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Evaluation of Certain Biochemical Changes in Celiac Patients

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ABSTRACT

The aim of the study was to evaluate and compare oxidase and ferroxidase ceruloplasmin activities (U L^{-1}) and their specific activities (U g^{-1}) in sera of celiac patients with different histopathological severity. This study included 75 celiac patients with different mean age (18.68±11.13) year, who had positive screen for celiac antibodies and who had gastrointestinal symptoms. In order to simplify the comparison with the healthy control group, celiac patients were divided into two groups according to their histopathological severity: Severe (marsh III a, b, c) and less severe (marsh 0,I). All these patients have been evaluating for S.CP. ferroxidase and S.CP. oxidase activities as well as its specific activities. Furthermore, the concentrations of total protein, albumin, copper and iron, were measured too. Non-significant increase ($p>0.05$) in serum ferroxidase activity of ceruloplasmin was found in all above mentioned patients groups in comparison to that of the control group, while its specific activity showed a significant increase for more severe mucosal histopathological damage (marsh III a, b, c) patients and ($p>0.05$) for less severe mucosal histopathological damage (marsh 0,I) patients in comparison to that of control group. As far as serum oxidase ceruloplasmin activities is concerned, a significant increase ($p<0.05$) was observed in all patients groups, while its specific activity showed non-significant increase ($p>0.05$) in sera of more severe mucosal histopathological damage (marsh III a, b, c) patients and a significant increase ($p<0.05$) for less severe mucosal histopathological damage (marsh 0,I) patients. Among the patients groups, serum copper levels showed non-significant increased ($p = 0.1$) and serum iron level was found to decrease significantly in patients with more severe mucosal histopathological damage (marsh III a, b, c) in comparison to that of control group. Meanwhile the mean values of patients total protein and their albumin were found to show non-significant increase ($p>0.05$) in comparison to that of the control groups.

Key words: Ceruloplasmin, celiac disease, mucosal histopathological damage, copper, anemia

INTRODUCTION

Celiac Disease (CD) is a syndrome characterized by damage of the small intestinal mucosa caused by the gliadin fraction of wheat gluten and similar alcohol-soluble proteins (prolamines) of barely and rye (Yadav and Singh, 2011) in genetically susceptible subjects. The presence of gluten in these subjects leads to self-continuous mucosal damage, whereas elimination of gluten results in full mucosal recovery (Trier *et al*., 1978; Stern *et al*., 2001). The clinical manifestations of celiac disease (CD) are changeable in nature and vary markedly with the age of the patient,

the duration and extent of disease and the presence of extra-intestinal pathological conditions (Catassi *et al.*, 1997; Richard and Kelly, 2001). In addition, to the classical gastrointestinal form, a variety of other clinical manifestations of the disease has been described, including atypical and asymptomatic forms (Trier, 1998). The keystone treatment of CD patients is a lifelong elimination diet in which food products containing gluten are avoided (Fasano and Catassi, 2001; Al-Tintas *et al.*, 2008).

Where oxidative stress plays an important role in the pathogenesis of many chronic diseases (Suresh *et al.*, 2008), Antioxidants have received increased attention by nutritionists and medical researchers for their potential effects in the prevention of chronic and degenerative diseases (Al-Humaid *et al.*, 2010). Several antioxidant maneuvers aim at modifying the oxidative status in CD patients like Ceruloplasmin (Ferroxidase; Iron (II): O_2 oxidoreductase, EC 1.16.3.1); the major blue copper containing glycoprotein (Holmberg and Luurell, 1948). It is a major enzymatic contributor to the antioxidant defense system of human plasma. It acts as an antioxidant by several mechanisms (Zowcza *et al.*, 2001; Cogalgil and Taysi, 2002; Taysi *et al.*, 2002) inhibiting iron-dependant lipid peroxidation and OH formation from hydrogen peroxide by its ferroxidase activity (Zowcza *et al.*, 2001; Shakour-Shahabi *et al.*, 2010), reacting and scavenging H_2O_2 and superoxide anion and inhibiting copper-induced lipid peroxidation by binding copper ions (Zowcza *et al.*, 2001; Taysi *et al.*, 2002). According to the literature over 90% of human copper is associated with the ceruloplasmin as a non dialyzable fraction and the remaining 5-10% of plasma copper is believed to be fairly loosely attached to albumin and histidine and only traces of copper is present as free Cu^{++} (Burtis and Ashwood, 1999).

