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Corneal thickness and intraocular pressure in pediatric patients with Celiac disease or Inflammatory Bowel Disease

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INTRODUCTION

The history of Celiac disease (CD) is intimately connected to the introduction of the cultivation of cereals for food purposes, dated to about 10,000 years ago, when it was introduced in the near Middle East, known for its climatic characteristics and those of the soil as the "Fertile Crescent" (Figure 1). However, the use of wheat, which constituted a fundamental moment in the switch of the population from nomadic to geographically stable, showed up the fact that a certain number of people could not tolerate this food, predominantly having intestinal problems.

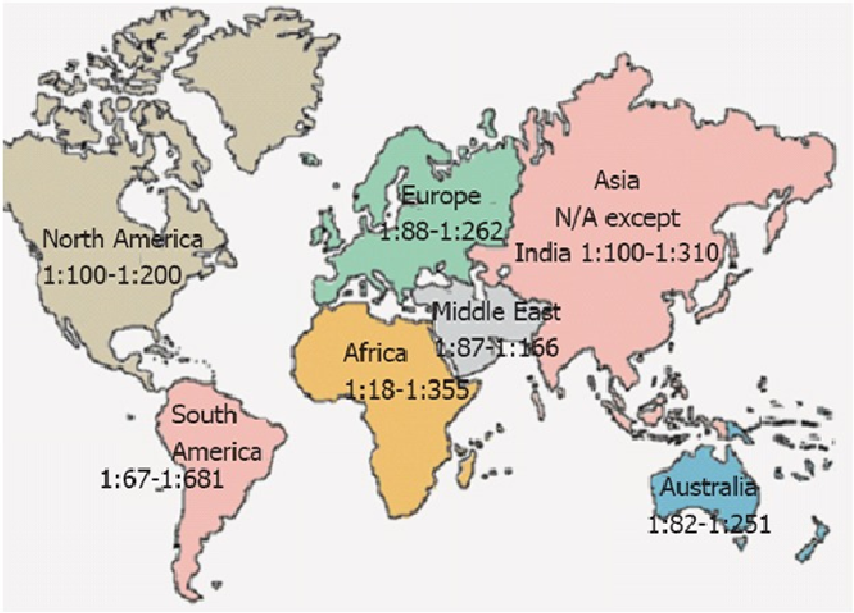
Figure 1: The Fertile Crescent

However, it was only in the first century AD that this morbid condition was recognized as a real illness with the name "celiac diathesis", from the Greek term *koiliakos* (of the bowel) to point out the specific place of the lesion responsible for the clinical pattern. In this connection, Arethaeus of Cappadocia (Fig. 2), a physician who lived in Rome at the time of Vespasian, hypothesized that it was a consequence of some intestinal alteration.

Figure 2 Arethaeus of Cappadocia

Interest in this disease was rekindled towards the end of the 19thcentury, in 1888,when an English paediatrician, Samuel Jones Gee, gave an excellent description of the illness in children, hypothesizing the fundamental role of feeding for the child's development and control. From then onvarious hypotheses were formulated to explain the nature and the causes of celiac disease but it was only in 1945 that the role of wheat flour was highlighted. Once again it was a paediatrician, Willem-Karel Dicke [1], that drew attention to that fact that suspension of the introduction of food stuffs based on wheat flour involved a dramatic improvement of the picture. This physician observed that his young patients clearly improved during the war period when they were forced to feed on a potato-based diet, while they again worsened when, at the end of the war, thanks to improved economic conditions, they went back to feedingon bread and other food stuffs containing gluten.

Since that time there has been a series of observations and discoveries [1,2,3] that have made it possible to clarify many of the aetiological-pathological aspects of this illness,which affects a rather large number of people. Epidemiological studies, which have gradually spread from the western world to the eastern world and to developing countries, have highlighted the fact that celiac disease is not an exclusive prerogative, as was mistakenly believed in the past, of the peoples of Europe and the countries of the Mediterranean area. It is found in all countries, especially those where immigration of populations of European origin has occurred. In Europe, where the consumption of wheat has constituted the basic food, the prevalence is estimated to be equal to 1/100 and in some studies even proves to be higher.



Prevalence of Celiac disease in the world

The epidemiological situation of Celiac disease drastically changed in the last decades of the previous century following the diffusion of very sensitive and specific serological tests, particularly endomysial antibody (EMA) and anti-tissue transglutaminase (anti-tTG)dosing.

In the scientific world interest in this disease has therefore also been accentuated by the complexity of the clinical pattern, whose pathogenesis is not entirely clear.

Table 1 shows some of the data that characterize the epidemiology of CD.

Prevalence in western countries: 1%
85,000 celiacs currently in Italy
5000 new diagnoses/year
Ratio of 1 to 7 between diagnosed and expected cases
Extreme variability of presentation
More and more frequent diagnosis in adults
Primarily extra-intestinal manifestations
Silent-asymptomatic forms 7-8 times more frequent than symptomatic ones

Table 1: Figures for Celiac disease (E.Ubaldi, Celiac disease in General Medicine)

Lastly, females are more affected than males, with a male-to-female ratio of 1:2.8 [4].

CELIAC DISEASE PATHOGENESIS

Celiac disease presents a complex multifactor pathogenesis, with a strong genetic component, to which there are added environmental and immunological factors;it can be defined an autoimmune pathology, which arises in genetically predisposed subjects.

Genetic factor: in the vast majority of patients (around 95%), there is present the biggest histocompatibility complex of class II HLA-DQ2 or HLA-DH8, which has marked affinity for peptides rich in glutamic acid that derive from deamination of gliadin. It is also to be noticed that in the last few years, using molecular genetics methods, various genetic loci have been highlighted that would appear to predispose the celiac subject to the disease, and precisely CELIAC1, on chromosome 6, CELIAC2, on chromosome 5, CELIAC3, on chromosome 2, and CELIAC4,on chromosome 19. It therefore appears evident that the genetic factor is fundamental but not sufficient, as in the case of diseases with Mendelian transmission.

Environmental factor: it is represented by the exposure of the intestinal mucosa to gluten, a protein component of cereals constituted by the peptides gliadin and glutenin, present in wheat as well as in a variety of cereals, like barley and rye.

Incomplete digestion of the proteins of gluten leads to the formation of gliadin, which, with mechanisms that are still not well defined, overcomes the barrier constituted by the layer of cells of the intestinal mucosa and has the function and task of separating the immuno-competent cells present in the submucosa from the large quantity of antigens that continually reach the intestinal lumen. The peptides of gliadin, particularly resistant to the digestive action of pancreatic enzymes and

intestinal peptidase, are exposed to deamination by the transglutaminase 2 (TG2) enzyme, becoming extremely adhesive to the APC cells responsible for antigenic presentation, and they are presented, linked to the molecules HLA-DQ2 or HLA-DQ8, by the dendritic cells of the T lymphocytes and by these to the B lymphocytes. This activates an immune reaction with the formation of anti-gliadinantibodies but also anti-transglutaminase and anti-endomysium ones. (Fig. 3)

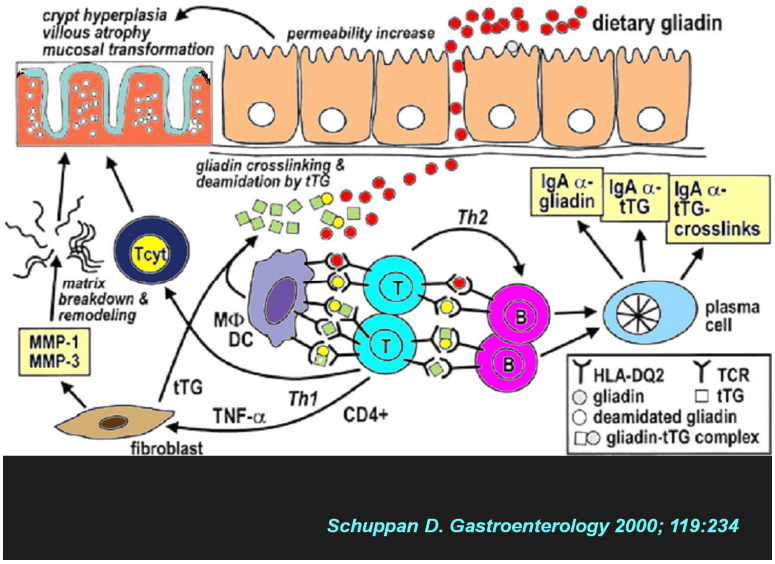


Figure 3: Pathogenetic mechanism of Celiac disease

There is thus an exaggerated response of cytokines and particularly of IFN-γ and Interleukin-15, the latter being responsible for migration and activation of IEL intrahepatic lymphocytes. These are responsible for the alterations that lead to apoptosis of enteric cells and remodelling of intestinal villi down to their disappearance. This harmful action is furthered by interferon-gamma activating the fibroblasts producing metalloproteinasis, which alter the ectocytic matrix, with further reduction of the mucosal barrier. (5)

In consequence of all this, there is atrophy of the villi, hypertrophy of the crypts and an increase in the number of intrahepatic lymphocytes. These lesions initially strike the duodenum and the proximal jejunum and then spread distally towards the ileum. This involves a reduction in the useful surface for absorption of the nutrients present in the intestinal lumen, with consequent malabsorption, which will be as serious as the lesions along the small intestine are extensive.

