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ORIGINAL RESEARCH ARTICLE**Correlation of Clinical, Magnetic Resonance Imaging, and Intraoperative Findings in the Preoperative Assessment of Fistula-in-Ano: A Prospective Observational Study**Varun Goswami¹ | Yuvraj Kumar Churendra^{2*} **Abstract**

Background: Accurate preoperative delineation of the fistulous tract, internal opening, and any secondary extensions is essential for successful surgical management of fistula-in-ano, since incomplete identification is a major cause of persistence and recurrence. Magnetic resonance imaging (MRI) has increasingly been adopted as the preoperative imaging modality of choice, but its diagnostic performance must be validated against intraoperative findings in the population in which it is used.

Objective: To assess and correlate clinical examination, MRI, and intraoperative findings in patients with fistula-in-ano, and to determine the diagnostic accuracy of MRI, using intraoperative findings as the reference standard, for the internal opening, tract complexity, secondary tracts, abscess, horseshoe extension, and supralelevator extension.

Methods: This prospective observational study enrolled 30 adults with a clinical diagnosis of fistula-in-ano at a tertiary-care teaching hospital over an 18-month period. All patients underwent digital rectal examination and proctoscopy, followed by pelvic MRI (1.0 Tesla, phased-array coil; T1 and T2-weighted turbo spin-echo sequences in axial, coronal, and optional sagittal-oblique planes) prior to surgery. Fistulas were classified using both the Parks classification and the St. James's University Hospital MRI-based grading system. All patients subsequently underwent examination under anesthesia and definitive surgery (fistulotomy or fistulectomy, with seton placement for high tracts), which served as the diagnostic reference standard. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of MRI were calculated for each finding.

Results: The cohort comprised 26 men and 4 women (mean age 35.6 years; most common age group 31–40 years). All patients had a palpable external opening on examination, most frequently at the 3 o'clock position (20%), while the internal opening was clinically palpable in only 4 of 30 patients (13.3%). MRI identified the internal opening in all 30 patients (100%), most commonly at the 6 o'clock position (26.7%). By Parks classification, intersphincteric fistulas predominated (63.3%), followed by transsphincteric (26.7%), suprasphincteric (6.7%), and extrasphincteric (3.3%) tracts. Intraoperatively, simple tracts were confirmed in 17 patients (56.7%) and complex tracts in 13 (43.3%); of the complex tracts, abscess was present in 5 (38.5%), secondary tracts in 4 (30.8%), horseshoe extension in 3 (23.1%), and supralelevator extension in 1 (7.7%). Overall MRI–surgical concordance was 86.7% (26/30). MRI showed a sensitivity/specificity/PPV/NPV of 100%/84.6%/89.4%/100% for simple tracts, 100%/89.5%/100%/84.6% for complex tracts, 75%/88.8%/75%/88.9% for secondary tracts, 80%/100%/100%/88.8% for abscess, and 33.3%/90%/50%/81.8% for horseshoe extension.

Conclusion: MRI is a highly accurate, noninvasive preoperative tool for delineating fistula anatomy and shows excellent diagnostic performance for the internal opening, tract complexity, and abscess, but more limited sensitivity for horseshoe extension in this cohort. Intraoperative findings remain the diagnostic reference standard, and MRI is best used as a complementary road map that informs, rather than replaces, careful intraoperative assessment.

Key words: fistulainano, magnetic resonance imaging, anal fistula, perianal sepsis, Parks classification, St James's University Hospital classification, diagnostic accuracy, fistulotomy

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1 | INTRODUCTION

A fistula is an abnormal communication between two epithelium-lined surfaces. Fistula-in-ano refers specifically to an abnormal tract connecting the epithelialized anal canal to the perianal skin, most commonly arising from suppuration of the anal glands within the intersphincteric space (the cryptoglandular hypothesis) (1). The condition has been recognized since antiquity, with descriptions attributed to Sushruta around 600 BCE and to Hippocrates approximately two centuries later; several of Hippocrates' management principles, including seton drainage, remain conceptually relevant today (1).

Fistula-in-ano is not rare: reported incidence ranges from approximately 1 to 2 per 10,000 population annually, with a male-to-female ratio of roughly 2:1 and peak incidence in the third and fourth decades of life (2, 3). Although many fistulas are straightforward to recognize and treat, a substantial minority are complex, with secondary extensions, horseshoeing, or supralelevator involvement that markedly increase the technical difficulty of surgery and the risk of recurrence or incontinence if unrecognized (4).