Copper is transported to the liver and unites with apoceruloplasmin to form ceruloplasmin, then ceruloplasmin is released into the blood. No copper exchange occurs in serum ceruloplasmin. Afterwards, ceruloplasmin is internalized in cells and copper is released from the ceruloplasmin by means of protein destruction reactions. Increased absorption of copper causes increased ceruloplasmin production. Cells capture ceruloplasmin to produce copper-containing enzymes such as mono- and diamine oxidases and ascorbate oxidase (Sato and Gitlin, 1991; Gitlin, 1998). Because of its oxidase activity, ceruloplasmin is also known as copper oxidase and this activity can be used for measurement of ceruloplasmin. Ceruloplasmin performs its ferro-oxidase activity at the cell surface by binding of iron to transferrin which is the first step in the transformation of Fe^{2+} to Fe^{3+}. Serum ceruloplasmin level was reported to increase during sport, pregnancy and estrogen supplement, as well as in states such as infections, malignities, Hodgkin's disease and cholangitis. While a decrease in this level was reported in malnutrition and malabsorbtion states, nephrotic syndrome and primary biliary cirrhosis (Beshgetoor and Hambidge, 1998; Shakour-Shahabi *et al.*, 2010). The objective of the study is to evaluate and compare different activities in oxidase and ferroxidase.

MATERIALS AND METHODS

Inclusion criteria: A total of 75 cases with different chief complaints and presentation like chronic diarrhea, bloating, chronic abdominal pain and short stature or if they were positive for a CD-antibody screen were included in this study. These patients attended to the center of Gastroenterology and Hepatology, they were referred from different hospitals in Baghdad and other governorates in Iraq during the period of May 2010 to June 2011.

The age of these patients ranged from 2 year to 43 years, all patients were subjected to a personal interview using especially designed questionnaire format full history with detailed information (age, sex, symptoms, autoimmune diseases, gluten diet if intake).

The control group consisted of 46 apparently healthy individuals who matched in age and gender with patients and had no history of any gastro-intestinal problem (from the friends and relatives), which refused to subject to Oesophago-gastro-duodenoscopy (OGD).

Endoscopic biopsy: A minimum of 3 biopsies were taken from different sites of the distal part of the duodenum, further examination of the duodenum, stomach and oesophagus were performed. Histological analyses of the biopsies were carried out by two blinded expert pathologists while withdrawing the scope, the biopsies were placed in 10% formalin in a ground glass tube (universal tube) (Richard *et al*., 2002).

The diagnosis of CD was based on the presence of villous atrophy (total, subtotal or partial) with increased intraepithelial lymphocyte (IEL) counts on initial endoscopic biopsies. These histological analyses were scored according to the Marsh (1992), classification (Marsh, 1992) revised in 1997: [(Marsh IIIa partial villous atrophy), Marsh IIIb (subtotal villous atrophy) and Marsh IIIc (total villous atrophy)] (Rostami *et al*., 1997).

Determination of the different enzymatic activities of ceruloplasmin: The oxidase activity of ceruloplasmin was determined using the modified Rice (1962) method where ceruloplasmin catalyzes the oxidation of p-phenylenediamine (which was used as a substrate) to give blue-violet color that measured at $\lambda = 540$ nm. Sera ceruloplasmin ferroxidase activity was determined, in term of the decrease in the concentration of the substrate (ferrous ion) upon its incubation with the enzyme and as described by Erel (1998).

