Celiac Disease
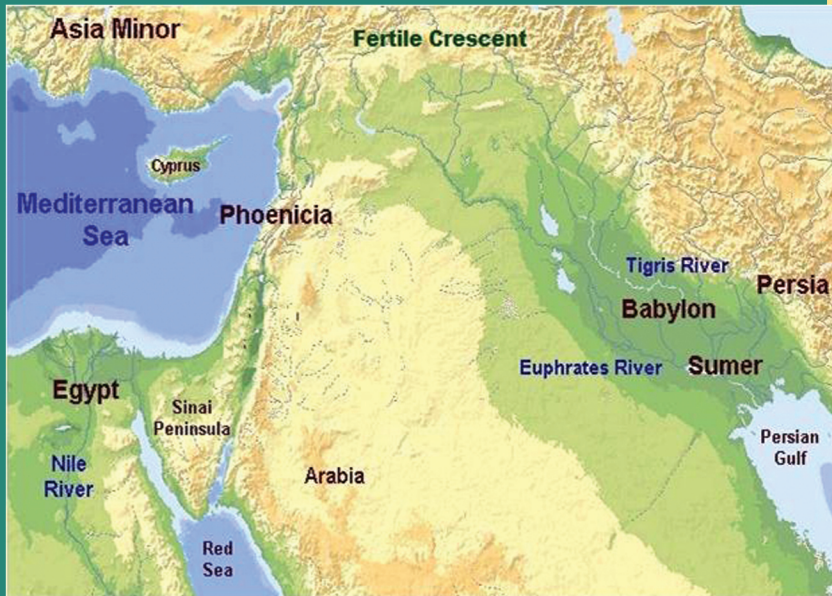
Epidemiology, Genetic and Clinical Behavior In Iran

Mohammad Rostami Nejad

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Thesis, RIGLD, with summery in English, Dutch, and Persian

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"When the door of happiness closes, another opens.
But often times we look so long at the closed door that we don't see the one which has been opened for us."

Mahatma Gandhi

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PART I

INTRODUCTION

&OUTLINE OF THE THESIS

Introduction

About 10,000 years ago, people discovered that if they settled in one place for long enough they could sow and then harvest crops of cereals like wheat instead that hunting animals and gathering wild berries and fruits. The discovery in the Neolithic age of ways to produce and store food was the greatest revolution humankind ever experienced. To our current knowledge, the origin of farming practices was located in the 'Fertile Crescent', the wide belt of Southeast Asia including Iran, Turkey Israel/Palestine, Syria, Lebanon and Iraq (1). This extends from the Mediterranean Coast on its western extreme to the great Tigris–Euphrates plain eastward (2,3). Cultivation of wheat and barley, first exploited and intensively developed in the Levant and western Zagros (Iran), slowly spread westward across northern Europe to reach Britain by Circa 4000 b.c. (1,4).

Not surprisingly, the major living and dietary changes caused by the agriculture revolution led to the appearance of 'new' diseases, such as coeliac disease (CD). This is an autoimmune disorder which affects genetically predisposed individuals upon the ingestion of gluten and is now recognized as a common disorder among Middle Eastern. Gluten is the major protein fraction in the cereals wheat, rye and barley. CD was already known in the most ancient times (5).

Genetic, immunological and environmental factors are involved in the pathogenesis of the celiac disease. Despite advances in investigations techniques, celiac disease remains a challenging problem that often eludes diagnosis and

receives sub-optimal attention (6). There are overwhelming reports and descriptions on celiac associated disorders but the severity and prevalence of such an involvement have not been systematically evaluated. Inflammation may lead to the malabsorption of several important nutrients. Malabsorption and nutritional deficiency are therefore causing a range of other disorders commonly associated with CD (table 1, 2).

Much has been learned about the immunology of CD in recent years, and there is overwhelming evidence that the immune response to gluten is central to the pathogenesis. This aspect provides evidence for association with some autoimmune disorders. Although there is a clear genetic association between CD, Dermatitis herpetiformis (DH) and Diabetes Mellitus Type I (DM), we are uncertain about a number of other disorders claiming an association with CD. The question is how far these conditions are in reality related to CD and what the underlying patho-mechanism for these associations is (7). What are the clinical implications? What is the management and what would be the role of gluten free diet (GFD) in treating these conditions?

Table 1. Complications and disorders, which are the results of nutritional deficiency following to malabsorption syndrome

Anemia, Folate & Iron Deficiency	Osteomalacia, Osteoporosis
Failure to Thrive (children)	Low Bone Mass
Infertility and Impotency	Vitamin K Deficiency
Neuropathies	Malignancy

Clinical spectrum of Coeliac Disease

There are plenty reports dividing CD in a range of subgroups in the current literature, but the most simple and comprehensive classification would be classical and atypical (8). Using the world's silent, latent and potentials are overlapping and confusing the readers (9). Now you may ask this question: why some celiac patients present with typical and with atypical signs and symptoms: limited knowledge (still we cannot explain, there must be other factors involved beyond our understanding), constitutional other factors, other environmental factors (drugs, infection, etc.).

Thirty years ago our understanding about CD was limited to the classical presentation and the prevalence of this form of gluten sensitivity (GS) was still low. By development of serological tests and advances in immuno-genetic, we also have been able to diagnose those atypical cases under the water line. The atypical forms comprise a range of various forms of this condition including those with typical histology with minimal symptoms, those with minimal or normal mucosal changes (microenteropathy) with positive or negative serology. Finally, those cases with typical mucosal changes and symptoms but non-responsive to GFD are known as refractory celiac disease.

A team of 16 physicians from seven countries designed a multidisciplinary task force during a meeting in Oslo and phone conferences (10). They suggest that different definitions for CD-related terms such as asymptomatic, atypical, classical, latent, non-classical, overt, paediatric classical, potential, refractory, silent, subclinical, symptomatic, typical, CD serology, CD autoimmunity, genetically at risk of CD, dermatitis herpetiformis, gluten, gluten ataxia, gluten intolerance, gluten sensitivity and gliadin-specific antibodies should be consensus on the use of terms related to coeliac disease and gluten. They finally concluded that classical CD should define as 'CD presenting with signs and symptoms of malabsorption including diarrhoea, steatorrhoea, weight loss or growth failure.' 'Gluten-related disorders' is the suggested umbrella term for all diseases triggered by gluten and the term gluten intolerance should not to be used.

Table 2. A list of diseases/disorders reported as associated with celiac disease

Primary Biliary Cirrhosis	Short Stature, Delayed Puberty
Recurrent Pericarditis	Sarcoidosis
Thrombocytosis (Hyposplenism)	Schizophrenia / Mental Problems
Systemic LupusErythematosus	Small-Intestinal Adenocarcinomas
Scleroderma	Chronic Fatigue Syndrome
Thrombocytopenic Purpura (ITP)	Spontaneous Abortion and Fetal Growth Retardation
Thyrotoxicosis	Vasculitis
Peripheral Neuropathy	Gastroparesis
IBD	Farmeris Lung
Head Aches (Migraine)	Myasthenia Gravis
Follicular Keratosis	Polyneuropathy
Autoimmune Hepatitis	Cystic Fibrosis
Dyspepsia	Fibromyalgia
Fibrosing Alveolitis	Pulmonary Hemosiderosis

Sapone et al. state that, besides celiac disease and wheat allergy, there are cases of gluten reactions in which neither allergic nor autoimmune mechanisms can be identified (11). These are generally defined as non-celiac gluten sensitivity (GS). Some

individuals who experience distress when eating gluten-containing products and show improvement when following a GFD may have GS instead of CD.

GS is a condition distinct from CD and is not accompanied by the concurrence of anti-tTG autoantibodies or other autoimmune comorbidities. The small intestine of GS patients is usually normal.

Epidemiology of celiac disease in the world and Iran

It is very likely that many associated disorders represent as a simple coincident rather than a real association. However, there are clearly many disorders in different way related to gluten sensitivity.

The theories behind this coexistence first of all bring to our attention that a good part of these associated disorders might be simple overlaps and/or coincidences as coeliac disease is quite a common and easily can overlap with these disorders (10). On the other hand the immunogenesis leading to CD is stimulated by an exogenous antigen with a different pathway compared to many of the associated disorders.

Many studies have shown that the total prevalence of coeliac disease throughout the world has increased significantly over the last three decades and despite previously belief, comparison of studies in European and Middle Eastern countries has shown that CD is common in both areas, with an almost similar prevalence (10-21). This discovery can be attributed to the judicious use of serological screening tests which measure anti-gliadin antibodies (AGA) and anti-endomysial (EMA), and more recently anti-transglutaminase (anti-

tTG) which has permitted the diagnosis of many subclinical CD cases that otherwise would not have been recognized (9, 22).

Nowadays, the map of CD prevalence in different areas of the world is much more detailed than in the past. For instance, Finland has one of the highest recorded rates, with recent estimates suggesting a prevalence of 2%, a near doubling of historical values (23). The prevalence of CD amongst different populations worldwide is varied and the actual prevalence of CD has been shown to be more frequent than in the past. However, there is limited data about the prevalence of CD in Middle Eastern countries. Recent studies of serum markers in blood donors have shown a prevalence of 1:250 in Sweden, 1:524 in Denmark, 1:333 in Holland, 1:157 in Israel, 1:250 in the USA, 1.5:100 in the Syria and 1:681 in Brasília. On the other hand most of the asymptomatic patients are still undiagnosed or misdiagnosed. A large UK population-based study (24) has suggested that more than 90% of coeliac disease in asymptomatic children remains undiagnosed.

Until the past decade CD was considered uncommon in Iran. Following the application of simple serological tests for diagnosis of CD in Western countries, some studies have been published on the prevalence and the importance of CD in Iran and showed that this prevalence ranges from 0.5% among schizophrenia patients (25) to 12% in patients with irritable bowel syndrome (IBS) (26). For instance, the prevalence of CD in low risk subjects was reported even higher than that of Western countries (1 out of 166 healthy Iranian blood donors) (27). Recent

screening studies performed on the general population and at-risk groups in different geographical areas in Iran have shown that the prevalence of CD is similar to that in Western countries.

In studies on apparently healthy blood donors in Northern (Sari and Gorgan) and Southeastern Iran (Kerman) have shown an overall prevalence of 1:91 to 1:120 for these cities (28). On the other study 1440 healthy individuals were screened in Shiraz and the result of this study showed that the prevalence of CD in this region was less than 0.5% which was much lower than reports from other areas of the country (29). The different prevalence between the northern versus the southern areas in Iran might be because of different genetic background of population in these regions (30). Although the prevalence of CD in some areas in Iran like Shiraz is very low, but other studies suggest a prevalence of 1% in the remaining areas of Iran which is similar to the frequency of this disorder in Western European countries (27, 30-32).

Genetic Associations

Coeliac disease is a polygenic disorder and HLA is the single most important genetic factor in this condition (33). The sharing of a similar HLA haplotypes may partly explain the strong association between DM, Dawn Syndrome, IgA-Deficiency, DH and CD. Evidence that there is a strong inherited predisposition to coeliac disease susceptibility comes from twin studies and studies of prevalence in relatives of affected individuals (34). There is, however, no discernable Mendelian inheritance pattern in families, and risk falls more rapidly in distant relatives than would be expected in a disease caused by a single gene defect. Exons of both CD28 and CTLA4 have been sequenced in large numbers of coeliac patients, and no evidence for mutations specific to coeliac disease has been found. Large scale studies in type I diabetes and autoimmune thyroid disease have recently clarified the haplotypic structure of the CD28-CTLA4-ICOS region, and a variant 6 kB 30 of the CTLA4 transcription start site (CT60) demonstrates the strongest autoimmune disease associations of over 100 variants studied from the region (35).

The finding that diseases such as type I diabetes, coeliac disease and multiple sclerosis are HLA-DQ associated is not easily explained by a simple hypothesis of DQ-restricted, autoreactive T cells, considering the generally marginal role of DQ in restricting responses. Thus, both HLA-DR and -DQ polymorphism exists for hTg in autoimmune thyroiditis.

We know that more than 30% of general population are HLADQ2/8 positive (36). Unfortunately there is no enough evidence to show whether this group are more prone to develop autoimmune disorders compared with those who have no HLA-DQ2/DQ8. As described for other populations (33, 35), the most frequent haplotype in Iranian CD patients was HLA-DQ2.5 (69.5%) and this frequency is very similar to that in European CD populations.

Aim of this thesis

This thesis consists of 5 parts. Introduction and outline of the thesis are presented in part I. The aim of part II was to present the epidemiological data regarding the celiac disease in Iran in different population and this part including

8 chapters. The aim of part III is describing the correlation between associated disorders and celiac disease and also this part including 6 chapters regarding this association. The aim of part VI was presented the data regarding the effect of gluten free diet in CD and this part include 3 chapters. Part V consists of Discussion, Summery, Acknowledgement and a brief CV.

PART I INTRODUCTION AND OUTLINE OF THE THESIS

PART II EPIDEMIOLOGY

Chapter II.1 consists of the review article which summarizes the epidemiological data on CD in Middle East and highlights different clinical presentations and management of CD in Iran.

Prevalence of CD in patients with GI symptoms is reported and the differentiation of typical manifestations from the atypical forms of CD was discussed in **Chapter II.2**.

In **Chapter II.3** we assessed the association between celiac disease and severe chronic depression and schizophrenia.

Chapter II.4 This is a multicenter study (European and middle eastern) in which the current practice in particular the number of biopsies taken by different center have been investigated and determined the incremental diagnostic yield of adherence to the recommended number of specimens.

The distribution of HLA-DQ2 and -DQ8 alleles in Iranian CD patients compared to healthy controls were explored and studied in Chapter **II.5**.

Chapter II.6; The result of this multi-center study indicated that upper abdominal disorders such as abdominal pain and dyspepsia were the most common primary complaints in European patients, while for Iranian patients, diarrhea and bloating were considered the classic presentations of CD.

Chapter II.7: Atypical forms of CD have increased considerably and the presence of dyspepsia as a unique symptom has been frequently attributed to CD. In classical CD with prominent malabsorptive features, dyspepsia may be one of the symptoms. It has been reported that the frequency of CD in people with dyspeptic complaints is 1.1-3%, which is two to nine times higher than in the general population. In the present study we described the prevalence of celiac disease in dyspeptic patients and compare the value of serology with histology in diagnosing CD.

Chapter II.8; In this Chapter, we have studied body mass index in celiac patients diagnosed in a population-based study, using a well-matched case-control analysis in order to obtain updated information regarding the underweight in celiac patients.

PART III ASSOCIATED DISORDERS

Chapter III.1 consist the review article. In this review we provide

information regarding liver disorders that may be seen in association with celiac disease and the effect of treatment of CD on these disorders.

In **Chapter III.2** we estimated the prevalence of undiagnosed CD and the co-existence of *Toxoplasma gondii* infection in a population of pregnant women and pregnancy outcome.

In **Chapter III.3** we assessed the prevalence, the related symptoms, and the endoscopic and histological feature of gastritis described in a simplified classification and celiac disease correlation in patients with *Helicobacter pylori* (Hp) was investigated.

We have evaluated the correlation of gluten related auto-antibodies in infertile couples and assessed in which proportion celiac disease is responsible for unexplained fertility disorders between men and women in **Chapter III.4**.

The prevalence of Rotavirus and CD serology among Iranian adults with non-specific gastrointestinal (GI) symptoms were shown in **Chapter III.5**.

Chapter III.6 was assessed the relation between clinical and demographic factors in coeliac patient, using logit and probit analysis.

PART IV GLUTEN FREE DIET

Chapter IV.1 consists of a review article on non-coeliac gluten sensitivity, and suggesting a simplified terminology called subclinical instead of silent and potential/latent.

Chapter IV.2: In this study the effects of a gluten free diet on hypertransaminasemia in patients with newly diagnosed celiac disease were studied.

Chapter IV.3: The aim of this study was to investigate the relation between CD and unexplained DUB in celiac women.

PART V: DISCUSSION, SUMMARY, ACKNOWLEDGEMENT, and a BRIEF CV.

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PART II

EPIDEMIOLOGY

PART II

Chapter 1

Epidemiology of Celiac Disease in Iran: A Review

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ABSTRACT

In the past, celiac disease (CD) was believed to be a chronic enteropathy, almost exclusively affecting people of European origin. Celiac disease is the permanent intolerance to dietary gluten, the major protein component of wheat. The availability of new, simple, very sensitive and specific serological tests (AGA, AEM and tTGA) has shown that CD is as common in Middle Eastern countries as in Europe, Australia and New Zealand where the major dietary staple is wheat. A high prevalence of CD has been found in Iran, in both general population and at-risk groups, i.e. patients with type 1 diabetes or irritable bowel syndrome (IBS).

In developing countries, serological testing in at risk groups is necessary for early identification of celiac patients. Clinical studies show that presentation with non-specific symptoms or a lack of symptoms is as common in the Middle East as in Europe. Wheat is a major component of the Iranian diet and exposure to wheat proteins induces some degree of immune tolerance, leading to milder symptoms that may be misdiagnosed with other GI disorders. The implementation of GFD is a major challenge for both patients and clinicians in Iran, especially since commercial gluten-free products are not available in this area.

Keywords: Celiac disease; Epidemiology; Iran.

Celiac disease, Epidemiology, Genetic and Clinical Behavior in Iran

Introduction

In the last few years, considerable changes in the epidemiology of celiac disease (CD) have been observed. A marked increase in CD prevalence and incidence has been reported, which can be at least partially explained by both the development of more sensitive serological tests and a high degree of disease suspicion.[1, 2] Although screening programs may discover some of the asymptomatic CD cases, if a clinical diagnostic approach is taken as well, a high proportion of under diagnosed patients may be detected.[3]

CD is the result of both environmental (gluten) and genetic factors (HLA and non-HLA genes). Distribution of these two components can probably be used to predict at risk areas of the world for gluten intolerance.[4] In this respect, the world geographical distribution of CD seems to have followed the spread of wheat consumption and migratory flows of mankind. Cultivation of wheat and barley, first exploited and intensively developed in Levant and western Zagros (Iran), slowly spread westward across northern Europe to reach Britain by circa 4000 B.C.[5, 6] The aim of this review is to investigate the epidemiology, different clinical presentations and management of CD in Iran.

Epidemiology of CD in the World

The age of presentation and prevalence of CD appears to have changed dramatically over the last 30-40 years.[7] Until a few years ago, gluten intolerance was thought to be a disorder almost exclusively affecting Europeans or people of European origin and they described typical features of celiac patients. Also until a decade ago, CD was considered to be very rare in Middle Eastern countries. A comparison of studies in European and Middle Eastern countries has shown that CD is common in both areas, with an almost similar prevalence (Table 1).[8-17] This discovery can be attributed to the judicious use of serological screening tests which measure anti-gliadin antibodies (AGA) and anti-endomysial (EMA), and more recently anti-transglutaminase (anti-tTG) which has permitted the diagnosis of many silent and subclinical CD cases that otherwise would not have been recognized. [18, 19]

Table 1. Prevalence of CD in Europe compared to Middle East population based on serological screenings.[8-17, 50, 58]

Europe	Prevalence	Asia	Prevalence
Italy	1:106	Iran	1:166
Czech	1:218	Israel	1: 157
Norway	1:262	Syria	1.5:100
Portugal	1:134	Turkey	1:87
Sweden	1:190	Anatolian adults	1:100
Netherlands	1:198	Kuwait (Chronic diarrhea)	1:18
United Kingdom	1:100	Saudi Arabia (Type1 diabetes)	12:100
Switzerland	1:132	Japan	1:20,000
Spain	1:118	India	1:500-2000

Nowadays, the map of CD prevalence in different areas of the world is much more detailed than in the past. However, there is limited data about the prevalence of CD in Middle Eastern countries. The prevalence of CD amongst different populations worldwide is varied and the

actual prevalence of CD has been shown to be more frequent than in the past. For instance, recent studies of serum markers in blood donors have shown a prevalence of 1:250 in Sweden, 1:524 in Denmark, 1:333 in Holland, 1:157 in Israel, 1:250 in the USA and 1:681 in Brasília.[20-24]

Epidemiology of CD in Iran

Until the past decade CD was considered uncommon in Iran; but following the application of simple serological tests for diagnosis of CD in Western countries, only few studies have been published on the prevalence and the importance of CD in Iran. This prevalence varies in different fields and ranges from 0.5% among schizophrenia patients [25] to 12% in patients with irritable bowel syndrome (IBS) in some areas of Iran.[26] For instance, the prevalence of CD in low risk subjects was reported even higher than that of Western countries (1 out of 166 healthy Iranian blood donors).[27] Recent screening studies performed by means of simple, sensitive and specific tests (AGA, AEM and AtTG) on the general population and at-risk groups in different geographical areas in Iran with a large consumption of wheat have shown that the prevalence of gluten sensitivity is similar to that in Western countries. However, there might be a different prevalence of CD between the northern versus the southern areas in Iran.[28]

The first study on CD ran from November 1998 through February 1999 in 2000 healthy blood donors in Tehran. Total serum AGA was measured and analyzed in all donors by an ELISA test and those with positive results were tested for EMA. All donors who had a positive serology for both AGA and EMA underwent small intestinal biopsies.

Of 2000 healthy blood donors, 49 were positive for IgA AGA (38 males, 11 females) and 12 were EMA positive. Gluten sensitive enteropathy was found in all subjects who had positive serology as follows: Marsh I (3), Marsh II (4) and Marsh IIIa (5) lesions. The results of this study showed that the prevalence of CD in this group was 1/166 and these results were similar to that of Western countries.[27]

In a study on apparently healthy blood donors in Northern Iran (Sari), CD was detected in 13 out of 1438 individuals. Small bowel biopsy histologies were consistent with Marsh 0 (1), Marsh I (8), Marsh II (2) and Marsh IIIa (2). At the same time, out of 1361 blood samples collected from apparently healthy blood donors in Southeastern Iran (Kerman), 16 were serologically CD positive. The histology of the small bowel biopsies was consistent with Marsh 0 (1), Marsh I (8), Marsh II (2) and Marsh IIIa (2) which represented an overall prevalence of 1:120 and 1:91 for these two cities, respectively.[29]

Saberi-Firouzi et al. also screened 1440 healthy individuals for EMA and tTG antibodies in Shiraz, Iran. Only 7 were positive for tTG antibody, of these 2 were also EMA positive. Five subjects with positive serologies agreed to undergo upper GI endoscopies. Small bowel intestinal abnormalities that included Marsh I-IIIc were noted in all patients with positive tTGA assays. The prevalence of CD in this study was less than 0.5% which was much lower than reports from other areas of the country.[30]

A seroprevalence study of 2547 healthy blood donors in Golestan Province showed 28 (1.1%) tTG positive cases [31]. This study had the same results as a study by Akbari et al.[29]

A case control study by Shahbazkhani et al. was undertaken at a university clinic in Tehran to determine the frequency of CD among patients diagnosed with IBS and consisted of 105 cases in each arm. Twelve IBS cases and no controls were diagnosed with CD. The result of this evaluation showed a prevalence of CD greater than 11% among IBS patients.[27] On the other hand, Emami et al. did not find any cases of CD based on serum IgA t-TG in a much larger sample size of IBS patients in Isfahan.[28]

Two different studies evaluated the prevalence of CD in iron deficiency anemia (IDA) patients in Iran. In a study by Nikpour and Hosseini, 126 patients with IDA underwent D2 biopsies during endoscopy. The average Hb level was 8.8 mg/dl. Serology was evaluated (AGA and EMA) for those who had a positive histopathology for CD. The researchers found that duodenal biopsies revealed histological features of CD in 8 (6.3%) patients according to modified Marsh criteria (6 Marsh IIIA; 2 Marsh IIIC). Six (75%) had positive serology for CD (2 positive EMA; 3 positive AGA; 1 positive for both).

In another study by Zamani et al., 206 patients were found to have IDA of obscure origin. Serology tests showed 31 positive tests (tTG and/or EMA). Thirty cases (14.6%) had abnormal duodenal histology of which 16 had Marsh III, 12 had Marsh II, and 2 had Marsh I lesions. After 4 to 6 months of gluten-free diet (GFD), the mean hemoglobin concentration of the patients rose from 8 g/dl to 13 g/dl. Both studies showed a high prevalence of CD in IDA of obscure origin and the efficacy of a GFD in patients who have mild to severe villous atrophy was demonstrated.[32, 33]

Behcet's disease (BD) is a chronic, relapsing inflammatory disease characterized by recurrent oral and genital aphthus lesions whose presentation is similar to CD. Based on a possible association, the sera of 288 patients with BD were screened with EMA and tTG antibodies for CD and D2 biopsies were taken from seropositive subjects.[34] Fourteen patients had positive tTG (2 positive EMA) but only 4 had histology compatible with CD (1, Marsh III; 3, Marsh I). The patients with CD were placed on a GFD to evaluate its efficacy on the improvement of their lesions. All 4 cases responded to the GFD. Although there seems to be a high percentage of false positive tTG results in BD, CD is approximately twice as common as seen in the general population.

A variety of neurological disorders such as epilepsy, ataxia and neuropathy have been reported in association with CD. Emami et al. studied 108 consecutive idiopathic epileptic patients.[35] The diagnosis of CD was determined by tTG antibodies and small intestinal biopsy. Histopathologic changes were interpreted according to the modified Marsh classification criteria.[36] The results of this study showed that 4 out of 108 (3.7%) epileptic patients were positive for IgA anti t-TG while the known prevalence of CD in the study area was 0.6%. The intestinal biopsy showed Marsh I lesions in all cases. The prevalence of CD is increased among patients with epilepsy of unknown etiology, justifying evaluation for CD in any patient with idiopathic

epilepsy even in the absence of digestive symptoms.

To assess the prevalence, related symptoms, endoscopic and histologic gastric features of CD in patients with *Helicobacter pylori* (HP), Rostami Nejad et al. investigated 450 dyspeptic patients by routine D2 biopsies.[37] HP was positive in 411 (91.3%) cases. Duodenal histology was normal in 385 (85.6%) patients, and positive in 28 (6.2%) who had Marsh I-IIIc lesions. In those with positive histology, 23 (82.1%) were also HP positive and 31 had positive CD serology. Serological analysis indicated that 12 out of 31 (38.7%) positive patients had abnormal histology (Marsh I - IIIc). The prevalence of CD in this group of patients was 2.6% and this finding indicated a false positive histology or low sensitivity of tTG in HP infection.

Celiac disease is a common chronic intestinal disease frequently associated with dyspeptic symptoms. It fulfils many of the disease criteria required for a screening program. From November 2007 to October 2008, 407 patients who underwent endoscopy for dyspeptic symptoms were studied. [38] _The results of this study showed that 10 out of 33 tTGA positive patients had abnormal histology (Marsh I-IIIc). In this study around 2.5% had small bowel mucosal abnormalities and positive CD serology. This may support routine serological screening for CD in dyspeptic patients.

We are probably far from an ideal screening serologic tool which relies on the antibody test as the sole method of screening for CD since the overall sensitivity and specificity of the IgA anti-tTG antibody has been determined to be 38% and 98%, respectively, in one Iranian study. The positive and negative predictive values for the anti-tTG antibodies were 57% and 96%, respectively. The sensitivity was 80% in patients with Marsh IIIC, which contrasts other reports that suggest a diagnostic accuracy of over 90% for anti-tTG antibody. Therefore serologic screening could result in many missed diagnoses, particularly in patients with lesser degrees of mucosal abnormalities.[39]

Rostami Nejad et al. evaluated 496 pregnant women for CD by serology. Thirteen (2.6%) cases had a positive serology for tTGA; 2 had low birth weight babies and 2 had a previous history of miscarriage. [40] This study showed a high incidence of unfavorable outcomes in pregnancy associated with positive serology for CD.

Different studies show that CD is the most common cause of chronic non-bloody diarrhea in adults and children in Iran, ranging from 6.5-19%.[41,42] Thus routine testing for CD is necessary in all patients who present with chronic non-bloody diarrhea.

Another study in 827 pregnant women showed 3.26% positive tTGA[43] which is much higher than the general population. Since we expect a lower pregnancy rate in CD cases, further evaluation is needed with other well designed studies.

EMA antibody was positive in 6 (2.4%) of 250 consecutive type I diabetes mellitus cases. D2 histology was compatible with Marsh I (2), Marsh II (3) and Marsh IIIb (1).[44] In another study by Fallahi et al., 96 children with type 1 diabetes mellitus were tested for tTG antibody.[45] Six (6.25%) were seropositive, and histopathological changes were compatible with CD in intestinal biopsies for all (5, Marsh IIIa; 1, Marsh IIIb). The results of both studies

show that the prevalence of CD in Iranian patients with type I diabetes mellitus is relatively high, justifying screening for CD in all patients with type I diabetes mellitus regardless of the presence or absence of symptoms. Patients should be screened at the onset of diabetes mellitus diagnosis and at regular intervals during follow up.

A total of 670 cases with non-specific GI symptoms were tested for serum IgA levels and tTG antibodies. Positive IgA tTG and IgG tTG were found in 22 cases as well as in 3 out of 8 IgA-deficient individuals. The prevalence of CD antibodies in serologically screened samples, excluding IgA-deficient cases, was 3.3% and 3.7% when IgA-deficient cases with positive tTG-IgG were included. This study indicated a high prevalence of CD antibodies among patients with non specific GI symptoms (3.7%). More awareness regarding the atypical presentation of CD could be the key step in identifying asymptomatic patients.[46]

Short stature is one of the most common causes of referrals to pediatric endocrinologists and is a well-known feature of pediatric CD.[47] Hashemi et al. studied 104 idiopathic short stature children (49 male, 55 female). All patients were investigated by serology and D2 histopathology. IgA tTG antibodies and IgA AGA were positive in 36 and 35 cases, respectively. Histological abnormalities compatible with CD were seen in 31 IgA tTG antibody positive and 28 IgA AGA positive subjects (26.9% (28 were positive for both anti-tTG and anti-AGA)).[48] This figure is very high and needs confirmation with other well designed studies.

High wheat consumption has been a major component of the Iranian diet for thousands of years[49] and the presence of about 57.6% frequency of HLA DQ2 and DQ8 in the general population suggest that the high percentage of our community could be susceptible to different presentations of CD.[50] There seems to be a lower prevalence of CD in Isfahan and Shiraz.[28, 30, 39]

The current wheat consumption per capita per year in Iran and other Middle Eastern countries is shown in Table 2. As this table shows, Iranians rank as one of the top wheat-consuming populations in the Asia–Pacific region with a per capita consumption of up to 150 kg/year.[51, 52]

Conclusion

Celiac disease was presumed to be rare in Iran because of lowered awareness and a low index of suspicion. However new epidemiological data show that CD is a common disorder in Middle Eastern countries, particularly Iran. Table 3 shows the current primary list of CD frequency in Iran [53-58]. This suggests a need for a more uniformly designed evaluation of CD for the entire country and a mapping of HLA DQ in the same areas along with a gluten consumption assessment, since a variable frequency of CD in different parts of Iran may exist as is the case for India.[59]

Although the prevalence of CD in some areas in Iran such as Shiraz Province is very low, a summary of the reviewed studies suggest a prevalence of 1% in the remaining areas of Iran which is similar to the frequency of this disorder in Western European countries.[27, 29, 60, 61]

Since commercial gluten-free products are not readily available and significantly more expensive than their gluten-containing products in this area,

therefore the main concern is the implementation of a GFD for Iranian patients.

Table 2. Wheat consumption (kg) per person per year (kg/year) for countries in the Asia–Pacific region.[19, 27, 51, 58]

< 25	25-74	75-150	> 150
Thailand	India	China	Iran
Singapore	Nepal	Saudi Arabia	Turkey
Indonesia	Malaysia	Oman	Kazakhstan
Myanmar	North Korea	Yemen	Turkmenistan
Bangladesh	Japan	Afghanistan	Iraq
Malaysia	Philippines	Pakistan	Syria
	Sir Lanka	Mongolia	Jordan
	Taiwan	South Korea	Uzbekistan
	Cambodia		Tajikistan
	Laos		Kyrgyzstan

Table 3. Prevalence of celiac disease among at risk groups in Iran (serological screenings)

Disease groups	Prevalence	Ref
Normal population	0.6	27
Chronic diarrhea (Children)	6.5-20	41,42
Inflammatory bowel diseases	7.8	53
Autoimmune hepatitis	3.6-10	54
Chronic psychiatric disorders	1.5	25
Irritable bowel syndrome	1-11.4	26
Short stature	4-33.6	48
Type 1 diabetes mellitus	2.4	44
Epilepsy	2.7	35
Dyspepsia	2.5	47
Infertility	1.5	55
Patients with non-specific GI symptoms	3.3	46
Mental retardation	1	56
Recurrent aphthous stomatitis	2.84	57
Behcet's	1.32	34
Iron deficiency anemia of unknown origin	14.6	58

This information will be useful to dietitians and gastroenterologists who counsel celiac patients, and to celiac advocacy groups for seeking financial support from the government.

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PART II

Chapter 2

Atypical presentation is dominant and typical for coeliac disease

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ABSTRACT

Objectives: Atypical presentation is the most prevalent form of coeliac disease (CD) and mostly clinically indistinguishable from other gastrointestinal (GI) disorders. The first objective of this study was to determine the prevalence of CD in patients with GI symptoms and the second objective was to characterize the typical manifestations of the atypical forms of CD.

Methods: This was a cross sectional study comprising 5,176 individuals by random sampling of self-referred people from the Tehran province, during the years 2006-2007 in a primary care setting. From 5,176 individuals, 670 with GI symptoms were selected for coeliac serology including total immunoglobulin A (IgA) and anti-tissue transglutaminase (tTG) antibodies. Those with IgA deficiency were tested with IgG tTG.

Results: This study shows that 13% (670/5176) of self-referred patients to a general practice suffer from GI symptoms. Dyspepsia was the most common symptom in 25 seropositive cases similar to the rest of the study group. A positive anti-tTG test was found in 22 from 670 investigated subjects (17 women, 5 men) (95% CI: 1.70-4.30) and 8/670 were IgA deficient. A positive IgG tTG was detected in 3/8 IgA deficient individuals. The prevalence of CD antibodies in serologically screened samples excluding IgA-deficient was 3.3% and 3.7% when including those IgA-deficient with positive tTG-IgG.

Conclusions: Non-specific GI symptoms seem to be the typical presentation of atypical CD. This study indicated that there is a high prevalence of CD antibodies among patients with GI symptoms (3.7%). More awareness regarding the atypical presentation of CD could be the key step in identifying asymptomatic patients.

Keywords: Prevalence, Coeliac disease, Anti-tissue Transglutaminase, Dyspepsia, Atypical presentation, Serology.

Introduction

Better awareness of "non-classical" coeliac disease (CD) and improved screening tests suggest that the prevalence of CD is underestimated in developed and developing countries [1-3]. The availability of serological tests for the diagnosis of CD during the last two decades, and a better knowledge of this disease, have permitted the identification of atypical CD [1, 4-8]. The symptoms of CD vary so widely among patients that there is no such thing as a "typical coeliac" as the individuals are affected differently. There is no correlation between the mode of presentation and the degree of mucosal damages [9]. There have been more than 200 signs and symptoms reported in association with gluten sensitivity, yet there are cases with this disorder which may have no symptoms at all [10, 11]. Terminologies such as latent, silent, potential and atypical are confusing and there is a need for a better definition to cover the spectrum of gluten sensitivity.

Increasing evidence of the adverse consequences relating to delays in diagnosis and easier screening assays such as tTG [12, 13] justifies the routine screening of high risk cases [14-20]. Some preliminary reports have shown the efficacy of a case-finding strategy in both adult and pediatric populations [21-23]. This approach relies on an active role being played by primary care physicians in selecting individuals to be tested for CD. The aim of this study was to explore the etiology of GI disorders in a large cohort of symptomatic patients and to identify the typical gastrointestinal (GI) pattern of atypical CD. The atypical extra-intestinal

symptoms have not been considered in this study.

Patients and Methods

Patients

This was a cross sectional study which involved 5,176 individuals randomly sampled from the population of the Tehran province, Iran during the period October 2006 – November 2007. Six hundred and seventy individuals with GI symptoms in their questionnaire were identified in a primary care setting and extensively investigated for a common GI pathology. From a total of 670 GI patients included in this study, 427 subjects were women (63.73%) and 243 subjects were men (36.27%) with a mean age of 41.61 and SD 16.55 years.The study was approved by the Institutional Medical Ethics Committees of Research Center for Gastroenterology and Liver Disease, Shaheed Beheshti University, M.C. and all participants signed a written informed consent.