MARSH'S CLASSIFICATION

In 1992, on the basis of the histological alterations detectable in the intestinal mucosa, Marsh [6] proposed a classification in 4 stages (Chart 1):

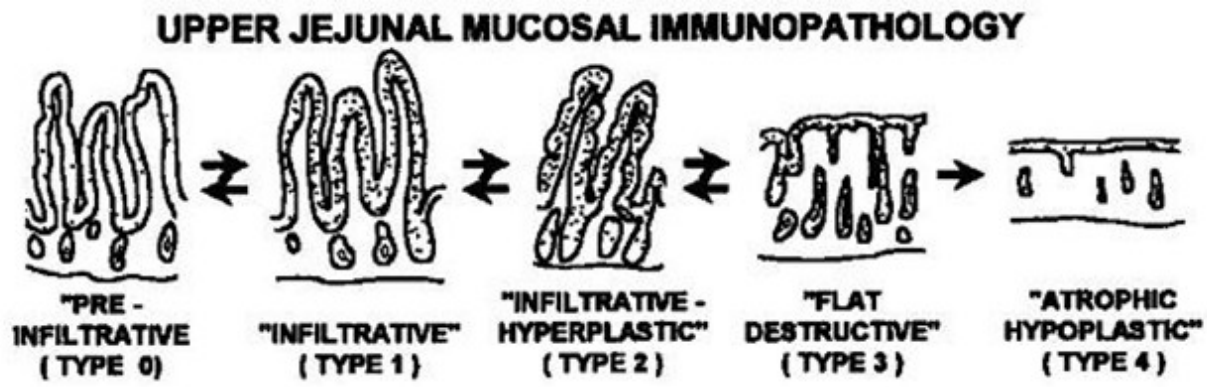


Chart 1: Marsh's classification

Stage 1: presence of slight alterations in the mucosas and deposits of IgA in the papillary derma. The crypts and the villi are normal, with the epithelium of the villi infiltrated by small lymphocytes. (Figure 4)

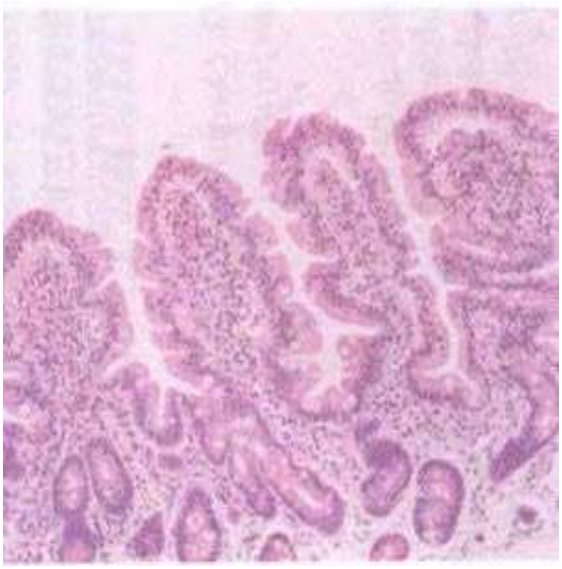


Figure 4: Celiac disease in stage 1: villi normal or showing slight atrophy and increase in the number of the intrahepatic lymphocytes.

Stage 2: the villi are still normal but the crypts contain small hyperplasic lymphocytes. (Figure 5)

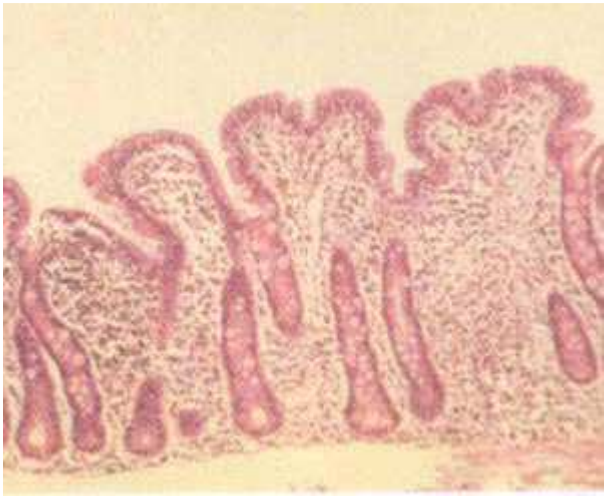


Figure 5: Celiac disease in stage 2, villi with moderate atrophy, increase in the number of intrahepatic lymphocytes, hyperplasic appearance of glandular elements.

Stage 3: the villi are atrophic with hypertrophic crypts containing large and mitotic lymphocytes (blasts).

Subsequently Oberhuber subdivided this stadium into:

3a) Slight : the villi have slightly inferior to normal height

3 b) Moderate : the villi are only sketched

3 c) Severe : one can only identify the glandular hosts

Stage 4 : the architecture of the intestine is completely lost. (Figure 6)

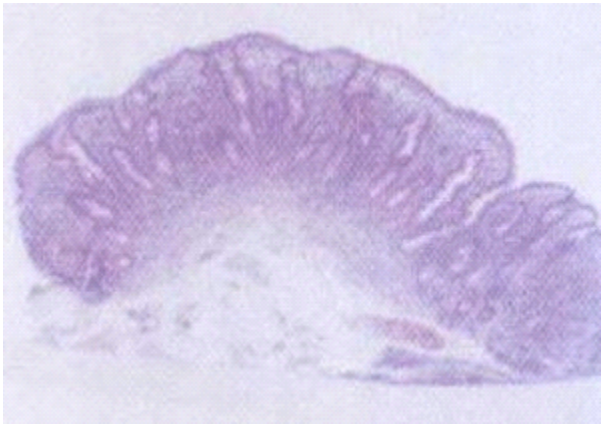


Figure 6: Celiac disease in stage 4 : total atrophy, increase in intrahepatic lymphocytes and hyperplasic glands

More recently, in 2005, Corazza and Villanacci (7) proposed a new classification in which the overall appearance of the mucosa is privileged, with a distinction into 3 degrees.

Degree A, not atrophic, unifying stages 1 and 2 of the classification by Marsh-Oberhuber;

Degree B1, with partial atrophy, unifying stages 3a and 3b;

Degree B2, with total atrophy, corresponding to stage 3c.

Stage 4 has been suppressed.

Marsh-Oberhuber Classification	Corazza-Villanacci Classification
Type 1	Grade A
Type 2	
Type 3a	Grade B1
Type 3b	
Type 3c	Grade B2
Type 4	Deleted

[a] Comparison of a new histopathologic classification scheme proposed by Corazza and Villanacci with the Marsh-Oberhuber classification in evaluation of celiac disease–associated mucosal lesions.

CELIAC DISEASE CLINICAL PRESENTATION

Classic Celiac disease is characterized by :

- bloating and abdominal pain
- diarrhea
- vomiting
- weightloss
- abundant , pale, smelly or oily stools

dependent on the functional and anatomical damage of intestinal mucosa

The absorption's changes can affect different elements and, consequently, may occur:

- iron deficiency anemia
- **growth retardation**
- defatigation caused by iron deficiency anemia and immune system activation
- osseous or articular dolor caused by Ca deficiency and/or Vitamin D deficiency

Atipical Celiac disease can be characterized by :

- depression or anxiety syndrome
- prickle and numbness in the hands and foots
- convulsions caused by IgA anti-tissue transglutaminase
- impotentia and/or decreased libido caused by testosterone lowered sensibility

- infertility or miscarriages caused by Vitamin E deficiency
- aphthous stomatitis caused by vitamins deficiency or due to mouth's celiac disease
- dermatitis herpetiformis caused by antibodies antitransglutaminase subtypes
- hemeralopia caused by Vitamina A deficiency.

In adult, Celiac disease can manifest as :

CLASSIC FORM : Malabsorption syndrome (diarrhea, abdominal pain, decrease of body weight, multiple nutritional deficiencies)

MONOSYMTOMATIC OR ATIPICAL FORM : with non-enteric symptoms (iron deficiency anemia resistant to treatment or macrocytic anemia, coagulation disorders and bleeding disorders, osseous or articular dolor, fractures, osteoporosis or rachitis, tetania, paraesthesias, aphthosus stomatitis, dental hypoplasia, peripheral neuropathy, depression syndrome, amenorrhea, miscarriages, infertility)

SILENT FORM : asymptomatic

CELIAC DISEASE AND THE EYE

Among observable clinical manifestations in subjects with Celiac disease little attention has so far been addressed to involvement of the eye, despite the reports present in the literature (14). Already towards the end of the last century, in 1982, Eliakim et coll. (15)described a case of keratocongiuntivitis in a subject with celiac disease and urged the medical world to pay attention to the ocular condition in young patients with celiac disease.

