



The Salivary Secretion and Biochemical Composition Analysis in Patients with *Helicobacter pylori* Infection

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ABSTRACT

Helicobacter pylori (*H. pylori*) infection defines as an agent who may involve in the occurrence of chronic gastritis, peptic ulcer, gastric cancer and also other known and unknown diseases. This study aims: to determine the salivary flow rate, pH and to compare alteration of salivary enzyme level like Alkaline Phosphatase (ALP), total protein (TP), salivary calcium and urea between subjects with *Helicobacter pylori* infection and healthy subjects. The saliva and blood samples were collected from patients and healthy control subjects. This study included 52 subjects in total that divided into thirty two subjects with *H. pylori* infection and twenty subjects were healthy control, this study carried out from (1 March 2017 until 30 July 2017) in the Gastroenterology clinic in Teaching Fallujah Hospital underwent gastrointestinal endoscopy. The salivary sample collection considers as a simple and easy method for analysis. The PH and flow rate in saliva were increased in study group in comparison with healthy controls; there was a highly significant difference between two groups ($p < 0.001$). The salivary ALP level was lowered, whereas salivary urea level was elevated in subjects with *Helicobacter pylori*, but statistically was non-significant ($P > 0.05$). On other hand the salivary total protein (TP) level in study group was decreased in comparison with control group with statistically significant difference, whereas the salivary calcium level was higher in study group in compared to healthy control with highly significant differences ($P < 0.001$). It was concluded that the *Helicobacter pylori* infection has obvious influence on salivary composition (like total protein and calcium), salivary flow rate and PH.

Keywords: Saliva, Digestive Pathologies, *Helicobacter pylori*, Total Protein, Calcium, Urea

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INTRODUCTION

The gastrointestinal pathogen that known by *Helicobacter pylori* is consider a one of the promoting factors of peptic ulcer and gastric diseases. It has also been delineated as a risk factor for gastric carcinoma [1]. The human stomach considers a natural habitat for the

microorganism, but it may also survive in other environments, like dental plaque, human and animal faces and aquatic systems [2, 3]. A potential extra-gastric reservoir for *Helicobacter pylori* which considers as a cause of infection or reinfection is the human oral cavity [4]. Many studies indicate the oral cavity is a transient or permanent location of *H. pylori*, especially in patients with gingival inflammation or chronic periodontal diseases [5].

The assay requires blood sample collection, human body materials such as feces and saliva,

which are collected by totally non-invasive procedures, have been subjected to ELISA examination. The saliva contains a hundreds of constituents that may work as evidence of systemic diseases or to detect the different harmful substances for disease status [6]. Stimuli for raised salivary secretion in the mouth involves the presence of irritating substances, smell of food and food. The connection between the gastric symptoms and *Helicobacter pylori* presence in the oral cavity stays unclear and because its presence in the mouth may be a first site of infection and re-infection, this study was performed to determine the saliva composition and secretion change from patients suffering digestive pathologies [7].

The objective of this study determines the salivary flow rate, pH and to compare alteration of salivary enzyme level (like Alkaline Phosphatase), total protein, salivary calcium and urea between subjects with *Helicobacter pylori* infection and healthy subjects.

MATERIALS AND METHODS

Patients & Method

Our study carried out from (1 March 2017 until 30 July 2017). The patients come to the Gastroenterology clinic in Teaching Fallujah Hospital underwent gastrointestinal endoscopy were selected with symptomatic criteria for endoscope examination (32 cases and 20 controls, respectively). The symptomatic criteria include unexplained vomiting, weight loss, recurrent abdominal pain and any signs of gastro-duodenal pathological disease. Exclusion criteria: patients who had taken antibiotics within one month prior to sample collection. In study group the *Helicobacter pylori* was diagnosed by using two tests (positive rapid test *H. pylori* for serum sample or stool antigen test).

In all patients, blood samples with positive rapid test *H. pylori* (antibody detection) or stool antigen test were obtained at morning before endoscopy examination after obtaining an informed consent from patient. Prior to the collection of saliva samples, the patients were informed no sanitizing the mouth, saliva was collected always in the morning after 8 hour of fasting. During the collection the subjects sat in an upright position in a quiet room and saliva was collected by spitting method for 10 minutes in a sterile plastic collector, about 3-4 ml of unstimulated saliva

(resting) was collected and kept frozen at -20°C until analysis. Blood and saliva were sent to laboratory under standard conditions, the spectrophotometer were used to measure the alkaline phosphatase, total protein, calcium and urea according to the instruction of manufacturer for each one. Alkaline phosphatase (BioMerieux Kit, France), total protein, calcium and urea (BIOLABO SAS, France) .The oral cavity of all patients was examined by using a mouth mirror and artificial light.

