IBBJ

Spring 2023, Vol 9, No 2

Short communication

Antioxidant Levels in the Saliva of Patients with Cirrhosis Compared with Patients Post-Transplantation

Suzan Cangul[1*], Begum Erpacal[1], Ozkan Adiguzel[2], Ibrahim Kaplan[3], Musa Acarturk[1], Omer Satici[4]

1. Department of Restorative Dentistry, Faculty of Dentistry, Dicle University, Diyarbakir,Turkey.

2. Department of Endodontics, Faculty of Dentistry, Dicle University, Diyarbakir, Turkey.

3. Department of Biochemistry, Faculty of Medicine, Dicle University, Diyarbakir, Turkey.

4. Department of Biostatistics, Faculty of Medicine, Dicle University, Diyarbakir, Turkey.

Submitted 25 Apr 2020; Accepted 27 Sep 2022; Published 3 Jul 2023

The aim of this study was to compare antioxidant levels of saliva in patients with cirrhosis and liver transplantation. This study was carried out on 45 subjects referred to the Gastroenterohepatology Clinic of Dicle University Medical Faculty. These subjects were composed of cirrhosis, transplanted patients, and control group with no liver problems. The saliva samples were taken in the morning hours after a fasting period, and were then stored at –80 ˚C until assay, when they were tested for total antioxidant capacity. Photometric analysis was performed with Erel's method. The statistical evaluation of the data was made using the Kruskal Wallis and Mann-Whitney tests. Total antioxidant level in saliva was significantly lower in patients with cirrhosis ($P < 0.05$). The post-transplant and control groups were statistically similar in terms of total antioxidant level. Our data suggest that dental caries and increased periodontal problems in cirrhosis may be related to the change in total antioxidant level in saliva. However, further studies are needed to clarify this issue.

Keywords: Liver disease, liver transplantation, oral health, saliva

Liver cirrhosis is a disease characterized by loss of parenchyma tissue, increased connective tissue, the formation of regeneration nodules, and impaired vascular structure as a result of the long-term clinical course of chronic liver diseases (1). This disease may become irreversible over time, but if treated in the early stage, it can result in regression of fibrosis. In contrast, at advanced stages of the disease, it enters an irreversible process, and liver transplantation is accepted as the only treatment option (2).

Several factors play a role in the etiology of cirrhosis, primarily viral hepatitis (B, C, Delta), and alcohol consumption. Other causes include biliary diseases, inherited metabolic diseases, drugs and toxins, heart failure, venous obstruction, cryptogenic factors, and obesity. In etiological evaluations in Turkey, viral hepatitis has been determined as the primary cause, whereas it has been reported to be hepatitis B infection in Asia and Africa, and alcohol in the USA (3). Although cirrhosis of the liver receded from the 10th to 12th cause of death according to the Centre for Disease Control data of 1998 and 2004, it remains a significant cause of mortality in many countries worldwide. As a result of the failure of metabolic functions of the liver, the disease manifests with several signs and symptoms such as fatigue, listlessness, oedema, anxiety, depression, itching and muscle cramps, and the mortality rate is significantly increased (4).

Listlessness and fatigue in particular increase throughout the day in the patient. As the disease

* Department of Restorative Dentistry, Faculty of Dentistry, Dicle University, Diyarbakir,Turkey. E-mail: suzanbali@outlook.com

progresses, additional symptoms of weakness start to emerge. The most important clinical findings of the disease in addition to these symptoms are hepatocellular failure and portal hypertension. In respect of treatment prognosis and duration, it is of greatest importance that symptoms of the disease are known.

Liver cirrhosis is classified as compensated or decompensated according to the clinical stage. The disease has an insidious course not showing symptoms in the short term (5). In the first stage of the disease, which is known as the compensated period, damage occurs with fibrosis and the formation of nodules in the normal structure, and portal hypertension develops. With the progression of portal hypertension, the disease enters the decompensated period. When the hepatic vein pressure gradient exceeds 12 mmHg, the disease becomes a systemic disease involving other organs (6). Important findings that differentiate these two clinical stages include acid, jaundice, and hepatic encephalitis. When any of these findings are present, the patient is evaluated as having entered the decompensated period.

