

Assessment of Salivary pH and Purisha Jala Nimajjana Pariksha as Non-Invasive Diagnostic Correlates of Doshaja Subtypes of Ajirna (Indigestion): An Observational Analytico-Correlational Study

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Abstract

Background: Ajirna (indigestion) is described in Ayurveda as a manifestation of Agnimandya (impaired digestive fire) leading to Ama formation. Diagnosis of its doshaja subtypes—Amajirna, Vidagdhajirna, and Vishtabdhajirna—primarily depends on subjective clinical assessment. Purisha Jala Nimajjana Pariksha (stool-water immersion test), a classical method for detecting Ama, lacks standardization, while salivary pH has not been adequately evaluated as a non-invasive biomarker in Ajirna.

Objectives: To standardize Purisha Jala Nimajjana Pariksha, evaluate salivary pH in different subtypes of Ajirna and healthy individuals, and determine the correlation between salivary pH and doshaja classification.

Materials and Methods: A two-phase analytico-correlational observational study was conducted at the Mahatma Gandhi Ayurved College, Hospital and Research Centre, Wardha, after ethics approval. Phase I standardized Purisha Jala Nimajjana Pariksha by evaluating water quality and quantity, container material, stool consistency, and drop height. Phase II included 232 participants (58 each with Amajirna, Vidagdhajirna, Vishtabdhajirna, and healthy controls; 18–60 years). Clinical symptoms were graded (0–3), salivary pH was measured in triplicate using a calibrated digital pH meter, and stool behaviour in water was documented. Pearson's and Spearman's correlation coefficients and multiclass ROC analysis were performed.

Results: Salivary pH increased progressively from Amajirna (predominantly 5.5–<6.0) to Vidagdhajirna (6.0–<6.5), Vishtabdhajirna (6.5–<7.0), and healthy controls (7.0–<7.5). A strong positive correlation was observed between salivary pH and health status (Spearman $\rho = 0.90$; $p < 0.001$). ROC analysis demonstrated excellent discrimination for healthy controls (AUC = 0.95), but limited discrimination among individual Ajirna subtypes.

Conclusion: Salivary pH objectively reflects the classical Agni–Ama continuum and effectively differentiates healthy from diseased states. However, it should be interpreted alongside standardized Purisha Jala Nimajjana Pariksha and classical Lakshana Pariksha for accurate subtype diagnosis.

Keywords: Ajirna, Agni, Ama, Salivary pH, Purisha Jala Nimajjana Pariksha, Dyspepsia.

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Introduction

Ajirna (indigestion) is described in Ayurveda as the consequence of impaired Jatharagni, resulting in incomplete digestion of food and the formation of Ama, the principal pathological factor responsible for gastrointestinal as well as systemic diseases.[1] Agni governs the transformation of Ahara into Ahara Rasa and subsequently into the Dhatus, thereby maintaining health, strength and longevity. Disturbance of Agni due to inappropriate diet, faulty lifestyle, suppression of natural urges, improper sleep, seasonal indiscretions and psychological factors such as anger, fear and jealousy culminates in Ajirna.[2] Classical texts describe three major doshaja subtypes—Amajirna (Kapha predominant), Vidagdhajirna (Pitta predominant) and Vishtabdhajirna (Vata predominant)—each characterized by distinct

symptom complexes.[4] These descriptions closely parallel the modern concept of functional dyspepsia, providing a rationale for developing objective diagnostic methods that complement traditional clinical assessment.

Among the classical diagnostic tools, Purisha Jala Nimajjana Pariksha (stool-water immersion test) is described as a method for assessing the presence of Ama, wherein Ama-associated stool sinks while properly digested (Nirama) stool floats.[5] Despite its classical importance, the test has not been adequately standardized for routine clinical application. Salivary pH, a simple, non-invasive biochemical parameter reflecting oral and gastrointestinal physiology, has also been shown to vary with several metabolic and digestive disorders and may serve as an objective indicator of Agni-Ama status. However, previous studies have largely evaluated Purisha Jala Nimajjana Pariksha only

in healthy volunteers, while the relationship of salivary pH with the individual doshaja subtypes of Ajirna has remained unexplored. Consequently, the diagnosis of Ajirna continues to rely predominantly on subjective symptom assessment.

The present study was therefore undertaken to standardize Purisha Jala Nimajjana Pariksha using a systematic methodology and to evaluate its clinical utility in patients with Ajirna. Simultaneously, salivary pH was assessed across the three classical doshaja subtypes of Ajirna and compared with healthy individuals to determine its correlation with disease status. The study also aimed to validate a symptom-based assessment scale for Amajirna, Vidagdhajirna and Vishtabdhajirna and to explore whether combining standardized Purisha Jala Nimajjana Pariksha with salivary pH could provide a more objective and reproducible diagnostic approach for Ajirna than symptom assessment alone.