In 2011 a wide-scale study by Mollazadegan K et coll. (16) highlighted the fact that the risk of onset of cataract in celiac patients is especially high in those with serious impairment of absorption; vitamin deficiencies and in particular lack of vitamin A could play a major role. The risk of cataract might also be due to increased oxidative and above all photo-oxidative stress consequent on alimentary disorders present in such patients. Chronic diarrhea can cause a malabsorption syndrome with a resulting from severe vitamin D deficiency. It can cause an hypocalcemia that contributes to the development of cataracts. In fact low levels of calcium in the aqueous humor change the permeability of the lens epithelium, causing an imbalance in the osmotic equilibrium and leading to lens opacification.

The frequency of cataracts, especially bilateral,in young subjects, according to Raina UK et al. (17) makes it necessity first of all to exclude the presence of celiac disease.

Vitamin A deficiency could lead to the appearance of a retinopathy. (18)

This Vitamin A, in fact, is important for the function of the cones. Nyctalopia is usually the first symptom in patients and the fundus examination can shows from yellowish to white punctate lesions in the peripheral retina

Typically, visual function improves after one to four months of vitamin A reposition.
Vitamin A deficiency may be associated with Pseudotumor cerebri that is characterized by the presence of papilledema. The Vitamin A deficiency can lead to dry eye because it is necessary to maintain the proper functioning of the ocular epithelium surface. In these cases it is possible to see Bitot spots resulting from keratinization of the perilimbal conjunctiva and sometimes liquefactive necrosis of the cornea (keratomalacia) too.
According to Mollazadegan K et coll. (19), the risk of uveitis would also be moderately increased in patients with celiac disease and, nevertheless, such as to involve, in cases of uveitis with unknown aetiology, the need to search for gluten intolerance. A gluten-freediet constitutes a fundamental element for treatment of this serious ocular manifestation (20.).

In the Celiac disease it is possible to see other ocular manifestations : orbital myositis, thyroiditis associated with orbitopathy and brain occipital calcification.
Recently, Thiago Gonçalves dos Santos et al. (21) have proposed to make a distinction between ocular manifestation of Celiac disease. They can be divided into two subgroups :

a) Manifestations related to malnutrion :
   Retinopathy:
   Cataract;
   Pseudotumor cerebri;
   Dry eye

b) Manifestations due to dysimmunity:
   Orbital myositis;
   Uveitis;
   Brain occipital calcification,.

Recently, Urganci N et al. (22) have also stressed the possible co-involvement of the various ocular structures in celiac disease. According to these authors, young celiac patients in particular should undergo careful eye examinations to show up involvement of this organ at the right moment and to avoid serious consequences for sight, with further worsening of their quality of life.

CROHN'S DISEASE

Crohn's disease, from the name of the American pathologist that described it in 1932, is a chronic inflammatory disease that can affect the whole alimentary tract, though it most frequently strikes the terminal segment of the small intestine (ileitis) alone or in association with the right colon (ileum-colitis) or the colon only (colitis). The disease affects both sexes without any preference and can arise at any age, though the largest number of patients, over 20%, are children and teenagers; a second peak appears between the ages of 50 and 70.

Its aetiology is not yet completely defined, though numerous risk factors have been highlighted. The various researchers agree in believing that the disease is a combination of ectogenous factors (variations in diet and/orin intestinal flora because of both geographical changes in the patient's life and the use of antibiotics) with a genetic predisposition, as the higher incidence in monozygotic twins suggests.

The primary function of the intestinal tract, as is well known, is constituted by absorption of nutritive substances, with excretion of waste products, effected by the intestinal epithelium, which is therefore directly exposed to the external environment. It constitutes the most extensive surface of our body, constantly exposed to a variety of environmental antigens, pathogenic microbes and a vast community of commensals. Bacteria, in particular, constantly pass through the epithelial state, and consequently the whole intestine is in a constant state of low-level inflammation; nevertheless, the regulatory mechanisms present usually limit

this inflammation to a subclinical state. In genetically predisposed subjects, alteration of the barrier function of the intestinal epithelium involves loss of immune tolerance towards the various possible intestinal antigens, with activation of the dendritic cells that transport them to the intestinal lymph nodes, with triggering of the antibody response, and subsequent production of proinfiammatory cytokines and chemokines causing alterations, down to necrosis of the intestinal epithelium, with alteration of its specific functions. (23,24)

From the clinical view point the picture is variable; not infrequently, for a long time patients only complain of altered bowel habit and above all diarrhoea, whose characteristics vary in relation to the place of the intestinal segment affected. In this connection, in ileal locations, there are raised volumes of watery faeces; in the colon the diarrhoea is often sanguinolent. In cases of widespread involvement of the alimentary tract there is constant decrease in weight associated with loss of appetite and, very often, fever. There is more or less constant abdominal pain primarily located in the lower right quadrant.

In the most serious forms extra-intestinal manifestations are quite frequent (25),such as:

Involvement of biliary ducts;

Nodous erythema, which can be the first manifestation associated with diarrhoea;

Peripheral arthritis, ankylosing spondylitis, and sacroileitis, particularly evocative of an autoimmune process in a young patient.

Even the eye , with a oscillating frequency in the various studies between 6 and 14%, is affected by the autoimmune process, although ocular complications are infrequent , can be associated with significant morbidity, including blindness. In additions, some ocular diseases, such as uveitis and scleritis, might precede a diagnosis of Crohn’s disease.

Clinical manifestations include blurred vision, teary, burning or itchy eyes, ocular pain, photophobia, conjunctival or sclera hyperaemia, loss of visual acuity and possible blindness.

The most common ocular findings were conjunctivitis and blepharitis followed by uveitis, cataract and episcleritis.

The anterior uveitis (26) requires special compliance and adherence therapy also because it can be often misunderstood.

Because the ocular complaints of IBD patients are often non-specific, it may be helpful to perfomed eye examinations as a routine component in the follow-up of these patients. It is well-known that early diagnosis and treatment of ocular involvement may prevent serious ocular complications that could be associated with significant visual morbidity.

AIM

Impairment of growth, a fairly common complication in patients with celiac disease and sometimes the only clinical manifestation, has a multifactor pathogenetic mechanism [27]. In this connection, according to some authors [28,29], to the chronic malnutrition, consequent on the serious histological damage to the intestinal mucosa that leads to malabsorption of essential nutrient substances, there is added the action of proinfiammatory cytokines like TNF-a and IL-6, which favour epithelial damage and directly interfere with bone growth. However, there are more and more numerous observations that in these patients, and in particular in those in whom a gluten-free diet does not lead to a return to normal bone growth, some alteration of the GH/IGF-1 system is present that, if not corrected, will make all therapeutic action vain.

Despite the various studies in the literature [30.31.32.33], there is no uniform opinion on the specific role of GH. Indeed, while for some there is a deficit of the latter, for others, instead, the failure to respond to hormonal action is to be ascribed, as in the case of reduced sensitivity to testosterone, to increased bone receptor resistance to GH [33].

As is well known, among the targets of GH we also have to consider the corneal cells [34,35,36]. In this connection, in patients with primitive hypopituitarism, reduced thickness of the corneal layer has been shown, which can be corrected by GH replacement therapy.

To contribute to clarifying the role played by GH in the pathogenesis of the low height of patients with CD, we therefore considered it appropriate to study in a group of celiac patients with reduced height the degree of corneal thickness and intraocular pressure (IOP). We also decided to compare the data observed with those obtained in subjects with Crohn's disease, since studies by Tietjen K and others [37] have shown that the somatic hypoevolutism detectable in subjects with this intestinal pathology cannot be related to impairment of the Gh - IGF-1 axis.

MATERIAL AND METHODS

After informed consent by their parents, 30 subjects were considered,aged between 4 and 16 years, 24 females and 6 males, presenting at the DIPROSAMI Operational Unit of the Paediatric Clinic of the University of Palermo.

The inclusion of patients up to 16 was motivated by the consideration that puberty in patients with Celiac disease may be delayed and by the decision to appraise, as suggested by other authors, the possible impact of puberal sexual hormones on IGF-1.

20 subjects had Celiac disease and 10 Crohn's disease.

The diagnosis of Celiac disease was confirmed through dosing of anti-gliadin, anti-endomysium and anti-transglutaminase antibodies and endoscopic examination with staging according to Marsh.

According to the standards of reference of our laboratory, antibody dosing above 10 U/ml was considered positive.

The diagnosis of Crohn's disease was confirmed through endoscopic examination and entero-MRI and specific serology.

