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Coimbra, 18 de Julho de 2014.

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Faculdade de Farmácia da Universidade de Coimbra

Monografia
"Vacinação contra a Hepatite B na Doença Celíaca"

Ano Letivo 2013/2014

Professora Responsável

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Agradecimentos

Começo por agradecer à minha orientadora, a Professora Olga Borges, por todo o apoio e assistência prestados ao longo da elaboração desta monografia.

Com o culminar deste percurso, tenho também que agradecer a todos aqueles que fizeram da minha estadia em Coimbra um período absolutamente incrível: aos amigos de infância, presentes desde sempre e para sempre; aos amigos da faculdade, companheiros de trabalho e aventuras académicas; aos amigos dessa magnífica instituição que é a Tuna Académica da Universidade de Coimbra, que me providenciou não só uma oportunidade de continuar envolvida na música mas também de fazer a minha parte pela histórica Academia de Coimbra.

Um muito obrigado ainda ao Fred, por me ouvir impreterivelmente e ser tão terrivelmente encantador.

E por fim, o maior agradecimento de todos: aos meus pais, que sempre me apoiaram incondicionalmente em tudo o que me propus a fazer e me moldaram na pessoa que hoje sou.

Coimbra foi, sem dúvida, uma lição.

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Abstract

Celiac disease is a common gastrointestinal auto-immune disease associated with the ingestion of gluten. It is strongly associated with specific human leukocyte antigen haplotypes that happen to be also linked with hepatitis B vaccine nonresponse. Studies support that amongst celiac patients hepatitis B vaccine response rates are lower when compared do healthy controls. Although multiple hypothesis have been suggested, such as gluten intake at time of vaccination or genetic predisposition, the phenomena is yet to be clearly explained. Further studies, with larger subject populations and stricter study design, are in order.

Resumo

A doença celíaca é uma patologia do trato gastrointestinal do foro autoimune ligada à ingestão de glúten. Está associada a determinados haplótipos do gene leucocitário humano, os mesmos que se crê estarem também ligados à ausência de resposta à vacina da hepatite B. Estudos mostram que na população celíaca as taxas de resposta à vacina da hepatite B são inferiores às demonstradas por controlos saudáveis. Ainda que várias hipóteses tenham sido sugeridas, como a ingestão de glúten concomitante com a vacinação ou predisposição genética, o fenómeno ainda não se encontra claramente explicado. São necessários mais estudos, executados em populações maiores e com *design* mais rigoroso.

Abbreviations

Anti-HBcAg IgG – Specific Hepatitis B Core Antigen Immunoglobulin G

APC – Antigen Presenting Cells

CD – Celiac Disease

CDP – Celiac Disease Patients

GFD – Gluten-Free Diet

HBsAb – Hepatitis B Surface Antibodies

HBsAg – Hepatitis B Surface Antigen

HBV – Hepatitis B Virus

HLA - Human Leukocyte Antigen

ID – Intradermal

IgA - Immunoglobulin A

IgG – Immunoglobulin G

IL-15 – Interleukin 15

IM – Intramuscular

tTG – Tissue transglutaminase

WHO – World Health Organization

Introduction

Celiac Disease

Celiac disease (CD) is an immunological disease triggered by the ingestion of gluten-containing foods. Its prevalence in Europe and the United States is estimated around 1 in 120-300 amongst the general population (PARK *et al.*, 2007). The illness is the result of an abnormal T-cell mediated response against components of the gluten protein. This immunologic response produces an inflammatory response and causes damage to small intestine mucosa, resulting in loss of absorptive villi and consequently, when the lesions are extensive, malabsorption of macro and micronutrients. Normally the disease presents itself during infancy and manifests as diarrhoea, abdominal distension, and occasionally severe malnutrition. Since these symptoms are not always present, the diagnosis might be delayed until adulthood. In adults, gastrointestinal symptoms include diarrhoea, constipation, bloating, flatus, or belching (PORTH, 2004).

The diagnosis of celiac disease is based on clinical manifestations and confirmed by intestinal biopsy and serological markers (tissue transglutaminase autoantibodies and antiendomysial antibodies (immunoglobulins A (IgA) and G (IgG)) are the most accurate, being fairly specific to CD). The treatment mostly consists in excluding gluten and all related proteins from the diet, that is, in a gluten-free diet (GFD). Complete elimination of dietary gluten generally results in the disappearance of the autoantibodies and consequently complete healing of the mucosa. Untreated CD is associated with long-term complications such as increased risk of oesophageal carcinoma and enteropathy-type T-cell lymphoma, as with the appearance of colitis and gastritis (ANTONIOLI, 2003; PORTH, 2004).