The definitive treatment of fistula-in-ano remains surgical, but outcomes depend critically on accurate preoperative localization of the internal opening and the full course of the primary and any secondary tracts (4). Historically, this assessment relied on clinical examination, Goodsall's rule, and examination under anesthesia alone; however, clinical examination frequently fails to identify the internal opening or to fully characterize complex tract anatomy, particularly in high or recurrent fistulas (5). Cross-sectional imaging, and MRI in particular, has therefore assumed an increasingly central role in preoperative assessment. MRI allows identification of infected tracts and abscess cavities that might otherwise remain occult, provides a detailed anatomic road map of the relationship between the fistula and the anal sphincter complex, and can thereby guide the surgeon toward the procedure best suited to eradicate sepsis while preserving continence (6, 7).

Two classification systems are in widespread use for describing fistula anatomy on imaging and at surgery: the surgical (Parks) classification, which

categorizes tracts by their relationship to the sphincter complex as intersphincteric, transsphincteric, suprasphincteric, or extrasphincteric (5); and the MRI-based St. James's University Hospital classification, a five-grade system that additionally incorporates the presence of secondary tracts, abscesses, and horseshoe or supralelevator extension, and which was specifically designed to standardize radiological-surgical communication (6). While several studies, discussed in detail below, have reported the diagnostic accuracy of MRI against intraoperative findings, results vary across populations, scanner protocols, and reader experience, underscoring the value of institution-specific validation.

This study was undertaken to prospectively assess and correlate clinical, MRI, and intraoperative findings in a cohort of patients with fistula-in-ano at a single tertiary-care center, and to determine the sensitivity, specificity, and predictive values of MRI for the internal opening, tract complexity, secondary tracts, abscess, horseshoe extension, and supralelevator extension, using intraoperative findings as the reference standard.

2 | MATERIALS AND METHODS

2.1 | Study Design and Setting

This prospective observational study was conducted jointly by the Departments of General Surgery and Radiodiagnosis at a tertiary-care teaching hospital in central India over an 18-month period (April 2018 to September 2019). The study protocol was approved by the Institutional Ethics Committee, and written informed consent was obtained from all participants prior to enrolment.

2.2 | Study Population

Adults (18 years of age or older) with a clinical diagnosis of fistula-in-ano who subsequently underwent surgery were eligible for inclusion. Patients with MRI-incompatible implants or devices, those who were hemodynamically unstable, uncooperative, or on life support, and those with claustrophobia precluding MRI were excluded. A total of 30 consecutive eligible patients were enrolled.

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2.3 | Clinical Assessment

All patients underwent a standardized clinical history and examination, including digital rectal examination and proctoscopy, to document the presence and clock-face location of the external opening and, where palpable, the internal opening, prior to imaging.

2.4 | MRI Protocol

Pelvic MRI was performed on a 1.0 Tesla scanner using a phased-array surface coil, without bowel preparation. Patients were imaged supine, with the field of view planned to include the distal rectum, anal canal, internal and external sphincters, ischiorectal fossae, levator plate, and supralelevator space. The imaging protocol comprised T1-weighted turbo inversion recovery magnitude (TIRM) sequences in the axial and coronal planes (with an optional sagittal-oblique sequence), and T2-weighted turbo spin-echo (TSE) sequences in the axial and coronal planes, the coronal plane being oriented parallel to the long axis of the anal canal as defined on the sagittal survey.

2.5 | MRI Interpretation and Classification

Fistulous tracts were defined as high-signal-intensity linear structures on T2-weighted and fat-saturated images in continuity with the sphincter complex, with surrounding hyperintensity interpreted as adjacent inflammatory change. The internal opening was localized on axial images using the anal clock convention (12 o'clock anterior, 6 o'clock posterior). Fistulas were differentiated from discrete abscess cavities using the criteria of Lunniss et al. (7), whereby fluid-filled tubular structures under 10 mm in diameter were classified as tracts and those 10 mm or larger as abscesses. Secondary tracts, abscesses, and horseshoe components were localized by anatomic compartment (intersphincteric, ischiorectal, or supralelevator), with horseshoe configuration defined as extension across the midline to the contralateral side. Every tract was classified according to both the surgical Parks classification (5) and the MRI-based St. James's University Hospital classification (6).

2.6 | Surgical Procedure and Reference Standard

All patients underwent standard preoperative preparation (rectal enema, urethral catheterization, antibiotic prophylaxis, and tetanus toxoid) followed by examination under spinal anesthesia in the lithotomy position. The external opening was inspected, and the internal opening was assessed by digital examination, proctoscopy, and anoscopy. The tract was delineated using methylene blue dye instillation and gentle probing with an olive-pointed probe (Figure 1A). Low tracts were managed by fistulotomy (lay-open technique) or complete fistulectomy (Figure 1B), while high tracts were managed by fistulectomy with placement of a draining or cutting seton (Figure 1C). Intraoperative findings, documented independently of the MRI report by the operating surgical team, served as the diagnostic reference standard against which clinical and MRI findings were compared.