Determination of trace elements: Serum iron and copper concentrations were measured by an GBC 933 plus atomic absorption spectrophotometer at $\lambda = 248.3$ and 324.7 nm for iron and copper, respectively. The results of the trace elements were expressed in μmol L^{-1}.

Protein determination: The serum total protein concentration was determined by using modified Lowery method by Hartree (1972). Bbovine Serum Albumin (BSA) was used as standard and the Protein concentration was expressed in g L^{-1}. Serum albumin concentration was estimated by the method employing bromocresol green as described by Doumas *et al*. (1971).

Statistical analysis: The data were analyzed by Duncan's multiple range test at ($p<0.05$) was accepted as statistically significant and highly significant when ($p<0.001$), using the SPSS software. All the analyses were repeated three times.

RESULTS

The mean ages of the patients included in the currant study were 14.58±9.77 for more severe histopathological celiac group (marsh III a, b, c), 17.807±11.707 for non and less severe histopathological celiac group (marsh 0,I) and 15.80±10.32 for the control group. Table 1 presented the marsh classification scheme.

The sex distribution of patients shows a statistical difference between the female (61%) and the male (39%). Meanwhile only 26.6% of all patients were in Gluten Free Diet (GFD), It is worth to

Table 1: Marsh classification scheme

Classification	Details
Marsh 0	Normal when villi were tall and finger-like, no crypt hyperplasia, no infiltration of inflammatory cells
Marsh I	There is increase IELs infiltrations, IELs >30 per 100 enterocytes
Marsh II	Increase IELs + crypt hyperplasia
Marsh III:	Influx of inflammatory cells, hyperplasia of crypts and villous atrophy
Marsh IIIa	Partial villous atrophy (PVA) (villous/crypt ratio <1:1), villi were short, broad or leafy with mild crypt hyperplasia
Marsh IIIb	Subtotal villous atrophy (SVA): villi were clearly atrophic, but separated villi were still recognizable
Marsh IIIc	Total villous atrophy (TVA); villi were rudimentary or absent
Marsh IV	Total villous atrophy (TVA); no significant inflammation atrophic- hypoplastic lesion

Table 2: The relation between the age and severity of histopathological changes

	Marsh III a		Marsh III b		Marsh III c	
Age groups (Y)	No.	%	No.	%	No.	%
≤10	9	34.0	15	58.0	2	08.0
11-20	10	53.0	5	26.0	4	21.0
21-30	3	30.0	6	60.0	1	10.0
31-40	0	00.0	3	50.0	3	50.0
> 40	1	100.0	0	00.0	0	00.0
Total	23		29		10	

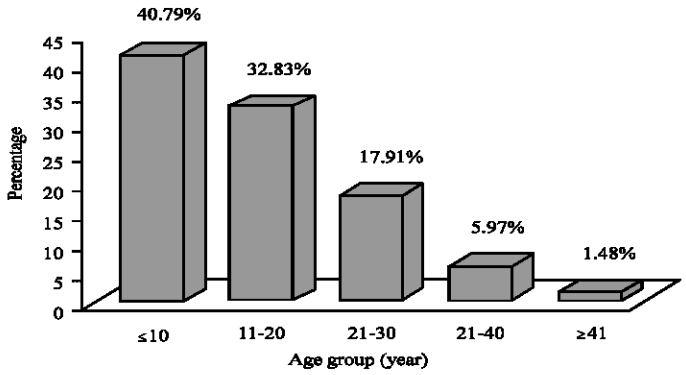

Fig. 1: Age distribution of celiac patients at time of diagnosis

mention that most celiac patients included in the present study were found to be at stage III (82%), with the higher ratio of 38% in marsh IIIb, then 31% in marsh IIIa and 13% in marsh IIIc; while the percentage of marsh I and 0 were 7 and 11%, respectively.

The patients group was divided into 5 subgroups according to their age at the time of diagnosis (Fig. 1). Table 2 shows the histopathological changes among the patients with different age.