The causes of CD are both genetic and environmental, making it a multifactorial disease. From a genetic point of view, it is known that more than 95 % of celiac patients share at least one of Human Leukocyte Antigen (HLA) class II molecules HLA DQ2, DR3, and B8 haplotypes (ERTEM *et al.*, 2010), with the presence of DQ2 in CD patients estimated around 90%. Although DQ2 and DQ8 can also be expressed by healthy individuals (FELDMAN, FRIEDMAN and BRANDT, 2010), these molecules are strongly associated with celiac pathogenesis: gluten-derived peptides such as gliadin, a set of large-sized peptides resistant to proteolysis, bind to DQ2, or DQ8 in DQ2 negative patients, after deamidation by the enzyme tissue transglutaminase (tTG), on the surface of antigen-presenting cells (APC). Deaminated residues bind more robustly to DQ2 (given that the highest affinity sites of gluten peptides are derived by deamidation), marking a stronger immune response. These

complexes are then recognized by gluten-specific T-cells, which will activate, proceed to invade small intestine lamina propria and initiate the inflammatory process (figure 1). The autoantigen recognized by disease specific antibodies (antiendomysial antibodies, used as serological markers) is, in fact, tTG. The local release of the interleukin 15 (IL-15), a pro-inflammatory cytokine, also has a role in pathogenesis: it contributes not only to induce epithelial cell apoptosis but also alters lymphocyte turnover. This fact may explain why not everyone carrying DQ2 and/or DQ8 develops celiac disease: dysregulated IL-15 expression may also be needed to support the development of T_H1 immunity to dietary gluten. (VAN BELZEN *et al.*, 2004; CAJA *et al.*, 2011; DEPAOLO *et al.*, 2011; VAN HEEL and WEST, 2006; LONDEI, QUARATINO and MAIURI, 2003).

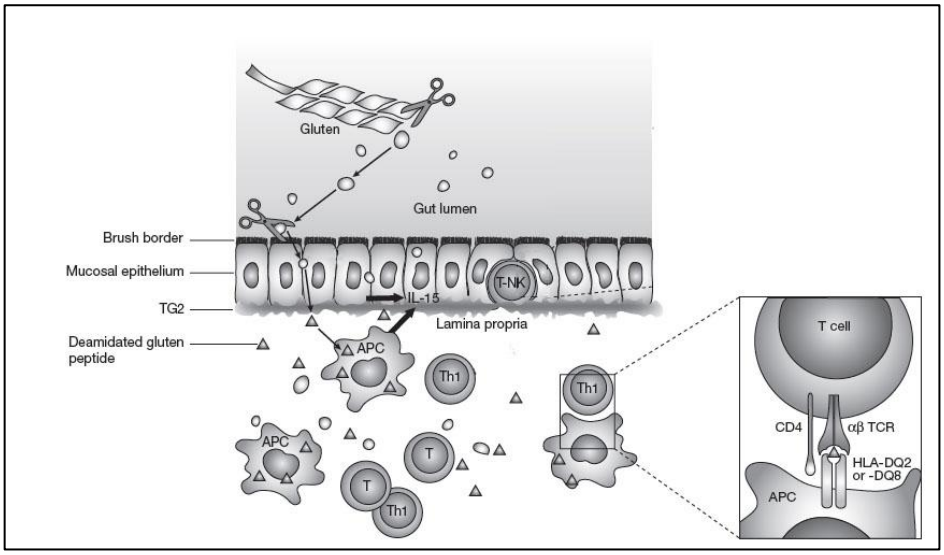

Figure 1: Celiac disease pathogenesis.

