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Biochemical markers in celiac disease

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1. ABSTRACT

Celiac Disease is a worldwide spread condition affecting 1:100-1:200 individuals. It is a permanent food intolerance to ingested gluten in genetically predisposed subjects. In this review we analyze the biochemical markers of the disease going from laboratory findings to histology passing through genetics. Gluten intolerance is a unique model of autoimmune disease in which we can recognize the main environmental factor (gluten) and the more complex genetic background. In additional way, serological markers for monitoring the disease and a safe and effective therapy (gluten free diet) are also available. In deed the environmental factor such as gluten intake is necessary to trigger the disease but genetics also matter. HLA genes are the most studied but in recent times also not HLA related genes are giving proof of additional relative risk to disease if present. From histological point of view intra epithelial cell infiltration by several lymphocyte subsets is becoming more and more important also for understanding pathogenesis of the disease.

2. INTRODUCTION

Celiac Disease (CD) is a permanent gluten triggered enteropathy in genetically susceptible individuals. Diagnosis of disease may be simple in overt classical forms, more challenging and difficult in atypical ones. Up to date several tools concur to perform a combined diagnosis. In deed clinicians have serological, genetic and histological means that embedded to clinical features may be helpful to diagnose CD. From a biochemical point of view each of the above mentioned diagnostic items has biological and chemical markers.

3. CLINICAL BIOCHEMISTRY

The total blood cell count may evidence increased number of platelets at onset of disease. Thrombocytosis is generally light to moderate with values ranging between 450,000 and 650,000. This finding is absolutely independent from the more well known and more well recognized iron deficiency anaemia (1). Iron deficiency

anaemia in active celiac disease is sustained by damage of distal duodenum with consecutive impaired iron absorption. For this reason this kind of anaemia is refractory to oral iron supplementation. Iron deposits may be reduced. Total iron binding capacity is increased.

4. SEROLOGY

Serology changed the diagnostic criteria for celiac disease after 1991 (2). The first antibodies detected in the serum of celiac patients were AGA, *id est* Anti-Gliadin Antibodies. They were used from 1983 and performed with ELISA method. They belong to IgA or IgG class of circulating immunoglobulines. Up to date they are almost completely replaced by anti human tissue trans-glutaminase antibodies (hTTG). Only in Pediatrics, in the younger children lesser than two years of age, AGA maintain a good sensibility and specificity in spite of the more recent ones anti hTTG.

Recent studies evaluated a new generation class of Anti Gliadin Antibodies against deamidated gliadin peptide (dgp) in monitoring children on gluten free diet (GFD). Their serological increase may precede than of anti hTTG if gluten is ingested (3). Anti Endomysium Antibodies (AEA) were introduced in the early 90's. They are performed by ELISA or, in the most of cases, by Indirect Immuno-Fluorescence (IIF) method. They have both sensitivity and specificity higher than 95% and near to 100%. First generation of AEA used monkey esophagus as biological substrate, while the modern ones use Human Umbilical cord. This kind of method strongly depends on performer experience if executed by IIF. ELISA is more reliable and reproducible. All the two possible methods give equivalent results provided that IIF AEA were correctly understood. The best and the more recent serological marker for detecting and monitoring CD is the assay of anti tissue transglutaminase antibodies. Discovered in 1997 by Dieterich and coll. (4), its use was largely widespread in all screening practices. Human transglutaminase is an ubiquitous enzyme which catalyses the cross linking transamination from a NH_2 containing donor peptide to an acceptor one. Antibodies to TTG are produced by B lymphocytes of the intestinal mucosa during active disease as attempt to block the above mentioned biochemical reaction responsible of magnifying T cell response against gluten peptides. Serological class of antibodies directed against hTTG, AEA and AGA are IgA or IgG ones. IgG class antibodies may be useful in IgA deficient CD affected patients. In the last years human recombinant TTG has replaced the elder one from guinea pig. IgA anti TTG are the highest specific and sensitive class of serological markers for CD diagnosis which has to be confirmed by duodenal biopsy (5, 6). Recently, studies have been set out to detect new serological markers but both anti actin antibodies and anti dgp have little to offer in screening for CD compared to anti transglutaminase assay (7-9).