The results in Table 3, 4a and b show the mean value of ceruloplasmin ferroxidase and its oxidase activity U L^{-1} and their specific activities U g^{-1}. While Table 5a and b show the mean value of ceruloplasmin oxidase concentration (mg dL^{-1}) in sera of control and patient groups.

The clinical signs and symptoms that observed in symptomatic celiac patients were listed in order of occurrence in Fig. 2.

Table 6 shows the presence of a significant increase in the mean values of ceruloplasmin concentration (mg dL^{-1}) and its oxidase activity in the sera of celiac patients who were on gluten diet. While, Table 7 shows the mean values of total protein concentration and albumin in the sera

Table 3: Mean values of ceruloplasmin (ferroxidase and oxidase) activities (U L^{-1}) and specific activities (U g^{-1}) in sera of control and celiac patients groups

	Groups			
Parameters	Control	Patients marsh III a, b ,c	Patients marsh 0,I	p-value
Age (years)	15.805±10.324 (4-40)	14.58±9.772 (2-43)	17.807±11.707 (4.5-38)	Non sig.
Ferroxidase activity (U L^{-1})	527.255±160.184	488.354±241.597	678.327±188.537	
	(287.23-821.54)	(118.48-956.12)	(434.59- 943.101)	Non sig. for all gp
Specific ferroxidase activity	8.940±3.299	6.785±3.419	9.639±3.555	Sig. for patients
(U g^{-1})	(4.206-14.933)	(1.584-14.272)	(4.522-13.633)	Marsh III and non sig. for marsh 0,I
Oxidase activity (U L^{-1})	76.201±39.781	97.991±45.585	106.677±32.068	Sig. for all gp
	(23.380-179.00)	(30.360-274.310)	(72.243-126.687)	Non sig. for patients
Specific oxidase activity	1.258±0.613	1.386±0.676	1.649±0.7489	Marsh III and sig.
(U g^{-1})	(0.442-2.896)	(0.391-3.350)	(0.916-3.216)	for marsh 0,I

Values are as Mean±SD, values within brackets are range

Table 4a: Mean values of ceruloplasmin ferroxidase activity (U L^{-1}) and specific activity (U g^{-1}) in sera of control and celiac patients group

			Specific ferroxidase activity (U g^{-1})		Ferroxidase activity (U L^{-1})	
Group	No.	Age (year) Mean±SD	Mean±SD	Range	Mean±SD	Range
Control	46	15.805±10.324	527.255±160.184	287.23-821.54	8.940±3.299	4.206-14.933
Patients marsh IIIa	22	13.721±9.289	486.696±253.963	118.48-956.12	6.959±3.652	1.584-14.272
Patients marsh IIIb	28	14.144±9.565	449.961±227.220	130.60-913.94	6.384±3.562	1.651-13.766
Patients marsh IIIc	10	19.400±12.130	602.312±248.465	327.43-944.10	7.481±2.567	4.314-12.101
Patients marsh 0,I	13	17.807±11.707	678.327±188.537	434.59-84.662	9.639±3.555	4.522-13.633
*p-value			Non sig. for all gp	Sig. (for a and b) and non sig. (for c and marsh 0,I)		

*Significant difference in comparison to control at ($p<0.05$)

Table 4b: Mean values of ceruloplasmin oxidase activity (U L^{-1}) and specific activity (U g^{-1}) in sera of control and celiac patients groups

			Specific oxidase activity (U g^{-1})		Oxidase activity (U L^{-1})	
Group	No.	Age (year) Mean±SD	Mean±SD	Range	Mean±SD	Range
Control	46	15.805±10.324	76.201±39.781	23.380-179.00	1.258±0.613	0.442-2.896
Patients marsh IIIa	23	13.956±9.479	94.971±31.100	55.140-171.361	1.357±0.479	0.744-2.327
Patients marsh IIIb	28	14.144±9.565	105.499±56.784	33.164-274.315	1.491±0.819	0.491-3.354
Patients marsh IIIc	10	19.400±12.130	83.018±38.004	30.368-140.653	1.142±0.627	0.392-2.212
Patients marsh 0,I	13	17.807±11.707	106.677±32.068	72.243-126.687	1.649±0.7489	0.916-3.216
Non sig. (for a+b and c) and sig (for marsh 0,I)			Sig. (for a+b and marsh 0,I) and non sig (for c)			*p-value