1. Materials and Methods

1.1 Study Design and Setting

This was a hospital-based, observational, analytico-correlational study conducted in two sequential phases at the Department of Roga Nidana evam Vikriti Vijnana, in the outpatient and inpatient services of the Mahatma Gandhi Ayurved College, Hospital and Research Centre, Salod (Hirapur), Wardha, Maharashtra, India, a constituent unit of Datta Meghe Institute of Higher Education and Research (Deemed to be University). The study was designed and reported in accordance with the Standards for Reporting Diagnostic Accuracy Studies (STARD 2015) guidelines to ensure methodological transparency and reproducibility.

Phase I comprised the standardization of Purisha Jala Nimajjana Pariksha on healthy volunteers. Phase II comprised the clinical application of the standardized test, together with salivary pH estimation and structured symptom scoring, in patients with the three doshaja subtypes of Ajirna and in healthy controls.

1.2 Ethical Considerations

The study protocol was approved by the Institutional Ethics Committee prior to commencement. Written informed consent was obtained from every participant after explaining the purpose, procedures and voluntary nature of participation, including the right to withdraw at any stage without providing a reason. Confidentiality of participant data was maintained throughout.

1.3 Phase I: Standardization of Purisha Jala Nimajjana Pariksha

Purisha Jala Nimajjana Pariksha is a classical diagnostic method for the detection of Ama in Purisha (stool); a stool mass that sinks in water is considered indicative of Ama, whereas a floating stool mass is considered indicative of Nirama (properly processed) matter. Because prior descriptions of this test were largely

qualitative, a structured standardization exercise was undertaken to establish reproducible testing conditions.

1.3.1 Participants

Healthy volunteers aged 18-60 years, free of gastrointestinal or other confounding disease, were recruited for this phase and were provided a low-fat diet for 48 hours prior to testing in order to minimize dietary confounders affecting stool consistency.

1.3.2 Sample Collection

Stool samples were collected in 30 ml wide-mouth propylene containers and tested within one hour of evacuation to prevent post-collection alteration of consistency. The presence of classical Saam Mala Lakshana was noted prior to testing, and stool consistency was graded using the Bristol Stool Scale (Type 1 to Type 7); Type 1 (Atisanghata, excessively hard) and Type 7 (Atidrava, excessively liquid) were excluded a priori, as classical texts contraindicate their use for this test owing to the likelihood of false-positive or false-negative results.

1.3.3 Variables Standardized

Building on and extending the pioneering work of Uikey and Kar,[6] the following pre-specified variables were systematically varied and tested: (i) quality of water (tap water, distilled water, mineral water); (ii) quantity of water (50 ml, 100 ml and 250 ml glass beakers); (iii) testing-container material (glass versus plastic); (iv) stool consistency (Bristol types 2, 3, 4 and 6); (v) quantity of stool sample (5 ml, 10 ml, 15 ml); and (vi) height from which the stool sample was dropped into water (2 cm, 5 cm, 10 cm and 15 cm). The finalized parameters, selected on the basis of reproducibility, feasibility and visual clarity of the sink/float endpoint, are summarized in Table 9.

Table 9. Standardized parameters of Purisha Jala Nimajjana Pariksha (Phase I)

Variable	Range tested	Finalized parameter
Quality of water	Tap water, distilled water, mineral water	No significant difference; potable tap/mineral water adopted
Quantity of water	50 ml, 100 ml, 250 ml	No significant difference; 100 ml glass beaker adopted for feasibility

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Testing container	Plastic, glass	No significant difference; glass container adopted for optical clarity
Stool consistency (Bristol scale)	Type 1-7	Types 1 and 7 excluded (Atidrava/Atisanghata contraindicated); types 2, 3, 4 and 6 retained
Quantity of stool sample	5 ml, 10 ml, 15 ml	No significant difference; minimum 2 ml, working volume 10 ml adopted
Height of drop	2 cm, 5 cm, 10 cm, 15 cm	No significant difference; minimum height of 2 cm adopted
Time to testing	Within 1 hour of collection	Retained to avoid post-collection alteration

1.4 \Phase II: Clinical Assessment in Ajirna

1.4.1 Participants and Setting

Patients aged 18-60 years presenting with classical symptoms of Ajirna to the OPD and IPD of the Department of Roga Nidana were screened and enrolled.

1.4.2 Inclusion Criteria

Patients of either sex, aged 18-60 years, exhibiting classical symptoms corresponding to one of the three doshaja subtypes of Ajirna (Amajirna, Vidagdhajirna, Vishtabdhajirna) as detailed in Table A (Supplementary Assessment Criteria), were included.

1.4.3 Exclusion Criteria

Patients with organic dyspepsia (structural gastrointestinal disease demonstrable on investigation) were excluded, in order to isolate functionally defined Ajirna from disease attributable to a demonstrable organic lesion.

