender	Male	33(30.6)	43(36.4)	0.4
	Female	75(69.4)	75(63.6)	
	Total	108(100)	118(100)	
Residence	Urban	80(74.1)	78(66.1)	0.2
	Rural	28(25.9)	40(33.9)	
	Total	108(100)	118(100)	

3.2-Clinical presentation of celiac patients:

Most of patients reveal *typical or intestinal clinical features, while

**atypical or extra-intestinal presentations account for small percentage (83.8% VS 16.2%). Three patients of total had a silent presentation and are known cases of type 1 Diabetes Mellitus (DM) and diagnosed by chance during screening tests for DM. Both adults (90%) and children (80%) clinical presentation is intestinal which shows no statistical significant difference between the two subgroups ($p > 0.05$) as demonstrated in the table below.

Another point is the presentation period (for how long the patient was complaining before diagnosis) which extended to years in more than half of cases.

In addition, CD patients often developed skin (mainly DH) and oral manifestations. The study group reveals that ***skin manifestations contributed to one quarter of the patients, while the ****oral manifestations account for about three quarters of cases. Among positive cases for skin manifestations, 15 are pediatric age group and 11 are adults, with macular/ papular rash, contact dermatitis, itching, are the most frequent manifestations. On the other hand, the pediatric age group constitutes a two third (67.5%) of the positive cases for oral manifestations, and aphthous stomatitis was the most frequent complaint.

***Typical or intestinal clinical features**: Include Diarrhea, steatorrhea, abdominal bloating or distension, abdominal pain, weight loss, dyspepsia, dysphagia, nausea and vomiting, constipation, and poor appetite.

****Atypical or extra-intestinal clinical features:** Include micronutrient deficiencies like Iron deficiency aneamia, growth retardation or failure to thrive, short stature, infertility and menstrual disturbances, osteoporosis, and lethargy.

*****Skin manifestations:** Contact dermatitis, macular or popular skin rash, urticaria, itching, dryness of skin, and hypo-or hyperpigmentation.

****** Oral manifestations:** Include aphthus**,** angular stomatitis, tongue fissures, and cracked lips.

Table-(3.2): The clinical presentations of celiac patients and associated intestinal and autoimmune diseases. The values are in percentage and numbers.

Clinical presentation	Number	%	*P* value
Intestinal	Children 52 Adult 36 Total 88	80 90 83.8	Chi square test was used
Extra intestinal	Children 13 Adult 4 Total 17	20 10 16.2	0.1
Total	105	100	
Skin manifestations	Number	%	
Negative	82	75.9	
Positive	26	24.1	
Total	108	100	
Oral manifestations	Number	%	
Negative	25	23.1	
Positive	Children 56 Adult 27 Total 83	51.8 25.1 76.9	
Total	108	100	
Period of presentation	Number	%	
Months	48	45.7	
Years	57	54.3	
Total	105	100	
Duration of diagnosis	Number	%	
Months	41	38	
Years	67	62	
Total	108	100	

3.3-Associated disorders:

Associated autoimmune disorders: For the study group, a 27 of the total 108 have DM type 1(25%) and a small percentage (9.3%) evolved other autoimmune diseases such as vitiligo, hypothyroidism, hyperprolactinaemia, SLE, psoriasis, and autoimmune hepatitis (3 patient of this category have

more than two autoimmune disease). In most of the patients, autoimmune disease started before CD and only two cases have simultaneous occurrence.

Regarding the distribution of autoimmune diseases among age groups, the table (3.2) illustrates that DM type 1 is more in children comparing to adults (16.7% VS 8.3%) with the reverse applied to the other autoimmune diseases which more in adults (6.5% VS 2.8%). Although these findings are important, but they not reach significance point (*P* value= 0.08).

Associated hereditary disorders: Like mengolism, selective IgA deficiency, hypogonadism, kidney agenesis and hydronephrosis, and esophageal stenosis, each distributed as single case between patients.

Associated intestinal diseases: reveals that one quarter of celiac patients had a history of worm infestation (24.1%), while only (5.5%) have different intestinal diseases like H-pylori infection, colitis, rectal fissure, acute pancreatitis with Amylase > 100U/L.

Table (3.3)-Associated intestinal, autoimmune, and hereditory disorders:

Associated intestinal diseases	Number	%	
Negative	76	70.4	
Worm infestation	26	24.1	
Others	6	5.5	
Total	108	100	
Associated autoimmune disorders	Number	%	*P* value
Negative	71	65.7	
DM type1	Children 18	16.7	0.08
	Adult 9	8.3	
	Total 27	25	
Others	Children 3	2.8	
	Adult 7	6.5	
	Total 10	9.3	
Total	108	100	

3.4- Family history of celiac disease:

More than half of the patients have no family history of CD, and approximately one quarter have first degree relatives with CD, while a small percentage have second degree relatives with CD as shown in figure (3.1)

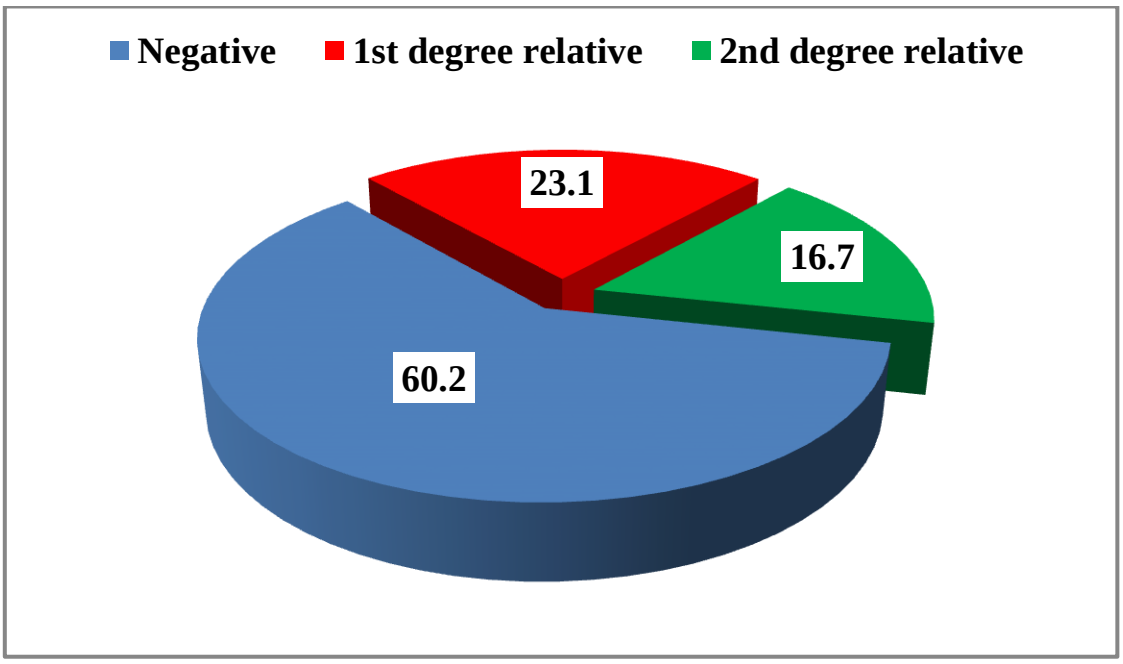

Figure (3.1): Family history of the disease of the patients.

Negative family history is more in pediatric age group comparing to adults (72.3% VS 27.7%), while the positive family history of first degree relatives is slightly more in adult age group (56.0% VS 44.0%) and the reverse for the second degree relatives. There is significant association between CD and family history (*P* value = 0.04) as shown below in the table (3.3).

Table-(3.4): Difference in family history of celiac disease among adults and children.

		Age groups		Total
		Children	Adult	
Family Hx	Negative	47	18	65
		72.3%	27.7%	100.0%
	Positive 1st degree relative	11	14	25
		44.0%	56.0%	100.0%
	Positive 2nd degree relative	10	8	18
		55.6%	44.4%	100.0%
Total		68	40	108
		63.0%	37.0%	100.0%

P=0.04*

3.5-Serological markers of CD:

For tissue TG IgA antibody titer as serological markers of CD, the study group reveals that the percentage of the 10 folds the upper limit of normal (ULN) range of the antibody (cut-off) i.e. 200 RU/ml and more, was positive in about three quarters of the patients.

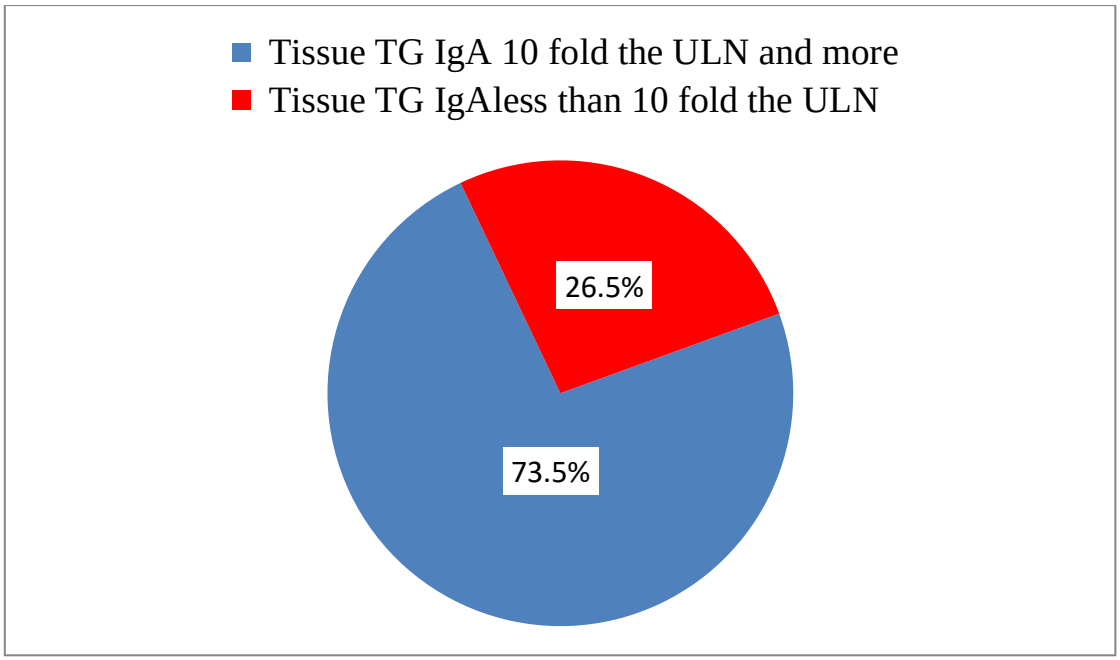

Figure (3.2): Percentage of tissue TG IgA titer of the patients.

Notably figure (3.3) below shows that there was no significant association of (difference in) tissue TG IgA titer between age groups (p=0.6).

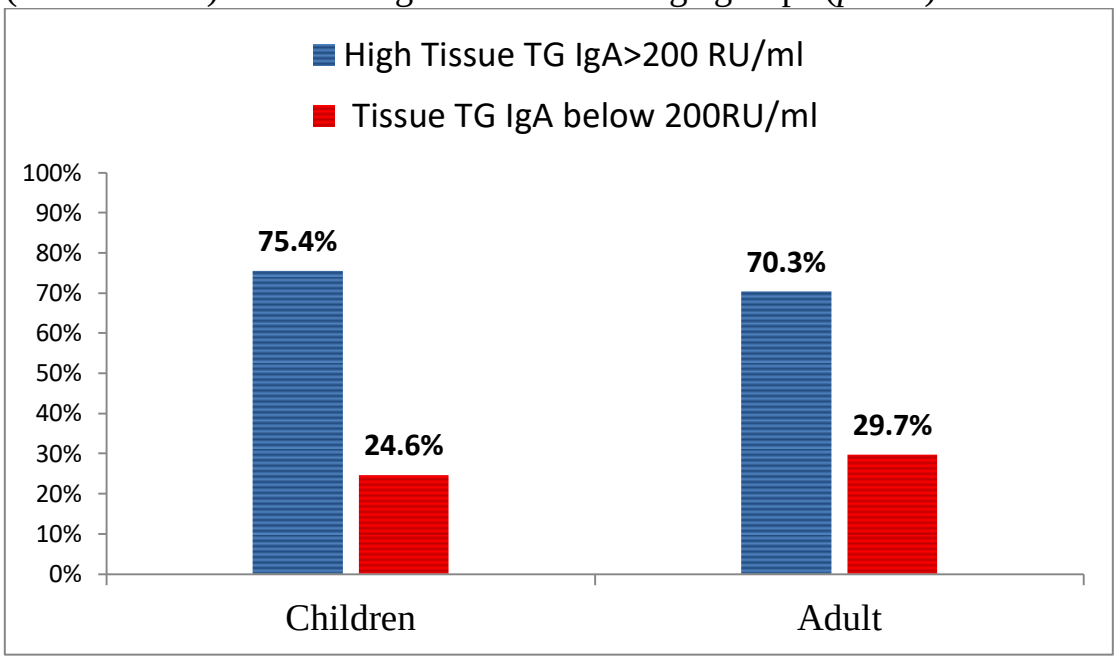

Figure (3.3): Association of tissue TG IgA titer with age groups.

3.6- Small intestine biopsy results:

A small intestine endoscopy and biopsy had been done for all adult age group and only three children and the results according to modified Marsh-Oberhuber classification demonstrates that M2 accounts for about half of cases shown in the figure (3.4).

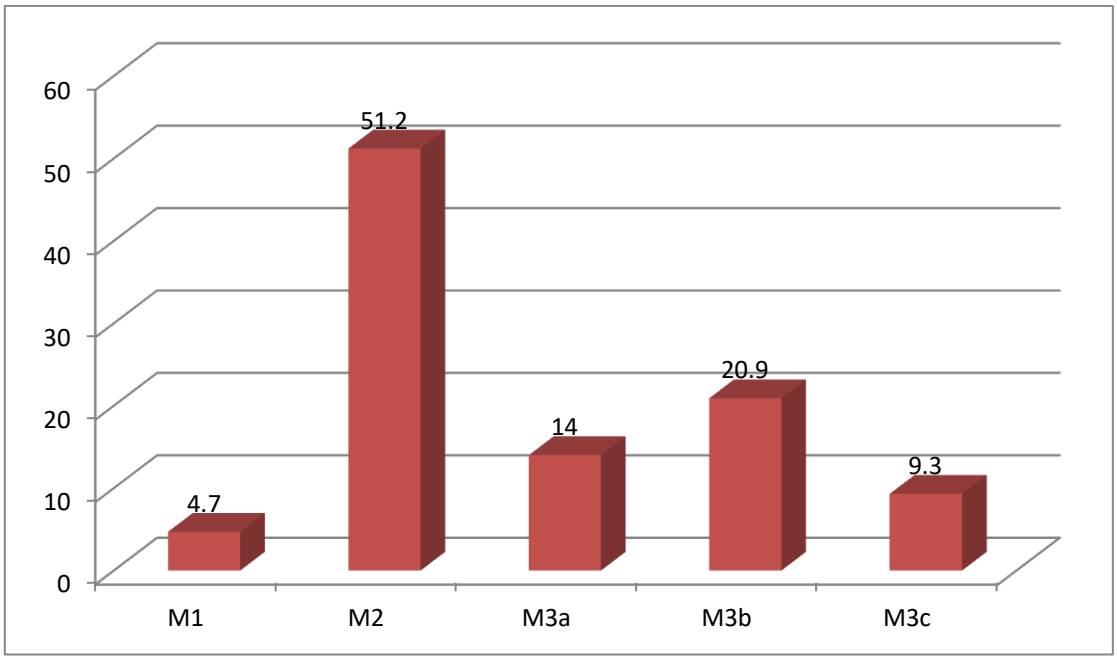

Figure (3.4): Small intestine biopsy results according to modified Marsh-Oberhuber classification.

*Notably, all other peadiatric patients were positive for challenge test.

3.7- Correlation of Tissue TG (IgA) level with small intestine biopsy results:

Those 43 patients who had undergone small intestine endoscopy and biopsy were divided into two categories: Patients with M3 (a, b, c) grade of modified Marsh-Oberhuber classification show mean autoantibody level more than that those with M2 grade. However, this positive correlation not reached significance (p value 0.07).

Table- (3.5): Correlation of Tissue TG (IgA) level with small intestine biopsy results according to modified Marsh-Oberhuber classification.

Biopsy findings		No.	Mean	SD	r	P value**
Tissue TG(IgA)	M2	22	252.56	131.07	0.084	0.07
	M3(a,b,c)	19	337.47	153.87		

** Pearson correlation was used. Correlation is significant at p value of 0.05.

3.8-Adherence to gluten free diet in celiac patients:

The majority of celiac patients had started gluten free diet (89.8%), with the period of GFD extended to months or years according to the duration of diagnosis of each. However, only a small percent of them (20.6%) had adequate adherence to GFD and the response to GFD demonstrates relief of symptoms in 39.2% of patients and both clinical improvement plus reduction of antibody titer in more than one half of them (56.7%).

Table-(3.6): Gluten free diet of celiac patients.

Starting Rx (GFD)	**Number**	**%**
Yes	97	89.8
No	11	10.2
Total	108	100
Period of GFD	**Number**	**%**
Months	37	38.1
Years	60	61.9
Total	97	100
Degree of adherence to GFD	**Number**	**%**
Adequate	20	20.6
Non adequate	77	79.4
Total	97	100
Response to adherence to GFD	**Number**	**%**
No response	4	4.1
Relief of symptoms	38	39.2

Relief of symptoms plus reduction of antibody titer	55	56.7
Total	97	100

3.9- Association between degree of adherence to GFD and response to GFD:

Although only small percent of celiac patients (20.6%) had adequate degree of adherence to GFD**,** the table (3.7) shows that they had better response than those with non-adequate degree of adherence (85% and 52% respectively) and this difference is statistically significant (p value 0.006).

Table-(3.7): Association between degree of adherence to GFD and response to GFD.

Response to GFD	adequacy		P value
	Adequate No. %	Non adequate No. %	
Relief of symptoms	3 15	35 48	0.006*
Relief of symptoms plus reduction of antibody titer	17 85	38 52	
Total	20 100	73 100	

3.10-Association between serum zoulin level and period of adherence to GFD:

Regarding serum zonulin level in association to the period of adherence to GFD, this study shows that celiac patients who adhered to GFD for more than one year had lower mean serum zonulin level than those adhered to GFD for months, however this difference not reached significance (p= 0.2).

Table-(3.8): Association between serum zoulin level and period of adherence to GFD.

Period of adherence to GFD		No.	Mean	SD	P value*
Serum Zonulin in ng/ml	Months				P=0.292
	1-6	37	7.81	6.56	
	7-12	4	6.47	4.55	
	Years				
	1-3	44	6.22	4.42	
	> 3	23	5.27	3.88	

*Independent-Samples t test was used.

3.11- Association between serum levels of Tissue TG (IgA) with period of adherence to GFD:

The difference in the period of adherence to GFD between celiac patients (years or months) shows a statistically significant association with their serum level of antibodies (p=0.001), where patients adhered >3 years had lowest serum level as shown in the table below.

Table-(3.9): Association between the serum levels of Tissue TG (IgA) antibodies with period of adherence to GFD.

Period of adherence to GFD		No.	Mean	SD	*P* value*
Tissue TG IgA	Months				
	1-6	37	298.9	81.87	
	7-12	4	209.63	58.31	*P*=0.001
	Years				
	1-3	47	212.76	66.44	
	>3	14	179.8	71.4	

3.12- Follow up of celiac patients:

From the total 108 celiac patients, only 41 (38%) of them had regular follow-

up, and the others have irregular follow up or only one time following diagnosis.

Table (3.10): Follow up of celiac patients.

Follow up	Number	%
Regular	41	38
Irregular	40	37
One time	27	25
Total	108	100

3.13- correlation of serum zonulin level with serum antibody titers in celiac patients

There was positive correlation shown between serum zonulin level and serum tTG (IgA), but didn't reach significance, while negative correlation revealed between serum zonulin and other serum antibodies level ($p > 0.05$), as demonstrated in table below.

Table-(3.11): correlation of zonulin level with antibody titers in celiac patients.

	Mean	SD	N	r	P value*
Serum Zonulin	6.59	5.28	108	0.14	0.4
TA	272.83	117.75	108		
Serum Zonulin	6.59	5.28	108	-0.08	0.3
TG	17.72	64.52	108		
Serum Zonulin	6.59	5.28	108	-0.069	0.2
GA	76.13	203.84	108		
Serum Zonulin	6.59	5.28	108	-0.18	0.1
GG	61.36	43.23	108		

* Pearson correlation was used with a significant p value of less than 0.05.

3.14- Correlation of serum IL15 level with serum antibody titers in celiac patients:

Also this study demonstrates that there was positive correlation shown between serum IL15 level and serum tTG (IgA) and AGA (IgA), but also didn't reach significance, while negative correlation revealed between serum IL15 and serum tTG (IgG) and AGA (IgG) antibodies level ($p > 0.05$), as shown in the table below.

Table-(3.12): Correlation of IL15level with antibody titers in celiac patients.

Serum level	Mean	SD	N	r	P value
IL15	748.88	1803.59	16	0.05	0.5
TA	272.83	117.75	108		
IL15	748.88	1803.59	16	-0.14	0.7
TG	17.72	64.52	108		
IL15	748.88	1803.59	16	0.087	0.3
GA	76.13	203.84	108		
IL15	748.88	1803.59	16	-0.03	0.2
GG	61.36	43.23	108		

* Pearson correlation was used with a significant p value of less than 0.05.