Methods

The optical density readings on enzyme-linked immunosorbent assay (ELISA) of 670 patients with GI symptoms received for tissue transglutaminase (tTG) antibody testing for CD were compared with their total IgA concentrations. Those with IgA deficiency were tested with IgG tTG. All serological investigations were performed without knowledge of the patient status. Human antitissue transglutaminase (tTG) antibody and Immunoglobulin A were measured. Determinations of IgA tTGA antibody were carried out using a commercially available kit (AESKULISA tTG, Germany) and an enzyme-linked

Table I. Clinical and laboratory features of tTG positive patients

Subjects	Gender Male/female	Age (yrs)	tTGA	tTGA level	Total IgA	tTGG Level	Gastrointestinal symptoms
Case 1	F	64	+ve	15.22	normal	-----	Constipation, heartburn
Case 2	F	14	+ve	19.97	normal	-----	Heartburn
Case 3	M	51	+ve	26.93	normal	-----	Heartburn, abdominal pain
Case 4	F	37	+ve	25.56	normal	-----	Heartburn
Case 5	F	69	+ve	24.4	normal	-----	Abdominal pain, constipation, bloating, weight loss
Case 6	F	63	+ve	17.44	normal	-----	Heartburn, abdominal pain, bloating
Case 7	F	22	+ve	102.7	normal	-----	Heartburn
Case 8	M	81	+ve	22.59	normal	-----	Abdominal pain, weight loss
Case 9	F	42	+ve	49.68	normal	-----	Abdominal pain, constipation, bloating
Case 10	F	21	+ve	286	normal	-----	Abdominal pain, bloating
Case 11	F	14	+ve	23.23	normal	-----	Diarrhea
Case 12	M	45	+ve	20.49	normal	-----	Weight loss
Case 13	F	46	+ve	20.93	normal	-----	Abdominal pain, bloating
Case 14	F	21	+ve	16.56	normal	-----	Abdominal pain, constipation
Case 15	M	68	+ve	17.61	normal	-----	Heartburn
Case 16	F	24	+ve	83.51	normal	-----	Heartburn, bloating, weight loss
Case 17	F	44	+ve	294.6	normal	-----	Constipation, bloating, weight loss
Case 18	F	41	+ve	15.50	normal	-----	Diarrhea,
Case 19	M	64	+ve	16.73	normal	-----	Abdominal pain, constipation
Case 20	F	43	+ve	21.99	normal	-----	Heartburn, bloating
Case 21	F	33	+ve	17.79	normal	-----	Bloating
Case 22	F	29	+ve	37.80	normal	-----	Heartburn
Case 23	M	23	-ve	0.07	deficient	80.25	Constipation
Case 24	M	71	-ve	3.25	deficient	37.85	Abdominal pain
Case 25	M	20	-ve	4.22	deficient	15.07	Abdominal pain

immunosorbent assay (ELISA) method. According to the manufacturer's instructions, when a value higher than 15.0 U/ml was recorded, the result was considered.

Total serum IgA values were measured by an immunoturbidometric assay (Pars Azmoon, Iran) and serum levels below 70 U/L were considered indicative of IgA deficiency. Immunoglobulin G (IgG) tTGG values were further obtained in individuals with IgA deficiency by an ELISA method, and using the commercially available kit (AESKULISA tTGG, Germany).

Statistical analysis

Descriptive statistics, the chi-square test and conditional logistic regression were carried out using SAS software in order to find significant associated factors.

Table II. Etiology of gastrointestinal symptoms in 290/670 GI patients positive

Etiology	Cases affected	Percentage
Blastocystis hominis	30/670	4.47
Giardia lamblia	41/670	6.11
Iodomoeba butchelii	13/670	1.94
Entamoeba Histolytica/ Entamoeba Dispar complex	11/670	1.64
Cryptosporidium parvum	3/670	0.44
Chilomastix mesenelli	13/670	1.94
Ascaris lumbricoides	2/670	0.3
Enterobius vermicularis	2/670	0.3
Rotavirus	150/670	22.38
tTG positive	25/670	3.7

Results

Around 670/5176 (13%) of cases who attended primary care for various reasons had GI symptoms (Figs. 1, 2). We found an etiology for 290/670 symptomatic cases who participated in this screening. A positive serology for coeliac disease was detected in 25/290 (Table I) and another 265/290 cases had an infectious etiology (Table II).

For 56.7% (380/670) symptomatic cases no organic etiology was found. 293/380 (77.3%) had functional symptoms like constipation, diarrhea and dyspepsia. A number of 43/380 cases (11.3%) fulfilled the Rome III criteria for Irritable bowel syndrome. The remaining 44/380 had only self-limited short term symptoms and responded to short term symptomatic treatment (Table III).

Table III. Functional bowel symptoms (n=380)

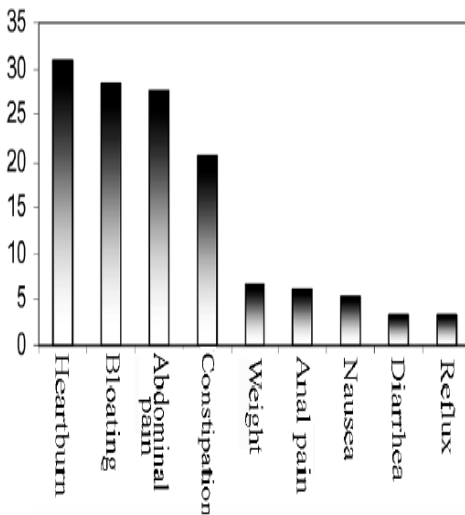|	Conditions	Number (%)
Functional bowel symptoms	IBS	43 (11.3)
Non-IBS	Heartburn	147 (38.68)
	Abdominal pain	145 (38.15)
	Diarrhea	12 (3.15)
	Constipation	94 (24.73)
	Short term	44 (11.57)

Figure 1. The frequency of gastrointestinal symptoms in 670 patients (percent)
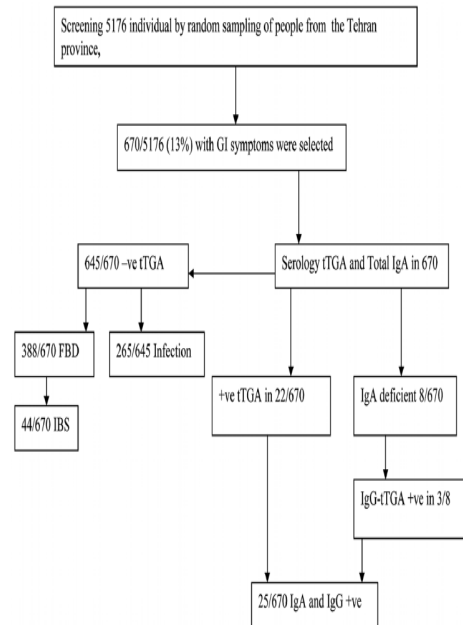

Figure 2. Flowchart or the study design. FBD functional bowel disorder, IBS irrritable bowel syndrome

The most prevalent symptoms in these 670 cases were dyspepsia (208/670), bloating (190/670), abdominal pain (185/670), constipation (139/670), weight loss (44/670), nausea (36/670), diarrhea (23/670) and reflux (23/670) (Fig.1). Constipation, heartburn, and bloating were significantly more prevalent in females compared to the male patients (Table IV).

Abdominal pain, heartburn, bloating and constipation were the most common symptoms found in tTG positive cases and diarrhea was found only in 2/25 cases (Table I). However, these symptoms were not specific for CD as the rest of the study group presented with similar symptoms.

A positive tTGA test was found in 22 out of 670 investigated subjects (17 women, 5 men) (95% CI: 1.70- 4.30) and 8/670 were IgA deficient. tTGG was positive in 3/8 IgA deficient. The most tTG positive patients

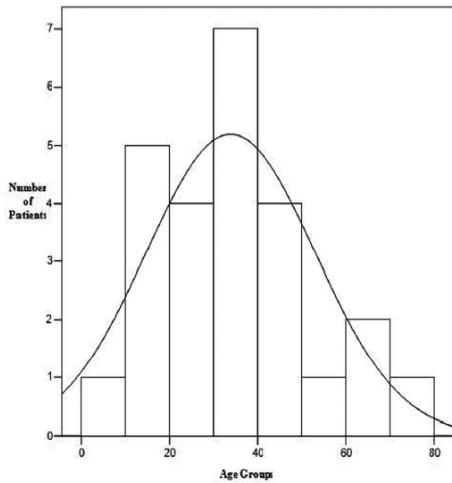ranged between 15- 45 years (14 patients) (Figs.3, 4).

Figure 3. Histogram of age (with normal curve) for patients with gastrointestinal symptoms

A multivariate logistic regression was performed to assess the relationship between GI symptoms and CD. Only weight loss (OR=3.45, 95% CI: 1.15-10.30) and constipation (OR=0.33, 95% CI: 0.13-0.82) appear to be correlated significantly with CD.

Table IV. The type and frequency of symptoms in all cases, actual number and (%)

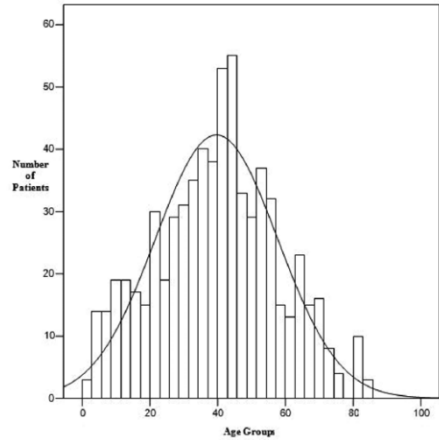| Symptoms	Females	Males	Total
Abdominal pain	142 (21.19)	43 (6.41)	185 (27.61)
Constipation	110 (16.41)	29 (4.32)	139 (20.74)
Diarrhea	13 (1.94)	10 (1.49)	23 (3.43)
Bloating	144 (21.49)	46 (6.86)	190 (28.35)
Heartburn	144 (21.49)	64(9.55)	208 (31.04)
Nausea	23 (3.43)	3 (0.44)	26 (3.88)
Weight loss	23 (3.43)	21 (3.13)	44 (6.56)
Dysphagia	18 (2.68)	6 (89)	24 (3.58)
Fecal incontinence	1 (0.14)	1 (0.14)	2 (0.29)

Figure 4. Histogram of age (with normal curve) for CD patients

Discussion

Coeliac disease is the major diagnosable food related disorder and often it is diagnosed late presenting with milder and more atypical symptoms [24]. Serologic screening studies suggest that it occurs in about 1-2.5% of the population around the world [25, 26]. However, serology does not detect a subgroup of atypical patients with milder mucosal abnormalities [27-30]. Our study included screening of patients with non-specific GI symptoms running a greater risk of CD, e.g., some of the subjects with dyspepsia and changing of bowel habits etc. Classically, the condition presented with malabsorption and failure to thrive in infancy, but this picture has now been overtaken by the much more common presentation in adults, usually with non-specific symptoms such as dyspepsia, disturbance in bowel habits or with symptoms outside the small bowel [31-39]. In this study, weight loss and constipation appeared to be correlated significantly with CD and dyspepsia was the most common

Table V. Typical presentation of atypical CD compared to classical CD

Investigations	Classical CD	Atypical CD outside small bowel	Atypical GI like this study
	Abdominal pain Chronic diarrhea Vomiting Weight loss Foul smelling stool Fatigue Failure to thrive or short stature Delayed puberty Osteoporosis Anaemia	Obesity Dyspepsia Constipation Depression and anxiety	Abdominal pain Dyspepsia Constipation
Biochemistry and haemathology	Iron deficiency Anaemia Low serum protein levels Low serum calcium levels	Macrocytic anaemia, Vitamin B12 deficiency, Abnormal liver function tests	
Serology	Positive serology, tTGA, EMA	Negative or +ve serology, IgA deficient, or only positive in small bowel mucosa or in stool samples	
Histology	Macroscopic lesion (Marsh IIIa-IIIc)	Microscopic and macroscopic lesions Marsh 0-IIIc affecting atypical site of small bowel: bulb or terminal ileum	

symptom in the whole study group (Fig. 1). This shows that a considerable number of coeliac patients do not have demonstrable clinical or functional characteristics of the disease [7, 40]. However, this atypical presentation especially with constipation has received considerably less attention than typical forms of disease such as diarrhea and malabsorption in clinical practice (Table V).

It was suggested several decades ago that symptomatology might be related to the extent to which the small intestine is structurally involved. In other cases, symptoms arise only when the compensating hypertrophied lower small bowel is defunctioned through other factors, such as an inter-current bowel infection. However, Murray et al and others clearly identified that the symptoms are not only predominately atypical but also they do not seem to be related solely to the degree of mucosal changes [41-43].

Similarly, the sensitivity of antibodies is not influenced by clinical presentation as it does not differ between patients with typical or atypical disease [44].

Interestingly, early CD has been shown to have gluten-dependent GI symptoms even at the microscopic stage of the mucosal lesion such as Marsh 0 or Marsh I [45-47]. The main issue is not the degree of mucosal changes, but the consistency of mucosal abnormalities with gluten sensitivity [48-51]. There are gluten-sensitised lymphocytes in the mucosa and this is what gluten sensitivity means, irrespective of the degrees of mucosal damage [46, 47, 49, 52]. Unfortunately, there are no facilities to look routinely for the subtle mucosal changes even in the most modern centers. In contrast to the current guidelines restrictions, we believe that symptomatic gluten sensitive cases with any degree of mucosal abnormalities

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would potentially benefit from a gluten free diet.

In this study, 380/670 presented functional and nonspecific bowel disorders. The symptomatology in this group was very similar to that of those with an organic etiology (Table VI). It is possible that there might be some unidentified gluten sensitive cases in this group which we have been unable to detect due to the lack of routine effective facilities [53]. Similar published analyses have shown that testing for CD in other symptomatic patients such as patients with suspected IBS is likely to be cost-effective even at a low CD prevalence (3–8%) [54-56]. Similar prevalence found in patients with dyspepsia and other atypical symptoms in this study would justify screening for gluten sensitivity in most patients with GI symptoms. Although a negative result of antibody screening does not exclude the CD diagnosis, a positive result of EMA/tTGA is associated with important histological changes. Therefore, with the limitations of serology [28, 29, 57] in detecting CD, one can assume that the prevalence of undiagnosed CD among patients with GI symptoms is even higher than the number of cases detected in this study.

Table VI. Gastrointestinal symptoms in 380 patients with functional symptoms and 290 with an organic GI disorder

Symptoms	with functional symptoms	with an organic etiology
Bloating	110 (28.9%)	80 (27.6%)
Heartburn	115 (30.30%)	93 (32.1%)
Nausea	16 (4.2%)	10 (3.4%)
Weight loss	30 (7.90%)	14 (4.8%)
Dyspepsia	18 (4.7%)	6 (2.1%)
Incontinence	1 (0.26%)	1 (0.3%)
Abdominal pain	102 (26.8%)	83 (28.6%)
Constipation	85 (22.3%)	54 (18.6%)
Diarrhea	11 (2.9%)	12 (4.1%)

One way to optimize the efficacy of screening would be by using the strategy suggested by Rashtak and Murray [5, 58]. They suggest using HLA typing as a high-sensitivity rule-out test when there is a high suspicion of CD and to use serologic testing a high-specificity rule-in test when the probability is low [5]. This strategy might be helpful in encouraging health professionals to use serology because the index of suspicion is generally low for atypical presentation. On the other hand, relying on serology alone might result in overlooking those patients with negative serology even when the suspicion is low [28-30, 57, 59]. Perhaps performing HLA typing in seronegatives would give some more degree of reassurance in ruling it out as suggested by Hadithi et al [58]. Finally, it is time to forget the classical GI presentation and focus on non-specific specificities of the CD spectrum when the health-related life quality of coeliac patients with atypical presentation is impaired. Implementing a new diagnostic strategy with a high index of suspicion based on recent evidence on atypical forms of CD would be the key step in identifying patients without symptoms.

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PART II

Chapter 3

Celiac disease in patients with chronic psychiatric disorders

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ABSTRACT

Aim: The aim of this study was to determine the prevalence of celiac disease in Iranian patients suffering from chronic depression or schizophrenia.

Background: Psychiatric disorders are common in untreated celiac disease.

Patients and methods: Two hundred Iranian inpatient men with in chronic phase of depressive disorders or schizophrenia, and 200 age-matched healthy male subjects were screened for celiac disease by anti-tissue transglutaminase IgA antibodies. The mean age of the study patients was 37 years.

Results: One (1%) schizophrenic and two (2%) depressive patients were positive for anti-tissue transglutaminase IgA antibodies; duodenal biopsy was not possible in these subjects. In the control group one (0.5%) individual was positive for anti-tissue transglutaminase IgA antibodies, but had normal duodenal histology. The difference between patients and controls was not statistically significant.

Conclusion: The frequency of celiac disease serology in schizophrenic and depressive inpatients was not significantly higher than that in the general population. We would therefore not advocate systematic serologic screening in these patients, but alertness to celiac disease should be kept in mind.

Keywords: Celiac disease, Depression, Schizophrenia, Serologic screening, Tissue transglutaminase antibodies.

Introduction

Celiac disease is an under diagnosed condition in which gluten ingestion in genetically susceptible individuals results in small-bowel mucosal inflammation and villous atrophy. Most patients are asymptomatic or suffer from mild symptoms only, (1) and many present with extraintestinal manifestations such as neurologic disorders (2). The anti-tissue transglutaminase antibody (tTGA) test is a sensitive and specific tool in disclosing celiac disease with overt villous atrophy (3). With a specificity of approximately 95%, false positive tests are uncommon, and tTGA may appear in serum at an early stage in the disease, in other words before the clinical manifestations and even before the development of villous atrophy (4). Psychiatric disorders are also common in untreated celiac disease, especially depressive symptoms (5-8). Hallert & Derefeldt reported that nine out of 42 studied subjects had attended a psychiatric clinic because of neurotic problems and most of them are involved with depressive disorders (7).

Celiac disease was considered relatively uncommon in Iran, until recently an estimated population prevalence of 1:166 was reported (9). Greater awareness of its varying presentation and the availability of new serologic tests have shown celiac disease to be relatively common (10). These observations prompted us now to assess the association between celiac disease and severe chronic depression and schizophrenia.

Patients and Methods

This cross-sectional study was carried out in 2006-2007. By random sampling, 200 inpatient men comprising 100 with chronic depression and 100 with schizophrenia (mean age 37 years, range 18-68 years) were enrolled in Razi Hospital, Tabriz, Iran. Chronic depression was defined according to DSM-IV criteria, 11 diagnosed and treated by semi-structural clinical interview by two psychiatric experts. The duration of the diseases was more than two years, and the diseases were unbearable without antipsychotic drugs. Patients with schizophrenia suffered from different types of the disease such as paranoid, phrenetic or undistinguished. Two hundred healthy males were selected as controls, matched for age and birthplace (mean age 32, range 4-77 years).

A written informed consent was obtained from patients (or from next of kin if necessary) and the study was approved by the Institutional Ethics Committees of the Research center for gastroenterology and liver disease, Tabriz Medical University. Blood samples were collected and the sera stored at −20°C until analysis. IgA class TTGA antibody was measured by enzyme-linked immunosorbent assay using a commercially available kit (Eu-tTG IgA, Eurospital, Trieste, Italy). A titer of > 7 U/mL was considered positive as recommended by the manufacturer. Serum IgA was measured in each subject.

Statistical analysis

A frequency of 0.6% celiac disease has been reported in Iran. 9 Assuming this frequency in 200 controls and a tenfold frequency in psychiatric patients (6%, as

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has been reported in many autoimmune conditions) the statistical power of 0.80 at a significance level of 0.05 would then be achieved. Percentages were compared by rates and proportion; 95% confidence intervals (CI) were reported.

Results

None of the 200 patients had a history of chronic diarrhea and all were taking antipsychiatric drugs (antipsychotic such as risperidone, haloperidol or perphenazine and anticholinegic for schizophrenic patients, and fluoxetine and tricyclic antidepressants for depressive patients). Three patients with chronic psychiatric disorders were TTGA positive, in which one (age 52 years) with schizophrenia and two (both 30 years of age) with chronic depression. Of these three patients, two refused duodenal biopsy and one died during the study period. In the control group, one (age 25 years) out of 200 individuals was positive, but duodenal histology proved normal. The prevalence of positive celiac disease serology in patients with chronic psychiatric disorders was thus slightly but not significantly higher than in controls; 1.5%, (95% CI: 0.38-4.03) and 0.5 % (95% CI 0.00025-2.44), respectively (*P*=0.0623).

Table 1. Clinical and laboratory features of male patients with schizophrenia and depression

	Schizophrenia (n=100)	Depression (n=100)	Healthy controls (n=200)
Age (yrs)	37±11[*]	37±8	32±14
tTGA+[†]	1	2	1
Constipation	12%	۱۲%	---
Heartburn	7%	10%	---

[*] Mean± standard deviation; [†] tTGA: IgA-class anti-tissue transglutaminase antibody

Clinical features and laboratory findings among the patients with schizophrenia and depression are shown in table 1. Five in the study group and none in the control group had selective IgA deficiency.

Discussion

In our reports, the frequency of positive celiac disease serology in Iranian inpatients suffering from depression or schizophrenia was 1.5%. By comparison, Pynnönen et al. have shown the prevalence of celiac disease in patients with depression to be 0.7% (5). The same authors have reported that in adolescent celiac disease patients the frequency of depression and disruptive behavioral disorders was higher than in controls, 31% and 7%, respectively (6).

The present observations support earlier findings that celiac disease is not increased in patients with schizophrenia. Eaton et al. (12) studied 7754 schizophrenia patients in Denmark and found a frequency of untreated celiac disease of 0.05%. In UK, West et al. showed that in subjects with celiac disease the prevalence of schizophrenia was 0.25%, the adjusted odds ratios showing no association between the two conditions (celiac disease *vs.* controls 0.76, 95% CI: 0.41-1.4) (13).

By contrast, a study in the UK revealed that patients with celiac disease developed schizophrenia 3 times more frequently than non-celiac controls (14). Some studies have suggested that schizophrenia and celiac disease may be associated with similar or adjacent genes (15,16). It has indeed been reported that genetic susceptibility in schizophrenia lies in human leukocyte antigen (HLA) DQ, similarly to autoimmune disorders such as celiac disease (17). By contrast, a recent

study showed no such HLA association in schizophrenia (18).

In a case report, the symptoms of schizophrenia were improved in a celiac patient aged 33 years after the introduction of gluten free diet (19). Here we had no opportunity to investigate the effect of gluten-free diet, since two patients refused and one died during the study.

In this present study, the frequency of positive celiac disease serology in patients with chronic depressive (1%) and schizophrenia (0.5%) was in fact similar to that found in healthy blood donors in Iran (0.6%) (9). In the latter study, the frequency of celiac disease in males (1.8%) was higher than in females (0.5%), although usually 60%-70% of celiac disease patients are female. We could not investigate females with psychiatric disorders, which may be considered as a limitation to the current study. On the other hand, all our patients were inpatients, indicating that they suffered from severe manifestations of chronic psychiatric disorders. Patients with selective IgA deficiency remain negative by tTGA IgA class screening, and we had no opportunity to test our 5 such subjects by IgG class tTGA. In blood donors positive IgG class tTGA was found in 9.8% (20). There may thus be additional celiac case in our study group, but we consider that this would not change our conclusions.

To conclude, mass screening for celiac disease in patients with depression or schizophrenia is not advocated. Despite this, alertness to celiac disease should be high, since early diagnosis and treatment by gluten-free diet may ameliorate the symptoms and quality of life of these patients.

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PART II

Chapter 4

Endoscopy and histological Pitfalls in Celiac disease diagnosis: Assessment of current practice, A Multicentre Study

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Submitted

ABSTRACT

Background and Study aim: The traditional diagnosis of celiac disease (CD) requires a small bowel biopsy to identify the characteristic mucosal changes. The current biopsy practice among endoscopists for CD is in most part unknown. The aim of this study was to compare the different diagnostic policies in various centers in their current practice. To measure the number of specimens submitted during duodenal biopsy among patients in Italy, Iran, Lithuania, Romania and the UK, and to determine the incremental diagnostic yield of adherence to the recommended number of specimens.

Patients and Methods: A total of 931 patients who underwent duodenal biopsy for CD were recruited retrospectively at nine centers in European and Middle Eastern countries. Small-bowel biopsies were obtained from the duodenal bulb and the second part of the duodenum. The histopathological appearances were described according to the modified Marsh classification.

Results: The most frequent degree of mucosal changes amongst Iranian subjects was Marsh IIIa whereas in the rest of the study population was Marsh IIIc. The most common number of biopsy specimens for Romanian subjects was 1 (52%) followed by 2 for Iranian (56%), 3 for Lithuanian (66.7%) and British patients (65%) and 4 for Italian patients (48.3%). The main presenting symptom was anaemia (18.7%) followed by malabsorption (10.5%), diarrhoea (9.3%) and dyspepsia (8.2%).

Conclusions: Despite the evidence based recommendations, this assessment revealed a poor compliance with major guidelines on diagnosis of CD. We emphasize that taking adequate number of duodenal biopsies is mandatory and essential for an accurate diagnosis or its exclusion of CD.

Keywords: Celiac disease, pitfall, biopsy specimen, multicenter.

Introduction

Celiac disease (CD) is an auto-immune disorder generated in genetically susceptible subjects via the ingestion of gluten containing grains such as wheat, rye and barley. The immune response to these grains leads to progressive damage in the small intestine and to the production of serum antibodies directed against tTGA (1-4).

Despite the availability of specific and sensitive serological tests diagnosis of atypical forms of CD could be very challenging (5). Intestinal biopsy is still considered a useful diagnostic tool for detection of CD in different stages (6). Histology also permits to evaluate the response to gluten-free diet, should there be any doubt about the diagnosis.

The sensitivity of the serological tests available does not correlate with the degree of mucosal abnormalities and according to many studies a negative serological test does not exclude the diagnosis of CD (7). The mucosal lesions in CD can be patchy, and if the sampling is insufficient, the risk of missing the diagnosis increases (8, 9). Low degrees of mucosal abnormalities like microscopic enteritis in small intestinal specimens are not specific for CD, but a combination of clinical, serological and genetic evaluation may help to confirm the diagnosis (10). If possible, it would be more desirable to avoid endoscopy completely. We might argue that endoscopy may be unnecessary in the small sub group of patients who have classic symptoms and positive serology, but we still find that taking a biopsy appears essential in the majority of cases

(11). A multicentre endoscopy database study found that most patients undergoing upper GI Endoscopy for indications such as anaemia, iron deficiency, and weight loss had no duodenal biopsy taken during the procedures (12). Smith et al. 2004 found that only one third of the patients with duodenal biopsies were actually diagnosed with CD; thus, pre-Endoscopy testing for CD is not specific (13). Moreover, a normal endoscopic appearance lacks sufficient sensitivity to exclude CD (14).

As mucosal abnormalities in CD are patchy and the orientation of biopsies are variable, multiple biopsies (4-6 biopsies) from the duodenal bulb and descending duodenum are usually recommended as indicated by most studies as the standard method for CD evaluation (10, 15-17). Study results by Pais and co-workers showed that 2 biopsies confirmed CD in 90 % of patients, while 4 biopsies established a diagnosis in 100% of cases (10). By using a large, international, pathology database we assessed the adherence to these proposed guidelines.

To address diagnostic yield of biopsies versus ecologic fallacy versus clinicopathologic correlation, we investigated the proportion of patients diagnosed with CD. We also investigated procedure-related factors associated with the submission of biopsy specimens and compared the different diagnostic criteria in various centers in Italy, Iran, Lithuania, Romania and the UK. This study elucidates how a guideline is exercised in clinical practice, both in terms of adherence to the recommendation as well as the incremental yield of adherence.

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Patients and Methods

In this retrospective multicentre study, data were collected in the period May 2009-May 2011 from Iran (Tehran), Romania (Bucharest), Lithuania (Vilnius), the United Kingdom (Birmingham) and 6 different areas in Italy, including Brescia, Bologna, Desio, Messina, Perugia and Empoli. For each center, at least 100 CD patients with endoscopic procedures and small bowel biopsies were included.

During the same period, the number of biopsy sample taken from each center and their clinical data were compared. For each specimen, the following information was available: sex and age of the patient, location and sample provider, summary of the clinical history, serology/genetic and histopathological findings.

GI symptoms such as abdominal pain, diarrhoea, constipation, nausea and vomiting, weight loss and flatulence as well as additional signs and symptoms such as iron deficiency anaemia, osteoporosis, hypertransaminasemia, and other related abnormalities were recorded.

To determine the number of duodenal biopsy specimens for each biopsy set, we used a free-text search of the pathologist's description of each sample. When present, specimens from the duodenal bulb were included in the total number of specimens submitted. In each lab, biopsies were interpreted by expert gastrointestinal pathologists (blinded to the clinical data). In Bucharest the patients suspected for CD are usually referred directly for the gastroenteroscopy because of lack of funding for serological tests and HLA-typing. The duodenal specimens in Bucharest were oriented using Endokit and the biopsies in some departments in Italy (Brescia, Desio and Messina) were oriented on acetate cellulose filters. Histopathological findings were evaluated in accordance with the original modified Marsh classification (1).

All centers used the cutoff of: >25 intraepithelial lymphocytes per 100 enterocytes for (Marsh I), increased intraepithelial lymphocytes accompanied by crypt hyperplasia (Marsh II), partial (Marsh 3A), subtotal (Marsh 3B) and total villous atrophy (Marsh 3C) as described in original classification (1). The final diagnosis of CD was considered when patients with abnormal mucosal findings were serologically positive for tTGA and/or EMA. Centers with the lowest and highest number of biopsy specimens were analysed separately and also compared in order to investigate causes for those differences. According to the follow up reports to ordering HLA typing, the genetic data available were incomplete and HLA DQ typing was carried out only in some centers.

Statistical analysis

We used the chi-square test to assess the association between adherence to the recommendation of submitting ≥ 4 specimens and the proportion of patients with pathological findings consistent with CD. The results with a p-value <0.05 were considered statistically significant. Because our data set did not contain information regarding HLA typing for all cases and clinical follow-ups, we defined a priority that results with either blunted villi (Marsh 3A) or flat villi (Marsh 3B/C) were meeting the pathological definition of CD. For assessing the relationship between ordinal categories such as the number of specimens and the pathologic diagnosis of CD, we used the Fisher's Exact Test for trend.

Results

Overall, 658 women and 273 men (mean age at diagnosis 35.4±17.25 years, median age 36 years, age range 1–72 years) with a diagnosis of CD were included. Around 70.7% of patients were women, and the mean (± standard deviation [SD]) age was 36.6-16.4 years. Lithuanian patients were the oldest, with a mean age of 43.7± 18.6 yrs, and Iranian patients the youngest (30±13.6 yrs). In all participating countries females were predominant. Sixty nine patients were excluded based on their incomplete data collection.

Marsh I and II lesions were noted in 152 individuals (16.3%), Marsh 3A was found in 108 (19.3%), Marsh 3B was found in 178 (19.1%) and Marsh 3C in 233 study subjects (25.2%) (Fig 1). When a pathological diagnosis of CD was defined as that of blunted or flat villi (Marsh 3A/B/C), a total of 519 individuals (55.7%) were categorized as having CD. In 18 individuals (1.9%) Marsh 0 was recorded (table 1). Regarding the number of biopsy specimens taken per CD case, we found that 1 biopsy specimen had led to a diagnosis in 63 (6.7%) cases (11 specimens were Marsh I and II and the rest was higher), 2 in 212 (22.7%) cases (96 specimens were Marsh II or lower), 3 in 306 (32.9%) cases (153 were Marsh II or lower), 4 in 299 (32.1%) cases (81 specimens were Marsh II or lower) and ≥ 5 in 51 (5.5%) cases (9 specimens were Marsh II or lower) (Fig 2). The average number of biopsy specimens per patient was 3 (Fig 3).

The most common number of biopsy specimens taken from Romanian subjects was 1 (52%) followed by 2 from Iranian patients (56%), 3 from Lithuanian (66.7%) and British patients (65%) and 4 from Italian

patients (48.3%). Villous atrophy (partial, subtotal or total) was the predominant histologic lesion in all studied countries (Fig 1).

Table 1. Frequency of different degrees mucosal abnormalities correlated to the number biopsies.

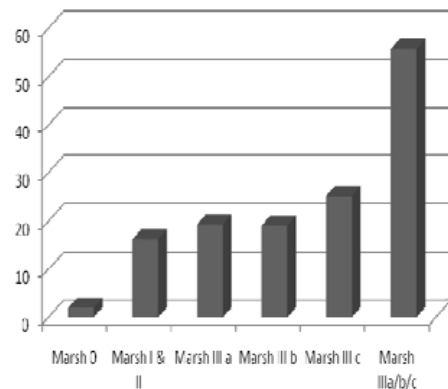| No. of Biopsy	Marsh 0	Marsh I	Marsh II	Marsh 3A	Marsh 3B	Marsh 3C
			Modified Marsh classification			
1	0	10	1	15	15	22
2	1	72	23	37	32	47
3	15	92	36	32	27	104
4	2	35	44	67	94	57
≥5	0	5	4	5	10	27

Fig 1. The percent of mucosal changes in this study group according to modified Marsh classification

The most frequent degree of villous atrophy for Iranian subjects was 3A and for the rest of the study population 3C (Table 2). According to the histological variability illustrated above, a statistical correlation was found between type and distribution of the histological lesions and the number of biopsy specimens (P= 0.0001). Four or more duodenal biopsies had been taken for 58.7% of the Italian, 37% of the British, 7% of the Romanian and 5% of the Iranian

studied subjects. None of the Lithuanian patients had 4 or more duodenal biopsies.

Routine immune-histochemical evaluation of T lymphocytes with CD3 and CD8 antibodies was used only in Iran and one center in Italy (Brescia). Serological data for anti-EMA was available in only 32.3% Iranian and Italian patients while serology results for anti-tTG IgA and anti gliadine IgA were available only in 39.3% and 6.3% of the Iranian, Italian and Lithuanian, respectively.

Table 2. Frequency of different degrees of mucosal abnormalities based on modified Marsh classification in different countries per 100 cases.

Country	No of Biopsy	Modified		Classification		Marsh		Total
		0	1	2	3A	3B	3C	
Iran	2	1	13	10	12	9	11	56
	3	1	6	7	13	4	8	39
	4	0	0	2	2	0	1	5
Italy	1	0	7	0	3	0	1	11
	2	0	25	2	21	17	16	81
	3	14	34	16	18	22	68	172
	4	2	34	42	64	93	56	291
	≥5	0	5	4	4	8	26	29
Lithuania	2	0	32	10	0	0	1	43
	3	0	51	12	0	0	23	86
Romania	1	0	3	1	12	15	21	52
	2	0	2	1	4	6	19	32
	3	0	1	1	1	1	5	9
	4	0	1	0	1	1	0	3
	≥5	0	0	0	1	2	1	2

HLA typing was not performed for Lithuanian subjects. If performed, there was no access to genetic results of the HLA DQ typing in this country. HLA typing was performed for very few selected cases in Romania. HLA-typing for Italian (72/602) and Iranian subjects (52/100)

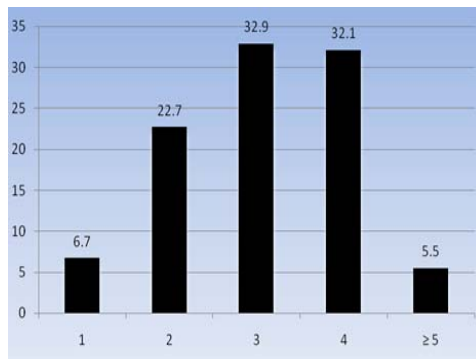showed that, HLA DQ2 is the predominant genotype in both countries.

Fig 2. Number of specimens submitted and the probability of the diagnosis of celiac disease

The clinical data available to pathologists also varied by region and the variability of this data show that, the main presenting symptoms of the majority of cases were anaemia (18.7%) malabsorption (10.5%), diarrhoea (9.3%) and dyspepsia (8.2%) (Fig 4).
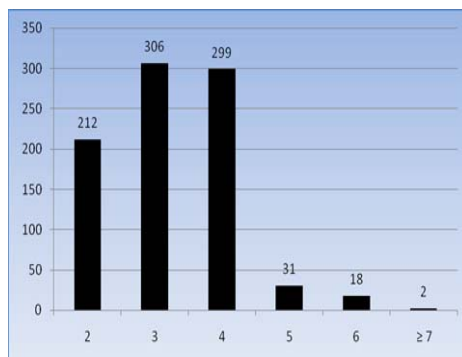

Fig 3. Histogram of number of specimens of small bowel biopsies among investigated patients (n=913)

There is a statistically significant correlation between these symptoms and abnormality in histology. As showed in fig 4 anemia and diarrhea is more correlated with Marsh 3c, malabsorbtion with Marsh

1 and dyspepsia with Marsh 3b. Table 3 shows most of the clinical symptoms for the countries investigated. 90 percent of the Lithuanian study subjects were affected by malabsorption. The most frequent symptom in Italian patients was dyspepsia (14.6%) (Probably related to high carbohydrate diet) followed by anaemia (14.2%) and diarrhoea (8.8%). For Iranian subjects predominant symptoms were anaemia (55%), diarrhoea (32%) and osteopenia (25%). Romanian subjects predominantly complained of diarrhoea (8%), abdominal pain (6%) and weight loss (5%). Statistically significant differences were observed when comparing the reported clinical symptoms in different countries.

In spite of the histological variability illustrated in this study, a statistical correlation was found between type and distribution of the histological abnormalities and some of the clinical symptoms such as abdominal pain, diarrhoea, low B12/Acid folic, malabsorption, IgA deficiency, dyspepsia, asthenia and failure to thrive (P $\geq$ 0.05). Table 4 shows the significant association between some of the clinical symptoms and the number of specimens submitted, with increased adherence to submitting $\geq$4 specimens and decreased adherence to submitting >4 specimens regarding a number of indications for patients undergoing endoscopy.

Among the 931 study subjects, 419 (45%) displayed typical presentation, 430 (46.2%) atypical presentation, and 64 (6.9 %) had no or only minor GI symptoms. A statistically significant correlation was found between abnormal histology and abdominal pain, dyspepsia and failure to thrive for Italian patients and between abnormal histology and anaemia for Lithuanian patients (P$\leq$ 0.5).
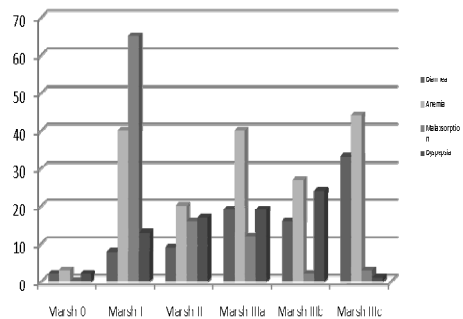

Fig 4. Compare the rate of anemia, diarrhea, dyspepsia and malabsorption in cases with abnormal histology (Marsh 0-II and Marsh IIIabc).

Discussion

Even though noninvasive antibody testing is an excellent screening tool for CD, endoscopic evaluation via duodenal biopsy in suspected patients remain crucial in the diagnosis of clinical atypical CD. The number of diagnosed patients has increased; this may be due to a greater proportion of outpatients being investigated for relatively minor symptoms, especially elderly people who visit their physicians more often (18).

