Cholinesterase Activity in Serum and Saliva of Patients with Inflammatory Bowel Disease

Original Article

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ABSTRACT

Background:
Inflammatory bowel disease (IBD) is a chronic relapsing inflammatory disorder of the gastrointestinal tract that affects millions of people worldwide. Since acetylcholine is one of the effective factors in reducing inflammation and considering the benefits of using saliva, in this study, the amount of cholinesterase activity in saliva and serum in patients with IBD and healthy people was investigated.

Materials and Methods:
Thirty patients with IBD who were referred to Imam Reza Hospital, as well as 30 healthy individuals, participated in this study. Saliva and serum samples were collected in the morning. Cholinesterase activity was evaluated using the photometric method, and data were analyzed using an unpaired Student's t-test or Mann-Whitney test.

Results:
The mean activity of cholinesterase and saliva flow rate in saliva and serum were not significantly different between the two groups. Xerostomia inventory score was significantly higher in the IBD group ($P<0.05$).

Conclusion:
It seems that cholinesterase activity does not change in patients with IBD, but patients feel more dry mouth than healthy people.

Keywords: Cholinesterase; inflammatory bowel disease; Saliva; Serum

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic relapsing inflammatory condition of the gastrointestinal tract that affects more than 6 million people worldwide (1). Although IBD can occur at any age, it most often occurs in early adulthood, and its incidence is increasing at all ages (2). IBD mainly includes ulcerative colitis (UC) and Crohn's disease (CD) (3). Although the etiology of these diseases has not been completely known, they may be caused by the interaction of several factors, including immune response, environmental factors, genetic factors, and intestinal microbiota (2,4).

Symptoms of IBD vary depending on the state and severity of inflammation and include bleeding ulcers, diarrhea, anemia, stomach pain, cramping, and weight loss, which may cause a debilitating condition with social and economic impacts (5). Differential diagnosis of IBD is based on history, endoscopy, and histopathological evaluation of inflamed tissue (6). Endoscopy is not without risks; it is costly and uncomfortable for patients, and there is a possibility of damage to the digestive system. Therefore, a relatively simple, accurate, and readily available test that reflects intestinal disease activity would be beneficial to patients and physicians (7).

Salivary compounds originate from the salivary glands and possibly from other oral tissues (8) and are also a biological liquid that is useful for novel approaches to prognosis, laboratory or clinical diagnosis, monitoring, and management of patients with both oral and systemic diseases (9). The potential use of saliva as a diagnostic fluid for certain diseases has been widely studied by researchers. However, there are few studies on saliva in IBD. Studies have shown that inflammation stimulates the vagus nerve to release acetylcholine (Ach) from its endings (10). Acetylcholine can inhibit the production of proinflammatory cytokines and suppress inflammation (11). There have been reports of decreased cholinesterase activity in various inflammatory diseases (12-14). It has also been shown that serum cholinesterase concentration decreased in patients with IBD (10).

Saliva, compared with blood sampling, is easily collected in a non-invasive and stress-free manner and does not carry the risk of infection spread (15). Unfortunately, it is still not commonly used for diagnostic purposes like other biological samples (such as blood plasma or urine). Evaluation of biomarkers in saliva can be considered in the rapid diagnosis of various diseases (16-19).

This study investigated the relationship of cholinesterase activity in serum and saliva samples of patients with IBD to answer whether this enzyme can be altered in IBD.

MATERIALS AND METHODS

The protocol of this study was approved by the Ethics Committee of AJA University of Medical Sciences (IR.AJUMS.REC.1402.130), and informed written consent was obtained from all subjects.

In this cross-sectional study, 30 patients with IBD who were referred to the gastroenterology clinic of Imam Reza Hospital in Tehran, Iran, in 2023 were selected. The diagnosis was based on clinical signs, endoscopic examination, and histopathology based on standard criteria. Also, 30 healthy subjects who were referred to a hospital for annual checkups participated in this study. Exclusion criteria were diabetes, fever, recent cold, cough, Sjogren's syndrome, symptoms of acute disease, and periodontal disease.

