ORIGINAL ARTICLE

UTILITY OF STOOL ANTIGEN TEST IN DIAGNOSIS AND PROGNOSIS OF *HELICOBACTER PYLORI* ASSOCIATED CHRONIC GASTRITIS FROM A TERTIARY CARE HOSPITAL, BALOCHISTAN

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ABSTRACT

Background: International literature has shown various investigational modalities used for *Helicobacter pylori* (*H. pylori*) associated gastritis diagnosis and its therapeutic response to eradication regimen. But there is paucity of availability of local data on this important issue from Balochistan.

Material and Methods: A cross-sectional observational study was carried out in The Pathology Department of The Combined Military Hospital Quetta from May 2021 to April 2022. Stool samples for H. *pylori* Antigen detection were received from all the clinically suspected cases of chronic gastritis irrespective of their gender, aged 2-60 years, which were later on confirmed by histopathology of gastric biopsy specimens**.**

Results: Total, 1450 HpSAg tests were performed, out of which 411(28.34%) specimens were found positive in the study. Our tested population was mainly male (69%). Majority of them belonged to the age group between 31-50 years (46-43%). Mean age of the patients in our studied populace was 39.45±31.9 years. The risk factors associated with chronic gastritis among males was (72%) smoking and (75%) excessive caffeine intake, where as in females, (68%) stress, (74%) excessive NSAIDS use and (41%) prolonged usage of steroids were the main contributing factors. (61%) Unhealthy lifestyle, (49%) positive family history and (58%) chronic diseases were more or less in same the proportion in both genders. 63% patients were found negative on repeat testing by HpSAg after 04 weeks of complete therapy. Whereas 37% patients who remained positive were treated for another 2 weeks for complete eradication. Only 3% were still positive despite repeated eradication therapies indicating treatment failure.

Conclusion: To conclude, HpSAg test is a rapid, easier, low cost, non-invasive and equally reliable test that not only aids in patient diagnosis but is invariably helpful in monitoring patient response to eradication therapy in resource poor settings.

Keywords: Chronic gastritis, *Helicobacter pylori* (*H. pylori*), *H. pylori* Stool for Antigen test (HpSAg), Eradication therapy

BACKGROUND

In 1982, *Helicobacter pylori* (H. *pylori*), the gram negative spiral shaped rods were first identified and cultured by Marshal and Warren. Since then, it has been recognized to be associated with chronic gastritis, most peptic ulcers and malignancies (gastric adenocarcinoma and lymphoma).[1] More than 20% of the population suffered from functional dyspepsia which is diagnosed on the basis of Rome IV criteria, may have H. *pylori* infection that needs eradication therapy for 6-12 months.[2] All those patients with no structural abnormality on imaging and endoscopy, no metabolic cause or that have one or more symptoms including epigastric pain or burning, early satiety, and postprandial fullness for a period of 3 months or more are suffering with functional dyspepsia according to above criteria.[3] *H. pylori* infection also has strong association with hematological disorders like idiopathic iron deficiency and Vitamin B12 deficiency anemias and some neurodegenerative disorders.[4] Severity pattern of the disease depends on host factors such as socioeconomic status, immune status, genetic susceptibility and various risk factors along with virulence of the strain causing H. *pylori* infection.[5] It has variable incidence in different parts of the world with higher prevalence in developing countries i.e. 87.7% in Nigeria and 92% in Bangladesh.[6] H. *pylori*

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infection can be diagnosed by both invasive and non-invasive techniques. Invasive techniques including endoscopic gastric biopsies which are sent for histopathology and culture and sensitivity which are costly, special techniques and need highly skilled human resource.[7] While among non-invasive tests radioactive carbon urea breath test, stool antigen test and serology are most commonly advised.[8] Urea breath test and serology have their pros and cons with limitations as they are not useful in monitoring treatment response.[9] Therefore it is better to use such a test which is a cheaper yet a helpful diagnostic tool in screening of masses. Stool antigen (Ag) test fulfils this basic criteria with better (more than 90%) sensitivity and specificity that can also be used in children and pregnant women safely to diagnose and monitor eradication of H. *pylori* infection.[10] This helps clinicians in timely treatment of the patients, thereby reducing the chances of complications due to *H. pylori* infection.[11] Limited local publications are available regarding the H. *pylori* diagnosis by using non-invasive techniques and their role in monitoring therapeutic response from Pakistan, particularly in Balochistan province. The purpose of our study is to ascertain the utility of H. *pylori* antigen testing in stool samples for diagnosis of H. *pylori* infection in patients with complaints of gastro duodenal disease refractory to proton pump inhibitors and antacids therapy from Balochistan province. We used non-invasive technique i.e. detection of H. *pylori* Ag in stool samples by lateral flow chromatographic immunoassay technique for the diagnosis and prognosis of the disease.