3.15- correlation of zonulin and IL15 levels in celiac patients:

A positive correlation demonstrated between the serum levels of the tow markers, however this correlation didn't reach significance (P=0.5).

Table- (3.13): correlation of zonulin and IL15 levels in celiac patients.

	Mean	SD	N	r	*P* value*
Serum Zonulin	6.59	5.28	108	0.13	0.5
IL15	748.88	1803.59	16		

* Pearson correlation was used with a significant p value of less than 0.05.

3.16- Serum zonulin and IL15 levels in children celiac patients compared to adults:

Here, the study demonstrated that adult celiac patients had lower serum zonulin level than children and this difference is statistically significant (p= 0.002). On the other hand, IL15 level was higher in adults but this difference is statistically not significant (p> 0.05) as seen in table (3.13).

Table (3.14): Zonulin and IL15 levels in children compared to adults.

Age groups		No.	Mean	SD	P value*
Zonulin	Children	68	7.80	5.75	0.002*
	Adult	40	4.52	3.59	
IL15	Children	13	509.69	1465.66	0.3
	Adult	3	1785.33	3086.23	

*Independent-Samples t test was used.

3.17- Association of serum zonulin and IL15 levels with complications in celiac patients.

Another association revealed in this study is the higher serum level of zonulin in patients with complications compared to those without complications, this statistically significant association (*p* value= 0.004). On the other hand serum IL15 levels show on significant association (*p* value > 0.05) as seen in the table below.

Table- (3.15): Association of serum zonulin and IL15 levels with complications in celiac patients.

	Complication	N	Mean	Std. Deviation	P value
Serum Zonulin in ng/ml	No Complications	76	5.65	3.90	0.004*
	Positive Complications	32	8.82	7.22	
Serum IL15 in pg/ml	No Complications	9	1192.61	2356.46	0.3
	Positive Complications	7	178.36	231.06	

3.18- Association of serum level of zonulin, IL15, and Tissue TG (IgA) with autoimmune diseases in celiac patients.

Also an attempt has done to look for an association between serum level of zonulin, IL15, and tTG (IgA) in CD's patients who have associated autoimmune diseases and those who haven't. None of these variables show statistically significant association (*p* value> 0.05).

Table- (3.16): Association of serum level of zonulin, IL15, and Tissue TG (IgA) with autoimmune diseases in celiac patients.

	Autoimmune diseases	N	Mean	Std. Deviation	P value
Serum Zonulin in ng/ml	Absent	71	6.89	5.87	0.4
	Present	37	6.01	3.93	
SerumIL15 in pg/ml	Absent	11	582.36	1593.08	0.6
	Present	5	1115.20	2368.49	
Tissue TG(IgA)	Absent	66	276.62	101.35	0.8
	Present	36	294.86	132.71	

3.19- Association of serum level of zonulin, IL15, and celiac-specific antibodies with clinical presentation of celiac patients.

In addition, the study associates between serum zonulin in ng/ml, serum IL15 in pg/ml, serum tTG (IgA and IgG), and serum DGP (IgA and IgG) with the

type of clinical presentation (intestinal or extra-intestinal). There was an association of tTG (IgG) with extra-intestinal manifestations, but it does not reach significance (*p* value =0.06), while DGP (IgA) reveals significant association also with extra-intestinal manifestations (*p* value =0.02). No significant association between other variables with the type of clinical presentation as shown in table (3.17).

Table- (3.17): Association of serum zonulin, IL15, and celiac specific antibodies levels with clinical presentation in celiac patients.

	Presentation	N	Mean	Std. Deviation	P value
Serum Zonulin in ng/ml	Intestinal	88	6.61	5.48	0.8
	Extra intestinal	17	6.21	4.61	
Serum IL15 in pg/ml	Intestinal	11	583.31	1592.70	0.4
	Extra intestinal	3	1783.50	3087.81	
Tissue TG(IgA)	Intestinal	82	286.68	104.83	0.5
	Extra intestinal	17	266.50	155.01	
Tissue TG(IgG)	Intestinal	88	12.98	42.52	0.06
	Extra intestinal	17	45.19	130.50	
DGP(IgA)	Intestinal	88	57.04	52.20	0.02*
	Extra intestinal	17	186.33	497.36	
DGP(IgG)	Intestinal	88	61.56	42.16	0.7
	Extra intestinal	17	65.40	50.56	

3.20- Association of serum level of zonulin, IL15, and Tissue TG (IgA) with skin manifestations in celiac patients.

Moreover, this study clarified the association of serum zonulin, IL15, and tTG(IgA) levels with skin manifestations appeared in about one quarter of our candidates, where only zonulin shows an association with positive skin manifestation, however this association also does not reach significance (*p*

value = 0.05). The presence or absence of skin manifestations had no statistical significance on the corresponding serum level of tTG (IgA) antibodies (p> 0.05), since no one revealed the significant dermatologic presentation of DH (strongly associated with antibody titer) as revealed in the table below.

Table (3.18): Association of serum level of zonulin, IL15, and Tissue TG (IgA) with skin manifestations in celiac patients.

Skin manifestations		N	Mean	Std. Deviation	P value
Serum Zonulin in ng/ml	Absent	82	6.01	5.17	0.05
	Present	26	8.39	5.31	
Serum IL15 in pg/ml	Absent	10	1154.70	2219.26	0.3
	Present	6	72.50	123.23	
Tissue TG(IgA)	Absent	77	275.91	114.83	0.3
	Present	25	305.06	106.74	

3.21-Serum zonulin and IL15 levels in celiac patients compared to controls:

This study demonstrates that there was significant statistical difference in serum zonulin and IL15 levels between celiac patients and their matched for age and gender controls ($p< 0.05$).

Table (3.19): Zonulin and IL15 levels in celiac patients compared to controls.

		Mean (SD)	P value*
Zonulin	Patients	6.59 (5.28)	0.0001*
	Control	0.28 (0.35)	
IL15	Patients	110.94 (726.26)	0.001*
	Control	0.18 (1.18)	

*Independent-Samples t test was used.

Chapter Four

Discussion

Chapter Four

4-Discussion:

Given the increasing rate in CD diagnosis, only a few Iraqi studies conducted about the predictive (inflammatory) markers orchestrating the disease pathogenesis, genetic predisposition, and the prevalence of the disease in different ethnic groups of Iraqi population. This study was conducted to uncover the role of zonulin as a biomarker for impaired IP and IL15 as a predictive marker for inflammatory process in CD patients comparing to normal controls matched for age and gender.

4.1- Socio-demographic characteristics of celiac patients and controls (age, gender and residence):

4.1.1 Age:

Two age groups are the candidate of the study (less than 18 years and 18 years and above), and their mean age ± (SD) is 18.39±14.08 year, where children account to about two third (N=68; 63%) of the celiac patients, and compared with the same age groups of controls (*P* value 0.07). These age groups correspond with many studies ensuring that CD can be diagnosed at any time of life, with most presentation in either early childhood (between 9 and 24 mo) or in the third or fourth decade of life (Mäki and Collin, 1997; Feighery, 1999; Ciclitira *et al*., 2001; Fasano and Catassi, 2001) Abadie and his team also reported that CD manifestations can evolve at any age (Abadie *et al*., 2011). These results are also consistent with the results of other studies which demonstrated that 64% of patients with CD in Karbala were among age group of 2-10 years (Al-Saadi and Abid, 2009; Hameed *et al*., 2016). In Iraq, the early introduction of gluten-rich diet (like biscuits and cakes) may be a contributing factor increase the risk of CD in childhood, while the awareness of pediatric specialists about CD might increase the rate of CD diagnosis in childhood comparing to adults.

4.1.2 Gender:

In this study group, females are more than males (69.4**%** VS 30.6**%**) with the female to male ratio being 2.27: 1, a results which usually observed in most autoimmune disorders, where CD is twice more frequent in women than in men (Polanco *et al*., 1981). Thomas *et al* in 2009 reported a proximal result with a male: female ratio of 1:2.8 (Thomas *et al*., 2009). Hameed and his team also concluded that CD is more in female than in male (Hameed *et al*., 2016).

Several factors may reflect the differential rates of diagnosis among sexes, of which are 1. In general there is a higher rate of autoimmune disease among females (Dixit *et al*., 2014). 2. Sex hormone differences among male and female account for the high rate of autoimmunity in females (Owen *et al*., 2013). 3. Some genetic loci are sex-influenced and the sex hormones role in regulating immune response might elucidate these differences (Green *et al*., 2001). Sex-dependent HLA associations are obvious as female patients often have DQ2.5 and/or DQ8 molecules (Megiorni *et al*., 2008).

4. The finding that males with CD were diagnosed at an older age may contribute to this sex difference (Hin *et al*., 1999). 5. Symptomatic disease and regular health care interaction noticed more in women than men (Dixit *et al*., 2014)

4.1.3 Residence:

Most of the study candidates were dwelling in urban areas (about a three quarters of patients) in comparison to those dwelling in rural ones (74.1**%** VS 25.9**%**), and most of them are educated. It was noted that CD occurred more commonly among areas with least socioeconomic deprivation (West *et al*., 2014). This point can be explained, where high social class people have protean food intake containing gluten, but at the same time they more aware of any complaints and always seek advice so diagnosed earlier.

4.2- Clinical presentation of celiac patients:

Most of patients (adults 90% and children 80%) revealed typical or intestinal clinical features, while atypical or extra-intestinal presentation account for small percentage (83.8% VS 16.2%) and only three patients of total had silent presentation and diagnosed during follow-up tests for DM type1. However these findings may agree with some studies which mentioned an eclectic clinical expression of the disease, with nutrient malabsorption (diarrhea, failure to thrive in children, anemia, etc.) be the most typical manifestations in 40 to 50% of patients (Green and Cellier, 2007). Leffler et al and Husby et al in 2012 found that classic gastrointestinal symptoms of CD are most common in early childhood , but other presentations including asymptomatic growth delay are also identified(Husby *et al.*, 2012; Leffler *et al.*, 2012). Also Rostami et al in 1999 revealed that CD is a relatively common cause of chronic diarrhea in Iran, Iraq and Kuwait (Rostami *et al*., 1999). Other studies had reported that the common clinical manifestations of CD include diarrhoea, malabsorption, emaciation, and aphthous stomatitis, however, only mild or no symptoms at all also exhibited by many patients(Fasano, 2005; Barker and Liu, 2008). In contrary, some studies revealed that extra-intestinal manifestations might be the dominating clinical presentation in adults comparing to children (Green and Cellier, 2007), but non-specific (uncommon) manifestations like headache, joint pain, and mood swings have also been revealed in some patients (Schuppan and Zimmer, 2013). Atypical (non-classical) manifestations are more in western patients (Bai *et al*., 2013).

A widely variable clinical presentation may attributed to age of onset, dietary habits, degree of mucosal injury, and gender, in addition to both genetical and immunological bases of each individual (Bai *et al*., 2005).

Another important point to mention is the period in which the patients were complaining before diagnosis which is ranging from months to years in more than half of cases (54.3%). Two possible explanations for this delay can be

illustrated, first is the clinical suspicion which directed toward irritable bowel in many patients and to a less degree inflammatory bowel disease with CD being the last in the list. Second is the low awareness of the extra intestinal manifestations of the disease and CD subtypes, while lack of sophisticated techniques, patients jumping among specialists can also be attributed. This make patients more prone to a myriad of CD complications as the unrecognized disease in young children with its chronic systemic inflammatory course that may increase the risk of long-term complications and other systemic inflammatory disorders associated in children with prolonged untreated CD, a point be accounted for when screening in young children is performed (Bjorck *et al*., 2015).

4.3- Skin and oral manifestations of celiac patients:

The skin manifestations contributed to about one quarter (24.1%) of the total patients. More than half of positive cases for skin manifestations are peadiatric age group with macular/ papular rash, contact dermatitis, itching, are the most frequent manifestations. At the time oral manifestations account for about three quarters (76.9%) of cases, the peadiatric age group also constitutes the larger category (67.5%) of the positive cases, and in most of the cases aphthous stomatitis was the predominant. A lot of studies demonstrated oral/cutaneous disease, including dermatitis herpetiformis as a part of CD atypical symptoms or unusual manifestations of it (Green and Jabri, 2003; Green, 2005; Green and Cellier, 2007; Caproni *et al*., 2009). Absence of DH cases could be due to small sample size or missed cases as people with skin lesions usually attend Dermatology clinic rather than Internal Medicine clinics.

Oral manifestations in large percent of candidates might reflect the systemic nature of the disease or might also be due to nutrients deficiency associated with CD, food habits, febrile illnesses or other unknown causes.

4.4- Associated diseases:

As mentioned above that a quarter of the study candidates have DM type 1(25%) and a small percentage (9.3%) affected with other autoimmune diseases, while a few cases having two autoimmune diseases in addition to CD. Regarding the distribution of autoimmune diseases among age groups, DM type 1 was more in children comparing to adults (66.7% VS 33.3%), a result coincides with other study results as the prevalence of CD in DM type 1 patients has been estimated up to 15% in children and 6% in adults (Holmes, 2002). The reverse applied to the other autoimmune diseases which more in adults (70.0% VS 30.0%). Although these findings are important, but they not reach significance point (*P* value= 0.08) which may be due to small sample size. In a very large cohort of children, Ludvigsson et al reported that type 1-diabetes poses increasing risk of 5- to 10-fold for celiac disease (Ludvigsson *et al*., 2006), while Cosnes et al in 2008 revealed a strong link among autoimmunity and CD given that 5%–10% of patients with DM type 1 evolve CD, while, 15%–20% of celiac patients have or will evolve autoimmune diseases (Cosnes *et al*., 2008). In spite of different phenotypes of CD and other autoimmune diseases, genetic studies have recently demonstrated shared loci between the two (Zhernakova *et al*., 2009; Kumar *et al*., 2012). The environmental factors together with these shared genetic components may rather elucidate the concurrent evolvement of various immune-mediated disorders in celiac patients and their relatives. Thus, HLA haplotypes has been prescribed to overlap between CD and other autoimmune diseases (Gutierrez-Achury *et al*., 2011; Larizza *et al*., 2012). The guidelines currently suggest considering celiac patients screening for autoimmune diseases (especially type 1 DM and Hashimoto thyroiditis)(Rubio-Tapia *et al*., 2013; Ludvigsson *et al*., 2014).

However the low proportion of other autoimmune diseases revealed by this study in general may not reflect the real situation as there are no available

screening tests for such disorders in all hospitals, symptomatic treatment of patients in many conditions, and loss of education about these disorders, all may contribute to the results and need further evaluation.

For hereditary disorders, only very small proportion of cases (n=7, 6.4%) have hereditary disorders like mengolism, selective IgA deficiency, hypogonadism, kidney agenesis and hydronephrosis, and esophageal stenosis. Two celiac cases have other autoimmune disease coincides with the hereditary disorder. These results consistent with the findings of many studies where genetic disorders like Down syndrome, Turner syndrome and William's syndrome, had been seen more in CD patients (Barker and Liu, 2008).

More over this study reveals that one quarter of celiac patients who < 18 year age (n=26, 24.1%) had a worm infestation, which is very common problem in school-age group children and not specific to CD. Only a small percent of candidates (n=6, 5.5%) have different intestinal diseases like H-pylori infection, non-specific colitis, rectal fissure, acute pancreatitis with Amylase > 100 reported mostly in adults, a findings that closely related to other study data which revealed that a lot of other immune- and non-immune-mediated diseases of the GI tract can be associated with CD like ulcerative colitis, Crohn's disease, microscopic colitis, and autoimmune liver diseases (Collin *et al*., 2002).

4.5- Family history of celiac disease:

More than half of cases (60.2%) had negative family history for CD with peadiatric age group has the more proportion comparing to adults (72.3% VS 27.7%). Family members with silent or potential CD, absence of screening tests, and low popular awareness about the disease may contribute the negative family history. The remaining 39.8% of cases had positive family history (23.1% first degree relatives and 16.7% second degree relatives). Among the latest category, the first degree relatives were more in adult age

group (56.0% VS 44.0%) and the reverse for the second degree relatives (more in children). Generally in this study, there is significant association between CD and family history (*P* value = 0.04), a finding that comes with the findings of many studies as there is substantial increase in frequency of CD in patients who have a first-degree family member affected with CD (Murray, 2005; Rubio - Tapia *et al*., 2008), while the second-degree relatives may apply a lower rate of risk for disease development (Fasano *et al*., 2003).

Other study demonstrated that first-degree relatives frequently have positive serology for celiac reflecting the importance of genetic predisposition (Aggarwal *et al*., 2012).

4.6-Serological markers of CD:

For tissue TG IgA antibody titer as serological markers of CD, this study group revealed that the percentage of the 10 folds the upper limit of normal (ULN) range of the antibody (cut-off) i.e. 200 RU/ml and more, was positive in about three quarters of the patients, with no significant difference in tissue TG IgA titer (10 folds ULN) between age groups (p value =0.6) i.e. both adults and children shows nearly the same results.

When reviewing the guidelines of the ESPGHAN which allow CD diagnosis without biopsies in children with obvious symptoms and levels of (TGA-IgA) antibodies 10-fold or more the ULN, with positive endomysium antibodies (EMA) in a different blood sample as a confirmatory test and positive HLA-DQ2/DQ8 typing.[triple test " ESPGHAN criteria"] (Husby *et al*., 2012). On verifying the study candidates with ESPGHAN approach for diagnosis, we have to explain some points:

1- There is no genetic study (HLA-DQ2/DQ8 typing) at least at the level of Kabala province to document the positivity of HLA-typing.
2- Anti- endomysium antibody (EMA) assay is also not available in Kabala province.

Nevertheless these points do not represent the stumbling block for the diagnosis as many studies data revealed the possibility to overcome these points, of which Werkstetter et al in 2017 in a prospective study to validate ESPGHAN approach on children who complaining of malabsorption symptoms instead of any symptom show that the inclusion of HLA analyses did not increase accuracy of dignosis (Werkstetter *et al*., 2017). Also Mubarak et al in 2011concluded that pediatric patients with a tTGA level ≥ 100 U/mL in whom symptoms improve upon consuming a GFD may not need a small intestinal biopsy to confirm CD. (Mubarak *et al.*, 2011)

Mubarak et al in another study in 2012 based on prospective data confirms omitting of a small intestinal biopsy for the diagnosis of CD in symptomatic patients with tTGA serum level ≥ 100 U/mL (Mubarak *et al*., 2012)

The WGO diagnostic protocol, especially in regions with limited resources (shortage of sophisticated techniques), considered tTG as an acceptable proxy of the EMA antibodies, ("The EMA test needs expert observers, and detection of tTG antibodies by ELISA tests should therefore be recommended in settings with low expertise") (Bai *et al*., 2013). The WGO also recommended the evaluation of the local resources available to develop a diagnostic protocol.

Measurement of antibodies serum levels was carried out using quantitative data for caliculation implying more accuracy for diagnosis in contrast to some laboratories that produce semi-quantitative data, making the assessment uncertain (Tucci *et al*., 2014).

The sensitivity of the TTG-IgA for untreated CD is about 95 % (Lewis and Scott, 2010), and the specificity is also 95 % or greater. The higher the titer of the test, the greater the likelihood of a true positive result (van der Windt *et al*., 2010), so tTG test has become the most common test for celiac diagnosis and monitoring due to its reliability and inexpensiveness (Dieterich *et al*., 1997; Rostami *et al*., 1999; Sugai *et al*., 2006). Ermarth and his team in a

population-based study found that serum level of tTG (IgA) can identify celiac disease patients with about 90% PPVs. The predictive values greatly increased as the circulating antibody levels are remarkably above the ULN or when the test is assessed in combination with other variables. However the measurement of tTG (IgG) or DGP (IgG) does not increase the accuracy of CD detection depending on tTG (IgA) levels (Ermarth *et al*., 2016).

4.7- Small intestine biopsy results:

A small intestine endoscopy and biopsy had been done for all adult age group and only three cases of peadiatric age group and the results according to modified Marsh-Oberhuber classification demonstrates that M2 accounts for about half of cases (n=22, 51.2%), while M3 (n=19) represents 44.2% of cases distributed among the subgrades (a, b, c), and only two cases show M1 class. The Gastroenterologists consider small intestine biopsy is a must for diagnosis of CD in adults. On the other hand, peadiatric specialists regarded challenge test as confirmatory test for children with clinical manifestations of CD with highly positive Tissue TG (IgA) level and their parents refused a small intestine endoscopy and biopsy as invasive procedure for diagnosis. Of note all the peadiatric patients were positive for challenge test.

When correlating Tissue TG (IgA) level with small intestine biopsy results, the study showed that patients with M3 (a, b, c) grade of modified Marsh-Oberhuber classification have mean tTG (IgA) levels (337.47RU/ml) more than that those with M2 grade (mean 252.56RU/ml), however, this positive correlation not reached significance (*p* value 0.07), an information that meriting further evaluation, where small sample size might be a confounding factor.

Generally these findings goes with Hawamdeh et al findings in 2016 who concluded a significant association between the degree of duodenal damage and anti-tTG titers, where anti-tTG titer more than 10 folds the ULN (≥ 180 U/mL) was significantly associated with Marsh III enteropathy; this

strong association of high anti-tTG titer and severity of intestinal damage might provide a remarkable evidence for diagnosis of CD when endoscopy is not applicable and fortified by positive EMA test or HLA typing (Hawamdeh *et al*., 2016).

4.8-Adherence to gluten free diet in celiac patients:

Most (n=97, 89.8%) of celiac patients in this study had started gluten free diet, with the period of restriction extended to months or years according to the date of diagnosis of each. However, only a small percent of them (n=20, 20.6%) had adequate adherence to GFD and the response to GFD demonstrates relief of symptoms in 39.2% of patients (n=38) and both clinical improvement plus reduction of antibody titer in more than one half of them (n=55, 56.7%).