1.4.4 Sample Size Estimation

Sample size was calculated using the formula for comparison of means, based on pilot-study values of salivary pH (Amajirna: mean 5.9 +/- SD 1.08; Vidagdhajirna: mean 6.5 +/- SD 1.12), giving a mean difference of 0.6 and a pooled standard deviation of 1.1. This yielded a calculated requirement of 53 participants per group; after adjusting for an anticipated 10% attrition, a final sample size of 58 participants per group was adopted. Accordingly, 58 patients each with Amajirna, Vidagdhajirna and Vishtabdhajirna, together with 58 age- and sex-comparable healthy volunteers, were enrolled, giving a total study population of 232.

1.4.5 Study Tools and Assessment Criteria

Data were collected using a specially designed and pre-validated case-record proforma covering demographic details, Ashtavidha Pariksha (eight-fold clinical examination), Dashavidha Pariksha (ten-fold constitutional examination), and Ahara-Vihara (dietary and lifestyle) history, in addition to disease-specific parameters.

Subjective assessment: A 21 item based Questionnaire was developed for assessing the classical symptoms specific to each doshaja subtype were scored on a 4-point ordinal scale (0 = absent, 1 = occasional, 2 = frequent, 3 =

continuous/severe). Amajirna was assessed on four cardinal symptoms - Udara Guruta (abdominal heaviness), Utklesha (nausea), Shotha of Ganda-Akshikuta (periorbital/facial oedema), and Udgara Bhuktavata-Avidagdhha (undigested eructation) - with a maximum composite score of 12. Vidagdhajirna was assessed on seven symptoms - Bhrama (giddiness), Trishna (excessive thirst), Murchha (syncope), Vividha Ruja (burning-type pain), Amla-Sadhuma Udgara (sour/fetid eructation), Daha (burning sensation) and Sweda (excessive perspiration) - with a maximum composite score of 21. Vishtabdhajirna was assessed on six symptoms - Adhmana (abdominal distension), Shula (abdominal pain), Angapeedana (body ache), Apravritti of Mala and Vata (obstruction to stool and flatus), Sthambha (stiffness) and Moha (mental clouding) - with a maximum composite score of 18.

Objective assessment: (i) Salivary pH was measured using a calibrated electronic India manufactured digital pH meter (Hanna Instruments); participants refrained from oral intake for at least one hour prior to measurement, and the mean of three consecutive readings was recorded for each participant.

(ii) Purisha Jala Nimajjana Pariksha was performed on each participant's stool sample using the standardized protocol established in Phase I, and the outcome (sinking or floating) was recorded. (iii) Stool routine and microscopic examination were performed to corroborate the presence of Ama-associated changes (mucus, undigested food residue) and to exclude organic or parasitic pathology.

1.5 \Statistical Analysis

Descriptive statistics (frequencies and percentages) were used to summarize demographic and clinical characteristics across the four study groups. The association between salivary pH and group (health status) was examined using Spearman's rank correlation coefficient (for monotonic association); a two-tailed p-value < 0.05 was considered statistically significant. One-way analysis of variance (ANOVA) was used to compare mean salivary pH across the four groups. The diagnostic discriminatory ability of salivary pH for each group was evaluated using multiclass receiver-operating-characteristic (ROC) curve analysis, with area under the curve (AUC), sensitivity and specificity computed at pre-specified pH cut-off bands. Statistical analyses were performed using standard statistical software.

2. Results

A total of 232 participants were enrolled and completed the study, comprising 58 patients each with Amajirna, Vistabdhajirna and Vidagdhajirna, and 58 healthy volunteers. No participant was lost to follow-up during data collection.

2.1 Demographic and Socio-Clinical Profile

The majority of participants across all groups belonged to the 29-38 year age band (Amajirna 41.38%, Vistabdhajirna 39.66%, Vidagdhajirna 43.10%, healthy volunteers 41.38%), indicating that Ajirna most commonly presents in early-to-middle adulthood (Table 1). Gender distribution was broadly balanced in Amajirna and Vistabdhajirna, while Vidagdhajirna showed a modest female preponderance (56.90% females versus 43.10% males).

Table 1. Age-wise and gender-wise distribution of study participants

Parameter	Category	Amajirna (n=58)	Vistabdhajirna (n=58)	Vidagdhajirna (n=58)	Healthy volunteers (n=58)
Age (years)	18-28	8 (13.79%)	9 (15.52%)	7 (12.07%)	10 (17.24%)
	29-38	24 (41.38%)	23 (39.66%)	25 (43.10%)	24 (41.38%)
	39-48	16 (27.59%)	15 (25.86%)	14 (24.14%)	15 (25.86%)
	49-60	10 (17.24%)	11 (18.97%)	12 (20.69%)	9 (15.52%)
Gender	Male	30 (51.72%)	29 (50.00%)	25 (43.10%)	30 (51.72%)
	Female	28 (48.28%)	29 (50.00%)	33 (56.90%)	28 (48.28%)

The socio-demographic profile (Table 2) showed a predominantly Hindu, middle-class, married population with secondary-level education across all groups, consistent with the catchment demographics of the study centre rather than a disease-specific association. Vidagdhajirna patients showed comparatively higher proportions of uneducated (17.24%) and primary-educated (31.03%) participants.

