CHANGES IN SALIVARY BIOCHEMISTRY ASSOCIATED WITH HPYLORI POSITIVITY IN PATIENTS WITH CHRONIC GASTRITIS

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Abstract:

Objective: The majority of the time, invasive endoscopic biopsies are employed as the current gold standard for diagnosing and tracking chronic gastritis. However, there is an increasing awareness of the need for patient-friendly, non-invasive diagnostic methods that are also economically viable. Along with playing a crucial protective function in the digestive system, saliva is also showing promise as a non-invasive diagnostic tool. This study intends to discover and characterize particular biochemical indicators in the saliva of patients with chronic gastritis, including both Helicobacter pylori-positive (HP+) and Helicobacter pylori-negative (HP-) persons, and compare these results with those of healthy individuals. With the eventual objective of using these indicators as a diagnostic tool, the research will also look into the association between the levels of these biochemical markers in both serum and saliva.

Methods: The cross-sectional study design was used in this research investigation, which was carried out at the Mayo Hospital in Lahore. The objective was to evaluate the disease activity in 60 patients with chronic gastritis (44 with Helicobacter pylori positive and 16 with Helicobacter pylori-negative) using subjective symptoms, serological data, and endoscopic results. The patients' ages varied from 12.08 to 58.73 on average. The individuals in the control group, who were healthy and did not smoke, were on average 56.86 8.67 years old. Examining serum and saliva samples that had not been stimulated was part of the study. Additionally, several components including albumin, total protein, uric acid, and secretory immunoglobulin A (sIg A) were examined in stool samples. Using an ELISA reader designed for oral fluid processes and a biochemical analyzer (Olympus AU 640), the effectiveness of the tests was assessed.

Results: When compared to the control group, the patients who got HP+ therapy had substantially greater levels of sIgA (secretory immunoglobulin A) and TP (total protein). The levels of UA (uric acid) didn't follow a similar trend, however. Our results revealed that only UA had a link with both saliva and blood levels. Intriguingly, endoscopic investigations showed an inverse correlation between UA levels

and the presence of inflammation. These alterations are thought to represent a defense mechanism against oxidative stress, irregularities in salivary flow, and inflammation that developed in the stomach.
Conclusions: Salivary characteristics are significantly altered in those with chronic gastritis brought on by Helicobacter pylori (HP+) infection. Even though saliva has significant limits, it may be a useful tool for detecting pathological alterations in the digestive tract, especially when an HP+ infection is present.
Keywords: saliva, uric acid, gastric albumin
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Introduction:
Continuous gastritis is an irritation of the lining of the stomach that persists for a long time and has no known cause. It is characterized by a reduction in the incretory, motor, and secretory activities. Both acute and chronic forms of gastritis are possible. Acute gastritis is less frequent than chronic gastritis. Numerous variables, including dietary practices, anxiety, the taking of various medicines, poor oral hygiene, contamination with H. pylori, smoking, and drinking in excess, may contribute to the widespread occurrence of this ailment in today's modern urban surroundings. The situation has reached the status of a serious public health catastrophe due to the ongoing demand for medical care. Diabetes, coronary artery disease, and ischemic illnesses are a few conditions that have been connected to infection with H. pylori but are unrelated to digestion (1). The main diagnosis method for chronic gastritis often involves invasive endoscopic examinations. Because medical treatments may be dreadful and painful, patients sometimes overstate the seriousness of their signs or medical issues (2). This mucosa's additional role is to stop hydrochloric acid from penetrating the digestive system further. The inside and outside of the mouth communicate constantly with each other, and the digestive system as a whole may be reached directly from the mouth's interior. Since saliva is the digestive system's initial point of contact with food, liquids, microbes, substances, and volatile substances before the stomach is involved, it serves as the first line of defense. Saliva is the first fluid to come into contact with food, liquids, microbes, chemicals, and volatiles. Saliva is essential to digestion, much as other body fluids. Some studies (3,4) suggest that disturbances of oral homeostasis may be the basis of many systemic diseases.