In the general population, the daily gluten consumption has been calculated to be approximately 15–20 g (van Overbeek *et al*., 1997). Several studies advocate the complete elimination of all food and food items and drugs that include gluten and its derivatives from diet as even small amounts of gluten (50 mg/day) can be immunogenic (Catassi *et al*., 2007) and mucosal lesions can be sufficiently induced in CD patients at daily doses of as low as <1 g and thus the safe threshold is 10–50 mg gluten per day (Collin *et al*.,2004; Hischenhuber *et al*.,2006; Catassi *et al*.,2007).This requires an educated patients, significant motivation, and follow-up to achieve good adherence to a GFD (Rubio-Tapia *et al*., 2013). Although most CD patients on a GFD have a good prognosis, the gluten ubiquity in all dietary products necessitates the avoidance of even minor contamination of otherwise foods known to be gluten-free, making gluten restriction a severe challenge (Ciacci *et al*., 2002; Pietzak, 2005; Hollon *et al*., 2013). Also unsteady adherence to GFD is seen in many patients and a treatment burden has been reported by most CD patients (Hall *et al*., 2009; Shah *et al*., 2014).

Although most of the study candidates are educated, complete restriction of gluten is a very difficult or impossible mission and they wish that non-dietary alternatives are present. Iraq is one of countries with high rank of gluten intake causing bad GFD compliance among candidates, where cost, poor palatability, availability, and social issues also could attribute.

Generally within a few weeks, patients can achieve clinical improvement, while recovery of the mucosal damage occurs in 1-2 years (Lionetti and Catassi., 2011), and although only small percent of celiac patients (n=20, 20.6%) had adequate degree of adherence to GFD**,** they had better response than those with non-adequate degree of adherence (85% and 52% respectively) and this difference is statistically significant (p value 0.006). However, 39.2% (n=38) of the candidates show response to incomplete gluten restriction demonstrated by some relief of symptoms and more than half (n=55, 56.7%) both by relief of symptoms and reduction of tTG antibody titer (mean titer=236.52) in spite of non- adequate adherence to GFD, reflecting the dose-related symptoms and serology.

Another point to mention is that the difference in the period of adherence to GFD between celiac patients (years or months) shows a statistically significant association with their serum level of antibodies (p=0.001), where patients adhered >3 years had lowest serum level despite the non- adequate adherence to GFD in many patients causing continuous gliadin translocation, persisting immunogenic process, and consequent autoantibodies production. Thus the amount of gluten intake during adherence certainly has an effect reflected by decreasing serum autoantibodies.

4.9- Follow up of celiac patients:

From the total 108 celiac patients, only n=41 (38%) of them had regular follow up where they attended their corresponding endocrine center for physical examination and serological testing and screening. Lacking of regular follow-up may be a part of poor compliance to GFD.

Periodic monitoring is important for CD patients and a routine testing for anti-transglutaminase antibodies required to assess the response to GFD (Monzani *et al*., 2011; Lerneret *et al*., 2017). Since type-1 DM and thyroiditis have an increased prevalence in CD, so patients should also be monitored by screening tests for the emergence of these entities (Lerneret *et al*., 2017).

4.10- Serum zoulin level a marker of dysfunctional IP:

Most of the research met on maintaining normal physiological processes of small intestine requires intact barrier, further delineating the importance of the proper function of IP.

Many studies reported the zonulin over expression in autoimmune diseases including CD in which defective TJs function is the major issue (Fasano *et al*., 2000; Drago *et al*., 2006**).** The CD peculiarity is that it is the only autoimmune disease for which gliadin the environmental trigger is known (Lammers *et al*., 2008). Gliadin induces a polarized luminal secretion of zonulin (El Asmar *et al*., 2002) increasing IP and allowing gliadin to reach the submucosa, where secretion of zonulin to the basolateral side can occur from zonulin-expressing immune cells (Jacob *et al*., 2005). The consequent deregulated zonulin signaling pathway disrupts normally sustained barrier function and alters immune responses to gliadin **(**Fasano *et al*., 2000; Fasano, 2011).

According to COSABIO User Manual, the minimum detectable dose of zonulin is less than 0.156ng/ml, while the detection range is 0.625ng/ml-40ng/ml. If we go back to the study group, we found that most of the candidates have an elevated serum zonulin level (5.42-34.12 ng/ml) comparing to normal controls (zero-1.41ng/ml). Inevitably the high serum zonulin levels are due to the improper restriction of gluten from diet revealed in more than two third of the patients (n=77, 79.4%) as mentioned above. The continuous zonulin release and consequent TJ disassembly resulting in loss

of mucosal integrity (Fasano *et al*., 2000; Cummins *et al*., 2001; Lammers *et al*., 2008; Tripathi *et al*., 2009), persisting the stimulation by non-self antigens [environmental triggers], (Fasano, 2008, Fasano, 2012). Through gliadin translocation into the submucosa, there would be a consequent activation of the local immune system allowing the pathogenic process to continue (Heyman *et al*., 2011).

Nevertheless, why serum zonulin level is still high (about 4.16-6.30 ng/ml) in some patients with adequate adherence to GFD? The answer of this question may be attributed to another factors causing zonulin up-regulatation like 1. Dysbiosis of gut microflora, as enteric bacteria are the second environmental trigger so far induce zonulin release from enterocytes(El Asmar *et al*., 2002; Lammers *et al*., 2008) 2. It could be due to the reported mucosal mast cell protease II, another serine protease that affects IP acting from both luminal and serosal sides causing a similar bilateral action as zonulin (Jacob *et al*., 2005). 3. Dietary habits, age, previous mucosal injury and other unknown causes that may offer the insight for more studies in this field in our population.

On viewing the serum zonulin level in association with the period of adherence to GFD, the study shows that celiac patients who adhered to GFD for years (>3years group and 1-3years group) had lower mean s. zonulin level (5.27ng/ml and 6.22ng/ml respectively) than those adhered to GFD for months (1-6M group and 7-12M group, 7.81ng/ml and 6.47ng/ml respectively) and this difference is statistically not significant (p=0.292) as the low level of zonulin in the first category not reaching the undetectable level seen in good adherent patients to GFD. This overlapping can be easily explained as the majority of the candidates were aware of gluten present only in the well-known food items and unaware of the gluten content in the other food products and drugs, and by that they are far of the widely accepted GF threshold [20 part per million (ppm)], recognized by food regulatory agencies

like the US Food and Drug Administration (Sharma *et al*., 2015). In general clinicians should be alert of high levels of serum zonulin which indicate an increased IP in their patients. Because zonulin is the only known modulator of IP, the therapeutic interference designed to restore intestinal barrier function by zonulin pathway downregulation, may offer novel approaches to treat these debilitating chronic disorders (Fasano, 2011)

4.11- IL15 a predictive marker for CD immunopathogenesis:

IL15 with its overall involvement in multiple steps of the NKG2D cytolytic pathway, in addition to its definite role in frustrating oral tolerance to dietary gluten and interfering with the intestinal regulatory T cells suppressive activity giving IL15 the role of "key player" for the immune responses deregulation in CD (Abadie and Jabri, 2014).

If we return back to the study results, you will find that the majority of patients are the same as the normal controls have zero pg/ml serum level of IL15 in different stages of the disease and in both study age groups (adults and children) and unrelated to other ADs associated with CD, while only small proportion (13 children and 3 adults) have elevated IL15 serum level ranging from 0.5- 5349pg/ml (normally undetectable as it part of innate immunity).These results might be conflicting at the first glance, but after reviewing some literatures, we noticed that Vicari and his team in 2017 also attempted to measure IL-15 serum levels in patients with eosinophilic esophagitis using either Mesoscale or ELISA techniques and they did not observe any substantial increase of circulating IL-15 levels in their patient cohorts , and IL-15 levels were very low or undetectable in all candidates (Vicari *et al*., 2017). Their explanation was attributed to the fact that multiple inflammatory molecules renowned to be locally contributed to the disease and may not be detected in serum yet they do not know the source of discrepancy with the previously reported results (Vicari *et al*., 2017). Dellon et al in 2015 also supports our finding where they concluded that a panel of inflammatory

mediators known to have a role in eosinophilic esophagitis pathogenesis were neither increased in the serum, nor were they responsive to therapy and they are unlikely candidates for a eosinophilic esophagitis serum test (Dellon *et al*., 2015) .Thus the same interpretation perhaps applied to our study group who revealed undetectable serum levels of IL15, since it is a unique cytokine (a cell surface-associated cytokine) that it is not secreted, and might be upregulated on the surface of all cell types under inflammatory and stressful conditions (Dubois *et al*., 2002; Bergamaschi *et al*., 2008; Bergamaschi *et al*., 2009). Many studies demonstrated that during synthesis IL-15 is bound to IL-15Rα intracellularly in the endoplasmic reticulum, with consequent surface shuttling, and trans presentation to responder cells expressing IL-15Rβ and γc, given the fact that IL-15 signaling acts in a cell contact-dependent manner(Dubois *et al*., 2002; Bergamaschi *et al*., 2008; Bergamaschi *et al*., 2009). In vivo this IL-15 transpresentation is the main mechanism by which IL-15 interacts with corresponding receptor, however alternative mechanism in which cis-presentation have been proposed, and involves the binding of soluble IL-15 to IL-15Rα permitting signaling of adjacent IL-2Rβ/γc on the same cell (Oh *et al*., 2004; Rowley *et al*., 2009; Ota *et al*., 2010).

Even though several studies contrasted starkly with the study result where variations in serum cytokine levels after starting a gluten-free diet have been revealed (Hansson *et al*., 1999; Romaldini *et al*., 2002; Lettesjo *et al*., 2005; Manavalan *et al*., 2010) compared to time of diagnosis, and some cytokines detected in serum continue to be increased when compared to normal controls after 1 year of treatment (Romaldini *et al*., 2002). Bjorck et al in 2015 also present a conclusion that a systemic inflammatory response signs reflected by increasing circulating levels of cytokines found in 3-years old children with screening-detected coeliac disease at the time of diagnosis (Bjorck *et al*., 2015). Also Manavalan and his team in 2010 demonstrated that patients with active CD and those on GFD with

positive antibodies had significantly higher levels of proinflammatory cytokines, such as interferon-gamma, interleukin (IL)-1beta, tumor necrosis factor-alpha, IL-6 and IL-8, and also Th-2 cytokines such as IL-4 and IL-10, compared with normal controls and patients on GFD without antibodies (Manavalan *et al*., 2010). Interestingly patients on GFD for less than 1 year had significantly higher levels of both proinflammatory cytokines and T(h)2 cytokines compared with the patients on GFD for more than 1 year (Manavalan *et al*., 2010). In human and murine IL-15 and IL-15Rα may present in a soluble form (cis-presentation) other than the membrane bound *(*trans-presentation). Thus, a plenty of soluble complexes of IL-15Rα/IL-15 could separate from the cells surfaces (Mortier *et al*., 2008; Bouchaud *et al*., 2010; Bergamaschi *et al*., 2012) and could also allow IL-15 effects being mediated. (Abadie and Jabri, 2014).

Regarding the small percent of patients with detectable circulating IL15 levels, yet the discrepancy source in relation to the other study candidates is unknown, however the DuoSet ELISA kits from R&D Systems that we used in the study measure the natural and recombinant human IL15, and it was not determined whether this kit would measure only free IL-15 or could detect both free IL-15 and IL-15/IL-15Rα heterodimers. It might be one of these ILs15 that detected in those 16 patients of total 108 enrolled in the study. Nevertheless the exact factors and mechanisms contributed to IL-15 upregulation are still to be defined, gliadin peptides has been suggested to promote IL-15 expression by IECs (Hue *et al*., 2004; Maiuri *et al*., 2003). However, this upregulation seems to be indirect, because several inflammatory mediators are upregulated as a consequence of T-cell activation. Induction of IL-15 can be achieved by many inflammatory stimuli, more likely cytokines and TLR ligands (Mattei *et al*., 2001; Dafik *et al*., 2012), and microbial ingredient richly present in the gut is another notable factor, might promote continuous IL-15 expression. However, it is significant to confess

that the mechanisms underlying IL-15 deregulation in CD and when this deregulation happens is still elusive (Abadie and Jabri, 2014).

Infections, dietary habits of each individual, genetic make-up and ethnicity may have a role in this discrepancy, and still another factors may be involved are beyond the scope of the study and to be evaluated.

A drawback of this study is that we did not explore the IL15 expression in the intestinal mucosa, which might enable us to compare circulating cytokine level and mucosal cytokine expression at individual level. Several reports describe the overexpression of IL-15 in tissues being the target of autoimmune processes makes the question regarding the role of IL-15 played in tissue immunity that could evolved into organ-specific autoimmune disorders (Jabri *et al*., 2000; Roberts *et al*., 2001; Green and Jabri , 2003).

Owing to this central role of IL-15 in CD immunopathogenesis, thus developing novel therapies is the growing interest in hope to abolish the effects of IL-15. Several agents have been emerged to dampen IL-15 activity and to prevent its baleful effect on oral tolerance and IELs activation. These agents include Jak inhibitors and antibodies specific for IL-15 or IL-2/15Rβ and so prohibit the IL-15 transpresentation by APCs to neighboring $CD8^+$ T cells and NK cells (Waldmann, 2006; Morris *et al*., 2006).

4.12- Other study associations and correlations:

Many associations and correlations between different variables have been made in this study, where some of which reveal statistically significant association($p< 0.05$), some have an association but not reach significance, and others show no significant association($p> 0.05$), and they are listed down :

1- A correlation of serum zonulin level has been made with serum antibody titers in celiac patients and there was positive correlation shown between serum zonulin level and serum tTG (IgA), but didn't reach significance, while negative correlation revealed between serum zonulin and other serum

antibodies level (p> 0.05). The local presence of zonulin and tTG (IgA) in the small intestine may have a role in this correlation, but small sample size and the improper restriction of gluten by candidates might be confounding factors. However, we didn't see such correlation in the literatures we reviewed.

2- Also this study demonstrates that there was positive correlation shown between serum IL15 level and serum tTG (IgA) and AGA (IgA), but also didn't reach significance, while negative correlation revealed between serum IL15 and serum tTG (IgG) and AGA (IgG) antibodies level (p> 0.05). A small size sample and the undetectable levels of IL15revealed by the study might influence this correlation.

3- A positive correlation also demonstrated between the serum levels of the zonulin and IL15, however this correlation didn't reach significance (*P*=0.5). More sample size and studying the intestinal mucosal cells expression of the two markers may become more informative about this correlation.

4- A comparison between serum zonulin and IL15 levels in children and adults celiac patients demonstrates that adult celiac patients had lower serum zonulin level than peadiatric group and this difference is statistically significant (p= 0.002),indicating better but still inadequate adherence to GFD in adults. While IL15 level was higher in in adult age group, but this difference is statistically not significant (p> 0.05).Generally there is no statistical significant association of serum zonulin and IL15 levels in celiac patients (p > 0.05) as whole, a finding that indeed requires simultaneous measuring of serum levels and intestinal mucosal cells expression of the two markers to bring the light on this association.

5- An association for serum zonulin in ng/ml and IL15 in pg/ml in patients who developed complications and those without complications. The serum zonulin level was higher (mean 8.82) in celiac patients with positive complications, which is statistically significant association (p value =0.004). On the other hand IL15demonstrates no significant association with

complications which may be attributed to the small proportion of patients (n=16) with increased serum IL15 level.

6- Association of serum level of zonulin, IL15, and Tissue TG (IgA) with autoimmune diseases in celiac patients present in the study group (DM type 1(25%) and a small percentage (9.3%) affected with other autoimmune diseases), and none of these markers reveal significant association with other autoimmune diseases (P> 0.05). More evaluation of this point is required.

7-Also in this work, we try to study the association of serum zonulin in ng/ml, serumIL15 in pg/ml, serum tissue TG (IgA and IgG), and serum DGP (IgA and IgG) with the type of clinical presentation (intestinal or extra-intestinal). There was no significant association between these variables with the type of clinical presentation except for tTG (IgG mean s. level=45.19) with extra-intestinal manifestations, but it does not reach significance (p value =0.06), while DGP (IgA mean s. level=186.33) reveals significant association also with extra-intestinal manifestations (*P* value =0.02). Further evaluation of these findings is required to justify these associations.

8-Another association made for of serum zonulin in ng/ml, serumIL15 in pg/ml, serum tissue TG (IgA) with skin manifestations. Of the three, only zonulin (mean 8.39) shows an association with positive skin manifestation appeared in about one quarter (n=26) of the candidates, however this correlation also does not reach significance (p value =0.05).

9-Finally the serum level of zonulin in all candidates and serum IL15 level in only 16 celiac patients were compared to normal controls and there was significant statistical difference in serum zonulin and IL15 levels between celiac patients and their matched for age and gender controls (p value = 0.0001)for **zonulin** and (p value = 0.001)for **IL15.** This reflects how much the degree of intestinal barrier integrity and stable immunity in the healthy controls compared to CD' patients.

4.13-Conclusions:

Through this study we have reached some conclusions that briefly listed below:

1. The patients are unaware of unintentional gluten intake causing inadequate adherence to GFD which reflected by high serum zonulin levels in the majority of patients. However an association revealed between zonulin and period of GFD as patients who adhered to GFD for years had lower mean serum zonulin level than those adhered to GFD for only months. Adult celiac patients also had lower serum zonulin level than peadiatric group, in addition zonulin was also high in patients with skin manifestations and patients who developed complications comparing to other patients.

2. Serum IL 15 level was undetectable in most patients suggesting local effects of this cytokine in the disease pathogenesis, and the mechanisms by which circulating IL15 elevated are still to be elucidated. The causes behind IL15demonstrates no significant association with any variable may be attributed to the small size of patients with detectable circulating IL15.

3. Serum zonulin and IL15 levels of candidates compared to normal controls matched for age and gender show significant difference as high levels only seen in patients.

4. Most of the patients had classical (intestinal) manifestations of CD, and the extra-intestinal manifestations were part of disease squeal in many patients. CD is more common in children and females affected with celiac two times more than males, while type1DM is the common autoimmune disease associated with CD. In addition, aphthous stomatitis is a common complaint in the patients.

5. In spite of shortcomings of genetic studies and EMA testing, the CD currently diagnosed depending on clinical presentation, strongly positive serological tests, and confirmation by intestinal biopsy in adults and challenge test in children. In general, a delay in diagnosis is expected due to prolonged period of presentation in most patients.

6. The 10 folds the upper limit of normal (ULN) range of the serum anti-tTG (IgA) level seen in about three quarters of the patients, a result making tTG (IgA) the best serological marker for the disease. There is an association between serum anti-tTG (IgA) level and degree of intestinal damage according to Marsh- Oberhouber classification, a finding that comes with many studies.

4.14-Recommendations:

Several suggestions are recommended to be evaluated in our population to uncover many elusive factors and mechanisms involved or evoking the immunopathogenic process in celiac patients, including:

1. Conducting a study about intestinal dysbiosis as bacterial colonization so far the second stimulant for zonulin release and TJs distruption.

2. Studying the expression of the chemokine receptors CXCR3 and transferrin receptor CD71 in the intestinal biopsies as they are the target intestinal receptor for gliadin translocation into submucosa.

3. Studying the expression of the IL15 and IL-15Rα receptor chain in the intestinal mucosal cells to evaluate the local IL15 effects.

4. Conducting a study about prehaptoglobin (HP) gene polymorphism in a biopsy-confirmed celiac patients as a genetic risk factor for CD development

5. Conducting a study on the mucosal mast cell protease II another serine protease that affects IP acting from both luminal and serosal sides causing a similar bilateral action as zonulin.

6. Studying the zonulin expression in the intestinal mucosal cells to clarify its' role in disease activity and correlated with lactulose/ mannitol permeability testing.

7. Testing the circulating level of a panel of pro-inflammatory cytokines in active phase and remission phase of biopsy-proved celiac patients.

REFERENCES

-Abadie V., Jabri B. 2014. IL-15: a central regulator of celiac disease immunopathology. Immunol Rev. 260: 221-34.

-Adams F. (translator). 1865. On the Coeliac Affection. The Extant Works of Aretaeus, the Cappadocian. London, Sydenham Society. 1856: 350-351.

-Adson A. *et al*. 1994. Quantitative approaches to delineate paracellular diffusion in cultured epithelial cell monolayers. J. Pharm. Sci. 83: 1529 - 1536.

-AGA Institute. 2006. Medical Position Statement on the Diagnosis and Management of Celiac Disease.Gastroenterology.131:1977–80.

-Aggarwal S., Lebwohl B., and Green P.H. 2012. Screening for celiac disease in average-risk and high-risk populations. Therap Adv Gastroenterol. 5: 37–47.

-Akirov A., Pinhas-Hamiel O. 2015. Co-occurrence of type 1 diabetes mellitus and celiac disease. World J Diabetes. 6:707–714.

-Akobeng A.K., Thomas A.G.2008. Systematic review: tolerable amount of gluten for people with coeliac disease. Aliment Pharmacol Ther. 27:1044-52

-Alarida K., Harown J., Ahmaida A. *et al*. 2011. Coeliac disease in Libyan children: a screening study based on the rapid determination of anti-transglutaminase antibodies. Dig Liver Dis. 43:688-91.

-AL-kenzawi A. H. 2006. Evaluation of immunological and pathological assays for diagnosis celiac disease in Iraqi patients. Msc. th. Baghdad U. p.45.

