

Changes in Salivary Biochemistry Associated with Helicobacter Pylori Positivity in Patients with Chronic Gastritis

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ABSTRACT

Introduction: The invasive endoscopic-biopsy approach is the one that is used the vast majority of the time for diagnosing and monitoring chronic gastritis. Finding non-invasive laboratory indicators would be beneficial for the patient in terms of both cost and convenience. It is becoming more and more well-known that saliva, in addition to its vital protective role for the digestive system, may also be employed as an efficient non-invasive diagnostic material.

Aim: To detect and define biochemical characteristics in the saliva of chronic gastritis patients (both HP+ and HP-) and compare these values to those observed in healthy people, and search for correlations between the levels of these biochemical in the serum and in the saliva in order to ultimately use these biochemical as a diagnostic tool.

Method: The design of this study was a cross sectional study design and this study was conducted at Bahawal Victoria Hospital, Bahawalpur. Subjective symptoms, serological data, and endoscopic findings were used to identify the disease activity in a total of 60 patients with chronic gastritis (44HP+ and 16HP-) from this research. The average age of the patients was anything from 58.73 to 12.08. The average age of the control group participants was 56.868.67, and they were all in excellent health and not smokers. Analyses made use of unstimulated saliva and serum. Stool samples are tested for albumin, total protein, uric acid, and secretory immunoglobulin A. (sIg A). To evaluate their efficacy, we employ a biochemical analyzer (Olympus AU 640) and an ELISA reader (an adaptation of the processes for oral fluid).

Results: Compared to the control group, HP+ patients had considerably greater sIgA and TP levels. However, UA did not show a comparable trend. We found that only UA had a correlation with both saliva and serum levels. It was shown that there was an inverse relationship between UA and endoscopic markers of inflammation. As a defense mechanism against the oxidative stress, salivary flow rate abnormalities, and inflammation that had occurred in the stomach, these alterations are being hypothesized.

Practical implication: The significance of this study is to see whether specific biochemical parameters may be utilised as a diagnostic tool in the future by comparing the levels in the blood and those detected in the saliva of those patients having chronic gastritis (both HP+ and HP-)

Conclusion: In patients with HP+ chronic gastritis, the findings show that there are considerable changes in the characteristics of their saliva. Saliva is a biological substance that has certain limitations, but it is an excellent indicator of the pathological processes occurring in the digestive system, particularly in the case of HP+ infection.

Keywords: Uric Acid, Total Protein, Saliva, Sig A, And HP+ Chronic Gastritis Albumin

INTRODUCTION

An inflammation of the stomach mucosa that lasts for an extended period of time but has no identifiable cause is called chronic gastritis, and it is associated with decreased secretory, motor, and incretory processes. Chronic gastritis may be classified as either acute or chronic. Chronic gastritis is more common than acute gastritis. The broad prevalence of this condition in today's contemporary metropolitan environments may be attributed to a variety of factors, including dietary practices, stress, the use of many drugs, improper dental hygiene, infection with *H. pylori*, as well as smoking and excessive alcohol use. The regular need for medical attention elevates the issue to the level of a severe public health crisis. An infection with *H. pylori* has been linked to a number of disorders that are not related to digestion, including diabetes, cardiovascular disease, and ischemic diseases⁽¹⁾. Endoscopic procedures that are invasive are often used as the primary diagnostic tool for chronic gastritis. Patients sometimes exaggerate the severity of their symptoms and health problems since medical procedures may be unpleasant and induce dread⁽²⁾. Another function of this mucosa is to block hydrochloric acid from entering further into the digestive system. The mouth's interior and exterior are in constant dialogue with one another, and the mouth's interior is directly linked to the rest of the digestive system. Saliva is the digestive system's first line of defense since it comes into touch with food, liquids, germs, chemicals, and volatiles before the stomach does. The first fluid to come into touch with food, liquids, microorganisms, chemicals, and volatile is saliva. As with other bodily fluids, saliva is crucial to digestion. Numerous systemic disorders may have their origins in oral homeostasis abnormalities, according to some research^(3,4).

The consistency of saliva may be described as viscous fluid. There are a wide variety of low-molecular-weight compounds that make up the remaining 1% of this complex system^(4,5,6). Some examples of these molecules include enzymes, hormones, antibodies, antimicrobials, and growth factors. Salivary glands create some, whereas diffusion, active transport, and ultrafiltration bring others from the bloodstream. One other technique is ultrafiltration. A person's emotional, hormonal, and hormonal status may all be seen in their saliva⁽⁵⁾. The mouth and throat are responsible for producing saliva.

