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

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ABSTRACT

Introduction: Invasive endoscopic-biopsy techniques are most frequently used for the diagnosis and monitoring of chronic gastritis. Finding non-invasive laboratory markers would enable the patient to save money and hassle. Saliva is progressively recognized as the useful non-invasive material of diagnosis because of its critical protective role for the digestive system. Aim: The parameters of biochemical in saliva of chronic gastritis patients (HP- & HP+), comparing their concentrations to those of healthy individuals and establishing correlations between their serum and salivary concentrations with the goal of potentially using saliva as the diagnostic tool.

Material and method: The study was conducted at the Dept. of Medicine, Pakistan Institute of Medical Sciences, Islamabad, Pakistan. There were 140 participants in the study, 60 of them had chronic gastritis (mean age, 58.73 ± 12.08 years; 44 HP+ and 16 HP-); disease activity was determined by endoscopic, serological, and subjective symptoms. Eighty healthy, non-smoking volunteers (mean age, 56.86 ± 6.7 years) made up the control group. Saliva and serum that had not been activated were analysed. Total Protein (TPo), Secretory IgA, Albumin (Alb), and Uric Acid (UA), are the variables we examine (sIg A). They are examined using pre-made B.Couter kits and an ELISA reader from DiaMetra Italy that adapts oral fluid procedures of the Olympus biochemical analyzer.

Results: In comparison to the control group, HP+ patients had significantly increased levels of sIgA (p=0.0001), Alb (p=0.0001), and TP (p=0.0434), but not the UA. For only the UA, we revealed the link between serum/saliva values (r=0.3389) (p=0.011). Endoscopic inflammatory alterations and UA had a moderately negative connection (r=−0.4203, p=0.016). The increased oxidative stress, changed salivary flow rate, and stomach inflammation are hypothesised to be compensated for by these modifications.

Practical implication: the parameters of biochemical in saliva of the chronic gastritis patients (HP- and HP+), comparing their concentrations to those of healthy individuals, and establishing correlations between their serum and salivary concentrations in order to potentially utilization of the data as the diagnostic tool.

Conclusion: The data show that HP+ chronic gastritis results in considerable alterations in salivary parameters. Saliva is a biological material with some limitations, but it is a good indicator of the pathological processes occurring in the digestive tract, particularly when there is an HP+ infection.

Keywords: Salivary Biochemistry; Total Protein; Chronic Gastritis; Helicobacter Pylori; HP+

INTRODUCTION

The non-specific, long-term inflammatory condition of gastric mucosa known as chronic gastritis is associated with dysfunctions of the motor, incretory, and secretory systems. The reasons for its prevalence in the contemporary urban environment include stress, eating habits, poor dental hygiene, various drugs, contamination with the H. pylori, alcohol misuse, smoking. It is a serious public health issue due to the frequent requirement for healthcare. Some extra-digestive disorders, including diabetes, cardiovascular disease, and ischemic diseases, are linked to H. pylori infection. (1)

Chronic gastritis is primarily diagnosed via invasive endoscopic techniques. Patients frequently underestimate their problems and conditions as a result of the unpleasant process and their fear. In the gastrointestinal tract, the mucosa that covers lumen of digestive system serves as mechanical barrier that prevents the passage of the proteolytic enzymes, antigenic structures (antigens of concomitant, food antigens, or pathogenic bacteria), and hydrochloric acid. (2) H. pylori infection of the stomach results in chronic inflammation of the stomach wall and sets off a chain of immune responses in the host. The entire digestive tract is tightly connected to the oral cavity, which is constantly in contact with the outside world. Saliva is an important digestive system protector since it is the first liquid to come into touch with the food, drinks, chemicals, bacteria, and volatiles. The significant number of systemic infections are caused by impairment in oral homeostasis. (3)

The 3-pairs of the salivary glands (the submandibular, sublingual, and parotid glands) plus several smaller glands in oral cavity generate the fluidly, viscous substance known as saliva. (4) It is an intricate system made up of 99% water and 1% of a variety of low molecular weight compounds, including growth factors, enzymes, hormones, antibodies, and antimicrobials. While several are synthesized locally by salivary glands, and others are transferred from blood-stream by the diffusion mechanisms, ultrafiltration or active transport. The bodily functions, metabolic processes, hormonal changes, and emotional state are all reflected in the saliva. (5)