2.7 | Statistical Analysis

Clinical, MRI, and intraoperative findings were recorded on a pre-designed proforma and tabulated in Microsoft Excel. Descriptive statistics (frequencies and percentages) were used to summarize demographic and anatomic findings. For each MRI-assessed parameter (internal opening, tract complexity, secondary tracts, abscess, horseshoe extension, and supralelevator extension), sensitivity, specificity, positive predictive value, and negative predictive value were calculated using intraoperative findings as the reference standard.

3 | RESULTS

Thirty patients with a clinical diagnosis of fistula-in-ano were enrolled and completed clinical assessment, preoperative MRI, and surgery within the study period.

3.1 | Demographic Characteristics

Of the 30 patients, 26 (86.7%) were male and 4 (13.3%) were female (Table 1), consistent with the well-described male predominance of this condition. Patient age ranged from 18 to 70 years, with a mean

of 35.6 years; the 31–40-year age group was most frequently represented (33.3%), followed by the 41–50-year group (30.0%) (Table 2, Figure 2).

Table 1. Sex distribution of the study population (n = 30).

Sex	Frequency	Percent (%)
Male	26	86.66
Female	4	13.33
Total	30	100

Table 2. Age distribution of the study population (n = 30).

Age group (years)	Number of patients	Percent (%)
18–20	2	6.66
21–30	6	20.00
31–40	10	33.33
41–50	9	30.00
51–60	2	6.66
61–70	1	3.33
Total	30	100

Figure 2. Baseline demographic characteristics of the study population

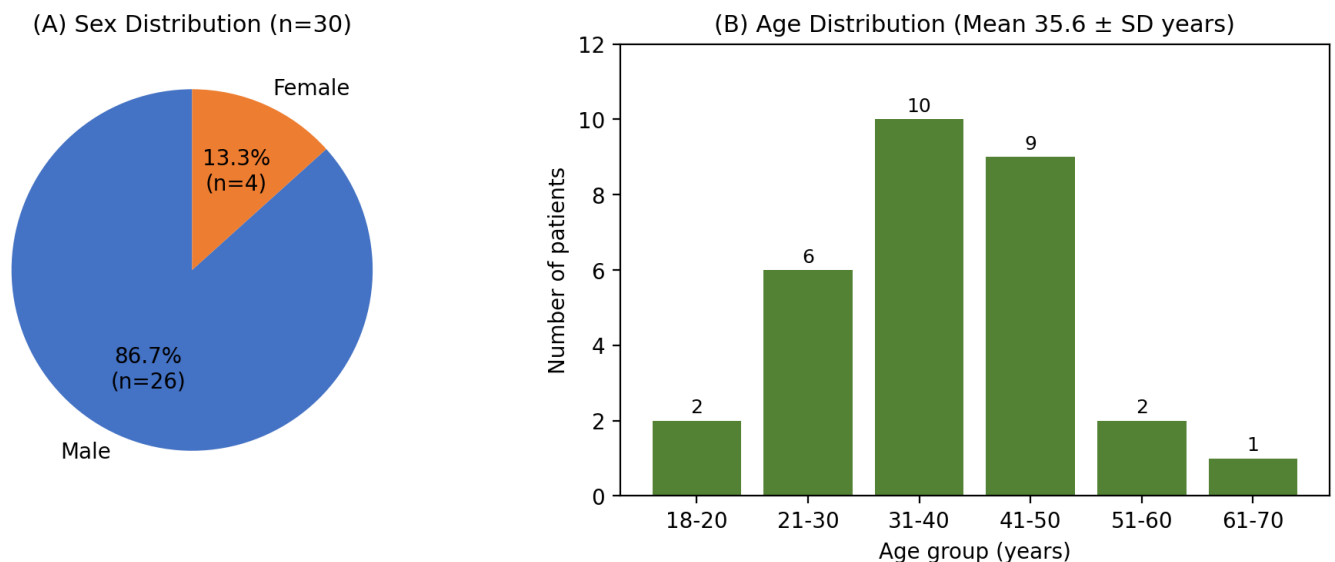


Fig. 1: Baseline demographic characteristics of the study population: (A) sex distribution; (B) age distribution.

3.2 | Clinical Findings

An external opening was identified on clinical examination in all 30 patients (100%) (Table 3). Its location was distributed across the anal clock, most com-

monly at the 3 o'clock position (20.0%); two patients (6.7%) had multiple external openings (Table 4). By contrast, the internal opening was palpable on digital rectal examination in only 4 of 30 patients (13.3%) (Table 5), underscoring the limited sensi-

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tivity of clinical examination alone for this critical anatomic landmark.