There are many different types of bacteria in the mouth, and the great majority of them are beneficial in maintaining oral homeostasis, or the state of health in the mouth. Saliva's various components may have both direct and indirect effects on oral microorganisms⁽⁷⁾. Salivary production and composition have been found to fluctuate in response to a wide range of physiological stimuli and oral and systemic pathological conditions. These changes have been reported. When it comes to the follow-up of relapse and remission, testing of oral fluid (OT) is becoming more significant since it provides the possibility to avoid uncomfortable and sometimes dangerous interventions like endoscopy and biopsy. Its extraction is simple and non-invasive, and the technique is designed with the patient in mind, all of which contribute to its growing popularity as a biological substance.

Significance: The significance of this study is to see whether specific biochemical parameters may be utilised as a diagnostic tool in the future by comparing the levels in the blood and those detected in the saliva of those patients having chronic gastritis (both HP+ and HP-).

MATERIAL AND METHOD

The design of this study was a cross sectional study design and this study was conducted at Bahawal Victoria Hospital, Bahawalpur. The patient group consisted of sixty individuals who were diagnosed with chronic gastritis and were between the ages of 30 and 78 years old on average. Eighty healthy nonsmokers who agree to have annual exams make up the Control Group (CG). 56.86.8 years was the median age ranging from 30 to 72 years. All study participants gave their informed permission and were found to have good dental health in general assessments. Subjective or objective signs of disease activity; endoscopic evidence of chronic gastritis; and the presence of HP infection were used to select patients for the research. For instance, a patient cannot participate if they have a history of recent surgery or if they have a condition that has a high risk of developing cancer as a result. Patients with active oral inflammation or who had recent dental surgery (within 48-72 hours) were not eligible to take part in this study. In the lab, we looked at both groups' levels of CRP, which is a sign of inflammation. The patient's venous blood is drawn in line with the preanalytical procedure. Centrifuging the blood at 3,500 RPM for 10 minutes was enough to separate the serum. After determining the levels of H. pylori antibodies (IgG) in the blood, we use a faecal qualitative HP antigen test to confirm the infection. Immunoglobulin A (IgA), Uric acid (UA), total protein (TP) and albumin (Alb) may all be measured in a blood sample (IgA). Between the hours of 8 and 10 a.m., saliva is passively and repeatedly drained from the mouth into specific graded sterile conical-bottomed containers. The serum parameters are measured using routine, standardized laboratory methods and are analyzed using an Olympus AU 640 biochemical analyzer. Within that time frame, we were able to extract 2-3 ml. Patients were asked to comply with the following requirements so that the findings could be relied upon: It has been more than thirty minutes since the last time you ate, drank (including coffee and other caffeinated beverages), chewed gum, or brushed your teeth with toothpaste and a toothbrush. Rinsing the mouth with saline or mineral water twice for ten seconds each and doing so five minutes before the test was required.

The material is processed very fast, usually between 30 minutes to an hour. The saliva is hypotonic, and it also contains good bacteria, both of which contribute to the lytic reactions that many different biomolecules undergo. We measure the quantity of saliva that has been collected using the container's built-in graduated scale. The biological samples are centrifuged for ten minutes at a speed of 3000 revolutions per minute. The aliquots of the supernatant were pipetted with extreme care and placed in microcontainers of the Eppendorf type. They are kept at a temperature of -20 degrees Celsius until the salivary parameters are analyzed. Beckman Coulter kits (Olympus AU 640) are used in conjunction with a modified version of the oral fluid technique in order to determine UA, TP, and Alb. Dia Metra kits were used in order to measure the level of secretory IgA.

Because the levels of protein and albumin in saliva are so much lower than those in serum, it is necessary to use procedures that are sensitive in order to analyse them. We use a dye called pyrogallol red for the salivary protein analysis, which, when it binds to proteins, causes a change in the dye's spectrum absorption. A spectrophotometric reading was taken at 570 degrees. This approach is sensitive in the range of low protein concentrations, which is less than 3.0 g/L. The amounts of albumin in saliva are between one hundred and one thousand times lower than those in serum. For the purpose of calibration, we have developed a series of calibrators consisting of an appropriate matrix (Artificial saliva for medical and dental research) and the addition of certified standard solutions (TP 1.0 g/dL, Alb 0.2 g/dL, and UA 8 mg/dL). These calibrators have been added to a series of artificial saliva samples. By using immunosorbent assay, we were able to quantify the total SigA levels in each of the samples (ELISA). As the substrate for the enzyme-linked immune reaction, hydro peroxide (H2O2) and TMB were employed. This caused the reaction to produce a blue color,

but when the Stop solution was added, the color changed to yellow. The amount of sigA present in the sample has a direct correlation with the degree of color intensity.