A vast variety of the micro-organisms, several of which are the natural and beneficial supporting oral homeostasis, “peacefully” coexist in the oral cavity. Oral bacteria are impacted directly and indirectly by saliva and its numerous elements. Numerous different microorganisms can thrive well in humid environments that are between 34 and 36 °C in temperature and with pH levels close to neutral. The host-microflora interaction is kept in a symbiotic state by the saliva's antibacterial activity, which is given by the range of the peptides and proteins, which fights off infections. (6)

Under a variety of physiological events as well as a number of oral and systemic pathological states, variations in salivary secretion and composition have been noted. In follow-up of the remission and relapse, oral fluid (OT) testing is becoming more and more significant as a chance to avoid unpleasant and dangerous interventions, including endoscopy and biopsy. It is becoming a more popular biological material because of its simple, non-invasive extraction and patient-friendly process.

MATERIAL AND METHOD

The group of patients consisted of the 60 patients with the chronic gastritis who were hospitalised to the Dept. of Medicine, Pakistan...
Institute of Medical Sciences, Islamabad, Pakistan with dyspeptic syndrome and pain. The average age of the patient group was 57.9±12.08 (range 30-78) years. Eighty healthy, non-smoking, volunteers who participate in the Control Group (CG) get yearly preventive exams on a regular basis. The average age of C-group was 56.8±8.67 (between 30 to 72). All the study partners gave their informed consent. The local ethics committee gave its approval to the study. The following inclusion criteria were used to choose patients: including subjective complaints, disease activity, endoscopic confirmation of HP infection and chronic gastritis. The presence of the malignant consequence or/and new surgery are exclusion factors. This study did not include patients who have the oral irritation or who undergone surgery of dental within the previous 48 to 72 hours. Laboratory measurements for both groups were made, including the inflammatory marker CRP. According to a conventional preanalytical technique, venous blood is drawn. Centrifugation was used to separate the blood serum at 3500 rpm for 10 minutes. We examine levels of blood of H. pylori (IgG) antibodies (LIAISON DiaSorin), and a faecal qualitative HP antigen test is used to confirm the infection. Total protein (TP), uric acid (UA), albumin (Alb), and the IgA levels in serum are all measured. For assessing serum parameters examined using an Olympus AU 640 biochemical analyzer, we use normal, routine laboratory techniques. After repeatedly extracting saliva that has accumulated in mouth from 8 to 10 in the morning, sterile graded containers are used to collect the oral fluid. 5 minutes were needed to obtain 2 to 3 ml. Patients were asked to adhere to the following requirements for trustworthy results: The time since last meal, beverage (including and other aperitifs and coffee), piece of the gum, or tooth-brushing session with the paste has passed more than 30 minutes. The mouth was washed two-time for the 10 seconds with mineral or saline water five minutes prior to the test.

The processing of the material takes about 30 to 60 minutes. Numerous biomolecules undergo lytic reactions as a result of the hypertonic saliva nature and presence of healthy microbiota. We count the quantity of saliva gathered on the container's graduated scale. The samples are centrifuged for the 10 minutes at 3000 rpm. The careful pipetting and aliquoting the supernatant into the micro containers. Until the parameters of the saliva are established, they are kept at -20°C. Utilizing a modification of the oral fluid method, Beckman Coulter kits (Olympus AU 640) are used to measure UA, TP, and Alb. A DiaMetra kit was used to determine secretory IgA.