Table 3. Presence of external opening on clinical examination (n = 30).

External opening	Frequency	Percent (%)
Present	30	100
Absent	0	0
Total	30	100

Table 4. Clock-face location of external opening on clinical examination (n = 30).

Location	Frequency	Percent (%)
1 o'clock	1	3.33
2 o'clock	1	3.33
3 o'clock	6	20.00
4 o'clock	3	10.00
5 o'clock	2	6.66
6 o'clock	3	10.00
7 o'clock	3	10.00
8 o'clock	3	10.00
9 o'clock	2	6.66
11 o'clock	1	3.33
12 o'clock	3	10.00
Multiple	2	6.66
Total	30	100

Table 5. Palpability of internal opening on digital rectal examination (n = 30).

Internal opening	Frequency	Percent (%)
Felt	4	13.33
Not felt	26	86.66
Total	30	100

Table 7. Tract complexity on MRI (n = 30).

Tract type	Frequency	Percent (%)
Simple	19	63.33
Complex	11	36.66
Total	30	100

3.4 | Intraoperative Findings

At surgery, a simple non-branching tract was confirmed in 17 patients (56.7%) and a complex tract in 13 (43.3%) (Table 8). Among the 13 complex tracts, abscess was the most frequent additional finding, in 5 patients (38.5%), followed by secondary tracts in 4 (30.8%), horseshoe extension in 3 (23.1%), and supralelevator extension in 1 (7.7%) (Table 9, Figure 4A).

3.3 | MRI Findings

MRI identified the internal opening in all 30 patients (100%), a marked improvement over clinical examination. The most common location was the 6 o'clock (posterior) position, seen in 8 patients (26.7%) (Table 6, Figure 3B), consistent with the posterior predilection expected from the cryptoglandular origin of most fistulas. On MRI, 19 patients (63.3%) had a simple linear tract and 11 (36.7%) had a complex tract with secondary extension (Table 7).

Table 6. Clock-face location of internal opening on MRI (n = 30).

Location	Frequency	Percent (%)
1 o'clock	2	6.66
2 o'clock	2	6.66
4 o'clock	1	3.33
6 o'clock	8	26.66
7 o'clock	3	10.00
9 o'clock	1	3.33
10 o'clock	2	6.66
11 o'clock	3	10.00
12 o'clock	3	10.00
Total	30	100

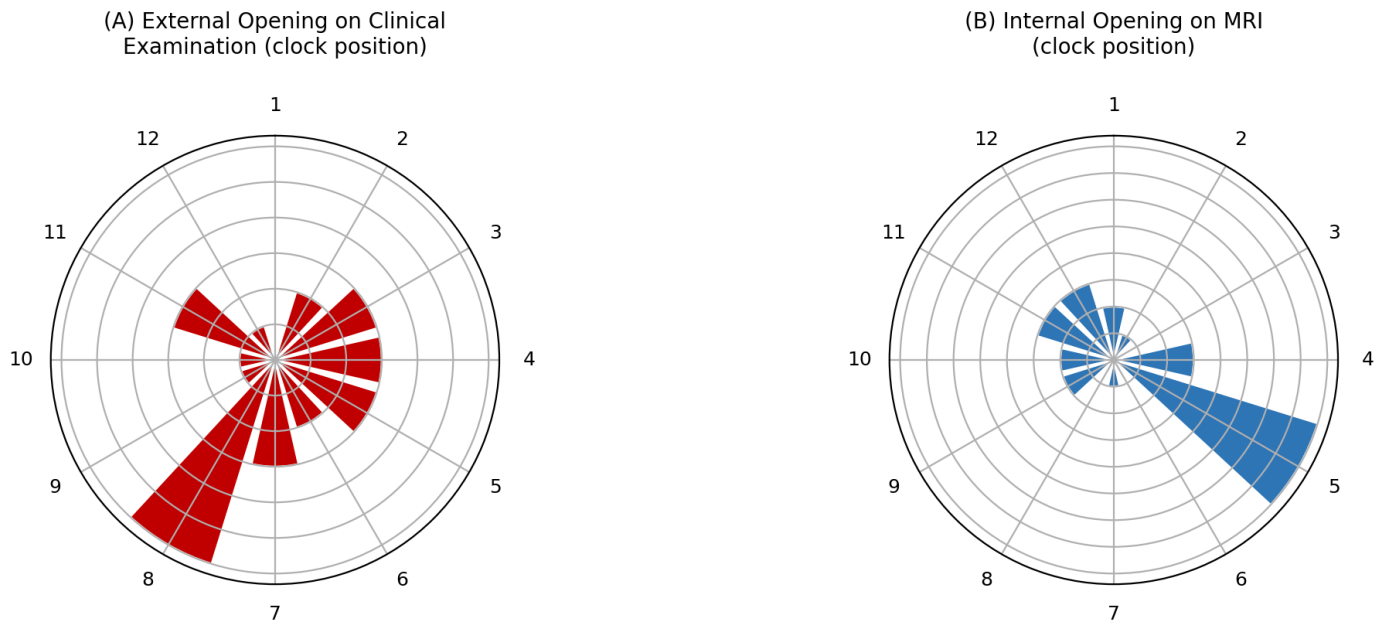
Table 8. Tract complexity confirmed intraoperatively (n = 30).