The consistency of saliva may be described as viscous fluid. There are a wide variety of low-molecular-weight compounds that make up the remaining 1% of this complex system (5,6). Some examples of these molecules include enzymes, hormones, antibodies, antimicrobials, and growth factors.

RESULTS

Patients are divided into two groups: those who have tested positive for Helicobacter pylori antibodies and antigen, and those who have not (Table 1).

Table 1: Analysis of Research Groups' Demographics

Gender HP(mean+SD)	Male (N)	Female (N)	Total (N)
HP +	26	18	44
Age	55.20±11.86	58.20±12.77	56.35±12.14
HP Negative	11	5	16
Age	63.55±10.88	58.0±12.23	63.77±10.05
Total patients group	37	23	60
Age	57.94±12.13	57.95±12.31	57.93±12.08
Control group	40	40	80
Age	58.21±8.52	55.52±8.77	56.86±8.67

Patients are given one of four grades (Table-2) based on whether or not endoscopic features of inflammatory alterations are present and how they are distributed topographically in the gastroduodenal mucosa. These grades are I, II, III, and IV.

Table 2: Changes in The Gastroduodenal Mucosa As Shown On Endoscopy Across The Board

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

Tables 3 and 4 provide the results of studies conducted on the parameters of saliva and serum, respectively.

Table 3: Comparison of Salivary Parameters Between Patients with And Without Herpes Simplex Virus (HP) And Healthy Volunteers (Controls).

Parameters	HP(+)	HP(-)	CG (Control Group)	P
Uric acid [umol/L]	209.9±55.95	220.9±58.45	223.2±36.8	0.4099
Total protein [mg/L]	892.9±345.9	787.0±238.1	724.9±394.1	0.0428
Albumin [mg/L]	89.89±63.02	68.03±24.94	51.04±20.02	<0.0001
sig A [g/L]	140.0±32.97	97.06±19.12	107.9±48.05	<0.0001

Table 4: Serum Parameters In HP+, HP-, And Control Groups

Parameters	HP(+)	HP(-)	CG (Control Group)	P-Value
Uric acid [umol/L]	375.15±92.91	337.03±82.10	343.16±74.04	Insignificant
Total protein [mg/L]	69.98±6.99	71.98±4.29	72.56±8.72	Insignificant
Albumin [mg/L]	44.11±5.92	46.34±4.18	47.12±4.31	Insignificant
Ig A [g/L]	2.70±1.23	1.95±0.76	1.91±0.78	0.003
CRP [mg/L]	19.14±15.06	5.68±9.28	2.42±1.62	0.001

Varying variables, including age, health, diet, and unhealthy behaviors (smoking, drug use, and excessive alcohol consumption), may cause the saliva's constituents to range widely within relatively narrow limits (8,9). Even if the host immune response is activated and IgA antibodies are produced, the reaction is ineffective. Persistent and persistent^(10,11) infection persists.

The major kind of antibody that is responsible for the particular immunological protection of mucosal surfaces is known as secretory IgA, abbreviated as S-IgA. IgA exists in two distinct structural forms: IgA1 (which accounts for 90% of the total) and IgA2 (which accounts

for 10%). IgA1 may be found in serum and is the end result of the production of B cells that takes place in the bone marrow. The production of IgA2 by mucosal B cells is an indicator of an efficient mucosal immune response. Mucosal B cells are situated in the mucosa. Approximately sixty percent of an adult's IgA1 is found in their saliva ^(12,13).

In the course of our research, we discovered that the HP + patient group had considerably higher mean sIgA levels in comparison to the HP and CG groups (Table 3). In a similar vein, increased serum concentrations of IgA were shown to be present (Table 4, Figure 1A, B). We conducted an investigation to see whether or not there was a significant association between serum (Ig A) levels and salivary (sIgA), but we discovered none (Table 5). It is possible that the function that sIgA plays in lowering the bacterial density and protecting the gastric mucosa is the reason for increased amounts of sIgA in saliva. Antibodies that are made locally, namely IgA, have been demonstrated in research to be effective in preventing H. pylori infection. ⁽¹⁴⁾ sIgA inhibits bacterial colonization as well as bacterial adherence and improves bacterial opsonization ⁽¹⁰⁾. [Citation needed] Infection with HP most likely triggers a variety of the host's innate oral immune defense systems, which work together to fight off the pathogenic microbe. Even in people who are otherwise healthy, the amount of IgA that is secreted has been shown to directly rise with advancing age ⁽¹⁵⁾. Medication (gastritis type C) or other co-morbidities are linked to ischemic alterations in the GET, and so create the HP- group. Although this subset is smaller than either the HP+ or CG subsets, they had a higher mean age and lower mean sIgA levels. Medications such as beta-blockers, diuretics, antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs), and antiarrhythmic might influence salivary flow and production, which may explicate the minor concentrations. A more precise evaluation of salivary immunoglobulin A synthesis requires an expansion of the HP-group.