MATERIAL AND METHODS

This prospective observational study was conducted in the Pathology department (Microbiology & Histopathology) of the Combined Military Hospital, Quetta from June 2021 to May 2022 after taking consent and approval from research and ethical committee of the hospital (IRB/038)**.** All those patients who presented with clinical findings of chronic gastritis, of either gender aged 2 years to 60 years were included in the study. Patients who did not give consent, those who took eradication therapy in the past six months or left their follow up during the study and duplicate samples were excluded from the study. Non-Probability consecutive sampling was done.

Onsite H. *pylori* Ag Rapid Test (HpSAg) CE-CTK Biotech Kit was used to look for H. *pylori* antigen in stool samples of clinically suspected cases for the diagnosis of H. *pylori* infection. It is a sandwich based lateral flow chromatographic immunoassay for the qualitative detection of Ag in stool with high sensitivity and specificity (98.8% and 100%) which makes it reliable and cost effective with rapid turnaround time of 15 minutes. Fecal specimens were collected in a clean, dry containers and were processed within 6 hours of delivery to the microbiology lab, in case of delay specimens were stored overnight at 2-8 ^{0}C. We processed both solid and liquid stool specimens as per kit manufacturer's instructions. Positive cases detected by Stool Antigen tests were further confirmed by Histopathology of gastric biopsies (taken as gold standard). The specimens were received in a leak proof sterilized container, immersed in 10% buffered neutral formalin, a tissue fixative. The biopsy specimen were then processed as per laboratory standard protocols and the slides were stained by Giemsa and Hemotoxylin/Eosin stains. The histopathological findings were noted on basis of upgraded Sydney criteria**.** Unfortunately only few patients were willing to underwent endoscopy for gastric biopsies and thus diagnostic accuracy was calculated considering those specimens only. The H. *pylori* stool antigen detection tests was carried out twice, first for diagnosis and then for monitoring the clinical response on completion of 2 weeks of eradication therapy. Clinicians advised first line quadruple therapy initially which comprised of Proton Pump inhibitor (Omeprazole 20 mg twice a day (BID), Amoxicillin 1000mg BID, Metronidazole 500mg bid, Clarithromycin 500mg BID.[25]The treatment regimen was given for 14 days and then after clinical improvement and stoppage of antibiotics for 02-04 weeks, repeat testing was performed. If post therapy test result turned out to be negative, it meant patient showed treatment response. But If HpSAg test remained positive despite completion of eradication regimen, patients were advised to repeat the same eradication regimen for another 2 weeks. If the test still remained positive after two courses, it meant patient showed treatment failure to first line therapy. Such patients were then advised second line therapy comprising of Levofloxacin 500mg once a day,

Amoxicillin 1000 mg twice a day with proton pump inhibitor (Esomeprazole 20 mg BID) along with Bismuth salts 6hourly (QID) for a period of 14 days.[25] Along with drugs, patients were also counselled about following a healthy routine that included dietary modification, daily exercise, avoidance of stress, reduction in coffee/tea intake and quit cigarette smoking. Documentation of variables like clinical features, age, gender, presence of risk factors, therapy and endoscopic findings of H. *pylori* positive patients were collected from patient hospital record (Gastroenterology department). Statistical analysis was done by using SPSS software version 24 and by applying correlations to ascertain significance. Frequencies and percentage of the qualitative variables like gender was calculated. The chi square test was applied to find P-value, (≤0.05) was considered statistically significant.

RESULTS

During this one year of study period, a total of 1450 stool specimens were received for H. *pylori* rapid antigen test from patients presenting with clinical features suggestive of chronic gastritis. Out of which, 411 (28.34%) specimens were found positive, with (69%) male predominance. Majority of samples belonged to age group between 31-50 years (46-43%) with mean age 40 ± 15 years irrespective of gender discrimination as shown in Table-1. The risk factors associated with chronic gastritis among males were (72%) smoking and (75%) excessive caffeine intake, where as in females, (68%) stress, (74%) excessive NSAIDS intake and (41%) prolonged steroids use were the main contributing factors as mentioned in Figure-1.