Table 2. Socio-demographic profile of study participants

Parameter	Category	Amajirna	Vistabdhajirna	Vidagdhajirna	Healthy volunteers
Religion	Hindu	55 (94.83%)	56 (96.55%)	57 (98.28%)	55 (94.83%)
	Muslim	2 (3.45%)	1 (1.72%)	1 (1.72%)	2 (3.45%)
	Others	1 (1.72%)	1 (1.72%)	0 (0.00%)	1 (1.72%)
Education	Uneducated	5 (8.62%)	6 (10.34%)	10 (17.24%)	7 (12.07%)
	Primary	10 (17.24%)	9 (15.52%)	18 (31.03%)	13 (22.41%)
	Secondary	25 (43.10%)	22 (37.93%)	10 (17.24%)	15 (25.86%)

		0%		)	%)
	Graduate	10 (17.24%)	12 (20.69%)	10 (17.24%)	13 (22.41%)
	Postgraduate	8 (13.79%)	9 (15.52%)	10 (17.24%)	10 (17.24%)
Marital status	Married	48 (82.76%)	49 (84.48%)	50 (86.21%)	55 (94.83%)
	Unmarried	10 (17.24%)	9 (15.52%)	8 (13.79%)	3 (5.17%)
Socio-economic class	Upper	1 (1.72%)	0 (0.00%)	0 (0.00%)	1 (1.72%)
	Middle	45 (77.59%)	38 (65.52%)	43 (74.14%)	37 (63.79%)
	Lower	12 (20.69%)	20 (34.48%)	15 (25.86%)	20 (34.48%)

2.2 Ahara and Vihara (Dietary and Lifestyle) Correlates

Adhyashana (eating before the previous meal is digested) and Vishamasana (irregular timing/quantity of meals) were the most prevalent dietary habits across all three Ajirna groups, in contrast to healthy volunteers, among whom good appetite (86.21%) and Samagni (48.28%) predominated (Table 3). Madhura Rasa (sweet taste preference) was strikingly more prevalent in Amajirna (58.62%) than in the other groups, consistent with the classical Kapha-dominant aetiology of this subtype, whereas Lavana and Katu Rasa preference and Tikshnagni (sharp digestive fire) were markedly more common in Vidagdhajirna (63.79% and 60.34% respectively), in keeping with its Pitta-dominant pathophysiology.

Table 3. Ahara (dietary) pattern of study participants

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Parameter	Category	Amajirna	Vistabdhajirna	Vidagdhajirna	Healthy volunteers	
Type of diet	Vegetarian	25 (43.10%)	24 (41.38%)	20 (34.48%)	28 (48.28%)	
	Mixed	33 (56.90%)	34 (58.62%)	38 (65.52%)	30 (51.72%)	
Dietary habit	Samasana	4 (6.90%)	5 (8.62%)	6 (10.34%)	5 (8.62%)	
	Adhyasana	29 (50.00%)	15 (25.86%)	30 (51.72%)	23 (39.66%)	
	Anasana	3 (5.17%)	4 (6.90%)	2 (3.45%)	0 (0.00%)	
	Vishamasana	22 (37.93%)	34 (58.62%)	20 (34.48%)	30 (51.72%)	
Dominant Rasa	Madhura	34 (58.62%)	11 (18.97%)	8 (13.79%)	15 (25.86%)	
	Amla	22 (37.93%)	23 (39.66%)	32 (55.17%)	26 (44.83%)	
	Lavana	12 (20.69%)	14 (24.14%)	37 (63.79%)	23 (39.66%)	
	Katu	25 (43.10%)	35 (60.34%)	36 (62.07%)	35 (60.34%)	
	Tikta	8 (13.79%)	3 (5.17%)	1 (1.72%)	5 (8.62%)	
	Kashaya	5 (8.62%)	10 (17.24%)	4 (6.90%)	6 (10.34%)	
	Appetite	Poor	13 (22.41%)	18 (31.03%)	10 (17.24%)	4 (6.90%)
		Good	30 (51.72%)	25 (43.10%)	13 (22.41%)	50 (86.21%)
	Agni status	Excessive	15 (25.86%)	15 (25.86%)	35 (60.34%)	4 (6.90%)
		Manda	13 (22.41%)	18 (31.03%)	10 (17.24%)	8 (13.79%)
Sama		18 (31.03%)	15 (25.86%)	10 (17.24%)	28 (48.28%)	
Tikshna		15 (25.86%)	15 (25.86%)	35 (60.34%)	10 (17.24%)	
	Vishama	12 (20.69%)	10 (17.24%)	3 (5.17%)	15 (25.86%)	