Patients submitted to growth hormone (GH) therapy or having a history of use of corticosteroids were excluded, because of possible suppression of the somatotrophic axis with interference in accurate evaluation of IGF-1.

All subjects, on recruitment, were also submitted to evaluation of body weight, height and GH and IGF-1 dosing, and to a complete ophthalmologic examination with determination of corneal thickness and intraocular pressure. The weight was measured using digital scales with an approximation of 0.1 kg; the height was measured with a stadiometer with an approximation of 0.1 cm.

Normal values were considered :

1-20 ng/ ml in the prepuberal child

$\leq$ 5 ng/ml in the male adult

≤10 ng/ml in the female adult

The IGF-1 was determined by ELISA (OCTEIA IGF-1 kit, IDS Inc. Fontana Hillis, AZ USA) and the values were expressed as mg / dL.

Normal values were considered:

In the female subiects:

2-5 years: 33.5 - 171.8 ng/dL (2-5 years)

6-8 years : 79.8 - 244.0

9-11 years: 87.4 - 399.3

12-15 years : 138.4 - 509.9

16.20 years : 267.5 - 470.8

In the male subiects :

2-5 years : 27.4 - 113.5 ng/dL

6-8 years : 54.9 - 206.4

9-11 years : 85.2 - 248.8

12-15 years : 115.4 - 498.2

16.20 years : 247.3 - 481.7

The corneal thickness was assessed by ultrasonic pachymetry (Pachpen Accutome 24-5100)

Fig. 7: Pachpen Accutome 24-5100

The measurements (38) were taken centrally as well as at four paracentral sites 3 mm from the corneal center at the 3, 6, 9, and 12 o'clock positions. The following values were considered normal :

538 ± 40micron lower than 2 years (< 2 years)

546± 41micron between 2 and 4 years (2 - 4 years)

565 ± 46 micron between 5 and 9 years (5 -9 years)

555 ± 33 micron higher than 9 years (> 9years).

- Intraocular pressure (IOP) was measured using a Perkins tonometer and the values were corrected in relation to the corneal thickness.

Every patient was submitted to a full ophthalmic examination that includes the measurement of the visual acuity and , if there is present, of the refractive power, eye examination both the anterior segment (conjunctiva, cornea, pupil, anterior chamber, lens) and the posterior segment (vitreous, fundus , vessels and optic disc).

Investigation also included a review of ocular motility to rule out any squint.

The ophthalmic examination was done with direct ophthalmoscopy, that provides about 15 times magnification and the image formed is virtual and erect, and/or with slit lamp examination, that was the most important of all the ophthalmological instruments.

The visual acuity of our little patients was always checked monocularly and the distance vision was measured using the Snellen (or equivalent) chart.

STATISTICAL ANALYSIS

The correlations between weight percentiles and corneal thickness and between the height percentiles and corneal thickness were examined through linear regression analysis and expressed as a r squared. Data were analyzed by Statistical Software Graph Padprism. $P<0.05$ was considered statistically significant.

RESULTS

The general eye examination was normal both for patients with Celiac disease and patients with chronic inflammatory illness of the bowel (Crohn's disease).

No ocular manifestations of inflammation was observed. Both the anterior segment and the fundus were normal.

18 patients (14 with Celiac disease and 4 with chronic inflammatory illness of the bowel) had refractive errors (Table 4). The others 6 subjects with Celiac disease and 6 subjects with Crohn's disease didn't have any refractive errors. 12 subjects with Celiac disease had hypermetropia and 2 had astigmatism.

All subjects with Crohn's disease had hypermetropia.

Description	Celiac disease	Crohn's disease
Hypermetropia	12	4
Myopia	0	0
Astigmatism	2	0

Table 2 : Classification of refractive errors of the patients both with Celiac disease and with chronic inflammatory illness of the bowel.

The table 3 shows data related to patients with Celiac disease.

	Age	Gender	Stage	Antibody titre.	Weight	Height	GH	IGF-1	CCT R	CCT L	IOP
1. C.G.	13	M	2	>10	50-75 %	50-75 %	2.8ng/ml	416 ng/ml	555	556	12
2.T.G.C.	14	F	2	>10	50 %	>50 %	0.28 ng/ml	410 ng/ml	518	512	13
3. C.C.	13	M	3	>10	50 %	75 %	3.6 ng/ml	388 ng/ml	540	544	11
4 P.M.	14	F	2	<10	50-75 %	50-75 %	7.9 ng/ml	315 ng/ml	512	536	13
5 C-E-M.	8	F	3	>10	75 %	75 %	5.86 ng/ml	207 ng/ml	512	508	12
6. G.F.	5	F	2	>10	50 %	50-75 %	2.4 ng/ml	142 ng/ml	518	519	10
7 C.G.	13	F	3	>10	50 %	75 %	6.1 ng/ml	410 ng/ml	516	518	12
8 F.D.	13	F	3	<10	50-75 %	50-75 %	4.94 ng/ml	412 ng/ml	520	518	11
9. C..E	14	F	2	>10	50 %	>50 %	0.46ng/ml	388 ng/ml	520	518	12
10.S. A.F	10	F	3	>10	75 %	50-65 %	0.20ng/ml	164 ng/ml	555	556	12
11 M-C.	13	F	3	>10	75 %	75 %	7.1ng/ml	387 ng/ml	544	544	14
12 P.L.	16	F	3	>10	3-10 %	97 %	16.7 ng/ml	248 ng/ml	553	559	13
13 M.M.	13	F	3	>10	25 %	10 %	2.1ng/ml	175 ng/ml	500	501	12
14. F.C.E.	4	F	3	>10	10 %	75 %	9.45 ng/ml	186 ng/ml	503	502	10
15 P.F.	15	M	2	<10	50-75 %	>5O°%	5.6 ng/ml	390 ng/ml	519	517	12
16 F.A.	11	F.	3	>10	25 %	50-75 %	0.46 ng/ml	326 ng/ml	520	518	12
17 S.N.	12	F	3	>10	25-50 %	25-50 %	2.9 ng/ml	157 ng/ml	500	500	14
18 P.M.	14	F	2	<10	50-75 %	50-75 5	8.2 ng/ml	415 ng/ml	526	540	13
19 M.G.	9	F	3	>10	25-50 %	50 %	12 ng/ml	219 ng/ml	505	507	14
20 D.C.	6	M	3	>10	25-50 %	25-50 %	14 ng/ml	188ng/ml	500	500	12

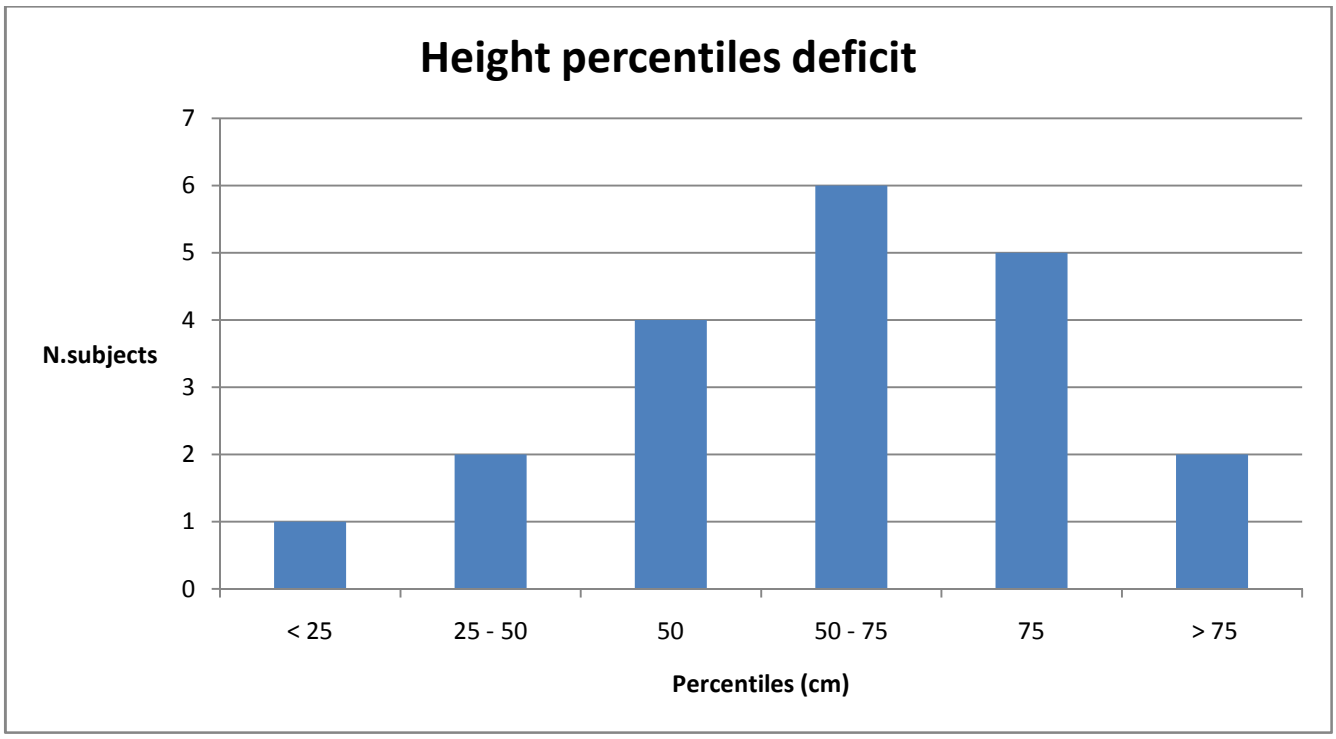
Table 3: Patients with Celiac disease

Legend: In red lower than normal values ; in boldfaced higher than normal values

Subjects 5,6,14,19 and 20 are naive patients; the others are patients submitted to a gluten-free diet.