Methods: Because of the salivary albumin and the protein levels are much low in comparison to the serum, it takes sensitive techniques to measure them. We use the dye pyrogallol red for salivary protein measurement because protein binding changes its spectrum absorption. Spectrophotometric reading at 570 nm. This technique is sensitive down to <3.0 g/L of protein content. Albumin levels in saliva are 100 to 1000 times lower than those in serum. We utilise an immunological turbidimetric assay for microalbumin to ascertain it. On an Olympus AU 640. UA was measured using the colorimetric method with a Coulter kit. Additionally, with approved standard solutions, we have developed a number of the calibrators with the proper matrix for calibration purposes. Immunosorbsent assay was used to measure each sample's total SlgA levels (ELISA). Before adding the samples to the microplate, they were diluted 1: 1000 with the proper buffer. Monoclonal anti-IgA is applied to the plate's wells (alpha-chain specific, DiaMetra Italy). Peroxidase is coupled to the second polyclonal antibody. Following incubation, washing was used to remove the free, solid-phase bound antibodies. The substrates for the enzyme-linked immune reaction were hydperoxide (H2O2) and TMB, which led to a blue colour response and a yellow colour change after the addition of Stop solution. The amount of slgA in the sample has a direct relationship to the cost concentration.

Statistical Methods: Utilizing the programme Graph Pad Prism V, data analysis was carried out. 6.0 using conventional statistical techniques. Mean and standard deviation (mean±SD) were used to present biochemical parametric data. At p<0.05, statistical significance was declared.

RESULTS

Depending on whether the patient group has demonstrated antibodies and antigen for Helicobacter pylori, the group is split into two sections. (Table 1)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Study Group</th>
<th>HP+</th>
<th>HP-</th>
<th>Control Group</th>
<th>Total Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>58.0±12.23</td>
<td>63.5±10.88</td>
<td>63.7±10.05</td>
<td>5.5</td>
<td>11</td>
</tr>
<tr>
<td>Control group</td>
<td>40</td>
<td>40</td>
<td>80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>55.0±12.77</td>
<td>55.2±11.86</td>
<td>56.3±12.14</td>
<td>18</td>
<td>26</td>
</tr>
<tr>
<td>HP Positive</td>
<td>23</td>
<td>37</td>
<td>60</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Patients are categorised as follows (Table 2) based on the features of endoscopy of the inflammatory alterations and the spread of topography in gastro-duodenal mucosa:

<table>
<thead>
<tr>
<th>Endoscopic Dx</th>
<th>Diffuse (n=31)</th>
<th>Regional (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrophic gastritis</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Erosive gastritis</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>Erythematous gastritis</td>
<td>10</td>
<td>15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>HP+</th>
<th>HP-</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>slg A</td>
<td>108.3±47.69</td>
<td>98.3±18.44</td>
<td>139.9±33.24</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Albumin</td>
<td>50.8±19.87</td>
<td>67.5±25.21</td>
<td>89.6±62.93</td>
<td>0.04853</td>
</tr>
<tr>
<td>Total protein</td>
<td>725.0±393.5</td>
<td>786.8±237.6</td>
<td>891.0±354.7</td>
<td>0.0434</td>
</tr>
<tr>
<td>Uric acid</td>
<td>222.9±36.8</td>
<td>219.8±58.45</td>
<td>210.6±56.68</td>
<td>0.4166</td>
</tr>
</tbody>
</table>

Table 3: Change in The Gastrointestinal Mucosae Of Endoscopic Characteristics Of Patient Group Study.

Table 4: Correlation Between Serum And Parameters In Unstimulated Saliva Of Control Group And Total Patient.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Serum-r</th>
<th>p-value</th>
<th>Serum-r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>slgA serum/saliva</td>
<td>0.01374</td>
<td>0.3389</td>
<td>0.0106</td>
<td></td>
</tr>
<tr>
<td>UA serum/saliva</td>
<td>-0.1569</td>
<td>-0.04853</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Alb serum/saliva</td>
<td>-0.06139</td>
<td>0.1777</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>TP serum/saliva</td>
<td>-0.02138</td>
<td>0.09266</td>
<td>ns</td>
<td></td>
</tr>
</tbody>
</table>

The most common type of the antibody that mediates the particular mucosal surfaces immune protection is secretory IgA (SIgA). IgA exists in two structurally distinct forms: IgA1 (which accounts for 90% of IgA) and IgA2 (which accounts for 10%). IgA1 is a by product of the production of B cells in the bone marrow and is found in serum. IgA2 is a by product of mucosal B cells.
serves as a symbol of the healthy mucosal immune system. IgA is present in adult saliva at a rate of about 60%. \(^{10}\)