Lifestyle correlates (Table 4) revealed that irregular or absent exercise, Divasvapna (daytime sleep) and disturbed sleep patterns were considerably more frequent in all three Ajirna groups than among healthy volunteers, among whom regular exercise (60.34%) and Samyak Nidra (normal sleep, 53.45%) predominated. Saam Mala (Ama-laden stool) was reported in the overwhelming majority of Ajirna patients (Amajirna 86.21%, Vistabdhajirna 82.76%, Vidagdhajirna 79.31%) but in only 20.69% of healthy volunteers, among whom Nirama Mala predominated (79.31%), providing an internally consistent clinical correlate for the subsequent objective Purisha Jala Nimajjana findings.

Table 4. Vihara (lifestyle) and bowel-habit correlates

Parameter	Category	Amajirna	Vistabdhajirna	Vidagdhajirna	Healthy volunteers
Vyayama (exercise)	Regular	17 (29.31%)	15 (25.86%)	16 (27.59%)	35 (60.34%)
	Irregular / occasional / none	41 (70.69%)	43 (74.14%)	42 (72.41%)	23 (39.66%)
Divasvapna (day-sleep)	Present	41 (70.69%)	35 (60.34%)	37 (63.79%)	13 (22.41%)
	Absent				
Nidra (sleep)	Samyak	16 (27.59%)	14 (24.14%)	16 (27.59%)	31 (53.45%)
	Disturbed (Alpa/Ati/Khandita)	42 (72.41%)	44 (75.86%)	42 (72.41%)	27 (46.55%)
Bowel regularity	Regular	25 (43.10%)	24 (41.38%)	22 (37.93%)	43 (74.14%)
	Irregular	33 (56.90%)	34 (58.62%)	36 (62.07%)	15 (25.86%)
Stool character	Sama	50 (86.21%)	48 (82.76%)	46 (79.31%)	12 (20.69%)
	Nirama	8 (13.79%)	10 (17.24%)	12 (20.69%)	46 (79.31%)
	Koshtha	30 (51.72%)	25 (43.10%)	18 (31.03%)	8 (13.79%)
Madhyama	18 (31.03%)	18 (31.03%)	20 (34.48%)	41 (70.69%)	
Mrudu	10 (17.24%)	15 (25.86%)	20 (34.48%)	9 (15.52%)	

2.3 Ashtavidha Pariksha, Prakriti and Manasika Avastha

Nadi (pulse) examination showed Kapha predominance in Amajirna (53.45%), Vata predominance in Vistabdhajirna (46.55%), and Pitta predominance in Vidagdhajirna (55.17%), corroborating the doshaja basis of subtype classification with an independent classical examination technique (Table 5). Sharirika Prakriti followed a similar pattern, with Kapha-Vata constitution more frequent in Amajirna (51.72%) and Vata-Pitta constitution more frequent in Vidagdhajirna (58.62%). Among Manasika Avastha (psychological states), Chinta (anxiety) was the most frequently reported disturbance across all three Ajirna groups, particularly in Vidagdhajirna (53.45%), while healthy volunteers reported markedly lower levels of anxiety, anger and despondency, consistent with the Ayurvedic postulate of a bidirectional Agni-Manas relationship.

Table 5. Ashtavidha Pariksha and Manasika Avastha findings

Parameter	Category	Amajirna	Vistabdhajirna	Vidagdhajirna	Healthy volunteers
Nadi predominance	Vata	11 (18.97%)	27 (46.55%)	16 (27.59%)	21 (36.21%)
	Pitta	16 (27.59%)	15 (25.86%)	32 (55.17%)	17 (29.31%)
	Kapha	31 (53.45%)	16 (27.59%)	12 (20.69%)	20 (34.48%)
	Predominant Manasika bhava	Chinta (anxiety)	25 (43.10%)	31 (53.45%)	4 (6.90%)
	Krodha (anger)	13 (22.41%)	21 (36.21%)	26 (44.83%)	11 (18.97%)

	Dainya (despondency)	16 (27.59%)	11 (18.97%)	25 (43.10%)	7 (12.07%)
Sharirika Prakriti	Kapha-Vata	30 (51.72%)	20 (34.48%)	13 (22.41%)	22 (37.93%)
	Vata-Pitta	15 (25.86%)	25 (43.10%)	34 (58.62%)	13 (22.41%)
	Pitta-Kapha	13 (22.41%)	13 (22.41%)	11 (18.97%)	23 (39.66%)
	Ahara Shakti	Pravara	15 (25.86%)	35 (60.34%)	8 (13.79%)
	Madhyama	30 (51.72%)	25 (43.10%)	13 (22.41%)	48 (82.76%)
	Avara	13 (22.41%)	18 (31.03%)	10 (17.24%)	2 (3.45%)