Graph 1 and 2 show the data relating to the height percentiles, with reference to the Italian percentiles, and to the weight deficit in subjects with Celiac disease, all being treated with a gluten-free diet, except 5 subjects diagnosed for the first time.

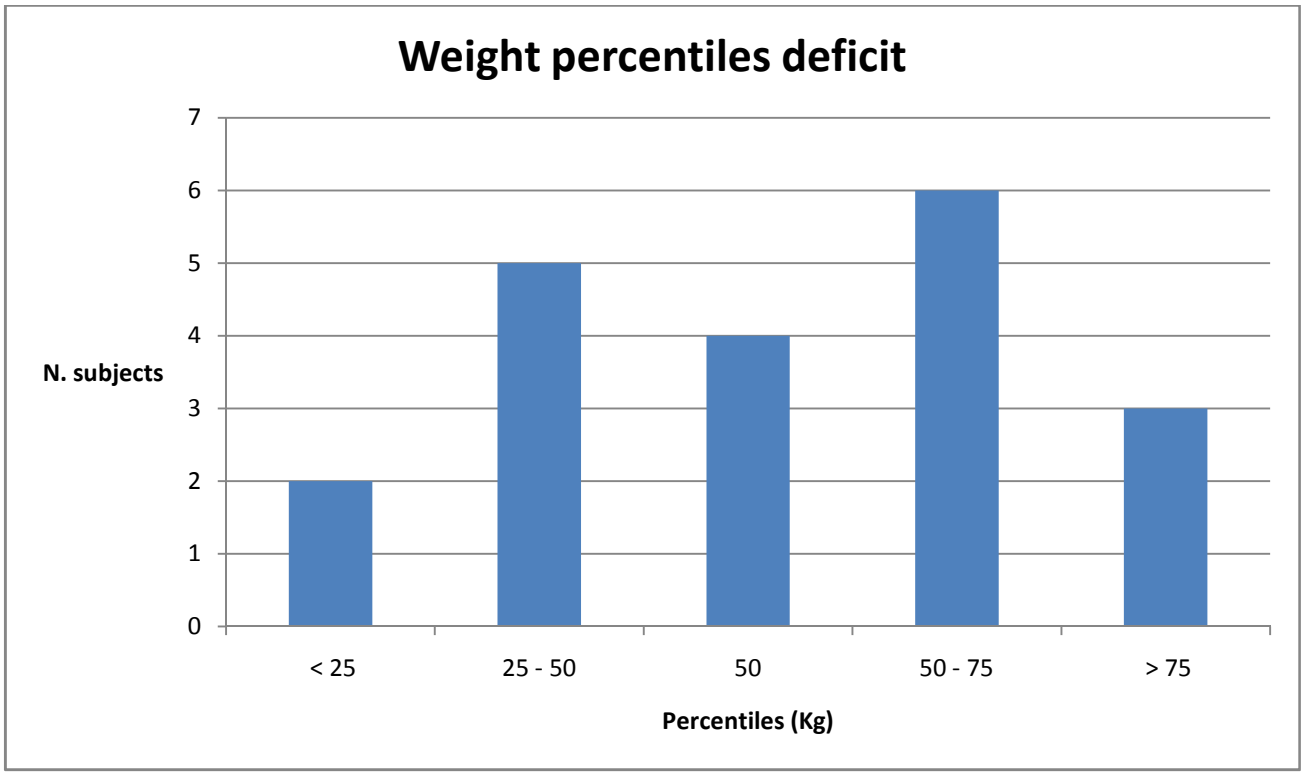
1 subject presented a height lower than 25 %;
2 subjects presented a height between 25 and 50 %;
4 subjects presented a height equal to 50 %;
6 subjects presented a height between 50 and 75 %
5 subjects presented a height equal to 75 %:
2 subjects presented a height higher than 75 %.



Graph 1 : Distribution of subjects with Celiac disease on the basis of height percentiles
On the x-axis there are height percentiles and on the y-axis there is the distribution of patients with Celiac disease.
The bar graph shows the distribution of subjects with Celiac disease on the basis of height percentiles. The frequency distribution is approximately equal to left asymmetrical distribution.

The subjects that presented a height between 50 and 75 % are the much more frequent than the other percentiles.
As a consequence, 3 subjects had a height percentile smaller than the height of the most of the children their age,4 subjects had a height percentile little bit smaller than the height of the most of the children their age. All the others had a height percentile that was within the normal range.

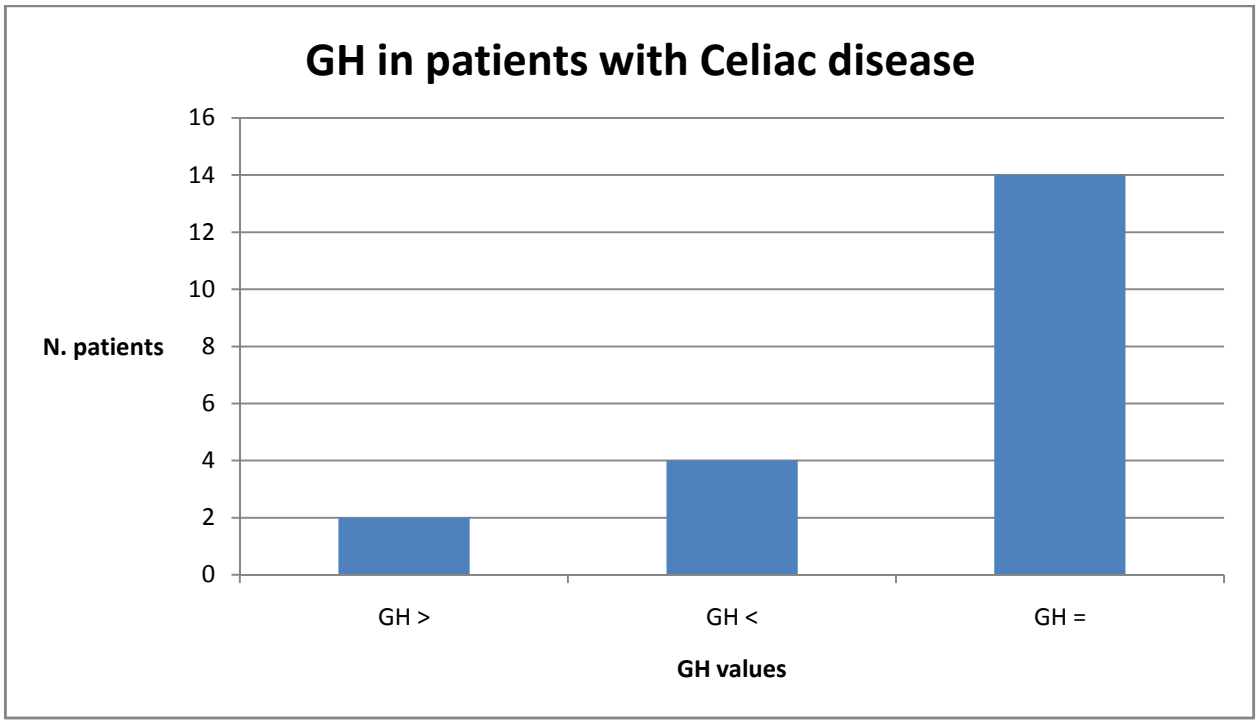
In consideration of the weight ,
2 subjects presented a weight lower than 25° %;
5 subjects between 25 and 50 %;
4 subjects equal to 50 %;
6 subjects between 50 and 75 %;
3 subjects higher than 75 %



Graph 2 : Distribution of subjects with Celiac disease on the basis of weight percentiles
On the x-axis there are weight percentiles and on the y-axis there is the distribution of patients with Celiac disease.