Tract type	Frequency	Percent (%)
Simple	17	56.66
Complex	13	43.33
Total	30	100

Table 9. Secondary tract, abscess, horseshoe extension, and supralelevator extension confirmed intraoperatively (among 13 complex fistulas).

Finding	Frequency	Percent (%)
Secondary tract	4	30.76
Horseshoe extension	3	23.07
Abscess	5	38.46
Supralelevator extension	1	7.66
Total	13	100

Figure 3. Anatomic (clock-face) distribution of fistula openings

**Fig. 2:** Anatomic (clock-face) distribution of (A) the external opening on clinical examination and (B) the internal opening on MRI.

3.5 | Fistula Classification

By the surgical Parks classification, intersphincteric fistulas were most common (19 patients, 63.3%), followed by transsphincteric (8, 26.7%), suprasphincteric (2, 6.7%), and extrasphincteric (1, 3.3%) tracts (Table 10, Figure 4B). By the MRI-based St. James's University Hospital classification, Grade 1 fistulas predominated (17, 56.7%), followed by Grade 4 (5, 16.7%), Grade 2 (4, 13.3%), and Grade 3 and Grade 5 (2 each, 6.7%) (Table 11).

Table 10. Fistula type by Parks classification (n = 30).

Parks type	Frequency	Percent (%)
Intersphincteric	19	63.33
Transsphincteric	8	26.66
Suprasphincteric	2	6.66
Extrasphincteric	1	3.33
Total	30	100

Table 11. Fistula grade by St. James's University Hospital MRI classification (n = 30).

Grade	Frequency	Percent (%)
Grade 1	17	56.66
Grade 2	4	13.33
Grade 3	2	6.66
Grade 4	5	16.66
Grade 5	2	6.66
Total	30	100

3.6 | Diagnostic Accuracy of MRI

Using intraoperative findings as the reference standard, MRI showed an overall concordance of 86.7% (26/30 patients); findings differed from surgery in 4 patients (13.3%) (Table 12, Figure 6). For simple tracts, MRI had a sensitivity of 100%, specificity of 84.6%, PPV of 89.4%, and NPV of 100%. For complex tracts, corresponding values were 100%, 89.5%, 100%, and 84.6%. For secondary tracts, sen-

sitivity was 75%, specificity 88.8%, PPV 75%, and NPV 88.9%. For abscess, MRI showed sensitivity of 80%, specificity of 100%, PPV of 100%, and NPV of 88.8%. For horseshoe extension, sensitivity was markedly lower at 33.3%, with specificity 90%, PPV 50%, and NPV 81.8%. For supralelevator extension, present in only a single patient, sensitivity and NPV were both 100%, while specificity and PPV could not be meaningfully calculated given the single positive case.

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Figure 4. Fistula tract complexity and anatomic classification

