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SERUM LEPTIN LEVEL IN CHILDREN WITH CELIAC DISEASE: RELATIONSHIP TO AGE, GENDER AND BODY MASS INDEX

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ABSTRACT
Celiac disease (CD) is a permanent intolerance of dietary gluten leading to mucosal damage in the proximal small bowel in genetically susceptible individuals. It is characterized by malabsorption of nutrients, chronic inflammation and damage of the small intestinal mucosa. The global prevalence ranges from 1% to 2%. In this study, thirty children (17 girls and 13 boys) with celiac disease (CD) at age range of 2-12 years and 15 apparently healthy controls were investigated for 3 autoantibodies [anti-gliadine IgA antibody (AGA), anti-gliadine IgG antibody and anti-tissue transglutaminase (tTG)] in addition to leptin in their sera. Serological screening tests revealed a non significant difference ($P>0.05$) in the level of antigliadin antibody IgA between the two groups (children with CD and control), while the levels of antigliadin antibody IgG and anti-tissue transglutaminase antibody were significantly higher ($P<0.01$) in children with CD as compared with the control. The results of serum leptin level showed that it was significantly lower ($P<0.01$) in children with CD as compared with the control. When the correlation was studied between levels of leptin with age, gender and BMI in children with CD, the results revealed a non-significant correlation ($P > 0.05$) with age and gender, while a significant positive correlation ($r = 0.63$, $P<0.001$) was found with BMI. From the result analysis we can conclude that serum leptin levels were markedly reduced in children with CD compared to controls and the values of BMI of these children were lower than the controls; these results may be explained on the ground that the children with CD were undernourished as compared to the controls.

KEYWORDS: Celiac Disease, Leptin, Serological Antibodies, Body Mass Index.

INTRODUCTION

Celiac disease (CD) or gluten sensitive enteropathy is a permanent intolerance of dietary gluten (a protein found in cereals such as wheat, barley or rye), leading to mucosal damage in the proximal small bowel in genetically susceptible individuals characterized by inflammation, crypt hyperplasia and villous atrophy which regress on withdrawal of gluten from the diet[1]. Celiac disease occurs in genetically predisposed individuals, both children and adults, and it affects approximately 1% of the world population[2]. A possible pathogenic mechanism involves increased permeability of the intestinal epithelium to gliadin, the immunogenic component of gluten, caused by dysregulation of the innate and adaptive immune systems [3]. Celiac disease is one of the most common causes of chronic malabsorption; this results from injury to the small intestine with loss of absorptive surface area, reduction of digestive enzymes, and consequential impaired absorption of micronutrients such as fat-soluble vitamins, iron, and potentially B_{12} and folic acid[4]. In addition, the inflammation exacerbates symptoms of malabsorption by causing net secretion of fluid that can result in diarrhea. Among children, CD has a varied presentation, impacted by factors such as age at presentation and duration of disease. Very young children present more often with classical CD, marked by diarrhea, abdominal distension, and failure to thrive[5]. Older children and adolescents are more likely to present with atypical gastrointestinal complaints such as pain, vomiting, or constipation; extra intestinal symptoms such as arthritis, neurologic symptoms, and anemia or may have silent disease without any apparent symptoms[6]. At present, the only treatment available is lifelong gluten free diet (GFD); the majority of children with CD show normalization of nutritional and growth parameters after GFD[7]. A diagnosis of CD is strongly suggested by the presence of sensitive and specific serological markers. Serologic testing is based on identifying IgA antibodies against gliadin, endomysium, and tissue transglutaminase. Anti-gliadin antibodies (AGA) [IgA antigliadin antibody (IgA AGA) and IgG antigliadin antibody (IgG AGA)] have been used for decades and are reasonably accurate when there is a high pretest prevalence of CD[8]. Also, an Anti-tissue transglutaminase (anti-tTG) antibody is the most practical test and is now widely used for diagnosing CD[9]. Leptin is a hormone that plays a central role in regulation of food intake and energy expenditure[10]. Leptin from adipocytes and gastric mucosa are released at different times after the onset of food intake. These two pools of leptin serve different purposes. Gastric leptin is involved in regulation of food absorption mostly at the level of the intestinal mucosa, whereas leptin from white adipose tissue controls satiety and energy storage[11]. Leptin research in CD is of interest for two major reasons; firstly, under physiological conditions, leptin affects intestinal function by reducing fat accumulation by balancing intestinal absorption of dietary proteins and fats, with impairment of this function leading to obesity, further, as the small intestinal mucosa is affected by inflammatory events, this likely interferes with the activity of leptin[12].

MATERIALS & METHODS

Subjects

A total of 30 children with CD were included in this study, which were referred to immunological lab in teaching laboratory in Baghdad Medical City. Carefully history was obtained from each patient; none of those patients was under medication at time of investigation. For the purpose of comparison, 15 apparently healthy controls were also enrolled, and matched patients for age and gender. Demographic features [gender, age and BMI (weight/height^2)] of all the subjects were recorded.

Collection of Blood

From each participant, 5 ml of blood was collected in a plain tube and allowed to clot at room temperature. The clotted blood was centrifuged at 3000 rpm for 10 minutes and then the serum was collected, distributed into aliquots and frozen at -20°C until assessment for autoantibody status and leptin.

Assessment

Anti-tissue transglutamase IgG antibody and anti-gladin IgG and IgM were evaluated by ELISA technique also according to leaflet manufactured company BIOHIT OYJ, Finland and Euroimmune, Germany; respectively. The leptin concentration was evaluated by ELISA technique according to leaflet manufactured company DRG, USA.