With recent developments in treatment approaches to cirrhosis, it has been seen that portal hypertension and liver failure have not developed in patients. Several studies have even shown that liver fibrosis has regressed with modern treatment methods. In particular, the control of inflammation, which has been shown to be one of the most significant causes of mortality, together with distancing from alcohol and toxic agents such as iron, and suppression of autoimmunity, regression of fibrosis can be obtained.

When the disease has entered the decompensated period and the patient does not respond to treatment, organ transplantation is performed (7). As attention to bacterial infections demands the greatest importance in organ transplantation candidates, all agents that can cause infections must be removed from the environment. Primary among these are dental infections. Previous studies have shown that dental caries and periodontal diseases are seen more in these patients (8).

The most important etiological factor of periodontal diseases is microbial dental plaque. Systemic, environmental, and genetic factors determine the severity of the periodontal disease. There are many factors that cause tissue destruction other than bacteria. The deterioration of homeostatic balance between proteolytic enzymes and inhibitors, and reactive oxygen species and antioxidant system is one of the possible degradation mechanisms (9). Therefore, antioxidant defense systems of saliva have begun to gain importance in recent years (10).

The damage caused by free radicals is repaired by antioxidants, which have the properties to prevent the formation of free radicals and reactive oxygen species, and prevent the oxidisation of lipids, proteins, and nucleic acids. The most important antioxidants are superoxide dismutase, catalse, and glutathione peroxidase (11). As it is difficult to measure the efficacy of each antioxidant separately, it is recommended to measure the total antioxidant capacity (12). Total antioxidant capacity is formed with the neutralisation of oxidants in a liter of solvent (13).

It is a matter of uncertainty whether the increase of dental caries and periodontal problems during decompensated cirrhosis is related to changes in antioxidant levels. Serum, plasma, saliva, and gingival gutter fluid are used to examine the antioxidant level in terms of periodontal aspect (14).

The aim of this study was to compare the effect of saliva antioxidant levels in the evaluation of oral health problems of patients with cirrhosis and liver transplantation.

Materials and Methods

The study included a total of 45 subjects of both genders referred to the Gastroenterohepatology Clinic of Dicle University Medical Faculty Hospital. Study groups were formed of patients with compensated and decompensated cirrhosis, and those who had undergone transplantation, and a

control group was formed of subjects with no liver problems. None of the patients included in the study smoked cigarettes or drank alcohol. The selection criteria for the control group included no systemic disease, no regular use of any medication, and for female participants that they were not pregnant or lactating. Ethics Committee Approval has received for this study from the Ethics Committee of Dicle University, Faculty of Dentistry (protocol no: 2019/7). Written informed consent was obtained from the patients who participated in this study.

Preparation of the saliva samples

The saliva samples were taken in the morning hours after a fasting period. The patients were instructed to rinse their mouth with water, then in an upright sitting position, to bend the head forward slightly and to spit into the given tubes, once a minute, 5 times. The patients were instructed not to move the tongue and lips while doing this. The saliva samples were then stored at -80 ˚C until assay, when they were sent to the biochemistry laboratory for total antioxidant capacity calculation.

Measurement of total antioxidant capacity

The saliva samples were centrifuged at 4000 rpm for 10 min and the supernatant fraction was taken. Total antioxidant capacity levels were measured by commercial kits (Relassay, Turkey) using photometric method by Erel's method.

Statistical analysis

The statistical evaluation of the data was made using the Kruskal Wallis and Mann-Whitney tests. P < 0.05 was considered significant.

Results

A statistically significant difference was determined between the cirrhosis patients, the transplantation patients and the control group in respect of total antioxidant capacity values (Table 1).

According to the Mann Whitney test, statistically significant differences were determined between the transplantation and cirrhosis groups (Z = -2.781, P = 0.005), and between the control and cirrhosis groups (Z = -2.158, P = 0.03).

The antioxidant levels of the cirrhosis patients were determined to be statistically significantly lower than those of the other groups.

The total antioxidant levels of the control group and the patients who had undergone transplantation were found to be statistically similar (Table 2). The total antioxidant levels of the groups are shown in Figure 1.