Gluten-derived peptides are transported across the epithelium and deamidated by tTG. T-cells recognize the deaminated peptides, presented by HLA-DQ2 or HLA-DQ8 molecules at the cell surface of APC. Locally produced IL-15 (either by enterocytes or lamina propria cells) stimulates T-cells to migrate to the epithelium. Adapted from SOLLID and KHOSLA (2005)

Celiac Disease and Hepatitis B Vaccination

Hepatitis B virus (HBV) infection is a major public health issue, affecting more than 350 million people worldwide, causing acute and chronic liver disease, cirrhosis, and hepatocellular carcinoma. The leading strategy for infection and viral transmission control is vaccination (NEMES *et al.*, 2008). There has been a safe and effective hepatitis B vaccine available since 1982. The first vaccines were plasma-derived, containing purified hepatitis B surface antigen (HBsAg) obtained from plasma of patients infected with HBV. In the following years, recombinant purified HBsAg was developed (obtained from genetically

engineered cells) and it is now largely used. Usually a sole course of three doses of HBV vaccine is administered, through one of several different schedules in use. Successfully vaccinated people who have lost antibodies after primary vaccination normally demonstrate rapid anamnestic responses when boosted, meaning HBsAg immunological memory can outlast antibody detection. Hence, consensually, no booster doses are needed to ensure long-term protection in immunocompetent patients (VITALITI *et al.*, 2013; ZANETTI *et al.*, 2004). Mass immunization has been recommended by the World Health Organization (WHO) since 1992 (WORLD HEALTH ORGANISATION, 2014).

Specific antibody levels ≥10 IU/L are considered as protective against HBV infection. Approximately 4 % to 10 % of healthy immunocompetent individuals fail to originate protecting levels of antibodies to recombinant HBsAg after completing the standard HBV vaccination schedule. Nongenetic factors such as age, obesity, smoking, drug abuse, alcoholism, infections, immunosuppression, or route of vaccination are associated with nonresponsiveness (NEMES *et al.*, 2008). On the other hand, HLA phenotype is considered the most important genetic marker associated with nonresponse, with nonresponders often carrying specific HLA haplotypes including B8, DR3 and DQ2 (PARK *et al.*, 2007). Studies have in fact demonstrated a genetic predisposition to HBV vaccine nonresponse. A study published in the nineties (STACHOWSKI *et al.*, 1995), for example, reported that homozygotes for HLA-A1, HLA-B8, HLA-DR3 and HLA-DQ2 were found almost exclusively in the non-responder group and that even heterozygotes for these alleles were found more frequently in the non-responder group when compared to the responders. Similar results were obtained by other research groups (ALPER, KRUSKALL and MARCUS-BAGLEY, 1989), namely a lower titer of produced antibodies within homozygotes for MHC haplotypes HLA-B8 and DR3 when compared to heterozygotes. In a more recent work (MARTINETTI *et al.*, 2000), HLA-DQ2 was associated with faulty immune response to HBV, with 40,6% of true nonresponders (<10 mIU/ml) carrying the allele. The authors defend that being homozygous or heterozygote for the gene determines the individual's level of responsiveness.

Since these same haplotypes are also overrepresented in patients with CD, it's hypothesized that they are less able to respond to HBV vaccine than the general population, in which the presence of HLA haplotypes B8, DR3 and DQ2 is much more uncommon (PARK *et al.*, 2007). HLA-DQ2 may predispose CD patients to fail in developing immunity after HBV vaccination through a T_H2 response which will be inadequate for B-cell differentiation and subsequently memory B cells formation (ZINGONE *et al.*, 2013). It has also been postulated that gluten intake at the time of vaccination may influence the immune

response to HBV vaccine (NEMES *et al.*, 2008). Gluten may be implicated because both HBsAg protein fragments and gliadin peptides bind to HLA-DQ2 molecules and induce proliferation of T lymphocytes. Competition between proteins might originate defective anti-HBsAg antibody production (ZINGONE *et al.*, 2013).

Studies have indeed showed that among celiac patients the rate of non response to hepatitis B vaccination is higher than within the healthy population (AHISHALI *et al.*, 2008; LEONARDI *et al.*, 2009; NOH, POLAND and MURRAY, 2003; PARK *et al.*, 2007), although the causes remain somewhat uncertain. This marks a significant public health problem, given the high prevalence of CD and the serious consequences inherent to an increase in HBV infection rates.

Decreased response to Hepatitis B vaccination in celiacs

The relationship between nonresponse to HBV vaccination and celiac disease has not been the subject matter of many studies, and additional investigation is required to obtain solider information.

Most of the works related to the topic share common traits.

The subjects were always CD patients, mostly children, usually recruited from hospital gastroenterology and paediatrics departments or celiac disease centres. Common exclusion factors included other immune diseases besides CD, chronic illnesses and previous HBV infection.