Only 125 patients, out of the total 411 HpSAg positive patients underwent endoscopy and submitted gastric biopsies for histopathology. On endoscopy, normal findings were reported in 48% and chronic atrophic gastritis in 40%. Among them, 95/125 (76%) were confirmed positive by both stool for H. *pylori* Antigen detection and Histopathology. One can appreciate inflammatory changes and presence of *H.pylori* on Hemotoxylin & Eosin and Giemsa stained slides of provided biopsies (Figure-2). 30 (24%) patients had a positive H. *pylori* stool antigen test but on histology had non-specific findings (Table-2). All positive patients received the complete eradication regimen for 02 weeks showed improvement in their symptoms. After 04 weeks of post therapy, the test was repeated, 63% were found negative and 37% patients were still found positive. Those patients were treated with the same regimen for another 2 weeks. 3% of patients showed no response even with second course of eradication therapy that depicted from a repeatedly positive HpSAg test. In those patients, clinicians advised second line regimen with certain life style changes like dietary modification, reduction of caffeine intake /cigarettes, avoiding stress, stopping NSAIDS and steroids. 99% of patients get benefit with that combined approach and finally had a negative result but 1% showed treatment failure needs further investigation like culture and susceptibility testing which was not available in our setup (Table-2). HpSAg test showed 92.3% specificity and 95.9% sensitivity with overall diagnostic agreement was up to 95% for the diagnosis of H. *pylori* infection, calculated for 125 patients with histological findings (Table -3 & 4).

Table-1: Prevalence of disease in relation to different age groups and gender by using chi square in 411 H. *pylori* stool Ag positive.

Gender: n (%)	02-12 years 19(5%)	13-30years 127(31%)	31-50 years 187(45%)	51- 60 years 78 (19%)	P-value (≤0.05)
Male: 285(69%)	13(4.5%)	95(33.3%)	132 (46.3%)	45(15.8%)	0.002
Female: 126(21%)	6(4.76%)	32 (25.4%)	55(43.65%)	33 (26.19%)	0.001

Table-2: Showing details of clinical, endoscopic and histopathological findings in 411 H. pylori stool Ag positive patients.

Clinical /Laboratory findings (411/ 1450= 28.34%)	**Endoscopic findings**	**Histopathological findings of 125 biopsies (upgraded Sydney criteria)**	**Treatment Response**
Indigestion/ Dyspepsia: 78%	Total endoscopy performed: 125/ 411 (30%)	Nonspecific findings: 30/ 125 (24%)	**First line quadruple therapy:** (4weeks after Post Initial course) Response: 63% (260/411)

			No response: 37% (151/411) **4weeks after repeated course:** Response: 97% (400/411) No response: 3% (11/411)
Epigastric pain: 55%	Chronic atrophic gastritis: 40%	H. pylori Present: 95/125(76%) **Among H. *pylori* positive following changes has been observed**:	**Second Line quadruple therapy with life style modification (after 4 weeks Post therapy):** Response: 99% (408/411) No response: 1% (410/411: treatment failure)
Abdominal pain radiating to back: 49%	Nodular gastritis: 7%	Inflammatory changes: 60% Activity: 40%	
Nausea/or vomiting: 11%	Erosive gastritis: 5%	Atrophy:25% Dysplastic changes: 1%	
Abdominal Fullness: 51%	Metaplastic gastritis: 0%		
Loss of Appitite: 21%	Normal findings: 48%		
Iron deficiency Anemia: 48%			
Vitamin B 12 deficiency: 33%			

Table-3: Showing comparison between HpSAg and histology among 125 gastric biopsies.

HpSAg	Histology Positive	Histology Negative	P-value
Positive	95 (TP)	2 (FP)	0.0001
Negative	4 (FN)	24 (TN)	

Note: TP=True positive, FP=False positive, FN=False negative, TN=True negative

Table-4: Diagnostic variables of H. *pylori* antigen stool test in relation to histology of 125 gastric biopsies.

S. No	Variable	Result
1.	Sensitivity (TP/TP +FN X100)	95.9%
2.	Specificity (TN/TN+FP X100)	92.3%
3.	Positive Predictive Value (TP/ TP+FP) X 100	97.9 %
4.	Negative Predictive Value (TN/TN+FN) X100	85.7%
5.	Overall agreement (TP+TN/TP+TN+FN+FP)	95.2 %

