Original Article

Oxidative stress and inflammatory parameters inpatients presenting celiac disease

Estresse oxidativo e parâmetros inflamatórios em pacientes com doença celíaca

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ABSTRACT

Objectives: Determine the presence of oxidative stress and inflammation in the gut of patients with celiac disease. **Methods:** Transversal study that included patients undergoing upper gastrointestinal endoscopy was performed. The study population consisted 24 cases and 26 controls. The duodenal levels of protein carbonyls, thiobarbituric acid reactive species, as well as catalase, superoxide dismutase (SOD) activities were measured. Gut levels of interleukin (IL) 6, 10 and 8 were also determined.The Marsh classification was recorded and used as a parameter of disease severity. **Results:** Both IL-6 and IL-10, but not IL8, were increased in celiac disease patients when compared to healthy individuals. Oxidative damage parameters were increased while antioxidant defenses were decreased in our sample. Both IL6 levels and SOD activity were related to Marsh score. **Conclusions:** Different markers of inflammation and oxidative stress are altered in the gut of celiac disease patients, and some of them are related to disease severity.

RESUMO

Objetivos: Determinar a presença de estresse oxidativo e inflamação no intestino de pacientes com doença celíaca. **Método:** Foi realizado estudo transversal que incluiu pacientes submetidos à endoscopia gastrointestinal. A população do estudo consistiu em 24 casos e 26 controles. Foram medidos os níveis duodenais de proteínas carboniladas, espécies reativas ao ácido tiobarbitúrico, bem como catalase (CAT), superóxido dismutase (SOD). Também foram determinados os níveis intestinais de interleucina (IL) 6, 10 e 8. A classificação de Marsh foi registrada e utilizada como parâmetro de gravidade da doença. **Resultados:** Tanto a IL-6 como a IL-10, mas não a IL8, aumentaram nos pacientes com doença celíaca quando comparados com indivíduos saudáveis. Os parâmetros de dano oxidativo foram aumentados,enquanto que as defesas antioxidantes foram reduzidas em nossa amostra. Os níveis de IL6 ea atividade do SOD foram relacionados com a pontuação de Marsh. **Conclusões:** Diferentes marcadores de inflamação e estresse oxidativo estão alterados no intestino de pacientes com doença celíaca, e alguns deles estão relacionados à gravidade da doença.

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INTRODUCTION

Celiac disease (CD) is a chronic small intestinal inflammatory condition caused by an inappropriate immune response to gluten of wheat, rye, barley and malt. The inflammatory process in the gut mucosa causes villous atrophy and bad absorption of nutrients. The disease can have multiple clinical manifestations, from a mild disease to severe forms associated with cancer[1]. The disease is common with a prevalence of about 1:100 in the Caucasian population. The histopathology of CD is characterized by villous blunting, crypt hyperplasia and increased number of intraepithelial lymphocytes. In treated patients gluten challenge induces an accumulation of CD14(+)CD11c(+) dendritic cells[2] and this could induce activation of lymphocytes.

This unique inflammatory response seems to involve also the innate immune system since CD patients presents high levels of Toll Like Receptor (TLR) 4 expression and interleukins (IL1, IL6, IL8, and IL17)[3].This kind of response could suggest that microbiota-associated factors may be important in the development of the disease[4]. Despite of this a more detailed description of the relationship between cytokines, chemokines and the cellular consequences of inflammation (such as oxidative damage) and DC severity is needed.

Within this perspective, this research aims to determine the presence of oxidative stress and inflammation in the gut of patients with CD, comparing these markers to healthy patients and histopathological markers of disease severity.

METHODS

This study was approved by the local Ethics committee(529 014/2014).

It was a transversal study that included patients undergoing upper gastrointestinal (GI) endoscopy in a tertiary hospital from February to June 2014. Adult patients (> 18 years-old) were included if they had complaints suggestive of celiac disease, and the diagnosis was confirmed after wards by histologic and serologic evaluation as suggested by the American College of Gastroenterology[5]. In this way, patients were not treated at the moment of sampling. In addition, it was included patients with normal histology who were examined with upper GI endoscopy as part of the routine diagnostic workup.