*Significant difference in comparison to control at ($p<0.05$)

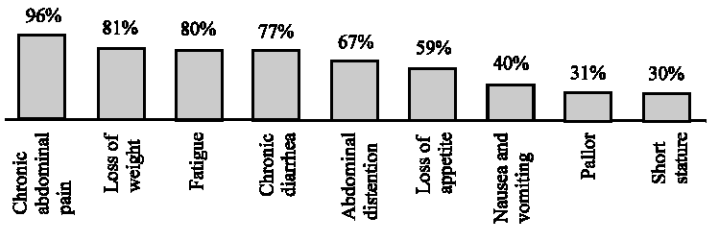

Fig. 2: Clinical manifestation in celiac patients

Table 5a: Mean value of CP oxidase concentration (mg dL^{-1}) in sera samples of control and patient groups

		Age (year)		Serum (mg dL^{-1})	
Group	No.	(Mean±SD)	Rang	(Mean±SD	Range
Control	46	17.805±7.324	(5-32)	14.703±7.137	4.55-26.78
Patients marsh III	61	14.58±9.772	(2-43)	18.9702±8.914	3.41-39.81
Patients marsh 0,I	13	17.807±11.707	(4.5-38)	20.513±7.592	13.56-35.87

*Significant difference in comparison to control at $p<0.05$, Sera: Sig. for both patients gp

Table 5b: Mean value of CP oxidase concentration (mg/dL) in sera samples of control and patient groups

		Age (year)		Serum mg dL^{-1}	
Group	No.	Mean±SD	Rang	Mean±SD	Range
Control	46	15.805±10.324	5-32	14.703±7.137	4.55-26.78
Patients marshIII a	23	13.956±9.479	2-43	17.289±7.098	4.29-30.49
Patients marshIII b	28	14.144±9.565	2.5-33	21.504±10.117	5.69-39.81
Patients marshIII c	10	19.400±12.130	5-39	15.569±7.910	3.41-27.56
Patients marsh 0,I	13	17.807±11.707	4-38	20.513±7.592	13.56-35.87

*Significant difference in comparison to control at ($p<0.05$), Sera: Sig. (for b and marsh 0,I) and non sig. (for a and c).

Table 6: Mean values of ceruloplasmin (ferroxidase and oxidase) activities (U L^{-1}) and specific activities (U g^{-1}) and cp conc. (mg dL^{-1}) in sera of control and celiac patients with or without gluten diet

	Groups			
Parameters	Control	Patients on gluten diet	Patients on gluten free diet	p-value
No. of causes	46	55	20	
Age (years)	15.805±10.324 (4-40)	14.14±10.14 (2-43)	15.69±9734 (3-33)	Non sig.
Ferroxidase activity (U L^{-1})	527.255±160.184	505.450±242.09	441.340±241.951	
	(287.23-821.54)	(122.55-944.10)	(118.48- 956.12)	Non sig. for both gp
Specific ferroxidase activity	8.940±3.299	6.897±3.283	6.472±3.911	Sig. for both gp
(U g^{-1})	(4.206-14.933)	(1.62-14.272)	(1.584-13.678)	
Oxidase activity (U L^{-1})	76.201±39.781	98.708±52.908	96.718±42.912	Sig. for GCD gp
	(23.380-179.00)	(60.38-274.31)	(30.360-200.15)	
Specific oxidase activity	1.258±0.613	1.459±0.650	1.343±0.688	Non sig. for both gp
(U g^{-1})	(0.442-2.896)	(0.86-3.34)	(0.391-3.23)	
Oxidase concentration (mg dL^{-1})	14.703± 7.137	18.844±9.309	16.322±4.353	Sig. for GCD gp
	(4.55-26.78)	(3.41-39.81)	(9.62-22.49)	