There is another methodology test that utilizes to discover the presence of *Helicobacter pylori* antigen by using a stool specimen (stool antigen testing). This test uses an enzyme immunoassay and considers a reliable way of diagnosing active infection; it has a sensitivity and specificity compared to other tests whereas the rapid *H. pylori* test has the advantages of being inexpensive, fast, and widely available [8]. Statistical analyses were done using SPSS version 21 computer software (Statistical Package for Social Sciences) in association with Excel version 5 and the statistical significance of difference in mean between 2 groups was assessed using the independent samples t-test.

RESULTS

A total of 52 patients [31 male (59.61%), 21 female (40.38%)] were participated in this study, twenty cases (38.46%) who were negative for *H. pylori* by either rapid test *H. pylori* or stool antigen test. The *H. pylori* were detected in thirty two cases (61.53%) by the same two tests.

The most frequent salivary biochemical composition change in study group is the total protein (TP) and calcium. In this study the result shows an obvious difference in total protein (TP) and calcium between two groups. A highly significant differences of salivary calcium ($P < 0.001$) and a significant difference of total protein (TP) in study group in comparison with control group ($P = 0.006$). However, slightly or no change levels of salivary alkaline phosphatase (ALP) and urea in study group in compared to the healthy control group, statistically no significant difference ($P > 0.05$) were established in (Table 2), also an obvious differences in salivary flow rate and PH between study and healthy control groups, there was highly significant differences between two groups ($P < 0.001$). (Table 2)(Figure 1)

Table 1: Descriptive Statistics (Mean + SD) of the studied Enzymes and saliva secretion in different samples (control and study group)

Group Statistics				
	groups	N	Mean	Std. Deviation
PH	study	32	8.0625	.35355
	control	20	7.0000	.00000
Flow rate of saliva (ml/min)	study	32	6.3500	1.80197
	control	20	2.4450	.38726
Alkaline phosphatase Iu/L	study	32	19.9275	2.12938
	control	20	20.0000	.00000
Urea (mg/dl)	study	32	33.9344	11.80423
	control	20	33.0150	6.62684
Total protein (g/dl)	study	32	1.0025	.52038
	control	20	1.4160	.46641
Calcium (mg/dl)	study	32	3.9781	1.37224
	control	20	2.6800	.24623

Table 2: t-test and significant levels of resting whole human saliva, flow rate, PH and biochemical Data between control & study groups

Independent Samples Test			
	t-test for Equality of Means		
	t	df	Sig. (2-tailed)
PH	13.390	50	.000
Flow rate of saliva (ml/min)	9.521	50	.000
Alkaline phosphatase Iu/L	-.152	50	.880
Urea (mg/dl)	.318	50	.752
Total protein (g/dl)	-2.898	50	.006
Calcium (mg/dl)	4.174	50	.000

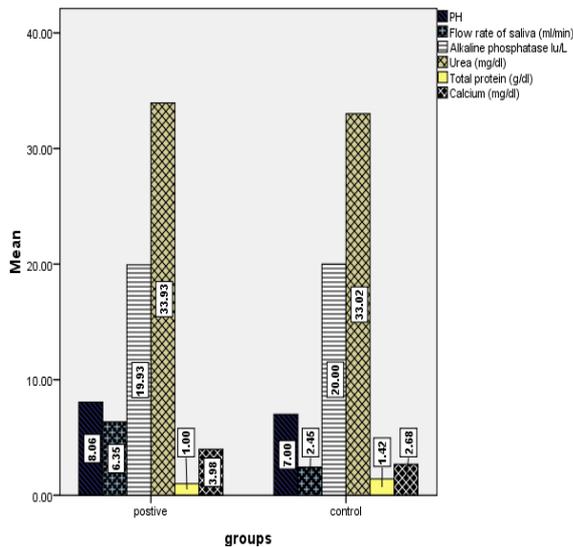


Figure (1): Mean values for selected salivary flow rate, PH and biochemical composition of study and control groups

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 current 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 of (7.8) 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 arise 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 data of this study showed the calcium level of saliva in study group was higher than control group; the elevation in the calcium level may be explained by the extremely used of antacid taken by the mouth to counterbalance the gastric acid, for example the calcium carbonate may cause rebound acid secretion about two hours after each dose and regular use may cause hypercalcemia, particularly if taken with sodium bicarbonate [22]. 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].

In the current study the alkaline phosphatase (ALP) and urea levels of saliva were founded to be slightly changes between two groups (salivary ALP was decreased while the urea become slightly increased), but the difference was failed to reach the significant this may be due to insufficient amount of subjects for statistical analysis, This result is disagreement with [20] who found the salivary (ALP) level was found to be elevated among patients with peptic ulcer and study [21] who found there was increased in the salivary ALP level but not statistically significant. 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]. This finding was in agreement with study [29] who found that the urea in saliva has a main influence on calculus formation that deposit in most people, also the result is in agreement with study [30] who reported the blood urea

nitrogen in saliva was increased with periodontal diseases.

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