CD diagnosis, had it been done previously to the study or reconfirmed after enrolment, was based in abnormal serological markers, such as anti-gliadin antibodies, tTG and anti-endomysial IgA antibodies, and typical intestinal histopathological findings of CD, including small intestine villous atrophy and increased numbers of intraepithelial lymphocytes (ERTEM *et al.*, 2010; LEONARDI *et al.*, 2009; PARK *et al.*, 2007).

Since most of the studies reviewed were done retrospectively (the subjects had been vaccinated against HBV in the past), it was regularly demanded that patients had been fully vaccinated against HBV at least six months before study enrolment (ERTEKIN, TOSUN and SELIMOGLU, 2011; LEONARDI *et al.*, 2009; PARK *et al.*, 2007). Vaccination records (when available) were analyzed to assure that all three doses of HBV had been administered and to check the elapsed time between the actual vaccination and the antibody tittering. HBV vaccine administration was done intramuscularly (IM), with the exception of one study

(LEONARDI *et al.*, 2012) where the IM and intradermal (ID) routes were compared. Patients and controls were generally vaccinated in accordance to the standard national HBV schedules. In the work of AHISHALI *et al.* (2008), however, there was no indication of previous vaccination (for neither the controls nor the CD patients), and a full HBV vaccination cycle was prospectively administered to the test subjects as part of the study.

In a more recent study, a booster dose was administered only to the nonresponder CD patients (ZINGONE *et al.*, 2011). Another research group studied only nonresponder subjects, who were also given a booster dose, randomly using IM or ID routes (LEONARDI *et al.*, 2012).

Controls had to be negative for blood autoantibody markers of CD such as antigliadin IgA and IgG autoantibodies (ERTEM *et al.*, 2010; PARK *et al.*, 2007).

In all the studies reviewed, the primary outcome variable was responsiveness to HBV vaccination, evaluated through anti-HBsAg antibodies blood analysis. Anti-HBsAg antibody titers <10mIU/ml were regarded as non-protective and consequently a negative response. The exception was the study conducted by PARK *et al.* (2007), where the cut-off value was 12mIU/ml.

In the majority of the studies, both controls and CD patients were tested for the presence of HBsAg, Anti-HBcAg IgG and other HBV markers (ERTEKIN, TOSUN and SELIMOGLU, 2011; ERTEM *et al.*, 2010; LEONARDI *et al.*, 2009; NEMES *et al.*, 2008) in order to assure there was no previous HBV infection.

Depending on the objective of the study, patients were in some occasions requested to have been compliant to a gluten free diet for a determined period of time before enrolment (ERTEM *et al.*, 2010; NEMES *et al.*, 2008) or had the time of their gluten free period checked (ZINGONE *et al.*, 2011, 2013). Compliance could be accessed not only by specific patient interrogation but also by measuring anti-tTG levels. The cut-off value for a positive test was 20 AU/ml (ERTEM *et al.*, 2010) and positive responses indicated noncompliance.

HLA-DQ2 typing was performed only in a few studies (ERTEM *et al.*, 2010; NEMES *et al.*, 2008; NOH, POLAND and MURRAY, 2003; ZINGONE *et al.*, 2011, 2013), by polymerase chain reaction amplification. Therefore, the absence of typing data in the majority of studies is a major drawback in establishing a relationship between HLA genotype and HBV vaccination nonresponse.

Despite significant differences in study design, the results retrieved by the studies reviewed reach one common conclusion: celiac patients show nonresponse considerably more frequently than healthy controls. Study results are displayed on table 1 below.

LEONARDI *et al.* (2009) examined, retrospectively, celiac patients, vaccinated within one year of age, to determine whether they failed to show a response to HBV vaccine more often than healthy control subjects. The results confirmed high numbers of seroconversion failure, but also showed that there were more responders within patients younger than 18 months at the time of diagnosis than within those older than 14 at diagnosis. Therefore, it was pointed out that not only that maybe revaccination should be recommended to CD patients, but also that CD diagnosis at a younger age may translate in higher response rates to HBV vaccination.

The results retrieved by PARK *et al.* (2007) added that patients vaccinated before completing one year of age revealed a higher failure rate in developing hepatitis B surface antibodies (HBsAb) than those vaccinated later. AHISHALI *et al.* (2008) also reached the outcome of diminished response rates among celiacs, even though the time between vaccination and antibody tittering was of only 4 weeks (given that subjects were only prospectively vaccinated). In this study, negative anti-HBsAg antibodies CD patients and controls prospectively received a full cycle of HBV vaccination. Although the response was positive for all of the controls, 32 % of the celiac patients did not show a protective titer of anti-HBsAg after vaccination, as shown on table 1. All subjects were adults, with a mean age of 38.