Figure 1: Hpylori Positivity

Saliva is a viscous fluid in terms of consistency. The remaining one percent of this complicated system (4,5,6) is composed of a large range of low-molecular-weight molecules. These compounds include, for instance, growth factors, hormones, antibodies, and antimicrobials. Some are produced by salivary glands, whereas others are brought from circulation through propagation, rapid movement, and ultrafiltration to purify Ultrafiltration is an additional method. Saliva may provide information about a person's emotional, endocrine, and hormonal health (5). Saliva production occurs in the mouth and throat.

The majority of the many distinct species of bacteria found in the mouth help to maintain dental homeostasis or the mouth's general condition of health. The different components of saliva may affect oral microbes directly or indirectly (7). Numerous physiological triggers, as well as oral and systemic disease states, have been identified to cause changes in salivary output and composition. There have been reports of these alterations. Screening of oral fluid (OT), which offers the chance to prevent painful and occasionally harmful operations like endoscopy and biopsy, is becoming increasingly crucial when related to the monitoring of recurrence and remission. It is becoming more and more well-liked as a biological material because of its straightforward and non-invasive extraction process, as well as the patient-centered design of the procedure.

Methods:

Study Design: This cross-sectional research was carried out at the Mayo Hospital in Lahore between December 2022 and May 2023. A patient group of sixty people with chronic gastritis was included in the trial; their ages ranged from 30 to 78 on average. The Control Group (CG) was made up of 80 healthy nonsmokers who consented to yearly physicals. The CG's age ranged from 30 to 72 years, with a median age of 56.8 years. All participants gave their free and voluntary permission, and their general oral health was deemed to be excellent. The selection of patients was based on the presence of Helicobacter pylori (HP) infection, endoscopic evidence of chronic gastritis, and subjective or objective symptoms of disease activity. Recent surgery, a high risk of developing cancer, current mouth inflammation, or recent dental surgery were all exclusion factors.

C-reactive protein (CRP), a sign of inflammation, was tested in both groups in the lab. Following the correct pre-analytical protocols, venous blood was drawn from the patients, and centrifugation was used to separate the serum from the blood. A fecal qualitative HP antigen test was used to establish the existence of infection and to measure the levels of H. pylori antibodies (IgG) in the blood. To test albumin, total protein, uric acid, and immunoglobulin A (IgA), additional blood samples were taken.

Between 8 and 10 in the morning, saliva samples from participants were passively drained into sterile conical-bottomed containers. For the duration of the collection procedure, it was necessary to abstain from eating, drinking, chewing gum, or cleaning your teeth with toothpaste (including coffee and other caffeinated drinks). Five minutes before the test, mouthwash with saline or mineral water was used. Using the graduated scale that is incorporated into the container, the amount of saliva was measured. After centrifuging the obtained saliva samples, the supernatant was carefully transferred to micro containers for salivary parameter analysis. Before analysis, the samples were kept at -20 degrees Celsius.

A modified version of the oral fluid method was used in conjunction with Beckman Coulter kits to analyze salivary parameters such as UA, TP, and Alb. We assessed secretory IgA levels using Dia Metra kits. Sensitive methods were used for their analysis since saliva contains less protein and albumin than serum does. Pyrogallol red dye, which changes in spectral absorption upon binding to proteins, was used for salivary protein analysis. At 570 degrees, a spectrophotometric measurement was collected to determine the protein contents. Salivary albumin concentrations were 100–1,000 times lower than those seen in serum. The samples were calibrated using calibrators made up of the appropriate matrix and certified standard solutions, and the levels of total secretory IgA were determined using the ELISA method. The quantity of sIgA present in the sample was directly associated with the reaction's color intensity.

Saliva was defined as a viscous fluid made up of a variety of low-molecular-weight substances, including growth factors, hormones, enzymes, and antibodies.