The bar graph shows the distribution of subjects with Celiac disease on the basis of weight percentiles. The frequency distribution consists of a curve with three portions : the first portion of graph is concave down, the second is concave up and the third is concave down again.
In this graph there are three summits at the percentiles between 25 and 50 %; at 50 % and at between 50 and 75 %, but the last (between 50 and 75 %) is more frequent than the others.
As a consequence, 7 subjects had a weight percentile smaller than the weight of the most of the children their age, 4 subjects had a weight percentile little bit smaller than the weight of the most of the children their age. All the others had a weight percentile that was within the normal range.

The study of GH (Graph 3) showed normal values in 14 patients (60%); in 2 patients (10%) the values were higher than those of healthy peers and only in 4 (20%) these values were indicative of an internal secretory deficit.



Graph 3 : Distribution of subjects with Celiac disease on the basis of GH values
GH > : higher than normal values
GH < : lower than normal values
GH = : values in the normal range
On the x-axis there are GH values and on the y-axis there is the distribution of patients with Celiac disease.

The bar graph shows the distribution of subjects with Celiac disease on the basis of GH values. The frequency distribution shows that most subjects had the values of GH in the normal range.

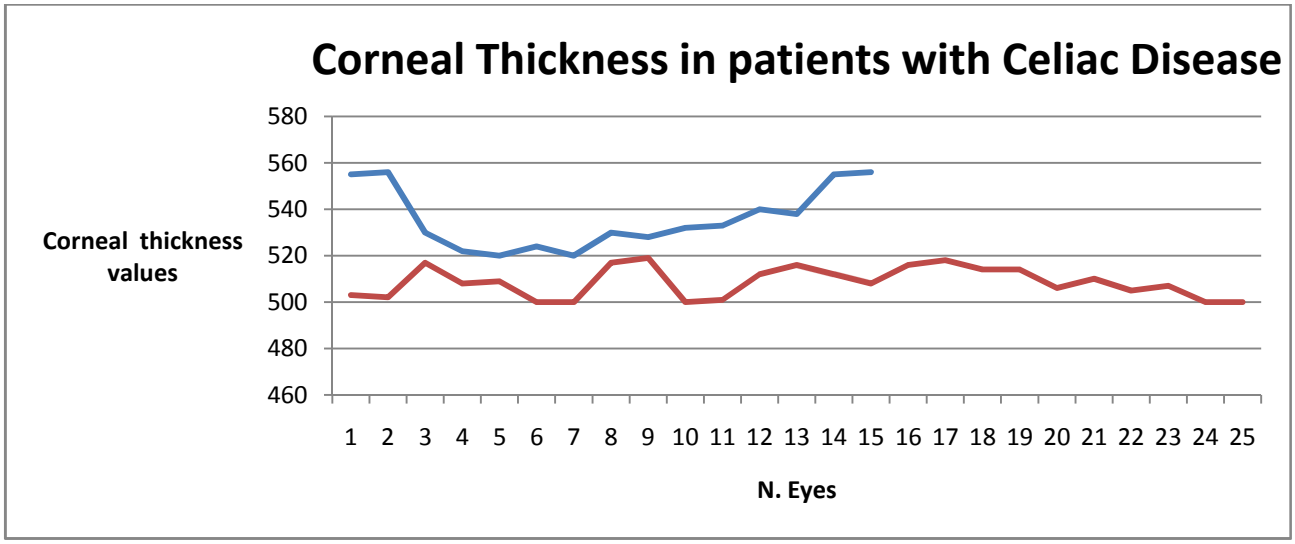
The values relating to IGF-1 were also in the normal range.

Corneal thickness (Graph 4) was within the normal range in 6 subjects; in 12 the values were lower than the normal minimum values.

Corneal thickness was within the normal range in 6 subjects; in 9 the values were lower than the normal minimum values. In 5 subjects the values were within the normal range in an eye and lower than the normal minimum values in the other.

In one (case 15) of the two subjects in which a higher than normal GH value was found, the corneal thickness proved to be lower than the expected average values for the relevant age.

IOP, after correction for the corneal thickness value, proved to be normal in all subjects.



Graph 4 : Corneal thickness in eyes of patients with Celiac disease

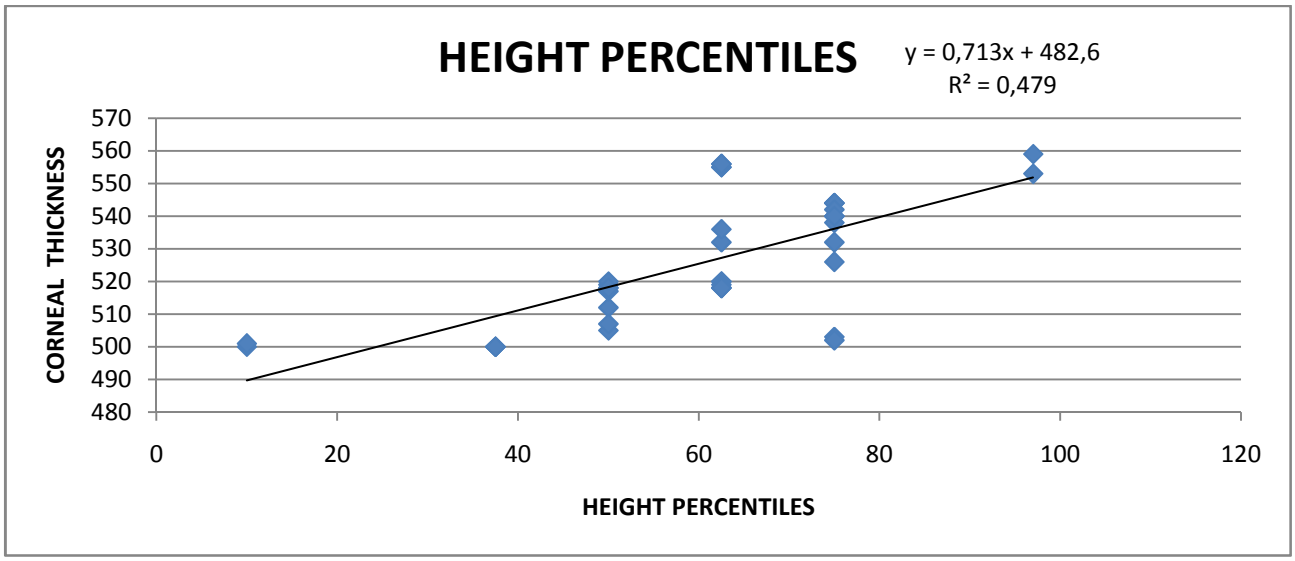
In blue: Eyes with normal corneal thickness

In red: Eyes with pathological corneal thickness

On the x-axis there are the distribution of the eyes of the patients with Celiac disease and on the y-axis there is the distribution of corneal thickness values. The line graph above shows the

distribution of the eyes of the patients with Celiac disease on the basis of the corneal thickness values. There are two lines : one in blue that represents the distribution of the eyes with normal corneal thickness and the second in red that represents the distribution of the eyes with pathological corneal thickness.

The Graph 5 shows a moderate positive correlation between height percentiles and reduced corneal thickness (r = 0.4793; p< 0.0001).