Some studies focus on the relationship between gluten intake at the time of vaccination and nonresponse to HBV vaccine. NEMES *et al.* (2008) compared vaccination efficiency between gluten-free patients immunized prospectively after diagnosis and standard mass immunized patients and controls with no regard for diet status or celiac disease diagnosis. Response rates in the first group were similar to those of controls, even though all patients carried HLA-DQ2. By the other hand, within those vaccinated when undiagnosed (and thus untreated), nonresponse was high (74,1 %).

ERTEM *et al.* (2010) found that post-vaccination anti-HBs antibody negativity was significantly higher in celiacs than in healthy population but also discovered response to HBV vaccination in gluten-free diet compliant CD patients to be similar to that of the healthy population.

ZINGONE *et al.* (2013) also related gluten dietary exposure with HBV vaccine nonresponse in celiac patients. Celiac patients had an inadequate response to vaccination

when compared to controls. Contrary to other studies, there was no significant difference between nonresponse within patients vaccinated at 12 years old who were exposed to gluten and within those with the same age who were on GFD. There was, however, a meaningful dissimilarity when comparing the second group with the one vaccinated as infants, the latter having a higher prevalence of nonresponse.

With the same objective, ERTEKIN, TOSUN and SELIMOGLU (2011) also found a higher prevalence of HBV nonresponders among celiac patients, when compared with healthy controls. In regard to the impact of GFD, however, the investigators concluded that GFD compliance might improve response to HBV vaccination in CD patients.

On the other hand, other works investigate the possible primary role of HLA haplotypes in nonresponse by celiac patients. NOH, POLAND and MURRAY (2003) established a relationship between CD and HBV vaccine nonresponse, studying 19 CD patients and finding that 13 of them had not responded to vaccination. All the subjects were HLA-DQ2 positive. It was postulated that nonresponsiveness in CD was due to a lack in Th2 response resulting from this particular HLA genotype.

The problem of unresponsiveness to HBV vaccination in CD has lead authors to investigate possible solutions, such as new vaccination strategies. LEONARDI *et al.* (2012) compared the efficacy of ID and IM HBV boosters in celiac patients who had not responded to previous standard vaccination and found no significant difference. Although both routes, IM and ID, were found to be effective in raising protective anti-HBs titres, ID was deemed as the most adequate, since it produced a larger number of high responders (anti-HBs > 1000 IU/L) and is also more cost effective (given that the dose of HBV required for ID administration is inferior to that of IM).

ZINGONE *et al.* (2011) administered a booster dose of HBV vaccine to 14 celiac patients on GFD and four controls lacking protective titres of anti-HBs. Only about a third of the celiacs achieved an anamnestic response (defined as a fourfold or greater rise in anti-HBs after vaccination). Given these results, the authors suggested there might be an impairment of the immune response due to memory cells and pointed out that CD patients might require higher doses of vaccine or a greater number of injections to achieve protection.

	Population	Schedule of vaccination	GFD at vaccination	Age when vaccinated (yo)	Mean time interval until testing	HLA-DQ2 presence	HBsAb <10IU/ml
(ZINGONE *et al.*, 2013)	Group A: 57 CDP	20 µg at 0, 1 and 6 months; IM	No	12	13 y	92,6 %	43,9 %
	Group B: 46 CDP		Yes	12	15 y		34,8 %
	Group C: 60 CDP	10 µg at 2, 5 and 11months; IM	Yes	At birth	19,5 y		58,3 %
	Group D: 48 controls	20 µg at 0, 1 and 6 months; IM	NA	12	10 y	25 %	8,3 %
(NEMES *et al.*, 2008)	Group 1: 22 CDP	10 µg at 0, 1 and 6 months; IM	Yes	4-12,5 (prospectively after diagnosis)	4 w after 2nd and 3rd vac.	100 %	4,5 %
	Group 2: 106 CDP	10 µg or 5 µg at 0, 1 and 6 months or 20 µg or 10 µg at 0 and 6 months	No exact data; only some subjects	14	28 months	56 % (out of only 53 typings available)	49,1 %
	Group 3: 113 controls		NA	14	23 months	ND	24,8 %
	Group 4: 37 CDP from group 2 w/o anti-HBs Ab	20 µg; IM	Yes	ND	4 w	ND	2,7 %
(PARK *et al.*, 2007)	26 CDP	3 full doses of recombinant HBV vaccination	ND	2,9 y (mean)	6,4 y	ND	53,9 %*
	18 controls		NA	3,5 y (mean)	6,9 y	ND	11,1 %*
(ERTEM *et al.*, 2010)	40 vaccinated CDP	3 full doses of recombinant HBV vaccination	Yes	ND	31 months	55% (HLA DQB1*02 only)	32,5 %
	28 anti-HBV negative CDP **	Prospectively vaccinated with same brand of vaccine (3 doses)		ND	4 w	ND	3,6 %
	54 controls	3 full doses of recombinant HBV vaccination	NA	ND	35 months	14,3 %	14,8 %