5. GENETICS

Genetics in CD is a more and more widely studied standpoint. The first evidence of gene linkage in CD consists of HLA region on chromosome 6 (CELIAC 1). In HLA identical siblings concordance rate of CD is around 35-40% suggesting importance of genes located outside HLA in disease susceptibility. HLA linkage of presentation in CD and also in type 1 diabetes and rheumatic diseases is well known since several decades. Probably it is related to strong linkage disequilibrium in HLA region proved by wide diffusion among healthy people (10, 11). In most populations over 90% of CD patients carry the DQ2 heterodimer, that is encoded by alleles DQA1*0501, DQB1*0201 (12). Almost the remaining CD patients DQ2 negative carry DQ8 aplotype consisting of allelic combination DQA1*0301, DQB1*0302. Only a small amount of CD patients is both DQ2 and DQ8 negative (13, 14).

Indeed these aplotypes are necessary for the disease onset but they cannot explain the whole genetic susceptibility. The evident difference in concordance between monozygotic and dizygotic twins is supported by existence of non-HLA related genes. In fact monozygotic twins sharing the whole human genome have the same relative risk (odd ratio) of getting ill. The concordance probability of only 70-80% maybe explained by differences in dietary gluten intake. Non-HLA genes also matter in determining predisposition to develop CD (15, 16). An higher significant linkage than the one of well known 5q31-32 (CELIAC 2) was present at 19p13.1 (CELIAC 4) in genome wide screen Dutch study (17). This MYO9B gene encodes a cytoskeleton protein involved in tight junction assembly and it may be responsible in increasing permeability of intestinal barrier (18). Other potential candidate gene linked to chromosome 19 (19p13.2) includes ICAM-1 precursor related to cell recruitment, adhesion and inflammation. Cytotoxic T-lymphocyte-associated protein (CTLA4) located on 2q33 (CELIAC 3) plays an important role in maintaining tolerance to self-antigens, both as a negative regulator of T-cell proliferation through the co-stimulating signal and as an inducer of clonal anergy (19-21).

Many reports indicate the involvement of CTLA4 in CD and also more recently in IBD pathogenesis (22). On the other hand a single report on Finnish population revealed in a genome-wide scan study an association between a region on chromosome 15q11-q13 (CELIAC 5) and celiac disease (23). The mapping of genome shows a strong association of the 4q27 (CELIAC 6) region with CD. This region contains IL-2 and IL-21 genes, cytokines involved in T-cell activation (24-26). Another significant genetic locus is located on the 1q31 region (CELIAC 7) encoding RGS1 (regulator of G-protein signalling 1), which is widely expressed in the intestinal intra-epithelial lymphocyte compartment (26-28). The Linkage Disequilibrium block contains two genes mapped on 2q11-2q12 (CELIAC 8) which are receptors for the IL-18 protein involved in the gamma-interferon synthesis by T-cell (26, 29). Mature IL-18 is expressed in the intestinal mucosa of active, treated and latent celiac patients but not in healthy ones. CELIAC 9 gene (3p21) encodes several chemokines which are critical for cellular recruitment and inflammation pathway. The 3q25-3q26 region (CELIAC 10) encodes the

Table 1. Genes involved in CD predisposition

Name	Locus	Product	Function
CELIAC 1	6p21-23	DQ2/DQ8	HLA II Heterodymer
CELIAC 2	5q31-32	???	???
CELIAC 3	2q33	CTLA 4	Maintaing tolerance
CELIAC 4	19p13.1-13.2	MYO9B/ICAM-1	Cytoskeletal Myosin /Adhesion molecule
CELIAC 5	15q 11-13	???	Role in Prader Willi-Angelman ???
CELIAC 6	4q27	IL 2 - IL 21	T cell Activation
CELIAC 7	1q31	RGS1	IEL
CELIAC 8	2q11-2q12	IL 18	Gamma IFN
CELIAC 9	3p21	Chemokines	Cellular recruitment
CELIAC 10	3q25-26	IL 12	Th response; Gamma IFN
CELIAC 11	3q28	???	???
CELIAC 12	6q25	???	???
CELIAC 13	12q24	SH2B3	Leucocyte recruitment and activation

IL-12, that has an important role in T-helper response. Relatively little is known about CELIAC 11 (3q28) and CELIAC 12 (6q25) and about their immunological role. SH2B3 (lymphocyte adaptor protein), encoded by 12q24 (CELIAC 13), is strongly expressed in the small bowel and involved in leukocyte recruitment and activation (26). CD, type 1 diabetes and rheumatoid arthritis sharing several genetic factors regulating innate and adaptive immunity are probably also able to trigger immunological activation against environmental factors.