Table 3. Clinical symptoms in different countries

Clinical symptoms	Country (n)				Total n (%)	P value
	Italy	Iran	Lithuania	Romania		
Abdominal pain	18	3	0	6	27 (2.9)	
Diarrhea	44	32	0	8	87 (9.3)	< 0.05
Anemia	71	55	37	0	174 (18.7)	< 0.05
Constipation	19	8	0	1	28 (3)	< 0.05
Family CD	49	1	0	0	56 (6)	< 0.05
Dermatitis herpetiformis	10	3	0	0	16 (1.7)	
IBS	10	0	0	0	10 (1.1)	
Osteoporosis	11	2	0	0	14 (1.5)	
Osteopenia	5	25	0	0	30 (3.2)	< 0.05
Malabsorption	6	0	90	2	98 (10.5)	< 0.05
Hypertransaminasemia	14	4	0	0	18 (1.9)	< 0.05
Thyroiditis	27	1	0	0	29 (3.1)	< 0.05
Autoimmune gastritis	2	0	0	0	2 (0.2)	
Weight loss	7	5	0	5	19 (2)	< 0.05
Aphthous	13	3	0	0	16 (1.7)	
Lymphoma		0	0	1	1 (0.1)	
Diabet	6	0	0	0	6 (0.6)	
Food allergy	1	4	2	0	7 (0.8)	
IgA deficiency	4	0	0	0	4 (0.4)	
Neurology symptoms	2	21	0	0	23 (2.5)	< 0.05
Menstrual abnormality	0	14	0	0	14 (1.5)	< 0.05
Bloating	2	11	0	0	13 (1.4)	< 0.05
Asthenia	9	0	0	2	11 (1.2)	
Failure to thrive	12	7	0	0	19 (2)	< 0.05
Low B12/Acid folic	8	0	0	0	8 (0.9)	
Dyspepsia	73	3	0	0	76 (8.2)	< 0.05
Gastritis	10	7	0	0	18 (1.9)	< 0.05

In this analysis of an international pathology database of duodenal biopsies, 37.6% of patients had ≥4 specimens submitted upon upper endoscopy. Adherence to the proposed standard remained low even for patients with typical CD, with fewer than 40% of such patients having ≥4 specimens submitted (1, 15, 18, 19).

As biopsy handling and experience of pathologists in different sites including duodenal bulb (17) can vary, it is suggested to take multiple biopsies from different duodenal sites in all cases where CD is suspected in order to minimize the risk of misdiagnosis (10). Biopsy specimens taken from the descending duodenum as well as jejunal specimens produce good results. However, contrary to common teaching and practice, duodenal bulb biopsy specimens appear adequate, and possibly should be taken as well (15, 17).

A duodenal biopsy should have a reasonable size, should be well orientated and adequately stained and should be thoroughly examined by an experienced GI pathologist (20). General pathologists are not aware of the variety of pathological changes seen in CD. Therefore, it appears of utmost importance in CD diagnosis that

all biopsies should be reviewed by an expert GI pathologist.

Villanacci et al. suggested that even a single biopsy taken from anywhere along the duodenum could theoretically lead to the diagnosis of CD if the specimen would have the quality and assessed according to the standards (20). More often, an impaired clinical state including the improvement of typical symptoms may be sufficient to provide a convincing clinical diagnosis. Having said this, some additional biopsies for complete diagnosis may be needed (21).

In a recent review, Yantiss & Odze 2009 referred to the "optimal method of obtaining biopsies in patients with celiac disease" as "controversial" (22). It may be stressed at this point that the proposed guideline entirely derives from the observation that the histopathologic abnormalities of CD are patchy, and can be missed if an insufficient quantity of specimens is submitted. The recommendation is supported by a single-centre retrospective study of 93 patients with CD, which found that 4 specimens led to a positive diagnosis in 100% of patients, whereas 2 specimens were diagnostic in only 90% of patients (10). The authors concluded that at least 4 duodenal biopsy specimens should be taken to rule out CD. A second study, investigating 56 patients with known CD, found that 3 biopsy specimens were sufficient as long as 1 specimen was obtained from the duodenal bulb; however, 5 biopsy specimens were necessary to recognize the most severe extent of villous atrophy. These studies are limited by small sample size and single-center settings (23). Evans et al. recently solve these problems. They investigated overall 461 patients including 126 newly diagnosed CD, 85 established CD, and 250 controls. Their study suggests that the optimal strategy for diagnosing celiac disease in both suspected patients with positive serology and those with established CD could only achieve 100 % sensitivity by always incorporating a duodenal bulb biopsy (17).

In this study, taking 1-2 biopsy specimens led to the confirmation of diagnosis in 29.5% of cases, while more than 3 biopsy specimens led to the diagnosis in 70.5%. We found a statistical correlation (P≤0.05) between the number of biopsies and histologic abnormalities. The variation in the number of biopsy samples in this study may be associated with endoscopists practice in each region. Endoscopists in Romania, Iran and Lithuania are more likely to perform less and fewer numbers of biopsies compared to endoscopists in Italy where CD is more common.

Because villous atrophy in CD is patchy and the orientation of the specimens is variable, 4 to 6 random biopsy specimens including at least one from the duodenal bulb are recommended in order to maximize the sensitivity and achieve a correct diagnosis of CD (10, 15, 17, 20). This may suggest that if additional biopsy specimens in our study had been taken from subjects CD may have been confirmed in more cases (10).

The incremental yield varied with indication and was greatest when the symptom was anaemia (18.7%). However, submitting ≥4 specimens also increased the diagnostic yield of CD even when the indication was dyspepsia (8.2%). Lecleire et al. suggest that endoscopic markers of

villous atrophy are not useful for the detection of celiac disease in patients with dyspeptic symptoms (24).

Retrospective analysis of pathology tissue databases produced high-quality analyses of GI epidemiology and quality measures (25, 26). In our study, conducted in a similar manner, we had some limitations. For instance, we did not have access to socioeconomic or racial data to determine whether these individual patient characteristics were associated with the submission of the recommended number of specimens. We also had no access to key variables that influence the likelihood of CD, such as data regarding family history of CD. We are looking at patients with confirmed diagnosis for most of the locations. We did not describe a yield of biopsies because the total number of patients biopsied for suspected CD was not included. Therefore, the false negative rate was not assessed. Moreover, information regarding the type of sedation used during the procedure and the degree of sedation, which may have impacted the ability to obtain ≥4 specimens, was not available. The degrees to which endoscopists adhere to such recommendations in clinical practice and the diagnostic yield of adherence to this standard have not been studied.

Since many of our CD patients did not have complete results of serology and clinical findings, we were not able to analyze the correlation between serology results and clinical findings regarding the degree of villous atrophy. Also, since the study was retrospective and many patients did not have uniform information regarding the degree of adherence to the diet in their medical record, we were not able to assess the correlation between seronegativity after treatment and the degree of compliance to the GFD (25). For example in Bucharest, for the majority of the patients that are suspected to have CD, endoscopy is performed prior to serological and genetic investigation; after the histological diagnosis of a picture compatible with CD, patients tend to have serological tests and, rarely, genetic testing. As methodological approach to the diagnosis of CD in this region may be attributed to high costs involved with genetic testing for DQ2/DQ8 (more than minimum salary/month), HLA typing is rarely performed. A similar situation is present in Iran and Lithuania; because HLA typing is expensive and access to laboratories that can perform genetic testing is limited, HLA typing is rarely carried out, and if performed, then it will be only in specific cases. This may represent the main obstacle for clinicians working without the facility for HLA-typing. However, the situation is not better in UK, as HLA typing is not popular between clinician again due to high cost and National Health Service strict cost saving policies.

Another obstacle for correct diagnosis in all investigated geographical locations may be that clinicians generally do not share clinical data or serology results of patients with pathologists. Most pathologists ignore the subtle histological changes like microscopic enteritis[27] in absence of such clinical data.

In our opinion this practice indicates the different levels of approach involved with the correct histological diagnosis of CD in different centers. Pathologists in general should know the subtle

manifestations and presentations of the disease. The evidence suggests that sharing the clinical data with pathologists might be essential toward a correct diagnosis as the pathologist is able to detect the majority of CD patients (26).

Our results indicate that we should implement the biopsy guidelines with ≥4 biopsy specimens into clinical practice including or with additional samples from bulb.[17] In our multicentre study only the minority of patients with upper GI endoscopy and duodenal biopsy had ≥4 specimens. In the light of the beneficial effect on the histological diagnosis of CD (28), in particular in atypical cases increased adherence to biopsy guidelines is warranted. In typical cases**,** as suggested by ESPGAN**,** the endoscopy and biopsy obviously might be avoided. However, the majority of celiac patients present with atypical manifestation where endoscopy and the correct sampling approach are paramount in diagnostic pathway (5).

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PART II

Chapter 5

The frequency of HLA DQ haplotypes in Iranian celiac disease patients

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ABSTRACT

Background: Celiac disease (CD) susceptibility has been strongly associated with HLA-DQ2 and -DQ8. No data are available on the distribution of these HLA alleles in Iranian CD patients, so our objective in this study was to assess the distribution of these alleles in Iranian CD patients and compare them to healthy Iranian controls.

Methodology: To predict the HLA-DQA1 and -DQB1 genes, we used the six previously reported HLA-tagging SNPs to determine HLA genotypes in 59 patients with CD and in 151 healthy individuals from Iran. To test the transferability of the method, 50 cases and controls were also typed using a commercial kit that identifies individual carriers of DQ2, DQ8 and DQ7 alleles.

Results: The results of the transferability test showed that the sensitivity and the specificity for DQ2 and DQ7 was 100% and 97%, respectively, and for DQ8 it was 86.4%. We observed that 96.6% of CD cases (n=57) and 57.6% of controls (n=87) were carriers of HLA-DQ2 and/or HLA-DQ8 heterodimers, either in the homozygous or heterozygous state. The HLA-DQ pattern of these 57 CD patients was: heterozygous DQ2.2 (n=14), and homozygous DQ2.2 (n=1), heterozygous DQ2.5 (n=33) and homozygous DQ2.5 (n=8), heterozygous DQ8 (n=13) and homozygous DQ8 (n=2). Twenty patients and controls were compound DQ2/DQ8 heterozygous. Two patients were negative for both DQ2 and DQ8 (3.4%).

Conclusion: We observed that the frequency of DQ2 was higher in CD patients than controls. The prevalence of DQ8 in our CD population was higher than that reported in other populations. Our results underline the primary importance of HLA-DQ alleles in the Iranian population's susceptibility to CD.

Keywords: HLA typing, validation, celiac disease, Iran.

Introduction

Celiac disease (CD) is characterized by malabsorption of nutrients in the small intestine after ingestion of wheat gluten or related proteins from rye and barley. The disease is characterized by villous atrophy of the small intestinal mucosa. With adherence to a strict gluten-free diet, most CD patients show prompt clinical and histological improvement, with relapse of the symptoms if gluten is re-introduced [1-3].

Many studies reflect the importance of genetic factors in the pathogenesis of CD [3]. The HLA-DQ2 heterodimer, which is coded by alleles DQA1*05 and DQB1*02, is present in approximately 95% of CD patients. The most important risk factor for CD is the DQ2.5 haplotype [4]. Alpha and beta chains of this heterodimer are encoded together in *cis* (DQA1*05, and DQB1*02) on a DRB1*03 haplotype. Other DQ heterodimers that are encoded in *trans* on DR7 haplotypes are related to the DQ2.2 haplotype (DQA1*0201/DQB1*02) [5-13]. The CD patients who do not carry the DQ2 heterodimer and are encoded by the DR4 haplotype carry HLA-DQ8 (α1*0301, β1*0302) [4]. HLA-DQ2 is common in Europeans and is expressed by 25-30% of the healthy European population. Consequently, the estimated HLA contribution to the development of CD is estimated to be approximately 35% [5-13].

Most of the CD patients who do not carry either DQ2.5 or DQ8, carry half of the DQ2.5 or DQ2.2 molecule (that is either HLA-DQA1*05 or HLA-DQB1*02), suggesting that carrying part of

the risk molecules still has functional implications for the risk of CD [13].

There are several ethnic groups, such as Persian, Kurd, Lur, Arab, Turkmen, Baloch, and Turk, living in Iran. Most Iranians are Muslims but Zoroastrians, Jews, Armenians, and Nestorians also live in this large country. Based on previous studies in Iran, the most common HLA haplotype in the different ethnic groups is DQ2 and DQ7 with prevalences of 22.1% and 25%, respectively [14-17] (fig 1).

These studies were performed in healthy populations in different parts of the country and showed considerable similarities in the distribution of HLA class II haplotypes with European countries. Recently, Monsuur et al. [13] described an HLA-tagging single nucleotide polymorphism (SNP) method for detecting the HLA risk alleles for CD. Using six SNPs, HLA genotyping for specific CD genotypes could be performed in a high throughput mode. The results of Monsuur et al. [13] and Koskinen et al. [12] showed that the sensitivity and specificity of this test in recognizing DQ2.2, DQ2.5, DQ7, and DQ8 haplotypes was >99% in the Dutch population and >98% in UK, Spanish, Finnish, Hungarian, and Italian populations.

We report the first attempt to type HLA in the Iranian population by this tagging SNP method, in both healthy individuals and in patients affected by CD.

Patients and Methods

Patients

A total of 59 CD patients (21 male and 38 female, median age 30.54 years, range 7–67 years) were included: 12

Figure 1. HLA haplotype frequencies in different ethnicities of Iran (refs 14-17).
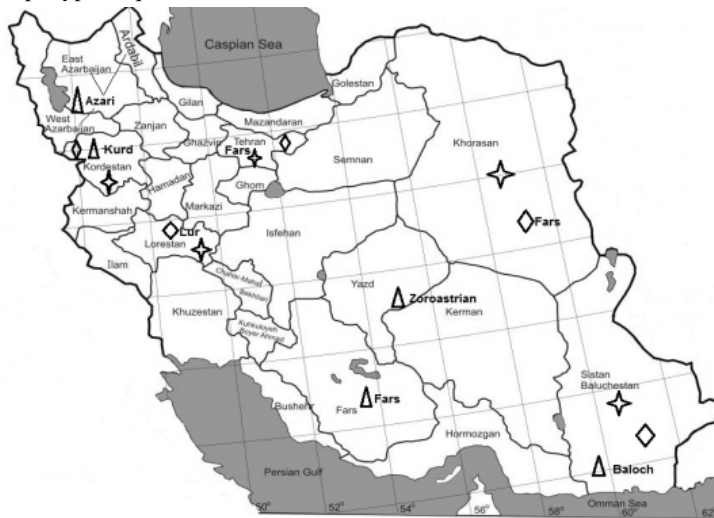
	Studied population	City	Ethnicity	HLA typing
✧				
◇	HLA typing in this study	Tehran	Fars	HLADQ2/7/8
		Mashhad	Fars	HLADQ2/7/8
		Lorestan	Lur	HLADQ2/7/8
		Kurdistan	Kurd	HLADQ2/7/8
		Sistan Baluchestan	Baloch	HLADQ2/7/8
⌂	HLA typing from previous studies	Fars	Fars	HLA DQ7
		Kurdistan	Kurd	HLA DQ7
		East and West Azarbaijan & Ardebil	Azari	HLA DQ7
		Sistan Baluchestan	Baloch	HLA DQ2
		Kerman & Yazd	Zoroastrian	HLA DQ2

patients from East Iran (Zahedan Province, Baloch ethnicity), 13 from North Iran (Mashhad Province, Fars), 10 from West Iran (Lorestan & Kurdistan Provinces, Lour and Kurd ethnic groups), and 24 patients from Tehran, in the center of Iran (Fars). All patients had positive tTGA and/or EMA antibodies and histology according to the Rostami-Marsh classification (Marsh I-IIIc) [18].

Controls

A total of 151 healthy controls (78 females and 73 males, median age 30.54 years, range 5–83 years) were selected from those who were negative for CD serological screening in the study areas (25 from Zahedan Province, 30 from Mashhad, 26 from Lorestan & Kurdistan provinces, and 70 from Tehran). None of them had a personal or family history of cancer or autoimmune diseases. The study was approved by the ethical committees of the Gastroenterology and Liver Diseases Research Centers, Shahid Beheshti University of Medical Sciences, Tehran, and all the participants (or their parents/guardians) were informed about the study according to the study protocol and gave their written informed consent.

HLA typing

DNA was extracted using the phenol chloroform method [19].

The six HLA-tagging SNPs reported by Monsuur et al. [13] were genotyped using 5 ng of DNA of the Iranian cohort, and using TaqMan chemistry and custom assays from Applied Biosystems (ABI, Foster City, CA, USA, www.appliedbiosystems.com), including DQ2.2 (rs2395182 and rs7775228 SNPs for DQ2.2 and rs4713586 SNP to exclude DQ4 from the DQ2.2 group), DQ2.5 (rs2187668 SNP), DQ7 (rs4639334 SNP), and DQ8 (rs7454108 SNP). DNA was genotyped using a standard protocol provided by Applied Biosystems. The PCR assays and allelic discrimination were run using an ABI PRISM 7900HT Sequence Detection System (SDS) machine. All individuals were run on the same 384-well plate to avoid biased results due to technical issues. The data was analyzed using SDS program 2.3 (Applied Biosystems).

Validation

The HLA alleles for 20 CD cases and 30 controls were also typed using a commercial kit (BAG, Germany). The BAG kit makes it is possible to identify individuals who are carriers of DQB1*02, DQB1*0301, DQA1*05, DQA1*0201 alleles for HLA-DQ2; DQB1*0302, DQA1*03 for HLA-DQ8; and DQB1*0301 for HLA-DQ7. We could thus distinguish DQ2.5-positive individuals from those who have DQ2.2.

Analysis

For the prediction of HLA alleles, we inferred the DQ types from the tag SNPs according to the method described by Monsuur et al. [13]. Only individuals with no missing data (59 cases) were entered in this study. The statistical analysis was performed using the Fisher exact test. A *P* value of less than 0.05 was considered statistically significant.

Results

Validation of the tag SNPs

Twenty random CD cases and 30 controls were first typed using both the BAG kit and the tag SNPs to test the validity of the tag SNP approach in the Iranian population. Nine cases and three controls showed discordant results between the two tests. Eight CD samples, which the tagSNP approach classed as non-DQ2/DQ8, were typed as DQ8 with the BAG kit. The results of the validation showed that the sensitivity to detect DQ2 and DQ7 was 100% and 84.6% for DQ8, while the specificity was 97% for DQ2 and DQ7, and 86.4% for DQ8 (table 1). These results suggest that DQ2 and DQ7 can be inferred using the tag SNP method, but that the detection of DQ8 is less sensitive and less specific. Heterozygotes and homozygotes of these haplotypes cannot be determined by the BAG kit for all samples, especially when the parallel haplotypes are unknown (DQX).

Testing the tag SNPs

We then evaluated the 59 CD patients and 151 controls for prediction of related HLA genotypes by TaqMan SNPs. We observed that 83.03% of cases and 42.4% of controls were carriers of an HLA-DQ2 heterodimer, either homozygously or heterozygously.

The patterns of the specific HLA-DQ haplotypes in the CD patients were as follows: 23.7% of CD patients were heterozygous DQ2.2 including DQ2.2/DQ2.5 compound heterozygosity, 1.7% of CD

patients were homozygous DQ2.2/DQ2.2, 28.8% of CD patients were heterozygous DQ2.5 including, DQ2.5/DQ8 compound heterozygosity, and 27.1% of CD patients were homozygous DQ2.5/DQ2.5.

Table 1. Validation results in the Iranian population using BAG kit compare to TagSNP

	Celiac patients		Controls	
	BAG Kit	Tag SNP	BAG Kit	Tag SNP
No. of patients	20	59	30	151
DQ 2.2				
Sensitivity	100	100	100	100
Specificity	100	100	100	100
False results	0	0	0	0
DQ 2.5				
Sensitivity	100	100	100	100
Specificity	97.8	97.8	96.7	96.7
False results	1	1	1	1
DQ 8				
Sensitivity	100	86.4	100	100
Specificity	100	86.4	93.3	97.1
False results	0	8	2	1

DQ8 heterozygosity was predicted in 13 patients, of which seven were DQ8/DQ2.5 carriers and two were homozygous DQ8/DQ8.

The HLA-DQ pattern in the controls was as follows: heterozygous DQ2.2 in 28.5%, non-homozygous DQ2.2/DQ2.2, and heterozygous DQ2.5 in 16.5%, and homozygous DQ2.5/DQ2.5 in one. DQ8 was positive in the heterozygous state in 30 controls and in the homozygous state in four controls.

Table 2 shows the frequency of HLA-related CD genotypes in the healthy controls and the prevalence of the different HLA-related CD genotypes in our Iranian CD patients. DQ2.2 and DQ7 can only confer risk to CD when both are present together or with DQ2.5 [13]. Based on this description, DQ2.2 and DQ7 haplotypes were present together or with DQ2.5 in

23.7% of CD patients and 11.9% of controls. A DQ2-DQ8-negative genotype was seen in 66 controls and two cases. DQ2.5/DQ2.2, DQ2.5/DQ2.5, DQ2.5/DQ8 and DQ2.5/DQX were detected as significant in the cases ($P < 0.05$) and DQ7/DQ7 and DQ7/DQX were observed as significant in the controls ($P < 0.05$). Accordingly, we can conclude that CD patients carry more HLA risk alleles than controls (table 2).

Table 2. The frequency of HLA genotypes in Iranian cases and controls[*]

Genotypes	Cases (%)	Controls (%)	*P-value*
DQ2.2/DQ2.2	1(1.7)	0	0.28
DQ2.2/DQ7	4(6.8)	8(6)	0.76
DQ2.2/DQX	3(5.1)	22(14.6)	0.06
DQ2.5/DQ2.2	7(11.9)	5(3.3)	0.04
DQ2.5/DQ2.5	8(13.6)	1(0.6)	<0.001
DQ2.5/DQ7	3(5.08)	5(3.3)	0.69
DQ2.5/DQ8	7(11.9)	5(3.3)	0.04
DQ2.5/DQX	16(27.1)	10(6.6)	<0.001
DQ7/DQ7	0	17(11.2)	0.004
DQ7/DQX	0	17(11.2)	0.004
DQ8/DQ2.2	0	8(5.3)	0.11
DQ8/DQ7	1(1.7)	9(6)	0.29
DQ8/DQ8	2(3.4)	4(2.5)	0.67
DQ8/DQX	5(8.5)	8(6)	0.54
DQX/DQX	2(3.4)	32(21.2)	0.001
Total	59(100)	151(100)	

[*] The frequency of DQ8/DQ2.2 in the cases was 0% and this number may be less reliable. This may be due to differences in the patterns of linkage disequilibrium blocks in Iranians or may result from low concentrations of DNA samples (the concentration of the samples was reduced when they were sent to UMCG for genetic analysis). We concluded that DQ2 worked well, but DQ8 needs to be interpreted more carefully with the tagSNP.

Risk groups

Based on the outcome of the HLA-DQ2/DQ8 prediction using tagging SNPs, we divided the Iranian population into three risk groups: low risk (these were DQ2/DQ8-negative) (13.5% of cases, 43.7% of controls), intermediate risk (these were homozygous for DQ2.2 and DQ8, or heterozygous for DQ8, DQ2.5 or DQ2.2)

(57.6% of cases, 39.07% of controls), and high risk (these were homozygous DQ2.5 or DQ2.5/DQ2.2) (25.4% of cases, 4% of controls) (Fig 2). The differences were statistically significant for all the risk groups when comparing cases to controls ($P < 0.05$). We also confirmed that CD patients carry more HLA risk alleles than healthy controls and this difference was statistically significant for the DQ2.5 haplotype distribution between cases and controls ($P = 0.0001$).

Ethnic heterogeneity

Based on sampling from four diverse areas in Iran (North, West, East and Center) with different ethnicity and in keep with previous studies which were introduced only the DQ2 for Baloch ethnic (15) and DQ7 for Kurd ethnic (17) both in healthy subjects of Sistan Baluchestan and Kurdistan provinces respectively, the outcome of the HLA prediction using tagging SNPs showed that HLA-

DQ2/DQ8/DQ7 are presented in all studied areas in both cases and controls. Although the HLA-DQ2 is principal genotype in cases and HLA-DQ7 in controls in these areas, but there is no statistically difference for the HLA haplotypes distribution between cases and controls according to their ethnic (P>0.5).

Marsh classification

Fourteen patients with Marsh I histology and 12 with Marsh II were compared to 33 patients with Marsh III (including 14 Marsh IIIa, 7 Marsh IIIb and 13 Marsh IIIc) in relation to their HLA-DQ haplotypes. Table 3 shows the distribution of HLA haplotypes according to the Rostami-Marsh classification [18]. Of the 25 patients demonstrating only minor small bowel mucosal changes (i.e. Marsh I and II histology), two (3.4%) were negative for DQ2 and DQ8. According to these findings, there were statistically significant correlations between the presence of

Table 3. Distribution of HLA haplotypes according to the degrees of mucosal changes

		Rostami-Marsh classification				
		Marsh I	Marsh II	Marsh III	Total	P-value
HLA	DQ2.2/DQ2.2	0	0	1	1	0.73
	DQ2.2/DQ7	0	2	2	4	0.46
	DQ2.2/DQX	1	0	2	3	0.2
	DQ2.5/DQ2.2	1	3	3	7	0.06
	DQ2.5/DQ2.5	1	2	5	8	0.46
	DQ2.5/DQ7	2	0	1	3	0.2
	DQ2.5/DQ8	1	1	5	7	0.78
	DQ2.5/DQX	5	2	9	16	0.96
	DQ8/DQ7	0	1	0	1	0.04
	DQ8/DQ8	0	0	2	2	0.53
	DQ8/DQX	0	2	3	5	0.13
	DQX/DQX	1	0	1	2	0.64
	Total	12	13	34	59	

DQ8/DQ7 haplotypes and a histology abnormality compatible with Marsh II ($P = 0.04$). Our result thus confirmed that patients with Marsh I or II histology carry low HLA risk alleles, such as HLA DQ8. We saw no statistically significant correlations between the other haplotypes and different Marsh classifications.

Discussion

Several epidemiological studies indicate that CD is a common disorder in the Iranian population with a prevalence of 1% [20-22], but there are no data on the frequency of HLA-related CD-predisposing alleles. We examined the frequency of the HLA-DQA1 and HLA-DQB1 haplotypes in Iranian CD patients and compared them to a matched, healthy control group.

A simple PCR-based SNP approach has been developed for large-scale population screening (Monsuur et al. 2008 [13]) and this method can be applied to different Caucasian populations [12].

A major advantage of this PCR-based SNP method is its ability to distinguish HLA-DQ2 and HLA-DQ8 homozygotes and heterozygotes which, in turn, translates into differences in the risk for CD. We tested the transferability of this method to the Iranian population and found that the sensitivity and specificity to detect the CD risk alleles was 100% and 97% for HLA-DQ2 and HLA-DQ7, respectively, but slightly lower for HLA-DQ8 (86.4%).

The lower sensitivity and specificity for HLA-DQ8 in the Iranian population might be attributable to differences in the patterns of linkage disequilibrium blocks in Iranians and Europeans. The frequency of the HLA haplotypes in Iran is not necessarily different from Europeans but the tagging SNP method might not be the ideal test to establish the frequency of CD-associated HLA-DQ8 haplotypes in this population.

As described for other populations [23-25], the most frequent haplotype in Iranian CD patients was HLA-DQ2.5 (69.5%) and this frequency is very similar to that in European CD populations.

Surprisingly, in contrast to the low population prevalence of HLA-DQ8 reported in the literature (0.6% in Cameroon, 2% in Italy, 2.3% in Hungary, 4.2% in USA, 6.4% in Finland and 7.6% in Japan) [12, 26], we found a rather high prevalence of this genotype in the Iranian CD population (25.4%). Our result is compatible with those presented by Catassi et al. in 2008 for Turkey (22%), North American Indians (25.3%), Mexico (28.3%) and Bushman (30%) [27].

Karell et al. and Polvi et al. suggested that only a small number of CD patients (6%) carry neither DQ2 nor DQ8 [28, 29]. Our study supports these figures, as we only found two CD patients (3.4%) who were DQ2-DQ8-negative.

Several studies have shown that individuals homozygous for HLA-DQ2.5 or heterozygous for HLA-DQ2.5/DQ2.2 genotypes have an increased risk for CD compared to those homozygous for HLA-DQ2.2 or heterozygous for HLA-DQ2.5 or for HLA-DQ2.2 [30-32]. Similarly, our results show that 25.4% of cases lie within the high risk group for CD compared to 44.9% in the low risk group. The frequencies of intermediate risk and high risk groups were, as expected, higher in cases than controls (Fig. 2).
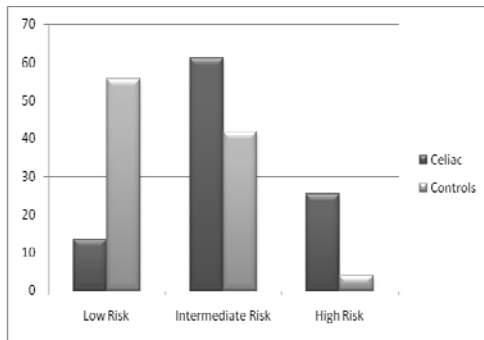
Figure 2. Distribution of the HLA risk groups in cases and controls according to tagSNP prediction. Low risk for those negative for DQ2/DQ8; intermediate risk for those homozygous for HLA-DQ2.2 or DQ8, heterozygous for DQ2.5 or DQ2.2; high risk for those homozygous for DQ2.5and DQ2.5/DQ2.2

In conclusion, despite a small study population as limitation of this study, the data presented in this study offer the first step towards defining the genetic structure of HLA in the Iranian population with celiac disease and provide information that can be used in family screening. In addition, our data suggest that the Iranian population, as a non-European Caucasian population, does share certain HLA class II genetic components with the populations of European countries. Iranians are more similar to Italian CD patients, given their increased frequency of HLA-DQ8 carriers. Further work, with larger sample sizes, on HLA polymorphisms among people living in different parts of Iran will provide more information about the genetic background of the Iranian population and its relationship to other populations in the world.

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PART II

Chapter 6

Gastrointestinal and non-gastrointestinal signs and symptoms in a large cohort of symptomatic patients with CD

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ABSTRACT

Background: Celiac disease (CD) may present in a variety of different ways. The aim of this study was to explore the etiology of gastrointestinal and non-gastrointestinal disorders in a large cohort of symptomatic patients with CD from a multi-center study which conducted across Italy and Romania from Europe and Iran from Middle East.

Methods: This is a multi-center study in which, data were collected in the period May 2009-May 2011 from Iran, Romania and Italy. For each center, only those confirmed CD with endoscopic procedures and small bowel biopsies and positive serology were included.

GI symptoms such as abdominal pain, diarrhoea, constipation, nausea and vomiting, weight loss and flatulence as well as additional signs and symptoms such as iron deficiency anaemia, osteoporosis, hypertransaminasemia, and other related abnormalities were collected.

Contingency tables, chi-square statistic and Fisher's Exact Test were employed to assess the association between the patient's country and the GI or Non-GI symptoms. T-test was used to comparison the numeric variable.

Results: Overall, 323 women and 127 men mean age at diagnosis 34.2±16.47 years, included in this study. 157 patients (34.9%) reported at least one gastrointestinal symptoms of disease. The main presenting GI symptoms of the majority of cases were diarrhea (13.6%), dyspepsia and constipation (4.0%). 168 patients (37.3%) reported other symptoms or diseases. The most presenting non-GI symptoms of the majority of cases were anaemia (20.7) and osteopenia (6%). Statistically significant differences between most symptoms were observed when comparing the reported clinical symptoms in different countries.

Conclusion: This multi-center study indicated that Upper abdominal disorders such as abdominal pain and dyspepsia were the most common primary complaints in European patients, while for Iranian patients, diarrhea and bloating were considered the classic presentations of CD. Between non-GI symptoms, anemia was highest for both Iranian and Italian patients, however in Iranian data, it was significantly higher.

Keywords: Celiac disease, multi-center, Clinical presentation.

Introduction

Celiac disease (CD) is the result of intestinal mucosal damage caused in susceptible subjects by the gluten content of some cereals. Celiac disease is often atypical or subclinical on clinical ground, so, many cases remain undiagnosed and become exposed to the increased risk of autoimmune disease (1). CD is a disease with the highest incidence (1in 100 to 1 in 300) in European countries (2, 3), and prevalence of 1 in 166 in apparently healthy blood donors in Iranian population (4). The frequency of CD in Iraqi type 1 diabetic patients was 11.2% (5). In another study from the Middle East, in Kuwait, CD accounted for 18.5% of cases of chronic diarrhea in children (6). CD may present in a variety of different ways: recurring abdominal pain and bloating, chronic diarrhea, constipation (in a few patients), excessive rectal gas, weight loss, mouth sores, fatigue, anemia (iron deficiency), osteopenia (osteomalacia, osteoporosis), swelling, fluid in the abdomen, behavior changes, mood disorders, growth retardation or with few or no apparent symptoms at all (7). This may have important health consequences, as dietary avoidance of gluten results in complete remission of the disease and avoids the two major complications, malignancy and osteoporosis (8) as well as resulting in a decreased mortality of patients with CD (9) and most of celiac patients are asymptomatic members of high-risk groups, such as patients with diabetes mellitus or thyroid disease and close relatives of patients with celiac disease (10). The recognition that there is a high prevalence of undiagnosed silent celiac disease in Western populations is already leading some experts to call for universal screening (11).

The aim of this study was to explore the etiology of gastrointestinal and non-gastrointestinal disorders in a large cohort of symptomatic patients with CD from a multi-center study which conducted across Italy and Romania from Europe and Iran from Middle East.

Patients and Methods

This is a part of a retrospective multi-center study in which, data were collected in the period May 2009-May 2011 from Iran (100 patients from Research institute of Gastroenterology and Liver Diseases, Taleghani Hospital, Tehran), Romania (100 patients from Fundeni Clinical Institute, Clinical of Gastroenterology and Hepatology, Bucharest), and 5 different areas in Italy, including 50 patients from Brescia (Spedali Civili Brescia), 50 patients from Bologna (St Orsola-Malpighi Hospital, University of Bologna), 50 patients from Messina (Regional Celiac Center, University Hospital ''G. Martino'', Messina), 50 patients from Perugia (Gastroenterology and Hepatology Section, Department of Clinical and Experimental Medicine, University of Perugia) and 50 patients from Milan. For each center, only those confirmed CD with endoscopic procedures and small bowel biopsies and positive serology were included.

All centers used the cutoff of: >25 intraepithelial lymphocytes per 100 enterocytes for (Marsh I), increased intraepithelial lymphocytes accompanied by crypt hyperplasia (Marsh II), partial (Marsh 3A), subtotal (Marsh 3B) and total villous atrophy (Marsh 3C) as described in

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modified classification by Rostami et al (12). The final diagnosis of CD was considered when patients with abnormal mucosal findings were serologically positive for tTGA and/or EMA.

During the same period, the number of biopsy sample taken from each center and their clinical data were compared. For each specimen, the following information was available: sex and age of the patient, location and sample provider, summary of the clinical history, serology/genetic and histopathological findings.

GI symptoms such as abdominal pain, diarrhea, constipation, nausea and vomiting, weight loss and flatulence as well as additional signs and symptoms such as iron deficiency anemia, osteoporosis, hypertransaminasemia, and other related abnormalities were collected.

Contingency tables, chi-square statistic and Fisher's Exact Test were employed to assess the association between the patient's country and the GI or non-GI symptoms. T-test was used to comparison the numeric variable.

Results

Overall, 323 women (71.8% of total patients) and 127 men mean age at diagnosis 34.2±16.47 years, median age (age range 1–87 years) with a diagnosis of CD were included in this multi-center study from Italy, Romania and Iran. Romanian patients were the oldest, with a mean age of 38.9± 12.3 yrs, and Iranian patients were the youngest (31±13.6 yrs). In all participating countries females were predominant (Table 1).

Marsh I and II lesions were noted in 26.4% of patients, Marsh 3A was found in 21.8%, Marsh 3B was found in 19.3% and Marsh 3C in 32.4% of study subjects.

There was a statistically difference for marsh classification across the countries (Table 1).

29 patients (6.4%) reported the family history of CD, however, in Romanian data no reported about family history was registered. 157 patients (34.9%) reported at least one gastrointestinal symptoms of disease. The main presenting GI symptoms of the majority of cases were diarrhea (13.6%), dyspepsia and constipation (4.0%). Up to 62% of Iranian patients reported GI symptoms and the most frequent GI symptoms in Iranians were diarrhea (32%), bloating (11%), constipation (8%), etc. In Italian patients about 32% had any GI symptoms in which abdominal pain (32%) was predominant followed by dyspepsia (14%) and diarrhea (8.4%). In Romanian patients with 15% reporting GI symptoms, diarrhea was more than other symptoms (8%) and after that, abdominal pain (6%) and weight loss (5%) were predominant (Table 2).