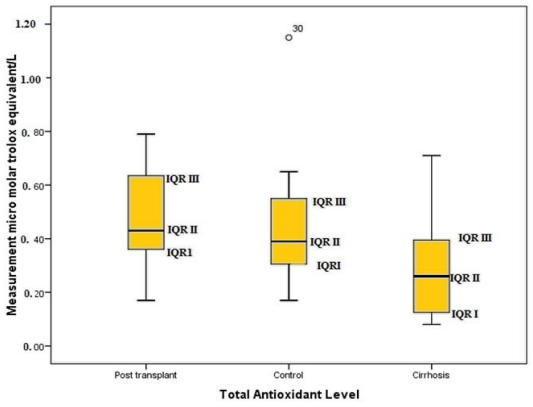

Figure 1. Total antioxidant levels of the groups. The antioxidant levels of the cirrhosis patients were significantly lower than those of the other groups (P < 0.05). IQRI: first interquartile range; IQRII: second interquartile range; IQRIII: third interquartile range.

Table 1. The mean total antioxidant capacity levels of the groups

Groups	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Post-transplant	15	0.4827	0.18418	0.04755	0.3807	0.5847
Control	15	0.4580	0.24364	0.06291	0.3231	0.5929
Cirrhosis	15	0.2747	0.17237	0.04451	0.1792	0.3701
Total	45	0.4051	0.21895	0.03264	0.3393	0.4709

Kruskal-Wallis Chi Square: 8.727; P = 0.01 significant.

Table 2. Statistically significant differences between the groups

Tukey Ba

Total Antioxidant Capacity	N	Subset for alpha = 0.05	
		1	2
Cirrhosis	15	0.2747	
Control	15		0.4580
Post-transplant	15		0.4827

Means for groups in homogeneous subsets are displayed. a: uses harmonic mean sample size = 15.

Discussion

Liver cirrhosis is considered to be one of the most important causes of death in the world and in Turkey. The etiology varies according to socioeconomic and cultural characteristics. Hepatitis and alcohol are among the most important factors that have been proven (15). Alcohol consumption leads to the induction of oxidative stress in liver and extra-liver tissues. Some enzyme systems are activated in chronic alcohol consumption. This increases the production of free radicals. The free oxygen radicals formed in the body are removed by the antioxidant defense system. The deterioration of this balance leads to a continuous increase in free oxygen radicals and to the formation of cell damage called oxidative stress.

Symptoms may be different in the clinical stages of cirrhosis. Oral manifestations of decompound cirrhosis, an advanced stage of liver dysfunction, consist of gingival bleeding, hematoma, glossitis, petechia, and jaundice mucosa (16). Since the immune system of cirrhosis patients is suppressed, their susceptibility to infections is high. It is of great importance in these patients yo consider infections caused by teeth (17). Systemic inflammation increases in dental infections that can cause bacteria and toxins to pass into the blood. These bacteria, which can be easily neutralized in healthy individuals, are difficult to clear due to liver dysfunction in cirrhosis patients (18). These problems have a negative effect on organ transplantation which may be performed for the treatment of liver cirrhosis. Therefore, the removal of dental infections is very important for the process of treatment. A number of drugs such as diuretics used in the treatment of cirrhosis reduce the flow of saliva, and lead to an increase in caries and gum problems (19).

Saliva is a body fluid that is critical in the protection of oral health and in the diagnosis of pathological conditions. In addition, due to its advantages such as being a non-invasive method and not requiring special equipment, it provides great convenience in evaluating large masses (20). Especially, the antioxidant systems in saliva are used in the diagnosis of changes in the mouth findings of diseases. Many researchers conducted studies on this subject. Maciejczyk et al. (21) evaluated oxidative stress indicators in stimulated and unstimulated saliva in children with chronic kidney disease and healthy controls, and found that total antioxidant level in stimulated saliva was significantly higher. In another study it was observed that peroxidase and uric acid from saliva antioxidants increased immediately after exercise in smokers and non-smokers (22).

Kavita et al. (23) reported statistically significant differences between autistic children and control groups. The antioxidant levels in the saliva of children in the control group were significantly higher than in the autistic individuals. Pendyala et al. (24) in a study evaluating the total antioxidant capacity of diabetes patients with and without periodontal disease, found that the antioxidant capacity of saliva was lowest in diabetic patients with periodontitis. Shetty et al. (25) investigated the antioxidant level of saliva and serum in women with and without periodontal disease, and observed significant differences between saliva antioxidants.