6. HISTOLOGY

Histological damage in CD consists of three different periods. The infiltration phase characterized by intra-epithelial lymphocytes infiltration, the hyperplastic phase with decrease of villous/crypt ratio and the destructive one consisting of villous atrophy and mucosal flattening. Up to date definitive diagnosis of CD rests upon histological findings of increased intra-epithelial lymphocytes of the small bowel associated or not to decrease of villous height-crypt depth (Vh/CrD) ratio. Crypt hyperplasia is the result of over expressed bowel cellular mitosis (30). Indeed immature enterocytes make an attempt to replace mature ones destroyed by immunological process. Immature epitheliocytes are lacking of lactase giving proof of secondary lactase deficiency typical of CD.

CD related histological lesions are present in the duodenal mucosa, but sometimes the bulb is the only site affected. The immunological process in CD is mediate by cytokines unbalance between pro-inflammatory chemokines (IFN-gamma, TNF-alpha, IL-12, IL-15) and anti-inflammatory ones (IL-10 and TGF-beta) (31). The IL-15, produced by enterocytes, promotes autoimmunity by arming cytotoxic T-lymphocytes to cause tissue destruction in CD. Intraepithelial T-lymphocytes CD8+ interplay with enterocytes by MHC I interaction (32). In fact epithelial cells behave like APC, presenting gliadin peptides to CD8+ lymphocytes in a way resembling that of heat shock proteins (33-37). In the *lamina propria* CD4 positive cells play a major role in determining damage, in particular a subset CD4+ CD25+ expresses FOXP3, that encodes a transcription factor essential for the development and function of T-regulatory cells (T-regs). T-regs are the main population involved in maintaining peripheral tolerance. The FOXP3 gene may play a role in commitment of Th17 cells, which are involved in pathogenesis of other autoimmune disorders and IBD (38-41). Total intraepithelial lymphocytes infiltration maybe detected in early phases of CD by immune-histochemical staining of CD3+ cells.

7. CONCLUSIONS

CD is a complex and multi-factorial disease in which environmental factors and genetic susceptibility are embedded. Several biochemical markers are available to detect CD and some of them (anti-TTG, AEA, AGA) are also useful to follow-up the disease. In the last years incidence of CD was world-wide increased: latest studies report incidence 1:100 in open population.HLA molecule know as DQ2 is very frequent in normal population, probably for evolutionary reasons. Indeed HLA susceptibility genes conferred in ancient times or in pre-antibiotic era a sort of protection against infectious diseases. This was responsible of positive selection of HLA-DQ2 carriers versus DQ2 negative subjects which were negatively selected by intractable infectious diarrhea. DQ2 carriers affected by mild enteropathy on a diet regimen based on foodstuffs derived by early grains containing small amount of gluten were protected by infectious enteritis. Nowadays DQ2 carriers have a rich gluten containing diet due to the fact that modern cultivars of grains are esaploid and wheaten flour contains up to 50% of gluten derived proteins. With the defeat of infectious diseases the positive selected DQ2 carriers have an increased probability of become gluten-intolerants. In this context non-HLA genes increase the relative risk of CD in gluten eating people.

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Abbreviations: CD: Celiac Disease, AGA: Anti Gliadin Antibodies, hTTG: human Tissue TransGlutaminase, dgp: deamidated gliadin peptide, GFD: gluten free diet, AEA: Anti Endomysium Antibodies, IIF: Indirect Immuno-Fluorescence, CTLA4: Cytotoxic T-lymphocyte-associated protein, IBD: Inflammatory Bowel Disease, IFN-gamma: Interferon-gamma, TNF-alpha: Tumor Necrosis alpha, TGF-beta: Transforming Growth Factor beta, APC: Antigen Presenting Cell.

Key Words: Celiac Disease, Gluten Enteropathy, Genetics of Celiac Disease, Celiac Disease Serology, Celiac Disease Histology, Celiac Disease Biomarkers, Review

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