

Oxidative Status and Plasma Prolidase Activity in Patients with Gallstones

Safra Kesesi Taşı Olan Hastalarda Oksidan Durum ve Plazma Prolidaz Aktivitesi

Alpaslan TERZİ¹, Sacit ÇOBAN², Fahrettin YILDIZ¹, Murat KAYA¹, Nurten AKSOY³, Taşkın ABDULLAH³

¹Harran Üniversitesi Tıp Fakültesi, Genel Cerrahi Anabilim Dalı, Şanlıurfa

²Gaziantep Üniversitesi Tıp Fakültesi, Genel Cerrahi Anabilim Dalı, Gaziantep

³Harran Üniversitesi Tıp Fakültesi, Klinik Biyokimya Anabilim Dalı, Şanlıurfa

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Objective: The aim of our study was to investigate the oxidative and antioxidative status of plasma and plasma prolidase enzyme activity on gallstone disease.

Material and Methods: The study group included 35 patient with gallstone disease and 30 healthy volunteers as control subjects. Plasma prolidase activity and oxidative-antioxidative parameters were measured. Antioxidative status of plasma was evaluated by measuring total antioxidant capacity. To determine oxidative status; total oxidant status and oxidative stress index were determined.

Results: Total antioxidant capacity levels of plasma were lower in the patients with gallstone disease than in controls ($p<0.001$). In contrast, total oxidant status levels and oxidative stress index values were higher in the patients than in controls ($p<0.05$ and $p<0.001$, respectively). Beside these, plasma prolidase activity was significantly lower in the patients than controls ($p<0.001$).

Conclusions: The present results indicated that the oxidant parameters increased and antioxidant parameters decreased in patients with gallstones, and that these patients were exposed to oxidative stress.

Key words: Gallstone disease; oxidative status; prolidase.

Amaç: Bu çalışmadaki amacımız safra kesesi taşı olan hastalarda oksidatif ve antioksidatif durumu ve plazma prolidaz enzim aktivitesini araştırmaktır.

Gereç ve Yöntemler: Çalışma grubu safra kesesi taşı olan 35 hastadan ve kontrol grubu sağlıklı 30 kişiden oluşturuldu. Plazma prolidaz aktivitesi ve oksidatif-antioksidatif parametre düzeyleri çalışıldı. Antioksidatif durum total antioksidan kapasite ölçülerek değerlendirildi. Oksidatif stresi değerlendirmek için total oksidan durum ve oksidatif stres indeksi çalışıldı.

Bulgular: Plazmada total antioksidan kapasite düzeyi safra kesesi taşı olan hastalarda kontrol grubuna göre daha düşüktü ($p<0.001$). Ayrıca total oksidan durum ve oksidatif stres indeksi kontrol grubunda daha düşüktü (Sırasıyla, $p<0.05$ ve $p<0.001$). Bunların yanında hastalarda plazma prolidaz aktivitesi kontrol grubuna göre anlamlı olarak düşüktü ($p<0.001$).

Sonuç: Mevcut bulgular safra kesesi taşı olan hastalarda oksidan parametrelerin arttığını ve antioksidan parametrelerin azaldığını ve bu hastaların oksidatif strese maruz kaldıklarını gösterdi.

Anahtar sözcükler: Kolelithiazis; oksidan durum; prolidaz.

INTRODUCTION

Gallstone disease is one of the most common gastrointestinal disorders in the world. Every year, 1-3% of people worldwide develop gallstones, and about 1-3% of these are symptomatic. The morbidity and fatality

are associated with symptomatic cholelithiasis, cholecystitis, or cholangitis. [1] It has been shown that there have been many changes in the wall of gallbladder, bile composition and plasma of patients with gallstones. [2-4] Gallstones are associated with various histopathological

changes in gallbladder mucosa, e.g. acute and chronic inflammation or glandular hyperplasia.^[2] Increased levels of an accelerated generation of reactive oxygen species (ROS) and toxic degradation products of lipid peroxidation have been reported in the plasma of individuals with gallstones.^[15]

It is well known that, in chronic diseases such as cholelithiasis, the active inflammatory response is induced with neutrophilic infiltration. These neutrophils, macrophages and/or monocytes produce ROS which may cause DNA damage to the adjacent cells^[6,7] Oxidative stress provoked by ROS plays an important role in the pathogenesis of many diseases such as hepatitis, cholecystitis, gallstones, gastroduodenal mucosal inflammation, peptic ulcer disease, and probably even gastric cancer.^[7-9] The organism has enzymatic and non-enzymatic antioxidant systems neutralizing the harmful effects of the endogenous ROS products. Under certain conditions, the oxidative or anti-oxidative balance shifts towards the oxidative status as a result of increase in ROS and/or impairment in antioxidant mechanism.^[10-12]