*Significant difference in comparison to control at $p<0.05$, GCD: Gluten contain diet

Table 7: Mean laboratory values with standard deviations of sera in patients and control groups

	Groups					
Serum	Marsh III a	Marsh III b	Marsh III c	Marsh 0,I	Control group	p-value
Protein g L^{-1}	70.88±8.47	70.23±8.89	75.8±8.77	67.323±10.2	64.06±9.1	0.651
Alb. g L^{-1}	40.14±5.01	39.5±4.51	39.86±5.44	38.32±6.81	40.64±6.48	0.06
Copper µmol L^{-1}	8.79±0.59	8.46±3.14	6.00±2.53	10.63±2.98	10.71±3.29	0.1
Iron µmol L^{-1}	9.45±4.31	7.52±4.55	8.94±3.86	14.56±5.39	15.22±4.68	0.025

Values are as Mean±SD

samples of all the studied groups and revealed a non- significant increase ($p>0.05$) in comparison to that of control group. Also it is clear from the results of the same table that a non-significant

increase is present in sera copper levels ($p = 0.1$), where its level was observed to be very low in sera of patients at marsh III c of the disease (6.00±2.53 μmol L^{-1}). When the sera iron levels in the patients were compared with that of the control, it was found clearly low in severe celiac (9.45±4.31, 7.52±4.55 and 8.94±3.86 μmol L^{-1} for marsh IIIa, marsh IIIb, and marsh IIIc, respectively) and 14.56±5.39 μmol L^{-1} in less severe histopathological celiac group (marsh 0.I), while it was 15.22±4.68 μmol L^{-1} in the controls ($p = 0.025$).

DISCUSSION

Previously CD was considered to be a disease of childhood because the majority of cases were found among individuals less than 10 years of age (41.79%) comparable to the results reported by Khuffash *et al.* (1987), Amara and Saghar (2000) and Savas *et al.* (2007). However, Savas *et al.* (2007) and his colleagues reported that CD is common in adults and can be diagnosed at any age. This difference in the age range affected by the disease may be attributed to the delay in diagnosis, the range of age selected for each study and to the total number of the patients included in each of these studies.

Sex distribution of CD patients shows excess in females (61%) than in males (39%), this was compatible to the results reported by Schmitz (1996) and Fasano and Catassi (2001). Many studies recorded that CD prevalence in females is more than in males (Sleisenger and Foratrans, 2002). Veghari and Jahanshahi (2007) observed in comparison with boys, malnutrition in girls is more, such result was also observed by many authors as reported by Veghari and Jahanshahi (2007).

The patients included in thee currant study, had classic symptoms of CD (weakness, weight loss, vomit and diarrhea, abdominal distention). Abdominal pain was high in celiac patients compared with control group because most of them have anemia this means, that they have upset, or flaw in a process of heme synthesis, which lead to accumulation of intermediate compounds in large amount like γ-aminolevulinate and porphobilinogen, which are associated with abdominal pain (Muray *et al.*, 2003). Among the results of the current study, it was found that about 77% of our studied patients were suffering from chronic diarrhea, this go well with what Amini-Ranjbar and Babak (2007) reported; Acute diarrhea is one of the common causes of malnutrition and mortality in children younger than 5 years of age, especially in developing countries. Most symptomatic patients have partial, subtotal or total villous atrophy, which are Marsh III lesions.

Copper is mostly absorbed from the duodenum and serum copper level increases in inflammatory conditions, so a decrease in serum copper levels is not expected in marsh (0,I) patients (10.63±2.98 μmol L^{-1}). Ince and his colleagues, reported that eventhough inflammation severity in celiac and Crohn's patients is generally more than in healthy people, these patients had been found to have serum copper less than that of the controls (Ince *et al.*, 2008).This result agrees with the results of the present study here the mean serum copper levels were found to be 8.79±0.59, 8.46±3.14, 6.00±2.53, 10.63±2.98 and 10.71±3.29 μmol L^{-1} for more severe histopathological celiac group (marsh III a, b, c), less severe histopathological celiac group (marsh 0,I) and healthy control groups, respectively. But disagrees with Hameed *et al.* (2001) results which were obtained with malnourished children with all ages.