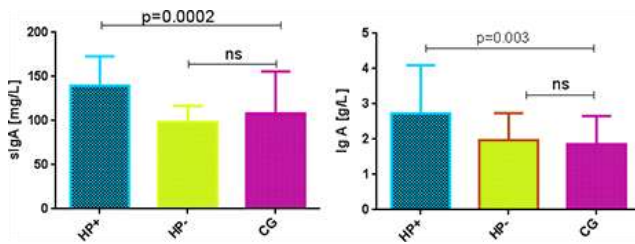


Figure 1: The Amount of Siga in The Saliva of Three Groups. 1B. Serum IGA in Groups With HP, Without HP, And as A Control

Table 5: Correlation Between Unstimulated Saliva and Serum of Patients and Controls

Parameters Saliva/Serum	Saliva R	P-Value	Serum R	P-Value
TP	0.089664	insignificant	-0.02098	insignificant
sIgA	-0.04915	insignificant	-0.1506	insignificant
Alb	0.1698	insignificant	-0.06093	insignificant
UA	0.3408	0.0105	0.01412	insignificant

Necil Kutukculer and his colleagues ⁽¹⁶⁾ thought that a lack of local defence systems in children and a lower level of resistance to HP infection go hand in hand based on their research. Saliva and stomach juice from infected individuals and healthy children with HP infection show very similar quantities of sIgA and SC component. Because of this oversight, the quality of our study isn't as high as it might have been. Researchers will have a better understanding of the role that saliva and dental homeostasis play in immune-inflammatory pathways and gastrointestinal lining alterations ⁽¹⁷⁾.

H. pylori has been linked to all of these health problems. If bacteria grow in dental plaque and saliva, it could cause an infection or re-infection of the stomach ⁽¹⁷⁾. It has also been shown that H. pylori infection is linked to a number of systemic diseases, such as hyperglycemia, dyslipidemia, and a number of heart conditions ⁽¹⁸⁻¹⁹⁻²⁰⁾.

Albumin serves as a transporter for substances that are not easily soluble in water (such as lipids, hormones, and unconjugated bilirubin), and it also keeps colloid-osmotic pressure stable. It accounts for more than half of all the proteins found in plasma ⁽²¹⁻²²⁾. Nutritional status, hormonal equilibrium, and osmotic pressure are the three determinants that influence albumin production ⁽²¹⁾. Albumin is a protein that serves as an antioxidant and is only present in very small amounts in whole saliva and serum from all of the patients and from the control group that had not been activated.

The values of serum TP levels were compared, and there was found to be no statistically significant difference between them. This rise in salivary protein most likely indicates that local glands are secreting proteins that are involved in saliva. It is referred to as serum ultrafiltrate and It has potential applications of performing an overall assessment of the function of the oral mucosa. Albumin is often regarded as a trustworthy indicator of mucositis or inflammation ^(23, 24). In order to rule out the possibility of active local inflammation, a thorough dental examination was carried out on each patient prior to their inclusion in the control group. Groups vary in age from 30 to 78 years, and it is only normal for the tooth status to alter throughout the course of one's lifetime ⁽²⁵⁾. The number of teeth is decreased, however, bridges and partial dentures may make up the difference. Several different researches point to an increase in the amount of albumin found in saliva as people become older. Because of the increased permeability of the basement membrane, there was also a rise in the use of radiation therapy, diabetic treatment, and immunosuppressive. Significantly greater albumin levels were seen in the HP-positive subset of patients compared to both the HP-negative and control groups (Figures 2,3).