Prolidase is a manganese-dependent cytosolic exopeptidase which cleaves imidodipeptides containing C terminal proline or hydroxyproline and plays an important role in the recycling of proline for collagen synthesis, matrix remodeling and cell growth.^[13] Prolidase enzyme activity has been investigated in various disorders such as chronic liver disease,^[14] osteoporosis,^[15] osteoarthritis^[16] and uremia.^[17] However, to the best of our knowledge, there is no information in the literature about prolidase activity in cholelithiasis. Our goal, therefore, in this study was to determine plasma prolidase activity in these patients and compare them with healthy individuals and to find whether its activity may reflect disturbances of collagen metabolism in this disease. We also evaluated the oxidative-antioxidative status and its relationship with prolidase activity in these patients and tried to establish whether there is a correlation between them.

MATERIALS AND METHODS

Subjects

The study group included 35 patients with cholelithiasis (group I) and 30 healthy volunteers as control subjects. (Group II). The ages and gender of the patients and controls were similar (Table 1). All patients' routine hematological and biochemical parameters were determined. Exclusion criteria were acute cholecystitis, pancreatitis, hepatobiliary disease, cirrhosis, malignant tumor, smoking and antioxidant usage. All of the subjects were examined by ultrasonography. The patients with signs of acute cholecystitis such as gallbladder wall thickness (over than 3mm), pericholecystic fluid and hydropic gallbladder were excluded. All subjects were informed about the study. The study protocol conforms to the principles of the Helsinki Declaration and was approved by the Medical Ethics Committee of Harran

University. All participants had poor socioeconomic status.

Samples

Blood samples were obtained in the morning from the cubital vein after an overnight fast. Fasting blood samples were drawn into heparinised tubes and centrifuged at 3000 rpm for 10 min. to separate the plasma. The samples were stored at -80°C until analysis

Determination of Prolidase Activity

Prolidase activity was determined by a photometric method based on the measurement of proline levels produced by prolidase.^[18] Plasma samples (100 µL) were mixed with 100µL of physiological serum. 25 µL of the mixture was preincubated with 75 µL of the preincubation solution (50 mmol/L Tris HCl buffer pH 7.0 containing 1 mmol/L GSH, 50 mmol/L MnCl₂) at 37°C for 30 min. The reaction mixture containing 144 mmol/L gly-pro, pH 7.8 (100 µL) was incubated with 100 µL of preincubated sample at 37°C for 5 min. To stop the incubation reaction, 1 mL glacial acetic acid was added. After adding 300 µL Tris HCl buffer, pH 7.8 and 1 mL ninhydrine solution (3 g/dL ninhydrine was melted in 0.5 mol/ L ortophosphoric acid), the mixture was incubated at 90°C for 20 min and cooled with ice and subsequently their absorbance was measured at a wavelength of 515 nm for determination of proline by the method proposed by Myara.^[19]

Determination of the Total Antioxidant Capacity

Total antioxidant capacity (TAC) of plasma was determined by using an automated measurement method.^[20] In this method, hydroxyl radical, which is the most potent biological radical, is produced. In the assay, ferrous ion solution, which is present in Reagent 1, is mixed with hydrogen peroxide, which is present in Reagent 2. The sequentially produced radicals such as brown colored dianisidiny radical cation, produced by the hydroxyl radical, are also potent radicals. The oxidation reactions progress among dianisidyl radicals and further oxidation reactions develop. The color formation is increased with further oxidation reactions. Antioxidants in the sample suppress the oxidation reactions and color formation. Using this method, the antioxidative effect of the sample against the potent free radical reactions, which is initiated by the produced hydroxyl radical, was measured. The results were expressed as mmol Trolox Equiv./L.

Measurement of Total Oxidant Status

Total oxidant status of plasma was determined by an automated measurement method developed by Erel.^[21] Oxidants present in the sample oxidise the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange

in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter ($\mu\text{mol H}_2\text{O}_2$ Equiv./L).

Oxidative Stress Index

Per cent ratio of TOS level to TAC level was accepted as the oxidative stress index (OSI). For calculation, the resulting unit of TAC was changed to mmol/l, and the OSI value was calculated according to the following Formula.^[22] OSI (Arbitrary Unit)=TOS ($\mu\text{mol H}_2\text{O}_2$ Equiv./L) / TAC (mmol Trolox Equiv. /L).