168 patients (37.3%) reported other symptoms or diseases. The most presenting non-GI symptoms of the majority of cases were anaemia (20.7) and osteopenia (6%). Iranian patients had reported up to 79% of any type of non-GI symptoms including anemia (55%), osteopenia (25%), Neurological symptoms (21%), menstrual abnormality (14%) etc. in Italian patients with 34.8% of non-GI symptoms the frequents ones were anemia (15.2%), osteopenia (4%) and thyroiditis (4%). In Romanian data the non-GI symptoms was only asthenia (2.0%) and other symptoms were not reported (Table 3). besides, 79 patients (17.9%) have had the combination of at least one GI symptom and non-GI symptom (Table 3). Statistically significant

Table1. Gastrointestinal symptoms in different countries

GI Diseases or Symptoms	Country (%)			Total	P-value
	Iran	**Italy**	**Romania**		
Abdominal pain	3 (3.0)	8 (32.0)	6 (6.0)	17 (3.8)	0.41
Diarrhea	32 (32.0)	21 (8.4)	8 (8.0)	61 (13.6)	<0.001
Constipation	8 (8.0)	9 (3.6)	1 (1.0)	18 (4.0)	0.04
Weight loss	5 (5.0)	6 (2.4)	5 (5.0)	16 (3.6)	0.33
IBS	0 (0.0)	5 (2.0)	0 (0.0)	5 (1.1)	0.13
Malabsorption	0 (0.0)	4 (1.6)	2 (2.0)	6 (1.3)	0.4
Bloating	11 (11.0)	1 (0.4)	0 (0.0)	12 (2.7)	<0.001
Dyspepsia	3 (3.0)	36 (14.0)	0 (0.0)	39 (8.7)	<0.001
Gastritis	7 (7.0)	6 (2.4)	0 (0.0)	13 (2.9)	0.01
Any GI Symptoms	62 (62.0)	80 (32.0)	15 (15.0)	157 (34.9)	<0.001

Table2. Non-Gastrointestinal symptoms in different countries

Non-Gastrointestinal Signs or Symptoms	Country (%)			Total	PValue
	Iran	**Italy**	**Romania**		
Anemia	55(55.0)	38(15.2)	0	93(20.7)	<0.001
Dermatitis herpetiformis	3 (3.0)	4 (1.6)	0	7 (1.6)	0.23
Osteoporosis	2 (2.0)	10 (4)	0	12 (2.7)	0.10
Osteopenia	25(25.0)	2 (0.8)	0	27 (6.0)	<0.001
Hypertransaminasemia	4 (4.0)	7 (2.8)	0	11 (2.4)	0.16
Thyroiditis	1 (1.0)	10 (4.0)	0	11 (2.4)	0.52
Aphtosis	3 (3.0)	7 (2.8)	0	10 (2.2)	0.23
Diabetes Mellitus	0 (0.0)	4 (1.6)	0	4 (0.9)	0.20
Food allergy	1 (1.0)	1 (0.4)	0	2 (0.4)	0.56
IgA deficiency	0 (0.0)	3 (1.2)	0	3 (0.7)	0.30
Neurological symptoms	21(21.0)	2 (0.8)	0	23 (5.1)	<0.001
Menstrual abnormality	14(14.0)	0 (0.0)	0	14 (3.1)	<0.001
Asthenia	0 (0.0)	4 (1.6)	2(2)	6 (1.3)	0.40
Failure to thrive	7 (7.0)	4 (1.6)	0	11 (2.4)	0.003
Low B12/Acid folic	0 (0.0)	3 (1.2)	0	3 (0.7)	0.29
Any non-GI Symptoms	70(79.0)	87(34.8)	2 (2)	168(37.3)	<0.001
Combination of GI and non-GI Symptoms	46(46.0)	32(12.8)	1 (1)	79(17.6)	<0.001

differences between most symptoms were observed when comparing the reported clinical symptoms in different countries (Table 2 & 3).

In spite of the variability of symptoms among different countries which illustrated in this study, no statistical correlation was found between total GI or non-GI symptoms and their combination with demographic factors including sex and age group in this multi-center study. Also this correlation was not significant for histology type too (Table 4).

Table4. Demographic factors: GI and Non-GI symptoms in Modified Marsh classification

	GI Symp.	P-value	Non-GI	P-value	Total	P-value
Age Group						
<15	26.3		38.6		10.5	
15-30	37.6	0.32	36.8	0.97	20.8	0.24
>30	35.4		37.3		17.5	
Sex						
Male	36.2	0.74	33.9	0.39	16.5	0.78
Female	35.4		38.7		18	
Marsh Classification						
I	31.7		34.9		15.9	
II	42.9	0.58	42.9	0.71	23.2	0.78
IIIA	37.8		39.8		18.4	
IIIB	31		39.1		17.2	
IIIC	33.6		33.6		15.8	

Discussion

In our study, females represented 72% of all patients, which is similar those observed in the United States, Europe and Middle-East with a female predominance (13-15). The reason behind this female predominance is unknown, but could be explained by the fact that the prevalence of immune mediated diseases in general is higher in women than in men (16).

In this study no difference was observed among male and female according to GI or non-GI symptoms, while some studies presented more GI symptoms for male and more non GI symptoms for female (16).

This multi-center study indicated that Upper abdominal disorders such as abdominal pain and dyspepsia were the most common primary complaints in Italian or Rumanian patients similar to other European countries (17), while for Iranian patients diarrhea and bloating were considered the classic presentations. Studies indicated that CD is the most common cause of adult chronic non-bloody diarrhea in Tehran (18) and this is a common presentation for CD in the Middle East (6, 19).

In non-GI symptoms, anemia was highest for both Iranian and Italian patients, however in Iranian data, it was significantly higher. Osteopenia was accounted for 25% in Iranian patients.

We know that wheat and barley are the major diet for most of Middle Eastern population and there are very few alternative diets to gluten-containing crops (4). Therefore the severity CD maybe higher in these area, compared to western countries in the form of anemia or osteopenia (20).

Some studies report a high number of neurological disorders in patients with CD (21, 22). In contrast to Italian and Romanian patients, neurological symptoms were the third highest non-GI presentation in Iranian database. The link between CD and neurological disorders might be attributed to the genetic background, most importantly the HLA region and other markers (22).

There are some limitations in this multi-center study; some information about symptoms for Romanian patients was not completed, we did not have access to socioeconomic or racial data and moreover, information regarding the type of sedation used during the procedure was absent.

This multicenter study was performed in different populations where CD is very common and shows that the clinical picture of CD might be variable. In conclusion, we found that the lower GI symptoms and anemia were higher in Middle Eastern population and upper abdominal symptoms such as abdominal pain and dyspepsia were the most common primary complaints in European patients. There was a significant association with non-GI conditions like diabetes type I, abnormal liver enzymes and other autoimmune conditions in Italian patients. It is, therefore, likely that a significant number of patients undergoing endoscopy for GI and non-GI symptoms, such as diarrhea, dyspepsia and anemia might have celiac disease. This study suggest, a high index of suspicion for CD would be required to detect CD in patients with chronic diarrhea, osteopenia and anaemia in Iranian patients. Similarly dyspepsia,

anaemia and other upper GI symptoms were more likely to be consistent with CD in European countries and may warrant screening for CD.

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PART II

Chapter 7

Gluten associated dyspepsia; serology and histological characteristics

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ABSTRACT

Aim: The aim of this study was to assess the prevalence of celiac disease (CD) in dyspeptic patients.

Background: Although severe mucosal abnormality with villous atrophy (lesions Marsh III) is the gold standard for the diagnosis of CD, non-specific microenteropathy (Marsh I-II) with positive serology is also common in patients with dyspepsia.

Patients and methods: From November 2007 to October 2008, 407 randomly chosen patients who underwent diagnostic upper gastrointestinal endoscopy for dyspeptic symptoms (193 male, 214 women; mean age 36.1 years) were studied. Small bowel biopsies were performed in all of them. Histologic characteristics in duodenal biopsy specimens for CD were evaluated according to the modified Marsh classification. All the patients were also tested for serum total immunoglobulin A and anti-transglutaminase (tTG) antibodies. Those with IgA deficiency were tested for IgG tTG.

Results: Duodenal histology showed Marsh I-IIIc lesions in 6.4% cases. Four patients (0.98%) were IgA deficient and none of them were positive for IgG tTG. Serology showed positive results for tTGA in 8% of the patients and 2.5% of them had abnormal histology (Marsh I-IIIc) compatible with CD.

Conclusion: The results of this study showed that milder enteropathy (Marsh 0-II) have a low specificity for CD. The prevalence of CD among dyspeptic individuals is significantly (2.5%) higher than in the general population (1%) and CD should be investigated in these patients.

Keywords: Dyspepsia, Celiac disease, Antibody.

Introduction

Coeliac disease (CD) and dyspepsia are common conditions, and consume considerable resources in both investigation and treatment. In the last years, a considerable change in epidemiology of CD has been observed. A marked increase in CD prevalence and incidence with milder enteropathy has been reported (1, 2), which can be at least partially explained by both the development of more sensitive serological tests and a high degree of disease suspicion (3, 4). The variability of in particular clinical (5) and histological aspects of CD may face the clinician often with uncertainty as some of the features might not quite fit in the diagnostic models in the current guidelines.

Malabsorptive symptoms, such as weight loss, diarrhea/steatorrhea and abdominal distension may not be necessarily observed in many celiac patients (6). Atypical forms of CD have increased considerably (7) and the presence of dyspepsia as a unique symptom has been frequently attributed to CD (8). In classical CD with prominent malabsorptive features, dyspepsia may be also one of the symptoms. It has been reported that the frequency of CD in people with dyspeptic complaints is 1.1-3%, which is two to nine times higher than in the general population (6, 8-12). The frequency of CD in the Iranian general population is considered to be around 1% (9).

In the present study we described the prevalence of celiac disease in dyspeptic patients.

Patients and Methods

Between November 2007 and October 2008, 5732 patients aged 15 years or more attended the Gastroenterology section of the Taleghani hospital of Tehran, Iran. Four hundred and seven patients (193 men and 214 women) randomly chosen patients with dyspepsia were prospectively studied. The study was approved by the institutional ethics committees of Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, and all participants signed a written informed consent.

Individuals were considered dyspeptic if they complained of persistent pain or uneasiness in the upper abdomen. Upper GI endoscopies were performed in these patients to diagnose common causes of dyspepsia including esophagitis, peptic ulcers, duodenitis and cancer. In addition, CD was identified by histological alterations characteristic of gluten sensitive enteropathy and by consistent CD serology.

Gastric biopsies were obtained for *H.pylori* detection and biopsies from the second part of the duodenum for histological processing.

Histological diagnosis of CD was based on the presence of intraepithelial lymphocytes, crypts hyperplasia and/or villous atrophy. Biopsy results were classified as absence of CD (Marsh 0) or suggestive of CD (Marsh II to IIIc), according to modified Marsh criteria (13, 14). The histological specimens were examined by two pathologists who did not know the endoscopic results and clinical history of the patients. The sera of these patients were analyzed for IgA

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class human antitissue transglutaminase (tTG) antibody and total serum IgA values according to standardized methods (15). Serological data were correlated to the endoscopic results and to the histological pattern observed in the small intestine. All patients with confirmed CD diagnosis were treated with a gluten free diet and followed.

Statistical analysis was performed using SPSS software, version 13.5. Descriptive variables such as mean, median and standard deviation were determined. Chi-square (χ2) test was performed to find out the association between CD and risk factors.

Results

The mean age of the patients was 36.1 years. The gastroenterology symptoms in the subjects were: 78% abdominal pain, 70% bloating, 58% heart burn, 46% early satiety, 32% nausea, 32% flatulence, 31% weight loss and 22% anorexia. Recurrent abdominal pain, heart burn and bloating were present in 60%, 45% and 31% of the patients, respectively (figure 1).
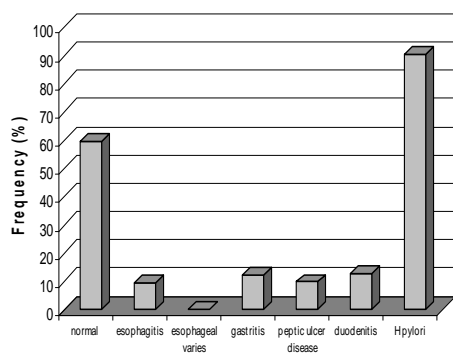
Figure 1.   Current endoscopy findings in study population

Helicobacter pylori was detected in 90.5% cases. There were 26 cases with enteropathy (12 Marsh I, 4 Marsh II, 2

Marsh IIIa, 6 Marsh IIIb and 2 Marsh IIIc). Four of 407 dyspeptic patients were IgA deficient and all of them were negative for IgG tTG. Thirty three (8.1%) of the 407 patients tested had tTGA level more than 15 u/ml and considered as tTGA positive. Twenty three of 33 seropositive had normal small bowel mucosa.

The demographic, histologic and serologic characteristics of 33 patients with serology positive and 26 with abnormal histology are shown in table 1.

In 10 of 33 tTGA positive patients, CD was confirmed by histological analysis of the intestinal biopsy samples, giving a prevalence of CD of 2.45%. Five of these 10 celiac patients were Marsh IIIa-c followed by 3 Marsh I and 2 Marsh II. The highest rate of histological abnormalities and of CD seropositivity was found in the age categories of 21-30 years and 10-20 years respectively (table 2).

Table 1. Clinical and laboratory features of seropositive patients

Subjects	Abnormal histology patients	Seropositive patients
No. of cases	26	33
Mean age	37.9	42.6
Gender		
Male	11	15
Female	13	20
GI symptoms		
abdominal discomfort	18	25
anorexia	6	8
weight loss	11	9
nausea	5	9
heart burn	14	10
early satiety	8	9
flatulence	7	8
bloating	12	15
H. pylori	21	26
Coeliac disease	10	10

Table 2. Cases with histology and serology consistent with celiac disease

Marsh classification	No. of patients	Gender		Mean age
		Female	Male	
Marsh I	3	2	1	27.3
Marsh II	2	1	1	39
Marsh III (a-c)	5	4	1	26.8
tTG +ve with normal histology	23	12	11	48.3

Discussion

Dyspepsia is a highly prevalent and heterogeneous disorder (16). We know that damages in CD are not confined to the small intestine (17) and not every celiac patient develop severe mucosal small bowel abnormality. Several studies have demonstrated that chronic exposure to gluten may damage the structure and function of the gastric mucosa in CD patients (18, 19). Other surveys indicate that approximately 20% of patients with dyspeptic symptoms have erosive esophagitis, 20% are estimated to have endoscopy-negative reflux disease, 10% have peptic ulcer, 2% have Barrett esophagus and 1% or less have malignancy (20) and the results of the present study suggest that at least 2-3% CD in dyspeptic patients should be e added to the list. However, the proportion of celiac autoantibodies in dyspepsia seems to be even higher (serology>8%) and the question is whether these antibodies are representing a different form of gluten related disorders or belong to the spectrum of false positivity.

The most important identifiable causes underlying dyspeptic symptoms in our study group were duodenitis (13%), gastritis (12%), esophagitis (9%) and peptic ulcer disease in 10% Malignancies

of the upper gastrointestinal tract were not found. Approximately, 60% of patients with dyspepsia showed no abnormality in their mucosa but the majorities were positive for *H. Pylori*.

It is important to note that serology at high level (when 10x >cut-off of normality) is a far more specific marker for atypical CD compared to microenteropathy (Marsh I-II) which seems to have a non-specific nature (23). With other words the specificity of serology at high level for CD seems to be close to 99% in many studies (24). Similarly histology represents the gold standard for CD diagnosis only in cases with severe mucosal abnormality (Marsh IIIa-c). A better definition and differentiation of true value of milder positivity of both histology and serology would be useful in clarifying the expectation of each test (25, 26).

We are aware that there is not a single perfect test available to diagnose CD in its own. Histological abnormalities ranging from mild to severe were found in the small bowel of 6.4% of our patients. Despite high specificity of autoantibodies, this finding would provoke the discussion on seronegative cases and question the sensitivity of serological tests. Although, microenteropathy could be a result of any other intestinal disorder, from previous experience we learned those negative serological tests were less reliable in symptomatic cases presenting with a milder enteropathy (21, 27, 28).

Serology at weak positive level and milder histology (microenteropathy) are both nonspecifics for CD. A combination of clinical presentation, histology, serology and HLA typing would contribute in making a more accurate diagnosis. The

limitation of this study was lack of second serological test in particular using Endomysial antibodies after tTG and lack of HLA typing for exclusion of non-celiac cases. Coeliac disease with flat mucosa based on which the gold standard was introduced >50 years ago is still a rare condition. It is time to recognize that for a good proportion of CD cases histology is non-specific and hence the pathologist is unable to make the definite diagnosis in his own. Serology at high level and histology with severe abnormalities are both reliable markers for CD. Milder enteropathy and low positive antibodies require a better identification. Future studies would be needed to assess whether dyspeptic patients presenting with positive antibodies and normal histology would benefit from a GFD?

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PART II

Chapter 8

Underweight in Adult's Celiac Patients in community; is screening program necessary in low weight individuals?

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To the Editor

Recently we studied celiac disease (CD) in patients with GI symptoms in Iranian community [1]. According to our findings and similar studies, related malabsorptive symptoms, such as diarrhea/steatorrhea and abdominal distension may not be observed in many cases necessarily [1, 2] but growth failure in terms of length (or height) or weight may be the earliest sign of the disease [3].

In this paper, we have studied body mass index in celiac patients diagnosed in a population-based study, using a well-matched case-control analysis in order to obtain updated information regarding the underweight in celiac patients [1, 4, and 5].

From total of 670 individuals with GI symptoms 25 individuals were positive for tTGA [1]. Total of 70 healthy people selected from the same database [5].

These healthy people were selected from the population in which CD patients drawn up but without any signs of GI symptoms. They matched according to demographic factors (sex and age group) with CD patients in order to compare height, weight and body mass index. The study was approved by the Ethics Committee of RCGLD.

A positive tTGA test was found in 22 out of 670 investigated subjects (17 women, 5 men), and positive tTGG test was found in 3 of 8 IgA deficient and matched with 70 healthy subjects (44 women, 22 men) entered to this case control study.

The mean±sd for body mass index in tTG positive patients and in healthy subjects were 23.7±2.38 and 26.8±4.3 respectively (P<0.001). Twenty two patients were observed with BMI<25, three patients in overweight group (BMI: 25-30) and no patient observed in obese group (BMI>30), in contrast of control group where 26 subjects were observed with BMI<25, 26 subjects in overweight group and 18 subjects in obese group (P<0.001). Table 1 showed anthropometric measurements including weight, height and BMI according to sex group, indicating that all anthropometric measurements were significantly low in CD patients compared to health controls in both men and women.

In contrast with Dickey and Kearney study [6] which state that few celiac patients are underweight at diagnosis time; our results indicated that a large group of CD patients in our community are underweight and there were differences in anthropometric measurements between CD patients and healthy controls, however other studies reported at least up to 33% of CD patients underweight [7, 8].

Given the fact that all patients who entered in this study were diagnosed for the first time and most of them are in adulthood, it can be concluded that the low weight and height in adult CD patients in our community are associated with a lower total daily energy intake because of misdiagnosed of disease.

Table 1. The mean ±sd of Anthropometric measurements for 22 CD patients and 70 sex- and age-matched healthy control subjects

	Men			Women		
	Patients	Control subjects	P-Value	Patients	Control subjects	P-Value
Weight (kg)	55.0 ± 5.5	76.8 ± 13.3	<0.001	62.0 ± 8.2	68.5 ± 11.1	0.03
Height (cm)	162.0 ± 5.9	173.1 ± 17.9	0.04	163.2±5.5	158.4 ± 8.5	0.02
BMI (kg/cm2)	20.9 ± 0.7	25.8 ± 4.1	<0.001	23.2 ± 2.4	27.4 ± 4.4	<0.001

We did not consider dietary components and life stress. This is potential limitations of this study.

On the other hand the high numbers of underweight individuals must draw the attention of physicians, during clinical practice, to consider celiac disease at the top of list of causes of low body weight and there is a real necessity for providing clinical practice and screening program for early diagnosis of celiac disease in the community and finally treatment with a gluten-free diet.

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PART III

ASSOCIATED DISORDERS

PART III

Chapter 1

Liver Complications In Celiac Disease

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ABSTRACT

Celiac disease (CD) is characterized by sensitivity to gluten, which is found in dietary wheat, barley, and rye. Many extra-intestinal manifestations have been described in association with CD. Liver disease and CD share widespread risk factors. Liver disorders such as autoimmune hepatitis, elevation of liver enzyme levels, primary biliary cirrhosis, nonspecific hepatitis, primary sclerosing cholangitis, and nonalcoholic fatty liver disease have been reported in patients with CD. In this review, we provide information regarding liver disorders that may be found in association with celiac disease and the effect of the treatment of CD on these disorders.

Keywords: Celiac disease, Liver damage, Epidemiology.

Introduction

Celiac disease (CD) is defined as a condition that affects the morphology of the mucosa of the small intestines, and it is improved if the patient consumes a gluten-free diet and relapses if gluten is reintroduced in the diet (1).

The prevalence of CD is high in the general Iranian population (1 in 166) (2), and the disease is currently considered the result of a complex interplay between inherent and environmental factors. The typical or classical form of CD is due to the interaction between gliadin and antibodies to tissue transglutaminase (tTG), and it results in the flattening of the villi in the small intestinal mucosa (3).

Although CD is known to affect the small intestine, it is a multisystem disorder and can involve other organs such as the skin, thyroid, pancreas, heart, liver, joints, muscles, bones, the reproductive system, the central and peripheral nervous systems (4-13).

The occurrence of liver impairment in CD is well established and must be regarded as one of the various extraintestinal presentations of gluten-sensitive enteropathy (14- 17). The association between CD and liver manifestations was first reported in 1977 (14). In this study, 30 of 74 adults newly diagnosed with CD had elevated levels of serum aminotransferase enzymes, which normalized after adherence to gluten-free diet in most cases. In this study, signs of reactive hepatitis were noted in 5 of 13 patients, and different types of histologic lesions were found in 7 patients.

Since the 1990s, a close association between CD and autoimmune liver disease has been clearly indicated in relevant studies (18-21).

Searches were performed in PubMed and SID (for Persian papers) for articles published in English- and Persian-language journals from 1977 to November 2010; the following keywords were used alone or in combination:

"celiac disease," "liver disorders," "liver abnormality," "liver injury," "hepatitis," "anti-tTG," "anti-endomysial," and "cholangitis." The aim of this review is to discuss the major forms of liver abnormalities associated with CD and to evaluate the prognosis of these abnormalities.

Liver Dysfunction related to Celiac Disease

Patients with CD have damaged gut mucosa, which can lead to malabsorption and increased permeability. A wide variety of liver injuries may occur in CD (22), and the principal conditions are listed in Table 1.

Table 1. Characterization of cryptogenic liver disorders related to CD

cryptogenic liver disorders	High liver enzymes due to gluten induced reactive hepatitis
Autoimmune liver disease	autoimmune hepatitis (AIH) autoimmune overlap syndrome primary sclerosing cholangitis (PSC) primary biliary cirrhosis (PBC) nonalcoholic fatty liver

Conditions such as nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), hepatitis C virus

(HCV)-related liver disease, and hepatitis B virus (HBV)-related liver disease are very common in the general Iranian population (23-25), and their incidence in patients with CD is likely a coincidence rather than a true correlation. Recently, 2 different types of liver injury, namely cryptogenic liver disorder (mild or severe type) and autoimmune liver disorder, have been found to be strongly related to CD. Cryptogenic liver disorder can be distinguished from autoimmune liver disorder on the basis of its positive response to gluten-free diet (GFD).

Cryptogenic liver disorder

1- Mild liver damage (gluten induced hepatitis)

The first report of gluten-induced hepatitis, published in The Journal of Pediatric Gastroenterology and Nutrition in 1986, was the case of a young girl with persistent cryptogenic elevation of serum aminotransferase levels and mild inflammation of the portal tract (26). A diagnosis of CD, suggested in this case by a high titer of antireticulin antibody, was confirmed by duodenal biopsy. Bardella *et al.* performed a similar study and found that 13 (9%) of 140 screened patients tested positive for antigliadin antibody (AGA) and anti-endomysial antibody (EMA). The relative risk of CD in these patients (18.6%) was significantly greater than that in the general population.

Antibodies associated with CD disappeared after 12 months of consuming a GFD, but liver enzyme levels normalized in only 1 patient (27). This form of CD was once called "gluten-induced hepatitis" (28) and is now suggested to be celiac hepatitis, which is characterized by mild periportal

inflammation with Kupffer cell hyperplasia, mononuclear cell infiltration, absence of any clinical features suggesting chronic liver disease, absence of hypergammaglobulinemia, absence of serum autoantibodies, presence of mild lobular and portal tract inflammation, and absence of hepatomegaly, splenomegaly, or both; this form is reversible if a GFD is consumed. In the majority of patients with hypertransaminasemia at diagnosis, liver enzymes returned to normal levels within 6 months of starting a strict GFD (29) and in almost all cases, returned to normal levels within 12 months of gluten withdrawal. Occasionally, hypertransaminasemia might be the only sign of CD, which is manifested without any gastrointestinal (GI) symptoms. The results of a study by Volta et al. showed that 9% of patients with increased levels of transaminase of unknown origin had asymptomatic CD (28). Furthermore, evaluation of 110 patients with cryptogenic hypertransaminasemia showed that 10% of patients with elevated transaminase levels were also positive for silent CD (29).

On the basis of the high prevalence of CD (9.3%) in patients with unexplained increases in serum transaminase levels, Bardella, et al. confirmed that cryptogenic hypertransaminasemia could be a possible extraintestinal sign of CD (30). Furthermore, investigators found that children with unknown causes of hypertransaminasemia had asymptomatic CD. By consuming a GFD, patients experienced rapid improvements in both hepatic and intestinal biochemical/histologic signs, thus confirming that the liver damage was gluten-dependent (31). In a few studies, it was reported that some patients on a GFD

for 12 months newly experienced increases in their liver enzyme levels, and this increase was probably because of the high quantities of lipids contained in some gluten-free foods (31, 32).

2- Severe liver damage

Severe histological diseases, including chronic hepatitis, severe fibrosis, and cirrhosis have been reported in adults and children (14, 25, 31). CD was detected in some patients with severe liver damage of unknown origin, and surprisingly, clinical improvement in the liver condition was noted when the patients consumed a GFD (33-35). The prevalence of CD in patients with chronic liver disease is higher than in the general population. Lindgren et al. reported that in 327 patients with chronic liver disease, the prevalence of CD was 1.5%, which is 15 times higher than that in the general population (36).

In a Finnish study, CD was reported in 4 adult patients (3 men and 1 woman) with severe liver disease, who were waiting for liver transplantation (33). Two of them had progressive hepatitis, congenital liver fibrosis, and massive hepatitis steatosis. Clinical symptoms, including ascites and jaundice, improved in all patients after 6 months of consuming a GFD. Of 185 Finnish patients who underwent transplantation, 8 (4.3%) were found to have CD; this rate is 4–10 times higher than that found in the general population (33). During the study for a transplant programin a 28-year-old woman with severe cryptogenic liver failure had a complete recovery of liver function after a few months of following a GFD (34).

3- Autoimmune liver diseases

3.1-Primary biliary cirrhosis

The relationship between CD and primary biliary cirrhosis (PBC) is well documented, and it was first reported by Logan, et al. in 1978 (37). Consequently, PBC patients have been extensively screened for CD. The reported prevalence of CD in PBC varies between 0 and 11%, and about 6% of individuals with CD may be affected by PBC (20, 38, 39). In a 12-year epidemiological study of a British population of 250,000, Kingham and Parker (38) accessed a large registry of patients with PBC and CD. They found that CD was identified in 4 of 67 (6%) patients with PBC and that 4 of 143 (3%) patients with CD were affected by PBC. In another study conducted in UK, in which 4,732 CD patients were matched with 23,620 controls, the prevalence of PBC was found to be 0.17% in patients with CD versus 0.05% in controls (39). This trend was confirmed by 2 large population-based studies from Danish and Swedish cohorts (40). In these studies, EMA antibody tests were positive in 11% of PBC patients. The titer of mitochondrial antibodies, which are markers of PBC, did not change after gluten removal. Various case reports on the association between CD and PBC have shown that a GFD induces a stable clinical and biochemical improvement because of the normalization of intestinal absorption, but it does not seem to adjust the course of the liver disorder (41-49). However, some studies have shown that there is no association between CD and PBC (50, 51), but these studies tended to have small numbers of studied patients. In addition, numerous investigations have reported the positive effect of a GFD in primary biliary cirrhosis, thereby suggesting that all

patients with PBC should be screened for CD (8, 13-20, 52-55).

3.2-*Primary sclerosing cholangitis*

No distinguishing autoantibody has been found in patients with primary sclerosing cholangitis (PSC). Using cholangiography as a diagnostic tool, the characteristics of the biliary lesion can be evaluated in biopsy tissue or the appearance of the intra- and extrahepatic biliary tree can be assessed (12). Several studies have found a positive correlation between PSC and CD; however, these studies involve small numbers of investigated patients, and also, surveillance bias has not been taken into account. This association was first suggested by Hay, et al. in 1988 (56), who described the cases of 3 PSC patients (confirmed by cholangiopancreatography retrograde endoscopy and liver biopsy) with steatorrhea as a severe form of malabsorption. Diarrhea was ameliorated after the diagnosis of CD and consumption of a GFD, but the course of PSC did not improve. In a recent study by Volta, et al. positive anti-EMA antibody was found in 1 of 61 patients with PSC, thus showing a prevalence of 1.6% for CD (20). The relationship between PSC and CD has not been extensively studied. In a study of 13,818 patients with CD and 66,584 age- and sex-matched individuals from the general Swedish population, the prevalence of PSC in CD patients was 4.46%, a rate 4–8 times higher than that of the general population (57).

In another survey, CD was found in 3% patients with PSC (58). Subsequently, the association between PSC and CD has been reported in several case reports (59-63). Liver enzyme levels in a 54-year-old man with CD, PSC, ulcerative colitis, and Hashimoto's thyroiditis were noticeably improved and finally normalized after 14 months of treatment with a GFD (64. A repeated liver biopsy showed marked improvement in liver histological characteristics.

In addition, in 2 studies, GFDs were reported to cause a significant improvement in hepatic histological characteristics and cholestasis in 3 patients. The small number of studied cases does not allow the formation of a definite conclusion as to whether diet slows down the progression of this autoimmune liver disorder (57, 60).

Further studies are needed to accurately determine the strength of the association between PSC and CD.

3.3- *Autoimmune hepatitis*

In the late 1970s, sporadic findings of CD in patients with autoimmune hepatitis (AIH) were reported for the first time (65-67). To evaluate the incidence of CD in patients with AIH, Volta, et al. studied the sera of 157 patients with type 1 AIH and 24 patients with type 2 AIH; CD was found in patients with both types of AIH and EMA antibody was identified in 8 AIH patients (4%). CD was diagnosed in 5/8 patients who underwent a duodenal biopsy (19). However, the benefit of a GFD to the clinical course of AIH patients was not reported. This study was performed through cooperation between the Mayo Clinic (Rochester, USA) and the University of Bologna (Italy).

In another study, the prevalence of CD in patients with AIH was 6.4% (21). In a multicenter study in Italy, AIH occurred in 1.1% of 909 children with CD and no case was found in either healthy populations or

in patients with an alternate gastrointestinal ailment such as Crohn's disease (68). Jacobsen, et al. studied 101 patients with CD (69) and detected chronic active hepatitis in 5 patients (2.3%) of 37 patients who underwent histologic evaluation. In addition, in a study by Novacek, et al. 3 (1.6%) of 178 patients with CD investigated for the presence of abnormal liver enzyme levels had documented AIH (70). In a study done at King's College, London, the prevalence of CD in 96 children with AIH was 3.4%, which was significantly higher than expected (71).

4.3- *Autoimmune cholangitis*

An association between CD and autoimmune cholangitis (AIC) has been described (72). Intestinal biopsies of patients with CD and AIC usually show either mild atrophy or an increased number of intraepithelial lymphocytes.

In a 60-year-old woman who was evaluated for chronic elevations of serum liver biochemical parameters and unexplained iron deficiency anemia, a diagnosis of CD was made; subsequently, treatment with a GFD led to resolution of these abnormalities (73). These studies suggest that CD should be considered in all patients diagnosed with AIC, as a GFD may avoid the need for immunosuppressive therapy.

Other liver disorders in CD

1-*Viral Hepatitis*

Hepatitis B and C are prevalent in Iran (74-77). It is estimated that between 1.2% and 19.7% of the general population have hepatitis B surface antigen (HBs Ag) and 0.12–0.89% have anti-hepatitis C virus antibodies, corresponding to 1.5–2.5 million HBV cases and 0.5 million chronic

carriers of HCV (75, 76). In a study recently published in Iran, 88 patients with chronic hepatitis B (CHB) were serologically tested for celiac autoantibodies, and 9 seropositive patients underwent duodenal biopsy (78). Compared with the general population, the prevalence of celiac autoantibodies in CHB (11.3%) is relatively high, and most autoantibody-positive patients were asymptomatic for CD.

Fifty-five percent of children and 68% of adults do not respond to standard vaccination regimens for hepatitis B virus. This lack of response to hepatitis B vaccine may be related to the genetic background of celiac patients, which seems to be linked to human leukocyte antigen (HLA) DQ2 (79, 80). Some authors have suggested that large follow-up studies with large sample sizes are necessary to clarify how HBV infection affects the development of CD and to identify principal prevention strategies (81- 83). There is no convincing evidence that patients with viral hepatitis are at increased risk of developing CD (84, 85), but an association between HCV infection and CD in adults has been reported. Indeed, HCV is suggested to be the most common liver disease associated with CD. Two hundred and fifty-nine patients who were consecutively evaluated with chronic hepatitis C (CHC) and 221 healthy volunteers underwent serologic screening for CD, and seropositive patients underwent duodenal biopsy (86). The results of this study showed that the prevalence of CD in patients with CHC was 1.2%, while only 0.4% of healthy volunteers had CD. In another study, the prevalence of antibodies to tTG in 462 patients with CHC was higher than that in

1,350 healthy controls (87). A third study showed that the prevalence of CD in 534 patients affected by CHC was 1.3% (88).

A recent study of patients having both CD and HCV described a well-defined route of transmission in most of these subjects, raising the hypothesis that the link between these diseases may be biased by the route of transmission of hepatitis C infection (89). An association between

HCV infection and CD has been hypothesized, but some studies show that there is no correlation between these 2 disorders. Thus, a clear association of CD and HCV is lacking. In a cross-sectional study, 827 multiparous pregnant women were serologically screened for CD and HCV antibodies by enzyme-linked immunosorbent assay (ELISA). Twenty-seven (3.26%) women had antibodies to tTG, but only 2 (0.24%) had antibodies to HCV and one of these also had antibodies to tTG. This result suggests that routine screening of HCV in CD patients is not efficient (90). Both interferon and ribavirin may enhance type 1 helper T cell immune responses via signal transducers and activators of the transcription-dependent pathway, which subsequently induces the expression of interferon (91-94). Cammarota, et al. reported 2 cases of CHC that displayed various features of CD during treatment with interferon. Symptoms and histological disorders improved after the interferon treatment was stopped and a GFD was consumed (92). Therefore, it is suitable to start treatment for HCV after CD diagnosis, and after a year of GFD, improvement of the intestinal disease can be achieved. Since HCV is a very common disorder and may be correlated to CD, the presence of

undetected CD should be ruled out before starting treatment of interferon in combination with ribavirin. Furthermore, CD should be considered in patients with unexplained diarrhea during or after interferon/ribavirin therapy.

2- *NAFLD/NASH*

Some conditions such as NAFLD and/or NASH are very common (up to 25%) in the general population (95), and its occurrence in patients with CD is likely to be a coincidence rather than a true correlation (96). Some studies declare a clear association between CD and fatty liver and suggest performing serological screening and continually evaluating biochemical abnormalities for CD in NAFLD patients (18, 96). Bardella, et al. investigated 59 patients with hypertransaminasemia and NAFLD, and 38 (64%) were diagnosed with NASH by anti-EMA and anti-tTG antibodies. HLA-DQ typing and endoscopy were performed in 2 anti-EMA positive patients (3.4%) and 6 anti-tTG positive patients (10%). On the basis of histological findings, CD was confirmed only in 2 patients (3.4%) who were positive for both anti-EMA and anti-tTG. After 6 months of a GFD, liver enzyme normalized in both cases (96). However, other studies do not recognize associations between CD and NAFLD (97). Nehra, et al. attempted to determine whether any relationship exists between NASH and CD by investigating the serology of 47 NASH patients, in whom NASH was confirmed by liver biopsy.

Only one patient was EMA-positive, thus indicating that there is no association between positive CD serology and NASH (97). In contrast, Valera, et al. found positive CD markers in 3 of 38 NAFLD

patients (7.9%), and histological evaluation showed Marsh I in 1 patient. The result of this study has indicated a high prevalence of positive tTG in patients with NAFLD (98). In an Italian study, the prevalence of silent CD in 59 consecutive patients with NAFLD was 3.4% (99), which suggested that screening with EMA is preferred to tTG antibodies since tTG positivity, in the absence of confirmatory anti-EMA antibodies, is not sufficient to perform diagnostic endoscopy.

3- CD and Haemochromatosis

CD and hemochromatosis are genetic disorders, but CD is paradoxically associated with iron deficiency anemia. Most published papers regarding these disorders together are case reports (100). Singhal *et al.* reported the cases of 2 patients who developed both CD and hereditary hemochromatosis (101). In the first case, CD was diagnosed first and 8 years later, a routine blood screen showed elevated mean corpuscular and alanine transaminase (ALT) levels. Additional examinations showed transferrin saturation and increased levels of ferritin and iron. Genetic analysis showed homozygosity for the C282Y gene mutation, which confirmed hemochromatosis. Liver biopsy showed an index consistent with hemochromatosis. The patient responded satisfactorily to venesections, with the serum ferritin levels presently maintained around 50 mg/L. The second case was that of a 54-year-old woman referred with abnormal ALT levels, but the rest of the liver profile and other biochemical tests were satisfactory. Screening tests for hepatitis B and C and the autoimmune profile were negative, but her serum ferritin level was noticeably raised. Heterozygosity for the H63D and C282Y mutations was shown in a genetic study. The patient rapidly responded to venesection, but the level of ALT remained high. Because of increasing upper abdominal discomfort, she underwent endoscopy, and histological evaluation confirmed CD. After 3 months of treatment with GFD, her liver disorders resolved completely and her ALT levels normalized (101).

