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Usefulness of Gastropanel for Validation of Efficacy of Drugs from Traditional Systems of Medicine in Functional Dyspepsia

Sharada Hendre[1], Poonam Gupte[2], Supriya Bhalerao[2], Madhavi Mahajan[1], Ajit S. Kolatkar[3]*

Abstract

Gastropanel, serological ELISA test comprising of stomach biomarkers; serum pepsinogen I, pepsinogen II, gastrin-17, and *Helicobacter pylori* antibody depicts clearly the morphological and functional status of stomach mucosa in patients suffering from dyspepsia. Although the traditional system of medicine is a huge resource of efficient formulations useful in gastrointestinal disorders such as functional dyspepsia, lack of robust scientific evidence, and qualitative/subjective parameters like symptom scores do not suffice the need for the same. This study was thus planned to assess usefulness of gastropanel tests to validate efficacy of *Avipattikar choorna*, well-known antacid remedy in functional dyspepsia. *A. choorna* was given to patients of dyspeptic disorders following which gastropanel was performed pre- and post-treatment. The gastropanel findings obtained, prior and post-interventions were compared using Wilcoxon MPSR test and a level of $P < 0.05$ was considered for statistical significance. It was observed that although symptom scores showed improvement in all patients after treatment, change in pre- and post-values of gastropanel was seen only in few patients. Gastropanel could differentiate between true responders and non-responders, identification of which was difficult merely with symptom scores. Another highlight was that *A. choorna* proved more effective in *H. pylori* IgG positive cases. Gastropanel may be used as an effective tool to understand and validate the efficacy of traditional medicines in functional dyspepsia and gastric disorders along with its effect on stomach physiology and acid regulation. Need for employment of quantitative/objective parameters for traditional system drugs validation is also highlighted.

Keywords: Gastrin, Gastropanel, *H. pylori*, Pepsinogen, Traditional system of medicine

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Introduction

Gastropanel or stomach function test is an Elisa based non-invasive serological test which uses serum pepsinogen I (PGI), PGII, gastrin-17 (G-17), and *Helicobacter pylori* antibody (HpAb). These stomach specific markers have been proposed to assess the morphological and functional status of the gastric mucosa and are used to diagnose various stomach diseases along with upper gastrointestinal (GI) endoscopy which remains the gold standard. Gastropanel is a proposed first-line diagnostic test for patients suffering from dyspepsia and can be used to differentiate between gastric disorders. The test helps to distinguish stomachs with a normal mucosa or acid dysfunction, from; *H. pylori* gastritis and chronic atrophic gastritis in a general population of dyspeptic patients.[1] It also helps to identify the patients at risk for stomach cancers, peptic ulcer and mal-absorption of Vitamin B12, iron, magnesium, calcium, and some drugs.[2-4] The test helps to non-invasively understand the extent of damage that *H. pylori* infection has caused to the stomach mucosa in terms of atrophy or inflammation. The most of the patients suffering from stomach diseases lack access to higher centers' having advanced endoscopy units required for an accurate diagnosis and therefore clinical evaluation remains the mainstay for diagnosing these diseases. Delayed diagnosis or misdiagnosis in primary healthcare due to over-reliance on clinical symptoms and various other reasons is well documented.[5,6] Accurate diagnosis for appropriation of treatment and early intervention is extremely critical and may require a combination of clinical acumen and diagnostic tests.[7]

There are studies reported wherein Pepsinogens have been used as a diagnostic tool in various gastric disorders.[8,9] Similarly, both Pepsinogens and Gastrin 17 (G17) provide a clear picture regarding the gastric mucosa[10] and its secretory function and evidence, suggest their use in early detection of precancerous lesions.[11] G17 and Pepsinogen levels have been used to assess the anti-secretory action of PPI and H2 blockers in gastroesophageal reflux disease (GERD).[12] Gastropanel has also been researched for its diagnostic accuracy in chronic atrophic gastritis.[13,14] Further Adamczyk *et al*. demonstrated the utility of gastropanel for assessment of effectiveness of *H. pylori* eradication therapy along with overview of inflammatory changes in gastric mucosa.[15] There is a study reported where in validation of these tests in morbidly obese patients before bariatric surgery has been done and concluded to be a good surrogate for the same.[16] Gastropanel is preferred at the initial diagnostic stage before or along with gastroscopy.[17]