Statistical Analysis

The Statistical Package for Social Sciences (SPSS 11.5, SPSS Inc, Chicago, IL) was used for all statistical analyses. Parametric statistical methods were used to analyze the data. The student's t-tests were used for pair wise comparisons. Bivariate comparisons were examined using Pearson rank correlation coefficients (r) and values for corrected for ties. The 2-tailed significance values were used. A p value of 0.05 or less was considered to be significant.

RESULTS

The social and demographic data of the patients and their controls showed homogeneity, and there were no statistically significant differences between the two groups with regard to age, gender, and BMI ($p>0.05$) (Table 1).

In this study, plasma TOS levels and OSI value were significantly higher in the patients with cholelithiasis than those of the control subjects ($p<0.05$ and $p<0.001$, respectively). We found that there were significant differences between cholelithiasis patients and healthy controls with respect to prolidase activity. Plasma prolidase activity and TAC levels were significantly lower in the patients with cholelithiasis than those of the control subjects ($p<0.001$). The results were summarized in Table 2. There were significant negative correlations between oxidant (OSI) and antioxidant (TAC) parameters ($p<0.01$, $r=-0.61$). There was no significant correlation between plasma prolidase activity and OSI values ($p>0.05$) in the study groups. However, a positive correlation was observed between plasma prolidase activity and TAC levels ($p < 0.05$, $r=0.21$). The results were summarized in Table 3.

DISCUSSION

Gallstones can induce inflammation in the gallbladder wall, when the composition of bile changes, at the same time the bilirubin metabolism, which is a potent antioxidant by radical scavenging and reducing activities, may be altered as cited by Sipos et al.^[23] The changes in bile composition can increase the biliary free radical formation.^[24] Increased levels of inducible NO synthesis activity was shown in inflamed gallbladders,

Table 1. Demographic characteristics of the patients in the gallstone and control groups

Parameters	Patients	controls	p
Case	35	30	
Age (years)	41±15	44±12	ns
Sex (Female/ Male)	23/12	17/13	ns
Body mass index (kg/m ²)	24.5±3.1	23.5±1.6	ns

Values are mean±SD; ns=non significant

Table 2. Prolidase activity and oxidative/antioxidative parameters in the patients in the gallstone and control groups

	Patients (n=35)	controls (n=30)	p
TAC (mmol Trolox Eq./L)	0.91±0.14	1.12±0.16	$p<0.0001$
TOS ($\mu\text{mol H}_2\text{O}_2$ Equiv./L)	8.92±1.86	7.85±1.45	$p<0.05$
OSI (Arbitrary Unit)	9.99±2.79	7.03±1.35	$p<0.0001$
Prolidase (U/L)	258.97±60.81	435.59±101.63	$p<0.0001$

Values are mean±SD;

TAC: Total antioxidant capacity, TOS: Total oxidant status, OSI: Oxidative stress index

Table 3. Correlations of prolidase activity with TAC and OSI levels, and TAC levels with OSI levels in the patient group

	TAC	OSI
Prolidase		
r=	0.21	-0.17
p	<0.01	>0.05
TAC		
r=		-0.61
p<		<0.01

which has an effect on elevation of oxidative stress and on fluid transport as well.^[25] Moreover, inflammatory changes of the gallbladder mucosa are associated with granulocyte infiltration; the activated phagocytes can produce reactive oxygen metabolites, and thus oxidative stress. One of the consequences of this kind of inflammatory disease is dysregulation in metabolism of collagen and its interaction with cell surface integrin receptors. Although extracellular metalloproteinases initiate the breakdown of collagen in tissues, the final step of its degradation is mediated by prolidase.^[1-4]

Oxidative stress activates a mechanism leading to synthesis of proinflammatory cytokines and cell adhesion molecules. Therefore, oxidative stress may contribute to an inflammatory response induced by cholelithiasis. In this study, we chose to measure oxidative stress with OSI, which was detected by using both oxidative and antioxidative parameters. We found that OSI significantly increased in cholelithiasis. Since the antioxidative

effects of antioxidant components of plasma are additive, the measurement of TAC reflects the antioxidative status of plasma. Free radicals and other peroxides derivatives produced physiologically in the human body and increased in many pathological conditions, diffuse into the blood.^[23] Here, antioxidant components of plasma overwhelm them and they are consumed. TAC, therefore, is detected as being significantly lower in the cholelithiasis patients than in the controls. We investigated the oxidative status of plasma by measuring TOS levels which were considerably high in the cholelithiasis patients. This is the first study evaluating plasma TAC and TOS levels together and implying a new parameter OSI in cholelithiasis.