Butterworth *et al.* investigated 145 CD patients and 187 matched controls for the presence of HFE gene mutations. The number of mutated C282Y and/or H63D genes were significantly higher in patients with CD (48.3%) compared to controls (32.6%). Nevertheless, none of the patients with CD showed a clinical presentation of hemochromatosis. The results of this study showed that HFE gene mutations are more frequent in the CD population and may provide a survival benefit by ameliorating the iron deficiency seen in these patients (102). The present studies show that there is a rare association between CD and hemochromatosis, and therefore, more studies in this field are necessary to substantiate this observation.

Pathogenesis of liver dysfunction in CD

The mechanism(s) of liver injury in CD is still undefined. Intestinal permeability is increased in CD; this may enable the entry of toxins, antigens, and inflammatory materials to the portal circulation, and these mediators may have a role in the liver involvement seen in patients with CD (14, 28, 29, 56, 99). Although liver dysfunction occurs not only in patients with CD but also in those with irritable bowel disease

(IBD), cow milk enteropathy, and food allergies, patients with tropical sprue and increased intestinal permeability do not present with liver enzyme abnormalities as frequently as CD patients (56) (Figure 1). Most patients with liver injury associated with CD have no symptoms or signs of liver disorder at the time of diagnosis, although nonspecific symptoms such as malaise and fatigue are common (14, 27, 28). The sensitivity and specificity of each sign is variable, but a combination of signs may otherwise raise the suspicion of CD. However, mild to moderate elevations in serum levels of aspartate aminotransferase (AST) and/or ALT are the most common and often the only laboratory manifestations of liver injury in patients with CD. In a study of 98 confirmed CD patients with varied LFT, there was an increase in only ALT levels in 8 patients and AST levels in 5 of these 8 patients (unpublished data). After adherence to a GFD for 6 months, elevations in serum aminotransferase levels were normalized, thus explaining the correlation between liver injury and intestinal damage.

Moreover, in view of the response to GFDs in hypertransaminasemia patients and abnormal ALP of suspected hepatic origin in CD patients, a liver biopsy may be useful. However, liver biopsy performance is dependent to some extent on factors such as patient age, associated co-morbid conditions, and the clinical significance of the liver test abnormality. Before interaction between environmental and genetic factors lead to irretrievable liver injury, early recognition of CD would allow complete recovery from hepatic lesions.

The following mechanisms have been suggested to have roles in establishing liver injury in CD: malabsorption and long-standing malnutrition, increased intestinal permeability, small intestine bacterial overgrowth, chronic intestinal inflammation, and a common genetic predisposition.

Furthermore, patients with CD who are exposed to gluten during a gluten challenge are at higher risk to develop autoimmune disease than those without further exposure (1, 103).
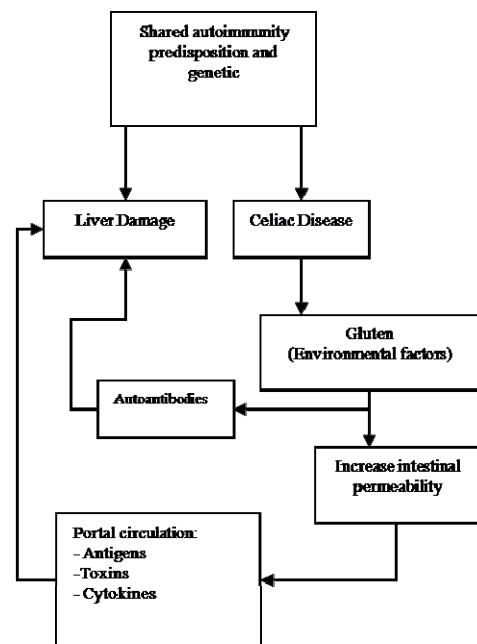
Figure 1. Possible pathogenesis mechanisms of liver abnormality in CD

Genetic Correlation

Genetic predisposition has an important role in the development of cryptogenic liver disorders in response to autoimmune hepatic injuries, and this might also contribute to the association between CD and liver disease (57). Different studies
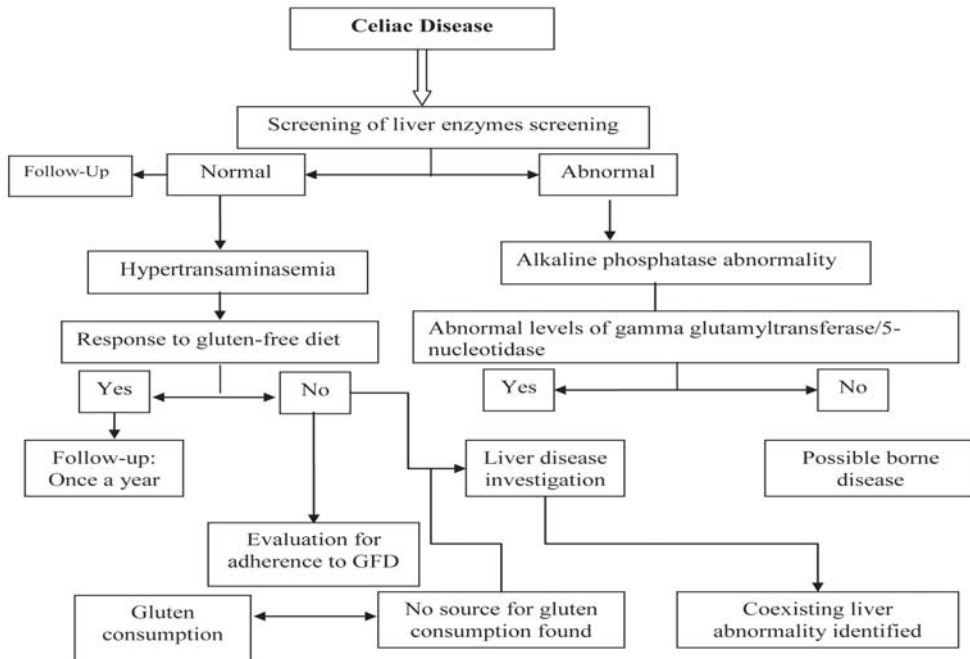
Figure 2. Approach to the diagnosis of liver abnormalities in celiac disease

reflect the importance of genetic factors in the pathogenesis of CD, which is associated with HLA class II molecules encoded by genes of the HLA complex on chromosome 6 (104). In this region, a large group of autoimmune diseases are linked to specific alleles or combinations of haplotypes (105). The main genetic marker of CD is the HLA-DQ2 (α1*0501, α1*0201) heterodimer, which is present in approximately 95% patients with CD, and the remaining patients have HLADQ8 (α1*0301, β1*0302) (106). HLA-DQ2 is in strong linkage with HLA-DR3, which is also the major HLA risk factor for AIH (107). In addition, AIH is associated with HLA-DR4 and HLA-DR52 (108, 109). The same HLA class II molecule has an important role in the pathogenesis

of PSC and CD. A shared immune-genetic predisposition to autoimmunity may account for the association between these diseases (33, 110, 111). In a multicenter study in Europe, it was found that the frequencies of the HLA-DR3, HLA-DR13, HLA-DQ2, HLA-DQ8, and HLA-DQB1*0603 haplotypes were higher in patients with PSC compared with matched controls (112). In Finnish adults who had undergone liver transplantation, the frequency of HLA-DQ2 and HLA-DQ8 in patients with PSC, autoimmune hepatitis, or acute liver failure was 56–75% compared with 39% in controls (33). Therefore, we suggest that there is a closer association between CD and AIH/PSC than between CD and other liver disorders.

Conclusions

Liver abnormality may be one of the associated extra-intestinal manifestations of CD. However, because of the high frequency of CD in the general population, an accidental association between CD and the liver cannot be excluded. The liver damage in CD ranges from mild hepatic abnormalities to severe liver disease and may be seen in 15–55% of patients. The mechanisms underlying liver abnormalities in CD are not defined clearly. However, consumption of a GFD is an effective treatment for most patients with CD and liver disorders. After excluding other causes of liver disease and because of the high prevalence of liver disorders in CD, the levels of liver enzymes should be evaluated in all patients at the time of CD diagnosis (Figure 2).

In cases of cryptogenic hypertransaminasemia, transaminase levels will be normalized, and it is recommended to re-check the liver enzyme levels after 6–12 months of a strict GFD. Fatty liver also may be associated with CD, although it is unclear whether this is a cause, an effect, or a serendipitous association. The important point is that 10–20% of this group may show hypertransaminasemia again after 2 or more years of GFD because of metabolic changes in the liver owing to the high amount of lipids found in commercially available gluten free foods. For this reason, monitoring of transaminase levels once a year is recommended, especially in patients gaining weight. Since the association of CD with liver autoimmunity has been largely validated, the first step is requesting a serology test for CD in all cases of autoimmune liver disorders such as AIH, PBC, and PSC. Indeed, CD can be present in 3–7% of these patients.

EMA is more specific but slightly less sensitive than anti-tTG (113), and as is well known, false-positive results for anti-tTG are found in patients with liver and autoimmune disorders (114). Therefore, determination of the levels of EMA is preferred to that of anti-tTG in patients with cryptogenic and autoimmune liver disease. An exception is in children with inflammatory liver disease of unknown cause, where investigation for CD should be started by determining the anti-tTG levels. In conclusion, serological evaluations for CD should be part of the general workup of patients with unexplained elevated liver enzyme levels when other causes of liver disease have been ruled out, and at the time of CD diagnosis, liver dysfunction should be concurrently evaluated.

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PART III

Chapter 2

Celiac Disease Increases the Risk of *Toxoplasma gondii* Infection in a Large Cohort of Pregnant Women

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To the Editor

It is well established that both celiac disease (CD) and *Toxoplasma gondii* (TG) infection induce a strong neutrophil-mediated immune response that can affect pregnancy outcome. Most cases of toxoplasmosis in immune-competent individuals are asymptomatic, but TG can cause a chronic infection and abortion in adults and a fatal illness in immune-deficient patients (1). On the other side, interleukin-8 (IL8) selectively stimulates the ability of neutrophil and T lymphocytes injured or inflamed tissue (2).

To establish possible association between CD and TG, 827 pregnant women were recruited from the Reproduction Section.

Samples were taken at mean pregnancy duration of 5.5 months. Enzyme-linked immunosorbent assay was used to determine *Toxoplasma* -specific immunoglobulin (Ig) G and IgM (Trinity-Biotech Toxo IgG & IgM, NY) and the level of IL8 (Human IL8/ NAP-1 enzyme-linked immunosorbent assay, Bender MedSystems, Vienna, Austria) according to the manufacturer ' s instructions as well as IgA anti-tissue transglutaminase antibodies for CD as described previously (3).

After childbirth, those anti-tissue transglutaminase- positive patients who were satisfied for follow-up submitted to biopsy specimens, and their biopsies were evaluated according to Marsh-Rostami classification (4). The results of this study show that 27 subjects (3.3 % confidence interval: 1.2 – 4.3) with a mean age of 27 years and mean pregnancy duration of 4.8 months were anti-tissue transglutaminase positive. Eight of the 27 CD-serology

positive subjects underwent endoscopy and 6 of them had abnormal histology, including two Marsh I, three Marsh IIIa, and one Marsh IIIc.

One hundred and fifty four (31 %) and 58 (7 %) pregnant women had positive total IgG and IgM for TG serology, respectively. Interestingly, 16 out of 27 (59 %) CD-serology positive subjects were infected by *T. gondii* compared with 57 out of 800 (32 %) non- CD pregnant women (odd ratio = 3.07, 95% confidence interval: 1.4 – 6.7), showing a significant relationship between CD-serology positive and TG incidence (P = 0.04).

Three patients with Marsh I, IIIa, and IIIc were also positive IgG for TG. On the other side, the level of IL8 was high in TG infected patients, especially in those IgM positive, irrespective of the CD status. In this study, CD-serology positive or TG serology was associated with poor pregnancy outcome.

As 9 out of the 27 (33.4 %) subjects with CD-serology positive had unfavorable medical history and more than half of them had history of miscarriage (4/9) and low birth weight (3/9), we can say that CD-serology positive was correlated with increased risk of low birth weight and miscarriages irrespective of *T. gondii* infection.

So according to the results, we suggest that both conditions predispose unfavorable pregnancy outcomes.

The results of this study show that the TG infection rate was also higher among subjects with CD-serology positive than among patients with negative CD serology, but CD did not cause increasing of proinflammatory cytokines, such as IL8.
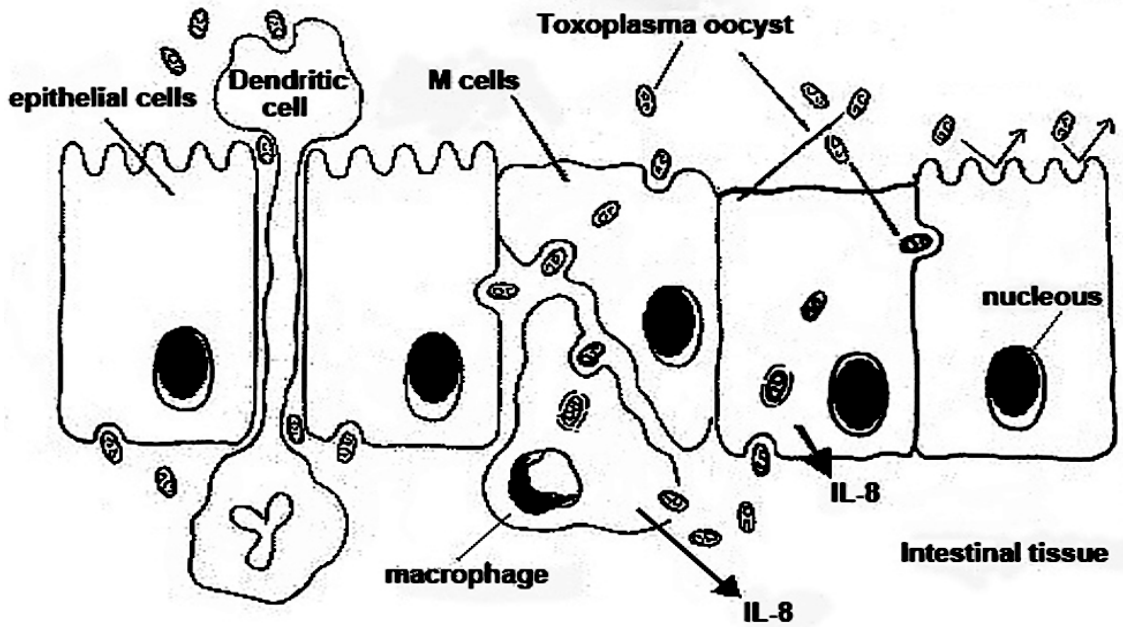
Figure 1. Sporozoites are released, penetrate the intestinal epithelium, and induce the secretion of IL8 in a variety of different cell types including macrophages, endothelial and epithelial cells, neutrophlis and other types of cells. Damaged tissue of CD patients may facilitate distribution of the ingested TG oocysts beyond small bowel lesions.

This suggests that CD may predispose the risk of TG infection. We postulate that CD predisposes to TG infection and may facilitate distribution of the ingested TG oocysts beyond small bowel lesions (Figure 1) and somehow could increase sensitivity to gluten. On the other hand, TG may precipitate the development of CD in susceptible individuals in keeping with studies that suggest that other enteric infections can predispose to the development of CD among susceptible individuals (5).

In conclusion, our results suggest that CD increases the risk of *T. gondii* infection during pregnancy and it is possible that the observed association in Iran may be dependent on geography, genetics, or other environmental factors.

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PART III

Chapter 3

Celiac disease and Hp infection association in Iran

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ABSTRACT

Background: we assessed the prevalence, the related symptoms, and the endoscopic and histologic gastric features of celiac disease (CD) in patients with Helicobacter pylori (Hp).

Methods: 450 dyspeptic patients were studied. Biopsies of gastric antrum and duodenum, CD serology, and total IgA were obtained. Histological findings were scored with the Marsh-Rostami criteria.

Results: 411 (91.3%) patients were Hp positive. Duodenal histology was normal in 385 (85.6%) patients, 124 (27.5%) had duodenitis and 28 (6.2%) showed duodenal abnormalities (Marsh I-IIIc). Twenty three/28 (82.1%) patients with malabsorption pattern were also Hp positive. Serological analysis: 12 of 31 (38.7%) positive patients had abnormal histology (Marsh I,-IIIc). Nine out 450 patients were IgA deficient; none of them was serologically positive for CD.

Conclusion: although a high prevalence of Hp infection was found in this study, the relationship between Hp infection and CD was similar to that reported in other geographic areas.

Keywords: Celiac disease, *H. pylori*.

Introduction

(CD) is frequently associated with abnormalities in gastric function and histology (1-8). Helicobacter pylori (Hp) is the causative agent in more than 90% of cases of chronic gastritis, peptic ulcer disease, primary gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer (9). Ahigh prevalence of this infection would be also expected in patients with celiac disease (1-8). Atrophic gastritis with an increased prevalence of parietal cell antibodies seems to be common in some studies (1), but others have found little evidence to support this in CD (10).

Although the pathogenesis of CD is relatively well understood, the possibility that a chronic gastric infection capable of inducing duodenal ulcers could influence the inflammatory and immune responses in the small intestine and, therefore, the development and evolution of CD, should be considered (11,12).

Hp is now recognized as a major etiological factor in most patients with non-autoimmune chronic gastritis (2). In developing countries, Hp infects the majority of the population; for instance, the Iranian population displays a rate of infection of more than 90% (13).

Even though epidemiological studies have failed to reveal a connection between gastritis and CD (11,12), some studies showed that patients with Hp gastritis are more likely to have increased numbers of intraepithelial lymphocytes in the duodenal mucosa, and that this can be reversed by the eradication of H. pylori (14,15). Other works have focused on Hp-related lymphocytic gastritis in CD (16-19) and on

the link between anemia and Hp infection in celiac patients (20, 21).

Purposes of the present study were to assess the prevalence of celiac disease, its related symptoms, and endoscopic features, and to compare the histopathological and clinical features in patients with associated Hp infection.

Patients and Methods

In the period January 2007-December 2008, four hundred and fifty patients (211 men, 239 women, mean age 36 years, range 15-83 years) who underwent upper endoscopy for dyspeptic symptoms were recruited in the out-patient clinic of Taleghani hospital. After obtaining a written informed consent from all subjects upper endoscopy with gastric and duodenal biopsies was carried out. Two biopsy specimens were obtained from the gastric antral mucosa and two to four samples from different portions of the duodenum. Biopsies were fixed overnight in buffered formalin, embedded in paraffin, cut to 3-μm thickness, and stained with hematoxylin-eosin for routine histological evaluation. Giemsa staining was also used for gastric specimens, to identify Hp. The slides were blindly evaluated by two expert gastrointestinal pathologists.

In accordance with the Updated Sydney System (22), the degree of gastric mucosal inflammation, polymorphonuclear cell infiltration, glandular atrophy, and intestinal metaplasia was classified into four grades as follows: 0 = none, 1 = mild, 2 = moderate and 3 = severe.

Hp infection was considered positive if present at least in one of the biopsies examined. The diagnosis of CD was based on the characteristic histological finding of

increased intra-epithelial lymphocytes, villous atrophy, and crypt hyperplasia, classified according to the standard classification proposed by Marsh (23,24) and subsequently modified by Rostami et al. (25) (Table I).

Before endoscopy, a blood sample was drawn for serum anti-tissue transglutaminase (tTGA) and stored at - 70 °C until tested. Patients who had normal biopsies, but tested positive for tTGA in their serum, were asked to undergo retest and an intestinal biopsy 12 months later.

IgA-tTGA levels were measured by a commercially available ELISA method (AESKULISA tTGA, Germany). Serum values of tTGA higher than 15 U/mL were considered positive. Total serum IgA values were measured by an immunoturbidometric assay (Pars Azmoon, Iran), and serum levels below 70 U/mL were considered indicative of IgA deficiency. Immunoglobulin G (IgG) tTG

values were further obtained in individuals with IgA deficiency by an ELISA method (AESKULISA tTGG, Germany).

Results

Out of 450 patients 411 (91.3%) were Hp positive; associated symptoms/diseases were diabetes 26 (5.8%), anorexia 99 (22%), weight loss 143 (31.5%), nausea and vomiting 145 (32.2%), heartburn 261 (58%), bloating 320 (71.1%), flatulence 140 (31.1%), a concomitant stressful condition 117 (26%), and abdominal discomfort 351 (78%). Nine out of 450 recruited patients were IgA deficient and none of them were positive for IgG tTG.

Duodenal histology was normal in 385 (85.6%) patients, 124 (27.5%) had duodenitis, and 28 (6.2%) showed mucosal abnormalities compatible with CD (Marsh I-IIIc) (Table II).

Twenty three out of 28 patients (82%)

Table I. Histological classification for coeliac disease

	Marsh 0	Marsh I	Marsh II	Marsh IIIa	Marsh IIIb	Marsh IIIc
IEL/100 enterocytes	< 25/100EC	> 25/100 EC	> 25/100 EC	> 25/100 EC	> 25/100 EC	> 25/100 EC
Crypt hyperplasia	-	-	Hyper plastic	Hyper plastic	Hyper plastic	Hyper plastic
Villous atrophy	-	-	-	PVA	STVA	TVA
	Microscopic enteritis			Macroscopic enteritis		

EC: enterocytes; PVA: partial villous atrophy; STVA: subtotal villous atrophy; TVA: total villous atrophy.

Table II. Serology and type of mucosal lesions in patients with small bowel mucosal abnormalities

	MCG	ModCG	ModACG	SCG	SACG	Total	tTGA	Hp
Marsh I	5	3	1	1	2	12	4/12	10/12
Marsh II	2	1	1			4	2/4	3/4
Marsh IIIa		1		1	1	3	1/3	2/3
Marsh IIIb	2	2	1	1	1	7	3/7	6/7
Marsh IIIc					2	2	2/2	2/2
Total	9	7	3	3	6	28	12	23

MCG: mild chronic gastritis; ModCG: moderate chronic gastritis; ModACG: moderate chronic active gastritis; SCG: severe chronic gastritis; SACG: severe chronic active gastritis; tTGA: tissue transglutaminase antibody; *Hp: H. pylori.*

with positive histology for CD had gastric biopsies positive for Hp (Fig. 1). The prevalence of Hp in patients without CD (86.2%) was slightly but not significantly higher. Hp infection was more prevalent in patients younger than 40; as expected, most (91.4%) Hp-positive patients had moderate to severe chronic gastritis, whereas only 12 out of 39 (30.7%) Hp-negative patients had moderate or severe chronic gastritis.

Serological analysis showed that 12 out of 31 (38.7%) tTGA-positive patients had abnormal histology (four Marsh I, two Marsh II (Fig. 2) one

Marsh IIIa, three Marsh IIIb (Fig. 3) and two Marsh IIIc (Fig. 4). In this group, one had mild chronic gastritis, three moderate chronic gastritis, two moderate active chronic gastritis, three severe chronic gastritis, and the last three had severe active chronic gastritis.

Figure 2. Marsh Type 1 lesion. **A**. H&E x 20. **B**. Immunohistochemistry for CD3 x 20

Discussion

In this study we found that, in this geographic area of the Middle East, the prevalence of Hp infection in dyspeptic patients was higher than 90%. Patients with a malabsorption pattern had 42.8% positive serologies for CD, with sensitivity values ranging from 33.4% in Marsh IIIa patients to 100% in Marsh IIIc, which is in agreement with previously published results (26-28).

According to literature results, in our serie also only 33.4% of Marsh I patients showed positive serology. The Marsh degree in 5 patients who were Hp-negative included one Marsh I, one Marsh II, two Marsh IIIa and one Marsh IIIc.

Figure 1. Active gastritis with positivity for Hp (arrows). H&E x 10

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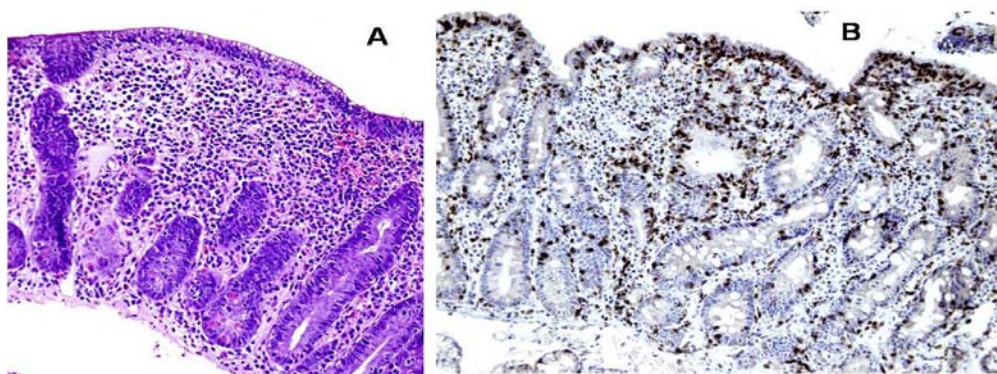
Figure 3. Marsh type 3A-3B. A. H&E x 20. B. Immunohistochemistry for CD3 x 20
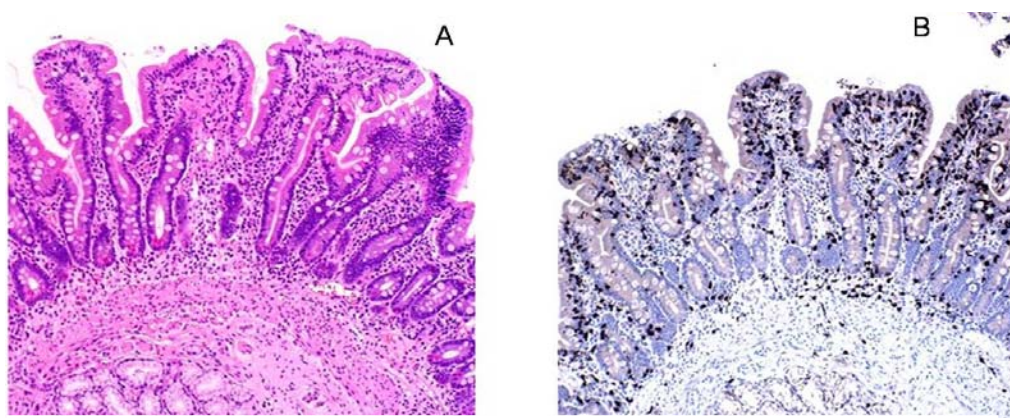

Figure 4. Marsh type 3C. A. H&E x 20. B. Immunohistochemistry for CD3 x 20

Moderate chronic gastritis was more prevalent in Hp-infected patients.

Out of 31 serology positive patients, 19 patients had normal histology. In contrast, in the 28 patients with a malabsorption pattern, 16 had negative serology. Therefore, only 12 patients had both abnormal histology and positive serology related to CD.

Hp has been positively identified as the main cause of active chronic gastritis and its major complications (peptic ulcer disease, gastric adenocarcinoma, and primary gastric MALT lymphoma), with numerous studies carried out to explore the possible etiological role of this bacterium in a variety of both gastrointestinal and extra-intestinal conditions (28, 29). Investigations on the relationship between Hp infection and CD have yielded conflicting results (11, 12), probably because of the different prevalence of Hp in the populations studied.

As shown in table II, in our series 23/28 patients with a malabsorption pattern (Marsh I-IIIc) were also Hp-positive. Histological findings in these patients included six mild chronic gastritis, eight moderate chronic gastritis, three moderate active chronic gastritis, two severe chronic gastritis, and four severe active chronic gastritis cases.

The four patients with Marsh I (two patients), Marsh II and IIIb who were positive for Hp and tTG-A had mild chronic gastritis, moderate active chronic gastritis, and severe active chronic gastritis, respectively. Two patients with

Marsh II had moderate chronic and moderate active chronic gastritis. For Marsh IIIa, one patient had severe chronic gastritis. Three patients with Marsh IIIb had moderate chronic gastritis, severe chronic gastritis and severe active chronic gastritis. Two patients with Marsh IIIc had severe active chronic gastritis. These results showed that 9 out of 12 with serology positive for CD were infected with Hp. On the other hand 24/31 tTG positive were also positive for Hp.

It should be noted that, concerning Hp positivity, the percentage of Hp-positive celiac patients is lower than that of non-celiac patients and, as recently shown (29), the clinical features of CD patients are unrelated to the simultaneous presence of Hp gastritis, and there is no relation between gastritis and severity of mucosal damage in CD.

In conclusion, the prevalence of Hp infection in Iranian patients complaining of dyspeptic symptoms is high, and the relationships between Hp and CD are similar to those described in other geographic areas.

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PART III

Chapter 4

Fertility disorder associated with celiac disease in male and female; fact or fiction?

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ABSTRACT

Background: The association between celiac disease and infertility is controversial in the literature. The aim of this study was to determine the prevalence of celiac disease among the couples with unexplained Infertility.

Methods: Serum samples from 100 Iranian couples with unexplained Infertility were evaluated for celiac disease by anti- tissue transglutaminase antibody (t-TGA). Two hundred couples not reporting reproductive problems and having delivered at least one uncomplicated birth served as controls. Total IgA was also obtained to investigate IgA deficiency. Those with IgA deficiency were tested with IgG tTG. Those cases with positive tTGA or tTGG (IgA deficients) underwent upper gastrointestinal endoscopy.

Results: Positive results of tTGA were detected in 13 infertile subjects (6.5%, 6 males and 7 females) and 11 controls (2.8 %, 4 males and 7 females) (P= 0.027). The odds ratio of celiac disease in unexplained infertile couples was 2.39 (95% CI: 1.15-5.01) compared with fertile couples. IgA deficiency was identified in 14 infertile cases and 11 controls. Only 5/24 tTGA-positive and 4/24 IgA-deficient infertile subjects and controls accepted to undergo duodenal mucosal biopsy. Celiac disease was confirmed by biopsy in 3 (1.5%) of unexplained infertile patients.

Conclusion: The results of this study show that there is a higher seroprevalence of celiac disease in those with infertility in comparison to those with normal fertility.

Keywords: Celiac disease, unexplained Infertility, Tissue transglutaminase antibodies (tTGA).

Introduction

Celiac disease may be accompanied with several extra intestinal manifestations/complications, with an adverse reproductive outcome (1, 2). Determining the cause of unexplained infertility is a challenge in reproductive medicine. When the results of standard infertility evaluation are normal, practitioners labeled the case as unexplained infertility (3). Some cases of unexplained infertility may be due to systemic diseases that have subtle effects on the reproductive system (4), among which celiac disease is of utmost importance. Some studies indicated that celiac disease may account for a significant percentage of unexplained infertility (5). However, other studies did not find any association between the two conditions (6). The likelihood for a causal relationship between celiac disease and reproductive problems including infertility, recurrent abortions and intrauterine growth retardation (IUGR) has received support in a number of reports (7). Some reports indicated a 4% to 8% prevalence of celiac disease in women with unexplained infertility (8, 9); others suggest that treating celiac disease with a gluten-free diet can improve fecundity (10, 11). Nevertheless, robust evidence has not yet been provided. Some researchers recommend that screening for celiac disease should be routine in all couples with unexplained infertility. Culturally, infertility is a significant health problem for young couples, while referring to infertility centers have been widely increased during the recent years. However, celiac disease has not found its role as a possible cause of unexplained infertility among couples. Therefore, we have evaluated the correlation of gluten related auto-antibodies in infertile couples and assessed in which proportion celiac disease may account for unexplained fertility disorders between men and women.

Patients and Methods

Infertile couples referred to the Infertility Department of Alzahra Hospital (Tabriz University of Medical Sciences, Tabriz, Azerbaijan) were evaluated by a standard infertility protocol between October 2006 and September 2007. Endocrine status was evaluated using serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, and thyroid hormone. Ultrasound, urinary LH, and/or luteal phase progesterone confirmed the presence or absence of ovulation. Tubal patency was assessed with hysterosalpingography. Hysteroscopy or laparoscopy was performed, when appropriate. All male partners underwent semen analysis according to the World Health Organization criteria (12). In case of abnormal findings in the above-mentioned investigations, couples were labeled unexplained infertility. Using this protocol, 100 unexplained infertile couples were randomly selected.

The control group consisted of 200 apparently healthy couples lacking reproductive problems with at least one child delivered and attending the hospital or gynecology offices for routine screening visits. All subjects were requested to complete a questionnaire regarding infertility duration, drugs consumption, history of diabetes, abortion, infertility

treatment, chronic diarrhea, anemia, autoimmune disease, hyper- and hypothyroidism and previous diagnosed celiac disease.

Five milliliters of fasting blood was obtained, and then serum was separated and divided into 2 aliquots and immediately stored at -20°C. Anti-tissue transglutaminase IgA antibodies (tTGA) were determined by enzyme-linked immunosorbent assay (ELISA) with human recombinant tTGA as antigen, using a commercial kit (Eu- tTG IgA, Eurospital, Trieste, Italy). Results were considered positive when higher than 7 AU/mL. tTGA is not appropriate for IgA-deficient patients and since 2-3% of celiac population is IgA-deficient (13), the serum IgA level should be determined before the serological tests such as tTGA. This would eliminate false-negative results. The total serum IgA level was determined as described previously (14).

The tTGA positive and IgA deficient subjects were asked to undergo an upper gastrointestinal endoscopy. Four biopsy samples, taking from distal duodenum, were evaluated by a pathologist who was unaware of the tTG test results. Intraepithelial lymphocytes, Crypt hyperplasia and villous atrophy were classified according to Marsh -Rostami criteria (15).

Symptomatic patients with positive serology and some degrees of mucosal abnormalities have been classified as celiac disease according to most recent studies. (16). Although histology has a non-specific nature specially in those with mild abnormality, serology has a very high positive and negative predictive value and currently those symptomatic patients with positive serology (tTG and or EMA) with lymphocytic enteritis (Marsh I-II) should be treated as celiac disease.

The Statistical Package for Social Science (SPSS, version 11.5, USA) was used for data analysis. Chi-square, t-test and logistic regression analysis were used, when appropriate. P values <0.05 were considered statistically significant.

The study protocol was approved by the Ethics Committee of the Tabriz University of Medical Sciences and an informed consent was obtained from each subject.

Results

Serological screening for celiac disease based on tTGA was performed on 100 unexplained infertile couples aged 29.06±6.09 years and 200 fertile couples aged 29.91±9.54 years (NS). The mean duration of infertility in study group was 5.25± 3.94 years (a range, 1 to 20 years).

Positive results of tTGA were respectively detected in 13 infertile subjects (6.5%, 5 males and 8 females) and 11 control (2.8%, 4 males and 7 females). The difference was statistically significant for tTGA positive in cases compare to the controls (P=0.027). IgA deficiency was identified in 14 (7%) infertile couples (6 male and 8 female) and 11 (2.8%) controls (4 male and 7 female); however, the difference did not reach a statistically significant level (NS). Three of 14 infertile cases (3 females) with IgA deficiency and three of 11 controls (2 females and one male) were serologically positive for tTGG. The odds ratio of celiac disease in unexplained infertile couples was 2.39 (95% CI: 1.15-5.01) when compared with fertile couples based on serological

Table 1. Clinical characteristics of unexplained infertile patients compared with healthy fertile controls.

Characteristics	Unexplained Infertile Patients	Control group	P
Number	200	400	
Age (mean±SD[#])	29.06±6.09	29.91±9.54	0.1[*]
tTG[¶] positive	13 (6 males and 7 females)	11 (4 males and 7 females)	0.027[†]
IgA deficiency	14 (4 males and 10 females)	11(4 males and 7 females)	0.15
History of chronic diarrhea	1 (0.5%)	0	0.3
History of anemia	5 (2.5%)	20 (5%)	0.14
History of hyperthyroidism	2 (1 %)	1(0.2%)	0.21
History of autoimmune disease	2(1%)	1(0.2%)	0.22

* Student's *t*-test; †χ^2 test; # Standard deviation; ¶ tissue transglutaminase

Table 2. Tissue-transglutaminase antibodies, IgA deficiency and histological aspects in infertile patients underwent duodenal mucosal biopsy

Case	Gender (male/female)	Clinical symptoms	tTGA	IgA deficiency	tTGG	Histological aspects	Endocrine status			
							FSH	LH	PL	TH
1	Female	Bloating	Positive	Negative	---	Marsh IIIb	2.6	8.1	12.6	2.2
2	Male	none	Positive	Negative	---	Normal	---	---	---	---
3	Female	Constipation	Positive	Negative	---	Normal	1.2	18.9	19.8	0.7
4	Male	Abdominal pain	Positive	Negative	---	Marsh I	---	---	---	---
5	Female	none	Positive	Negative	---	Normal	7.2	4.6	9.4	0.4
6	Female	none	Negative	Positive	---	Normal	4.8	10.2	8	0.3
7	Female	Abdominal pain	Negative	Positive	Positive	Marsh I	5.4	8.2	16.4	0.9
8	Female	Abdominal pain, weight loss	Negative	Positive	Positive	Marsh I	3.7	8.5	10.8	3.1
9	Female	none	Negative	Positive	Positive	Normal	6.7	9.2	17.3	1.3

FSH; follicle-stimulating hormone, LH; luteinizing hormone, PL; prolactin, and TH; thyroid hormone

screening adjusted for age. There was no significant association between positive tTGA results and gender in either case or control group. Table 1 represents results of serologic screening together with age and extra intestinal manifestations of celiac disease in cases and controls.

Totally, 5 tTGA-positive and 4 IgA-deficient cases underwent duodenal mucosal biopsy (3 tTGA-positive and 2 IgA-deficient were belong to infertile patients and 2 tTGA-positive and 2 IgA-deficient patients were from control group). Due to no remarkable clinical presentation, the rest of seropositive patients were unwilling to undergo duodenal mucosal biopsy. The biopsy samples revealed Marsh IIIb in one of tTGA-positive and Marsh I in two IgA-deficient subjects in infertile patients and Marsh I in one tTGA positive subjects in control group (table 2). Therefore, celiac disease was suggested by biopsy and auto-antibodies test in 3 (1.5%) cases of unexplained infertility compare to 1 (0.25%) in control group. All infertile celiac disease patients were female versus those control fertile was male.