-Al-Saadi H A, Abid A H. 2009. Celiac disease in karbala .J Karb Unv;7(2):51-61.

-Amil Dias J. 2017. Celiac Disease: What Do We Know in 2017? GE Portuguese Journal of Gastroenterology. 24(6): 275–278. http://doi.org/10.1159/000479881.

-Anderson J.M., Van Itallie C.M. 1995. Tight junctions and the molecular basis for regulation of paracellular permeability. Am. J. Physiol. 269:G467–G475.

-Ansaldi N., Tavassoli K., Faussone D. *et al*. 1988. Clinico-histological behavior of celiac patients after gluten load following the definitive diagnosis . Pediatr Med Chir. 10: 3-6.

-Antoni L., Nuding S., Wehkamp J., Stange E.F. 2014. Intestinal barrier in inflammatory bowel disease. World J Gastroenterol. 20:1165–1179.

-Armitage R.J., Macduff B.M., Eisenman J., Paxton R., Grabstein K.H. 1995. IL-15 has stimulatory activity for the induction of B cell proliferation and differentiation. J Immunol. 154:483–90.

-Arrieta M.C., Bistritz L., Meddings J.B. 2006. Alterations in intestinal permeability. Gut. 55:1512-1520.

-Aziz I., Sanders D. S. 2012. The irritable bowel syndrome-celiac disease connection. Gastrointest Endosc Clin N Am. 22: 623- 37.

-Bai D., Brar P., Holleran S., Ramakrishnan R., Green PH. 2005. Effect of gender on the manifestations of celiac disease: evidence for greater malabsorption in men. Scand J Gastroenterol. 40: 183-187.

- Biagi F., Klersy C., Balduzzi D., Corazza G. R. 2010. Are we not over-estimating the prevalence of coeliac disease in the general population? Annals of Medicine. 42 (8):557–561.

-Bai J.C., Fried M., Corazza G.R., Schuppan D., Farthing M., Catassi C., Greco L.,Cohen H., Ciacci C., Eliakim R., Fasano A., González A., Krabshuis J.H., LeMair A. 2013. World gastroenterology organization global guidelines on celiac disease. J Clin Gastroenterol. 47: 121–126.

- -Bai J., Zeballos E., Fried M., Corraza G. 2013. World Gastroenterology Organisation Practice Guidelines: Celiac Disease; World Gastroenterology Organisation: Milwaukee, WI, USA. 48: 1–18.

-Bao F., Green P. H. R., and Bhagat G.2012. “An update on celiac disease histopathology and the road ahead,”Archives of Pathology & Laboratory Medicine.136(7): 735–745.

-Barker J.M., Liu E. 2008. Celiac disease: pathophysiology, clinical manifestations, and associated autoimmune conditions. Adv Pediatr. 55:349–365.

-Barone M.V., Zanzi D., Maglio M., Nanayakkara M., Santagata S., Lania G., Miele E., Ribecco M.T., Maurano F., Auricchio R., Gianfrani C., Ferrini S., Troncone R., Auricchio S. 2011. Gliadin-mediated proliferation and innate immune activation in celiac disease are due to alterations in vesicular trafficking. PLoS One 6:e17039.

-Basso D., Guariso G., Fogar P. *et al*. 2009. Antibodies against synthetic deamidated gliadin peptides for celiac disease diagnosis and follow-up in children. Clin Chem. 55:150–157.

-Baslund B., Tvede N., Danneskiold-Samsoe B. *et al*. 2005. Targeting interleukin-15 in patients with rheumatoid arthritis: a proof-of-concept study. Arthritis Rheum;52:2686–2692.

-Bauer H, Zweimueller-Mayer J, Steinbacher P, Lametschwandtner A, Bauer HC. 2010. The dual role of zonula occludens (ZO) proteins. J Biomed Biotechnol. 402593

-Baum B, Georgiou M. 2011. Dynamics of adherens junctions in epithelial establishment, maintenance, and remodeling. J Cell Biol 192:907-917

-Bayrak S., Mitchison N.A., 1998. Bystander suppression of murine collagen-induced arthritis by long-term nasal administration of a self type II collagen peptide, Clin. Exp. Immunol.113: 92–5.

-Ben Ahmed M., Belhadj Hmida N. *et al*. 2009. IL-15 renders conventional lymphocytes resistant to suppressive functions of regulatory T cells through activation of the phosphatidylinositol 3-kinase pathway. J Immunol. 182:6763-70

Bergamaschi C. *et al*. 2008. Intracellular interaction of interleukin-15 with its receptor alpha during production leads to mutual stabilization and increased bioactivity. J Biol Chem. 283: 4189–99.

-Bergamaschi C. *et al*. 2009 Secretion and biological activity of short signal peptide IL-15 is chaperoned by IL-15 receptor alpha in vivo. J Immunol. 183: 3064–72.

-Bergamaschi C. *et al*., 2012. Circulating IL-15 exists as heterodimeric complex with soluble IL-15R alpha in human and mouse serum. Blood. 120:e1–8.

-Bischoff S.C., Barbara G., Buurman W., Ockhuizen T., Schulzke J.D., Serino M., Tilg H., Watson A., Wells J.M. 2014. Intestinal permeability a new target for disease prevention and therapy. BMC Gastroenterol. 14: 189.

-Bischoff S.C. 2011. Gut health: a new objective in medicine? BMC Med, 9: 24.

-Bjorck S., Lindehammer S.R., Fex M., Agardh D., 2015. Serum cytokine pattern in young children with screening detected celiac disease. Clin. Exp. Immunol. 179:230–235.

-Bjorkman P.J., Saper M.A., Samraoui B., Bennett W.S., Strominger J.L.,Wiley D.C. 1987. Structure of the human class-I histocompatibility antigen ,Hla-A2. Nature. 329:506 –512

-Bolotin D, Petronic-Rosic V. 2011. Dermatitis herpetiformis. Part I. Epidemiology, pathogenesis, and clinical presentation. J Am Acad Dermatol. 64:1017-24; quiz 1025-6.

-Bonamico M., Mariani P., Thanasi E. *et al*. 2004. "Patchy villous atrophy of the duodenum in childhood celiac disease," Journal of Pediatric Gastroenterology & Nutrition. 38(2): 204–207.

- Borrelli M. *et al*. 2010. High density of intraepithelial gammadelta lymphocytes and deposits of immunoglobulin (Ig)M anti-tissue transglutaminase antibodies in the jejunum of coeliac patients with IgA deficiency. Clin Exp Immunol. 160: 199–206.

-Bouchaud, G. *et al*. 2010. Interleukin-15 and its soluble receptor mediate the response to infliximab in patients with Crohn's disease. Gastroenterology. 138:2378–87.

-Brandtzaeg P. 2011. The gut as communicator between environment and host: immunological consequences. Eur J Pharmacol, 668(Suppl 1): S16–S32.
-Branski D, Fasano A, Troncone R.(2006): Latest developments in the pathogenesis and treatment of celiac disease. J Pediatr. .149:295–300.
- Burkett P.R., Koka R., Chien M., Chai S., Boone D.L., Ma A. 2004. Coordinate expression and trans presentation of interleukin (IL)-15Ralpha and IL-15 supports natural killer cell and memory CD8+ T cell homeostasis. J Exp Med. 200:825–834.
-Burton J.D. *et al*. 1994. A lymphokine, provisionally designated interleukin T and produced by a human adult T-cell leukemia line, stimulates T-cell proliferation and the induction of lymphokine-activated killer cells. Proc Natl Acad Sci U S A. 91:4935–9.

- Catassi C., Fabiani E., Iacono G. 2007: A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease. Am J Clin Nutr. 85:160–166.
-Camarca M.E., Mozzillo E., Nugnes R., Zito E., Falco M., Fattorusso V., Mobilia S., Buono P., Valerio G., Troncone R. *et al*. 2012. Celiac disease in type 1 diabetes mellitus. Ital. J. Pediatr.; 38: 10.

-Campanella J., Biagi F., Bianchi P.I., Zanellati G., Marchese A., Corazza G.R., 2008. Clinical response to gluten withdrawal is not an indicator of coeliac disease. Scand. J. Gastroenterol. 43:1311–1314.

-Caproni M., Antiga E., Melani L., *et al*. 2009. Guidelines for the diagnosis and treatment of dermatitis herpetiformis. J Eur Acad Dermatol Venereol. 23:633-8.

-Caproni M., Antiga E., Melani L., Fabbri P. 2009. Guidelines for the diagnosis and treatment of dermatitis herpetiformis. Journal of the European Academy of Dermatology and Venereology.23 (6):633–638.

-Castillo E.F., Acero L.F., Stonier S.W., Zhou D., Schluns K.S. 2010. Thymic and peripheral microenvironments differentially mediate development and maturation of iNKT cells by IL-15 transpresentation. Blood. 116: 2494–2503.

-Castillo N.E., Theethira T. G., Leffler D. A. 2015. The present and the future in the diagnosis and management of celiac disease. Gastroenterology Report, *3*(1), 3–11.

-Catassi C., Fabiani E., Iacono G. *et al*. 2007.A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease. Am J Clin Nutr. 85:160-6

-Catassi C., Fasano A. 2010. Celiac disease diagnosis: simple rules are better than complicated algorithms. Am J Med. 123: 691-693.

-Catassi C., Gobellis G., 2007. Coeliac disease epidemiology is alive and kicking, especially in the developing world. Dig Liver Dis. 39: 908-910.

-Catassi C., Kryszak D., Louis-Jacques O., Duerksen D.R., Hill I., Crowe S.E., Brown A.R., Procaccini N.J., Wonderly B.A., Hartley P., Moreci J., Bennett N., Horvath K., Burk M., Fasano A., 2007. Detection of Celiac disease in primary care: a multicenter case-finding study in North America. Am J Gastroenterol. 102: 1454-1460.

-Cereijido M, Valdes J, Shoshani L, *et al*. (1998). Role of tight junctions in establishing and maintaining cell polarity. Annu Rev Physiol. ;60:161–177.

-Chen Y, Chou K, Fuchs E, Havran WL, Boismenu R., 2002. Protection of the intestinal mucosa by intraepithelial gamma delta T cells. Proc Natl Acad Sci U S A. 99(22):14338-43.

-Choung, R. S., Larson, S. A., Khaleghi, S., Rubio-Tapia, A., Ovsyannikova, I. G., King, K. S., … Murray, J. A. (2017). Prevalence and Morbidity of Undiagnosed Celiac Disease From a Community-based Study.Gastroenterology, 152(4), 830–839.e5.

-Ciacci C, Cirillo M, Cavallaro R, Mazzacca G., 2002. Long-term follow-up of celiac adults on gluten-free diet: prevalence and correlates of intestinal damage. Digestion. 66:178–185.

-Ciacci C., Cirillo M., Sollazzo R., Savino G., Sabbatini F., Mazzacca G.1995. Gender and clinical presentation in adult celiac disease. Scandinavian Journal of Gastroenterology. 30(11):1077–1081.

-Ciaccio E.J., Bhagat G., Lewis S.K., Green P.H., 2015. Quantitative image analysis of celiac disease. World J Gastroenterol. 21:2577–2581

-Ciclitira P.J., King A.L., Fraser J.S., 2001. AGA technical review on Celiac Sprue. American Gastroenterological Association. Gastroenterology. 120: 1526-1540

-Clarke L.L. 2009. A guide to Ussing chamber studies of mouse intestine.Am J Physiol Gastrointest Liver Physiol. 296: G1151–G1166.

-Clemente M.G., De Virgiliis S., Kang JS, Macatagney R, Musu MP, Di Pierro MR, Drago S, Congia M, Fasano A. 2003. Early effects of gliadin on enterocyte intra-cellular signalling involved in intestinal barrier fun-tion. Gut. 52:218-23.

-Clemente M.G., Musu M.P., Troncone R., Volta U., Congia M., Ciacci C., Neri E., Not T., Maggiore G., Strisciuglio P. *et al*. 2004. Enterocyte actin autoantibody detection: a new diagnostic tool in celiac disease diagnosis: results of a multicenter study. Am J Gastroenterol. 99:1551-6.

-Codex Alimentarius. (1979 rev. 2008). Codex standard for foods for special dietary use for persons intolerant to gluten. CODEX STAN 118-1979.

-Collin P., Kaukinen K.,Valimaki M., Salmi J. 2002.Endocrinological disorders and celiac disease. Endocr Rev. 23: 464–483.

-Collin P., Reunala T. 2003. Recognition and management of the cutaneous manifestations of celiac disease: a guide for dermatologists. Am J Clin Dermatol. 4:13-20.

-Collin P., Thorell L., Kaukinen K., Maki M. 2004. The safe threshold for gluten contamination in gluten-free products. Can trace amounts be accepted in the treatment of coeliac disease? Aliment Pharmacol Ther.19:1277–1283.

-Colpitts S. L., Stonier S. W., Stoklasek T. A., Root S. H., Aguila H. L., Schluns K. S., & Lefrançois L. 2013. Transcriptional regulation of interleukin-15 expression during hematopoiesis. Journal of Immunology (Baltimore, Md. : 1950). 191(6): 3017–3024.

-Commins S.P. 2015. Mechanisms of Oral Tolerance. Pediatric Clinics of North America. 62(6): 1523–1529.

-Conrad K., Roggenbuck D., Ittenson A. *et al*. 2012. A new dot immunoassay for simultaneous detection of celiac specific antibodies and IgA-deficiency. Clin Chem Lab Med. 50: 337-43.

-Constantin C., Huber W. D., Granditsch G., Weghofer M., Valenta R. 2005. Different profiles of wheat antigens are recognised by patients suffering from coeliac disease and IgE-mediated food allergy. Int Arch Allergy Immunol. 138:257-66.

-Cosnes J., Cellier C., Viola S., Colombel J.F., Michaud L., Sarles J., Hugot J.P., Ginies J.L., Dabadie A., Mouterde O. *et al*. Groupe D'Etude et de Recherche Sur la Maladie Coeliaque. 2008. Incidence of autoimmune diseases in celiac disease: protective effect of the gluten-free diet. Clin. Gastroenterol. Hepatol. 6, 753–758.

-Coulon S., Dussiot M., Grapton D., Maciel T.T., Wang P.H., Callens C.,Tiwari M.K., Agarwal S., Fricot A., Vandekerckhove J. *et al*. 2011. Polymeric IgA1 controls erythroblast proliferation and accelerates erythropoiesis recovery in anemia. Nat. Med.17: 1456–1465

-Crowe SE. 2011. In the clinic. Celiac disease.Ann Intern Med. 154:ITC5-1-15; quiz ITC5-16.

-Cukrowska, B., Sowińska, A., Bierła, J. B., Czarnowska, E., Rybak, A., & Grzybowska-Chlebowczyk, U. 2017. Intestinal epithelium, intraepithelial

lymphocytes and the gut microbiota - Key players in the pathogenesis of celiac disease. World Journal of Gastroenterology. 23(42): 7505–7518.

-Cummings J.H., Antoine J.M., Azpiroz F., Bourdet-Sicard R., Brandtzaeg P., Calder P.C., Gibson G.R., Guarner F., Isolauri E., Pannemans D., Shortt C., Tuijtelaars S., Watzl B. 2004. PASSCLAIM–gut health and immunity. Eur J Nutr.43 (2):II118–II173.

-Cummins A.G., Thompson F.M., Butler R.N., Cassidy J.C., Gillis D., Lorenzetti M., Southcott E.K., Wilson P.C. 2001. Improvement in intestinal permeability precedes morphometric recovery of the small intestine in coeliac disease. Clin. Sci. (Lond.) 100:379–386.

-Cuvelier C., Barbatis C., Mielants H., Devos M., Roels H., Veys E. 1987. Histopathology of intestinal inflammation related to reactive arthritis.Gut. 28:394 – 401

-Czaja-Bulsa G. 2015. Non coeliac gluten sensitivity. A new disease with gluten intolerance. Clinical Nutrition. 34 (2): 6.

-Dafik L., Albertelli M., Stamnaes J., Sollid L.M., Khosla C. 2012. Activation and inhibition of transglutaminase 2 in mice. PLoS One. 7:e30642.

-Dahlbom I., Olsson M., Forooz N.K., Sjöholm A.G., Truedsson L., Hansson T. 2005. Immunoglobulin G (IgG) anti-tissue transglutaminase antibodies used as markers for IgA-deficient celiac disease patients. Clin. Diagn. Lab. Immunol. 12: 254–258.

-Dalgic B., Sari S., Basturk B. *et al*. 2011. Prevalence of celiac disease in healthy Turkish school children. Am J Gastroenterol. 106:1512-7.

-Dalton J.E., Cruickshank S.M., Egan C.E., Mears R., Newton D.J., Andrew E.M., Lawrence B., Howell G., Else K.J., Gubbels M.J. *et al*. 2006. Intraepithelial gammadelta+ lymphocytes maintain the integrity of intestinal epithelial tight junctions in response to infection. Gastroenterology. 131(3):818-29

-Daum S., Cellier C., Mulder C.J. 2005. Refractory coeliac disease. Best Pract Res Clin Gastroenterol. 19:413-24

-Dawood F.H., Jabbar A.A., Al-Mudaris A.F., Al-Hasani M.H. 1981.Association of HLA antigens with coeliac disease among Iraqi children. Tissue Antigens. 18: 35-39.

-Day L., Augustin M. A., Batey I. L., and Wrigley C. W. 2006. Wheat-gluten uses and industry needs. Trends in Food Science and Technology. 17: 9.

- Dellon E. S., Rusin S., Gebhart J. H., Covey S., Higgins L. L., Beitia R., Speck O., Woodward K., Woosley J. T., and Shaheen N. J. 2015. Utility of a noninvasive serum biomarker panel for diagnosis and monitoring of eosinophilic esophagitis: A prospective study. Am J Gastroenterol. 110: 821-7.

-De Re V., Caggiari L., Tabuso M., Cannizzaro R. 2013.The versatile role of gliadin peptides in celiac disease. Clin Biochem. 46:552–560

-Depaolo R.W., Abadie V., Tang F. *et al*. 2011. Co-adjuvant effects of retinoic acid and IL-15 induce inflammatory immunity to dietary antigens. Nature. 471:220-4.

- Diaz-Amigo C., Popping B. 2013. Accuracy of ELISA detection methods for gluten and reference materials: a realistic assessment, J. Agric. Food Chem. 61 (24):5681–568.

-Dicke W.K., Weijers H.A., Van De Kamer J.H. 1953. Coeliac disease. II. The presence in wheat of a factor having a deleterious effect in cases of coeliac disease. Acta Paediatr. 42:34–42

-Dieterich W., Ehnis T., Bauer M., Donner P., Volta U., Riecken E.O., Schuppan D. 1997. Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat. Med., 3, 797–801.

-Di Niro R., Mesin L., Zheng N.-Y., Stamnaes J., Morrissey M., Lee J.-H., Sollid, L. M. 2012. Abundance and unique repertoire of plasma cells secreting IgA autoantibodies to transglutaminase 2 in the intestinal lesion of celiac disease. Nature Medicine, 18(3), 441–445. http://doi.org/10.1038/nm.2656

-Di Sabatino A., Calarota S.A., Vidali F., Macdonald T.T., Corazza G.R. 2011. Role of IL-15 in immune-mediated and infectious diseases. Cytokine Growth Factor Rev. 22:19–33.

-Di Sabatino A., Ciccocioppo R., Cupelli F. *et al*. 2006. Epithelium derived interleukin 15 regulates intraepithelial lymphocyte Th1 cytokine production, cytotoxicity, and survival in coeliac disease. Gut. 55: 469–77.

- Di Sabatino A., Corazza G.R. 2009. Coeliac disease.Lancet. 373: 1480–1493.

-Di Sabatino A. *et al*. 2012. The function of tissue transglutaminase in celiac disease. Autoimmun. Rev.11(10): 746–53.

-Dixit R., Lebwohl B., Ludvigsson J.F., Lewis S.K., Rizkalla-Reilly N., Green P.H.2014. Celiac disease is diagnosed less frequently in young adult males. Dig Dis Sci. 59:1509-12

-Dominguez-Bello, M.G.; Costello, E.K.; Contreras, M.; Magris, M.; Hidalgo, G.; Fierer, N.; Knight, R. 2010. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc. Natl. Acad. Sci. USA, 107: 11971–11975.

- Dorum S., Arntzen M.O., Qiao S.W., Holm A., Koehler C.J., Thiede B. *et al*. 2010. The preferred substrates for transglutaminase 2 in a complex wheat gluten digest are peptide fragments harboring celiac disease T-cell epitopes. PLoS ONE. 5(11):e14056

-Drago S. *et al*. 2006. Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. Scand J Gastroenterol 41:408–419.

- Dubois P.C., Trynka G., Franke L., Hunt K.A., Romanos J., Curtotti A., Zhernakova A., Heap G.A., Adány R., Aromaa A. *et al*. 2010 Multiple common variants for celiac disease influencing immune gene expression. Nat Genet. 42:295–302.

- Dubois S.P., Waldmann T.A., Muller J.R. 2005. Survival adjustment of mature dendritic cells by IL-15. Proc Natl Acad Sci U S A. 102:8662–7.

-Dubois S., Mariner J., Waldmann T. A., Tagaya Y. 2002. IL-15R alpha recycles and presents IL-15 In trans to neighboring cells. Immunity. 17:537–47.

-Duitman E.H., Orinska Z., Bulanova E., Paus R., Bulfone-Paus S. 2008. How a cytokine is chaperoned through the secretory pathway by complexing with its own receptor: lessons from interleukin-15 (IL-15)/IL-15 receptor alpha. Mol Cell Biol. 28: 4851–4861.