Ceruloplasmin is one of the metalloproteins, which has a critical role in copper homeostasis and functions as a storage reservoir and/or chaperone for this essential trace metal (Mizzen *et al.*, 1996). This may be used to explain the significant differences that observed in ceruloplasmin ferroxidase specific activities in celiac patients with more severe mucosal histopathological damage (marshes IIIa and b), even though they had non significant differences in their serum copper levels and the

significant increase in ceruloplasmin oxidase specific activities that was found to be present in patients with less severe histopathological damage of the disease (marsh 0,I), who were found to have non significant differences in their serum copper levels.

From the data presented in Table 6, one can conclude that patients with CD, can benefit from a GFD, this apparent from the decline in Cp conc. and its oxidase activity in GFD patients group, It is well known that patients with CD recover completely if they adhere strictly to a Gluten-Free Diet (GFD) for life (Cooke and Holmes, 1984; Strober, 1986). Such results can be explained by Adewumi *et al.* (2007) in which they reported that oxidative destruction of sub-cellular membrane lipids was implicate along with other types of intracellular oxidative damage in pathophysiology of a number of chronic illnesses. Complex antioxidant mechanism, including antioxidant vitamins and minerals exists to limit the effects of these reactions. GFD may protect against malignancy of the gut, which is a known sequel of untreated celiac patients. In untreated CD patients, the small intestinal intraepithelial T-cell lymphomas is increased (Holmes *et al.*, 1989), in addition, many studies indicate that direct cytotoxic effect of gluten on enterocytes may be one of the mechanisms underlying pathogenesis of CD. In the last decade the results of several investigations showed that gluten corrupts the pro-oxidant-antioxidant balance in intestinal mucosa, probably by an overproduction of free radicals. Nevertheless the data concerning antioxidant status of celiac patients are scare (Stojiljkovic *et al.*, 2009).

It is clear from the results in Table 7 that total protein at $p = 0.654$ and alb. level at $p = 0.06$ were shown non-significant increase. The cause of the observed results may be due to the role of albumin as one of the extracellular antioxidants where albumin constitute up to 49% of total plasma antioxidant status (Emerson, 1989). Meanwhile albumin acts as sacrificial antioxidant by inhibiting the generation of free radicals through an immediate attacks of albumin molecule itself, so the radical reaction continue on albumin surface and cause damage to albumin molecule (Gutteridge and Wikins, 1983; Marx and Chevion, 1985) such damage is probably biologically insignificant, due to that the albumin is present in plasma in high concentration (Halliwell and Gutteridge, 1986). The values in normal subjects were relatively lower than those graded into various degrees of malnutrition (Zaidi *et al.*, 1999).

Iron deficiency has been reported to be the most prevalent nutritional deficiency caused from diminished absorption (Ebuehi and Oyewole, 2011). This was confirmed by the present study results where statistically, iron levels were found to be significantly lower in patients at the severe stage of the disease (marsh III a, b, c) than that of patients with less severe mucosal damage (marsh 0,I) and of the control group ($p = 0.025$). Present result here is compatible with Thorvardur *et al.* (2007) result, who reported such low values may originate from diminished absorption of iron since it is usually absorbed by the proximal small intestine; the site of the greatest damage in celiac disease. Such conclusion was based on Andrews (2004) results, which indicated that the iron absorption depends on several factors among them, an intact mucosal surface and intestinal acidity.

CONCLUSION

To our knowledge no report is available in the literatures concerning studying the enzymatic activities of cp in patients with CD. Our present study highlights the relationship between this disease at its different stages and cp different enzymatic activities. Further study is carried out in our laboratories to investigate this relationship more deeply in these patients. GFD for celiac patients may decline the oxidative stress, throughout decline cp conc. and activity.

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