2.4 Salivary pH Across Study Groups

Salivary pH values demonstrated a clear graded distribution across the four groups (Table 6). Amajirna patients clustered predominantly in the acidic range of 5.5 to <6.0 (62.07%); Vistabdhajirna patients clustered predominantly in the near-neutral range of 6.5 to <7.0 (68.97%); Vidagdhajirna patients clustered predominantly in the moderately acidic range of

6.0 to <6.5 (67.24%); and healthy volunteers clustered predominantly in the slightly alkaline range of 7.0 to <7.5 (79.31%). This progressive rightward shift in salivary pH from Amajirna through Vistabdhajirna and Vidagdhajirna to the healthy state was consistent and monotonic.

Table 6. Distribution of salivary pH across the four study groups (n = 232)

Salivary pH range	Amajirna (n=58)	Vistabdhajirna (n=58)	Vidagdhajirna (n=58)	Healthy volunteers (n=58)
5.5 - <6.0	36 (62.07%)	2 (3.45%)	8 (13.79%)	0 (0.00%)
6.0 - <6.5	13 (22.41%)	14 (24.14%)	39 (67.24%)	1 (1.72%)
6.5 - <7.0	9 (15.52%)	40 (68.97%)	10 (17.24%)	11 (18.97%)
7.0 - <7.5	0 (0.00%)	2 (3.45%)	1 (1.72%)	46 (79.31%)

Correlation Between Salivary pH and Health Status

Both parametric and non-parametric correlation analyses confirmed a strong, statistically significant positive relationship between salivary pH and health status across the ordered categories of Amajirna, Vistabdhajirna, Vidagdhajirna and healthy volunteers (Table 7). Spearman's rank correlation yielded $\rho = 0.90$ ($p < 0.001$), indicating a very strong monotonic association.

Table 7. Correlation between salivary pH and clinical group (health status)

Statistical test	Coefficient	p-value	Inference
Spearman rank Correlation	$\rho = 0.90$	< 0.001	Very strong positive monotonic correlation

2.5 Diagnostic Performance of Salivary pH (ROC Analysis)

Multiclass ROC curve analysis was performed to evaluate the ability of salivary pH to discriminate each group from the remaining three (Table 8). Healthy volunteers were discriminated with excellent accuracy (AUC = 0.95), and neutral-to-alkaline salivary pH (7.0-7.5) demonstrated 79.31% specificity for the healthy state. Vistabdhajirna showed fair discriminatory performance (AUC = 0.58), with peak sensitivity (68.97%) at pH 6.5-6.9. In contrast, Amajirna (AUC = 0.15) and Vidagdhajirna (AUC = 0.33) showed poor discrimination when considered against all other groups combined, although low salivary pH (5.5-6.0) retained a sensitivity of 62.07% for Amajirna specifically, and moderately acidic pH (6.0-6.5) retained a sensitivity of 67.24% for Vidagdhajirna specifically. These findings indicate that while salivary pH is highly effective at distinguishing diseased from healthy states, and demonstrates a consistent directional (ordinal) relationship with disease severity, its stand-alone ability to differentiate between individual doshaja subtypes of Ajirna - particularly between Amajirna and Vidagdhajirna - is limited, reflecting the

overlapping Ama-dominant pathology shared by these two subtypes.

Table 8. Diagnostic performance (multiclass ROC analysis) of salivary pH across study groups

Group	AUC	Discriminatory ability	Peak sensitivity (pH band)
Amajirna	0.15	Very poor	62.07% at pH 5.5-6.0
Vistabdhajirna	0.58	Fair	68.97% at pH 6.5-6.9
Vidagdhajirna	0.33	Poor	67.24% at pH 6.0-6.5
Healthy volunteers	0.95	Excellent	79.31% specificity at pH 7.0-7.5

2.6 Pilot Observations Bridging Phase I and Phase II

Pilot data obtained from 12 participants per group during the transition from standardization (Phase I) to clinical application (Phase II) supported the subsequent full-scale findings (Table 10). Purisha Jala Nimajjana Pariksha showed a predominance of the sinking pattern (indicative of Ama) in Amajirna (11/12) and Vidagdhajirna (8/12), and a predominance of the floating pattern (indicative of Nirama) in Vistabdhajirna (9/12), providing preliminary triangulating evidence between the classical stool-water test and the modern salivary-pH marker, and informing the sample-size calculation for the definitive study.