The traditional systems of medicine (TSM) are a great resource for many medicinal formulations and have been found to be

[1]Department of Internal Medicine, College of Ayurveda, Bharati Vidyapeeth (Deemed to be) University, Pune - Satara Road, Katraj – Dhankawadi, Pune, Maharashtra, India

[2]Obesity Diabetes Lab, Interactive Research School for Health Affairs, Bharati Vidyapeeth (Deemed to be) University, Pune – Satara Road, Katraj – Dhankawadi, Pune, Maharashtra, India

[3]Department of Integrative Research, Director, I-AGNI (Integrative Ayurveda, Gastroenterology and Nutrition Initiative), Pune, Maharashtra, India.

Corresponding Author: Ajit S. Kolatkar, Director, I-AGNI (Integrative Ayurveda, Gastroenterology and Nutrition Initiative), Founder and Director, GastroLab India Pvt. Ltd, Pune, Maharashtra, India. E-mail: ajitkolatkar@gmail.com

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effective as per literature. There is a general perception about lack of efficacy studies and clinical data to substantiate the claims objectively about usefulness of traditional medicinal formulations in effective management of gastrointestinal diseases. There is hardly any biomarker based clinical studies done on these drugs used for treating upper GI disorders such as functional dyspepsia, GERD, peptic ulcers, *H pylori* infection, atrophic gastritis, or to understand their influence on stomach physiology and acid regulation.

Therefore, it was thought to be of interest and relevance to evaluate the efficacy of formulations from Ayurveda, the Indian traditional system of medicine, using these stomach specific biomarkers. The present study was, therefore, carried out to assess the usefulness of gastropanel for validation of efficacy of Ayurvedic formulations and to understand its influence on stomach specific biomarkers, namely, PGI, PGII, G17, and *H. pylori* antibodies (Hpab). We selected functional dyspepsia as a representative condition of the dysregulated gastroduodenal system and Avipattikar choorna (AVP), a well-known Ayurvedic remedy[18] for treating the same.

Materials and Methods

Patients

Thirteen patients of either sex in the age group of 18–60 years suffering from dyspeptic disorder were identified. Since the study involved use of novel biomarker, gastropanel, permission from the Institutional Ethics Committee (BVDU/COA/2601/2016-17) was obtained. A written informed consent for undergoing gastropanel was obtained from each patient.

Study Drug Details

The study drug AVP was prepared as per the classical method. It consists of 14 ingredients which have been shown in tabular format [Table 1].

All the ingredients were taken in mentioned quantity, powdered in a pulverizer separately, sieved (80 mesh size), mixed, and a homogenous blend was obtained. The raw material used was authenticated and standardized before preparation of the formulation. Since the objective of our work was not to evaluate efficacy of AVP, but to prove the utility of gastropanel for evaluation of Ayurvedic medicines, no control group was used. The drug was administered to the participating individuals at a dose of 4 g twice a day with warm water before meals for 1 month.

Table 1: Composition of AVP

Ingredients	*Ratio*
Amalaki (*Phyllanthus emblica*)	1 part
Haritaki (*Terminalia chebula*)	1 part
Bibhitaki (*Terminalia bellerica*)	1 part
Sunthi (*Zingiber officinale*)	1 part
Marich (*Piper nigrum*)	1 part
Pippali (*Piper longum*)	1 part
Mustaka (*Cyperus rotundus*	1 part
Vidanga (*Embelia ribes*)	1 part
vida lavana (black salt)	1 part
Ela (*Elettaria cardamomum*)	1 part
Tvak (*Cinnamomum tamala*)	1 part
Lavang (*Syzgium aromaticum*)	11 parts
Trivrit (*Operculina turpethum*	44 parts
Sugar	66 parts