Discussion

In the present study, the frequency of celiac disease autoantibodies among unexplained infertile patients serologically was 8% (13 tTGA+ & 3 tTGG +) which was significantly higher than controls (3.5%, [11 tTGA+ & 3 tTGG +]). Furthermore, the likelihood of celiac disease in infertile patients was 2.39 times higher than controls. We used tTGA for screening which is a highly sensitive and specific serological marker (17).

Similarly, an increased prevalence of celiac disease in infertile patients has been reported in previous studies in Europe and Middle East (6-9, 18, 19). Collin et al. in Finland investigated the prevalence of sub clinical celiac disease in women with infertility or recurrent miscarriage by serological screening tests (9).

In their series, no cases of celiac disease were identified in the control group of 150 fertile women, however, 2.7% (four of 150) of infertile women were found to have sub clinical celiac disease. When infertile couples in Italy were evaluated for sub clinical celiac disease, an increased prevalence of 3% was found (3/9). This rate of disease was much higher compared with the general population (17 cases among 1607 women; 1.06%) (8). Celiac disease has been also found to be more prevalent among the Arab infertile female. When a group of 192 Arab women suffering from unexplained infertility were tested for serologic markers of celiac disease, 2.65% were affected. This figure was five times higher than the controls (0.5%) (18). A study from Israel investigated pregnancy outcome in patients with celiac; no statistically significant differences were noted between the groups regarding fertility treatments (0% among patients with known celiac versus 2.5% among patients without known celiac sprue; p=0.267) (19).Tiboni et al (20) investigated the prevalence of celiac disease in women undergoing assisted reproduction techniques by tTGA. Five (2.5%) cases and 2 (1.0%) controls were revealed to have celiac disease (P=0.44). In a cohort study among women with unexplained infertility in United State population, tissue transglutaminase and endomysium antibodies (EMA) were assessed among whom only EMA was positive in one (0.8%) which is approximately as prevalent as the general population in United State (<1%) (6).

In present study, none of the infertile patients with celiac disease had remarkable gastrointestinal complaints consistent with studies performed by Tiboni et al (20) and Collin et al (9). Moreover, there was no significant association between positive TGA results and gender. Regardless of risk factors such as GI symptoms, individuals experiencing unexplained infertility could significantly benefit from awareness of disease status as management has demonstrated improved fertility outcomes (7). There are growing evidence to prove that celiac disease may affect reproduction at various points in both men and women. Farthing's study (21) of men with celiac disease demonstrated an increased incidence of hypogonadism, sexual dysfunction and poor semen quality in almost half of his

cohort, resulting in an increased incidence of infertility. Women with celiac disease can also have major menstrual problems. One report from Italy showed 34 newly diagnosed celiac women suffered from significantly delayed menarche (13.5 vs. 12.1 years of age; P = 0.000), more frequent secondary amenorrhoea (38.8% vs. 9.2%; P = 0.001) and a trend towards earlier menopause (45.5 vs 49.5 years of age) (22). In our study the prevalence of celiac disease was not same in males and females and all of the confirmed celiac disease in infertile group was female.

Two recent studies by Kurppa, et al have elegantly demonstrated that even those who are serology positive but having no villous abnormality do respond to gluten free diet (23, 24). Likewise, some studies have suggested that treating celiac disease with a gluten-free diet can improve fecundity in these patients (10, 11). In a study examining the effect of celiac disease on reproduction, patients on a normal diet were found to be at increased risk for infertility in comparison to patients on a gluten-free diet (11). The potential of a gluten-free diet to exert a positive effect on reproduction is rationalized by the possibility that nutritional imbalance especially malabsorption of selective nutrients including zinc, selenium, iron and folate may underlie celiac disease-mediated reproductive disorders (2). There is little doubt that untreated celiac disease adversely affects both male and female reproduction. It also seems that patients with minimal symptoms have a considerably increased risk of problems. However, good evidence exists regarding the effectiveness of GFD in returning reproduction to normal. Several studies have indicated a potential association between celiac disease and infertility (6, 10, 11, 19, and 25), often recommending celiac disease screening of some or all patients with unexplained infertility.

The present study, in agreement with prior reports, showed higher frequency of celiac disease among unexplained infertile couples compare to fertile couples in Tabriz, Iran.

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PART III

Chapter 5

Rotavirus and Coeliac Autoimmunity among adults with non-specific gastrointestinal symptoms

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ABSTRACT

Objectives: Study in children suggests that Rotavirus (RV) may be linked to coeliac disease (CD). The aim of this study was to determine CD serology and rotavirus by PCR in adults with non-specific gastrointestinal complaints. **Methods:** The study comprised 5176 randomly selected individuals living in Tehran, Iran during 17 Sep 2006- 17 Sep 2007. 670 Individuals with GI symptoms were identified with a questionnaire and invited for a further study comprising blood and stool sampling. Stool samples were examined for detection of RV by amplification of specific gene (VP6) and by light microscopy and formalin-ether concentration methods for parasite detection. The subjects also tested for CD including anti-transglutaminase (tTG) antibodies and total immunoglobulin A (IgA). **Results:** VP6 gene was detected in 150(22.3%) individuals. IgA tTG antibody was positive in 22 individuals (95% CI 2.3-5.1) and IgG tTG antibody in 3 individuals who were IgA deficient. Amplification of VP6 gene was positive in 8 (32%) out of the 25 patients with positive, and in 142(22%) out of the 645 with negative CD serology. This difference was not statistically significant (p=0.2). **Conclusion**: This study shows that RV infection is common among Iranian patients with non-specific gastrointestinal symptoms. However, in contrast to studies in children, this study shows that active rotavirus infection was not statistically significantly different between individuals who were tTG antibody positive and those who were tTG antibody negative.
Keywords: Rotavirus, Coeliac Disease, Gastrointestinal Symptoms, VP6.

Introduction

Coeliac disease (CD) is an autoimmune disorder characterized by gluten sensitivity in genetically susceptible individuals.[1] The classic manifestations appear in the small intestine, but CD patients may also suffer from extraintestinal manifestations such as neurological, reproductive, and endocrinological disorders.[2]

An increased intestinal permeability permits the absorption of intact gliadin molecules which may initiate the immune process leading to CD.[3] On the other hand, intestinal infection and inflammation may increase the intestinal permeability. Few studies have investigated the role of specific infectious agents in the development of CD.

The prevalence of rotavirus (RV) infection is ranged from 3.3% to 63.6% in different parts of the world.[4-6] This infection is one of the most common causes of acute gastroenteritis worldwide.[7] A prospective study by Stene et al.[8] suggests that rotavirus predisposes to celiac disease in genetically susceptible host by either molecular mimicry or by repeated infections in childhood.

The objective of this study was to assess the prevalence of rotavirus and coeliac serology among Iranian adults with non-specific gastrointestinal symptoms.

Patients and Methods

This cross sectional study in Iran, Tehran province was designed to assess the association between RV and CD.[9-12] During 17 Sep 2006- 17 Sep 2007, a total of 5176 adults drawn up randomly from the population of Tehran on the basis of the list of postal cods in which the random

samples of these postal codes and their related address were drawn from the databank registry of Tehran central post office, approximately 1000 households selected and all members (5176 persons) surveyed in each corresponding address.

Interviewers asked them regarding 8 gastrointestinal symptoms (yes or no) including abdominal pain, constipation, diarrhea, bloating, dyspepsia, nausea and vomiting, weight loss and Heart burn. Those who reported at least one of these 8 gastrointestinal symptoms were selected for participation in this study. Altogether 670 (mean age 40, range 14 to 83 years) with GI symptoms comprised the actual study group; 427 (63.7%) were women (mean age 42 years). Those with similar symptoms but a stabilised diagnosis like IBD, pancraetitis or GI malignancy were excluded.

Blood and stool samples were collected from each of them: the samples were transferred to National Research Department of Food borne Diseases (NRDFD) located in Research Center of Gastroenterology and Liver Diseases, Shaheed Beheshti University, M.C., Tehran, Iran.

The sera were assayed within 24 hours after collection or stored at −80°C until analysis. Determinations of IgA anti-tTG antibody were carried out using a commercially available kit (AESKULISA tTG, Germany) and an enzyme-linked immunosorbent assay (ELISA) method and total serum IgA values were evaluated by an immunoturbidometric assay (Pars Azmoon, Iran). IgA class human antitissue transglutaminase (tTG) antibody and Total serum IgA values for CD were measured as described previously.[13]

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Stool sample were kept in closed containers, refrigerated at 4°C, rapidly sent to NRDFD and stored at -20°C until processed.

Viral RNA-extraction:

For preparation stool samples, the cured fecal samples (0.5ml) were diluted with 150 µl of phosphate-buffered saline, vortex for 10 sec and incubated at room temperature with gentle mixing for 15 min. For RNA extraction QIAamp viral RNA mini kit (Qiagen/ Westburg, Leusden, the Netherlands) was used according to the manufacturer's instruction.

RV VP6-specific PCR:

A reverse transcriptase polymerase chain reaction (RT-PCR) was performed to amplify a 382 bp of segment 6 RNA. This region was chosen to determine group a specific VP6 protein of RV as previously described by Gomara et al. [15]

Parasites Detection:

Specimens preserved in SAF (sodium acetate, glacial acetic acid and formalin) were sent to NRDFD where the stools were examined using light microscopy and the formalin–ether concentration method for detection of protozoa and of geohelminth eggs. [16] Also modified acid-fast staining was used to identify *Cryptosporidium parvoum.*

The study was approved by the Institutional Ethics Committees of Research Center for Gastroenterology and Liver disease, Shaheed Beheshti University, M.C. and all participants signed a written informed consent.

Statistical analysis

Descriptive statistics was performed to depict the results. Fisher exact test was carried out using SAS software P value

0.05 was considered statistically significant.

Results

Of the total of 670 symptomatic GI patients, who participated in this screening, we found an organic etiology in 290 cases; 380 reported self-limiting symptoms of short duration. tTG antibody was found in 17 women and 5 man (22 subjects (3.3%)). Also three IgA deficient were positive for IgG tTG. The prevalence of tTG antibody in the screened samples was thus 3.7% when including IgA-deficients with positive tGG.

One hundred and twenty six (19 %) isolates were positive for pathogen and non-pathogen parasites separately 53 pathogen, 73 non-pathogen and 544 negative.

Among pathogen parasite, *Giardia labmlia* was the most common intestinal pathogen (41/670) followed by *Blastocystis hominis* (30/670), *Iodomoeba butchelii* (13/670), *Entamoeba histolytica/ Entamoeba dispar complex* (11/670) and *Cryptosporidium parvoum* (3/670). Among non-pathogen parasites *Entamoeba coli* was the most prevalent species (39/670) followed by *Endolimax nana* (34/670).

Altogether 150 (22.3 %) out of 670 subjects had positive RT-PCR testing for the RV VP6 gene in their stool samples (76 female and 74 male). Eight (32%) of 25 positive CD serology cases were infected by RV; the frequency was not statistically significantly higher.

The most frequent GI symptom in 150 cases positive for RV were heartburn (47/150, 31%), and in 25 positive CD serology were abdominal pain (n=10, 40%). Table 1 shows the clinical signs and

symptoms, and associated conditions in RV-infected patients and positive CD serology patients.

Table 1. Clinical signs and symptoms, and associated conditions in RV-infected patients and positive CD serology patients[*]

Symptom, Sign, Associated Condition or, Test	Rotavirus-positive (N = 142)	positive CD serology (N = 17)	P value
Diarrhea	4 (2.8%)	1 (5.9%)	0.43
Abdominal pain	36(25.3%)	10(58.8%)	<0.001
Nausea & vomiting	3 (2.1%)	8 (47%)	0.07
Constipation	27 (19%)	6 (35.2%)	0.12
Weigh loss	7 (4.9%)	4 (23.5%)	0.02
Heart burn	46 (39.4%)	9 (52.9%)	0.10
Bloating	32 (22.5%)	8 (47%)	0.01
Dyspepsia	3 (2.1%)	0	

* 8 patients whom diagnosed with both CD and RV excluded from the analysis

There was a statistically significant relationship between the weight loss and abdominal pain and positive serological marker for CD autoimmunity (P<0.05) and between the RV positive and bloating (P<0.05). There was no obvious difference in the symptoms between RV infected and non-infected individuals.

Discussion

Rotavirus is an important cause of diarrhea in children but asymptomatic carriage state is also known. This is the first report of RV infection in Iranian adults and our results are similar to those obtained in other population studies. But in this study what has been shown is just reflects the prevalence of rotavirus infection among adults with non-specific GI symptoms and not with only diarrhea.

22.3% of our subjects were positive to RV, compared to the prevalence of 20–40% of RV reported elsewhere such as Brazil, Tunisian and India.[16-18]

From birth, individuals can be infected by RV and upon reaching the age of 3 years, the majority have been in contact with the virus and have developed antibodies.[6] In one study from Tehran, from total of 1250 stool samples that were collected from children under five years old, RV was detected in 32.3%.[19] Comparing the results of our study with the above study in children in Tehran, RV seems to be more prevalent in children. This may partly explain why RV found to be associated with CD in children. Repeated infections are also common, detectable by an increase in the level of RV specific antibodies and RT-PCR (PCR).[20, 21]

Spread by fecal-oral transmission, RV infection in adults typically manifests with nausea, malaise, headache, abdominal pain, diarrhea, and fever. Infection can also be symptomless.[22] The most common symptoms for patients with evidence of RV in the current survey were heartburn and abdominal pain, suggesting atypical carriage. There was, however, a significant correlation between RV and bloating. Bloating is a non-specific symptom, but it is possible RV infection may be the causative factor for some of these individuals. There were statistically significant difference in incidence of weight loss (P= 0.2) and abdominal pain (P= 0.001) in patients with positive CD serology compared to infected individuals with RV.

Altogether 3.7% of our subjects were positive CD serology. We have no histological confirmation for CD in these

subjects, but tTG antibodies are highly specific for the condition. [22] Within the limitations of the present study (lack of performing small bowel biopsy), one can infer that the prevalence of undiagnosed CD autoimmunity among adults with non-specific GI symptoms is 2 to 3 times higher than the general population in Iran. [23]

In contrast to the study in children this study did not find a significant correlation between CD and RV infection. Nevertheless, the infected cases with RV scored higher in patients with positive tTG with 32% versus 22% in those with negative CD serology (p=0.2). One of the reason for the lack of this association might be the lower prevalence of RV infection in adult with non-specific symptoms compared to children with specific symptoms like diarrhea.[8] Further studies in larger population in adults with specific symptoms would be required to identify the possible triggering role of RV in CD.

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PART III

Chapter 6

Implementation of statistical analysis in the clinical research of Coeliac Disease: Use of Probit and Logit Analysis

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ABSTRACT

Background: Coeliac disease (CD) is an autoimmune disorder of the small intestine that occurs in genetically predisposed people of all ages. The objective of this study is to investigate the relation between demographic and clinical factors with coeliac disease using probit and logit models. **Methods:** During the period of January to December 2008, 396 patients were recruited for this study. Histologic characteristics in duodenal biopsy specimens were evaluated in those with serology positive for coeliac disease according to the modified Marsh Classification1999. **Results:** From all of 369 cases in our study 26 (7.1%) had coeliac disease according to pathology results. Both of models had the same result indicated a negative association between BMI and CD but other factors were not significant. **Conclusion:** Coeliac patient had less BMI compare with rest of study population. Results from probit and logit models were similar to each others, indicated that probit analysis can be employed as a logit model to analyze the relationship between clinical and demographic factors and CD.

Celiac disease, Epidemiology, Genetic and Clinical Behavior in Iran

Introduction

Coeliac disease (CD) is an autoimmune enteropathy characterized by chronic inflammation of the small intestinal mucosa and by presence of typical auto antibodies (1). It develops in genetically predisposed individuals after mucosal contact with gluten and secondarily hitherto unknown triggering factors (1, 2, and 3). Because CD is caused by a reaction to gliadin, a gluten protein found in wheat, the only effective treatment is a lifelong gluten-free diet. According to last study the prevalence of CD increased in last few years (4, 5) and as well as in Iran (6). So there are lots of studies which investigate different clinical and demographic factors that may be influence on CD.

The most frequent statistical tool to address the relationship among a dichotomous response and other covariates are log linear models such as logistic regression and probit regression. Logit analysis used for analyses in which there are one or more categorical dependent variables. Probit regression is an alternative log-linear approach to handle categorical dependent variables. Its assumptions are consistent with having a categorical dependent variable assumed to be a proxy for a true underlying continuous normal (7). The aim of this study was to assess relation between clinical and demographic factors in coeliac patient, using logit and probit analysis.

Materials and Methods

Patients with dyspeptic symptoms, attended at the Gastroenterology ward of the Taleghani Hospital of Tehran, Iran for diagnostic endoscopy of the upper gastrointestinal tract, were prospectively evaluated between Januarys to December 2008. The Ethnic committee of research center for gastroenterology and liver disease, Shahid Beheshti University, M.C approved the protocol of study and informed consent was obtained from all participants in this study.

Patients who were informed of their high possibility of having CD and underwent gastrointestinal endoscopies with duodenal biopsies. Two or three mucosal biopsies were obtained. The specimens were read by two pathologists. Biopsy results were evaluated according to Marsh criteria (8) and subsequently modified by Rostami et al (9).

Relation between age, sex, body mass index and some clinical factors including diabetes, chronic obstwctiv, hepatitis, renal failure, cardiovascular disease, abdominal pain, anorexia, increasing weight, weight loss, nausea-vomiting, heart burn, bloating, early satiety, flatulence and stress with CD analyzed retrospectively using logit and probit. Logistic regression is a technique for making predictions when the dependent variable is a dichotomy, and the independent variables are continuous and/or discrete logit analysis is based on log odds while probit uses the cumulative normal probability distribution and the function used is the inverse of the standard normal cumulative distribution. SAS program version 9.1 was employed for analysis.

Results

From all of 369 cases in our study 26 patients (7.1%) had coeliac disease (8 men and 18 women). The result of probit and

logit models appeared in Table 1. According to both logit and probit analysis a significant relation was fund between BMI and CD. The p-value of abdominal pain in both models was very close with a criterion (0.05) which means that maybe it had a significant effect on CD. Other demographic and clinical factors were not significant. The coefficient of BMI was negative which means the relation between BMI and CD was inversing and all cases with positive CD had lees BMI than the others. According to the results, tow models were more similar to each other but in logit model odds ratio estimated and can be used for interpretation the coefficients.

Table 1. Demographic and laboratory features of celiac patients

subjects	Gender Male/female	Age (y)	Weight (kg)	Height (cm)	tTGA level
Case 1	F	27	55	158	44.3
Case 2	M	18	66	159	34.5
Case 3	M	45	77	180	67.2
Case 4	M	25	58	17	111.2
Case 5	F	68	66	155	53.1
Case 6	F	17	48	155	42.9
Case 7	M	35	55	150	94.5
Case 8	M	45	65	177	31.2
Case 9	M	35	55	164	71.8
Case 10	F	51	77	154	84.3
Case 11	F	24	59	157	42.1
Case 12	F	40	85	178	145.6
Case 13	F	20	75	168	19.9
Case 14	F	25	80	170	25.7
Case 15	F	29	50	155	93.5
Case 16	F	47	72	160	67.8
Case 17	M	30	67	170	194.9
Case 18	F	67	50	152	69.3
Case 19	F	60	51	155	55.6
Case 20	F	50	88	179	79
Case 21	F	21	67	160	64.7
Case 22	M	55	92	177	18.4
Case 23	M	24	60	177	81
Case 24	F	40	50	167	49.5
Case 25	F	50	75	167	69.4
Case 26	M	50	57	70	70.2

Discussion

Logit and probit models are special cases of general linear models to better treat the case of dichotomous and categorical variables. Although Probit is a variant of Logit modeling based on different data ssumptions, results of probit analysis are rarely reported in the original units. Logit is the more commonly used, based on the assumption of equal categories. Probit may be the more appropriate choice when the categories are assumed to reflect an underlying normal distribution of the dependent variable, even if there are just two categories (7). Coeliac disease is a genetically mediated autoimmune proximal enteropathy triggered by the ingestion of gluten. Last study in Tehran province showed that the prevalence of CD in this population in the serologically screened samples excluding IgA- deficient was 3.3% and 3.7% when including those IgA deficient with positive tTGG (5).

This paper examined relation between CD and demographic and clinical factors Using logit and probit models. Although probit model is not well-known for researchers in the filed of medical science, there are some studies using these models to interpret the relation between prognostic factors and a binary response. Costa-Font et al examined the influence of obesity jointly with other determinants on the prevalence of four chronic conditions, type 2 diabetes, cardiovascular disease, hypertension, and high cholesterol, using probit regression (10). Shelton Brown et al investigated the relation between diabetes and employment with probit model (11) and Shahrabani et al studied the impact of vaccinated in the past perceive influenza to prevent flu infection by probit analysis (12). In addition

Table2. Relation between Dempgraphic and Clinical Factors with CD with by Logit and Probit Analysis

Factors	Logit				Probit		
	Confidence limit	P-Value	Odds ratio	coefficient	Confidence limit	P-Value	Coefficient
Sex	0.910-5.487	0.0795	2.234	0.8039	0.925-5.678	0.0731	0.3825
Age	0.618-1.735	0.8943	1.036	0.0350	0.589-2.567	0.8989	0.0161
BMI	0.799-0.994	0.0387	0.891	-0.1154	0.674-0.997	0.0278	-0.0603
Diabetes	254.0-4.123	0.5810	1.365	0.3113	0.480-4.678	0.5737	0.1692
Chronic diarrheal	Not compatible	0.9735	<0.001	-12.2154	Not compatible	0.9911	-4.1920
Hepatitis	0.709-198.897	0.0853	11.875	2.4744	0.684-197.678	0.1126	1.4209
Renal failure	0.260-21.470	0.4449	2.363	0.8601	0.389-22.356	0.4687	0.4509
Cardiovascular disease	0.294-6.624	0.6745	1.396	0.3337	0.567-6.567	0.6798	0.1686
Abdominal pain	0.481-4.338	0.5124	1.444	0.3676	0.456-4.489	0.5054	0.1743
Anorexia	0.692-4.312	0.2411	1.728	0.5472	0.678-4.245	0.2500	0.2699
Increasing Weight	Not compatible	0.9850	<0.001	-12.1296	Not compatible	0.9882	-3.7375
Weight loss	0.756-4.009	0.1931	1.740	0.5541	0.658-4.763	0.1975	0.2710
Nausea and vomiting	0.615-3.244	0.4158	1.412	0.3453	0.619-3.896	0.4186	0.1678
Heart burn	0.689-4.550	0.2356	1.770	0.5712	0.574-4.678	0.2264	0.2712
Bloating	0.480-3.618	0.5925	1.318	0.2758	0.678-3.980	0.5881	0.1317
Early satiety	0.613-3.146	0.4308	1.389	0.3287	0.745-3.659	0.4298	0.1585
Flatulence	0.367-2.086	0.7624	0.875	-0.1341	0.329-2.843	0.7616	-0.0645
Stress	0.382-2.559	0.9822	0.989	-0.0108	0.573-2.678	0.9822	-0.00522

Pourhoseingholi et al examined the relation between gastrointestinal cancers and demographic factors with these two models (13). In reality, the difference between two models was not too important: both models need to have diagnostics done afterwards to check that the assumptions of the model have not been violated and use maximum likelihood. In general, the logit coefficients are larger than the probit coefficients. Because of the probit function is the inverse cumulative distribution function (CDF), or quantile function associated with the standard normal distribution. It has applications in exploratory statistical graphics and specialized regression modeling of binary response variables (4). In probit model we don't estimate odds ratio and describe models with coefficient directly. Negative and positive coefficients and significance are very important in describe of models. So probit regression can be one of useful statistical method for describe relationship between binary response and explanatory factors.

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PART IV

EFFECT OF GLUTEN FREE DIET

PART IV

Chapter 1

Subclinical Celiac Disease and Gluten Sensitivity

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ABSTRACT

Atypical presentation is the most common form of celiac disease (CD). Although the terminologies like *latent, silent* and *potential* have expressed different aspects of clinical and pathological behaviour of CD, they also have contributed in some extent to confusion between clinicians and patients due to the multiple definitions and uncertainty around them. In the light of new advances and the discovery of entities such as non-celiac gluten sensitivity, using *subclinical* instead of *silent* and *atypical* instead of *potential / latent* may simplify the understanding behind the clinical behaviour of atypical CD. The evidence behind a lower threshold for starting a gluten free diet (GFD) in non-celiac gluten sensitive patients would strongly support applying a GFD treatment strategy in any forms of CD.

Keywords: Subclinical, Celiac disease, Atypical, Microscopic enteritis, Gluten sensitivity.

Introduction

Using multiple terminology in defining atypical celiac disease (CD) has confused many of the clinicians to recognise atypical forms of this common disorder. CD is not considered an uncommon disorder any longer and is not a disease of essentially European origin (1). Nevertheless, recognising the existence of atypical forms known under the old terminologies like *latent*, *silent* and *potential* CD has introduced a new insight on clinical behaviour of this condition. That way the age of presentation of the disease has changed dramatically (1, 2) and the factors responsible for this change are mainly attributed to advances in diagnostic tools in recognising atypical and subclinical forms of disease. Adult presentation is increasingly common, and subclinical CD can occur at any age. Population screening with serological tests, have shown a CD screening prevalence of the order of 1% in the western hemisphere (3). European and Asian studies involving healthy blood donors found a prevalence rate of 1 in 166-330 (4, 5) subclinical CD. According to the previous studies, screening based on antibodies only would underestimate the prevalence of CD due to false-negative results caused by the low sensitivity of tests (6-10). However, some studies suggest that the overestimation of CD frequency could also result from antibody based screening programmes due to a high rate of false positives (11).

Sub-clinical Celiac Disease

Terminologies like *latent*, *silent* and *potential* celiac disease can be confusing for clinicians and patients. *Silent* CD is not absolutely silent after all; patients show signs of CD with no significant symptoms. *Potential* and *latent* are defined differently in different studies. T-cell-mediated autoimmune processes are initiated by gluten exposure, leading to both intestinal and atypical extraintestinal manifestations. More and more diseases are proven to be associated with CD. In these conditions, screening is strongly recommended. However, a typical CD patient today has merely mild abdominal symptoms. Malabsorption can be silent like a mild anaemia (better defined under subclinical), or there is usually only moderate malabsorption, if any at all. Diagnostic difficulties may further emerge when minor mucosal changes are found (12). Should the presence of CD be ascertained in every symptomatic patient with atypical presentation? Since gluten sensitivity is no longer limited to overt villous atrophy, and given the results of many studies (13-15), we believe the answer is yes.

Subclinical or so called silent CD cases are being detected in increasing numbers because of raised awareness of the disease. Presentations with atypical symptoms are the dominant form of disease manifestation and these comprise the sole and main part of the celiac iceberg (16). Whether they have positive serology with negative biopsy or increased $\gamma\delta$ T Cells receptors with symptoms compatible with CD they could be classified as atypical CD. We are moving towards a lower threshold in implementing a gluten free diet (GFD). Villous atrophy is not mandatory any longer to qualify a patient for GFD. In fact a large number of patients present with non-celiac gluten sensitivity with completely normal biopsy and negative
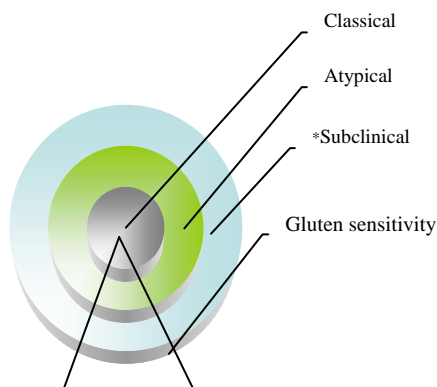
Figure 1. Celiac and non-celiac gluten sensitivity
*Suclinical: previously known as silent and atypical as known under latent and potential CD

serology. They also seem to benefit from a GFD. In such circumstances there is a need to re-define the terminologies according to the modified treatment strategy in gluten related disorders. The main strategy for treatment should target symptomatic typical or atypical patients, and not asymptomatic cases. We propose a simplified classification by dividing and replacing previous terminology to typical, atypical and subclinical instead of *silent/latent* and *potential*. (See figure 1).

Immunogenetics involvement in Celiac Disease

The spectrum of gluten related disorders seems to be beyond HLA DQ2-8. Our knowledge of CD pathogenesis has made significant progress in the last decade. The disorder is now considered the result of a complex interaction between genetic and environmental factors such as gluten that classified from subclinical to severe malabsorption. In contrast to gluten sensitivity celiac disease development has

a strong genetic component with a sibling relative risk (lambda (s)) of 30. Recent studies using the human genome screening technique in families with multiple siblings suffering from CD have suggested the presence of at least 4 different chromosomes in the predisposition to suffer from CD (17). One susceptibility locus is the MHC (major histocompatibility complex) region, with a particular association with the HLA-DQ alleles DQA1*0501 and DQB1*0201. However, shared-haplotype studies suggest that genes within the MHC complex contribute no more than 40% to the sibling familial risk of disease. Early studies showed that gliadin elicits an inflammatory T-cell reaction when added to intestinal biopsy specimens of celiac patients *in vitro* and a link to the genetic predisposition was provided by the isolation of gliadin-specific HLA-DQ2-restricted T-cell clones from CD mucosa (18, 19). However, the prevalence of HLA-DQ2 is high in the normal population (25-45%), suggesting the involvement of additional, and probably non-HLA-linked genes in CD pathogenesis.

Microscopic enteritis

The pathologic spectrum of the mucosal abnormalities seen on small intestinal biopsies, range from microscopic enteritis (Marsh 0-II) to macroscopic forms (Marsh IIIa-c) (20). Not every gluten-sensitised individual inevitably develops CD and not every celiac patient develops the destructive lesions such as Marsh III. Celiac disease is not exclusively due to antibody production either. A large proportion of the patients present with microscopic enteritis (Marsh I-II) whose

diagnoses may actually be missed (20-23). Five major histopathological features that define CD have been recognized in the previous study. These 6 distinct and sequential phases of the CD are microscopic enteritis (ME) Marsh (0-II). Marsh 0 with normal small bowel mucosa where intraepithelial lymphocytes are below 25/100 entrecotes. Some patients could still have subtle abnormalities at this stage like increased γδ T cells receptors or alteration of enterocytes and microvillis. i) Recruitment of T-lymphocytes >25/100 enterocytes (intestinal-intraepithelial lymphocyte or IEL; Marsh I), ii) lymphocyte infiltration and crypt hyperplasia (Marsh II), iii) macroscopic enteritis (Marsh IIIa-c) partial villous atrophy (Marsh IIIa), iv) subtotal villous atrophy (Marsh IIIb) and v) total atrophy (Marsh IIIb) and total villous atrophy (Marsh IIIc) (6,8). This sequential cascade suggests that a T-cell response to gliadin precedes, and very likely produces, the complete pattern of CD. The statistical comparison between antibody-positive and antibody-negative cases shows that the appearance of antibodies was seen predominantly in cases with serious mucosal damage in which IELs was highly increased. However, it is hard to rule out the contribution of antibodies in genesis of an autoimmune condition like CD.

The screening value of autoantibodies has been too optimistically overestimated, especially those on tissue transglutaminase antibodies (tTGA) (24, 25). However, comparing the tTGA to EMA and AGA, the sensitivity of tTGA does not offer any advantages over EMA for screening of the populations at high risk of CD (26- 28). It is time to re-evaluate our perception of

intestinal pathology (29) in such terms, rather than by continued use of subjective degrees of villous atrophy (VA), since absence of VA is not evidence of absence of CD. Such terminology obscures the recognition of fundamental changes occurring within small bowel mucosa. In simple words, increased density of IEL's and crypt hyperplasia form an essential phase in the disease pathogenesis sequence of progression. As CD with milder enteropathy is the most common form, histology cannot be considered as the gold standard any longer. Therefore, treatment should target the symptoms and not the immunohistology (29-32).

Gluten sensitivity and Celiac disease

Gluten sensitivity (GS) is characterised by negative antibodies and normal histology; it is defined as a non-allergic and non-autoimmune condition in which the consumption of gluten can lead to symptoms similar to those seen in CD. Until recently the terms GS and CD were used synonymously in literature (33) and it is not clear yet whether patients affected by GS will have some subtle intestinal and mucosal changes consistent with microscopic enteritis. Yet we know very little about the pathogenic mechanism behind gluten sensitivity. Some GS patients would tolerate even more than 5g gluten/day and still remain symptom free with negative serology (34, 35).

GS patients are gluten intolerant and gluten consumption does not lead to small intestinal damage, so it is not accompanied by the concurrence of tTG

autoantibodies or autoimmune disease. In the study by Kaukinen et al. out of 94 adults with GI symptoms, 63% were affected by gluten foods and did not classified as CD or as allergic (36).

On the other hand around 50% of the GS patients were DQ2/DQ8 positive, which is similar to that of the general population, while celiac patients carry more than 95% in most regions of the word. However, while the prevalence of CD is roughly 1% within the general population, GS is thought to affect 6 to 10% of the general population (37). In some cases GS can present with normal or milder enteropathy seen as increased intestinal permeability, IBS, abdominal discomfort, pancreatic disorders, pain or diarrhoea; or it may present with a variety of extraintestinal symptoms including lymphoma, attention deficit disorder and neuropathy, autism and schizophrenia, infertility, IBD, muscular disturbances as well as osteopenia and osteoprosis (38-42). Conform with current literature a GFD is recommended to gluten sensitive cases with/without enteropathy. This policy includes a range of symptomatic gluten sensitive cases with atypical presentation including those with small bowel microscopic changes (Marsh 0-II) who have negative antibody or characteristic feature for other conditions.

Conclusion

The spectrum of gluten related disorders is widening. This is because these common systemic disorders have multifactorial etiology with a multitude of symptoms and complications inside and outside the small bowel. We still don't know how seriously subclinical CD will be affected by the long term complications if they are not treated with GFD.

A marked increase in the prevalence of CD, especially the subclinical CD forms and non-celiac gluten sensitivity, seem to become a major health problem (43-46). The clinician may often face the variability of histological and clinical aspects of CD (46) with uncertainty, as they might not quite fit in the diagnostic models in the current guidelines. Accumulated evidence supports decreasing the treatment threshold for atypical CD and those with gluten sensitivity as the life quality of these cases will improve with GFD and the long-term health benefit of this strategy would perhaps be also cost effective.

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PART IV

Chapter 2

The effects of gluten-free diet on hypertransaminasemia in patients with celiac disease

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ABSTRACT

Background and Study Aims: Celiac disease is an immune mediated condition that leads to small bowel atrophy that resolves with a gluten free diet. Extra-intestinal manifestations of celiac disease include hypertransaminasemia. In this study the effects of a gluten free diet on hypertransaminasemia in patients with newly diagnosed celiac disease were studied.

Patients and Methods: Ninety eight consecutive patients with a new diagnosis of celiac disease were studied. All patients with celiac disease were treated with a gluten free diet Patients with hypertransaminasemia, at diagnosis, had a cirrhosis screen performed. Patients with a negative cirrhosis screen were reviewed, 6 months after the introduction of a gluten free diet, and serum levels of liver transaminases were measured again.

Results: Nine patients had hypertransaminasemia. One patient was HBsAg positive and was excluded from this study. The 8 remaining patients had no obvious cause for the hypertransaminasemia. Mean ($\pm$ SD) AST and ALT levels were 42.6 ± 16.5 IU/L (range: 16-66 IU/L) and 69.3 ± 9.3 IU/L (range: 52-81 IU/L). Six months after treatment with a gluten free diet, mean AST and ALT levels decreased to 24.5 ± 5.1 IU/L (range: 18-31 IU/L) ($p < 0.05$) and 24.6 ± 6 IU/L (range: 17-32 IU/L) (p: 0.01), respectively. In 7 patients the hypertransaminasemia, at diagnosis had resolved.

Conclusion: This study provides further evidence that some patients with celiac disease have a reversible hypertransaminasemia that resolves with a gluten free diet.

Keywords: Celiac disease; Gluten-free diet; Hypertransaminasemia; Liver.

Introduction

Celiac disease (CD) is an immune-mediated disease, leading to small bowel atrophy that resolves upon the introduction of a gluten free diet. Symptoms can include steatorrhea, weight loss, and fatigue. CD can also be asymptomatic. CD affects approximately 1 % of the Iranian general population (1) (2). CD can affect extraintestinal organs, such as the skin, pancreas, heart and liver (3). Previous studies have suggested that up to 9% of patients with hypertransaminasemia and negative investigations to identify chronic liver disease, have CD (4). Studies have reported that patients with CD have an increased incidence of auto-immune and cryptogenic liver disease compared to the general population (5) (6). The cytogenetic liver disorder associated with CD is characterized by hypertransaminasemia with non-specific histological changes (7). The mainstay of treatment of CD is adherence to a gluten-free diet (GFD)(2). Mild liver dysfunction often improves with introduction of a GFD. Kaukinen K, et al. showed that GFD may inhibit progression to hepatic failure, even in cases being considered for liver transplantation (8). The aim of this study was to evaluate the prevalence of liver dysfunction in patients with newly diagnosed CD patients and the effects of a GFD were studied.

Patients and Methods

Patients with newly diagnosed with CD, referred to Taleghani Hospital, Tehran, Iran, between September 2007 and September 2010 were recruited. The diagnosis of CD was based on duodenal biopsies with histological abnormalities characteristic of CD (the presence of any of the following: intraepithelial lymphocytes, crypts hyperplasia and villous atrophy) and serology consistent with a diagnosis of CD (positive IgA class human anti-tissue transglutaminase (tTGA) antibody, endomysial antibody (EMA) and total serum IgA (If IgA deficient and tTGG was positive)) (9, 10). Liver function tests (LFTS), serum bilirubin, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase, were measured in all patients. The serum levels of bilirubin (normal range: 0.2-1.3), aspartate aminotransferase (AST, normal range: 15–35 IU/L), alanine aminotransferase (ALT, normal range: 11–35 IU/L) and alkaline phosphatase (ALP, normal range: 64-306 for adults and 180-1200 for children) were measured by routine laboratory methods.