Duodenal samples were collected during upper GI endoscopy in the same duodenal area taken for histopathological examination. During the histopathological analyses, the number of lymphocytes was quantified as well as the Marsh classification recorded. Relevant clinical information was collected directly from the patients or reviewing medical charts.

METHODS

Oxidative damage to proteins was assessed by determining the carbonyl groups of the sample, based on the reaction with dinitrophenylhydrazine (DNPH). Briefly, proteins were precipitated by addition of 20% trichloroacetic acid, dissolved in guanidine and mixed with DNPH. Protein carbonyls were determined by the absorbance at 370 nm and expressed as nmol/mg protein[6]. As an evidence of lipid peroxidation it was measured thiobarbituric acid reactive species (TBARS) levels in a heated acidic reaction. Briefly, samples were mixed with 10% trichloroacetic acid and of thiobarbituric acid.The solution was boiled for 15 minutes, and the amount of TBARS determined by absorbance at 535 nm[7]. TBARS levels were expressed as MDA equivalents (nmol/mg protein).

Superoxide dismutase (SOD) activity was measured by the inhibition of autoxidation adrenaline followed spectrophotometrically at 420 nm. A calibration curve was made using as a standard purified SOD in order to calculate the specific activity of SOD present in the samples. A 50% inhibition of the autoxidation was defined as one unit of SOD and the specific activity was represented as units per mg protein[8]. The activity of catalase (CAT) was determined by the rate of H_2O_2 clearance at 240 nm. A unit of CAT is defined as one mole of hydrogen peroxide consumed per minute and the specific activity was reported as units per mg protein[9].

As inflammatory parameters,two cytokines (IL6 and IL10) and one chemokine (IL8) were measured by ELISA kits as recommended by the manufacturer (Peprotech, FUNPEC Brazil - SP). The unit of measure was pg/mg protein.

After collecting data,it was designed a database on IBM software Statistical Package for Social Sciences (SPSS) version 21.0. Quantitative variables were expressed as mean and standard deviation and qualitative by frequency and percentages. An association between qualitative variables and the presence of DC was investigated by chi-square and Fisher exact tests. The magnitude of the association was estimated by calculating the odds ratio (OR).

The difference between quantitative variables and the presence of DC was investigated by the Mann-Whitney U test, preceded by the Shapiro-Wilk test. Correlation between quantitative variables was determined by Spearman test. The relation of the biomarkers with the Marsh classification was determined by Kruskal-Wallis test. Statistical tests were performed with a significance level $\alpha=0.05$ and 95% confidence.

RESULTS

The study population consisted of 50 patients, of whom 24 had a diagnosis of CD. CD patients are significantly older when compared to healthy individuals (Table 1). When it was analyzed the presence of GI symptoms, only the presence of diarrhea was significantly more prevalent in CD patients (Table 1).

Since it is believed that inflammation have a central role in the pathogenesis of CD it was measured the duodenal levels of IL6, IL10 and IL8. Both IL-6 and IL-10 were increased in CD patients when compared to healthy individuals (Table 2). This was not true IL-8 level.

One of the major consequences of inflammation is oxidative damage, thus it was also determined oxidative damage levels in our sample. Both TBARS and protein carbonyls levels were increased in the duodenum of CD patients (Table 2). Oxidative damage could also be secondary to an imbalance in antioxidant defenses. In this way, the activity of both CAT and SOD were decreased in patients with CD (Table 2).

It was further investigate if there is any correlation between histopathological markers of CD severity and inflammatory or oxidative stress biomarkers. There was not any significant correlation between the numbers of lymphocytes in duodenal biopsies and either inflammatory or oxidative parameters (data not shown). In contrast, patients with Marsh score 1 presented higher SOD activity and lower IL6 levels when compared to patients with Marsh score 2 and 3 (Figure 1). In our sample we didn't have any patient with Marshal score 0 or 4. There was no significant variation between either catalase, IL10 or IL8 when comparing different Marshal scores.