Study Evaluation

The symptoms of dyspepsia were evaluated on a weekly basis. However, the focus was on stomach specific biomarkers (gastropanel) which were evaluated prior and post-intervention period, that is, 1 month after the treatment. For estimation of gastropanel, 3 ml blood sample was collected from each patient in EDTA bulbs. It was subjected for centrifugation and 1.5–2 ml plasma was separated. To this G17 stabilizer was added (1 ml/1 drop) to prevent degradation at ambient room temperature and stored in plain bulbs at −20°C. This was followed by ELISA estimation. The serological biomarker test (GastroPanel®, Biohit Oyj, Helsinki, Finland) which was performed on an automated ELISA test measured the plasma levels of the following biomarkers: PgI and PgII, fasting (basal) and stimulated amidated G17 (G17b and G17 s), and HP antibodies (HPAb). The following reference values of the four biomarkers were used: PgI 30–160 μg/l; PgII 3-15 μg/l; PgI/PgII ratio 3–20, G17b 1-7 pmol/l; G17 s 3–30 pmol/l; and HPAb<30EIU.[19] The patients were followed up every week to assess clinical symptomatology. The results obtained were interpreted through Gastrosoft® software.

Statistical Analysis

The parametric data are shown as mean ± SD while non-parametric data are shown as Median (range). The findings obtained from the gastropanel prior and post-intervention are compared using Wilcoxon MPSR test. A level of $P < 0.05$ was considered for statistical significance.

Results

Out of 13 patients who were started on AVP, only 10 patients completed the treatment for 1 month. The average age of individuals was 37.44 ±10.23 years. There were seven females and three males in the study. Patient flow pertaining to the study is given here [Figure 1].

It was observed that the stomach specific biomarkers showed variations following the treatment with AVP. The levels of serum PG-I marginally decreased while PG-II levels remained largely unchanged. G17 levels also marginally reduced and *H. pylori* IgG levels reduced compared to baseline values [Table 2].

To further assess utility of gastropanel on the clinical outcomes, we studied the comprehensive effect on the gastric markers in individual patients. Table 3 represents the pre- and post-treatment

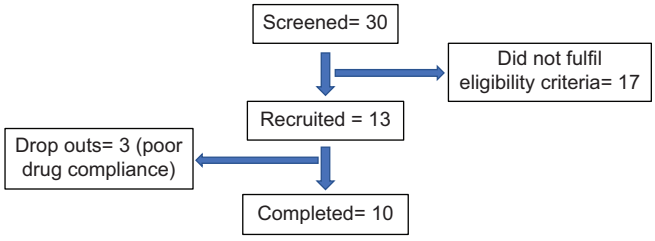

Figure 1: Consort flow diagram

Table 2: Effect of AVP on gastropanel markers

Gastric biomarker	*Pre-treatment*	*Post-treatment*	*P value*
Pepsinogen I (μg/l)	51.4 (6.6–110.3)	32.9 (21–88.5)	0.4316
Pepsinogen II (μg/l)	6 (1.6–12.8)	4.6 (2.2–15.6)	0.2500
PG I/PG II	7.8 (2.7–16.1)	8.5 (5.6–11.4)	0.8457
Gastrin 17(pmol/l)	4.7 (0.8–28.7)	2.9 (0.8–28.7)	0.7344
HpAb IgG (EIU)	69.1 (16–362.9)	21.3 (17.5–237)	0.0840