Results:

Based on the findings of tests for Helicobacter pylori (HP) antibodies and antigens, the patients were split into two groups. (Table 1) Based on the existence and distribution of inflammatory alterations in the gastroduodenal mucosa, four grades (I, II, III, and IV) were given to the patients. (Table 2) Variations in saliva composition may be brought on by several variables, including age, health, food, and bad behaviors. In contrast to the HP- and control groups, patients in the HP+ group in our research showed greater amounts of secretory IgA (sIgA) in their saliva. IgA serum concentrations were likewise higher in the HP+ group. However, we were unable to detect a connection between salivary sIgA levels and serum IgA.

Saliva's elevated sIgA concentrations may be connected to their ability to decrease bacterial density and defend the stomach mucosa. It has been shown that IgA antibodies produced locally are beneficial in avoiding H. pylori infection. The HP- group had lower mean sIgA levels despite having gastritis type C and other co-morbidities. Lower sIgA concentrations may result from changes in salivary flow and production brought on by medications and certain medical conditions.

Table 1: Demographics of the study population

	Gender								
Age	**Female**			**Male**			**Total**		
	n	Mean	SD	n	Mean	SD	n	Mean	SD
HP -	5			11			16		
		58	12.23		63.55	10.88		63.77	10.05
HP +	18			26			44		
		58.2	12.77		55.2	11.86		56.35	12.14
Control Group	40			40			80		
		55.52	8.77		58.21	8.52		56.86	8.67
Total	23			37			60		
		57.95	12.31		57.94	12.13		57.93	12.08

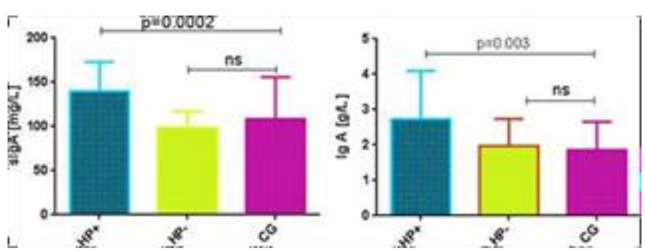

Figure 2: The Siga Content of Three Groups' Saliva

Children have been reported to be more susceptible to HP infection and to have lesser levels of local defense mechanisms. Our research may have been of higher quality if saliva and stomach juice from infected people and healthy kids had been compared. Understanding the significance of saliva and oral health in immune-inflammatory pathways and gastrointestinal alterations should be the main goal of future studies.

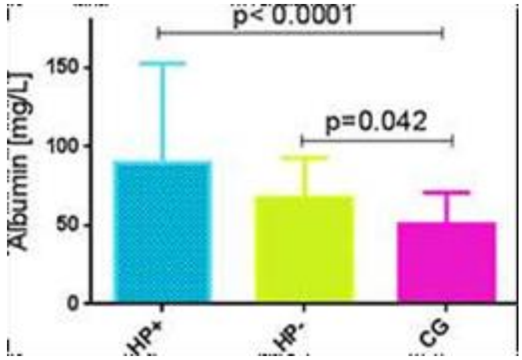

Figure 3: Albumin Levels, Positive, Negative, and Control for HP

Table 2: Endoscopy Results Across The Board Showing Modifications in The Gastroduodenal Mucosa

Endoscopic Dx	Erythematous	Erosive	Atrophic
Regional	15	14	0
Diffuse	10	17	4

Table 3: Evaluation of Salivary Factors Between Herpes Simplex Virus (HP) Patients and Healthy Volunteers

Parameters		sIg A	Albumin	Total protein	Uric acid
HP(Negative)	Mean	97.06	68.03	787	220.9
	SD	19.12	24.94	238.1	58.45
HP(Positive)	Mean	140	89.89	892.9	209.9
	SD	32.97	63.02	345.9	55.95
Control Group	Mean	107.9	51.04	724.9	223.2
	SD	48.05	20.02	394.1	36.8
P		0.0001	0.0001	0.0428	0.4099