Table 10. Pilot observations of Purisha Jala Nimajjana Pariksha and salivary pH (n = 12 per group)

Parameter	Amajirna (n=12)	Vidagdhajirna (n=12)	Vistabdhajirna (n=12)
Salivary pH - mean	5.9	6.5	7.1
Salivary pH - range	5.6-6.2	6.4-6.8	7.0-7.4
Purisha Jala Nimajjana - sinking	11	8	3
Purisha Jala Nimajjana - floating	1	4	9

3. Discussion

3.1 Demographic and Clinico-Ayurvedic Correlates

The predominance of Ajirna in the 29-38 year age group in the present cohort is consistent with the greater exposure of this age group to erratic dietary and occupational schedules, and mirrors previously reported epidemiological patterns of functional dyspepsia in economically active adults. The striking concordance between reported dietary Rasa preference and doshaja subtype

- Madhura Rasa predominance in Amajirna and Lavana/Katu Rasa predominance with Tikshnagni in Vidagdhajirna - lends face validity to the classical Ayurvedic construct that habitual Ahara shapes Agni and, consequently, disease phenotype. Similarly, the high prevalence of Chinta (anxiety), Krodha (anger) and Dainya (despondency) among Ajirna patients, contrasted with their marked rarity among healthy volunteers, reinforces the classical description of Manasika Nidana in the aetiology of Ajirna and is consonant with the modern understanding of the gut-brain axis, wherein psychological stress modulates gastric motility, secretory function and visceral sensitivity.

3.2 Standardization of Purisha Jala Nimajjana Pariksha

The standardization exercise undertaken in Phase I addressed a longstanding gap in Ayurvedic diagnostic methodology. Consistent with the earlier observations of Ukey and Kar in healthy volunteers,[7] variation in water quality, water quantity, testing-container material, stool quantity and drop-height did not materially alter the sink/float outcome, suggesting that the test's underlying physical principle - the relative density and cohesion of Ama-laden versus Nirama stool - is robust to procedural variation. The present study extended this standardization by explicitly excluding Bristol stool types 1 and 7 (correlating with the classical contraindications of Atisanghata and Atidrava), thereby reducing the likelihood of false-positive or false-negative readings and rendering the test more reproducible for future application in diverse clinical settings. This methodological rigor is a necessary precondition for Purisha Jala Nimajjana Pariksha to be considered a viable adjunct diagnostic tool alongside modern investigations.

3.3 Salivary pH as an Objective Correlate of the Agni-Ama Continuum

The central finding of this study - a consistent, graded elevation in salivary pH proceeding from Amajirna (most acidic) through Vidagdhajirna and Vistabdhajirna to the healthy state (most alkaline) - provides an objective biochemical correlate for a spectrum of digestive derangement that has historically been characterized only through subjective clinical assessment.[8] In Ayurvedic terms, this gradient parallels the classical description of Agni states: Manda Agni

(weak digestive fire, driven by Kapha) in Amajirna, Vishama Agni (irregular digestive fire, driven by Vata) in Vistabdhajirna, Tikshna Agni (excessively sharp digestive fire, driven by Pitta) in Vidagdhajirna, and Sama Agni (balanced digestive fire) in health. The acidic salivary environment observed in Amajirna may be explained by heightened fermentative and putrefactive activity secondary to Manda Agni and consequent Ama accumulation, producing organic acids that lower salivary pH; whereas the near-neutral to alkaline environment of the healthy state reflects intact salivary buffering capacity and undisturbed systemic acid-base homeostasis. These observations are in keeping with prior reports linking components of metabolic dysfunction to altered salivary pH and flow rate, and with earlier case-control evidence associating a coated tongue (Saam Jivha), a clinical proxy for Ama, with reduced salivary pH.[9]

The strong Spearman ($\rho = 0.90$) correlations, both significant at $p < 0.001$, substantiate the hypothesis that salivary pH is not an incidental finding but a biologically meaningful, quantifiable correlate of the Agni-Ama status underlying Ajirna. The rejection of the null hypothesis in favour of H1 indicates that classical Ayurvedic nosology, though qualitative in origin, maps onto a measurable biochemical continuum - an observation of translational relevance for integrating Ayurvedic diagnostics into evidence-based clinical practice.