There are conflicting reports concerning prolidase activity. Some studies indicated that prolidase activity decreased in several diseases, such as chronic uremia and type 2 diabetes mellitus.^[15-17] In chronic liver diseases, increased prolidase activity has been reported. This increase is especially seen in the early stage of fibrosis.^[14] Several *in vitro* studies have reported the degradation of cartilaginous tissue slices by ROS-generating systems. The damage was supposed to be secondary to direct attack of proteoglycan and collagen molecules by free radicals. Free radicals degrade collagen and prevent formation of fibrils by this collagen. As stated earlier, prolidase plays the main role in this mechanism. In the study, we showed that there was a significant decrease in plasma prolidase activity in patients with cholelithiasis, which may be interpreted as evidence of decreased collagen re-synthesis.

One of the main factors for gallstone formation is gallbladder hypomotility. The role of plasma prolidase deficiency in gallbladder hypomotility is not known at present. However, there may be two important points to note. First, cholelithiasis probably decreases prolidase activity by leading to a chronic inflammation of gallbladder which causes dysregulation in collagen metabolism. Second, decreased prolidase activity may occur due to increased TOS, together with OSI and decreased TAC levels in cholelithiasis patients. To our knowledge, there is no study in the literature reporting prolidase activity and/or collagen metabolism in cholelithiasis. In our cases, the cause of gallstone is not very clear and should be investigated. It is known that gallstones cause changes on the bladder wall. Decreased prolidase activity may cause a decline in collagen turnover. Changes in collagen turnover stages may be one of the reasons for the persistence in chronic cholecystitis due to gallstone. Gallstone consists of the majority of gallbladder diseases. It also plays a role in the formation of other gallbladder diseases. Further, the role of chronic inflammation due to gallstone is prominent in gallbladder cancers.^[26]

Many studies on this topic have been concentrated on the effects of gallstone on the gallbladder wall and on bile composition in the gallbladder. Many antioxidants

and oxidants were studied to determine these effects. In particular, vitamin C, which is a strong external antioxidant^[27] and is very important for the maintenance of collagen synthesis, since it is involved in hydroxylation of the amino acid proline in collagen, may be very effective in the relation of prolidase activity and oxidative stress. ROS can be considered as the regulatory factor of cartilage homeostasis. Therefore, decreased prolidase activity may reflect low collagen and proteoglycan re-synthesis as a result of oxidative stress. However, we could not find a correlation between oxidative stress parameters and prolidase activity. There is a positive correlation between plasma TAC and prolidase activity. It may be assumed that, in the case of oxidative stress, not only are plasma antioxidants affected but also collagen turnover is affected adversely.

In this study, none of the patients had any symptoms related to gallbladder within the last 6 weeks. Increased TOS, particularly OSI evaluating total oxidant-antioxidant status together, and decreased TAC, despite the symptom-free 6 weeks, were quite important. Since they indicate the presence and continuation of oxidative stress in the symptom-free period, they may provide evidence for the progression of inflammation and possible some other pathological alterations in the gallbladder. In addition, it is possible to argue that these inflammatory changes are not localized to the gallbladder and have some systemic effects, since these parameters have been measured in plasma.

In the light of these findings, we may conclude that the patients with gallstone are exposed to a potent oxidative stress, and increased oxidative stress may play a role in the progression of inflammatory changes in the gallbladder. Decreased prolidase activity reflects decreased collagen turnover, which may be one of the causes of hypomotility in the gallbladder. However, more comprehensive work should be undertaken to clarify this. On the other hand, it is possible to say that supplementation of antioxidant-enriched diet to the therapy might shed light on the development of novel therapeutic strategies for cholelithiasis.

Conflict of Interest

No declared.

REFERENCES

1. Weltman DI, Zeman RK. Acute diseases of the gallbladder and biliary ducts. *Radiol Clin North Am* 1994;32:933-50.
2. Baig SJ, Biswas S, Das S, Basu K, Chattopadhyay G. Histopathological changes in gallbladder mucosa in cholelithiasis: Correlation with chemical composition of gallstones. *Trop Gastroenterol* 2002;23:25-7.
3. Seki S, Kitada T, Yamada T, Sakaguchi H, Nakatani K, Onoda N, et al. Immunohistochemical detection of 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage, in human chronic cholecystitis. *Histopathology* 2002;40:531-5.