Table 4: Serum Measurements in the HP-, HP+, and Control Groups

Parameters		CRP	Ig A	Albumin	Total protein	Uric acid
HP(Negative)	Mean	5.68	1.95	46.34	71.98	337.03
	SD	9.28	0.76	4.18	4.29	82.1
HP(Positive)	Mean	19.14	2.7	44.11	69.98	375.15
	SD	15.06	1.23	5.92	6.99	92.91
Control Group	Mean	2.42	1.91	47.12	72.56	343.16
	SD	1.62	0.78	4.31	8.72	74.04
p		0.001	0.003	Insig	Insig	Insig

Hyperglycemia, dyslipidemia, and cardiac disorders are only a few of the systemic illnesses and health issues that are linked to H. pylori infection. Compared to HP-negative and control groups, patients with HP positivity had greater quantities of albumin, a protein that helps to regulate osmotic pressure, in their saliva. This shows that saliva-production-related proteins are being secreted. The presence of albumin is thought to signal mucositis or inflammation. Compared to the HP- and control groups, CRP levels were significantly higher in the HP+ group. However, there was no discernible relationship between CRP and the variables found in the serum and saliva.

Table 5: Unstimulated Patients' and Controls' Serum and Salivary Correlation

Parameters	UA	Alb	sIgA	TP
Serum R	0.01412	-0.06093	-0.1506	-0.02098
p	Insig	Insig	Insig	Insig
Saliva R	0.3408	0.1698	-0.04915	0.089664
p	0.0105	Insig	Insig	Insig

Table 6: It was discovered that the evaluated serum parameters and the inflammatory marker CRP correlated.

CRP vs	Alb	UA	IgA	TP
p	0.0982	0.8822	0.477	0.0712
r	-2198	-0.02204	0.1012	-0.2518

Table 7: It was discovered that the evaluated salivary parameters and the inflammatory marker CRP correlated.

CRP vs	sIG A	Alb	TP	UA
p	0.1412	0.0895	0.0144	0.9625
r	-0.2002	-0.2291	-0.3308	0.0076

Table 8: It has been shown that the salivary parameters that have been examined and the degree of endoscopic inflammatory changes correlate.

vs	sIG A	Alb. saliva	TP saliva	UA saliva
p	0.8892	0.9016	0.5671	0.0017
r	0.01792	-0.01831	-0.07778	-0.4195

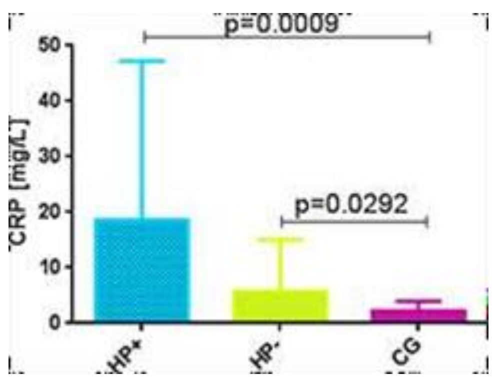

Figure 4: CRP Concentrations in Control, Positive, and Negative HP Serum

Antioxidant uric acid (UA) binds metal ions and displaces free radicals. The bulk of saliva's antioxidant capability is provided by UA. Heart disease, diabetes, metabolic syndrome, and malignant tumors have all been related to elevated levels of UA. However, our research did not uncover any significant variations in UA levels across the groups. The lowest UA levels were seen in the HP+ group, potentially as an adaptive response to oxidative stress in the gastrointestinal tract. The levels of UA in serum and saliva were somewhat correlated in the ill group but not in the control group.

Conclusion: In comparison to HP-negative and control groups, patients who tested positive for HP had greater amounts of sIgA and albumin in their saliva. The HP+ group had increased CRP values, which suggests inflammation. The groups' UA levels did not vary significantly from one another. These results imply that sIgA and albumin may have a role in the immunological response to H. pylori infection and gastrointestinal tract inflammation.