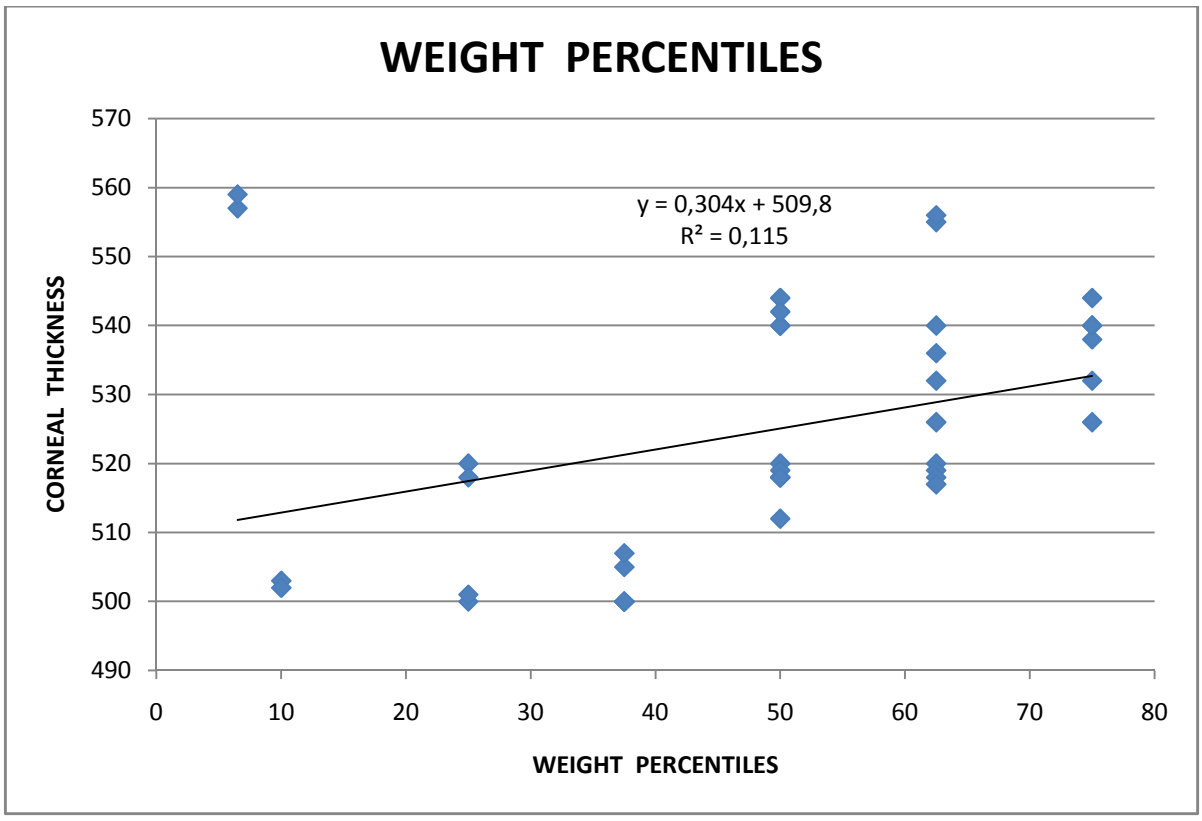


Graph 5 : Linear regression line between height percentiles and corneal thickness

On the x-axis there are the distribution of the height percentiles and on the y-axis there is the distribution of corneal thickness values.
This graph shows a moderate positive correlation between height percentiles and reduced corneal thickness.

The Graph 6 shows a very weak positive correlation between weight percentiles and corneal thickness

(r = 0.1150; p= 0,0323).



Graph 6 : Linear regression line between weight percentiles and corneal thickness

On the x-axis there are the distribution of the weight percentiles and on the y-axis there is the distribution of corneal thickness values.

This graph shows a very weak positive correlation between weight percentiles and corneal thickness.

In the control subjects (Table 4) the body weight proved to be markedly deficient in 9 subjects; only in one patient was it almost normal.

	Age	Gender	Weight	Height	GH	IGF-I	CCT		IOP
							R	L	
1.M.V. .	19	F	3-5 %	75 %	3.5ng/ml	326 ng /ml	560	562	13
2. P.G.	15	M	5 %	50 %	4.8 ng/ml	210 ng/ml	528	530	12
3.C.D.	12	F	3-5 %	50 %	6.4 ng/ml	328 ng /ml	530	527	11
4. B.S.	11	F	5 %	10-25 %	9.5 ng/ml	360 ng/ml	520	522	12
5.G.A.	18	F	3-5 %	50-75 %	8.6 ng/ml	310 ng/mL	534	536	13
6. G.S.	17	M	95 %	95 %	4.4 ng/ml	282 ng/ml	528	527	13
7.F.M.	17	F	3-5 %	50-75 %	6.2 ng/ml	278 ng/ml	532	534	12
8.C.D.	9	F	10 %	25-50 %	1.99ng/ml	388ng/ml	510	508	10
9.M.A.	13	F.	3-5 %	50 %	7.8ng/ml	260 ng/ml	526	524	12
10. A.D.	12	F	25 %	40 %	6.77ng/ml	318 ng/ml	532	534	11

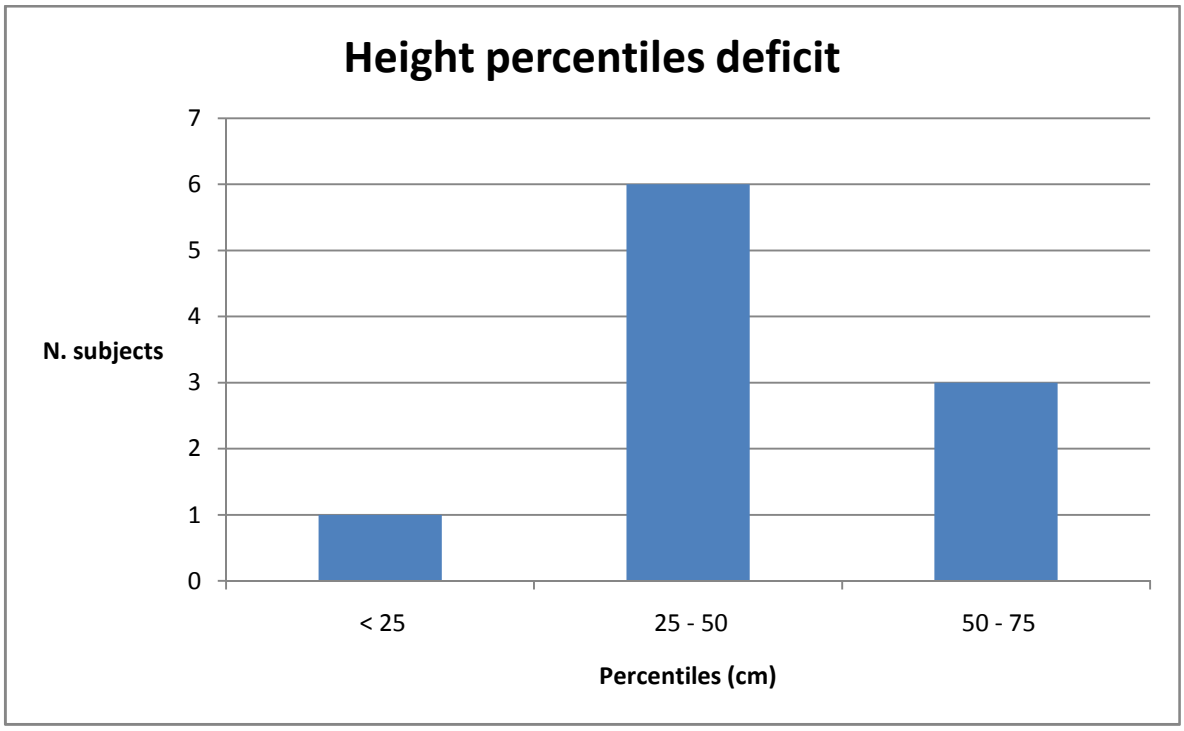
Table 4. Patients with chronic inflammatory illness of the bowel (Crohn's disease)

Regarding height (Graph 7),

1 subject presented a height between 10 and 25 %

6 subjects presented a height between 25 and 50 %

3 subjects presented a height between 50 and 75 %



Graph 7: Distribution of subjects with Crohn's disease on the basis of height percentiles

On the x-axis there are height percentiles and on the y-axis there is the distribution of patients with Crohn's disease.
The bar graph shows the distribution of subjects with Crohn's disease on the basis of height percentiles. The frequency distribution is approximately equal to bell-shaped curve distribution. The subjects that presented a height between 25 and 50 °C are most frequent than the other percentiles.
As a consequence, 7 subjects had a height percentile smaller than the height of the most of the children their age, all the others had a height percentile that was within the normal range.

The GH and GF-1 values proved to be within the normal limits in all subjects.

Also within the normal range were the corneal thickness values ; only in one subject (Case 8) was a value below the norm detected (510 in Odx and 508 in Osx).

In this group ,also, the IOP values were in the normal range.

DISCUSSION

Low height and weight deficit constitute two of the commonest manifestations of Celiac disease and in some patients hypoevolutism can be the manifestation and the only symptom of the illness (10).

Reduced growth in children with Celiac disease is traditionally considered a consequence [39] of nutritional deficits due to the profound alterations that occur in the intestinal mucosa and significantly alter its capacity for absorption. Indeed, the more marked the alterations of the intestinal mucosa, the greater is the damage to the digestive process and absorption of nourishing substances. In our subjects too it was possible to highlight the fact that in those with reduced alteration of the mucosa, to be set in Marsh's stage 2, there is slighter impairment of the body weight. The antibody titre too, an expression of the activity of the aggressive autoimmune process, can be indicative of a weight deficit. In subjects with a titre above 10 U/ml the decrease in body weight on average was 50%, and in six subject sit was below 50%.

Elimination of gluten from the diet involves if not an arrest at least a deceleration of the autoimmune process. It is associated with an improvement in body weight and, often, also with an increase in height, especially in the first year of gluten restriction. In some patients, nevertheless, dietary correction does not replace catch-up growth and, therefore, other mechanisms must intervene in determining it.

Some studies had lower than normal GH serum values, suggesting precisely in the internal secretory deficit of this hormone the possible cause of the marked deceleration of bone growth [30,31,32].

The association between somatic hypoevolutism and GH alteration, according to Giannatasio et al. [40], would constitute an association that cannot be considered casual, instead, must recognise the pathogenetic mechanisms in the inflammatory disease itself.