-Eckburg P.B., Bik E.M., Bernstein C.N., Purdom E., Dethlefsen L., Sargent M., Gill S.R., Nelson K.E., Relman D.A. 2005. Diversity of the human intestinal microbial flora. Science. 308:1635–1638.

-Edelblum K.L., Shen L., Weber C.R., Marchiando A.M., Clay B.S., Wang Y., Prinz I., Malissen B., Sperling A.I., Turner J.R. 2012. Dynamic migration of gammadelta intraepithelial lymphocytes requires occludin. Proc Natl Acad Sci U S A. 109(18):7097-102

-El Asmar R. *et al*. (2002) Host-dependent zonulin secretion causes the impairment of the small intestine barrier function after bacterial exposure. Gastroenterology 123:1607–1615

-El Asmar R., Panigrahi P., Bamford P., Berti I., Not T., Coppa G.V., Catassi C., Fasano A. 2002. Host-dependent activation of the zonulin system is involved in the impairment of the gut barrier function following bacterial colonization. Gastroenterology 123: 1607–1615.

-Elli L., Branchi F., Tomba C., Villalta D., Norsa L., Ferretti F., Roncoroni L., Bardella M.T. 2015. Diagnosis of gluten related disorders: Celiac disease, wheat allergy and non-celiac gluten sensitivity. World J Gastroenterol. 21: 7110–7119.

-Erkan T., Kutlu T., Yilmaz E., Cullu F., Tümay G.T. 1999. Human leukocyte antigens in Turkish pediatric celiac patients. Turk J Pediatr1. 41: 181-188.

-Ermarth A., Bryce M., Woodward S., Stoddard G., Book L., Jensen M.K. 2017. Identification of Pediatric Patients With Celiac Disease Based on

Serology and a Classification and Regression Tree Analysis. Clin Gastroenterol Hepatol. 15(3):396-402.e2.

-Fallang L.E., Bergseng E., Hotta K., Berg-Larsen,A., Kim C.Y., and Sollid L.M. 2009. Differences in the risk of celiac disease associated with HLA-DQ2.5 or HLA-DQ2.2 are related to sustained gluten antigen presentation. Nat. Immunol.10: 1096–1101.

-Farquhar M. G., Palade G. E. 1963. Junctional complexes in various epithelia. *J. Cell Biol.* 17: 375–412.

-Fasano A. 2005. Clinical presentation of celiac disease in the pediatric population. Gastroenterology.128: S68–S73.PMID:15825129

-Fasano A. 2012. Intestinal Permeability and its Regulation by Zonulin: Diagnostic and Therapeutic Implications. Clinical Gastroenterology and Hepatology : The Official Clinical Practice Journal of the American Gastroenterological Association.10(10): 1096–1100.

-Fasano A. 2008. Physiological, Pathological, and Therapeutic Implications of Zonulin-Mediated Intestinal Barrier Modulation: Living Life on the Edge of the Wall. The American Journal of Pathology. 173 (5): P 1243-1252.

-Fasano A. 2000. Regulation of intercellular tight junctions by zonula occludens toxin and its eukaryotic analogue zonulin. Ann N Y Acad Sci. 915:214–222.

-Fasano A. 2012. Zonulin, regulation of tight junctions, and autoimmune diseases. Ann N Y Acad Sci. 1258:25–33.

-Fasano A. 2011. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. Physiol Rev. 91:151–175.

-Fasano A. *et al*. 2000. Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease. Lancet 355:1518–1519.

-Fasano A., Baudry B., Pumplin D.W., Wasserman S.S., Tall B.D., Ketley J.M., Kaper J.B. 1991. Vibrio cholerae produces a second enterotoxin, which affects intestinal tight junctions. Proc Natl Acad Sci U S A. 88:5242-6

- Fasano A., Berti I., Gerarduzzi T. *et al*. 2003. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. Arch Intern Med. 163(3): 286 – 92.

-Fasano A., Catassi C.2001. Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. Gastroenterology.120: 636-651.

-Fasano A., Fiorentini C., Donelli G., Uzzau S., Kaper J.B., Margaretten K., Ding X., Guandalini S., Comstock L., Goldblum S.E.1995. Zonula occludens toxin modulates tight junctions through protein kinase C-dependent actin reorganization, in vitro. J Clin Invest. 96:710-20

-Fasano A., Shea-Donohue T. 2005. Mechanisms of disease: The role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases. Nat Clin Pract Gastroenterol Hepatol 2:416–422.

-Fasano A., Not T., Wang W., Uzzau S., Berti I., Tommasini A., Goldblum S.E. 2000: Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease. Lancet. 355:1518–1519.

-Fasano A., Uzzau S., Fiore C., Margaretten K. 1997.The enterotoxic effect on zonula occludens toxin on rabbit small intestine involves the paracellularpathway. Gastroenterology. 112:839–46.

-FDA. 2013. Food labelling; Gluten-free labelling of foods. Food and Drug Administration Agency, Federal Register/Rules and Regulations. 78 (150).

-Fehniger T.A., Caligiuri M.A. 2001. Interleukin 15: biology and relevance to human disease. Blood. 97:14–32.

-Fehniger T.A., Suzuki K., Ponnappan A., VanDeusen J.B., Cooper M.A., Florea S.M., Freud A.G., Robinson M.L., Durbin J., Caligiuri M.A. 2001. Fatal leukemia in interleukin 15 transgenic mice follows early expansions in natural killer and memory phenotype CD8+ T cells. J Exp Med.193:219–231.

-Feighery C. 1999. Fortnightly review: coeliac disease. BMJ. 319: 236-239

-Fihn B.M., Sjoqvist A., and Jodal M. 2000. Permeability of the rat small intestinal epithelium along the villus-crypt axis: effects of glucose transport. Gastroenterology. 119: 1029 – 1036.

- Fleckenstein B., Molberg O., Qiao S.W., Schmid D.G., von der Mulbe F., Elgstoen K., *et al*. 2002. Gliadin T cell epitope selection by tissue transglutaminase in celiac disease. Role of enzyme specificity and pH influence on the transamidation versus deamidation process. J Biol Chem. 277(37):34109–16.

- Ford A.C., Chey W.D., Talley N.J. *et al*. 2009.Yield of diagnostic tests for celiac disease in individuals with symptoms suggestive of irritable bowel syndrome: 169(7):651-
8.

- Frost A. R., Band M. M., and Conway G. S. 2009. Serological screening for coeliac disease in adults with Turner's syndrome: prevalence and clinical significance of endomysium antibody positivity. European Journal of Endocrinology. 160(4): 675–679.

-Forster C. 2008.Tight junctions and the modulation of barrier function in disease. Histochem Cell Biol. 130:55–70.

-Freeman H.J., Chopra A., Clandinin M.T., Thomson A.B. 2011. Recent advances in celiac disease. World J Gastroenterol.17: 2259–2272.

-Freeman H.J. 1995. Clinical spectrum of biopsy-defined celiac disease in the elderly. Can J Gastroenterol. 9: 42-46.

-Freeman H.J. 2013. Non-dietary forms of treatment for adult celiac disease. World J Gastrointest Pharmacol Ther. 4: 108-11.

-Freeman H.J. 2010. Risk factors in familial forms of celiac disease. World J Gastroenterol. 16: 1828-1831.

-Fry L., Keir P., McMinn R.M. *et al*. 1967. Small-intestinal structure and function and haematological changes in dermatitis herpetiformis. Lancet. 2:729-33.

-Fry L., Seah P.P., Riches D.J. *et al*. 1973. Clearance of skin lesions in dermatitis herpetiformis after gluten withdrawal. Lancet. 1 :288-91.

- Fuchs A. *et al*. 2013. Intraepithelial type 1 innate lymphoid cells are a unique subset of IL-12- and IL-15-responsive IFN-gamma-producing cells. Immunity. 38: 769–81.

-Furuse M., Hirase T., Itoh M., Nagafuchi A., Yonemura S., Tsukita S., Tsukita S. 1993. Occludin: a novel integral membrane protein localizing at tight junctions. J Cell Biol. 123:1777-1788.

-García-Manzanares A., Lucendo A.J. 2011. Nutritional and dietary aspects of celiac disease. Nutr Clin Pract. 26: 163–73.

-Garioch J.J., Lewis H.M., Sargent S.A *et al*. 1994. 25 years' experience of a gluten-free diet in the treatment of dermatitis herpetiformis. Br J Dermatol. 131:541-5.

-Garrett W.S., Gallini C.A., Yatsunenko T., Michaud M., DuBois A., Delaney M.L. *et al.*2010. Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. Cell Host Microbe.8: 292–300.

-Garrote J.A., Gomez-Gonzalez E., Bernardo D., Arranz E., Chirdo F. 2008. Celiac disease pathogenesis: The proinflammatory cytokine network. J. Pediatr. Gastroenterol. Nutr. 47(Suppl. 1): S27–S32.

-Gasbarrini G., Miele L., Corazza G.R., Gasbarrini A. 2010. When was celiac disease born? : The Italian case from the archeologic site of Cosa. J. Clin. Gastroenterol. 44:502–503.

-Gee S.J. 1888. On the coeliac affection. St. Bartholomew's Hospital Report. 24: 17-20.

-Gerace E., Resta F., Landucci E., Renzi D., Masi A., Pellegrini-Giampietro D. E., Mannaioni G. 2017. The gliadin peptide 31-43 exacerbates kainate neurotoxicity in epilepsy models. Scientific Reports 7, 15146.

-Gianfrani C., Auricchio S., Troncone R. 2005. Adaptive and innate immune responses in celiac disease. Immunol Lett. 99: 141-145.

-Grabstein K.H. *et al*. 1994. Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor. Science. 264:965–8.

-Green P.H., Cellier C. 2007. Celiac disease. N. Engl. J. Med. 357:1731–43

-Green P.H., Fleischauer A.T., Bhagat G., Goyal R., Jabri B., Neugut A.I. 2003. Risk of malignancy in patients with celiac disease. Am J Med. 115:191-5

-Green P.H., Jabri B. 2003. Coeliac disease. Lancet.362:383–91.

-Green P.H. 2005.The many faces of celiac disease: clinical presentation of celiac disease in the adult population. Gastroenterology.128:S74–78.

-Green K.J., Simpson C.L. 2007. Desmosomes: new perspectives on a classic. J. Invest. Dermatol. 127: 2499–2515.

-Green P., Stavropoulos S., and Panagi S.*et al*. 2001. Characteristics of adult celiac disease in the USA: result of a national survey. Am.J. Gastroentero. 96(1):126-131.

-Greetje J. Tack, Wieke H. M. Verbeek, Marco W. J. Schreurs, and Chris J. J. Muldel. 2010. The spectrum of celiac disease: epidemiology, clinical aspects and treatment, Nature Reviews Gastroenterology & Hepatology. 7: 204–213.

-Groschwitz K.R., Hogan S.P. 2009. Intestinal barrier function: molecular regulation and disease pathogenesis. J Allergy Clin Immunol. 124:3-20; quiz 21-2.

-Gutierrez-Achury J., Coutinho de Almeida R., Wijmenga C. 2011. Shared genetics in coeliac disease and other immune-mediated diseases. Journal of Internal Medicine. 269(6):591–603.

-Hadithi M., von Blomberg B.M., Crusius J.B. *et al*. 2007. Accuracy ofserologic tests and HLA-DQ typing for diagnosing celiac disease. Ann Intern Med.147(5):294-302.

- Hall N.J., Rubin G., Charnock A. 2009. Systematic review: adherence to a gluten-free diet in adult patients with coeliac disease. Aliment. Pharmacol. Ther.30:315–330.

-Halpern M. D., Denning,P. W. (2015). The role of intestinal epithelial barrier function in the development of NEC. Tissue Barriers, 3(1-2), e1000707.

- Halstensen T., Hvatum M., Scott H., Fausa O., Brandtzaeg P. 1992. Association of subepithelial deposition of activated complement and immunoglobulin G and M response to gluten in celiac disease. Gastroenterology. 102:751–759.

-Hameed W.S., Abdul-Mehdi R.J., Tarish H.R. and Alsherees, H.A.A., 2016. Prevalence of DQ2, DQ8 and DR4 Alleles in Iraqi Celiac Patients. *International Archives of* BioMedical and Clinical Research. 2(3): 118-121.

-Han A., Newell E.W., Glanville J., FernandezBecker N., Khosla C., Chien Y.H., Davis M.M. 2013. Dietary gluten triggers concomitant activation of CD4+ and CD8+ αβ T cells and γδ T cells in celiac disease. Proc Natl Acad Sci USA.110: 13073–13078.

-Hansson T., Dannaeus A., Klareskog L. 1999. Cytokine-producing cells in peripheral blood of children with coeliac disease secrete cytokines with a type 1 profile. Clin Exp Immunol. 116:246–250

-Harhaj N.S., Antonetti D.A. 2004.Regulation of tight junctions and loss of barrier function in pathophysiology.Int. J. Biochem. Cell Biol. 36:1206–1237.

-Harris K.M., Fasano A., Mann D.L. 2010. Monocytes differentiated with IL-15 support Th17 and Th1 responses to wheat gliadin: Implications for celiac disease. Clin. Immunol. 135: 430–439.

-Hartsock A., Nelson W.J. 2008. Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. Biochim Biophys Acta. 1778:660-669

-Hassan K., A-Kader H. 2014. Celiac disease: the search for adjunctive or alternative therapies. Expert Rev Gastroenterol Hepatol. 8:313–321.

-Haugen T.H., Hanley J.M., Heath E.C. 1981. Haptoglobin. A novel mode of biosynthesis of a liver secretory glycoprotein. J Biol Chem. 256:1055–1057.

-Hawamdeh H., Al-Zoubi B., Al Sharqi Y., Qasrawi A., Abdelaziz Y., and Barbar M. 2016. Association of Tissue Transglutaminase Antibody Titer with Duodenal Histological Changes in Children with Celiac Disease. Gastroenterology Research and Practice, 2016, 6718590.

-Helander H.F., Fandriks L. 2014. Surface area of the digestive tract—revisited. Scand J Gastroenterol.49:681–689.

-Hell D. A., West J. 2006. Resent advances in celiac disease.

-Hering N.A., Fromm M., Schulzke J.D. 2012. Determinants of colonic barrier function in inflammatory bowel disease and potential therapeutics.J Physiol. 590:1035–1044.

-Heyman M., Abed J., Lebreton C., and Cerf-Bensussan, N. 2011. Intestinal permeability in coeliac disease: insight into mechanisms and relevance to pathogenesis. *Gut*, gutjnl-2011.

-Hill D.A., Artis D. 2010. Intestinal bacteria and the regulation of immune cell homeostasis. Annu Rev Immunol. 28: 623–667.

-Hill I.D., Dirks M.H., Liptak G.S. *et al*. 2005. Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr.40:1-19.

-Hin H., Bird G., Fisher P., Mahy N., Jewell D.1999. Coeliac disease in primary care: case finding study. BMJ. 318: 164-167

-Hischenhuber C., Crevel R., Jarry B. *et al*. 2006: Review article: safe amounts of gluten for patients with wheat allergy or coeliac disease. Aliment Pharmacol Ther. 23:559–575.

- Hoffmann B.R., Annis D.S., Mosher D.F. 2011. Reactivity of the N-terminal region of fibronectin protein to transglutaminase 2 and factor XIIIA. J Biol Chem. 286 (37):32220–32230.

-Hollon J., Leonard Puppa E., Greenwald B., Goldberg E., Guerrerio A., Fasano A. 2015. Effect of Gliadin on Permeability of Intestinal Biopsy

Explants from Celiac Disease Patients and Patients with Non-Celiac Gluten Sensitivity. Nutrients. 7(3): 1565–1576.

-Hollon J., Puppa E.L., Greenwald B., Goldberg E., Guerrerio A., Fasano A.2015. Effect of gliadin on permeability of intestinal biopsy explants from celiac disease patients and patients with non-celiac gluten sensitivity. Nutrients.7:1565–76.

- Hollon J.R, Cureton P.A., Martin M.L., Puppa ELL, Fasano A. 2013. Trace gluten contamination may play a role in mucosal and clinical recovery in a subgroup of diet-adherent non-responsive celiac disease patients. BMC Gastroenterol. 13:40.

-Holmes G., Catassi C., Fasano A. 2009. Dermatitis Herpetiformis in Celiac disease. Oxford: Health Press. 83-90.

-Holmes G.K. 2001. Coeliac disease and Type 1 diabetes mellitus the case for screening. Diabet Med. 18: 169-177.

-Holmes G. 2002. Screening for coeliac disease in type 1 diabetes. Archives of Disease in Childhood. 87(6): 495-498.

-Holmes S. 2010. Coeliac disease: symptoms, complications and patient support. Nurs Stand. 24:505–506: quiz 508.

-Holthofer B., Windoffer R., Troyanovsky S.*et al*. 2007. Structure and function of desmosomes. Int Rev Cytol. 264: 65–163.

-Hoontrakoon R. *et al*. 2002. Interleukin-15 inhibits spontaneous apoptosis in human eosinophils via autocrine production of granulocyte macrophage-colony stimulating factor and nuclear factor-kappaB activation. Am J Respir Cell Mol Biol. 26: 404–12.

-Horton R.E., Vidarsson G. 2013. Antibodies and their receptors: different potential roles in mucosal defense. Front Immunol. 4:200

-Houlston R.S., Ford D. 1996. Genetics of coeliac disease. QJM. 89: 737-743.

-Houlston R.S., Tomlinson I.P., Ford D. *et al*. 1997. Linkage analysis of

- Ho-Yen C., Chang F., van der Walt J., Mitchell T., Ciclitira P. 2009. Recent advances in refractory coeliac disease: a review. Histopathology. 54: 783–795.

-Hue S., Mention J.J., Monteiro R.C. *et al.* 2004. A direct role for NKG2D/MICA interaction in villous atrophy during celiac disease 1. Immunity. 21: 367-77.

- Humbert P., Pelletier F., Dreno B., Puzenat E., and F. Aubin F. 2006. Gluten intolerance and skin diseases. European Journal of Dermatology. 16 (1):4–11.

-Hunt K.A., Zhemakowa A., Turner G., Heap G.A., Franke L., Bruinenberg M. *et al*. 2008. Newly identified genetic risk variants for celiac disease related to the immune response. Nat Genet 40:395-402.

- Husby S., Koletzko S., Korponay-Szabo I.R. *et al*. 2012. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr. 54: 136-60.

-Ianiro G., Bibbo S., Bruno G. *et al*. 2016. Prior Misdiagnosis of Celiac Disease Is Common Among Patients Referred to a Tertiary Care Center: A Prospective Cohort Study. Clinical and Translational Gastroenterology. 7: e139; doi:10.1038/ctg.2015.48

-Ianiro G., Bibbo S., Pecere S., *et al*. 2015. Current technologies for the endoscopic assessment of duodenal villous pattern in celiac disease. Comput Biol Med. 65: 308–14

-Imamichi H., Sereti I., Clifford Lane H. 2008. IL-15 acts as a potent inducer of $CD4^{+}CD25^{high}$ cells expressing forkhead box protein P3. European Journal of Immunology. 38(6): 1621–1630.

-Ismail A.S., Severson K.M., Vaishnava S., Behrendt C.L., Yu X., Benjamin J.L., Ruhn K.A., Hou B., DeFranco A.L., Yarovinsky F. *et al*. 2011. Gammadelta intraepithelial lymphocytes are essential mediators of host-microbial homeostasis at the intestinal mucosal surface. Proc Natl Acad Sci USA. 108(21):8743-8

-Ivanov A.I., Naydenov N.G. 2013. Dynamics and regulation of epithelial adherens junctions: recent discoveries and controversies. Int Rev Cell Mol Biol. 303: 27–99.

-Ivarsson A., Persson L.A., and Nystrom L.2000. Epidemic of coeliac in Swedish children. Acta Paediatr. 89(2):165-71.

-Jabbar A.A. 1993. HLA and disease associations in Iraq. Dis Markers.11: 161-170.

-Jabri B., Abadie V. 2015. IL-15 functions as a danger signal to regulate tissue-resident T cells and tissue destruction. Nat Rev Immunol. 15: 771–783.

-Jabri B., de Serre N.P., Cellier C., Evans K., Gache C. *et al*. 2000. Selective expansion of intraepithelial lymphocytes expressing the HLA-E-specific natural killer receptor CD94 in celiac disease. Gastroenterology118:867–79

-Jackson J. R., Eaton W. W., Cascella N. G., Fasano A., Kelly D. L. 2012. Neurologic and psychiatric manifestations of celiac disease and gluten sensitivity. Psychiatr Q. 83(1): 91–102.

-Jacob C. *et al*. 2005. Mast cell tryptase controls paracellular permeability of the intestine. Role of protease-activated receptor 2 and beta-arrestins. J Biol Chem 280:31936–31948.

-Jeon M.K., Klaus C., Kaemmerer E., Gassler N. 2013. Intestinal barrier: Molecular pathways and modifiers. World J Gastrointest Pathophysiol. 4: 94–99.

-Johansson M.E., Thomsson K.A., Hansson G.C. 2009. Proteomic analyses of the two mucus layers of the colon barrier reveal that their main component, the Muc2 mucin, is strongly bound to the Fcgbp protein. J Proteome Res. 8:3549–3557.

-Jorge S.E., Abreu C.F., Guariento M.E., Sonati Mde F. 2010. Haptoglobin genotypes in Chagas' disease. Clin Biochem. 43:314-6

- Jabri B. *et al*. 2000. Selective expansion of intraepithelial lymphocytes expressing the HLA-E- specific natural killer receptor CD94 in celiac disease. Gastroenterology. 118:867–79.