In our study involving HP + and CG patient group in our study displayed considerably higher mean sIgA values (Table 3). Similar to that, increased serum IgA concentrations were seen. There was no discernible relationship between salivary IgA levels and serum Ig A levels after analysis. (Table 4) Salivary IgA concentrations may have increased as a result of its function in lowering density of bacterial and defending stomach mucosa. Studies have demonstrated that the naturally occurring antibodies, particularly IgA, could prevent infection of H. pylori. \(^{11}\) The sIgA improves bacterial opsonization and inhibits bacterial adherence and colonisation. \(^{12}\) In order to fight off the pathogenic bacteria, the host's adaptive immune mechanisms are likely involved in HP infection. Even in healthy people, the secretory amount of IgA directly rises with age. \(^{13}\) Despite having a smaller sample size than HP + and CG, the HP group has the greatest age and the lowest mean IgA levels. Drug-induced gastritis of type C or other concomitant conditions that result in ischaemia alterations in the GET are what are categorised as HP -. The lower values could be explained by the effects of diuretics, B-blockers, NSAIDs, antibiotics, and antianitrythmics on the salivary secretion and flow. The HP- group should be increased to allow for a more accurate evaluation of global immunoglobulin secretion.

In their study, it is claimed that a lack of and diminished local defence mechanisms predispose youngsters to HP infection. \(^{14}\) When compared to kids who don't have an organic condition and HP infection, the concentrations of the sIgA and the SC component in saliva and the stomach juice of the patients with infection of HP don't differ significantly. The limitation of our study in this regard is that we did not assess the concentration of sIgA in gastric juice. The information will fully illustrate how saliva and ocular homeostasis are involved in the immune-inflammatory pathways and the changes to gastric mucosa. \(^{15}\)

It has been established that H. pylori may have a role in the aetiology of a number of oral disorders, including periodontal disease, recurring aphthous stomatitis, burning mouth syndrome, halitosis, and squamous cell carcinoma. The development of bacteria in saliva and dental plaque seems to serve as a reservoir for infection of stomach and the reinfection. \(^{16}\) Additionally, it has been suggested that H. pylori infection is linked to a number of systemic conditions, including dyslipidaemia, hyperglycaemia, and a number of cardiovascular diseases. \(^{17}\)

Saliva contains essential amounts of total protein. The majority of its tasks, including taste and digestion, lubrication, physical protection, cleansing, buffing, and tooth integrity preservation, are carried out by it. The rate of the salivary flow, crevicular fluid and release of protein from the glands are main variables controlling concentration of protein in the saliva. \(^{18}\) The mostly smaller protein amounts, enzyme-rich saliva and mucin are excreted. \(^{18}\) Because of how the medications used to treat chronic gastritis affect the body, xerostomia is a typical symptom in patients with this condition. (Butyl scopolamine, drotaverine – antimuscarinic agents, hydrochloride, antibiotics, Omeprazole) In our investigation, the HP + patient group had statistically significant higher salivary protein values than the CG group (Table 3). While the HP- group and CG have not shown any evidence of a similar reliance. The values of serum TP levels were not found to differ significantly. This rise in salivary protein is probably a sign of local glandular protein secretion involved in non-specific immune protection in response to the host. \(^{19}\) In both the group of patient and CG (control group), there was no association between serum protein levels and salivary protein levels.

Lipids, hormones, and unconjugated bilirubin are carried by albumin, which also helps to keep the colloid-osmotic pressure constant. It accounts for more than half of all plasma proteins. Nutrients, hormones, cellular harmony, and the sodium pressure are all present in albumin production. \(^{20}\) Whole saliva contains only a little amount of albumin, an antioxidant protein. Generally speaking, the albumin is regarded as an accurate indicator of mucositis or inflammation. \(^{20}\)

Therefore, to exclude active local inflammation, a general dental examination was carried out before choosing the control group and patients. In our study involving HP + and CG, the HP- group has the greatest age and the lowest mean IgA levels. Drug-induced gastritis of type C or other concomitant conditions that result in ischaemia alterations in the GET are what are categorised as HP -. The lower values could be explained by the effects of diuretics, B-blockers, NSAIDs, antibiotics, and antianitrythmics on the salivary secretion and flow. The HP- group should be increased to allow for a more accurate evaluation of global immunoglobulin secretion.