Participants were forbidden from eating and drinking for one hour before sampling; then they were asked to pour whole unstimulated saliva samples into sterile 10 mL polypropylene falcon tubes. Then, laboratory experts took 5 ml of blood from the antecubital vein and poured it into a clot tube. Blood and saliva samples were centrifuged at 4000 rpm for 5 minutes, then serum and supernatant of saliva were carefully transferred into a microtube and stored at −70°C. Cholinesterase activity was measured by a photometric method using an affiliated kit (Biorex, Shiraz, Iran) according to manufacturer guidelines. The saliva flow rate was calculated by dividing the saliva volume by the time of saliva collection.

Also, all participants completed questionnaires to determine their dry mouth status (20). The answers to each question included never (score 1), hardly (2), sometimes (3), fairly often (4) and often (5). Then, the overall score of the dry mouth questionnaire was calculated as a measure to evaluate the intensity of the feeling of dry mouth (xerostomia) for each person. The maximum possible score is 55, and the minimum is 11.

The data were analyzed using an unpaired Student's t test or Mann-Whitney test using SPSS software version 22.

RESULTS

In this present study, 30 patients with IBD (17 men, 13 women) with an average age of 37.7±2.3 years and 30 healthy subjects (16 men, 14 women) with an average age of 32.5±2.2 years participated in the study.

Our data showed that there were no significant differences between IBD and control groups in mean serum and saliva cholinesterase activity ($P>0.05$, Table 1). There was also no significant difference in the salivary flow rate between patients with IBD compared with the control group. However, the xerostomia inventory score was significantly higher in the IBD group than in the healthy group (Table 1).

Table 1. Serum and saliva cholinesterase activity (CEA), flow rate, and xerostomia inventory score in patients with IBD

Variable	Control	IBD	P value
Serum CEA(U/L)	4957±636	5361±630	0.68 9 [a]
Saliva CEA(U/L)	362±12	377±20	0.592 [a]
Salivary flow(mL/min)	0.65±0.06	0.62±0.07	0.33[a]
xerostomia inventory score	15.7±0.67	24.7±1	0.04[b]

Data are expressed as a mean ±s.e.m analyzed by student t test, b median ±inter quartile range (IQR), analyzed by Mann-Whitney test. IBD: inflammatory bowel disease

DISCUSSION

IBD is a severe chronic inflammatory disease of the gastrointestinal tract and is divided into CD and UC, the incidence of which is increasing worldwide, and it strongly affects the patients' quality of life (3,21). The present study examined serum and saliva cholinesterase activity in patients with IBD and healthy volunteers. Our data showed no significant difference in cholinesterase enzyme activity in serum and unstimulated saliva between the group of patients and healthy people.

A study showed a significant decrease in serum cholinesterase concentration in patients with IBD, which contradicts the results of our study. The reason for this mismatch could be that we examined the activity of the cholinesterase while Shao and colleagues measured its concentration (10). Another reason for this difference is that the study included patients in the active and remission phases of IBD, but our patients were all in the remission phase.

It has been presented that the cholinesterase activity is significantly reduced in some inflammatory diseases such as multiple sclerosis (13), Parkinson's disease (22), irritable bowel syndrome (23), gastroesophageal reflux disease (19), IBD (10), hepatitis (24), cervical cancer (25), and stroke (26) that all are in disagreement with our study. However, A study conducted on the saliva of patients with Alzheimer's showed no significant difference in the acetylcholinesterase enzyme activity between the control group and the patients. Since one of the proposed theories for the cause of this disease is inflammation, it can be concluded that the results of that study are in agreement with our study (27,28).

Our data presented that patients with IBD had dry mouth feeling compared with the control group, which is in agreement with the results of Goldinova and others (29), Tan cx and colleagues (30), and Oltulu and co-workers (31). Since in most diseases, patients suffer from stress and stress can cause dry mouth (32), stress is probably the cause of xerostomia in these patients.

The major reason for this study is the ease and non-invasiveness of saliva sampling, which allows for a safe, inexpensive, easy, effortless, and regular sampling of patients. On the other hand, saliva is believed to reflect the entire body and will be a diagnostic fluid in the future; thus, it is included in the health maintenance program for the diagnosis, follow-up, and screening of diseases (13). Because of these important features, finding biomarkers in saliva to diagnose serious systemic diseases has been the focus of researchers (20).

CONCLUSIONS

It seems that cholinesterase activity does not change in patients with IBD, but patients feel more dry mouth than healthy people.

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