Discussions:

The stomach mucosa is predominantly impacted by the prevalent chronic bacterial illness known as Helicobacter pylori. This illness affects more than half of the world's population (8,9). Saliva is an appealing choice for epidemiological investigations since it has been discovered to be a non-invasive sample for identifying H. pylori. Salivary samples are simple to get and analyze; the procedure is quick, painless, and handy, and there is no chance of being hurt by a needle (10).

When compared to healthy controls, those with H. pylori infections had greater saliva pH and flow rates. Saliva without stimulation typically has an acidic pH between 5.75 and 7.05. With a higher flow rate, it can grow more acidic and perhaps reach a high pH. Salivary protein content, phosphate ions, and bicarbonate all serve as buffers to keep pH levels stable and affect salivary pH (11). Due to the H. pylori infection, acidic stomach contents that reflux into the esophageal lumen might disrupt the esophagogastric reflex, increasing salivary production and altering the composition of saliva. This may indicate that the stomach's acidity is having an impact on the saliva's flow rate (12). These results agree with those of an earlier investigation (13).

In comparison to the control group, those with H. pylori infections had somewhat lower amounts of total protein (TP) in their salivary samples, according to the current research. Salivary glands, mucosal cells, immune cells, blood, and microbes are the sources of salivary proteins. Salivary proteins are involved in immunological activation, non-immune elimination of germs (such as salivary amylase preventing microbial development), antimicrobial defense, and immune activation. The interference of salivary proteins with bacterial colonization, enamel demineralization-remineralization processes, tooth caries development, and plaque formation may be the cause of the decline in TP. Furthermore, since H. pylori may be present in the oral cavity, the decreased TP levels can be linked to antimicrobial defense mechanisms against bacterial colonization. The decline in TP levels may also be attributed to nutritional and immunological alterations that occurred throughout the disease's progression (14). This result conflicts with studies that found higher amounts of TP in saliva in people with peptic ulcers (15).

Common antacid treatments include sodium bicarbonate and calcium carbonate, which are both silicates and phosphates. Elevated calcium levels may result from hypercalcemia, worsened nausea, vomiting, lack of appetite, and constipation, all of which may be brought on by increased stomach acid. When correct oral hygiene procedures are not practiced, dental plaque builds up on tooth surfaces and is thought to be a reservoir for H. pylori colonization. Plaque may develop into calculus, which is covered with a biofilm made up of both organic and inorganic elements including glycoproteins and calcium. Higher amounts of calcium in the saliva and chronic periodontal disease are both caused by calculus development (16). These results agree with those of earlier investigations (17,18).

Research on salivary urea activity in people with H. pylori infection is scarce. However, the alkaline pH seen in the study group relative to the control group may be responsible for the slightly elevated salivary urea levels. Calcium phosphate deposition and plaque mineralization are encouraged by alkaline pH. The nitrogenous substance urea, which is found in saliva, functions as a buffer and may increase the pH of biofilm by releasing ammonia and carbon dioxide. The small rise in salivary urea levels in H. pylori patients may be explained by the need for alkaline pH conditions in plaque for the development of natural calculi. This increase may be linked to plaque buildup and calculus formation brought on by mineralized dental plaque rather than to H. pylori infection (19,20).

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Conclusions:

For the diagnosis of chronic gastritis, a disorder marked by inflammation and difficult symptoms, there are few unreliable laboratory tests available. Certain biomarkers may alter as a result of this inflammation. Over half of the world's population is affected by the most widespread illness, Helicobacter pylori (HP) infection. HP-related illnesses may be expensive to treat and may have a big effect on one's finances. We have discovered that saliva analysis, a non-invasive alternative biological sample, may provide important information. According to our study, individuals with chronic gastritis and HP infection have considerably altered salivary parameters. It is widely known that there is a link between poor dental health, the disturbance of the homeostasis of the mouth cavity, and the invasion of germs and inflammation in the gastrointestinal system. Although saliva is not the ideal biological matrix for diagnostics, there are still some crucial issues that need to be addressed, such as standardizing the process and improving evaluation procedures. Nevertheless, saliva analysis is a superb method for identifying and tracking digestive tract pathologies, especially when HP infection is present.

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