Table 3: Individual-wise details of markers prior and post-treatment

Age (years)	*PG I (µg/l) Pre*	*PG I (µg/l) Post*	*PG II (µg/l) Pre*	*PG II (µg/l) Post*	*PG I/ PG II Pre*	*PG I/ PG II Post*	*G 17 (pmol/l) Pre*	*G 17 (pmol/l) Post*	*H. pylori IgG (EIU) Pre*	*H. Pylori Igg (Eiu) Post*	*Remarks Pre*	*Remarks Post*
30	71.5	26.2	9.6	4.6	7.4	5.6	5.9	9.3	116.5	237	*H. Gastropanel Pylori* infection but normal acid output	Reduction in PG 1 levels indicate loss of function of Chief cells. Transient increase in *H. pylori* IgG levels, increase in G 17 levels suggestive of reduced acid output
43	48.3	36.1	12.8	4.6	3.8	7.8	0.8	0.8	294.2	23.6	*H. pylori* Infection with increased acid output	Reduction in *H. pylori* IgG levels
41	60.6	58	9.7	7.4	6.2	7.8	1.4	3.5	362.9	27	*H. pylori* infection slightly increased acid output	Reduction in *H. pylori* IgG levels. Normal stomach mucosa with normal acid output
40	25.2	29.6	1.6	3.1	16	9.7	0.8	1.3	20.1	18.3	Corpus Atrophy (Loss of function of chief cells)	A rise in PG1 levels may be indicative of a slight improvement in function of parietal cells. G 17 levels normalized indicates normal acid secretion
19	54.6	29.6	4.3	2.9	12.6	10.4	1.2	2.2	16	19.4	Normal Stomach mucosa normal acid output	Drop of PG1 levels below normal values suggest s a transient reduction in function of chief cells, Normal acid output
59	35.3	42.7	5.1	4.7	7	9.2	28.7	1.7	23.2	18.5	Reduced acid output	The acid secretion normalized with a significant drop in G 17 levels
38	110.3	60.9	6.8	5.3	16.1	11.4	11.5	23.4	22.2	17.5	Reduced acid output	Increase in G17 levels indicates a further reduction of acid output
40	18	21	2.2	2.2	8.2	9.4	3.5	0.8	27.3	18.2	Corpus atrophy (loss of function of chief cells)	Loss of function of Chief Cells, A drop in G 17 levels suggestive of increase in acid output
33	6.6	88.5	2.5	15.6	2.7	5.7	19.9	28.7	110.8	43.6	Corpus atrophy (loss of function of chief cells) due to *H. pylori* Infection with reduced Acid output	Significant drop in *H. pylori* levels with a simultaneous rise in PG 1 levels suggestive of improved function of chief cells with no change in acid output status with raised G 17 values
34	64.2	28.4	7.9	3.8	8.1	7.4	9.8	4.9	119	23.1	*H. pylori* Infection reduced acid output	Significant drop in *H. pylori* levels, reduction in G 17 levels indicative of Normal acid output. A reduction in PG 1 levels is observed suggestive of a transient loss of function of Chief Cells

*PG I: Pepsinogen I, PG II: Pepsinogen II, G 17: Gastrin 17, *H. pylori* IgG: *Helicobacter pylori* Immunoglobulin G, µg/l: microgram/litre, pmol/l: picomoles/litre

levels in each patient along with the clinical remarks. The response to therapy was positive with considerable improvement in the clinical symptomatology [Table 4].

Discussion

The present study was carried out to explore utility of gastropanel tests in validating efficacy of traditional medicine formulations. We observed that there was considerable improvement in the symptoms of dyspepsia in all patients. However, there was huge variation in the changes observed in the median values of the gastropanel.

Of the different markers in gastropanel, pepsinogen is an inactive precursor of pepsin and comprises of different isoenzymes, PG I and II. They are secreted from different mucosal sites of the stomach and are activated after HCL cleaves pepsinogen and gets converted to pepsin at a stomach pH < 3. It is most active at a pH of 2 while is completely inactivated at a pH > 6.5.[20] PG I is secreted by gastric chief cells in the corpus while PG II by the chief cells and the Brunner's glands in the corpus as well as the antral stomach mucosa.[21] Reduction in PG I levels below the normal cutoffs correlates with loss of function of chief cells of the stomach(corpus) while PG II levels decrease with amelioration of inflammation and is a useful tool for monitoring the outcome of anti *H. Pylori* therapy.[22]

H. Pylori infection is a common reason for elevation in the levels of PG II, characterized by increase in neutrophil and mononuclear cell infiltration resulting in mucosal damage with alteration in mucosal integrity,[23,24] a phenomenon common to chemical induced gastritis.[25] G17 is a gastrointestinal hormone secreted by the antral G cells of gastric antrum and regulates the gastric acid secretion.