Statistical Analysis

Statistical analysis was done using Statistical Package for Social Science (SPSS), version 16, computer software. Data are expressed as mean ± SE. Student's t-test was used to compare means of patients and controls. Relationships between variables were estimated by Pearson correlation coefficient (r). P value <0.05 was considered significant.

RESULTS & DISCUSSION

Thirty children with CD (17 girls and 13 boys) and 15 healthy children (8 girls and 7 boys) as control group were enrolled in this study. As shown in table (1), the demographic features of the two different groups revealed that there were non-significant differences (P>0.05) in age and gender of the children with CD and healthy children. While the mean level of BMI was significantly lower (P= 0.006) in children with CD as compared with healthy children.

TABLE 1: Demographic features of children with celiac disease and control groups

Features \ Groups	Patients (n=30)	Control (n=15)	P value
Gender (girls/ boys)	17/13	8/7	0.713
Age (years)	4.80 ± 0.46	5.40 ± 0.82	0.505
Body mass index (BMI) kg/m^2	12.87 ± 0.55	16.23 ± 0.86	0.006

Values are means ± SE

Concerning the age, this is due to the selection of subjects who are nearly within the same age. In fact, this is an important aspect for the comparison of other parameters especially those which vary with age. Regarding the gender, the current findings disagree with several previous studies[13,14] which stated that the disease has been shown to have a female predominance. The results of BMI values are in agreement with those observed by Brambilla *et al.* (2013) who have reported that CD children were less frequently overweight or obese and more frequently underweight than controls[15].

Serological screening tests showed a non significant difference (P>0.05) in the level of antigliadin antibody IgA between the two groups (children with CD and control), while the levels of antigliadin antibody IgG and anti-tissue transglutaminase antibody were significantly higher (P<0.01) in children with CD as compared with healthy children. The results of serum level of leptin revealed that it was significantly lower (P<0.01) in children with CD as compared with healthy children. The above data documented in table (2).

TABLE 2: Serum levels of antigliadin antibodies (IgA and IgG), anti-tissue transglutaminase and leptin in children with celiac disease and control groups

Tests \ Groups	Patients (n=30)	Control (n=15)	Student test (t-test)	
			P-value	Sig.
Antigliadin antibody IgA (AU)	16.04 ± 3.01	16.42 ± 2.23	0.939	Non Sig. (P>0.05)
Antigliadin antibody IgG (AU)	41.08 ± 3.77	16.90 ± 2.22	0.000	Highly Sig. (P<0.01)
Anti-tissue transglutaminase antibody (RU/ml)	21.62 ± 1.31	10.81 ± 0.76	0.000	Highly Sig. (P<0.01)
Leptin (ng/ml)	14.52 ± 0.38	17.76 ± 0.59	0.000	Highly Sig. (P<0.01)

Values are means ± SE

A diagnosis of CD is strongly suggested by the presence of antibodies against gliadin peptides as well as autoantibodies against transglutaminase. It has been reported that immunoglobulin A (IgA) anti-tissue transglutaminase antibody is the preferred single test for detection of CD in individuals over the age of 2 years[16]. The immune response of CD is directed against gliadin; once across the intestinal barrier, the gliadin remains in an immunogenic state, resulting in a immune response and an up-regulation of peptides and proteins normally involved in controlling gut permeability [17], allowing the facilitation of further gliadin absorption. Once absorbed into the lamina propria this peptide is then exposed to the ubiquitous, predominantly cytoplasmic enzyme tissue

transglutaminase[18].This enzyme catalyses deamidation of the peptide to form glutamic acid and ammonia[19].

Concerning the current results of leptin, they are agree with the results of Quiros *et al.* (2001) who found that leptin concentrations were reduced in patients with CD[20]. Likewise, Ertekin *et al.* (2006) also reported low leptin levels in active CD[21]. It has been stated that the serum concentration of leptin reflects body fat mass; in some cases, such as under nutrition, leptin levels fall as fat mass decreases[22]. When the correlation was studied between levels of leptin with age, gender and BMI in children with CD, the results (table-3) revealed a non-significant correlation ($P > 0.05$) with age and gender, while a significant positive correlation ($r = 0.63$, $P<0.001$) was found with BMI.

TABLE 3: Correlation coefficient between levels of leptin and age, gender and body mass index in children with celiac disease (n=30)

Parameters	Correlation coefficient	Level of sig.
Age	0.21	0.326
Gender	0.02	0.948
Body Mass Index (BMI)	0.63 *	0.001

* Correlation is significant at the 0.001 level

Gender differences in leptin levels have been reported, especially in late puberty and adolescence, with lower levels in males [23]. This has been explained at least in part by differences in body composition and a suppressive effect of androgens on leptin levels[24]. However, as in a previous report[20], we found that age and gender had no significant influence on leptin levels. In healthy individuals, leptin is positively correlated with indices of nutritional status like BMI[25] and body weight[21]. There have been controversial reports regarding the positive correlation of leptin with nutritional indices in disease states. Some researchers have reported that a positive correlation is maintained despite the disease state[26]. In the current study, a positive correlation was found between the BMI and serum leptin levels. Similarly, Ertekin *et al.* (2006) found a positive correlation between BMI and leptin in children with CD before and one year after GFD [21]. While Quirósa *et al.* (2001) have reported no positive correlation of BMI with leptin in active CD but were able to elicit it in remission of the disease[20].

CONCLUSION

Serum leptin levels were markedly reduced in children with CD compared to controls. Also, the values of BMI of these children were lower than the controls; these results may be explained on the ground that the children with CD were undernourished as compared to the controls.

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