-Kagnoff M.F. 2007. Celiac disease: pathogenesis of a model immunogenetic disease. J Clin Invest. 117:41–49.

- Kalinski P., Hilkens C.M., Wierenga E.A., Kapsenberg M.L. 1999. T-cell priming by type-1 and type-2 polarized dendritic cells: the concept of a third signal. Immunol Today. 20:561–567.

- Khalesi M., Jafari S.A.., Kiani M, Picarelli A., Borghini R., Sadeghi R., Eghtedar A., Ayatollahi H., Kianifar H.R. 2016. In Vitro Gluten Challenge Test for Celiac Disease Diagnosis. J Pediatr Gastroenterol Nutr. 62:276–283.

-Kamada N., Seo S.U., Chen G.Y., Nuñez C. 2013. Role of the gut microbiota in immunity and inflammatory disease. Nat. Rev. Immunol. 13: 312–335.

-Kamekura R. *et al*. 2015. Inflammation-induced desmoglein-2 ectodomain shedding compromises the mucosal barrier. Mol. Biol. Cell 26, 3165–3177.

-Karell K. A., Louka S., Moodie S.J., Ascher H., Clot F., Greco L., Ciclitira P.J., Sollid L.M., Partanen J., European Genetics Cluster on Celiac Disease. 2003. HLA types in celiac disease patients not carrying the DQA1*05-DQB1*02 (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease.Hum. Immunol. 64: 469–477.

-Kaswala D.H., Veeraraghavan G., Kelly C.P., Leffler D.A. 2015. Celiac Disease: Diagnostic Standards and Dilemmas. Diseases. 3(2):86-101.

-Kaukinen K., Lindfors K., Maki M. 2014. Advances in the treatment of coeliac disease: an immunopathogenic perspective. Nat Rev Gastroenterol Hepatol. 11:36–44.

-Ke Y., Pearce K., Lake J.P., Ziegler H.K., Kapp J.A. 1997. Gamma delta T lymphocytes regulate the induction and maintenance of oral tolerance. J Immunol. 158(8):3610–3618.

-Kim Y.S. and Ho S.B. 2010. Intestinal goblet cells and mucins in health and disease: recent insights and progress. Curr. Gastroenterol. Rep. 12: 319–330.

- Kim S.M., Mayassi T., Jabri B. 2015. Innate immunity: actuating the gears of celiac disease pathogenesis. Best Pract Res Clin Gastroenterol. 29: 425–435.

-Király R. *et al*. 2009. Functional significance of five non-canonical Ca^{2+}-binding sites of human transglutaminase 2 characterized by site directed mutagenesis. FEBS J. 276:7083–7096.

-Klemenak M., Dolinšek J., Langerholc T., Di Gioia D., Mi˘ceti´c-Turk D. 2015. Administration of Bifidobacterium breve Decreases the Production of TNF-α in Children with Celiac Disease. Dig. Dis. Sci. 60(11):3386-92. doi: 10.1007/s10620-015-3769-7.

-Koning F. 2005. Celiac disease: caught between a rock and a hard place. Gastroenterology 129:1294-1301

-Koskinen O., Villanen M., Korponay-Szabo I. *et al*. 2009.Oats do not induce systemic or mucosalautoantibody response in children with coeliac disease. J Pediatr Gastroenterol Nutr. 48:559–65.

-Kotler BM, Kerstetter JE, Insogna KL (2013) Claudins, dietary milk proteins, and intestinal barrier regulation. Nutr Rev 71:60-65

-Kumar V., GutierrezAchury J., Kanduri K., Almeida R., Hrdlickova B., Zhernakova D. V. *et al*. 2015. Systematic annotation of celiac disease loci refines pathological pathways and suggests a genetic explanation for increased interferon-gamma levels. Hum. Mol. Genet. 24: 397–409.

- Kumar V., Wijmenga G., Withoff S. 2012. From genome-wide association studies to disease mechanisms: celiac disease as a model of autoimmune diseases. Seminars in Immunopathology. 34(4):567–580.

-Kurashima Y., Goto Y., Kiyono H. 2013. Mucosal innate immune cells regulate both gut homeostasis and intestinal inflammation.Eur. J. Immunol. 43: 3108–3115

-Kurppa K., Salminiemi J., Ukkola A., Saavalainen P., Löytynoja K., Laurila K. *et al*. 2012. Utility of the new ESPGHAN criteria for the diagnosis of celiac disease in at-risk groups: A large family-based cohort study. J Pediatr Gastroenterol Nutr. 54:387–391.

-Kutlu T., Brousse N., Rambaud C., Le Deist F., Schmitz J., Cerf-Bensussan N. 1993. Numbers of T cell receptor (TCR) αβ+ but not of TCR γδ+ intraepithelial lymphocytes correlate with the grade of villous atrophy in coeliac patients on a long term normal diet. Gut 34:208–14.

-Lacy B.E., Talley N.J., Locke G.R. III *et al*. 2012. Review article: current treatment options and management of functional dyspepsia . Aliment Pharmacol Ther. 36 : 3 – 15 .

- Lahdeaho M. L., Mak i M., Laurila K. *et al*. 2011. Small- bowel mucosal changes and antibody responses after low- and moderate-dose gluten challenge in celiac disease . BMC Gastroenterol.11:129.

-Lal-Nag M . & Morin P . J. 2009. The claudins . Genome Biol.10: 235.

-Lammers K.M., Lu R., Brownley J., Lu B., Gerard C., Thomas K., Rallabhandi P., Shea-Donohue T., Tamiz A., Alkan S. *et al*. 2008. Gliadin induces an increase in intestinal permeability and zonulin release by binding to the chemokine receptor CXCR3. Gastroenterology. 135:194–204.e3.

-Lammi A., Arikoski P., Simell S., Kinnunen T., Simell V., Paavanen-Huhtala S., Hinkkanen A., Veijola R., Knip M., Toppari J., Vaarala O., Simell O., Ilonen J. 2015. Antibodies to deamidated gliadin peptide in diagnosis of celiac disease in children. J Pediatr Gastroenterol Nutr. 60(5):626-31.

-Lanzini A., Lanzarotto F., Villanacci V. *et al*. 2009. Complete recovery of intestinal mucosa occurs very rarely in adult coeliac patients despite adherence to gluten-free diet. Aliment Pharmacol Ther. 29: 1299-308.

-Larizza D., Calcaterra V., Klersy C. *et al*. 2012. Common immunogenetic profile in children with multiple autoinmune diseases: the signature of HLA-DQ pleiotropic genes. *Autoimmunity*. 45(6):470–475.

-larke L.L. 2009. A guide to Ussing chamber studies of mouse intestine.Am J Physiol Gastrointest Liver Physiol. 296: G1151–G1166.

-Laukoetter M. G., Bruewer M., Nusrat A. 2006. Regulation of the intestinal epithelial barrier by the apical junctional complex. Curr. Opin. Gastroenterol. 22, 85–89

-Lavelle E.C., Murphy C., O'Neill L.A. *et al*. 2010. The role of TLRs, NLRs, and RLRs in mucosal innate immunity and homeostasis. Mucosal Immunol. 3:17–28.

-Lee S.K., Lo W., Memeo L., Rotterdam H., Green P.H. 2003. Duodenal histology in patients with celiac disease after treatment with a gluten-free diet. Gastrointest Endosc. 57:187–91.

-Leffler D.A., Schuppan D. 2010. Update on serologic testing in celiac disease. Am J Gastroenterol. 105: 2520-4.

-Leffler D., Schuppan D., Pallav K., Najarian R., Goldsmith J.D., Hansen, J.; Kabbani, T.; Dennis M., Kelly C.P. 2012. Kinetics of the histological, serological and symptomatic responses to gluten challenge in adults with coeliac disease. Gut. 62: 996–1004.

-Lerner A., Jeremias P., Matthias T. 2017. Gut-thyroid axis and celiac disease. Endocr Connect. 6: R52–R58.

-Lettesjo H., Hansson T., Bergqvist A., Gronlund J., Dannaeus A. 2005. Enhanced interleukin-18 levels in the peripheral blood of children with coeliac disease. Clin Exp Immunol. 139:138–143.

-Levy A.P., Asleh R., Blum S., Levy N.S., Miller-Lotan R., Kalet-Litman S., Anbinder Y., Lache O., Nakhoul FM, Asaf R, *et al*. 2010. Haptoglobin: basic and clinical aspects. Antioxid Redox Signal. 12:293-304.

-Levy A.P., Hochberg I., Jablonski K., Resnick H.E., Lee E.T., Best L., Howard B.V.2002. Strong Heart Study. Haptoglobin phenotype is an independent risk factor for cardiovascular disease in individuals with diabetes: The Strong Heart Study. J Am Coll Cardiol. 40:1984-90http

- Lewis N.R., and Scott B.B. 2010. “Meta-analysis: deamidated gliadin peptide antibody and tissue transglutaminase antibody compared as screening tests for coeliac disease,” Alimentary Pharmacology and Therapeutics. 31(1):73–81.

-Li M., Yu L., Tiberti C. *et al*. 2009. A report on the International Transglutaminase Autoantibody Workshop for Celiac Disease . Am J Gastroenterol. 104 : 154-63 .

-Lionetti E., Castellaneta S., Francavilla R., Pulvirenti A., Tonutti E., Amarri S., Barbato M., Barbera C., Barera G., Bellantoni A., Castellano E. 2014. Introduction of gluten, HLA status, and the risk of celiac disease in children. New England Journal of Medicine. 371(14):1295-303.

-Lionetti E., and Catassi C. 2011. New clues in celiac disease epidemiology, pathogenesis, clinical manifestations, and treatment. Int Rev Immunol. 30:219–231.

-Liu E., Li M., Emery L. *et al*. 2007. Natural history of antibodies to deamidated gliadin peptides and transglutaminase in early childhood celiac disease. J Pediatr Gastroenterol Nutr. 45:293–300.

-Liu Z., Li N., Neu J. 2005.Tight junctions, leaky intestines, and pediatric diseases. Acta Paediatr. 94: 386–393.

-Lohi S., Mustalahti K., Kaukinen K. *et al*. 2007. Increasing prevalence of coeliac disease over time. Aliment Pharmacol Ther. 26: 1217–25.

-López-Vázquez A., Fuentes D., Rodrigo L., González S., More-no M., Fernández E., Martínez-Borra J., López-Larrea C. 2004. MHC class I region plays a role in the development of diverse clinical forms of celiac disease in a Saharawi population. Am J Gastroenterol. 99: 662-667.

-Lorand L., and Graham R.M. 2003. Transglutaminases: crosslinking enzymes with pleiotropic functions. Nat Rev Mol Cell Biol. 4: 140–156.

- Losowsky M.S. 2008. A history of coeliac disease. Dig. Dis. 26:112–20

-Ludvigsson J.F., Bai J.C., Biagi F. *et al*. 2014. Diagnosis and management of adult coeliac disease: guidelines from the British Society of Gastroenterology. Gut. 63:1210–28.

Ludvigsson J.F., Leffler D.A., Bai J.C. *et al* .2013. The Oslo definitions for coeliac disease and related terms. Gut .62(1):43–52.

- Ludvigsson J.F., Green P.H. 2011. Clinical management of coeliac disease. J Intern Med. 269:560–571.

-Ludvigsson J.F., Ludvigsson J., Ekbom A., Montgomery S.M. 2006. Celiac disease and risk of subsequent type 1 diabetes: a general population cohort study of children and adolescents. Diabetes Care. 29:2483–2488.

-Luissint A.C., Parkos C. A., Nusrat A. 2016. Inflammation and the Intestinal Barrier: Leukocyte–Epithelial Cell Interactions, Cell Junction Remodeling, and Mucosal Repair. Gastroenterology. 151(4), 616–632

-Lundin K E, Sollid L M, Qvigstad E, Markussen G, Gjertsen H A, Ek J and Thorsby E. (1990). T lymphocyte recognition of a celiac disease-associated cis- or trans-encoded HLADQ alpha/beta-heterodimer. Journal of immunology;145: 136-139.

-Lundin K.E., Wijmenga C. 2015.Coeliac disease and autoimmune disease-genetic overlap and screening. Nat Rev Gastroenterol Hepatol. 12:507–15.

-Madara J.L., Moore R., Carlson S.1987. Alteration of intestinal tight junction structure and permeability by cytoskeletal contraction. Am J Physiol. 253:C854-61

-Madara J.L. Warner-Lambert/Parke-Davis Award lecture. 1990. Pathobiology of the intestinal epithelial barrier.Am J Pathol. 137(6):1273-81.

-Maeda N., Yang F., Barnett D.R., Bowman B.H., Smithies O. 1984. Duplication within the haptoglobin Hp2 gene. Nature. 309:131-5.

-Maglio M., Tosco A., Paparo F., Auricchio R., Granata V., Colicchio B., Indolfi V., Miele E., Troncone R. 2010. Serum and intestinal celiac disease-associated antibodies in children with celiac disease younger than 2 years of age. J. Pediatr. Gastroenterol. Nutr. 50: 43–48.

-Maiuri L., Ciacci C., Ricciardelli I., Vacca L., Raia V., Auricchio S., Picard J., Osman M., Quaratino S., Londei M. 2003. Association between innate response to gliadin and activation of pathogenic T cells in coeliac disease. Lancet 362:30-37.

- Makharia G.K. 2014. Current and emerging therapy for celiac disease. Front Med (Lausanne). 1:6.

-Mäki M., Collin P . 1997. Coelia c disease . Lancet. 349: 1755-1759.

-Malamut G., Afchain P., Verkarre V., Lecomte T., Amiot A., Damotte D.,Bouhnik Y., Colombel J.F., Delchier J.C., Allez M. *et al*. 2009. Presentation and long-term follow-up of refractory celiac disease: comparison of type I with type II. Gastroenterology.136: 81–90.

-Malamut G., Cellier C. 2014. Refractory celiac disease. Expert Rev Gastroenterol Hepatol. 8:323-8.

-Malamut G., El Machhour R., Montcuquet N. *et al*. 2010. IL-15 triggers an antiapoptotic pathway in human intraepithelial lymphocytes that is a potential new target in celiac disease-associated inflammation and lymphomagenesis. J Clin Invest. 120: 2131-43.

- Mamone G., Picariello G., Addeo F., Ferranti P. 2011. Proteomic analysis in allergy and intolerance to wheat products, Expert Rev. Proteomics. 8(1): 95–115.

-Manavalan J. S., Hernandez L., Shah J. G. *et al*. 2010. Serum cytokine elevations in celiac disease: association with disease presentation. Hum Immunol. 71:50–7.

- Mandel L.J., Bacallao R., Zampighi G. 1993. Uncoupling of the molecular “fence” and paracellular “gate” functions in epithelial tight junctions. Nature. 361:552–555.

-Marchiando A.M., Shen L., Graham W.V. 2011. The epithelial barrier is maintained by in vivo tight junction expansion during pathologic intestinal epithelial shedding. Gastroenterology. 140:1208–1218.

-Marine M., Farre C., Alsina M., and Vilar P. 2011. The prevalence of celiac disease is significantly higher in children compared with adults.Aliment Pharmacol Ther. 33 (4):477-484

- Marsh M.N. 1992. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity (‘celiac sprue’), Gastroenterology. 102 (1): 330–354.

-Marsh MN. 1992. Mucosal pathology in gluten sensitivity. In Coeliac Disease, ed. M Marsh, pp. 136–91.Oxford, UK: Blackwell Sci.

-Martin A. *et al*. 2012. Pathophysiological roles of transglutaminase - catalyzed reactions in the pathogenesis of human diseases. Inflamm. Allergy Drug Targets. 11(4): 278–84.

-Martini E., Krug S. M., Siegmund B., Neurath M. F., Becker C. 2017. Mend Your Fences: The Epithelial Barrier and its Relationship With Mucosal Immunity in Inflammatory Bowel Disease. Cellular and Molecular Gastroenterology and Hepatology, 4(1): 33–46.

-Martín-Pagola A., Pérez-Nanclares G., Ortiz L., Vitoria J.C., Hualde I., Zaballa R., Preciado E., Castaño L., Bilbao J.R. 2004. MICA response to gliadin in intestinal mucosa from celiac patients. Immunogenetics. 56: 549-554.

-Massey V.L., Arteel G.E. 2012. Acute alcohol-induced liver injury. Front Physiol. 3:193.

- Mattei F., Schiavoni G., Belardelli F., Tough D.F. 2001. IL-15 is expressed by dendritic cells in response to type I IFN, double-stranded RNA, or lipopolysaccharide and promotes dendritic cell activation. J Immunol. 167:1179–87.

-Matteoli G., Mazzini E., Iliev I.D., Mileti E., Fallarino F., Puccetti P., *et al*. , Gut CD103+ dendritic cells express indoleamine 2,3-dioxygenase which influences T regulatory/T effector cell balance and oral tolerance induction., Gut. 59 (2010) 595–604.

-Matthias T., Neidhöfer S., Pfeiffer S., Prager K., Reuter S., Gershwin M.E. 2011. Novel trends in celiac disease.Cell Mol Immunol. 8:121–125.

-Matysiak-Budnik T., Moura I.C., Arcos-Fajardo M. *et al*. 2008. Secretory IgA mediates retrotranscytosis of intact gliadin peptides via the transferrin receptor in CD. J Exp Med. 205:143–154.

- Mauro Piacentinia, bVittorio Colizzi. 1999. Tissue transglutaminase: apoptosis Versus autoimmunity. Immunol. Today. 20(3): 130–134.

-Mayer L. Mucosal immunity. Immunol Rev. 2005: 206:5.

management of celiac disease. Gastroenterology 131:1977-80.

- McGowan K.E., Lyon M.E., Butzner J.D. 2008. Celiac disease and IgA deficiency: complications of serological testing approaches encountered in the clinic. Clin Chem. 54: 1203 – 9.

-McNeish A.S.,Harms H.K., Rey J,*et al*. 1979. The diagnosis of coeliac disease. A commentary on the current practices of members of the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN). Arch Dis Child. 54:783-6.

- Meeuwisse GW. 1970. Diagnostic criteria in coeliac disease. Acta Paediatr Scand. 59:461–463.

-Megiorni F., Mora B., Bonamico M., Barbato M., Montuori M. *et al*. 2008. HLA-DQ and susceptibility to celiac disease: evidence for gender differences and parent of origin effects. Am J Gastroenterol.103:997–1000.

- Megiorni F, Pizzuti A. 2012. HLA-DQA1 and HLA-DQB1 in Celiac disease predisposition: practical implications of the HLA molecular typing. J Biomed Sci 19:88-0127-19-88.

-Menard S., Cerf-Bensussan N., Heyman M. 2010. Multiple facets of intestinal permeability and epithelial handling of dietary antigens. Mucosal Immunol 3:247-259.

-Meresse B., Ripoche J., Heyman M., Cerf-Bensussan N. 2009. Celiac disease: from oral tolerance to intestinal inflammation, autoimmunity and lymphomagenesis. Mucosal Immunol. 2: 8-23.

-Meresse, B., Malamut, G., Cerf-Bensussan, N. 2012 .Celiac disease: an immunological jigsaw. Immunity. 36:907-919.

*Mestecky J., Strober W., Russell M. W. (eds.). 2015. Mucosal Immunology (ForthEdition), chapter 41, Oral tolerance to soluble antigens (p

-Mestecky J., Bienenstock J., Lamm M.E., McGhee J., Strober W., Mayer L. (eds.). 2005. Mucosal Immunology. 3rd ed. Amsterdam: Elsevier–Academic Press.

-Mills J.R., Murray J.A. 2016. Contemporary celiac disease diagnosis: is a biopsy avoidable? Curr Opin Gastroenterol. 32: 80–85.

-Mirenda V., Millington O., Lechler R.I. *et al*. 2005.Tolerant T cells display impaired trafficking ability. Eur J Immunol. 35(7):2146–2156

-Moens E., Veldhoen M. 2012. Epithelial barrier biology: good fences make good neighbours. Immunology.135:1–8.

-Molberg O., Mcadam S.N., Körner R., Quarsten H., Kristian-sen C., Madsen L., Fugger L., Scott H., Norén O., Roepstorff P., Lundin K.E., Sjöström H., Sollid L.M. 1998.Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. Nat Med. 4: 713-717.

-Monsuur A.J., Wijmenga C. 2006. Understanding the molecular basis of celiac disease: What genetic studies reveal. Ann. Med. 38: 578–591.

-Monzani A., Rapa A., Fonio P., Tognato E., Panigati L., and Oderda G. 2011. Use of deamidated gliadin peptide antibodies to monitor diet compliance in childhood celiac disease. Journal of Pediatric Gastroenterology and Nutrition. 53(1): 55–60.

-Moog F. 1981. The lining of the small intestine. Sci Am. 245(5):154–158.

-Moreno M.L., Vazquez H., Mazure R. *et al*. 2004. Stratification of bone fracture risk in patients with celiac disease. Clin Gastroenterol Hepatol. 2:127–34.

-Morita K ., Furuse M ., Fujimoto K ., Tsukita S . 1999. Claudin multigene family encoding four-transmembrane domain protein components of tight junction strands . Proc. Natl Acad. Sci. USA. 96: 511 – 516.

- Morón B., Verma A.K., Das P., Taavela J., Dafik L., Diraimondo T.R. *et al*. 2013. CYP3A4-catalyzed simvastatin metabolism as a non-invasive marker of small intestinal health in celiac disease. Am J Gastroenterol. 108:1344–51.