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<table>
<thead>
<tr>
<th>p</th>
<th>-0.0915</th>
<th>0.0154</th>
<th>-0.1515</th>
<th>0.9559</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>0.2319</td>
<td>0.3282</td>
<td>0.1979</td>
<td>0.007703</td>
</tr>
</tbody>
</table>

When compared to HP- and CG groups, the HP + group had statistically significant higher levels of CRP, an inflammatory marker. The association between HP- and SG is seen to be comparable. The acute-phase protein has a wide range of the reactivity in the HP+ patients, despite the fact that it is neither selective nor sensitive to chronic gastritis. We compared the CRP values to the levels of the investigated parameters in the serum and saliva. We discovered a weakly negative association between albumin levels in the serum and the total protein levels in the saliva (r=–0.3282, p=0.0154) and the saliva (r=–0.2283, p=ns) but not as much in the serum (r=–0.2491, p=ns). Numerous proteins are secreted by the liver and are distributed differently throughout the body as a result of inflammation. A protein in the reverse-phase is albumin. Regardless of greater amounts of salivary albumin, it appears that local oxidative stress, inflammatory process, and differences in filtration of albumin via capillaries of salivary gland explain the results. \(^{20}\) (Table 5, Table 6).

<table>
<thead>
<tr>
<th>p</th>
<th>0.8977</th>
<th>0.0016</th>
<th>0.8967</th>
<th>0.5717</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>0.01792</td>
<td>0.4203</td>
<td>0.01821</td>
<td>0.07869</td>
</tr>
</tbody>
</table>
According to Ndebi ME and colleagues, a persistent H. pylori infection is associated with a rise in serum uric acid. Although there was no statistical significance, our patient HP+ group similarly displayed increased UA levels. The comparative analysis showed that the endoscopic inflammatory alterations of the gastroduodenal mucosa most closely connect with uric acid. (r=−0.4203, p=0.0016) We found a moderately negative correlation dependence. The beginning of the imbalance with loss of the AOC in chronic course and the damage to stomach mucosa are factors that we attribute to oxidative stress (Table 7).

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 affected by this disease. Various studies have reported that saliva is a non-invasive sample for detection and attractive option for epidemiologic studies because saliva can be analyzed and obtained easily, collection and testing salivary specimen is fast, painless, convenient, and carries no risk of needle stick injury. 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−) and bicarbonate (HCO3−) that have considerable buffering capacity for maintaining the PH level in saliva. 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 in patients with H. pylori, thus their salivary secretion and composition could be partly under esophago -salivary reflex control. 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. 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. The salivary protein has an important role for carcinogens, part of this protein is implicated in activation of immunity like salivary immunoglobulin’s, while others protein are responsible for non-immune elimination of microbes like salivary amylase by inhibitory effect on microorganism growth. 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”.

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. 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-remineralization and dental caries formation as well as plaque formation 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 result from the nutritional and immunological changes that occur during the disease course, this result is disagreement with studies by 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. 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, 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.

CONCLUSION

There aren’t many, and they're not specific enough, laboratory indicators for the diagnosis of chronic gastritis. The clinical symptoms, inflammatory response, and the complications of their course all affect some markers. The most prevalent infection in people is caused by Helicobacter Pylori (HP). With almost 50% of the world's population affected, it is pervasive. High morbidity and mortality rates and costs of care are connected with HP-related illnesses. Another biological substance that is easily extracted using a non-invasive process is saliva. We discovered significant alterations in salivary parameters in HP+ chronic gastritis in our investigation. Pathogen penetration and GET inflammation are intimately related to oral illnesses and compromised oral cavity homeostasis. The biological matrix that is saliva has some limitations. The procedure’s standardisation and the approaches employed to look at its indicators both have unresolved problems. However, it accurately depicts the diseases processes in the gastrointestinal tract, particularly when HP + infection is present, and in the future, it will be used more frequently in the diagnostic and monitoring process.

REFERENCES