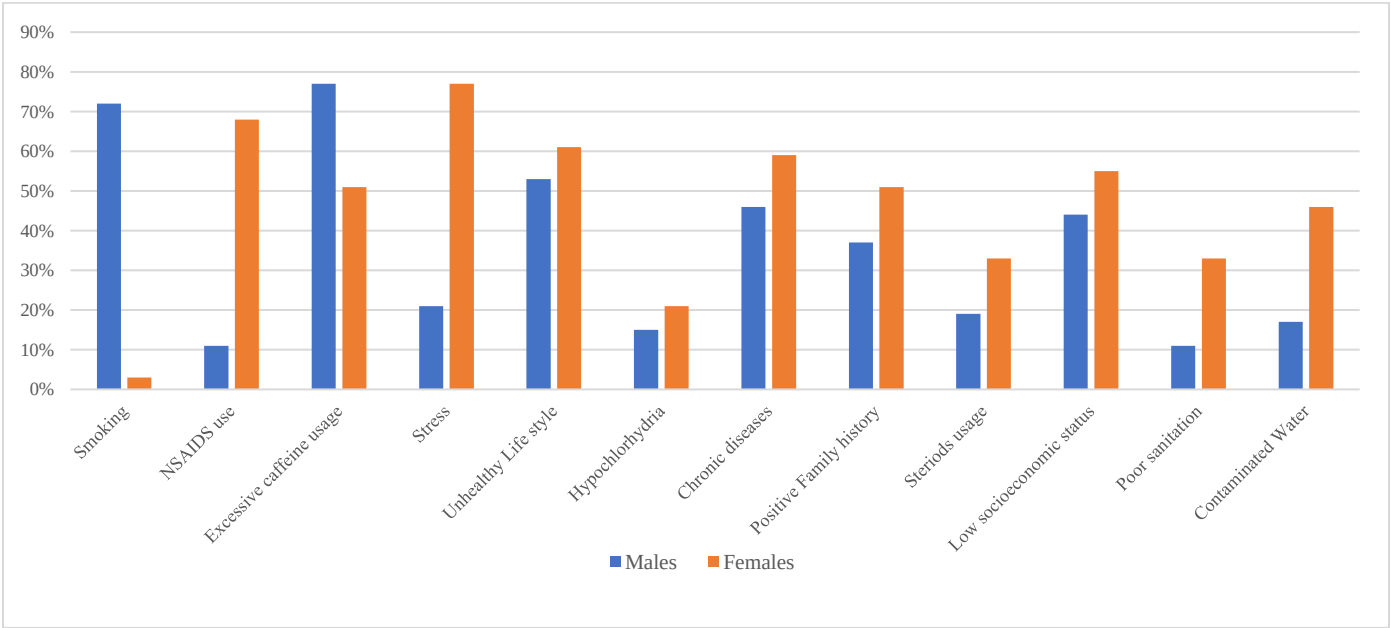

Figure-1: Association of risk factors with H. *pylori* associated chronic gastritis in relation to gender.

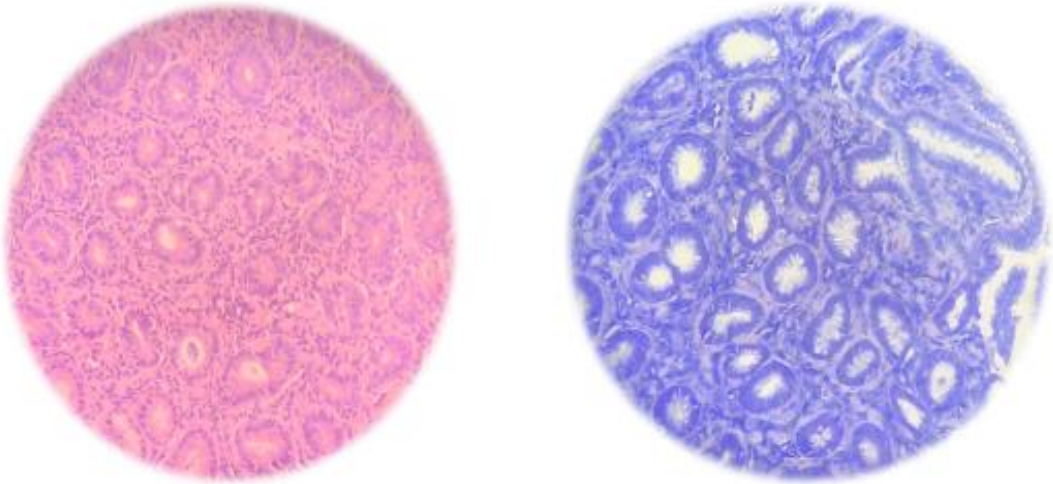

Figure-2: **Showing histological changes noted in 95 H. *pylori* infected gastric biopsy on Hemotoxylin & Eosin (a) and Giemsa (b) stained slides**

DISCUSSION

Limited literature is available regarding association of *H. pylori* with chronic gastritis and its eradication therapy response in the people of Balochistan. In order to improve management and outcome of H. *pylori* related gastro duodenal disorders continuous surveillance of infected populations from various regions of the world including Pakistan needs to be carried out regularly. Many studies found stool HpSAg detection test as an equally reliable and easier alternative to gold standard invasive tests for diagnosis and post treatment monitoring. Our results reflected the same.