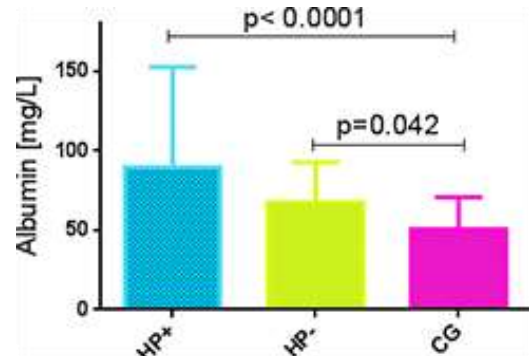


Figure 2: HP Positive, Negative and Control Albumin Levels

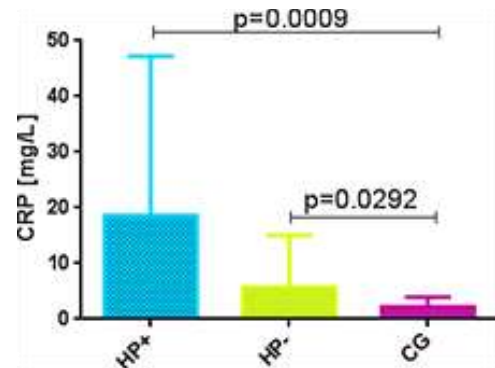


Figure 3: CRP Levels In HP-Positive, HP-Negative, And Control Serum

When compared to the HP- group and the control group, the CRP levels of the HP+ group were significantly higher in statistical analyses. An analogous relationship holds between HP- and SG. Reactivity among HP+ individuals varies widely, with values ranging from 1.6% to 112% of the normal range. Although the acute-phase protein is not sensitive nor selective for chronic gastritis, this is nonetheless the case. We looked for correlations between CRP and the parameters we detected in saliva and serum. Although there

was a weak negative link between serum and albumin, there was a substantial negative correlation between total salivary protein and serum. We also found a marginally negative correlation between albumin in serum and cell density. and When inflammation is present, it alters the liver's ability to synthesize and distribute proteins. Albumin is a protein that is created during the "reverse-phase" phase. There was probably an alteration in the filtration of albumin via the capillaries of the salivary gland, inflammation, and oxidative stress, and this led to higher albumin levels in the saliva ⁽²⁵⁾. See (Tables 6,7).

Table 6: The Inflammatory Marker CRP Was Shown to Have a Correlation With The Serum Parameters That Were Evaluated.

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

Table 7: The Inflammatory Marker CRP Was Shown to Have A Correlation With The Salivary Parameters That Were Evaluated.

	r	p-value
CRP vs. UA	0.007603	0.9625
CRP vs. TP	-0.3308	0.0144
CRP vs. Alb.	-0.2291	0.0895
CRP vs. sIG A	-0.2002	0.1412

Uric acid functions as an AOC, or antioxidant, since it doesn't need enzymes. It neutralizes free radicals and binds metal ions ⁽²⁶⁾. Reductase of xanthine oxide speeds up the oxidation of xanthine and hypoxanthine, producing this byproduct ⁽²⁷⁾. Most of the AOC found in saliva comes from UA, which accounts for around 70%-80% of the total. Heart disease ⁽²⁸⁾, diabetes ⁽²⁹⁾, metabolic syndrome ⁽³⁰⁾, and malignant tumors ⁽³¹⁾ are all associated with elevated levels. ^(28,29,30) The pro-oxidant and pro-inflammatory effects of UA might be to blame for this. ⁽³¹⁾ We thank Lyngdoh T and his colleagues' research shows that UA makes mononuclear cells make more of the inflammatory cytokines that cause inflammation. TNF-, IL-1b, and IL-6 are all types of cytokines. In our research, we could not find any statistically significant differences between the groups that were studied, despite the fact that the HP+ patient group had the lowest results overall. It is possible that, as a kind of adaptive mechanism, its elevation might be detected in order to deal with the oxidative stress that is happening in the GET. One such possibility is that the length of the sickness, the geography, and the inflammatory activity were all different. The majority of our patients have recurring symptoms that have been present for an average of 2.9 years (range 1-10 years). Particularly in individuals suffering from gout, several writers have discovered a strong link between the amounts of uric acid detected in the saliva and the serum. They advise using UA levels as a means of monitoring its therapy ⁽²⁾, based on the findings they just presented. Researchers Fawaz Pullishery and colleagues looked for evidence of a similar reliance in their work, but they came up empty ⁽³³⁾. In the sick group, our findings indicated that there was a moderate association between UA saliva and serum, but in the control group, we did not find any reliance of this kind.