H pylori is a Class 1 carcinogen and long-term exposure to *H. pylori* infection may result in atrophic gastritis and subsequently stomach cancers.[26] *H. pylori* being a chronic infection, the IgG antibodies estimation is a well-established marker for diagnosis of *H. pylori* infection and measuring the therapeutic outcome of anti *H. Pylori* therapy.[27]

It was observed that AVP choorna influenced all these markers inconsistently. AVP choorna marginally decreased median levels of serum PG I indicating that the drug may have influenced acid secretion. The levels of PG II remained largely unchanged suggesting that the ayurvedic formulation was safe for gastric mucosa, did not cause any inflammatory response, and was safe even at a high dosage. None of the patients had prior higher levels of PG II to indicate inflammation in gastric mucosa. Interestingly, Hpab levels were reduced compared to baseline values indicating potential role of AVP in decreasing *H. Pylori* infection. This was congruent with the finding of an increase in the ratio of PG I/PG II in some patients, which is a significant indicator of eradication of *H. pylori*,[28] thus underlining the effect of Ayurvedic formulation. There was a drop in median levels of G17, but to understand the true effect of the choorna on Gastrin17, higher sample size and appropriate patient selection may be required. G17 stimulates parietal cells to secrete HCl and, therefore, it may be of interest to understand the influence of various Ayurvedic formulations on the peptide. A low G 17 suggests a higher acid output or hyperchlorhydria and high levels of G 17 suggest lower acid output or hypochlorhydria.

Table 4: Effect of AVP on clinical symptoms

Symptoms	*Pre-treatment*	*Post-treatment*	*P value*
Acidic eructation	2 (0–3)	0 (0–1)	0.0016
Heartburn	2 (2–3)	0 (0–1)	<0.0001
Throat burn	2 (1–3)	0 (0–1)	0.0002
Regurgitation	2 (2–3)	0 (0–1)	<0.0001
Indigestion	3 (2–3)	1 (0–1)	<0.0001

Grading pattern: 0-nil, 1-mild, 2-moderate, 3-severe. AVP: Avipattikar choorna

The difference in any of the biomarkers did not reach to statistical significance probably due to small sample size and huge inter-individual variation. It is important to note that we did not employ gastropanel for diagnosis/prognosis or to select patients according to some cutoffs. We used gastropanel only as a tool to understand the influence of AVP in symptomatically diagnosed dyspeptic patients.

We also studied changes in gastropanel in individual patients (not as grouped values). It was seen that of the 10 patients, one patient had negative response to therapy, two patients had moderate response, and one patient did not show much alteration. The remaining six patients responded positively to treatment. Out of 10 symptomatic patients, one patient had a normal stomach mucosa and normal acid output and two patients had reduced acid output. Five patients had *H pylori* infection with or without acid dysfunction and one out of these five, had corpus atrophy (loss of function of chief cells). The remaining two cases were of corpus atrophy, a pre-cancerous condition that warrants advanced endoscopic follow-up. This condition would have got missed if the diagnosis would have been made on the basis of symptoms only. There were only six individuals in the study who had altered acid secretion status, of which four were with reduced acid output and two were with increased acid output. The choice of AVP for these different pathologies is doubtful. Interestingly, *H. pylori* infection was found significantly reduced after the treatment with AVP. This may provide a new indication for the time-tested formulation of Ayurveda.

Our observations thus bring forth the critical importance and usefulness of gastropanel in diagnosis and prognosis of patients suffering from uninvestigated or investigated dyspepsia. The future studies using different formulations recommended for stomach related disorders can strengthen the use of gastropanel further. One of the commonly posed issues in case of research on TSM is use of "soft" end points such as symptom complexes diluting the quality of research findings. Gastropanel can prove as an effective "hard" end point for research in the area of GI diseases using TSM formulations,[29] thereby suggesting its utility for validation of efficacy of these formulations in functional dyspepsia and potentially other gastric disorders as well.

Conflict of Interest

The authors declare no potential conflicts of interest with respect to research, authorship, and/or publication of this article.

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