Antioxidants are responsible for preventing the formation of free radicals and limiting the damage they may cause (26). Free radicals are normally

produced continuously, but in liver diseases, this production rate increases. Under normal conditions, the body is protected against reactive oxygen metabolites and their toxic products. The liver is the main organ attacked by reactive oxygen species (27). Excessive reactive oxygen species cause hemostasis to deteriorate, leading to oxidative stress in liver diseases. Oxidative stress is one of the pathological mechanisms involved in liver diseases such as chronic viral hepatitis and alcoholic liver diseases (28), and causes damage to the parenchymal cells in the liver. The main factors that cause oxidative stress in the liver are alcohol, drugs, environmental pollution, radiation, and temperature. Chronic alcohol use, especially in alcoholic liver disease, is one of the predisposing factors affecting dental caries and periodontal tissues (29).

Oxidative damage caused by free oxygen radicals plays a role in the pathology of many diseases. Recent studies on the antioxidant capacity of saliva indicate that some diseases may affect the saliva antioxidants. In particular, the imbalance between free radicals and antioxidants can cause significant changes in physiological and metabolic functions, which may result in some inflammatory oral pathologies. Therefore, many studies have been carried out to investigate the total antioxidant capacity of saliva.

The determination of saliva antioxidant levels in individuals with systemic disease is expected to be an alternative technique for early diagnosis (22,30). The present study considered the cumulative effect of antioxidants, and investigated the total antioxidant capacity of saliva in individuals with cirrhosis and liver transplantation in comparison with healthy individuals. The antioxidant capacity of saliva increased in healthy individuals and after organ transplantation, and this level decreased in compensatory and decompensated cirrhosis process.

Decrease in the antioxidant level in the cirrhosis period of the disease, after the transplantation and in healthy individuals, was associated with increased oxidative stress at the stage of disease. Changes in the oral manifestations of the disease may be related to the decrease in the total antioxidant capacity in the saliva. Because periodontal tissue destruction is caused by deterioration in the harmony between reactive oxygen species and antioxidant system.

In conclusion, our data suggest that dental caries and increased periodontal problems in cirrhosis may be related to the change in total antioxidant level in saliva. The level of saliva antioxidant in patients who underwent transplantation was at the level of individuals without liver disease, and liver transplantation was beneficial not only for liver disease but also oral health problems. However, further studies are needed to clarify this issue.

Conflict of interest

The authors declared no conflict of interest.

References

1. Lackner C and Tiniakos D. Fibrosis and alcohol-related liver disease. J Hepatol. 2019;70:294-304.

2. Siciliano M, Parlati L, Maldarelli F, et al. Liver transplantation in adults: Choosing the appropriate timing. World J Gastrointest Pharmacol Ther. 2012;3:49-61.

3. Ayubi E, Safiri S, Sani M, et al. Heavy daily alcohol intake at the population level predicts the weight of alcohol in cirrhosis burden worldwide: Methodological issues of confounding and prediction models. J Hepatol. 2017;66:864-5.

4. Kim S H, Oh E G, Lee W H. Symptom experience, psychological distress, and quality of life in Korean patients with liver cirrhosis: a cross-sectional survey. Int J Nurs Stud. 2006;43:1047-56.

5. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. J Hepatol. 2018; 69:406-60.

6. Pinzani M, Rosselli M, Zuckermann M. Liver cirrhosis. Best Pract Res Clin Gastroenterol. 2011;25:281-90.

7. Hytiroglou P S D, Alves V, Et Al. Beyond "Cirrhosis": A Proposal from the International Liver Pathology Study Group. Am J Clin Pathol. 2012;137:5-9.

8. Anand A C, Pardal P K, Sachdev V P. Dental caries and periodontal disorders in chronic liver disease. Medical journal Armed Forces India. 2001;57:26-30.

9. Chapple I L C, Brock G, Eftimiadi C, et al. Glutathione in gingival crevicular fluid and its relation to local antioxidant capacity in periodontal health and disease. Mol pathol. 2002;55:367-73.