In this connection, it is known that in patients with Celiac disease there is internal secretion of ghrelin, due to alterations of the P/D1 cells located in the mucosa of the gastric fundus. This hormone, the internal secretion of which is precisely the task of these cells, in addition to favouring the absorption of food has a stimulatory action on the cells of the anterior lobe of the hypophysis, favouring GH secretion[38]. Reduced secretion of ghrelin might therefore influence growth, both through an insufficient supply of nourishing substances and through reduced GH secretion[41,42].

According to Iughetti L et al. [43], reduced GH secretion might also be due to a form of autoimmune hypophysitis with formation of somatotrophic anti-cell auto-antibodies. Indeed, research by these authors appears to have shown in celiac patients with a GH deficit a high rate of anti-hypophysitis antibodies.

On the other hand, it is known that patients with celiac diseases are frequently exposed [44,45,46] to autoimmune pathologies like hypothyroidism and type 1 diabetes mellitus; the fact is that all these three conditions share a similar HLA with HLA DR3-DQ2 and DR4-DQ8 genotype, and therefore the possibility of an autoimmune hypophysitis must possibly be taken into consideration.

Other authors, however, not having found reduced internal secretion of GH in subjects with retarded or failed bone growth, have hypothesized [33] possible increased resistance to the hormone, having noticed, in some cases, GH values above the norm or normal values but with low levels of IGF-1 [47,48,49].

As is well known, the insulin-like growth factor-1(IGF-1) is an effector hormone,essential for normal growth in human beings, with an important role in mediating the effects of growth hormone (GH). Its internal secretion(Fig. 7) mainly occurs in the liver cells and it is GH-dependent; indeed, its serum concentrations are decreased in patients with a GH deficit and increased in those with acromegaly.

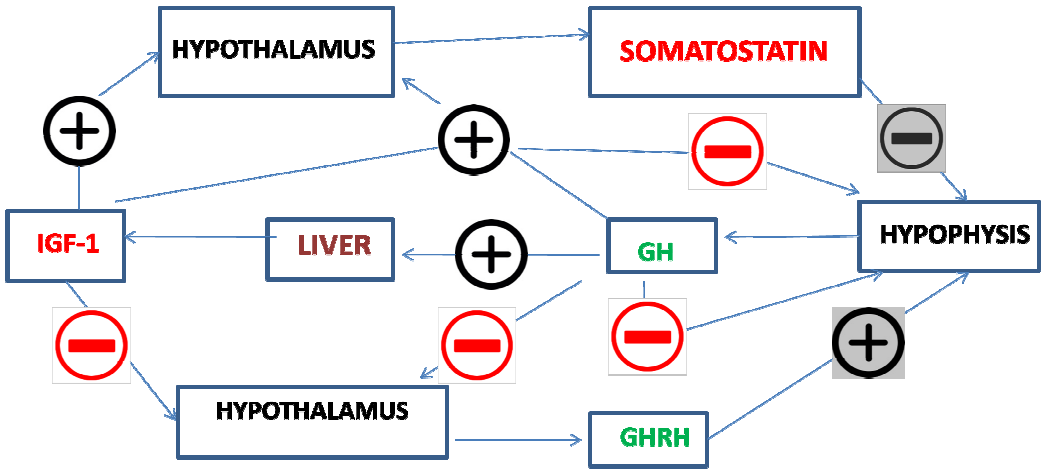


Fig. 7: Regulation of IGF-1secretion

On the other hand, hepatic expression of IGF-1 is negatively influenced by increased production of proinfiammatory cytokines, as shown by Sanderson [28] in children with inflammatory bowel disease and in Celiac disease, in which, as already mentioned, an increase in cytokines is present, particularly TNF-a and IL-6. The latter, as also highlighted by Griffiths, also seem to constitute potential mediators of GH resistance [39].

Hence from the various studies in the literature no univocal answer emerges on the pathogenetic process at the basis of somatic hypoevolutism.

A contribution to the explanation of the pathogenetic mechanism can be made, in our opinion, by studying corneal thickness.The fact is that GH, through IFN-1, acts on the cellular matrix of the cornea, increasing its thickness without causing alterations of its transparency. The corneal cells therefore constitute a valid specific target for the action of this hormone. Corneal thickness, in subjects with acromegaly, is constantly above the norm and could therefore condition refraction disorders, while in subjects with GH deficiency, it is more or less markedly reduced and tends to normalization as soon as corrective hormonal treatment is begun [34, 35].

Determination of corneal thickness in the patients with CD examined by us proved to be at the lower limits of the norm (Graph 4) in 14 subjects out of 20 (70%), as is noticed in cases of reduced action of hormonal activity, and proved to be reduced in 1 patient that had raised GH values.

In fourteen subjects the GH serum values proved to be in the normal range and only in two were they above the norm. One of these two subjects had a corneal thickness that was markedly reduced, confirming probable receptor resistance to the hormone. The IGF-1 serum values were in the normal range in all subjects, expressing a normal response to the GH stimulus.

Hence the data found could be particularly indicative of reduced receptor sensitivity of osteoblasts but also of corneal receptors to the GH-IGF-1 system, like the finding of markedly reduced corneal thickness in 1 of the 2 patients with raised GH values.

This hypothesis would also be confirmed by the fact that a moderate positive correlation is present (Graph 5) between height percentiles and reduced corneal thickness ($r = 0.4793$) and a very weak positive correlation is present (Graph 6) between weight percentiles and corneal thickness ($r = 0.01150$).

The height deficit therefore seems to be primarily due to the reduced receptor sensitivity to GH and the weight deficit to impairment of the digestive function of the intestinal epithelium.

The fact that the height and weight deficit involves a different pathogenetic mechanism finds further confirmation in the fact that, at least in our subjects, there is no correlation between the two values ($r = 0.087$). The two deficits are independent of one another and consequently involve different pathogenetic mechanisms that in some subjects may overlap.

Nevertheless, the finding of a raised GH value in 2 subjects (10%) confirms the complexity of the pathogenetic mechanism at the basis of the height deficit. This imposes the need for careful study of the hormonal conditions of the single patient before beginning substitutive hormonal treatment,which may not be ethically correct.

While in the majority of the subjects reduced receptor sensitivity was ascertained, the pathogenetic mechanism at its basis is not yet well defined.

According to Griffiths [39], as already mentioned, it would be due to the action of proinfiammatory cytokines, which would mediate the action of IGF-1. However,this hypothesis does not appear very probable, also considering the fact that subjects with Crohn's disease, in whom proinfiammatory cytokines are constantly found, do not present, at least in our case study, corneal thickness alterations. The latter constantly proves to be normal, as do the GH and IGF-1 values.

The reduced receptor sensitivity, in our opinion, could have a specific genetic component.

In the last decade it has been shown that in patients with Down's syndrome [50,51,52] Celiac disease is also quite frequent; and a recent study by Marild K et coll. [51] has shown that the risk of Celiac disease in subjects with Down's syndrome is 6 times higher than in normal subjects. Furthermore, in patients with Down's syndrome, reduced corneal thickness is frequently found and it can evolve down to formation of a keratoconus. This alteration appears to be due to genetic impairment, as is suggested by the studies by Vincent et al. [53]. With computerized corneal topography the latter authors detected an altered parameter in 39% of the parents of children with Down's syndrome, an alteration that would be indicative of the presence of a genetic anomaly responsible for the reduced corneal thickness. In this connection, most researchers suggest complete penetration of the factors but with varying phenotypic expression. In some patients heterozygous mutations of gene VSX1 have been described as a basic genetic defect. This gene, located on chromosome 20 (20p 11-21) is implicated in the synthesis of proteins that regulate normal corneal trophism, and indeed mutations of it are responsible for polymorphous corneal dystrophy and keratoconus.

In subjects with Celiac disease too, as in those with Down's syndrome, genetic mutations are quite frequent [54,55], affecting genetic loci located on different chromosomes. It would therefore be useful to verify whether in subjects with height deficit and reduced corneal thickness this gene is affected.

Lastly, the specificity and precocity of corneal impairment make this element a sign that could be fundamental in the diagnosis of reduced receptor sensitivity to GH, making it possible not only to start more expensive and invasive investigations for confirmation but also monitoring of the response to the therapy.

It follows that ophthalmologic examination with attached cornea measurement should be an integral part of the clinical study of patients with Celiac disease and of the response to dietary and, if appropriate, pharmacological treatment.

CONCLUSION

From our work, whose significance is nevertheless limited by the small size of the sample, it is possible to draw the following conclusions:

- In children with Celiac disease, failed or limited recovery of growth, despite careful dietary treatment, very probably implies reduced receptor sensitivity to action of the GH-IGF-1 system;
- Study of corneal thickness constitutes a valid *in vivo* test to appraise receptor response to the action of the GH-IGF-1 system;
- Precocity of corneal impairment could constitute an easy indicator of the altered receptor response to the GH-IGF-1 system.

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