Patients with chronic H. pylori infection exhibited higher than normal levels of uric acid in their blood, as reported by Ndebi ME et al. Our HP+ patient cohort had higher UA levels as well, however this difference did not reach statistical significance (4). Among the variables studied, the correlation between uric acid and endoscopically detected inflammatory alterations in the gastroduodenal mucosa was the greatest. It seems that there is a negative association (r=-0.4203, p=0.0016), however it is only marginally significant. We attribute this to oxidative stress, as well as the initiation of an imbalance due to a sustained decrease in AOC and damage to the stomach lining (Table 8).

Table 8: The Degree of Endoscopic Inflammatory Alterations Has Been Shown to Correlate with The Salivary Parameters That Have Been Investigated.

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

DISCUSSION

Infection by Helicobacter pylori stays the most prevalent chronic bacterial disease and mainly colonizes the mucosa of gastric, more than half of the world population are affecting by this disease ⁽⁹⁾. Various studies have reported that saliva is a non-invasive sample for detection and attractive option for epidemiologic studies because it has been analyzed and obtained easily, collection and testing salivary specimens is fast, painless, convenient, and carries no risk of needle stick injury ⁽¹⁰⁾. The salivary PH and flow rate in study were higher in study group than normal values in healthy control group, the pH value of unstimulated saliva is acidic which ranges between (5.75 - 7.05), it becomes more when the flow rate was increased and may reach a PH at high flow rate, in addition to the flow rate, the pH also depends on the salivary proteins concentration, phosphate (PO43-) ions and bicarbonate (HCO3-) that have considerable buffering capacity for maintaining the PH level in saliva ⁽¹¹⁾. The esophago-salivary reflex may be affected by the acidic gastric content that refluxing into esophageal lumen which causes damage to esophageal mucosa, all these changes lead to stimulates salivary secretion and changes the concentration of some of saliva constituents. The salivary secretion stimulation is relay on PH, the intra-gastric pH is usually (1 – 2) in patients with H. pylori, thus their salivary secretion and composition could be partly under esophago - salivary reflex control ⁽¹²⁾. Thus, the increase in flow rate of saliva and PH in H. pylori patients may perform a sign that the acidity in stomach has ability to effect on flow rate of saliva. Data of this result is in agreement with study ⁽¹³⁾. The result of the present study illustrated the levels of total protein (TP) was observed to be slightly lowered in study group in compared to control group. The concentration of salivary protein is not change and self-reliant from the salivary flow rate, about (30-40 %) of salivary proteins are performed by salivary glands, while other proteins are arisen from mucosal, immune cells, blood and /or from microorganisms ⁽¹⁴⁾. The salivary protein has antimicrobial defense, part of defense are implicated mainly in activation of immunity like salivary immunoglobulin's ⁽¹⁵⁾, while others protein are responsible for non-immune elimination of microbes like salivary amylase by inhibitory effect on microorganism growth ⁽¹⁶⁾. It is believed that the infection with gastric H. pylori mainly occurs at the same time when the dental plaque pathogen was founded "when the pathogenic strains are shared in mucosa of human stomach and dental plaque" ⁽¹⁷⁾. However, the association between gastric symptoms and existence of H. pylori in the oral cavity is not obvious. Many study found the positive correlation of oral samples and gastric biopsies for Helicobacter pylori were statistically significant, so the data of this results indicated the patients with positive H. pylori were also with positive results in dental plaque ^(2, 4, 18). So the lowered level of TP may explained by the fact that the salivary proteins interfere with bacterial colonization and these proteins effect on the process of enamel demineralization-rem mineralization and dental caries formation as well as plaque formation ⁽¹⁹⁾ and because of various research finding the oral cavity is H. pylori reservoir especially with periodontal disease so the lower level of TP is related to antimicrobial defense mechanism against bacterial colonization, another explanation about the decreased level of TP may be result from the nutritional and immunological changes that occur during the disease course, this result is disagreement with studies by ^(20, 21) who found the salivary TP was increased in patients with peptic ulcer. The sodium bicarbonate and calcium carbonate are common components with silicates and phosphates of antacid preparations, also the hypercalcemia is produced with increased stomach acid as