Available literature review reveals that invasive test like endoscopic observation for the presence of antral nodularity has variable sensitivity (39.8 to 96.4%) and specificity (83.6% to 100%).[12] Though histology of gastric mucosal biopsy is still considered as gold standard with high sensitivity (95%) and specificity (98%) provided when obtained from multiple sites (two antral and two corpus).[13,14] The prevalence of *H. pylori* in our country is 28% which is lower than many developing countries like Nigeria (87.7%) and Bangladesh (92%) and some developed countries like China (83%) and United states (40%).[7,15] We found out that in our study only 5% of children between 2-12 years of age were affected by the disease while the prevalence is much higher in other countries like US state of Alaska (86%) and Bolivia (80%).[16] Zhou X and Ozbey G *et al* commented on the significance of non-invasive test i.e. HpSAg among children and its higher prevalence in low socioeconomic groups living in squalid conditions without proper sanitation and availability of clean drinking water similar to our study.[16,17] The results of the test are equally reliable in pre and post eradication therapy as mentioned by GOLD BD *et al* including the extra gastric manifestations like growth retardation, iron-deficiency anemia, and idiopathic thrombocytopenic purpura which were not noted by us as it was beyond the scope of our study.[18] In this study, histopathological data for children was not available as their parents were reluctant for endoscopy so we only had to rely on HpSAg test in those cases, the same observation was made by Elvira G *et al*.[19] We observed that majority of HpSAg test positive patients were males (69%) and middle aged person belonging to age group between 31-50years (46.3%) with mean age 40 ± 15 years which were in contrast to study done by Lee Y-C and Zubair *et al* in which females (56.3%) were affected more.[20,22] The mean age in our study group was 35 ± 18 similar to a study conducted by Qadir A.[20] There is abundance of literature available regarding HpSAg test as a screening tool for population in which the sensitivity and specificity of HpSAg test was more than 90% with variable clinical accuracy upto 94%, the findings were similar to our study.[14,20,21] Our results were significantly different from Zubair *et al* in which sensitivity was noted around 80% with diagnostic accuracy of 75.5%.[22]

The major challenge after diagnosis is treatment, as combination of two different antibiotics along with either a proton pump inhibitor or bismuth salts were administered in form of triple or quadruple regimen for

a minimum period of 14 days. More than 20% failure rate was reported with first line therapy which was usually based on triple drug regimen,[23] so we treated our patient with quadruple therapy in order to minimize the development of resistance and for optimal clinical results.[25] When patients do not respond to eradication therapy twice then culture and sensitivity on endoscopic gastric biopsies is recommended to evaluate susceptibility trends of the bacteria against the current regime, this was seen in 1% of our patients. The major drawback of culture technique is special growth requirements and prolonged incubation time for availability of result that makes it unfavorable for many laboratories especially in resource poor countries like us.[24] There is a need for future studies to look into various other preventable risk factors like smoking, stress, NSAIDS over usage, unsafe drinking water. By removing or at least minimizing these risk factors we can get maximum successful results of the eradication therapy in our setup. Another study by Schulz TR *et al* also commented upon the cost effectiveness of HpSAg test in comparison to other tests available for early diagnosis and treatment of H. *pylori* infections to reduce the development of complications like peptic ulcer disease and gastric cancers.[24] The confirmation of eradication can easily be performed after 4 weeks of completion of therapy as we did which helped us in detecting the success or failure of treatment.[25]

LIMITATIONS

Small study group, Single Centered, gastric biopsies were not received from all HpSAg test positive patients.

CONCLUSION

To conclude, Stool for *H. pylori* Antigen test is a low cost, rapid and an important non-invasive test that can aid in timely diagnosing the cases of chronic gastritis due to *H. pylori* infection. It is user friendly that requires no technical expertise that make it ideal test for clinical laboratories of underdeveloped countries with no histopathology and endoscopy set-ups. It is a point of care test with a beneficial prognostic value that also guides the clinicians regarding the treatment selection.

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