Patients with hypertransaminasemia had a cirrhosis screen performed to investigate possible causes of liver dysfunction. The cirrhosis screen consisted of measuring serological markers for viral hepatitis, metabolic liver diseases and serum protein electrophoresis. Serologic markers for viral hepatitis, including HBsAg (Hepatitis B surface antigen) and HCVAb (Hepatitis C virus antibody) were determined with commercially available ELISA kits (DIA PRO Diagnostic Bioprobes, Srl., Italy). Serum levels of Ferritin, TSH, caeruloplasmin, copper, ANA (anti-nuclear antibody), ASMA (anti smooth muscle antibody), AMA (anti mitochondrial antibody) and aFP (alpha-fetoprotein) were evaluated. History of taking medications, using alcohol, exposure to hepatic toxins and co-morbidities, such as diabetes mellitus, were considered. Body mass index (BMI) was

calculated in patients to detect overweight or obese patients. Ultrasonography of hepato-biliary system was done. Levels of AST and ALT higher than 35 IUL, increased serum bilirubin greater than 2 mg/dL and/or elevated serum alkaline phosphatase above 306 IU/L for adults and 1200 for children were considered as evidence of liver dysfunction. In patients with abnormal ALP values, serum levels of calcium and phosphate were determined. Patients with a specific diagnosis that might be responsible for altering LFT were excluded. Liver biopsy was performed in those patients with hypertransaminasemia and a negative cirrhosis screen.

All CD patients were treated with a gluten free diet (GFD). Patients with undiagnosed causes of abnormal LFT were reviewed after 6 months and Serum levels of AST, ALT, billirubin and ALP were rechecked. We have asked all patients about continuing GFD each month.

The patients were assured that their private information would be kept confidential and a written informed consent was obtained from them. The study was approved by the Ethics Committee of the Research Center for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences.

Data were analyzed by SPSS software ver.16 (Chicago, USA). Wilcoxon signed-ranks test used to compare mean before and after treatment. $P < 0.05$ was considered significant.

Results

Ninety eight patients with confirmed CD (40 Males and 58 females) with mean age of 32 ± 17.1 were studied. Nine of these patients had hypertransaminasemia. One patient with HBsAg was exclude in this survey and referred to gastroenterologist for further evaluation. Therefore 8 patients (five females and 3 males) with mean age of 26.4 ± 4.8 years (range: 19-33 years) were studied and maintained GFD. Marsh II was reported in two patients, Marsh IIIa in three, Marsh IIIb in two and Marsh IIIc in one. None of the patients with abnormal LFT had deficiency in total IgA serum. There was no medical history of significant co-morbidities, excess alcohol consumption or exposure to knwon hepatotoxic agents. None of the patients were overweight or morbidly obese. Ultrasonographic evaluation of these 8 patients had no significant findings. Liver biopsy was unremarkable. None of the biopsies showed evidence of steatohepatitis.

At the beginning of study, All 8 (8.1%) CD patients with undiagnosed liver dysfunction had normal billirubin levels with mean value of 0.95 ± 0.2 mg/dL (range: 0.7-1.3 mg/dL). Eight patients had abnormal ALT level and 5 of these 8 patients had abnormal AST. Also ALP levels were abnormal in 2/8 patients. Mean ($\pm$ SD) AST and ALT levels were 42.6 ± 16.5 IU/L (range: 16-66 IU/L) and 69.3 ± 9.3 IU/L (range: 52-81 IU/L). Mean ($\pm$ SD) concentration of ALP was 240.3 ± 118.7 IU/L (range: 108-428 IU/L). Two of 8 patients with elevated levels of ALP had low levels of calcium, representing metabolic disorder.

Six months after using GFD, mean AST and ALT levels decreased to 24.5 ± 5.1 IU/L (range: 18-31) (p: 0.04 IU/L) and 24.6 ± 6 IU/L (range: 17-32 IU/L) (p: 0.01), respectively. Billirubin levels decreased to

Table 1. Serum levels of billirubine, AST, ALT and ALP among CD patients with hypertransaminasemia and no other causes of hypertransaminasemia, before and 6 months after GFD

Patients			Total billirubin (mg/dL) Normal range: 0.2-1.3		AST (IU/L) Normal range: 15–35 IU/L		ALT(IU/L) normal range: 11–35 IU/L		ALP(IU/L) normal range: 64-306	
	Age	Sex	Before GFD	6 months after GFD	Before GFD	6 months after GFD	Before GFD	6 months after GFD	Before GFD	6 months after GFD
1	21	M	0.8	0.7	66	24	81	31	243	218
2	19	F	1.1	0.9	31	22	76	24	145	164
3	24	F	0.7	0.7	48	30	63	27	428	216
4	29	M	0.9	1	52	31	72	19	255	260
5	33	F	1	0.8	16	24	65	17	108	124
6	27	F	1.3	1.1	46	18	77	32	398	166
7	27	M	0.7	0.9	27	29	52	29	137	108
8	31	F	1.1	1.1	55	18	68	18	208	186

[*] AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; CD: Celiac Disease

0.9 ± 0.2 mg/dL (range: 0.7-1.1 mg/dL), although not significant differences was found (p: 0.33). Concentration of ALP reduced to 180.3 ± 50.6 IU/L (range: 108-260 IU/L) 6 months after using GFD and there was no significant differences with ALP values before GFD (p: 0.09). Except one case with persistent abnormal LFT's, the rest of 7 cases LFT's have normalized after 6 month of GFD. The prevalence of reactive hepatitis in this cohort was 8% with almost complete resolution following GFD. Table 1 represents the serum levels of billirubin, AST, ALT and ALP before and after following GFD.

Discussion

This study showed that CD may be associated with hypertransaminasemia in the absence of other possible causes of liver dysfunction. Furthermore introduction of a GFD can improve the hypertransaminasemia and normalise the liver enzyme abnormality.

The pathogenesis of hypertransaminasemia in CD is unidentified, several mechanisms have been proposed. We are aware of a link between CD and autoimmune disorder like autoimmune hepatitis, sclerosing cholangitis and primary biliary cirrhosis. However, most CD patients with elevated serum transaminases have no evidence of autoimmune disorder (11). It has been suggested that the hypertransaminasemia in CD patients may be multifactorial and a complication of prolonged existence of malabsorption, small intestinal bacterial overgrowth, iron overload disorder, hepatic steatosis, chronic intestinal inflammation and enhanced absorption of toxic substances (6, 11 and 12).

In this study, 8 percent of adult CD patients had cryptogenic liver disorder and we did not find any evidence of autoimmune liver diseases. In a comparable study, Dicky W, et al. reported a hypertransaminasemia in 15% of patients with newly diagnosed CD, (13). In previous studies, the frequency of elevated transaminases in patients with CD was 36-55% (7, 14, 15). Possible explanations for this high prevalence of a hypertransaminasemia in other studies may

include the very diferent populations studied. European patients may be more likely toocnsume excess alcohol and have a greater prevalence of non-alcoholic staetohepatitis than our Iranian population.

The prevalence of hypertransaminases among children with CD is higher than adult patients (14, 16, 17). Di Biase, et al evaluated a large population of children with CD in a prospective study. They found that isolated hypertransaminasemia is present in 40% of CD patients and that autoimmune liver hepatitis is present in 2% of cases, while no other autoimmune liver diseases were found (16). It has been reported that hypertransaminasemia might be an initial presentation of CD (18). Assessment of hypertransaminasemia in some studies has led to a diagnosis of CD in 10 % of patients, using serological methods (14, 19). Bardella *et al* study, 13 out of 140 patients with elevated transaminases were seropositive for gliadin and endomysial antibodies (14).

In a similar study, Volta et al. reported that in 55 patients with unexplained hypertransaminasemia, 5 had CD. In a similar studiy, Abdo et al. reported that CD serology was positive in 9% of patients with unexplained hypertransaminasemia (4). We feel these studies suggest that clinicians should consider CD in the evaluation of patients with hypertransaminasemia (19). In our study, 2 of the patients with newly daignosed CD patients had elevated ALP values, a result comparable to the Bardella et al, study (14). This was secondary to hypocalcaemia induced by chronic malabsorption.

Conclusion

Our study suggests that the hypertransaminasemia associated with CD resolves with the introduction of a GFD. This is in keeping with other studies that have reported an improvement in effects in the hypertransaminasemia associated with CD provided there was no evidence of underlying autoimmune, metabolic or viral liver disease (8, 16, 14, and 19). We suggest that clinicians need to consider the diagnosis of CD, when patients present with hypertransaminasemia. Furthermore we suggest that this hypertransaminasemia resolves with the introduction of a GFD.

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PART IV

Chapter 3

Celiac disease and Dysfunctional Uterine Bleeding; The efficiency of gluten free diet

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ABSTRACT

The aim of this study is to investigate the relation between Celiac disease (CD) and unexplained dysfunctional uterine bleeding (DUB) in celiac women. The celiac patients were selected from women who referred to celiac department. Controls were selected from those women without any signs of celiac and matched with age. Meanwhile, a trained physician was ready to explain the study, and then in case of their allowance, a questionnaire was completed by the physician. 24% of celiac women reported a past history of at least one menstrual cycle disorder v.s 10% of controls reported these problems (P=0.038) and higher percentage of unexplained DUB has been observed in celiac women. All celiac patients were undergoing with gluten free diet for at least 3 months and the celiac patients who reported the history of DUB again interviewed for any signs of unexplained DUB. From 12 celiac women with DUB, 10 patients reported no more unexplained DUB after getting gluten-free diet (83.3%). The occurrence of a significant correlation between CD and DUB suggests the possibility of considering CD as one of the potential causes of abnormal uterine bleeding. Therefore, celiac disease must be seriously considered in the screening of patients with reproductive disorders.
Keywords: Celiac disease, Dysfunctional uterine bleeding, Gluten Free Diet.

Introduction

Celiac disease (CD) is an autoimmune disorder characterized by gluten sensitivity in genetically susceptible individuals (1, 2). CD causes small bowel inflammation and is associated with increased small bowel permeability. It is associated with both intestinal and several extra-intestinal manifestations/complications, including infertility and adverse pregnancy outcomes (2-4). Celiac disease can affect women's reproductive life. Previous studies have suggested that women with undiagnosed CD to have 9-fold relative risk of multiple abortions and low birth weight babies compared to women with treated CD (5). Aside from adverse pregnancy outcomes, CD may present with a persistent iron deficiency and abnormal weight loss during a first, but more often, second pregnancy (2).

Dysfunctional uterine bleeding (DUB) is a common problem amongst women and accounts for 20% of gynecology office visits (6). It is defined as abnormal uterine bleeding in the absence of organic disease (excessively heavy, prolonged, or frequent intervals of bleeding), complications of pregnancy or systemic disease (7, 8).

The spectrum of abnormal uterine bleeding comprises of menorrhagia (heavy periods; blood loss >80 mL), metrorrhagia (prolonged, irregular periods), polymenorrhoea (frequent periods), oligomenorrhoea (scanty and infrequent periods), amenorrhoea (absent menstrual periods), intermenstrual bleeding and postcoital bleeding.

There is limited data on reproductive implications in Iranian women with CD.

The aim of this study is to investigate the relation between CD and unexplained DUB in celiac women who were referred to celiac department of Taleghani hospital. We also studied the effect of gluten-free diet on reducing the complications of DUB in women diagnosed with CD.

Patients and Methods

This study was designed as a case-control study. The celiac patient's group was selected from those who referred to celiac department of Taleghani hospital with diagnosis of celiac disease with or without dysfunctional uterine bleeding. Celiac disease was diagnosed based on positive serology and confirmation by histological assessment of small bowel biopsies. For this purpose, 5cc heparinized blood were be obtained. Blood sample was delivered to the laboratory within 2 hours. IgA class human anti-tissue transglutaminase (tTG) antibody using recombinant human tTG and Total serum IgA values were measured. Determinations of IgA anti-tTG antibody were carried out using a commercially available kit (AESKULISA tTG, Germany) and an enzyme-linked immunosorbent assay (ELISA) method. According to the manufacturer's indications, the result was considered positive when a value higher than 15.0 U/ml was recorded. Total serum IgA values were measured by an immunoturbidometric assay (Pars Azmoon, Iran) and serum levels below 70 U/L were considered indicative of IgA deficiency. Immunoglobulin G (IgG) tTG values were further obtained in individuals with IgA deficiency by an ELISA method, and using the commercially available kit (AESKULISA tTGG, Germany). Those

serology positive for CD will be underwent biopsy specimens processing too. Histological diagnosis of CD was based on the presence of intraepithelial lymphocytes (Marsh I), crypts hyperplasia and/or villi atrophy (Marsh II to IIIc). Biopsy results were classified according to modified Marsh criteria by Rostami et al. (1).

Control group was selected from those women who had not celiac disease with or without dysfunctional bleeding and referred to GI clinics and matched with age. Inclusion criteria for enrolling case and control group were; - Age range of 18-45 years old; - celiac disease which diagnosed and confirmed by serology and biopsy after that in symptomatic cases; - dysfunctional uterine bleeding that diagnosed when abnormal uterine bleeding ruled out any pathology in endometrial biopsy, normal hormonal levels of prolactin and thyroid stimulating hormone (TSH) and normal pelvic ultrasound. Exclusion criteria were: -any pathology contributed in abnormal uterine bleeding; - abnormal levels of TSH or prolactine; systemic disease confounding in diagnosis of celiac disease or DUB. Meanwhile, a trained physician was ready to explain the aim of study, and then in case of their allowance, a questionnaire was completed. The study was approved by the institutional ethics committees of Research Institute for gastroenterology and liver disease, Shahid Beheshti University of Medical sciences, and all participants signed a written informed consent.

Results

A total number of 50 celiac women who diagnosed for the first time (mean age±sd: 32.1±15.2) and 70 healthy control (mean age±sd: 31.7±12.7) were entered to this study. All celiac patients were new cases who diagnosed in Taleghani hospital, Research Institute for Gastroenterology and Liver Diseases and referred to department of celiac disease for intervention. Celiac patients and the age-matched healthy control were interviewed for clinical and demographic factors. 59.4% of patients and 67.3% of controls were married and the mean±sd of BMI in patients was 21.3±4.4 and in healthy controls was 21.5±5.9 (no statistically difference). Infertility was reported in 4% of celiac women and 2.9% of controls respectively (P=0.73). Also 6% of patients and 8.6% of controls reported a past history of at least one abortion (P=0.59) respectively (Table 1).

Table 1. The demographic factors and characteristics of celiac and non celiac groups

Characteristics	Celiac (n=50)	Non-Celiac (n=70)	P-Value
Age	32.1±15.2[*]	31.7±12.7	0.90
BMI	21.3±4.4	21.5±5.9	0.89
Married	33 (67.3)[†]	41 (59.4)	0.38
DUB	12 (24.0)	7 (10.0)	0.03
Infertility	2 (4.0)	2 (2.9)	0.73
History of Abortion	3 (6.0)	6 (8.6)	0.59

[*] Mean±SD; [†] percent

A higher percentage of unexplained DUB has been observed in celiac women. Twelve out of 50 cases of celiac group (24%) had DUB but in control group 7 out of 70 controls (10%) had DUB that is significant (P vaue=0.038). logistics regression analysis indicated that the crude risk of DUB was increasing 2.84 times for women with celiac disease compared to healthy women (95% CI: 1.03-7.84). Also

the adjusted risk of DUB was 3.83% for celiac women according to multivariate logistics analysis (95% CI: 1.19-12.33).

According to the histology of CD patients who reported DUB, 1 patient was in Marsh 0; four patients were in Marsh I, two patients in Marsh II and three patients in Marsh IIIc.

We also investigated the distribution of gastrointestinal complications and other diseases (which had been registered in CD patient's documents) with DUB (Table 2). The results indicated that there is a significant association between DUB and weight loss (P=0.03), which means that patients with lower weight, experiencing higher risk of unexplained DUB. Other symptoms and diseases were not significance.

All celiac patients were undergoing with gluten free diet for at least 3 months. After 3-4 months, the celiac patients who reported the history of DUB interviewed again for report of repeated abnormal bleeding. In fallow up of the patients on gluten free diet, approximately 10 out of 12 celiac cases did not complain of any abnormal uterine bleeding (83.3%). The menarche age in patients with CD on a GFD was decreased but was higher in the untreated CD patients; we concluded that the age at menarche in women with CD is regulated by a GFD.

Discussion

This study indicated that the risk of DUB is increasing in women with CD. The exact mechanism of DUB is uncertain but is thought to be caused by dysfunction of hypothalamic-pituitary-ovarian axis (9). Weight loss is another issue that has negative impact on reproduction. On the other hand, the spectrum of gluten related disorders is widening. This is because these common systemic disorders have multifactorial etiology with a multitude of symptoms and complications (10).

Table 2. Association with dysfunctional uterine bleeding and clinical symptoms in celiac group

Symptoms (%)	DUB (n=12)	Non-DUB (n=38)	P-value
Diarrhea	4 (33.3)	7 (18.4)	0.28
Bloating	6 (50)	18 (47.4)	0.87
Heart Burn	4 (33.3)	7 (18.4)	0.27
Weight Loss	8 (66.7)	12 (31.6)	0.03
Neuse & Vomiting	4 (33.3)	4 (10.5)	0.06
Anemia	10 (83.3)	22 (57.9)	0.11
Bone Disease	6 (50)	9 (23.7)	0.08

Several studies have shown that celiac disease can impair women's reproductive life eliciting delayed puberty, infertility, amenorrhea and early menopause. Some clinical and epidemiological studies have demonstrated that women with celiac disease are at a higher risk of miscarriage, low birth weight of the newborn (11-14).

In particular, celiac women, have their menarche at an older age, compared to that of healthy controls, whereas the average age at menopause of celiac women has been observed to be younger than that of healthy women; in the same studies an increased frequency of cases of secondary amenorrhea has also been registered among celiac women (15).

In the study by Ferguson et al. the reproductive life of 74 celiac patients including 54 patients on normal diet and 20 under gluten free diet were studied. They found that delayed menarche and early menopause were more common in the untreated group than the controls (16). In

compatible with Ferguson et al. study, Molteni et al. (15) examined 54 celiac patients and 54 healthy women and detected that the mean age at menarche was significantly later in CD patients (15). In another study, the mean age of menarche in 59 girls with CD was significantly higher in untreated girls compared to those who were on a GFD (17). Delayed menarche and earlier menopause in CD that cause a decreased number of children may be interpreted as sub-fertility (18-20).

Previous studies suggested that women with CD have higher risk of multiple abortions (5). An Italian case-control study on 62 celiac women and 186 healthy controls showed a higher percentage of menstrual cycle disorders in celiac women (21). In our study there was no association between CD and infertility or abortion which may be due to small sample size. But the results indicated that risk of DUB was higher for women with CD.

The gluten itself, could explain the disturbances and malnutrition would worsen the disease in a consequent vicious cycle (22). So these reproductive disorders may be a consequence of the endocrine derangements caused by selective nutrient deficiencies (14).

The occurrence of a significant correlation between CD and DUB suggests the possibility of considering CD as one of the potential causes of abnormal uterine bleeding. Dysfunctional uterine bleeding can be treated with medical therapy, a levonorgestrel releasing IUD and hysterectomy (23).

Our results revealed that the gluten free diet could decrease the risk of DUB in CD women. Nowadays, the early diagnosis and treatment of CD is possible and not very costly. Therefore, celiac disease must be seriously considered in the screening and treatment of patients with reproductive disorders.

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PART V

DISCUSSION

Discussion

Increasing awareness and publication of recent data has left no doubt that Celiac Disease (CD), is affecting not only European but also the Middle Eastern countries with the same prevalence.

As CD was considered to be very rare previously in Iran, based on this assumption; it was not generally considered as a possibility in the differential diagnosis of patients coming with nonspecific gastrointestinal symptoms. By the development of more epidemiological studies using sensitive and specific serological tests with biopsy verification established higher prevalence of CD (up to 1: 166 in healthy population) in recent decade in Iran.

People of so-called 'fertile crescent' in South West Asia like Iran share a genetic background with European populations. It is therefore, not surprising to find a high incidence of CD in this region, where the HLA haplotype is present and large quantities of wheat are consumed.

Important points extracted from this thesis:

• According to presented studies in this thesis, the prevalence of CD in different ethnic groups in Iran was reported to be almost similar to the prevalence of CD in Western countries.

Publication of our data has generated a significant interest between Iranian and Middle Eastern health professional in considering CD in their differential diagnosis and investigation the high risk group suspected for CD

• The advise generated form extracted data have been formulated in Persian Language in Iranian Celiac website that is served as a valuable resource and information frequently used by patients and health professional. It has become a popular policy to follow up the cases where the diagnosis of gluten sensitivity or celiac disease is in doubt, in addition, observing the effect of either gluten withdrawal or gluten challenge in appropriate way is considered.

• The Iranian health professional become more familiar with atypical presentation of CD and the behavior of this condition in this country. The policy of taking appropriate number of biopsies from appropriate sites and following a reasonable diagnostic pathway to detect the sufferers has become a practical tool in most of Iranian medical centers.

• Some clinical behavior of CD is reflected in our studies indicating that atypical presentation, specially with dyspepsia, could be the main presentation of CD. However, this is also common presentation in non-celiacs.

• Using serological tests with a more realistic insight on their sensitivity and

specificities, appropriate use of other diagnostic tools like HLA typing in challenging cases has been promoted appropriately.

- The results of our studies on patients at risk like in iron deficiency anemia, osteoporosis, infertility, recurrent miscarriages, liver enzyme abnormalities, seizures, chronic diarrhea, insulin-dependent diabetes mellitus, autoimmune thyroid disorders and malignancies have been widely published in Iranian media.

- Clinicians are aware that CD is more prevalent among patients with atypical symptoms.

- Similar to European our epidemiological data demonstrate that CD can occur at any age but in adults the peak incidence is in the forth decade. Females are more commonly affected than males, and of those patients presenting during their fertile years.

- We produced some valuable data on HLA typing in Iranian celiac and non celiac. We observed that the frequency of DQ2 was higher in CD patients than controls. The prevalence of DQ8 in our CD population was higher than that reported in other populations.

- Wheat is a major component of the Iranian diet and exposure to wheat proteins induces some degree of immune tolerance, leading to milder symptoms that may be misdiagnosed with other GI disorders.

Finally

The availability of serological tests for screening and diagnosis of CD has resulted in an increase rate of diagnosis of this disease in Middle East, specially in Iran where CD was traditionally considered to be very rare. CD is now reported to be as common in Iranian population, as in Western countries. This has important implications for clinicians, as well as for the authorities in Iran, who will need to address the supply of gluten-free diets to those diagnosed with CD. This certainly raises important issues for the health of Iranian population where the major staple diet is wheat. Since commercial gluten-free products are not readily available and significantly more expensive than their gluten-containing products in this area, this will represent a serious challenge for patients and health professionals.

SUMMARY

Summary

Celiac associated disorders and the questions around these associations seems to be one of the hottest topics in the world of patients with Celiac disease and the health professional who deal with this condition. This is because this common systemic disorder has a multifactorial etiology with a multitude of symptoms and complications inside and outside small bowel. Almost all those conditions as reported to have an association with coeliac disease are or must be HLA-DQ2/DQ8 positive. That means that they all have a common characteristic sharing a similar genetic background. The environmental factors associated with a complex genetics are leading to destructions of the small intestinal villi resulting in malabsorption syndrome. The co-morbidity between celiac disease and other disorders has been clearly established and there have been many reports of numerous intestinal and extra intestinal coexistent disorders with CD. In this short systematic review the nature of these associations has been explored and classified according to their pathogenesis.

Study on epidemiology, clinical behavior in Eastern countries and challenges in diagnosis and treatment of celiac disease are the outline of my research and training over the past few years. The study we performed so far includes 15 Original Article, 4 Review Articles, 5 Case Reports/ Letters and one Editorial. According to these studies coeliac disease (CD) is as common in Iran as in Western countries with similar association pattern with a prevalence of around 1% in Iranian population. Our preliminary study on genetic showed that the proportion of HLA-DQ2 in coeliac patients was higher than control and this finding is in agreement with other studies in Western populations. A short summery of the studies we published or under preparation is presented below.

PART I
Chapter I.1: INTRODUCTION and OUTLINES

PART II
EPIDEMIOLOGY

Chapter II.1: In the past, celiac disease (CD) was believed to be a chronic enteropathy, almost exclusively affecting people of European origin. The availability of new, simple, very sensitive and specific serological tests (AGA, AEM and tTGA) has shown that CD is as common in Middle Eastern countries as in Europe, Australia and New Zealand where the major dietary staple is wheat. A high prevalence of CD has been found in Iran, in both general population and at-risk groups. In developing countries, serological testing in at risk groups is necessary for early identification of celiac patients. Clinical studies show that presentation with non-specific symptoms or a lack of symptoms is as common in the Middle East as in Europe. The implementation of GFD is a major challenge for both patients and clinicians in Iran, especially since commercial gluten-free products are not available in this area. This review provides evidence and summarizes the epidemiological data on CD in Middle East and highlights different

clinical presentations and management of CD in Iran.

Chapter II.2: Non-specific GI symptoms seem to be the typical presentation of atypical CD. This study had two objectives: first objective was to determine the prevalence of CD in patients with GI symptoms and the second objective was to characterize the typical manifestations of the atypical forms of CD. The results indicated that there is a high prevalence of CD antibodies among patients with GI symptoms (3.7%). Therefore, more awareness regarding the atypical presentation of CD could be the key step in identifying asymptomatic patients.

Chapter II.3 and II.4: Among the most common neurology problems associated with CD are peripheral neuropathy, cerebellar ataxia, epilepsy, multiple sclerosis, psychiatric disorders and mental retard.

Nutritional factors have been suspected in association with neurological defects but are rarely found and their correction does not seem to influence in the prognosis. Some reports show certain neurologic symptoms that respond to a GFD, especially if it is started in the first few months after their appearance. It is now evident that the link between CD and neurologic disorders results, in part, from common genetic background, most importantly, the HLA region on chromosome 6, and other markers. In addition to genetic predisposition, immunologic factors probably also play a role.

Depression, intellectual disabilities, schizophrenia and other psychiatric symptoms have been reported as common complications of CD, occurring in about one third of patients. Common symptoms include apathy, excessive anxiety and irritability. All of them clearly improve after few months of adherence to a GFD.

Chapter II.5: In this study CD was not associated with a high incidence of unfavorable outcomes. Overall, 1/66 (1.5%) pregnant women had a confirmed CD. Despite pregnancy is acting as a triggering factor for manifestation of CD in susceptible individuals, it seems that CD severity is variable in different individual and not every untreated pregnant woman affected by CD is at high risk for reported complications. This may suggests that gluten free diet could be avoided in some of patients who had a normal pregnancy.

Chapter II.6: In this multicenter study 931 patients who underwent duodenal biopsy for celiac disease were studied at nine centers from west, south and east European and Middle Eastern countries. The results indicated that because the proportion of individuals undergoing duodenal biopsy who have ≥4 specimens increased recently, we should diffusing this guideline into clinical practice. This study shows that there is significant gap between evidence based medicine and the reality in clinical practice. Only a minority of individuals undergoing upper GI endoscopy had ≥4 duodenal biopsies. Therefore, given the high incremental yield of submitting ≥4 specimens, efforts to increase adherence to this standard are warranted.

Chapter II.7: This is the first study conducted on the frequency of HLA typing in Iranian celiac disease patients compared to general population. We found that the frequency of DQ2 was higher in CD patients compared to controls. The prevalence of DQ8 in our CD population

was higher than the frequency reported in other populations. Our results underline the primary importance of HLA-DQ alleles in susceptibility to CD in the Iranian population. This can be used as a diagnostic modality, especially, in whom the diagnosis is uncertain. This paper is under submission.

Chapter II.8: The metabolic changes in serum of celiac patients and control were explored using a metabonomics approach based on Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. In order to find the important variables influencing the zinc level of the cases and control group, Classification and Regression Trees (CART) method was employed. Serum levels of zinc were measured using Atomic absorption method. To make a non-linear model between zinc and the ^{1}HNMR matrix an artificial neural networks (ANN) model was employed.

Our descriptors consisted of Lipid, Choline and Histidine. Based on the square of correlation coefficients (R^2) for training, test and validation, there is good agreement between experimental and the theoretical zinc values.

Chapter II.9: The experimental data consists of 56 blood serum samples, employing prometab software, NMR spectra data are reduced to 205 variables and then the orthogonal signal correction (OSC) effect on the celiac disease metabonomics as well as following the separation by Principle Component Analysis (PCA) was investigated. Partial least squares Discriminant Analysis (PLS-DA) method was used for supervised study. PCA and PLS-DA models calculated following OSC showed enhanced separation between classes and tighter clustering within each class.

Chapter II.10: In this paper, we have studied body mass index in celiac patients diagnosed in a population-based study, using a well-matched case-control analysis to assess the BMI in celiac patients. This study showed that up to 33% of CD patients were underweight.

PART III
ASSOCIATED DISORDERS

Chapter III.1: Liver disease and CD share widespread risk factors. Different liver disorders such as autoimmune hepatitis, elevations of liver enzyme levels, primary biliary cirrhosis, nonspecific hepatitis, primary sclerosing cholangitis and nonalcoholic fatty liver disease have been reported in patients with CD. In this review we provide information regarding liver disorders that may be seen in association with celiac disease and the effect of treatment of CD on these disorders.

Chapter III.2: It appears non-responsiveness to HBV vaccine should lead to a routine assessment in patients with CD in those non-responsive to HBV vaccination. Non-responsiveness to vaccination might be a sign of a possible undiagnosed celiac disease or suggest the lack of compliance with gluten free diet.

Chapter III.3: Microscopic enteritis (ME) is the stage of microscopic and sub-microscopic changes (microenteropathy) associated with the symptoms of gluten sensitive enteropathy leading to micronutrient deficiencies. It is characterized by subtle mucosal abnormalities without prominent inflammation, villous affacement, erosions or ulcerations on conventional light

microscopy. This review summarizes the patho-mechanism of Malabsorption in milder and severe enteropathy and shows that there is no correlation between the severity of Malabsorption and the degrees of mucosal changes.

Chapter III.4: Coeliac disease and *Toxoplasma gondii* (TG) infection are associated with infertility and adverse pregnancy outcomes. The result of this study show that CD increases the risk of *T.gondii* infection as CD may facilitate distribution of the ingested TG oocysts beyond small bowel lesions during pregnancy and that this infection, but not CD, increases the serum levels of pro-inflammatory cytokines such as IL8. CD was behind the increased risk of low birth weight and miscarriages irrespective of *T.gondii* infection. It is also possible that TG could theoretically increase sensitivity to gluten. This may precipitate the development of CD in susceptible individuals. This would be in keeping with studies that suggest that other enteric infections can predispose to the development of CD among susceptible individuals.

Chapter III.5: Dyspepsia is a major health problem in Iranian population. It has been reported that the frequency of CD in people with dyspeptic complaints is 1.1-3%, which is two to nine times higher than in the general population. Although a high prevalence of *H pylori* infection was found in this study, the relationship between Hp infection and CD was similar to that reported in other geographic areas. In this study we have shown that there are no differences between clinical presentation of coeliac disease and *H. pylori*.

Chapter III.6: This is the first report of a case with unexplained nail deformity associated with CD. The result of this study show that celiac disease should be considered and ruled out in patients with persistent nail abnormalities who are not responding to the conventional treatment.

Chapter III.7: Celiac disease is frequently associated with abnormalities of gastric histology and gastric function, including gastritis, peptic ulceration and atrophic gastritis. Although our knowledge of the pathogenesis of CD is rapidly expanding, the possible role of chronic *Helicobacter pylori* (HP) infection, known to be capable of inducing duodenal ulcers, needs further examination. In This study we examined the association of CD with chronic gastritis associated with *Helicobacter Pylori* (HP) infection. This manuscript introduced a way to simply the interpretation of gastric lesions classifying them in macroscopic and microscopic stages for clinical studies.

Chapter III.8: Some discrepancies could stem from the heterogeneity of the studies. Regarding a potential pathogenic mechanism, since CD causes malabsorption of folic acid and other nutrients, this pathway has been proposed to explain the unfavourable outcomes of pregnancy. As serology scores a far higher sensitivity and specificity compared to histology for CD, our study suggests a higher prevalence of CD among unexplained infertile men and female. Regular screening for CD in men and females with unexplained infertility is recommended.

Chapter III.9: It is important to recognize that there are a number of possible ways that infections might trigger autoimmunity, and many of these immunological

Celiac disease, Epidemiology, Genetic and Clinical Behavior in Iran

mechanisms have been proven in experimental systems. Some recent studies provide evidence that a rotavirus protein may be linked to celiac disease through a molecular mimicry mechanism and that the risk of developing celiac disease appears to increase in children in relation to the number of rotavirus infections. This is not specific for as some infection may also be persistent and can mimic or trigger different autoimmune inflammatory disorders. This study shows that Rotavirus infection is common among Iranian patients with non-specific gastrointestinal symptoms. However, in contrast to studies in children, this study shows that active rotavirus infection was not statistically significantly different between individuals who were tTG antibody positive and those who were tTG antibody negative.

Chapter III.10: Logit and probit models are special cases of general linear models to better treat the case of dichotomous and categorical variables. Although Probit is a variant of Logit modeling based on different data ssumptions, results of probit analysis are rarely reported in the original units. Logit is the more commonly used, based on the assumption of equal categories. Probit may be the more appropriate choice when the categories are assumed to reflect an underlying normal distribution of the dependent variable, even if there are just two categories. . Results from probit and logit models were similar to each others, indicated that probit analysis can be employed as a logit model to analyze the relationship between clinical and demographic factors and CD.

PART IV

EFFECT OF GLUTEN FREE DIET IN THE IMPROVEMENT OF CELIAC DISEASE

Chapter IV.1: In the light of new advances and the discovery of entities such as non-coeliac gluten sensitivity, using *subclinical* instead of *silent* and *atypical* instead of *potential* / *latent* may simplify the understanding behind the clinical behaviour of CD. Accumulated evidence supports decreasing the treatment threshold for atypical CD and those with gluten sensitivity as the life quality of these cases will improve with GFD and the long-term health benefit of this strategy would perhaps be cost effective.

Chapter IV.2: The prevalence of hypertransaminasemia (HT) in children is increased in CD. Studies show that patients with HT were younger than patients with normal aminotransferases. A significant percentage of adult patients with non-alcoholic fatty liver disease (NAFLD) have no metabolic risk factors and may be related with the concomitant presence of CD. Mild liver abnormalities are common in adult patients with celiac disease and usually resolve with a GFD. The possible presence of celiac disease should be investigated in patients with severe liver disease. Dietary treatment may prevent progression to hepatic failure, even in cases in which liver transplantation is considered. Our study on 98 coeliac cases showed that CD may affect liver function and induce hypertransaminasemia in the absence of other possible causes of liver dysfunction. Moreover, GFD can improve liver function and decrease liver enzymes to normal values.

Chapter IV.3: Celiac disease is an autoimmune disorder which can affect

women's reproductive life. Previous studies have suggested that women with undiagnosed CD to have 9-fold relative risk of multiple abortions and low birth weight babies compared to women with treated CD. Dysfunctional uterine bleeding (DUB) is a common problem amongst women. It is defined as abnormal uterine bleeding in the absence of organic disease (excessively heavy, prolonged, or frequent intervals of bleeding), complications of pregnancy or systemic disease. The occurrence of a significant correlation between CD and DUB suggests the possibility of considering CD as one of the potential causes of abnormal uterine bleeding. Our results revealed that the gluten free diet could decrease the risk of DUB in CD women.

Chapter IV.4: This is a case report of a male adult who presented with oral aphthous ulcerations, autoimmune hepatitis and mistaken with fatty liver disease. In this case, where the patient presented with high BMI and evidence of fatty liver disease, grade I, CD was suspected due to mild abnormal bloating, cryptogenic hypertransaminasemia, abnormal LFT and poor response to fatty liver treatment. This presentation type is not uncommon; diagnosis was confirmed by the presence of subtotal villous atrophy in the biospy specimen, by the positive specific antibody screening (AGA, tTG and EMA antibodies), by negative antibody screening and the normalisation of liver enzymes on a gluten-free diet.

Chapter IV.5: A full blown CD presentation is rare. This case highlights classical presentation of CD with multiple deficiencies in modern life. In patients who suspected of having CD barium evaluation of the small bowel are seldom required. But by barium investigation of this patient we found abnormal roentgen findings included straightening of the valvulae conniventes, replacement of the normal delicate feathery mucosal pattern with either marked thickening or complete obliteration of the mucosal folds dilation of the small intestine.

PART V
DISCUSSION, SUMMERY, ACKNOWLEDGEMENT AND A BRIEF CV.

DEEL V

SAMENVATTING

SAMENVATTING

Aandoeningen geassocieerd met coeliakie evenals vragen die zich voordoen in dit verband lijken erg belangrijk te zijn voor de gezondheidsspecialisten die zich bezig houden met deze aspecten. Dit komt doordat algemene systemische aandoeningen een multifactoriële etiologie hebben, met een veelheid aan symptomen en complicaties binnen en buiten de dunne darm. Bijna alle gemelde aandoeningen die verband houden met coeliakie zijn of zouden HLA-DQ2/DQ8 positief moeten zijn. Dit betekent dat ze allemaal een gemeenschappelijk kenmerk hebben, namelijk dezelfde genetische achtergrond. Omgevingsfactoren geassocieerd met een immuno-genetisch complex leiden tot een enteropathie die als resultaat een malabsorptie syndroom zal hebben. Co-morbiditeit tussen coeliakie en andere aandoeningen is duidelijk vastgesteld en er zijn vele meldingen geweest van darm- en extra-intestinale aandoeningen, samengaand met coeliakie. In deze systematische studie werd de aard van deze verbandingen onderzocht en gerangschikt volgens hun pathogenese.