	Population	Schedule of vaccination	GFD at vaccination	Age when vaccinated (yo)	Mean time interval until testing	HLA-DQ2 presence	HBsAb <10IU/ml
(NOH, POLAND and MURRAY, 2003)	19 CDP	ND	ND	37 y (mean)	5,6 y	100 % (out of 15 typings)	68,4 %
(ERTEKIN, TOSUN and SELIMOGLU 2011)	52 CDP	3 full doses of recomb. HBV vaccination at 0,1 and 6 months; IM	84,6 % of CDP	At birth	More than 6 months after vac.	ND	38,5 %
	20 controls		NA				10 %
(ZINGONE *et al.*, 2011)	51 CDP	20µg at 0, 1 and 6 months	5,9 % of CDP	12y	11,08 y	90,2 %	31,4 %
	48 controls		NA		11,52 y	25 %	8,3 %
	14 non responder CDP	Booster dose	Yes	ND	2w	100 %	71,4 % w/o anamnestic response
	4 non responder controls		NA	ND		ND	25 % w/o anamnestic response
(LEONARDI *et al.*, 2009)	60 CDP	10 µg at 3, 5 and 11 months; IM	Yes	At birth	More than 6 months after vac.	ND	50 %
	60 controls		NA				11,6 %
(LEONARDI *et al.*, 2012)	30 non responder CDP	10 µg at 3, 5 and 11 months; IM Booster: 2 µg ID	Yes (when boosted)	At birth/ booster at 10,45 y (mean)	More than one year after vac./ 4 w after booster	ND	23,3 %
	28 non responder CDP	10 µg at 3, 5 and 11 months; IM Booster: 10 µg IM		At birth/ booster at 9,3 y (mean)			21,4 %
(AHISHALI *et al.*, 2008)	25 unvac. CDP	20 µg/0.5 ml at 0, 1 and 6 months; IM	ND	40,4 (mean)	4w	ND	32 %
	20 controls			35,85 (mean)			0 %

Table 1: State of the art on the link between celiac disease and hepatitis B vaccine nonresponse, displaying study results and specifics of the works above reviewed.

CDP = Celiac Disease Patients; ND=No Data; NA=Not Applicable
HBsAb cut-off value of 10 mIU/ml, except in (Park *et al.*, 2007), as noted with *
** between vaccinated, unvaccinated and with no records available

Discussion

These study results contribute towards establishing a definite link between CD and HBV vaccination nonresponse, given that the fraction of HBV non responders among celiac patients is in all works higher than that among matching controls. However, it is not yet possible to attribute an explicit cause to this phenomenon.

The influence of GFD in vaccination response, for one, remains uncertain. Since celiac disease is frequently diagnosed only later in life, patients vaccinated as newborns or as adolescents might not have been diagnosed yet and consequently did not follow a GFD at the time. Therefore, and although some authors (LEONARDI *et al.*, 2009) defend that early diagnosis and introduction of GFD has positive influence on vaccination response, not enough data regarding this matter is available when it comes to primary vaccination. When it comes to revaccination the data is clearer, since the vaccination is done prospectively and GFD compliance can thus be assured. However the information is still scarce (ZINGONE *et al.* (2011), for example, defended vaccination under GFD based only in positive results from three patients, who had received a revaccination cycle) and inconclusive, for while there were authors defending the benefits of HBV vaccination under GFD and proposing booster administration, in this conditions, to CD patients (ERTEKIN, TOSUN and SELIMOGLU, 2011; ERTEM *et al.*, 2010; NEMES *et al.*, 2008), there were also contrary opinions, based on results that did not confirm a favourable role for GFD (ZINGONE *et al.*, 2013).