4. Jünger C, Sreejayan, Eder M. I, von Stillfried N, Zündt B, Spelsberg F. W, Kullak-Ublick G. A, Jünger D, von Ritter C. Lipid peroxidation and mucin secretagogue activity in bile of gallstone patients. *European Journal of Clinical Investigation* 2007;37:731-6.
5. Geetha A. Evidence for oxidative stress in the gall bladder mucosa of gallstone patients. *J Biochem Mol Biol Biophys.* 2002;6:427-32.
6. Matsui A, Ikeda T, Enomoto K, Hosoda K, Nakashima H, Omae K, et al. Increased formation of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine, in human breast cancer tissue and its relationship to GSTP1 and COMT genotypes. *Cancer Lett.* 2000;151:87-95.
7. Galli F, Piroddi M, Annetti C, Aisa C, Floridi E, Floridi A. Oxidative stress and reactive oxygen species. *Contrib Nephrol* 2005;149:240-60.
8. Aslan M, Nazligul Y, Horoz M, Bolukbas C, Bolukbas FF, Aksoy N, et al. Serum prolidase activity and oxidative status in *Helicobacter pylori* infection. *Clin Biochem.* 2007;40:37-40.
9. Kavalcı C, Durukan P, İlhan N, Güzel A, Ömürlü Kİ. Kafa içi yaralanmanın tanısında serum MDA'nın yeri. *Trakya Univ Tıp Fak Derg.* 2008;25:209-13.
10. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *Lancet* 1994;344:721-4.
11. Aruoma OI Characterization of drugs as antioxidant prophylactics. *Free Radic Biol Med* 1996;20:675-705.
12. Sontakke AN, Tare RS. A duality in the roles of reactive species with respect to bone metabolism. *Clin Chim Acta* 2002;318:145-14.
13. Palka JA, Phang JM. Prolidase activity in fibroblasts is regulated by interaction of extracellular matrix with cell surface integrin receptors. *J Cell Biochem* 1997;67:166-75.
14. Myara I, Myara A, Mangeot M, Charpentier C, Lemonnier A. Plasma prolidase activity: a possible index of collagen catabolism in chronic liver disease. *Clin Chem* 1984;30:211-5.
15. Erbagci AB, Araz M, Erbagci A, Tarakcioglu M, Namiduru ES. Serum prolidase activity as a marker of osteoporosis in type 2 diabetes mellitus. *Clin Biochem* 2002;35:263-8.
16. Altındag O, Erel O, Aksoy N, Selek S, Celik H, Karaoglanoglu M. Increased oxidative stress and its relation with collagen metabolism in knee osteoarthritis. *Rheumatol Int* 2007;27:339-44.
17. Gejyo F, Kishore BK, Arakawa M. Prolidase and prolidase activities in the erythrocytes of patients with chronic uremia. *Nephron* 1983;35:58-61.
18. Ozcan O, Gultepe M, Ipcioglu OM, Bolat B, Kayadibi H. Optimization of the Photometric Enzyme Activity Assay For Evaluating Real Activity of Prolidase. *Turk J Biochem* 2007; 32:12-6.
19. Myara I, Cosson C, Moatti N, Lemonnier A. Human kidney prolidase--purification, preincubation properties and immunological reactivity. *Int J Biochem.* 1994;26:207-14.
20. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin Biochem* 2004;37: 112-9.
21. Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem* 2005;38:1103-11.
22. Bolukbas C, Bolukbas FF, Horoz M, Aslan M, Celik H, Erel O. Increased oxidative stress associated with the severity of the liver disease in various forms of hepatitis B virus infection. *BMC Infect Dis* 2005;5:95.
23. Sipos P, Krisztina H, Blázovics A, Fehér J. Cholecystitis, gallstones and free radical reactions in human gallbladder. *Med Sci Monit.* 2001;7:84-8.
24. Chamulitrat W, Carnal J, Reed NM, Spitzer JJ. In vivo endotoxin enhances biliary ethanol-dependent free radical generation. *Am J Physiol.* 1998;274: 653-61.
25. Nilsson B, Delbro D, Hedin L, Conradi N, Thune A, Friman S, et al. Role of nitric oxide induction of inflammatory fluid secretion by the mucosa of the feline gallbladder. *Gastroenterology.* 1996;110: 598-606.
26. Schottenfeld D, Beebe-Dimmer J. Chronic inflammation: a common and important factor in the pathogenesis of neoplasia. *CA Cancer J Clin.* 2006;56:69-83.
27. Kundak AA, Erbas H, Gülen S, Dökmeci G, Celik H, Özcan T. C ve E vitaminlerinin, kronik olarak alkolle beslenen sıçanlarda beyin dokusu arjinaz aktivitesi, ornitin ve üre düzeylerine etkileri. *Trakya Univ Tıp Fak Derg.* 2007;24:49-55.