well as the intensify nausea, vomiting, loss of appetite and constipation may result from the dehydration can cause calcium level to rise⁽²³⁾. Many of previous studies that examined a dental plaque in mouth as a carrier for *H. pylori* carriage have proposed that the plaque is the first place for accumulation of microorganisms that embedded in an intracellular matrix which consist of inorganic components like calcium in addition to other minerals and organic components like glycoprotein's⁽²⁴⁾, usually the dental plaque adheres to supra-gingival and sub-gingival tooth surfaces when the good oral hygiene measures is absent, it will form quickly and by the time it will advances into calculus that is superficially coated by the biofilm plaque which progress to chronic periodontal disease and causes higher level of salivary calcium due to the calculus formation. This fact may be related to the high level of calcium in subjects with *H. pylori* infection. This result is in agreement with other studies^(25, 26). There is no study was performed on saliva to measure urea activity in patients with *H. pylori*, but the slightly increased in salivary urea level may be related to the alkaline PH which increased in study group in compared to control group. Wong et al.,⁽²⁷⁾ found the importance of alkaline PH for deposition of calcium phosphate and plaque mineralization, the urea is a nitrogenous products present in saliva and considers a buffer present in oral fluid that causes a rapid increased in PH of biofilm by releasing carbon dioxide and ammonia⁽²⁸⁾. When the PH was alkaline the deposition of calcium phosphate is high due to the variable pH conditions in plaque which considers an essential factor in natural calculus formation, so the possible explanation for slightly increased in salivary urea in *H. pylori* patients not related to the infection with *H. pylori* bacteria, but it may be related to plaque deposition and calculus formation that result from mineralized dental plaque⁽²⁷⁾.

CONCLUSION

There are few and not very accurate laboratory tests that can be used to diagnose chronic gastritis. Inflammation, clinical symptoms, and a challenging course may produce alterations in some biomarkers. In humans, Infection with *Helicobacter pylori* (HP) is the most prevalent disease worldwide. More over half of the world's population has it. High morbidity and mortality from HP-related diseases, as well as the expenditures of treating such illnesses, may put a significant dent in your savings. Saliva is a non-invasive alternative biological material that may be readily extracted. We discovered that salivary parameters were significantly altered in patients with HP + chronic gastritis. The invasion of microorganisms and inflammation of the GET are intimately linked to oral illnesses and the disruption of homeostasis in the oral cavity. As a biological matrix, saliva isn't perfect. The procedure's standardization and the methodologies employed to assess its indications are both open questions that have yet to be addressed. However, it is an excellent diagnostic and monitoring tool since it accurately portrays the pathological processes occurring in the digestive system, particularly in the presence of HP + infection.