Table 1 – Clinical characteristics of patientes.

Variable	Celiac disease		OR	IC 95%	*p* value
	Yes (n=24)	No (n=26)			
Age (years)	37.50±13.54	31.27±11.17			
Sex					
Female	21 (87.5)	22 (84.6)	0.786	0.157-3.938	1.00
Race					
Caucasian	21 (87.5)	24 (92.3)	0.583	0.089-3.833	0.661
Diarrhea					
Yes	18 (75.0)	12 (46.2)	3.500	1.051-11.660	0.038
Pain and abdominal distention					
Yes	(20 (83.3)	23 (88.5)	0.652	0.130-3.271	0.697
Flatulence					
Yes	14 (58.3)	13 (50.0)	1.400	0.458-4.281	0.555
Anemia					
Yes	6 (25.0)	6 (23.1)	1.111	0.303-4.071	0.874
Stomatitis					
Yes	10 (41.7)	10 (38.5)	1.143	0.368-3.547	0.817

Table 2 – Oxidative stress and inflammatory parameters in Celiac Disease patients.

Variable	Celiac disease		*p* value
	Yes (n=24)	No (n=26)	
Carbonyl (nmol/mg protein)	0.021±0.009	0.015±0.003	0.002
TBARS (inmol/mg protein)	0.007±0.001	0.003±0.001	<0.001
SOD (U/mg protein)	1.464±0.188	5.239±1.410	<0.001
CAT (U/mg protein)	0.517±0.234	1.013±0.345	<0.001
IL 10 (pg/mg protein)	7.175±1.740	4.729±1.701	0.002
IL 6 (pg/mg protein)	16.989±7.342	9.654±3.831	0.002
IL 8 (pg/mg protein)	7.438±2.294	5.882±1.729	0.15

Carbonyl - protein carbonylis; SOD - superoxide dismutase; TBARS - thiobarbituric acid reactive species; CAT - catalase; IL - interleukin

Figure 1A:

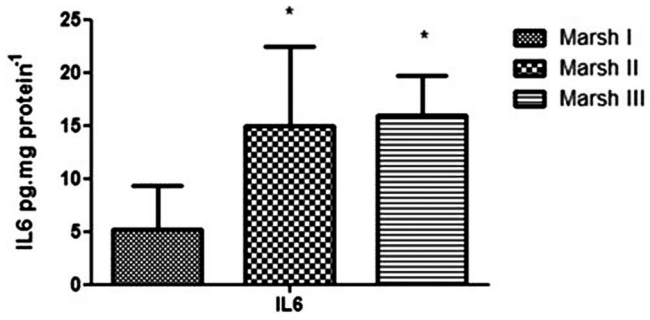

Figure 1B:

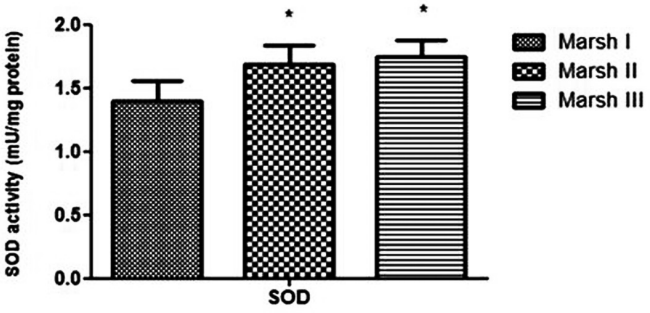

***Figura 1** – (A and B) – Histopathological severity index and its relation with inflammatory response (IL-6) and oxidative stress markers (SOD).*
** significantly different from Marsh I, p <0.05*

DISCUSSION

We here demonstrated that both oxidative stress and inflammatory parameters are associated with CD, and both SOD and IL6 levels are related to Marsh score. IL-10 is a cytokine with anti-inflammatory properties and plays an important role in inflammation, regulating the immune system[10]. It is secreted by Th2 and T regulatory lymphocytes. CD was originally described as a purely Th1 disease. Probably it involves Th17 and T regulatory lymphocytes as well[11]. It is proposed that new therapies to CD involves the suppression of Th1 / Th17 activation by a cross-regulation of the immunological response by concurrent Th2 activation or IL10 production[12].