-Morris J. C. *et al*. 2006. Preclinical and phase I clinical trial of blockade of IL-15 using Mikbeta1 monoclonal antibody in T cell large granular lymphocyte leukemia. Proc Natl Acad Sci U S A. 103:401–6.

-Mortier E., Woo T., Advincula R., Gozalo S., Ma A. 2008. IL-15Ralpha chaperones IL-15 to stable dendritic cell membrane complexes that activate NK cells via trans presentation. J Exp Med. 205: 1213–25.

-Mowat A.M. 2003. Anatomical basis of tolerance and immunity to intestinal antigens. Nat Rev Immunol. 3(4):331–341.

-Mubarak A., Wolters V.M., Gerritsen S.A., Gmelig-Meyling F.H., Ten Kate F.J., Houwen R.H. *et al*. 2011. A biopsy is not always necessary to diagnose celiac disease. J Pediatr Gastroenterol Nutr. 52(5):554-7.

-Mubarak A., Wolters V.M., Gmelig-Meyling F.H., Ten Kate F.J., Houwen R.H. *et al*. 2012. Tissue transglutaminase levels above 100 U/mL and celiac disease: a prospective study. World J Gastroenterol. 28; 18(32):4399-403.

-Murray J.A. 2005. Celiac disease in patients with an affected member, type 1 diabetes, iron-deficiency, or osteoporosis? Gastroenterology.128:S52 – 6.

-Mustalahti K1, Catassi C, Reunanen A, Fabiani E, Heier M, McMillan S, Murray L, Metzger MH, Gasparin M, Bravi E, Mäki M. 2010. The prevalence of celiac disease in Europe: results of a centralized, international mass screening project. Ann Med. 42:587–595.

-Musu M.P., Di Pierro M.R., Drago S., Congia M., Fasano A. 2003. Early effects of gliadin on enterocyte intracellular signalling involved in intestinal barrier function. Gut. 52:218-23.

-Myléus A., Hernell O., Gothefors L. *et al*. 2012. Earlyinfections are associated with increased risk for celiac disease: an incident case-referent study. BMC Pediatr. 12:194.

-Naiyana Gujral, Hugh J Freeman, Alan BR Thomson.2012. Celiac disease: Prevalence, diagnosis, pathogenesis and treatment World J Gastroenterol. 18(42): 6036-6059

-Nava, P., Kamekura, R. & Nusrat, A. (2013). Cleavage of transmembrane junction proteins and their role in regulating epithelial homeostasis. *Tissue Barriers* 1, e24783

-Nekrasova, O., Green, K. J. 2013. Desmosome assembly and dynamics.*Trends Cell Biol.* 23, 537–546

-Nikulina M., Habich C., Flohe S.B., Scott F.W., Kolb H. 2004.Wheat gluten causes dendritic cell maturation and chemokine secretion. J.Immunol. 173(3):1925-33.

- Nistico L., Fagnani C., Coto I. 2006. Concordance, disease progression, and heritability of coeliac disease in Italian twins. Gut. 55:803–808.

- Norström F., Lindholm L., Sandström O., Nordyke K., Ivarsson A. 2011. Delay to celiac disease diagnosis and its implications for health-related quality of life. BMC Gastroenterol. 11:118.

- Nusrat A., Turner J.R., Madara J.L. 2000. Molecular physiology and pathophysiology of tight junctions. IV. Regulation of tight junctions by extracellular stimuli: nutrients, cytokines, and immune cells. Am J Physiol Gastrointest Liver Physiol 2000; 279(5):G8517

-Oberhuber G. 2000. Histopathology of celiac disease. Biomed Pharmacother. 54(7):368-372.

-Oberhuber G., Granditsch G., Vogelsang H. 1999. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. Eur J Gastroenterol Hepatol. 11:1185–1194.

-Ohno H. 2016. Intestinal M cells. J Biochem. ;159:151–160.

- Oh S., Perera L. P., Burke D. S., Waldmann T. A., Berzofsky J. A., 2004. IL-15/IL-15Ralpha-mediated avidity maturation of memory CD8+ T cells. Proc Natl Acad Sci U S A. 101:15154–9.

-Olivares M., Castillejo G., Varea V., Sanz Y. 2014. Double-blind, randomised, placebo-controlled intervention trial to evaluate the effects of bifidobacterium longum cect 7347 in children with newly diagnosed coeliac disease. Br. J. Nutr. 112: 30–40.

- Ota N., Takase M., Uchiyama H., Olsen S. K., Kanagawa O. 2010. No requirement of trans presentations of IL-15 for human CD8 T cell proliferation. J Immunol. 185:60418.

*Owen J., Punt J., Stranford S. (eds.) 2013. Kupy Immunology (Seventh Edition). Chapter 16, Tolerance, autoimmunity and transplantation (p517-550).

-Owens S.R, Greenson J.K. 2007. The pathology of malabsorption: current concepts . Histopathology. 50 : 64 – 82 .

-Ozgör B., Selimoğlu M.A. 2010. Coeliac disease and reproductive disorders. Scand J Gastroenterol. 45:395–402.

-Pabst O., Mowat A.M.M. 2012. Oral tolerance to food protein. Mucosal Immunol. 5: 232–239.

-Paiatto L. N., Silva F. G. D., Bier J., Brochetto-Braga M. R., Yamada Á. T., Tamashiro, W. M. S. C., Simioni,P. U. 2017. Oral Tolerance Induced by OVA Intake Ameliorates TNBS-Induced Colitis in Mice. PLoS ONE. 12(1): e0170205.

-Palma G.D., Capilla A., Nova E., Castillejo G., Varea V., Pozo T., Garrote J.A., Polanco I., López A., Ribes-Koninckx C. *et al*. 2012. Influence of milk-feeding type and genetic risk of developing coeliac disease on intestinal microbiota of infants: The proficel study. PLoS ONE. 7: e30791.

-Papp M., Foldi I., Nemes E., Udvardy M., Harsfalvi J., Altorjay I., Mate I., Dinya T., Varvolgyi C., Barta Z. *et al*. 2008. Haptoglobin polymorphism: a

novel genetic risk factor for celiac disease development and its clinical manifestations. Clinical Chemistry. 54:697-704

-Park C.S., Yoon S.O., Armitage R.J., Choi Y.S. 2004. Follicular dendritic cells produce IL-15 that enhances germinal center B cell proliferation in membrane-bound form. J Immunol. 173:6676–83.

- Park D., Choi S.S., Ha K-S. 2010. Transglutaminase 2: a multi-functional protein in multiple subcellular compartments. Amino Acids. 39: 619–631.

-Parzanese I., Qehajaj D., Patrinicola F., Aralica M., Chiriva-Internati M., Stifter S., Grizzi F. 2017. Celiac disease: From pathophysiology to treatment. World Journal of Gastrointestinal Pathophysiology. 8(2): 27–38.

-Paterson B.M., Lammers K.M., Arrieta M.C., Fasano A., Meddings J.B. 2007. The safety,tolerance, pharmacokinetic and pharmacodynamic effects of single doses of AT-1001 in coeliac disease subjects: a proof of concept study. Aliment Pharmacol Ther. 26:757–66.

-Paulley J.W. 1954. Observation on the aetiology of idiopathic steatorrhoea: jejunal and lymph-node biopsies. Br. Med. J. 2:1318–21.

-Pendyala S., Walker J.M., Holt P.R. 2012. A high-fat diet is associated with endotoxemia that originates from the gut. Gastroenterology. 142: 1100–1101.

-Perez-Moreno M., Fuchs E. 2006. Catenins: keeping cells from getting their signals crossed. Dev Cell. 11:601-612

-Perrier C., Corthesy B. 2011. Gut permeability and food allergies. Clin Exp Allergy. 41(1):20–8.

- Perera P.Y., Lichy J.H., Waldmann T.A., Perera L.P. 2012. The role of interleukin-15 in inflammation and immune responses to infection: implications for its therapeutic use. Microbes Infect. 14: 247–61.

-Piacentini M., D'Eletto M., Farrace M.G., Rodolfo C., Del Nonno F., Ippolito G., Falasca L. 2014. Characterization of distinct sub-cellular location of transglutaminase type II: changes in intracellular distribution in physiological and pathological states. Cell Tissue Res. 358: 793–805.

-Pietzak M.M. 2005. Follow-up of patients with celiac disease: achieving compliance with treatment. Gastroenterology. 128:S135–S141.

-Platt S.G., Kasarda D.D. 1971. Separation and characterization of -gliadin fractions. Biochim Biophys Acta. 243:407–15.

-Pletinckx K., Döhler A., Pavlovic V., Lutz M.B. 2011. Role of dendritic cell maturity/costimulation for generation, homeostasis, and suppressive activity of regulatory T cells, Front. Immunol. 2:39

-Ploski R., Ek J., Thorsby E., Sollid L.M. 1993. On the HLA-DQ (α1*0501, β1*0201)-associated susceptibility in celiac disease: a possible gene dosage effect of DQB1*0201. Tissue Antigens 41:173–177.

- Plugis N.M., Khosla C. 2015.Therapeutic approaches for celiac disease. Best Pract Res Clin Gastroenterol. 29: 503–521.

-Polanco I.B.I., van Leeuwen A., Schreuder I, Khan PM, Guerrero J, *et al*. 1981. Gluten-sensitive enteropathy in Spain: genetic and environmental factors. In The Genetics of Coeliac Disease, ed. RB McConnell, pp. 211–34. Lancaster, UK: MTP

-Powell D.W. 1981. Barrier function of epithelia. Am J Physiol; 241: G275-88.

-Prince, H.E. 2006. Evaluation of the INOVA diagnostics enzyme-linked immunosorbent assay kits for measuring serum immunoglobulin G (IgG) and IgA to deamidated gliadin peptides. Clin. Vaccine Immunol.13: 150–151.

-Qiao S.W., Bergseng E., Molberg O., Xia J., Fleckenstein B., Khosla C., Sollid L.M. 2004. Antigen presentation to celiac lesion-derived T cells of a 33-mer gliadin peptide naturally formed by gastrointestinal digestion. J Immunol 173:1757-1762.

-Qiao S.W., Sollid L. M., Blumberg R. S. 2009. Antigen presentation in celiac disease. Current Opinion in Immunology, 21(1),111–117.

-Rampertab S.D., Pooran N., Brar P., Singh P., Green P.H. 2006.Trends in the presentation of celiac disease. Am J Med. 119: 355.e9-355.14

-Rashtak S., Ettore M.W., Homburger H.A., Murray J.A. 2008. Combination testing for antibodies in the diagnosis of coeliac disease: Comparison of multiplex immunoassay and ELISA methods. Aliment. Pharmacol. Ther. 28: 805–813.

-Rashtak S., Murray J.A. 2012. Review article: coeliac disease, new approaches to therapy. Aliment Pharmacol Ther. 35:768–781.

-Reilly N.R., Aguilar K., Hassid B.G., Cheng J., Defelice A.R., Kazlow P., Bhagat G., Green P.H. 2011. Celiac disease in normal-weight and overweight children: clinical features and growth outcomes following a gluten-free diet. J Pediatr Gastroenterol Nutr. 53 (5):528-31. doi: 10.1097/MPG.0b013e3182276d5e.

-Reilly N.R., Fasano A., Green P.H. 2012. Presentation of celiac disease. Gastrointest Endosc Clin N Am. 22(4):613-621.

-Remely M., Aumueller E.. Merold C., Dworzak S., Hippe B., Zanner J., Pointner A., Brath H., Haslberger A.G. 2014. Effects of short chain fatty acid producing bacteria on epigenetic regulation of FFAR3 in type 2 diabetes and obesity.Gene, 537:85–92.

-Rescigno M. 2010. Functional specialization of antigen presenting cells in the gastrointestinal tract. Curr Opin Immunol. 22(1):131–136.

-Rescigno M., Rotta G., Valzasina B., Ricciardi-Castagnoli P. 2001. Dendritic cells shuttle microbes across gut epithelial monolayers . Immunobiology. 204: 572 – 581

-Revised criteria for diagnosis of coeliac disease. 1990. Report of Working Group of European Society of Paediatric Gastroenterology and Nutrition . Arch Dis Child 65: 909 – 11.

- Richir M., Songun I., Wientjes C., Snel P., Dwars B. 2010. Small Bowel Adenocarcinoma in a Patient with Coeliac Disease: Case Report and Review of the Literature. Case Rep Gastroenterol. 4: 416–420.

-Roberts A.I. *et al*. 2001. NKG2D receptors induced by IL-15 costimulate CD28-negative effector CTL in the tissue microenvironment. J Immunol. 167:5527–30.

-Rochman Y., Spolski R., Leonard W.J. 2009. New insights into the regulation of T cells by gamma(c) family cytokines. Nat Rev Immunol. 9: 480–90.

-Romaldini C.C., Barbieri D., Okay T.S., Raiz R., Jr, Cancado E.L. 2002. Serum soluble interleukin-2 receptor, interleukin-6, and tumor necrosis factor-alpha levels in children with celiac disease: response to treatment. J Pediatr Gastroenterol Nutr. 35:513–517.

-Roshan B., Leffler D.A., Jamma S. *et al*. 2011.The incidence and clinical spectrum of refractory celiac disease in a north american referral center. Am J Gastroenterol.; 106: 923–8.

-Rossi M., Maurano F., Luongo D. 2005. Immunomodulatory strategies for celiac disease. Int Rev Immunol. 24: 479-499.

-Rostami K., Kerckhaert J., Tiemessen R., von Blomberg B.M., Meijer J.W., Mulder C.J. 1999. Sensitivity of antiendomysium and antigliadin antibodies in untreated celiac disease: disappointing in clinical practice. Am J Gastroenterol. 94: 888-894

- Rostom A., Dubé C., Cranney A. *et al*. 2005. The diagnostic accuracy of serologic tests for celiac disease: a systematic review.Gastroenterology. 128(4):S38–46.

-Rostom A., Murray J.A., Kagnoff M.F. 2006.American Gastroenterological Association (AGA) Institute technical review on the diagnosis and management of celiac disease. Gastroenterology. 131:1981–2002.

-Rowley J., Monie A., Hung C. F., Wu, T. C. 2009. Expression of IL-15RA or an IL-15/IL-15RA fusion on CD8+ T cells modifies adoptively transferred T-cell function in cis. Eur J Immunol. 39:491–506.

-Rubio-Tapia A., Hill I.D., Kelly C.P. *et al*. 2013. American College of G. ACG clinical guidelines: diagnosis and management of celiac disease. Am J Gastroenterol. 108:656–76.

-Rubio-Tapia A., Kyle R.A., Kaplan E.L. *et al*. 2009. Increased prevalence and mortality in undiagnosed celiac disease. Gastroenterology.137: 88–93.

-Rubio-Tapia A., Ludvigsson J.F., Brantner T.L., Murray J.A., Everhart J.E. 2012. The prevalence of celiac disease in the United States. Am J Gastroenterol. 107(10):1538-1545.

-Rubio-Tapia A., Murray J.A. 2010. Classification and management of refractory coeliac disease. Gut. 59:547-57

- Rubio-Tapia A., Van Dyke C. T., Lahr B. D. *et al*. 2008. Predictors of family risk for celiac disease: a population-based study. Clinical Gastroenterology and Hepatology. 6(9): 983–987.

-Rujner J., Socha J., Romanczuk W. *et al*. 2002. Individual sensitivity of jejunal mucosa to small doses of gluten in coeliac disease. Wiad Lek. 55: 554-60.

-Ruoff, M. 1996. Chapter 5- Small Bowel. In A. Gel b (Ed.), Clinical Gastroenterology in the Elderly (pp. 78-80). New York: Marcel Dekker.

-Russell M.W., Ogra P.L. 2010. Mucosal decisions: tolerance and responsiveness at mucosal surfaces. Immunol Invest.39: 297–302.

- Salmi T.T., Collin P. Korponay-Szabó I.R. *et al*. 2006. Endomysial antibody-negative coeliac disease: clinical characteristics and intestinal autoantibody deposits. Gut. 55:1746–1753.

-Salvati V.M., Mazzarella G., Gianfrani C., Levings M.K., Stefanile R. *et al*. 2005. Recombinant human interleukin 10 suppresses gliadin dependent T cell activation in ex vivo cultured coeliac intestinal mucosa. Gut. 54: 46–53.

-Sandau M.M., Schluns K.S., Lefrancois L., Jameson S.C. 2004. Cutting edge: transpresentation of IL-15 by bone marrow-derived cells necessitates expression of IL-15 and IL-15R alpha by the same cells. J Immunol. 173:6537–6541.

-Sanz Y., De Pama G., Laparra M. 2011. Unraveling the ties between celiac disease and intestinal microbiota. Int Rev Immunol. 30:207-18.

-Sardy M., Karpati S., Merkl B., Paulsson M., and Smyth N. 2002. Epidermal transglutaminase (TGase 3) is the autoantigen of dermatitis herpetiformis. J. Exp. Med.195, 747–757.

- Sattar N., Lazare F., Kacer M. *et al*. 2011. "Celiac disease in children, adolescents, and young adults with autoimmune thyroid disease," The Journal of Pediatrics. 158(2): 272–275.e1.

-Scaldaferri F, Pizzoferrato M, Gerardi V, Lopetuso L, Gasbarrini A. 2012. The gut barrier: new acquisitions and therapeutic approaches. J Clin Gastroenterol.; 46 (Suppl): S12–S17.

-Schlegel, N. *et al*. 2010. Desmoglein 2-mediated adhesion is required for intestinal epithelial barrier integrity. *Am. J. Physiol. Gastrointest. Liver Physiol.* 298, G774–G783

-Schumann, M., Siegmund, B., Schulzke, J. D., & Fromm, M. (2017). Celiac Disease: Role of the Epithelial Barrier. *Cellular and Molecular Gastroenterology and Hepatology*, *3*(2), 150–162.

-Schuppan D., Junker Y., Barisani D. 2009.Celiac disease: from pathogenesis to novel therapies. Gastroenterology.137:1912–1933.

-Schuppan, D., Zimmer, K. P. 2013. The diagnosis and treatment of celiac disease. Dtsch Arztebl Int 110: 835–846.

-Sender R., Fuchs S., Milo R. 2016. Revised Estimates for the Number of Human and Bacteria Cells in the Body.PLoS Biol. 14:e1002533.

-Shahbazkhani B., Malekzadeh R., Sotoudeh M., Moghadam K.F., Farhadi M., Ansari R., *et al*. 2003. High prevalence of coeliac disease in apparently healthy Iranian blood donors. Eur J Gastroenterol Hepatol. 15:475-8.

- Shah S., Akbari M., Vanga R., Kelly C.P., Hansen J., Theethira T., *et al*. 2014. Patient perception of treatment burden is high in celiac disease compared with other common conditions. Am. J. Gastroenterol. 109:1304–1311.

-Shan L., Molberg Ø., Parrot I., Hausch F., Filiz F., Gray G.M., Sollid L.M., Khosla C. 2002. Structural basis for gluten intolerance in celiac sprue. Science. 297: 2275-2279

-Sharaiha RZ, Lebwohl B, Reimers L, Bhagat G, Green PH, Neugut AI. Increasing incidence of enteropathy-associated T-cell lymphoma in the United States, 1973-2008. Cancer 2012;118:3786-92.

-Sharma G. M., Pereira M., Williams K.M. 2015. Gluten detection in foods available in the United States–A market survey. Food chemistry. 169: 120-126.

-Sicherer S.H., Sampson H.A. 2014. Food allergy: epidemiology, pathogenesis, diagnosis, and treatment. J Allergy Clin Immunol. 133:291-307

-Silano M., Agostoni C., Guandalini S. 2010. Effect of the timing of gluten introduction on the development of celiac disease. World J Gastroenterol.16:1939–1942

-Simon-Vecsei, Z., Király R., Bagossi P., Tóth B., Dahlbom I., Caja S., Korponay-Szabó, I. R. 2012. A single conformational transglutaminase 2 epitope contributed by three domains is critical for celiac antibody binding and effects. Proceedings of the National Academy of Sciences of the United States of America. 109(2): 431–436.

-Simoons F.J. 1981. Celiac disease as a geographic problem. In Food, nutrition, and evolution, ed. D. Walcher and N. Kretchmer, 179-99. New York: Masson.

-Singh P., Arora S., Lal S. 2015. Risk of celiac disease in the first- and second-degree relatives of patients with celiac disease: a systematic review and meta-analysis. Am J Gastroenterol. 110:1539–1548.

-Sjöberg, V., Sandström, O., Hedberg, M., Hammarström, S., Hernell, O., Hammarström, M.-L. 2013. Intestinal T-cell Responses in Celiac Disease – Impact of Celiac Disease AssociatedBacteria. *PLoS ONE. 8(1), e53414*. http://doi.org/10.1371/journal.pone.0053414

-Skovbjerg H., Koch C., Anthonsen D., Sjostrom H. 2004. Deamidation and cross-linking of gliadin peptides by transglutaminases and the relation to celiac disease. Biochim. Biophys. Acta. 1690: 220–230.

- Smedby K.E., Akerman M., Hildebrand H., Glimelius B., Ekbom A., Askling J. 2005. Malignant lymphomas in coeliac disease: evidence of increased risks for lymphoma types other than enteropathy-type T cell lymphoma. Gut. 54:54–59.

-Smyth D.J., Plagnol V., Walker N.M. *et al*. 2008. Shared and distinct genetic variants in type 1 diabetes and celiac disease. N Engl J Med. 359:2767–77.

- Sollid L. M., Jabri B. 2013. Triggers and drivers of autoimmunity: lessons from coeliac disease. Nat. Rev. Immunol. 13: 294–302.