3.4 Diagnostic Utility and Its Limits

The ROC analysis clarifies both the strength and the boundary of salivary pH as a diagnostic tool. Its near-excellent performance in identifying the healthy state ($AUC = 0.95$) and its fair performance in identifying Vistabdhajirna ($AUC = 0.58$) indicate genuine clinical utility as a rule-out test and, to a lesser extent, as a rule-in test for Kapha dominant indigestion. However, the poor discriminatory performance for Amajirna ($AUC = 0.15$) and Vidagdhajirna ($AUC = 0.33$) when tested against all other groups reflects the considerable pathophysiological overlap between these two Ama-dominant subtypes - both are characterized by incomplete digestion and Ama accumulation, differing chiefly in the dosha (Pitta versus Vata) driving the obstructive process, a distinction that a single biochemical parameter such as pH may not fully capture. This finding is clinically important: it indicates that salivary pH should not be used as a stand-alone diagnostic or subtyping tool but rather as one component of a composite diagnostic strategy that also incorporates Lakshana Pariksha (symptom-based scoring, as operationalized in the present study through the 0-3 grading scales) and standardized Purisha Jala Nimajjana Pariksha, in keeping with the Ayurvedic principle of multi-parametric (Bahudha) examination.

3.5 Integration with the Modern Understanding of Dyspepsia

The findings of this study resonate with the modern biomedical understanding of functional dyspepsia, wherein impaired gastric accommodation, delayed gastric emptying, visceral hypersensitivity and psychosomatic dysregulation converge to produce a clinical syndrome with substantial symptomatic overlap with Ajirna. The demonstration that a simple, non-invasive, chair-side biomarker such as salivary pH varies systematically with Ayurvedic doshaja diagnosis lends support to the broader proposition that classical Ayurvedic disease categories, despite their qualitative origin, are amenable to objective biochemical validation. This has practical implications for integrative gastroenterology practice, where salivary pH could serve as a low-cost, repeatable screening adjunct in primary-care and Ayurvedic OPD settings, particularly in resource-limited environments where endoscopic or laboratory-based work-up may not be immediately accessible.

3.6 Strengths

The principal strengths of this study include: (i) the adoption of STARD 2015-compliant methodology for standardizing a classical diagnostic test prior to its clinical application; (ii) an adequately powered sample size derived from pilot data; (iii) the inclusion of a healthy control group, enabling contrast between pathological and physiological states; (iv) the use of both parametric and non-parametric correlation statistics together with ROC analysis, providing a comprehensive and statistically rigorous evaluation of diagnostic performance; and (v) the integration of classical Ayurvedic examination techniques (Ashtavidha and Dashavidha Pariksha) with a modern, quantifiable biomarker, exemplifying a genuinely translational research design.

3.7 Limitations

This study has several limitations that merit consideration. First, the cross-sectional, single-centre design limits generalizability and precludes assessment of temporal or treatment-related changes in salivary pH. Second, although Purisha Jala Nimajjana Pariksha was standardized in Phase I, its group-wise sink/float outcomes in the full Phase II cohort require confirmation through a dedicated large-sample analysis; the present results in this regard are supported chiefly by pilot data. Third, potential confounders of salivary pH - including oral hygiene status, recent food or beverage intake beyond the one-hour restriction, smoking, and systemic comorbidities - were not exhaustively controlled. Fourth, the poor discriminatory performance of salivary pH for Amajirna and Vidagdhajirana individually indicates that this biomarker alone cannot substitute for comprehensive clinical

assessment. Finally, as with most Ayurvedic clinical research of this nature, inter-observer variability in classical symptom grading, despite the use of a structured proforma, cannot be entirely excluded.

4. Conclusion

This study demonstrates, for the first time in a structured clinical cohort, that salivary pH exhibits a consistent, statistically significant, graded relationship with the doshaja subtypes of Ajirna, rising progressively from the most acidic values in Amajirna, through intermediate values in Vidagdhajirna and Vistabdhajirna, to near-neutral/alkaline values in healthy volunteers. This gradient provides objective, quantifiable support for the classical Ayurvedic understanding of a continuum linking Agni derangement, Ama accumulation and disease severity. Concurrently, the standardization of Purisha Jala Nimajjana Pariksha in accordance with STARD 2015 guidelines represents a methodological advance that renders this ancient diagnostic test more reproducible and amenable to future clinical validation studies. Taken together, salivary pH and standardized Purisha Jala Nimajjana Pariksha constitute simple, non-invasive, low-cost tools with genuine potential to complement classical Lakshana Pariksha in the objective assessment of Ajirna, though salivary pH alone is best suited to distinguishing diseased from healthy states rather than differentiating between individual doshaja subtypes.

4.1 Scope for Future Research

Future multi-centric studies with larger sample sizes are warranted to validate these findings, to establish population-specific reference ranges for salivary pH across doshaja subtypes of Ajirna, to determine the responsiveness of salivary pH to Ayurvedic (Deepana-Pachana and Shodhana) interventions, and to formally validate the standardized Purisha Jala Nimajjana Pariksha protocol against modern reference standards such as faecal calprotectin or stool microbiome analysis. Combining salivary pH with standardized Purisha Jala Nimajjana Pariksha and validated symptom-scoring instruments in a composite diagnostic algorithm may

further enhance the objectivity and clinical utility of Ayurvedic diagnosis in Ajirna and allied Annavaha Srotas disorders.

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