Thus, the observed increased in IL10 levels could be related to an adaptive response to the Th1/Th17 response naturally associated with the development of CD. This is supported by the finding that potential CD patients (those subjects with a normal small intestinal mucosa who are at increased risk of developing CD) show activation of regulatory mechanisms (such as increasing IL10 mRNA levels) and is believed that this is a mechanism to prevent the progression toward a mucosal damage[13].

IL-6 is a cytokine secreted by T cells and macrophages to stimulate the immune response mainly during infection. It is well known that both Th1 and Th17 lymphocytes are able to secrete IL6, thus it is expected that during CD development there is an increase in gut IL6 levels. As we demonstrated here, Eiró et al.[3] showed that both children and adult CD patients have increased gut levels of several Th1/Th17 related cytokines, including IL6.In treated CD patients a challenge with gliadin induces the expression of IL6 in gut mucosa[14].

IL-8 is produced by macrophages and other cell types such as epithelial cells, airway smooth muscle cells and endothelial cells. Its main function is to induces the chemotaxis of neutrophils. The role for IL8 during CD development is not well understood. IL8 is up-regulated, for example, in ulcerative colitis, and the remission of the disease is associated with a normalization of IL8 gene expression[15]. To the best of our knowledge no study determined IL8 levels in the gut of CD patients.

One study found an increase in IL8 gene expression in the gut of CD patients[3], but we are not able to demonstrate any significant difference in protein levels between CD and control patients. IL8 is mainly produced by the innate immune system, and despite some evidence for a role of this response in CD, it

is classically related to the activation acquired immune system. Certain gliadin peptides are able to induce an innate immune response probably through the activation of TLRs. TLR activation could then increase the secretion of several cytokines, including IL6 and IL8. A better understanding on the modulation of TLR responses is needed to explain these non-concordant findings[16].

In addition to immunogenic effects, gliadin may directly affect intestinal cell structure and function. One of these mechanisms seems to be related to oxidative stress. Gliadin exposure induces an oxidative imbalance, and some markers of oxidative stress, such as 4-hydroxy-2-nonenal and an increase in the oxidised (GSSG)/reduced (GSH) glutathione ratio have been demonstrated in vitro[17].

Duodenal biopsies of CD patients also demonstrated markers of oxidative damage, as we here demonstrated[18,19]. Differently from what we found, in children SOD activity seems to be increased in the gut of CD patients, CAT did not change and there was a decrease in the glutathione-related antioxidant defenses[19]. Thus, it seems that oxidative damage could be related to antioxidant defenses alterations, opening the perspective of an antioxidant-based treatment for CD[20].

Some limitations must be taken in consideration when interpreting our results. It were included newly recognized CD patients, thus we are not able to determine the time-point of the course of the disease that each patient is at the moment of gut biopsies. Due to the transversal design of the study we are not able to determine if CD treatment would interfere in the analyzed parameters, nor if they could predict disease outcomes. The longitudinal observation of CD patients is of pivotal importance to a better understanding of the role of inflammation and oxidative stress on disease progression.

CONCLUSIONS

Different markers of inflammation and oxidative stress are altered in the gut of CD patients, and some of them are related to disease severity. Inflammatory markers, most often associated with Th1/Th17 and Th2 balance, seems to be relevant to CD development, as well as oxidative damage and antioxidant defense imbalance.

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