De studie over de epidemiologie, de verbanden met andere aandoeningen zoals het klinische gedrag, *H.pylori*, Rotaviros, *Toxoplasma gonadii* in Oosterse landen, evenals de uitdagingen in de diagnose en behandeling van coeliakie vertegenwoordigt het plan van mijn onderzoek en voorbereiding gedurende de afgelopen 4-5 jaren. De studie die ik tot nu toe uitgevoerd heb, omvat 12 originele artikelen, 3 beoordelingen, 2 brieven. Volgens deze studies is coeliakie net zo gewoon in Iran als in de Westerse landen, met een vergelijkbaar patroon van associaties met een prevalentie van ongeveer 1% onder de Iraanse bevolking. Onze voorafgaande studie met betrekking tot genetica toonde aan dat het aandeel van HLA-DQ2 bij coeliakie patiënten hoger was dan bij controle, en deze bevinding vertegenwoordigt een overeenkomst met andere studies onder Westerse bevolkingsgroepen. Hieronder volgt een korte samenvatting van deze studies die wij gepubliceerd hebben of welke in publicatie.

DEEL I
Hoofdstuk I.1. INLEIDING   en SCHETSEN van Het Proefschrift

DEEL II
EPIDEMIOLOGIE

Hoofdstuk II.1. In het verleden dacht men dat coeliakie een chronische enteropathie was die bijna uitsluitend mensen van Europese oorsprong trof. De beschikbaarheid van eenvoudige, zeer gevoelige en specifieke, nieuwe serologische testen (AEM, en tTGA) toonde aan dat coeliakie even gebruikelijk is in het Midden-Oosten landen als in Europa, Australië en Nieuw-Zeeland waar de basisvezels tarwe zijn. Een grote prevalentie van coeliakie werd geïdentificeerd in Iran, zowel onder de algemene bevolking als bij risicogroepen. In ontwikkelingslanden is serologisch testen van risicogroepen noodzakelijk voor de vroege identificatie van coeliakie patiënten. Klinische studies tonen aan dat de presentatie met niet-specifieke symptomen of geen symptomen even gewoon is in het Midden-Oosten als in Europa. De implementatie van GVD (glutenvrij dieet) is een grote uitdaging

voor zowel patiënten als voor artsen in Iran, vooral sinds commerciële glutenvrije producten niet meer beschikbaar zijn in dit gebied. Deze analyse levert het bewijs en verzamelt de epidemiologische gegevens over coeliakie in het Midden-Oosten en benadrukt de verschillende klinische verschijnselen en het beheer van coeliakie in Iran.

Hoofdstuk II.2: Niet-specifieke GI symptomen schijnen de typische manifestatie van atypische coeliakie te zijn. Dit onderzoek had twee doelstellingen: het eerste doel was de prevalentie van coeliakie vast te stellen bij patiënten met gastro-intestinale symptomen en het tweede doel was de typische uitingen van atypische vormen van coeliakie te karakteriseren. De resultaten gaven aan dat er een hoge prevalentie van coeliakie-antilichamen is bij patiënten met atypische GI symptomen (3,7%). Zo kan een groter bewustzijn betreffende atypische verschijnselen van coeliakie de belangrijkste stap zijn in het identificeren van deze patiënten met atypische manifestaties.

Hoofdstuk II.3: Tussen de meest voorkomende neurologische problemen in verband met coeliakie zijn perifere neuropathie, cerebrale ataxie, epilepsie, multiple sclerose, psychiatrische stoornissen en mentale retardatie. Voedingsfactoren zijn verdacht in verband met neurologische afwijkingen maar worden zelden geïdentificeerd en de correctie hiervan schijnt niet te worden beïnvloed in de prognose. Sommige rapporten tonen bepaalde neurologische symptomen aan die reageren op een glutenvrij dieet, vooral als deze wordt

gestart binnen een paar maanden na hun verschijning. Nu is het duidelijk dat het verband tussen coeliakie en neurologische aandoeningen gedeeltelijk uit de gemeenschappelijke genetische achtergrond resulteert, vooral het HLA gebied op chromosoom 6 en andere merkers. Naast genetische aanleg, kunnen immunologische factoren misschien ook een rol spelen.

Depressie, verstandelijke handicap, schizofrenie evenals andere psychiatrische symptomen zijn gemeld als zijnde gemeenschappelijke verbanden/complicaties van coeliakie die zich bij ongeveer een derde van de patiënten voordoen. Gemeenschappelijke symptomen zijn onder andere apathie, angst en overmatige prikkelbaarheid. Sommigen hiervan kunnen na een aantal maanden verbeteren na het volgen van een glutenvrij dieet.

Hoofdstuk II.4: In deze multicenter studie werd een totaal van 931 patiënten geanalyseerd die zijn onderworpen aan een duodenale biopsie voor coeliakie in negen centra in de westerse, zuidelijke en oostelijke Europese landen, evenals landen in het Midden-Oosten. Deze studie toont aan dat er een groot verschil bestaat tussen de geneeskunde op basis van bewijzen en de realiteit van de klinische praktijk. Slechts een minderheid van gevallen die een GI endoscopie onderging, had 4 of meer dan 4 duodenale biopsiën. Dus, gezien de toenemende efficiëntie van de aangeboden monsters, zijn de inspanningen tot verhoging van de naleving van deze standaard gegarandeerd.

Hoofdstuk II.5: Dit is de eerste studie verricht op de frequentie van HLA-

typering bij Iraanse patiënten die aan coeliakie lijden, in vergelijking met de algemene bevolking. We vonden dat DQ2 frequentie hoger was bij coeliakie patiënten in vergelijking met controles.

De prevalentie van DQ8 in onze coeliakie bevolking was hoger dan de frequentie gemeld bij andere volken. Onze resultaten benadrukken het primaire belang van HLA-DQ allelen bij susceptibiliteit voor coeliakie bij de Iraanse bevolking. Dit kan gebruikt worden als een diagnosemethode, vooral bij personen bij wie de diagnose onzeker is. Dit document is in afwachting van de indiening.

Hoofdstuk II.6: Het doel van deze multicenter studie was de etiologie van gastro-intestinale en niet-gastro-intestinale stoornissen te onderzoeken in het kader van een grote groep van symptomatische coeliakie patiënten uit Italië en Roemenië op Europees niveau en uit Iran in het Midden-Oosten. De resultaten van deze studie toonden aan dat bovenbuik aandoeningen zoals buikpijn en dyspepsie, de meest voorkomende primaire chronische ziekten waren bij Europese patiënten, terwijl bij Iraanse patiënten diarree en het opgeblazen gevoel werden beschouwd als uitingen van de klassieke coeliakie.

Hoofdstuk II.7: Het doel van deze studie is de prevalentie van coeliakie te bespreken bij dyspeptische patiënten. Tussen november 2007 en oktober 2008, zijn er 407 patiënten onderzocht die willekeurig een bovenste gastro-intestinale endoscopie hebben ondergaan in het diagnostisch onderzoek voor dyspeptische symptomen. Duodenale histologie heeft Marsh-IIIc laesies aangetoond in 6,4% van de gevallen en de serologische testen bevestigden de aanwezigheid van coeliakie in 2,5% van hen. De resultaten van deze studie wijzen op een hoge prevalentie van coeliakie bij mensen met dyspeptische symptomen en als zodanig dient de dyspepsie te worden beschouwd als een basisreferentie voor coeliakie.

Hoofdstuk II.8: In dit artikel bestudeerden we de lichaamsmassa-index bij coeliakie patiënten die gediagnosticeerd ware als gevolg van een bevolkingsgebaseerde studie, gebruikmakend van een gepaste case-control analyse met het oog op de evaluatie van lichaamsmassa-index bij coeliakie patiënten. Deze studie heeft aangetoond dat 33% van de coeliakie patiënten ondergewicht hadden.

DEEL III
GEASSOCIEERDE AANDOENINGEN

Hoofdstuk III.1: Leverziekte en coeliakie delen wijdverspreide risicofactoren. Diverse leverstoornissen zoals auto-immune hepatitis, de toename van leverenzymen niveau, primaire biliaire cirrose, niet-gespecificeerde hepatitis, primaire sclerotische cholangitis en niet-alcoholische leververvetting zijn gemeld bij patiënten met coeliakie. In deze analyse hebben we leveraandoeningen onderzocht die gemeld zijn als zijnde geassocieerd met coeliakie en het effect van coeliakie-behandeling op deze aandoeningen.

Hoofdstuk III.2: Zowel coeliakie als de infectie *Toxoplasma gondii* (TG) worden geassocieerd met onvruchtbaarheid

en ongunstige uitkomsten van de zwangerschap. De uitkomst van deze studie toont aan dat coeliakie het risico op *T. gondii* infectie verhoogt omdat coeliakie de verspreiding van de ingenomen TG oocysten verder dan dunne darm-letsels tijdens de zwangerschap zou kunnen vergemakkelijken en dat deze infectie, en niet coeliakie, de serumconcentraties van pro-inflammatoire cytokines zoals IL8 verhoogt. Coeliakie bevond zich achter het verhoogde risico op een laag geboortegewicht of een miskraam, ongeacht de aan- of afwezigheid van *T.gondii* infectie. Het is ook mogelijk dat TG theoretisch de sensitiviteit voor gluten kan verhogen. Dit kan de ontwikkeling van coeliakie versnellen bij gevoelige personen. Dit zou in overeenstemming zijn met studies die suggereren dat andere enterische infecties de ontwikkeling van coeliakie kunnen versnellen bij gevoelige personen.

Hoofdstuk III.3: Dyspepsie is een belangrijk gezondheidsprobleem onder de Iraanse bevolking. Er werd gemeld dat de frequentie van coeliakie bij mensen met dyspeptische ziekten 1 tot 1,3% is, hetgeen 3 keer zoveel betekent dan bij de algemene bevolking. Hoewel een wijdverspreide *H pylori-infectie* (*Helicobacter pylori*) gevonden werd in deze studie, was de relatie tussen Hp-infectie en coeliakie onbeduidend in vergelijking met het gerapporteerde in andere geografische gebieden. In deze studie hebben we laten zien dat er geen verschillen zijn tussen de klinische manifestatie van coeliakie en *H. pylori*.

Hoofdstuk III.4: Gezien coeliakie malabsorptie van foliumzuur en andere voedingsstoffen produceert, kan dat ook de

ongunstige zwangerschapsuitkomsten verklaren. Sinds serologie een veel te hoge sensitiviteit en specificiteit scoort in vergelijking met milde histologie van coeliakie, suggereert onze studie een bredere prevalentie van coeliakie bij mannen en vrouwen met onverklaarbare onvruchtbaarheid. Het wordt aanbevolen om de mannen en vrouwen met onverklaarbare onvruchtbaarheid regelmatig te onderzoeken op coeliakie.

Hoofdstuk III.5: Het is belangrijk te erkennen dat er een aantal mogelijke methoden zijn waardoor infecties auto-immuniteit kunnen uitlokken, en veel van deze immunologische mechanismen werden bewezen in experimentele studies. Enkele recente studies tonen aan dat een rotavirus eiwit kan worden gekoppeld aan coeliakie door middel van een moleculair mechanisme van imitatie en het risico op het ontwikkelen van coeliakie lijkt te verhogen onder kinderen in verband met het aantal rotavirus infecties. Dit is niet specifiek, omdat sommige infecties tevens persistent kunnen zijn en diverse auto-inflammatoire aandoeningen kunnen imiteren of uitlokken. Deze studie toont aan dat rotavirus-infectie veel voorkomt bij Iraanse patiënten met niet-specifieke gastro-intestinale symptomen. Echter, in tegenstelling tot studies bij kinderen, toont deze studie aan dat actieve infectie met het rotavirus statistisch niet significant verschillend was tussen degenen die positieve tTG antilichamen hadden en degenen met negatieve tTG antilichamen.

Hoofdstuk III.6: Logit- en probitmodellen zijn speciale gevallen van algemene lineaire modellen om het geval van dichotome en categorische variabelen

optimaal te behandelen. Hoewel Probit een variant is op Logit model op basis van verschillende gegevenssimulatie, worden probit analyseresultaten zelden gemeld in de oorspronkelijke eenheden. Logit wordt vaker gebruikt, gebaseerd op de aanname van gelijke categorieën. Probit kan een betere keuze zijn wanneer de categorieën verondersteld worden een normale basisverdeling van de afhankelijke variabele te reflecteren, zelfs indien er slechts twee categorieën zijn. De resultaten uit Probit en logit modellen waren vergelijkbaar met elkaar, aangevend dat de probit-analyse kan worden toegepast als een logit model voor het analyseren van de relatie tussen demografische en klinische factoren enerzijds en coeliakie anderzijds.

DEEL IV
HET EFFECT VAN HET GLUTENVRIJE DIEET

Hoofdstuk IV.1: In het licht van de nieuwe vooruitgang en van de ontdekking van entiteiten zoals niet-coeliakie gluten sensitiviteit, gebruikmakend van de term *subklinisch* in plaats van *stil* en *atypisch* in plaats van *potentieel/latent*, kan het inzicht achter het klinische gedrag van coeliakie vereenvoudigd worden. De verzamelde bewijzen ondersteunen de verlaging van behandelingsdrempel voor atypische coeliakie en die met gluten sensitiviteit dienen aangegeven te worden omdat de levenskwaliteit van deze gevallen zal verbeteren tegelijk met GVD en de lange termijn voordelen van deze strategie zouden het meest waarschijnlijk de rentabiliteit zijn, in wat betreft de kosten.

Hoofdstuk IV.2: De prevalentie van hypertransaminasemie (HT) bij kinderen is verhoogd in coeliakie. Studies tonen aan dat patiënten met HT jonger waren dan die met normale aminotransferasen. Een aanzienlijk percentage van de volwassen patiënten met niet-alcoholische leververvetting (NAFLD) heeft geen metabole risicofactoren en kan worden gerelateerd aan de gelijktijdige aanwezigheid van coeliakie. Onze studie op 98 coeliakiegevallen heeft aangetoond dat coeliakie de leverfunctie kan beïnvloeden en hypertransaminasemie induceren in de afwezigheid van andere mogelijke oorzaken van leverfunctiestoornissen. Bovendien kan GFD de leverfunctie verbeteren en kan leverenzymen naar normale waarden brengen.

Hoofdstuk IV.3: Disfunctioneel bloeding van de uterus (DUB) is een veelvoorkomend probleem bij vrouwen. Het wordt gedefinieerd als abnormale bloedverlies uit de baarmoeder in de afwezigheid van een organische ziekte (uiterst moeilijke, langdurige of frequente periodes van bloeding), complicaties van de zwangerschap of systemische ziekte. Het optreden van een significante correlatie tussen coeliakie en DUB suggereert de mogelijkheid om coeliakie in aanmerking te nemen als een van de mogelijke oorzaken van abnormaal uterus bloedverlies. Onze resultaten laten zien dat een glutenvrij dieet het risico op disfunctionele baarmoederbloeding kan verminderen bij vrouwen die aan coeliakie lijden.

DEEL V
DISCUSSIE, SAMENVATTING, ERKENNING en een korte CV.

Coeliakie, epidemiologie, genetisch- en klinisch gedrag in Iran

بخش ۵

خلاصه

خلاصه

اختلالات مرتبط با بیماری سیلیاک و سئوالات پیرامون این بیماری برای متخصصان سلامتی که به این وضعیت رسیدگی می‌کنند بسیار با اهمیت است. علت آن این است که این اختلال سیستمیک شایع علل مختلفی دارد که با علایم و عوارض روده‌ای و خارج روده‌ای همراه است.

تقریباً تمام کسانی که شرایط مشابهی در ارتباط با بیماری سیلیاک در آنها گزارش شده یا HLADQ2/8 مثبت هستند یا در آینده خواهند بود. این بدان معنی است که همه این بیماران دارای زمینه ژنتیکی مشترکی هستند. عوامل محیطی مرتبط با کمپلکس ایمنی– ژنتیک در نهایت منجر به انتروپاتی منفک شده از سندرم سوء جذب خواهد شد.

عوارض مشترک بین بیماری سیلیاک و دیگر اختلالات به خوبی ثابت شده است و گزارشات مختلفی از اختلالات همزمان روده‌ای و خارج روده‌ای با بیماری سیلیاک در دست است.

در این مطالعه مروری سیستماتیک ماهیت این ارتباطات با توجه به پاتوژنز بیماری بررسی و طبقه‌بندی شده است. بررسی اپیدمیولوژی، ارتباط با دیگر بیمارها مانند هلیکوباکترپیلوری، روتاویروس، توکسوپلاسما گوندی، برخوردهای کلینیکی در کشورهای اروپایی و چالش‌های تشخیصی و درمانی بیماری سیلیاک که حاصل ۴ تا ۵ سال مطالعات ما تاکنون بوده، هدف اصلی من در انجام این پایان‌نامه بوده است. مطالعات انجام شده

ما شامل ۱۲ مقاله پژوهشی، ۳ مقاله مروری، ۲ مقاله به سردبیر بوده است. بر پایه مطالعات انجام شده، شیوع بیماری سیلیاک در ایران دارای الگوی مشابه کشورهای غربی با شیوعی در حدود یک درصد است.

مطالعات اولیه ما در بحث ژنتیک نشان داد که نسبت HLADQ2 در بیماران مبتلا به سیلیاک بالاتر از گروه شاهد است و این یافته‌ها مشابه نتایج مطالعات انجام شده در کشورهای غربی است. خلاصه‌ای کوتاه از مطالعات منتشر شده و یا در حال انتشار در ذیل ارائه شده است:

بخش اول

فصل I.1: مقدمه و اهداف پایان‌نامه

بخش دوم:

اپیدمیولوژی

فصل II.1: در گذشته اعتقاد بر این بود که بیماری سیلیاک یک انتروپاتی مزمن است که تقریباً بطور انحصاری در مردم اروپا وجود دارد. در دسترس بودن آزمایش‌های ساده، بسیار حساس و اختصاصی سرولوژیکی (AGA، EMA و tTG) نشان داد که بیماری سیلیاک همانند کشورهای اروپایی، استرالیا و نیوزلند که عمده رژیم غذایی آنها گندم است در کشورهای خاورمیانه نیز شیوع دارد و شیوع بالای

این بیماری در هر دو جمعیت نرمال و گروه‌های در معرض خطر بالا دیده شده است.

در کشورهای در حال توسعه، آزمون‌های سرولوژیکی در گروه خطر برای شناسایی زود هنگام بیماران مبتلا به سیلیاک لازم است. مطالعات بالینی نشان می‌دهد که تظاهرات با علایم غیراختصاصی و یا عدم وجود علایم در کشورهای خاورمیانه دارای شیوع برابر کشورهای غربی است. انجام و پیگیری رژیم غذایی بدون گلوتن مهم‌ترین چالش بیماران و پزشکان ایرانی است. بخصوص از آنجایی که محصولات فاقد گلوتن تجاری در این زمینه در دسترس نیست. این بررسی شواهد و خلاصه اطلاعات اپیدمیولوژیک منتشر شده در زمینه بیماری سیلیاک را در خاورمیانه و همچنین علایم مختلف بالینی بارز و مدیریت این بیماری در ایران را نشان می‌دهد.

فصل II.2: به نظر می‌رسد علایم غیراختصاصی گوارشی نشان دهنده علایم بارز غیرپپتیک بیماری سیلیاک باشد. این مطالعه دو هدف داشت: هدف اول تعیین شیوع بیماری سیلیاک در بیماران مبتلا به علایم گوارشی و هدف دوم افتراق علایم پپتیک از غیر پپتیک بیمار سیلیاک است. نتایج مطالعه نشان دهند شیوع بالای آنتی‌بادی مرتبط با سیلیاک در بیماران با علایم غیرپپتیک بود (۳/۷٪) بنابراین آگاهی بیشتر در رابطه تظاهرات غیرمعمول بیماری سیلیاک می‌تواند کلید شناسایی این بیماران با تظاهرات غیرمعمول باشد.

فصل II.3: از رایج‌ترین مشکلات مغز و اعصاب که با بیماری سیلیاک در ارتباط هستند می‌توان نوروپاتی محیطی، آتاکسی مخچه، صرع، مالتیپل اسکروزیز، اختلالات روانی و عقب‌ماندگی ذهنی را نام برد. ارتباط مشکوکی بین بین عوامل تغذیه‌ای و نقایص نورولوژیکی دیده شده که به نظر نمی‌رسد بهبود این وضع پیش‌آگهی بیماری را تحت تأثیر قرار دهد.

برخی گزارش‌ها نشان می‌دهد برخی علایم عصبی بخصوص اگر در ماه‌های اولیه ظهور باشند با شروع رژیم غذایی بدون گلوتن بهبود می‌یابند. امروزه نشان داده شده که رابطه بین بیماری سیلیاک و نتایج اختلالات عصبی مربوط به زمینه ژنتیکی مشترک و مهم‌تر از همه ژن HLA بر روی کروموزوم ۶ و دیگر نشانگر هاست. علاوه بر زمینه ژنتیکی، عوامل ایمنولوژی نیز احتمالاً در این رابطه را دارای نقشی مشخص هستند. افسردگی، معلولیت فکری، اسکیزوفرنی و سایر علایم روانی به عنوان عوارض و علایم مشترک با بیماری سیلیاک گزارش شده‌اند که در حدود یک سوم از بیماران اتفاق می‌افتد. علایم رایج این بیماری شامل بی‌تفاوتی، اضطراب و تحریک‌پذیری بیش از حد است که برخی از آنها ممکن است پس از چند ماه رعایت رژیم غذایی فاقد گلوتن بهبود یابند.

فصل II.4: در این مطالعه چند مرکزی ۹۳۱ بیمار که بیوپسی دئودنوم برای بیماری سیلیاک انجام داده بودند از ۹ مرکز واقع در غرب، جنوب و شرق اروپا و کشورهای خاورمیانه مورد مطالعه قرار

در حالی که برای بیماران ایرانی اسهال و نفخ به عنوان علایم کلاسیک شایع دیده شدند.

فصل II.7: هدف از انجام این مطالعه بررسی شیوع بیماری سیلیاک در بیماران مبتلا به سوءهاضمه است بین سالهای ۲۰۰۸-۲۰۰۷ تعداد ۴۰۷ بیمار که تحت انجام آندوسکوپی تشخیصی دستگاه گوارش قرار گرفته بودند به صورت تصادفی وارد مطالعه شدند. بافت‌شناسی دئودنوم ضایعات Marsh I تا Marsh IIIc را در ۶/۴ درصد موارد نشان داد که با انجام غربالگری سرولوژی بیماری سیلیاک در ۲/۵ درصد آنها تأیید گردید. نتایج این تحقیق نشان دهنده شیوع بالای بیماری سیلیاک در میان مبتلایان سوءهاضمه دارای علامت است. در نتیجه سوءهاضمه بایستی به عنوان علامت اصلی بیماری سیلیاک مد نظر باشد.

فصل II.8: در این مطالعه BMI بیماران سیلیاکی تشخیص داده شده در مطالعه بر پایه جمعیت در مقایسه گروه کنترل همسان بررسی شد. نتایج این مطالعه افزایش وزن را در حدود ۳۳ درصد از بیماران سیلیاکی نشان داد.

بخش سوم

اختلالات مرتبط

فصل III.1: بیماری‌های کبدی و بیماری سیلیاک دارای فاکتورهای خطر مشترک و گسترده‌ی

گرفتند. این مطالعه نشان داد که شکاف قابل توجهی بین پزشکی مبتنی بر شواهد و واقعیت در عملکرد بالینی وجود دارد. تنها در اقلیتی از افراد تحت آندوسکوپی دستگاه گوارش فوقانی راهنماهای پیشنهاد شده جهت اخذ بیشتر از ۴ بیوپسی از دوازدهه را رعایت کرده بودند. بنابراین با توجه به نتایج فوق افزایش ارسال بیشتر یا مساوی ۴ نمونه بیوپسی لازم و ضروری است.

فصل II.5: در این مطالعه برای اولین بار در ایران فراوانی تایپ HLA در بیماران مبتلا به سیلیاک در مقایسه با جمعیت عمومی بررسی شد. نتایج مطالعه نشان داد که DQ2 شیوع بالاتری در میان بیماران سیلیاکی در مقایسه با جمعیت نرمال داشت. شیوع DQ8 در میان بیماران سیلیاکی مورد مطالعه بالاتر از فراوانی گزارش شده در جمعیت‌های دیگر بود. نتایج ما بر اهمیت بررسی آل‌های HLA در افراد مضنون به بیماری سیلیاک در جمعیت ایرانی تأکید دارد. این نکته می‌تواند به عنوان یک روش تشخیصی بخصوص در افرادی که در آنها تشخیص نامشخص است مورد استفاده قرار گیرد.

فصل II.6: هدف این مطالعه چند مرکزی بررسی علت‌شناسی اختلالات گوارشی و غیرگوارشی در جمعیت Cohort بیماران دارای علایم گوارشی مبتلا به سیلیاک در ایتالیا و رومانی از اروپا و ایران از خاورمیانه بود. نتایج این مطالعه نشان داد اختلالات گوارشی شکمی بالارونده مانند درد شکم و سوءهاضمه شایع‌ترین شکایت بیماران اروپایی بود

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هستند. اختلالات کبدی متنوعی مانند هپاتیت اتوایمیون، افزایش سطح آنزیم‌های کبدی، سیروز اولیه صفراوی، هپاتیت غیراختصاصی، اسکلروز کولانژیت اولیه و کبد چرب غیرالکلی در بیماران مبتلا به سیلیاک گزارش شده است. در این مطالعه مروری ما کلیه اختلالات کبدی که با بیماری سیلیاک در ارتباط هستند را بررسی و تأثیر درمان بیماری سیلیاک در بهبود این بیماری‌ها را به بحث می‌گذاریم.

فصل III.2: هر دو بیماری سیلیاک و عفونت توکسوپلاسماگوندی با نازایی و عوارض جانبی بارداری همراه هستند. نتایج این مطالعه نشان داد بیماری سیلیاک خطر ابتلا به عفونت توکسوپلاسما را افزایش داده و همچنین در طی حاملگی اووسیت‌های هضم شده توکسوپلاسما را روی زخم‌های روده کوچک بطور وسیعی می‌گستراند. از طرفی عفونت توکسیوپلاسما بدون توجه به بیماری سیلیاک باعث افزایش مقدار سایتوکاین‌های پیش-التهابی مانند IL8 می‌شود. بیماری سیلیاک سبب افزایش خطر تولد نوزادان با وزن کم هنگام تولد و سقط جنین بدون درنظر گرفتن عفونت توکسوپلاسماگوندی است.

همچنین ممکن است توکسوپلاسما گوندی بطور نظری باعث افزایش حساسیت به گلوتن گردد و این ممکن است باعث گسترش بیماری سیلیاک در افراد حساس گردد. این امر در راستای مطالعاتی است که نشان می‌دهد دیگر عفونت‌های روده‌ای می‌توانند به توسعه بیماری سیلیاک در افراد مستعد و حساس کمک کند.

فصل III.3: سوءهاضمه از مهم‌ترین علل مشکلات سلامت در جمعیت ایرانی است. شیوع بیماری سیلیاک در جمعیت مبتلا به سوءهاضمه بین ۱/۱ تا ۳ درصد گزارش شده که این میزان ۳ برابر شیوع این بیماری در جمعیت نرمال است. اگر چه شیوع بالای از عفونت هلیکوباکترپیلوری در مطالعه حاضر گزارش شد اما همانند دیگر مطالعات ارتباط معنی‌داری بین این عفونت و بیماری سیلیاک یافت نشد. در این مطالعه همچنین اختلاف بین علایم بالینی بیماری سیلیاک و هلیکوباکترپیلوری دیده نشد.

فصل III.4: از آنجایی که بیماری سیلیاک باعث سوءجذب اسیدفولیک و سایر مواد مغذی می‌گردد، ممکن است نتایج نامطلوبی بارداری را نیز توضیح دهد. از طرفی چون نتایج سرولوژی حساسیت و اختصاصیت بالاتری در مقایسه با بافت-شناسی خفیف در بیماری سیلیاک دارد، مطالعه ما شیوع بالای این بیماری را در میان مردان و زنان نابارور با علت ناشناخته نشان می‌دهد. غربالگری منظم بیماری سیلیاک در مردان و زنان مبتلا به ناباروری با علت ناشناخته توصیه می‌گردد.

فصل III.5: برخی از مطالعات اخیر شواهدی ارائه می‌کند که نشان می‌دهد پروتئین روتاویروس از طریق یک مکانیسم تقلید مولکولی با بیماری

سیلیاک ارتباط داشته و به نظر می‌رسد که خطر ابتلا به بیماری سیلیاک در کودکان با افزایش عفونت روتاویروس مرتبط باشد. البته این مطلب خاص این عفونت نیست زیرا برخی از عفونت‌ها می‌توانند با حضور خود و تقلید مولکولی باعث اختلالات التهابی خودایمنی متنوعی گردند. مطالعه ما نشان داد که عفونت روتاویروس از شایع‌ترین علامت‌های گوارشی غیراختصاصی در بیماران ایرانی است هر چند در مقایسه با مطالعات انجام شده در کودکان، بررسی حاضر نشان داد عفونت فعال روتاویروس تفاوت معنی‌داری در میان افراد آنتی‌بادی ITG مثبت و منفی ندارد.

فصل III.6: Logit و Probit مدل‌های موارد مخصوص مدل‌های عمومی خطی هستند که متغیرهای دو بخشی و مطلق بهتر شناسایی شوند اگر چه مدل Probit متفاوت از مدل Logit برپایه نمونه سازی است اما نتایج حاصل از تحلیل مدل Probit بندرت در واحد های اصلی گزارش شده است. برپایه فرض طبقه‌های یکسان مدل Logit معمولاً بیشتر به کار برده شده است. مدل Probit ممکن است هنگامی که طبقه‌بندی‌ها منعکس کننده توزیع یک متغیر وابسته حتی اگر فقط دو دسته مناسب‌ترین انتخاب باشد استفاده گردد. نتایج مدل‌های Probit و Logit به یکدیگر شباهت داشت و نشان داد که آنالیز مدل Probit می‌تواند به عنوان جایگزین مناسبی برای مدل Logit جهت آنالیز ارتباط بین فاکتورهای بالینی و دموگرافیک بیماری سیلیاک باشد.

بخش چهارم

تأثیر رژیم غذای بدون گلوتن

فصل IV.1: در پرتو پیشرفت‌های جدید و کشف عناوین مانند حساسبت به گلوتن غیرسیلیاکی و استفاده از عبارت Subclinical به جای خاموش و Atypical به جای بالقوه و نهفته می‌تواند درک رفتارهای بالینی بیماری سیلیاک را ساده‌تر کند. شواهد فراوانی کاهش رفتار آستانه درمان بیماران سیلیاکی Atypical و افراد حساس به گلوتن را نشان می‌دهد. کیفیت زندگی این افراد با رعایت رژیم غذایی بدون گلوتن بهبود خواهد یافت و استراتژی سلامت این افراد در دراز مدت در کاهش هزینه‌ها مؤثر خواهد بود.

فصل IV.2: شیوع افزایش آنزیم‌های کبدی در کودکان مبتلا به سیلیاک بالا است. مطالعات مختلف نشان می‌دهدکه بیماران مبتلا به Hypertramsaminasemia جوانتر از بیماران با سطح نرمال آمینوترانسفرازها هستند. درصد بالایی از بیماران مبتلا به بیماری کبد چرب غیرالکلی فاقد فاکتورهای خطر متابولیک هستند که علت این بیماری را بایستی در ابتلا به بیماری سیلیاک جستجو کرد. مطالعه ما روی ۹۸ بیمار مبتلا به سیلیاک نشان داد که بیماری سیلیاک می‌تواند روی عملکرد کبدی تأثیر گذاشته و در صورت عدم وجود علت خاصی برای مطالعه عملکرد بد کبد افزایش ترانس آمیناز را القا نماید.

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هر چند رژیم غذای بدون گلوتن می‌تواند عملکرد کبد و همچنین بازگرداندن سطح آنزیم‌های کبدی را به سطح نرمال بهبود بخشد.

فصل IV.3: خونریزی رحمی بدون علت (DUB) یکی از معمول‌ترین مشکلات در میان زنان است. این علامت به صورت خونریزی رحمی غیرطبیعی در غیاب بیماری ارگانیک، عوارض بارداری و یا بیماری‌های سیستمیک ثابت می‌شود. ارتباط معنی‌دار بین بیماری سیلیاک و DUB

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بخش پنجم

بحث، خلاصه، تقدیر و تشکر و رزومه کوتاه و مختصر

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Celiac disease, epidemiology, genetic and clinical behavior in Iran

CURRICULUM VITAE

Celiac disease, Epidemiology, Genetic and Clinical Behavior in Iran

CURRICULUM VITAE

The author of this thesis was born in Aleshtar (Lorestan Province), Iran, on 23 September 1979. He passed his secondary school exam in 1997 at the Emam Ali high school in Aleshtar Lorestan. His AS degree was Veterinary and took place at the Azad University, Shahrekord, Iran from 1998-2000. After that he started his BS degree on laboratory of veterinary medicine in the period 2003 until 2005 at the Azad University, Research and Science Tehran.

From April 2005 he has been working as research fellow at the Research Center for Gastroenterology and Liver Diseases (RCGLD), Shahid Behehsti University of Medical Sciences, Tehran. He started his research on coeliac disease on 2007 under support and supervision of his brother Kamran Rostami.

He undertook a short training course at University Medical Center Groningen under supervision of Prof. Cisca Wijmenga, with further inspirational visit at VU University Medical Center, Amsterdam facilitated by Prof Mulder and University Medical Center Leiden by Prof. Luisa Mearin.

Based on publications and presentations during 2010 he was awarded with Superior Scholar price in the 11th Research Festival of Shahid Beheshti University of Medical Science, Tehran, Premier Investigator in Sciences & Research Festival of Lorestan province on the June 2011 and has obtained research grants from WHO (2007) and Iran National Science Foundation 2011. In addition he had scientific collaboration with different national and international centers and universities and published 2 books on basic nutrition and diagnosis of celiac disease in Persian in 2009 and 2010 respectively.

He initiated the first celiac diseases website in Persian (www.celiac.ir). Currently he is managing coeliac disease department at RCGLD and the International affairs of Gastroenterology and Hepatology From Bed to Bench (GHFBB) journal.

At the same time he has been supporting the research activities at Parasitology Department RCGLD resulting in a significant number of peer reviewed publications in this field.

From April 2009 at the 13th International Coeliac Disease Symposium in Amsterdam, he was assigned to the objectives of this PhD project by Prof. Chris Mulder. During this period he worked on the research projects described in this thesis under supervision of Kamran Rostami, Prof. Mohammad Reza Zali and Prof. Chris Mulder.

He is married to Somayeh Jahani in April 2012.

De auteur van dit proefschrift werd geboren in Aleshtar (Provincie Lorestan), Iran, op 23 september 1979. Hij is geslaagd voor zijn middelbare school examen in 1997 op de Emam Ali school in Aleshtar, Lorestan. Hij studeerde diergeneeskunde op Azad Universiteit in Shahrekord, Iran tussen 1998-2000. Daarna begon hij zijn BS studie in het laboratorium van de diergeneeskunde in de periode 2003 tot 2005, aan de Azad Universiteit in Teheran.

Vanaf april 2005 is hij werkzaam als onderzoeker verbonden aan het Onderzoeks Centrum voor Maag-, Darm- en Leverziekten (RCGLD) op Shahid Behehsti Universiteit van Medische Wetenschappen, Teheran. Hij begon zijn onderzoek naar coeliakie in 2007 met ondersteuning en met begeleiding van zijn broer, Kamran Rostami.

Hij kreeg een korte training op Universitair Medisch Centrum Groningen onder leiding van Prof. Cisca Wijmenga, met verder een inspirerend bezoek aan VU Medisch Centrum, Amsterdam ondersteund door Prof. Chris Mulder en aan het Universitair Medisch Centrum Leiden door Prof. Luisa Mearin.

Op basis van publicaties en presentaties in 2010 werd hij onderscheiden met de Superior Scholar prijs op het 11e Onderzoek Festival van Shahid Beheshti Universiteit van Medische Wetenschap, Teheran, "Premier Onderzoeker" op het Wetenschaps & Onderzoek Festival van provincie Lorestan in juni 2011 en heeft hij subsidies voor onderzoek ontvangen van de WHO (2007) en van de Iran National Science Foundation 2011. Daarnaast had hij een wetenschappelijke samenwerking met verschillende nationale en internationale centra en universiteiten en publiceerde twee boeken in het Perzisch over de basisvoeding en diagnose van coeliakie, in 2009 en 2010.

Hij initieerde de eerste coeliakie website in het Perzisch (www.celiac.ir). Momenteel leidt hij de Coeliakie afdeling op RCGLD en de Internationale betrekkingen van "Gastroenterology and Hepatology From Bed to Bench Research" (GHFBB) een nieuw MDL tijdschrift.

Tegelijkertijd heeft hij onderzoeksactiviteiten ondersteund op de Parasitologie Afdeling van RCGLD wat resulteerde in een aanzienlijk aantal publicaties op dit gebied.

Vanaf april 2009, op het 13e Internationale Coeliakie Symposium in Amsterdam, werd dit PhD project met Prof. dr. Chris Mulder verder doorgesproken. Tijdens deze periode werkte hij aan de onderzoeksprojecten beschreven in dit proefschrift, onder de leiding van Kamran Rostami, Prof. Mohammad Reza Zali en Prof. Chris Mulder.

Hij trouwde Somayeh Jahani in april 2012