Studies lacked a group of newly diagnosed celiacs, who had not yet started GFD, for more accurate comparison with compliant patients. It cannot be ruled out that gluten free patients may have generated better study results simply because they had been vaccinated recently, with antibody tittering done short after vaccination.

It is also difficult to attribute the impaired immune response exclusively to HLA typing. The reviewed studies focused mostly on the HLA-DQ2 allele, since it is both extensively present in celiac disease and one of the known HLA haplotypes connected with HBV impaired immune response. The authors advocating for GFD deemed HLA-DQ alleles as not preponderant for HBV vaccination response, since within the diet compliant celiac subjects the seroconversion was similar to that of healthy subjects, even though HLA-DQ2 was, for the most part, present (ERTEM *et al.*, 2010; NEMES *et al.*, 2008; ROSTAMI and ROSTAMI NEJAD, 2013). Typing was only seldom performed and for that reason it did not provide enough data to draw a tangible conclusion. There were, however, some suggestive data contradictory to the latter point of view, where celiacs did indeed demonstrate a

significant diminished response to vaccination and the prevalence of the haplotype (either as homozygotes or heterozygotes) was on every occasion higher when compared to controls.

More recently, literature has been searching for new vaccination strategies as a possible way to mitigate the problem of HBV vaccine nonresponse. Booster doses or full revaccination cycles were administered to small groups of CD patients, while on a GFD, but results were once more contradictory. While the works of ZINGONE *et al.* (2011), for example, showed that 71,4 % of CD patients did not have an anamnestic response after booster, a result markedly inferior to that of controls, NEMES *et al.* (2008) found that only 2,7 % did not have a proper response to the booster. Therefore, there is a need for further investigation on this subject, with a greater number of test subjects and a more rigid and specific study design. Only this way can a conclusion be drawn on the benefits and efficacy of CD patient's revaccination. And if revaccination becomes a valid option when it comes to celiacs, vaccination parameters such as dosage, periodicity or model (single booster doses or full revaccination cycles) need also to be accessed.

A systematic review and meta-analysis conducted on standard ID HBV vaccination (SANGARÉ *et al.*, 2009) pointed out that, overall, ID HBV vaccination had induced seroprotection in a lesser number of individuals when compared to the IM route, although it had appeared to be more immunogenic in school-aged children and in women. It was thereupon suggested that this route of vaccination was a fairer alternative to the standard one in these specific populations. In a study conducted by LEONARDI *et al.* (2012), where IM and ID HBV boosters were administered to celiac patients, the efficacy of both routes was proven similar. ID was, however, considered the best choice for its favourable cost-benefit relation. This vaccination route has the advantage of introducing directly a dendritic-cell-mediated immune response and thus needing a lower dose of antigen. It is also easier to assess immune responsiveness, since a skin reaction is developed at the injection site (VITALITI *et al.*, 2013). Larger prospective randomized studies with CD patients as subjects are needed to validate this method for nonresponder revaccination.

Several limitations affected the reviewed studies. For instance, all of them had very limited study populations, non representative of the high prevalence of CD, and serological testing was mostly done several years after vaccination. Hence it becomes harder to attribute nonresponse to CD, as it is not clear nonresponse is not due to the age of the subjects (older individuals tend do have a weaker response to vaccination), or simply to the diminishing of immunity over time. Although there is the possibility that celiacs may lose their antibody protection at a faster pace than healthy individuals but retain immunological

memory and consequently show an anamnestic response, clear supporting evidence has not yet been provided. These issues could be addressed by only enrolling subjects recently vaccinated, and by testing them at a fixed period of time after vaccination.

Until there is a clear answer for all the questions addressed above, and the most adequate strategy of vaccination in CD can be assessed, HBV nonresponse remains a concerning threat to public health. Therefore, and considering that there is no assurance CD patients maintain immunological memory and would show an anamnestic response in case of infection, all celiacs should be routinely tested for HBV seroprotection after diagnosis and revaccinated in case of negative antibody serology results, preferably while having been on a GFD for a solid period of time. Information concerning possible response impairment in celiac patients should be included on the product characteristics summaries for the various hepatitis B vaccines currently on the market.

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