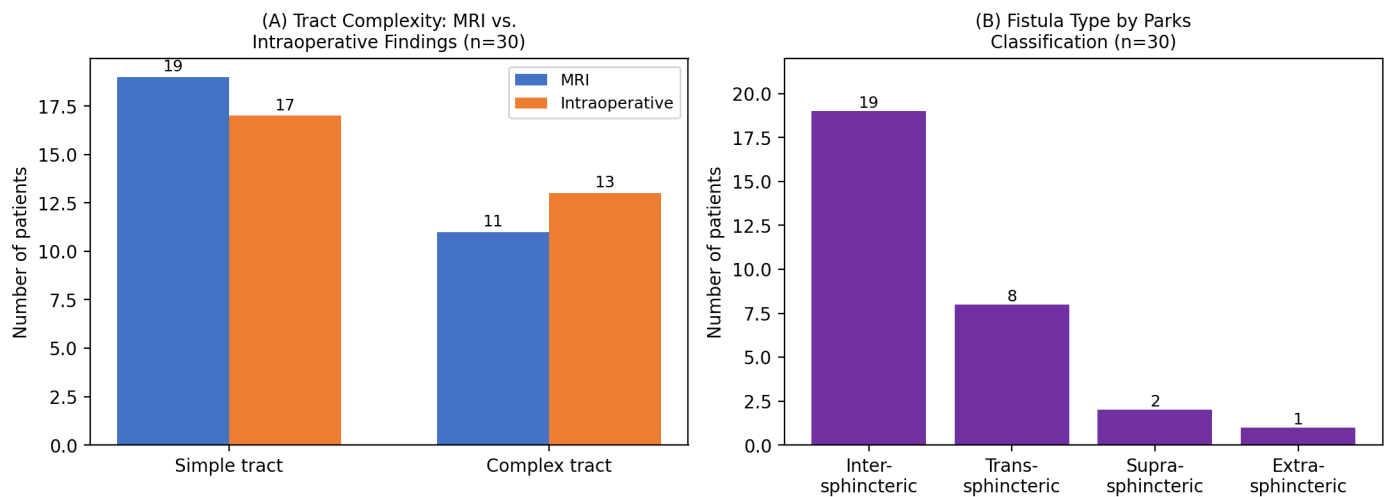


Fig. 3: (A) Tract complexity on MRI versus intraoperative findings; (B) fistula type by Parks classification.

Table 12. Diagnostic accuracy of MRI relative to intraoperative findings (reference standard).

Finding	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Simple tract	100.0	84.6	89.4	100.0
Complex tract	100.0	89.5	100.0	84.6
Secondary tract	75.0	88.8	75.0	88.9
Abscess	80.0	100.0	100.0	88.8
Horseshoe extension	33.3	90.0	50.0	81.8
Supralelevator extension*	100.0	—	—	100.0

*Specificity and PPV not calculable for supralelevator extension owing to the single positive case in this cohort.

Table 13. Overall corroboration of MRI and intraoperative findings (n = 30).

Concordance	Frequency	Percent (%)
Findings same as MRI	26	86.66
Findings different from MRI	4	13.33
Total	30	100

4 | DISCUSSION

This prospective study of 30 patients confirms that MRI provides substantial incremental diagnostic value over clinical examination alone in the preoperative assessment of fistula-in-ano, while also illustrating the specific findings for which MRI performs less reliably in this cohort.

The demographic profile observed here, a marked

male predominance (86.7%) with a mean age of 35.6 years and peak incidence in the fourth decade of life, closely mirrors the pattern described by Halligan and Stoker, who similarly reported that the disease predominantly affects young to middle-aged men (4). The mean age in our cohort was somewhat older than the 28 years reported by H AI P Badder (8), a difference that may reflect population-specific variation in age at presentation or referral patterns rather than a difference in underlying disease biology.

Figure 5. Diagnostic Performance of MRI Relative to Intraoperative Findings (Reference Standard), by Fistula-Related Finding