10. Nagler R M, Klein I, Zarzhevsky N, et al. Characterization of the differentiated antioxidant profile of human saliva. Free Radic Biol Med. 2002;32:268-77.

11. Chen X and Scholl T O. Oxidative stress: changes in pregnancy and with gestational diabetes mellitus. Curr Diab Rep. 2005;5:282-8.

12. Hosseini-Zijoud S M, Ebadi S A, Goodarzi M T, et al. Lipid Peroxidation and Antioxidant Status in Patients with Medullary Thyroid Carcinoma: A Case-Control Study. J Clin Diagn Res. 2016;10:Bc04-7.

13. Niki E. Assessment of antioxidant capacity in vitro and in vivo. Free Radic Biol Med. 2010;49:503-15.

14. Chapple I L C, Milward M R, Dietrich T. The Prevalence of Inflammatory Periodontitis Is Negatively Associated with Serum Antioxidant Concentrations. J Nutr. 2007;137:657-64.

15. Møller S, Henriksen JH. Cirrhotic cardiomyopathy. J Hepatol. 2010;53:179-90.

16. Golla K, Epstein J B, Cabay R J. Liver disease: current perspectives on medical and dental management. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004;98:516-21.

17. Hung T-H, Hsieh Y-H, Tseng K-C, et al. The risk for bacterial endocarditis in cirrhotic patients: a population-based 3-year follow-up study. Int J Infect Dis. 2013;17:e391-e3.

18. Aberg F, Helenius-Hietala J, Meurman J, et al. Association between dental infections and the clinical course of chronic liver disease. Hepatol Res. 2014;44:349-53.

19. Guggenheimer J, Eghtesad B, Close J M, et al. Dental health status of liver transplant candidates. Liver Transpl. 2007; 13:280-6.

20. Kaufman E and Lamster I B. The diagnostic applications of saliva--a review. Crit Rev Oral Biol Med. 2002;13:197-212.

21. Maciejczyk M, Szulimowska J, Skutnik A, et al. Salivary Biomarkers of Oxidative Stress in Children with Chronic Kidney Disease. J Clin Med. 2018;7:209.

22. Arazi H, Simaei E, Taati B. Comparison of responses of salivary antioxidant markers to exhaustive aerobic exercise in smoker and non-smoker young girls. J Sports Med Phys Fitness. 2016;56:1132-8.

23. Rai K, Hegde A M, Jose N. Salivary antioxidants and oral health in children with autism. Arch Oral Biol. 2012;57:1116-20.

24. Pendyala G, Thomas B, Joshi S R. Evaluation of Total Antioxidant Capacity of Saliva in Type 2 Diabetic Patients with and without Periodontal Disease: A Case-Control Study. N Am J Med Sci. 2013;5:51-7.

25. Shetty M S, Ramesh A, Shetty P K, et al. Salivary and Serum Antioxidants in Women with Preeclampsia with or Without Periodontal Disease. J Obstet Gynaecol India. 2018;68:33-8.

26. Wojtunik-Kulesza K A, Oniszczuk A, Oniszczuk T, et al. The influence of common free radicals and antioxidants on development of Alzheimer's Disease. Biomed Pharmacother. 2016;78:39-49.

27. Sánchez-Valle V, Chávez-Tapia N C, Uribe M, et al. Role of oxidative stress and molecular changes in liver fibrosis: a review. Curr Med Chem. 2012;19:4850-60.

28. Feng Y, Wang N, Ye X, et al. Hepatoprotective effect and its possible mechanism of Coptidis rhizoma aqueous extract on carbon tetrachloride-induced chronic liver hepatotoxicity in rats. J Ethnopharmacol. 2011;138:683-90.

29. Singal A K, Jampana S C, Weinman S A. Antioxidants as therapeutic agents for liver disease. Liver Int. 2011;31:1432-48.

30. Zamani-Ahari U, Zamani-Ahari S, Fardi-Azar Z, et al. Comparison of Total Antioxidant Capacity of Saliva in Women with Gestational diabetes mellitus and Non-diabetic Pregnant Women. J Clin Exp Dent. 2017;9:e1282-e6.