REFERENCES

- Chung GE, Heo NJ, Park MJ, Chung SJ, Kang HY, et al. (2013) Helicobacter pylori seropositivity in diabetic patients is associated with microalbuminuria. *World J Gastroenterol* 19: 97-102.
- Corfield AP (2015) Mucins: a biologically relevant glycan barrier in mucosal protection. *Biochim Biophys Acta* 1850: 236-252.
- Edgar M, Dawes C, O'Mullane D (2012) Saliva and oral health fourth edition, Published by Stephen Hancocks Limited, Little Steine, Hill Farm Lane, Duns Tew, OX25 6JH © Stephen Hancocks Limited 2012.
- Lee ES, Adhikari N, Jung JK, An CH, Kim JY, et al. (2019) Application of Developmental Principles for Functional Regeneration of Salivary Glands, *Anat Biol Anthropol* 32: 83-91.
- Greabu M, Battino M, Mohora M, Totan A, Didilescu A, et al. (2009) Saliva - a diagnostic window to the body, both in health and in disease. *J Med Life* 2: 124-132.
- Nunes LA, Mussavira S, Bindhu OS (2015) Clinical and diagnostic utility of saliva as a non-invasive diagnostic fluid: a systematic review., *Biochem Med (Zagreb)*: 25: 177-192.
- Lynge Pedersen AM, Belström D (2019) The role of natural salivary defences in maintaining a healthy oral microbiota, *J Dent* 80:S3-S12.
- Gillum T, Kuennen M, Miller T, Riley L (2014) The effects of exercise, sex, and menstrual phase on salivary antimicrobial proteins. *Exerc Immunol Rev* 20: 23-38.
- Akhiani AA, Stensson A, Schön K, Lycke NY (2005) IgA antibodies impair resistance against *Helicobacter pylori* infection: studies on immune evasion in IL-10-deficient mice. *J Immunol* 174: 8144-8153.
- Srivastava R, Kashyap A, Kumar M, Nath G, Jain AK (2013) Mucosal IgA & IL-1β in *Helicobacter pylori* Infection. *Indian J Clin Biochem* 28: 19-23.
- Wilson KT, Crabtree JE (2007) Immunology of *Helicobacter pylori*: insights into the failure of the immune response and perspectives on vaccine studies. *Gastroenterology* 133: 288-308.
- Gleeson M, Hall ST, McDonald WA, Flanagan AJ, Clancy RL (1999) Salivary IgA subclasses and infection risk in elite swimmers. *Immunol Cell Biol* 77: 351-355.
- Kheirmand Parizi M, Akbari H, Malek-Mohamadi M, Kheirmand Parizi M, Kakoei S (2019) Association of salivary levels of immunoglobulin-a and amylase with oral dental manifestations in patients with controlled and non-controlled type 2 diabetes. *BMC Oral Health* 19: 175.
- Medina ML, Medina MG, Merino LA (2017) Correlation between virulence markers of *Helicobacter pylori* in the oral cavity and gastric biopsies. *Arq Gastroenterol* 54: 217-221.
- Adachi K, Notsu T, Mishiro T, Yoshikawa H, Kinoshita Y (2019) Influence of *Helicobacter pylori* infection on periodontitis, *Journal of Gastroenterology and Hepatology* 34: 120-123.
- Shaila M, Pai Gp, Shetty P (2013) Salivary protein concentration, flow rate, buffer capacity and pH estimation: A comparative study among young and elderly subjects, both normal and with gingivitis and periodontitis. *J Indian Soc Periodontol* 17: 42-46.
- Keremi B, Beck A, Fabian TK, Fabian G, Szabo G, et al. (2017) Stress and Salivary Glands. *Curr Pharm Des* 23: 4057-4065.
- Rantonen PJF, Meurman JH (2000) Correlations between total protein, lysozyme, immunoglobulins, amylase, and albumin in stimulated whole saliva during daytime. *Acta Odontol Scand* 58: 160-165.
- Karthikeson PS, Gayathri R, Vishnu Priya V (2018) Evaluation of salivary total proteins, albumin, globulin, and A/G ratio among healthy individuals and patients with chronic periodontitis, *Drug Invention Today* 10: 2018.
- Shahbaz S, Katti G, Ghali SR, Katti C (2015) Evaluation of salivary albumin in diabetic children. *J NTR Univ Health Sci* 4: 253-256.
- Vaziri PB, Vahedi M, Abdollahzadeh SH, Abdolsamadi HR, Hajilooi M, et al. (2009) Evaluation of Salivary Albumin in Diabetic Patients Iranian *J Publ Health* 38: 54-59.
- Al-Muhtaseb SI (2014) Serum and saliva protein levels in females with breast cancer. *Oncol Lett* 8: 2752-2756.
- Bakhtiar S, Toosi P, Samadi S, Bakhtsh M (2017) Assessment of Uric Acid Level in the Saliva of Patients with Oral Lichen Planus., *Med Princ Pract* 26: 57-60.
- Salian V, Demeri F, Kumari S (2015) Estimation of salivary nitric oxide and uric acid levels in oral squamous cell carcinoma and healthy controls. *Clin Cancer Invest J* 4: 516-519.
- Lamacchia O, Fontana A, Pacilli A, Copetti M, Fariello S, et al. (2017) On the non-linear association between serum uric acid levels and all-cause mortality rate in patients with type 2 diabetes mellitus. *Atherosclerosis* 260: 20-26.
- Li C, Hsieh MC, Chang SJ (2013) Metabolic syndrome, diabetes, and hyperuricemia. *Curr Opin Rheumatol* 25: 210-216.
- Farmer RG, Mir-Madjlessi SH, Kiser WS (1973) Proceedings: Urinary excretion of oxalate, calcium, magnesium, and uric acid in inflammatory bowel disease and relationship to urolithiasis. *Gut* 14: 828-829.
- Lyngdoh T, Marquesvidal P, Paccaud F, Preisig M, Waeber G, et al. (2011) Elevated serum uric acid is associated with high circulating, inflammatory cytokines in the population-based colaus study. *PLoS One* 6: e19901.
- Soukup M, Biesiada I, Henderson A, Idowu B, Rodeback D, et al. (2012) Salivary uric acid as a noninvasive biomarker of metabolic syndrome. *Diabetol Metab Syndr* 4: 14.
- Pullishery F, Panchmal GS, Siddique S (2015) Salivary Thiocyanate, Uric Acid and pH as Biomarkers of Periodontal Disease in Tobacco Users and Non-Users- An In-Vitro Study. *J Clin Diagn Res* 9: ZC47-50.
- Ndebi ME, Guimtsop YAT, Tamokou JD (2018) The assessment of risk factors, lipid profile, uric acid and alanine aminotransferase in Helicobacter pylori-positive subjects. *International Journal of Research in Medical Sciences* 6: 2889-2894.