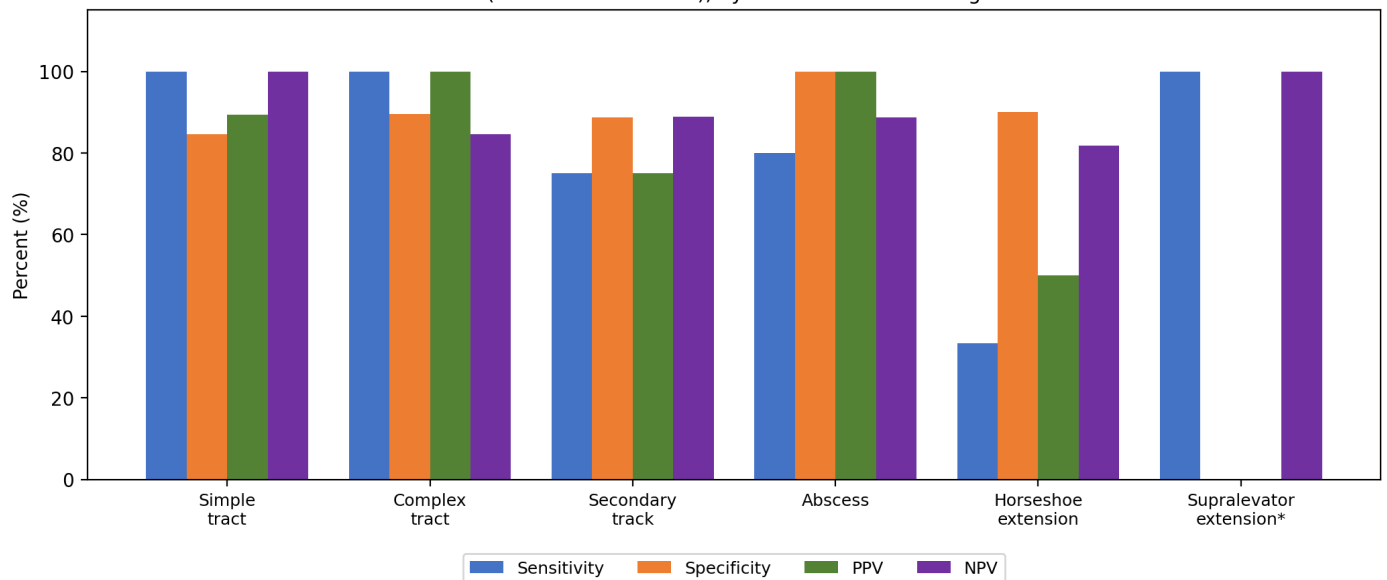


Fig. 4: Diagnostic performance of MRI (sensitivity, specificity, PPV, NPV) relative to intraoperative findings, by fistula-related finding.

Figure 6. Overall Concordance Between MRI and Intraoperative Findings (n=30)

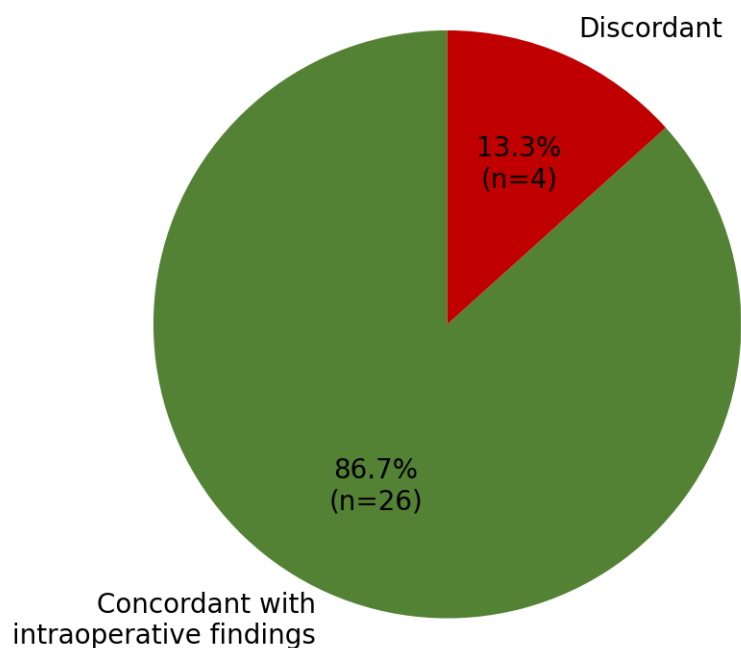


Fig. 5: Overall concordance between MRI and intraoperative findings.

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Figure 1. Intraoperative photographs from the study cohort

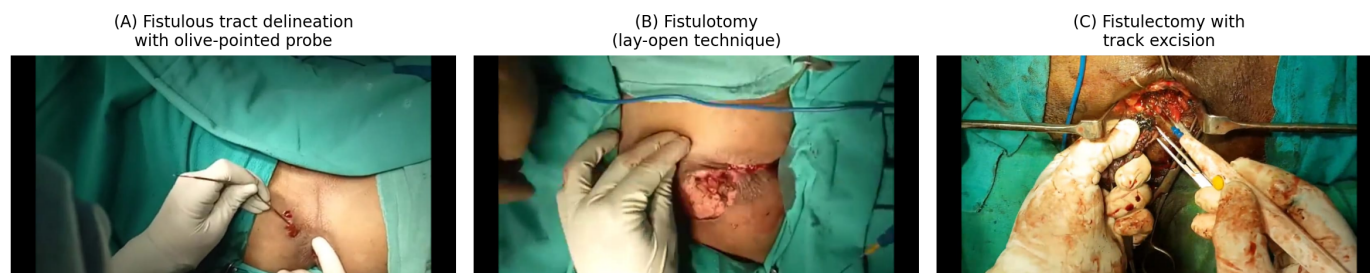


Fig. 6: Intraoperative photographs from the study cohort: (A) delineation of the fistulous tract with an olive-pointed probe following methylene blue instillation; (B) fistulotomy (lay-open technique) for a low tract; (C) fistulectomy with excision of a complex tract.

The disparity between clinical and MRI detection of the internal opening was striking: only 13.3% of internal openings were palpable on digital rectal examination, compared with 100% detection on MRI. This finding reinforces the well-established observation that the internal opening, despite being the anatomic key to surgical cure, is frequently occult to clinical examination alone, particularly for deeper or more complex tracts (4, 6). Correct identification of the internal opening is essential, since failure to address the true source of sepsis is a recognized cause of recurrence after apparently adequate surgery (4). The predominance of the 6 o'clock (posterior) position for the internal opening in our series (26.7%) is consistent with the posterior midline predilection expected under the cryptoglandular hypothesis and broadly consistent with the 50% posterior predominance reported by Rania et al. in a comparable population (9), although the lower proportion in our cohort may reflect a wider anatomic spread of disease in our patients.