- Sollid L. M., Iversen R., Steinsbo O., Qiao S. W., Bergseng E., Dorum S., du Pre M. F., Stamnaes J., Christophersen A., Cardoso I., Hnida K., Chen X., Snir O., and Lundin K. E. 2015. Small bowel, celiac disease and adaptive immunity. Dig. Dis. 33: 115–121.

--Sollid L. M., Markussen G., Ek J., Gjerde H., Vartdal F., Thorsby E. 1989. Evidence for a primary association of celiac disease to a particular HLA-DQ α/β heterodimer. The Journal of Experimental Medicine. 169(1):345–350

-Sollid L. M., Molberg O., McAdam S., and Lundin K. E. 1997. Autoantibodies in coeliac disease: tissue transglutaminase–guilt by association? Gut 41: 851–852.

-Sollid, L.M., Qiao, S.W., Anderson, R.P., Gianfrani, C., and Koning, F. 2012. Nomenclature and listing of celiac disease relevant gluten T-cell epitopes restricted by HLA-DQ molecules. Immunogenetics. 64: 455–460.

-Sollid L. M., Thorsby E. 1990. The primary association of celiac disease to a given HLA-DQ α/β heterodimer explains the divergent HLA-DR associations observed in various Caucasian populations. Tissue Antigens. 36(3):136–137.

- Song F., Guan Z., Gienapp I.E., Shawler T., Benson J., Whitacre C.C. 2006. The thymus plays a role in oral tolerance in experimental autoimmune encephalomyelitis. J Immunol. 177(3):1500–1509.

-Sood A, Midha V, Sood N, Malhotra V. 2003. Adult celiac disease in northern India. Indian J Gastroenterol. 22:124-6.

spectrum. Gastroenterology. 2001;120:636–651

-Spencer J., MacDonald T.T., Diss T.C., Walker-Smith J.A., Ciclitira P.J., Isaacson P.G. 1989. Changes in intraepithelial lymphocyte subpopulations in coeliac disease and enteropathy associated T cell lymphoma (malignant histiocytosis of the intestine). Gut 30:339–46

-Spurkland A., Sollid L.M., Polanco I., Vartdal F., Thorsby E. 1992. HLA-DR and DQ genotypes of celiac disease patients serologically typed to be non-DR3 or non-DR5/7. Human immunology. 35: 188-192.

*-Srivastava M. 2006. Gastrointestinal mucosal immunology and mechanisms of inflammation. In Pediatric Gastrointestinal and Liver Disease (Third Edition) (pp. 85-107).

-Stadnyk A.W. 2002. Intestinal epithelial cells as a source of inflammatory cytokines and chemokines. Can J Gastroenterol. 16:241–246.

-Stamnaes J., Iversen R., du Pré M. F., Chen X., Sollid, L. M. 2015. Enhanced B-Cell Receptor Recognition of the Autoantigen Transglutaminase 2 by Efficient Catalytic Self-Multimerization. PLoSONE, 10(8):e0134922. http://doi.org/10.1371/journal.pone.0134922

-Stamnaes J., Sollid L. M. 2015. Celiac disease: Autoimmunity in response to food antigen. Semin Immunol. 27(5): 343-52.

-Stein H., Dienemann D., Sperling M., Zeitz M., Riecken E.O. 1988. Identification of a T cell lymphoma category derived from intestinal-mucosa-associated T cells. Lancet. 2:1053–54

-Stein J., Schuppan D. 2014. Coeliac Disease – New Pathophysiological Findings and Their Implications for Therapy. Viszeralmedizin. 30(3): 156–165.

–Stene L.C. *et al*. 2006. Rotavirus infection frequency and risk of celiac disease autoimmunity in early childhood: a longitudinal study. Am. J. Gastroenterol.101: 2333 – 2340.

-Stern M., Ciclitira P.J., van Eckert R., Feighery C., Janssen F.W., Mendez E., Mothes T., Troncone R., Wieser H. 2001. Analysis and clinical effects of gluten in coeliac disease. Eur J Gastroenterol Hepatol. 13:741-747

-Stevens L., Rashid M. 2008. Gluten-free and regular foods: a cost comparison. Can J Diet Pract Res. 69: 147–50.

-Stoven S., Murray J.A., Marietta E. 2012. Celiac disease: advances in treatment via gluten modification. Clin Gastroenterol Hepatol 10:859-862 Immunol. 173: 1925–1933.

-Sugai E., Vázquez H., Nachman F., Moreno M.L., Mazure R., Smecuol E., Niveloni S., Cabanne A., Kogan Z., Gómez J.C.

-Suzuki T. 2013. Regulation of intestinal epithelial permeability by tight junctions. Cell Mol Life Sci. 70:631-659

-Sverker A., Hensing G., Hallert C. 2005. “Controlled by food” — lived experiences of coeliac disease. J Hum Nutr Diet. 18:171-80

-Takai Y., Nakanishi H. 2003. Nectin and afadin: novel organizers of intercellular junctions. J Cell Sci. 116:17–27.

-Tanabe S. 2008. Analysis of food allergen structures and development of foods for allergic patients. Biosci Biotechnol Biochem. 72:649-659.

-Tavelin S. *et al*. 2003. Prediction of the oral absorption of low-permeability drugs using small intestine-like 2/4/A1 cell monolayers . Pharm. Res. 20: 397 – 405.

-Thomas H.J., Ahmad T., Rajaguru C., Barnardo M., Warren B.F., Jewell D.P. 2009. Contribution of histological, serological, and genetic factors to the clinical heterogeneity of adult-onset coeliac disease. Scand J Gastroenterol. 44: 1076-1083.

- Thomas K.E., Sapone A., Fasano A., Vogel S.N. 2006. Gliadin stimulation of murine macrophage inflammatory gene expression and intestinal permeability are MyD88-dependent: role of the innate immune response in Celiac disease. J Immunol. 176:2512-21.

-Tim Vanuytsel, Séverine Vermeire, Isabelle Cleynen. 2013. The role of Haptoglobin and its related protein, Zonulin, in inflammatory bowel disease, Tissue Barriers. 1:5, e27321.

-Tonutti E., Visentini D., Picierno A. *et al*. 2009. Diagnostic efficacy of the ELISA test for the detection of deamidated anti-gliadin peptide antibodies in the diagnosis and monitoring of celiac disease. J Clin Lab Anal. 23:165-71

-Tosco A., Salvati V.M., Auricchio R., *et al*. 2011. Natural history of potential celiac disease in children. Clin Gastroenterol Hepatol. 9:320-5.

-Tripathi A., Lammers K.M., Goldblum S. *et al*. 2009. Identification of human zonulin, a physiological modulator of tight junctions, as prehaptoglobin-2. Proc Natl Acad Sci USA. 106:16799–804.

-Troncone R., Greco L., Mayer M., Paparo F., Caputo N., Micillo M., Mugione P., Auricchio S. 1996. Latent and potential coeliac disease. Acta Paediatr Suppl. 412:10–14.

-Trynka G., Hunt K.A., Bockett N.A., Romanos J., Mistry V., Szperl A. *et al*. 2011. Dense genotyping identifies and localizes multiple common and rare variant association signals in celiac disease. Nat. Genet. 43: 1193–1201.

- Tsukita S., Furuse M., Itoh M. 2001. Multifunctional strands in tight junctions. Nat Rev Mol Cell Biol. 2:285–293.

-Tucci F., Astarita L., Abkari A., Abu-Zekry M., Attard T., Ben Hariz M., Greco L. 2014. Celiac disease in the Mediterranean area. BMC Gastroenterology. 14: 24.

-Tully M.A. 2008. Pediatric celiac disease. Gastroenterol Nurs. 31:132–140; quiz 141-142.

-Ukkola A., Maki M., Kurppa K. *et al*. 2011. Diet improves perception of health and well-being in symptomatic, but not asymptomatic, patients with celiac disease. Clin Gastroenterol Hepatol. 9 : 118–23.

-Upchurch H.F, Conway E., Patterson M.K., Birckbichler P.J.,Maxwell M.D. 1987. Cellular transglutaminase has affinity for extracellular matrix. In Vitro Cell Dev Biol 23(11): 795–800.

- van der Windt D.A. , Jellema P., Mulder C.J. *et al*. 2010. Diagnostic testing for celiac disease among patients with abdominal symptoms: a systematic review. JAMA. 303(17):1738-46. doi: 10.1001/jama.2010.549.

- van de Water J.M., Cillessen S.A., Visser O.J. *et al*. 2010. Enteropathy associated T-cell lymphoma and its precursor lesions. Best Pract Res Clin Gastroenterol.; 24:43–56.

- Vanuytsel T., Vermeire S., Cleynen I. 2013. The role of Haptoglobin and its related protein, Zonulin, in inflammatory bowel disease. Tissue Barriers. 1:e27321

*-Votavova P., Tomala J., Kovar M. 2014. Increasing the biological activity of IL-2 and IL-15 through complexing with anti-IL-2 mAbs and IL-15Rα-Fc chimera. Immunology Letters, 159 (1-2): 1-10.

- Volta U., Villanacci V. 2011. "Celiac disease: diagnostic criteria in progress,"Cellular and Molecular Immunology. 8(2):96–102.

- Volta U., Tovoli F., Caio G. 2011. "Clinical and immunological features of celiac disease in patients with Type 1 diabetes mellitus," Expert Review of Gastroenterology and Hepatology. 5(40): 479–487.

-Vader L.W., de Ru A., van der Wal Y., Kooy Y.M., Benckhuijsen W., Mearin M.L. *et al*. 2002. Specificity of tissue transglutaminase explains cereal toxicity in celiac disease. J Exp Med. 195(5):643–9.

-van Berge-Henegouwen G.P., Mulder C.J. 1993. Pioneer in the gluten free diet: Willem-Karel Dicke 1905-1962, over 50 years of gluten free diet. Gut. 34(11):1473-1475.

-Van Bergen J., Mulder C.J., Mearin M.L., Koning F. 2015. Local communication among mucosal immune cells in patients with celiac disease Gastroenterology.148 (6) : pp. 1187-1194.

-Van de Kamer J., Weijers H., Dicke W. 1953. Coeliac disease V. Some experiments on the cause of the harmful effect of wheat gliadin. Acta Paediatr. Scand. 42, 223–231.

-van de Wal Y., Kooy Y., van Veelen P., Pena S., Mearin L., Papadopoulos G., Koning F .1998. Selective deamidation by tissue transglutaminase strongly enhances gliadin-specific T cell reactivity. J Immunol 161:1585–1588.

-van der Flier L.G., Clevers H. 2009. Stem cells, self-renewal, and differentiation in the intestinal epithelium.Annu Rev Physiol. 71:241–260.

-van der Meer J.B. 1969. Granular deposits of immunoglobulins in the skin of patients with dermatitis herpetiformis. An immunofluorescent study. Br J Dermatol. 81:493-503

-van der Windt D.A., Jellema P., Mulder C.J. *et al*. 2010. Diagnostic testing for celiac disease among patients with abdominal symptoms: a systematic review . JAMA. 303: 1738 – 46.

-van Overbeek F.M., Uil-Dieterman I.G., Mol I.W., Köhler-Brands L., Heymans H.S., Mulder C.J. 1997. The daily gluten intake in relatives of patients with coeliac disease compared with that of the general Dutch population. Eur J Gastroenterol Hepatol. 9:1097–1099.

-Vanheel H., Vicario M., Vanuytsel T., Van Oudenhove L., Martinez C., Keita A.V., Pardon N., Santos J., Soderholm J.D., Tack J., Farre R. 2013. Impaired duodenal mucosal integrity and low-grade inflammation in functional dyspepsia. Gut. 63(2):262-71. doi: 10.1136/gutjnl-2012-303857.

-Vicari A. P., Schoepfer A. M., Meresse B., Goffin L., Léger O., Josserand S., Chvatchko, Y. 2017. Discovery and characterization of a novel humanized anti-IL-15 antibody and its relevance for the treatment of refractory celiac disease and eosinophilic esophagitis. mAbs, 9(6): 927–944.

Villalta D., Alessio M.G., Tampoia M. *et al*. 2007. Testing for IgG class antibodies in celiac disease patients with selective IgA deficiency. A comparison of the diagnostic accuracy of 9 IgG anti-tissue transglutaminase, 1 IgG anti-gliadin and 1 IgG anti-deaminated gliadin peptide antibody assays . Clin Chim Acta. 382 : 95 – 9 .

Villalta D., Tonutti E., Prause C., *et al*. 2010. IgG antibodies against deamidated gliadin peptides for diagnosis of celiac disease in patients with IgA deficiency. Clin Chem. 56: 464–468.

-Villanacci V., Ceppa P., Tavani E., Vindigni C., Volta U. 2011. Coeliac disease: the histology report. Dig Liver Dis. 43 Suppl 4:S385–S395.

-Volta U., Villanacci V. 2011. Celiac disease: diagnostic criteria in progress. Cell Mol Immunol. 8:96–102.

-Wacklin P., Laurikka P., Lindfors K., Collin P., Salmi T., Lähdeaho M.L., Saavalainen P., Mäki M., Mättö J., Kurppa K., Kaukinen K. 2014. Altered duodenal microbiota composition in celiac disease patients suffering from persistent symptoms on a long-term gluten-free diet. Am J Gastroenterol. 109: 1933–1941.

-Wada M., Tamura A., Takahashi N., Tsukita S. 2013. Loss of claudins 2 and 15 from mice causes defects in paracellular Na + flow and nutrient transport in gut and leads to death from malnutrition.Gastroenterology. 144: 369–380.

-Walcher, Dwain N., Kretchmer, Norman. 1981. Food, nutrition, and evolution: food as an environmental factor in the genesis of human variability. International Organization for the Study of Human Development (USA) [Corporate Author].

-Waldmann T.A.2004. Targeting the interleukin-15/interleukin-15 receptor system in inflammatory autoimmune diseases. Arthritis Res Ther. 6: 174–7.

-Waldmann, T. A. 2006. The biology of interleukin-2 and interleukin-15: implications for cancer therapy and vaccine design. Nat Rev Immunol. 6:595–601

-Wang W., Uzzau S., Goldblum S.E., Fasano A. 2000. Human zonulin, a potential modulator of intestinal tight junctions.J Cell Sci.113(Pt 24):4435–4440.

-Wapenaar M.C., Monsuur A.J., van Bodegraven A.A., Weersma R.K., Bevova M.R., Linskens R.K., Howdle P., Holmes G., Mulder C.J., Dijkstra G., van Heel D.A., Wijmenga C. 2008. Associations with tight junction genes

PARD3 and MAGI2 in Dutch patients point to a common barrier defect for coeliac disease and ulcerative colitis. Gut.57:463–467.

-WeberC. R.., Turner J. R. 2017. Dynamic modeling of the tight junction pore pathway. Annals of the New York Academy of Sciences. 1397(1): 209–218.

-Weiner H.L., da Cunha A.P., Quintana F., Wu H. 2011. Oral tolerance. Immunol. Rev. 241 : 241–59.

-Werkstetter K.J., Korponay-Szabó I.R., Popp A., Villanacci V., Salemme M., Heilig G., Lillevang S.T., Mearin M.L., Ribes-Koninckx C., Thomas A. *et al*. 2017. Accuracy in Diagnosis of Celiac Disease Without Biopsies in Clinical Practice. Gastroenterology; 153(4):924-935.

-West J., Fleming K. M., Tata L. J., Card T. R., Crooks C. J. 2014. Incidence and Prevalence of Celiac Disease and Dermatitis Herpetiformis in the UK Over Two Decades: Population-Based Study. The American journal of Gastroenterology. 109(5): 757-768.

-Wolters V.M., Wijmenga C. 2008. Genetic background of celiac disease and its clinical implications. Am JGastroenterol. 103:190–195.

-Worbs T. *et al*. 2006. Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. J. Exp. Med. 203:519 – 527.

- Wouters J., Weijerman M.E., van Furth A.M. *et al*. 2009. "Prospective human leukocyte antigen, endomysium immunoglobulin antibodies, and transglutaminase antibodies testing for celiac disease in children with Down syndrome," Journal of Pediatrics. 154 (2): 239–242.

-Yan D., Holt P.R. Willem Dicke. 2009. Brilliant clinical observer and translational investigator. Discoverer of the toxic cause of celiac disease. Clin Transl Sci. 2:446–8.

-Yang H., Antony P.A., Wildhaber B.E., Teitelbaum D.H. 2004. Intestinal intraepithelial lymphocyte gamma delta-T cell-derived keratinocyte growth factor modulates epithelial growth in the mouse. J Immunol. 172(7):4151-8

-Yantiss R.K., Odze R.D. 2009. Optimal approach to obtaining mucosal biopsies for assessment of inflammatory disorders of the gastrointestinal tract. Am J Gastroenterol. 104: 774 – 83.

-Zeissig S., Blumberg R.S. 2014. Life at the beginning: Perturbation of the microbiota by antibiotics in early life and its role in health and disease.Nat. Immunol.15: 307–310.

-Zemskov E.A., Mikhailenko I., Hsia R.C., Zaritskaya L., Belkin A.M. 2011. Unconventional secretion of tissue transglutaminase involves phospholipid-dependent delivery into recycling endosomes. PLoS ONE. 6 (4):e19414.

-Zhernakova A., van Diemen C.C., Wijmenga C.2009. Detecting shared pathogenesis from the shared genetics of immune-related diseases. Nature Reviews Genetics. 10 (1):43–55.

-Zihni C., Mills C., Matter K., Balda M.S. 2016. Tight junctions: From simple barriers to multifunctional molecular gates. Nat. Rev. Mol. Cell Biol.17:564–580.

-Zintzaras E., Germenis A.E. 2006. Performance of antibodies against tissue transglutaminase for the diagnosis of celiac disease: meta-analysis. Clin Vaccine Immunol. 13:187-92.

-Zone J.J., Meyer L.J., Petersen M.J. 1996. Deposition of granular IgA relative to clinical lesions in dermatitis herpetiformis. Arch Dermatol. 132 :912-18.

-Zone J.J. 2005. Skin manifestations of celiac disease. Gastroenterology

-Zone J.J., Schmidt L.A., Taylor T.B., Hull C.M., Sotiriou M.C., Jaskowski T.D., Hill H.R., and Meyer L.J. 2011. Dermatitis herpetiformissera or goat anti-transglutaminase-3 transferred to human skin-grafted mice mimics dermatitis herpetiformis immunopathology. J. Immunol.186: 4474–4480.

الخلاصة:

مرض السيلياك (الزلاقي) هو اعتلال معوي مزمن مناعي مع مجموعة واسعة ومتنوعة من الأعراض الشديدة ، التي يسببها الغلوتين الغذائي الموجود في القمح والشعير والشوفان في الأشخاص الذين لديهم استعداد وراثيا من جميع الأعمار. مرض السيلياك يعتبرنمط مرض فريد لدراسة التسبب في اضطرابات المناعة الذاتية لدى البشر. وبصرف النظر عن البناء الوراثي وإثارة العوامل البيئية ، فإن العنصر الرئيسي الثالث في تسبب المرض هو زيادة نفاذية الأمعاء. الهدف من هذه الدراسة هو النظر في دور بروتين الزونولين كمؤشر حيوي لاضطراب نفاذية الامعاء والانترلوكين15 (IL15) كعلامة تنبؤية لعملية التهاب الأمعاء الدقيقة عند مرضى السيلياك في الشعب العراقي حيث أن كلتا العلامتين تتم إعادة تنظيمهما في مراحل مختلفة من المرض. الطريقة: تم إجراء دراسة لاختبار 108 من مرضى السيلياك من مجموعات عمرية مختلفة ومراحل مختلفة من المرض عن طريق قياس مستوى المصل من العلامتين باستخدام تقنية ELISA ومقارنتها بـ 118 من الاشخاص السليمين الملائمين للعمر والجنس. النتائج: كان لدى غالبية مرضى السيلياك الذين يعانون من مراحل مختلفة من المرض تركيز أعلى ل بروتين الزونولين في مصل الدم حيث لم يكن هناك قيود كافية على الغلوتين من النظام الغذائي ، في حين لم يكن هناك أي مستوى قابل للكشف للانترلوكين 15 في مصل المرضى باستثناء نسبة صغيرة (16 مريض) وهي نتيجه قد تدل على التاثير الموضعي لهذا السايتوكين . لكن هذه الدراسة توضح أن هناك فروق ذات دلالة إحصائية في مستوى الزونولين في المصل ومستويات IL15بين مرضى السيلياك والمضاهين لهم في العمر والجنس من الاشخاص السليمين ($p < 0.0001$ للزونولين و $p<0.001$ للانترلوكين 15). ان ارتفاع مستويات بروتين الزونولين عند الغالبية يوضح مدى معاناة المرضى في اتباع الحميه الغذائيه من الغلوتين (GFD)، وقد يوفر بعض المنطق للبحث عن بدائل غير غذائية. كما تدعو هذه المراجعة إلى توسيع معرفتنا الحالية حول IL15 لكسب المزيد من الأفكار حول الإجراءات الحيوية لهذا السيتوكين في CD وغيرها اضطرابات المناعة الذاتية على مستوى سكان بلدنا.

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء

كلية الطب

فرع الأحياء المجهرية

دراسة مستوى الدم لبروتين الزونيولين والانترلوكين (15) عند المرضى المصابين بداء الزلاقي

رسالة مقدمة الى

كلية الطب ولجنة الدراسات العليا / جامعة كربلاء وهي جزء من متطلبات نيل درجة الماجستير في علم الاحياء المجهرية

من قبل:

اسراء حسين هجوج الجراح

بكلوريوس طب وجراحة عامة

اشراف:

أستاذ مساعد

د . آلاء سعد العتابي

2019 ميلادية 1440 هجرية