Regarding classification, the predominance of intersphincteric fistulas by Parks classification (63.3%) and Grade 1 fistulas by St. James's classification (56.7%) in our series is broadly consistent with the expectation that most cryptoglandular fistulas are relatively simple, low tracts. However, our grade distribution differed from that reported by Rania et al. (9), who found a comparatively even spread across Grades 1 through 5, and from Naglaa et al. (10), who reported a predominance of Grade 3 and 4 (higher-grade) fistulas in a population with a substantial proportion of perianal Crohn's-related sep-

sis. These differences most plausibly reflect differences in underlying case mix, particularly the proportion of Crohn's-associated and recurrent fistulas, which tend toward higher-grade, more complex disease, rather than a fundamental difference in MRI performance across studies.

The diagnostic accuracy figures observed in our study are broadly consistent with, though not identical to, those reported in the foundational validation literature. Beets-Tan et al. (11) reported MRI sensitivity, specificity, PPV, and NPV of 96%, 90%, 90%, and 96%, respectively, for the internal opening; while we did not calculate formal accuracy statistics for the internal opening given its detection in all patients, our 86.7% overall concordance with surgery is in the same broad range. For simple tracts, our sensitivity and NPV of 100% closely matched the 100% sensitivity reported by both Beets-Tan et al. (11) and Villa et al. (12), and our specificity of 84.6% was comparable to the 86% reported in both of those studies. For abscess detection, our sensitivity of 80% was somewhat lower than the 96% reported by Beets-Tan et al. (13) and Villa et al. (14), though our specificity and PPV of 100% each exceeded their reported figures; this pattern, higher specificity but lower sensitivity, may reflect our relatively small sample size (only 5 abscesses were present intraoperatively), such that a single missed or over-called case has an outsized effect on the calculated accuracy statistics.

The most notable limitation identified in our data was the comparatively low sensitivity of MRI for horseshoe extension (33.3%), substantially below the sen-

sitivity reported for other complex features. Beets-Tan et al. (15, 16) specifically highlighted horseshoe abscesses as an area of particular additional value for MRI, and our finding of reduced sensitivity for this feature, based on only 3 positive cases, likely reflects both the genuine technical difficulty of distinguishing a horseshoeing secondary tract from an isolated unilateral extension on axial imaging, and the instability of accuracy estimates calculated from very small numbers of positive cases. In our series, one of the three intraoperatively confirmed horseshoe extensions had been characterized on MRI as a unilateral complex trans-sphincteric secondary tract, illustrating the specific type of misclassification underlying this reduced sensitivity.

A prospective study by Gordon et al. concluded that MR imaging is the most accurate preoperative technique for fistula classification and performs particularly well in delineating the primary tract and secondary extensions (17–19), while Dariusz et al. similarly concluded that MRI is accurate for soft-tissue assessment of perianal fistulous tracts and recommended its wider adoption as the diagnostic method of choice (20–23). Our findings are broadly consistent with this body of literature: MRI in our cohort was highly sensitive for tract complexity and abscess, and provided a preoperative road map that was concordant with surgical findings in the substantial majority of patients, while horseshoe extension emerged as a specific area warranting cautious interpretation and continued intraoperative vigilance.

4.1 | Limitations

This study has several limitations. First, the sample size of 30 patients, while adequate to characterize overall diagnostic patterns, is small for calculating stable accuracy estimates for less common findings such as horseshoe and supralelevator extension, where the diagnostic accuracy statistics are each based on fewer than five positive cases and should be interpreted with corresponding caution. Second, this was a single-center study using a 1.0 Tesla scanner without an endoanal coil; higher-field-strength magnets and dedicated endoanal coils have been reported to further improve spatial resolution and might yield different accuracy estimates. Third, MRI interpretation was not performed by multiple

independent, blinded readers, so inter-observer variability in image interpretation could not be assessed. Finally, as a single-institution study conducted over a defined period, the case mix (predominantly cryptoglandular fistulas, with relatively few complex or Crohn's-associated cases) may not be representative of all populations in which MRI is used for fistula assessment.

5 | CONCLUSION

In this prospective study of 30 patients with fistula-in-ano, MRI substantially outperformed clinical examination for localizing the internal opening and provided high diagnostic accuracy for tract complexity and abscess, with an overall concordance with intraoperative findings of 86.7%. MRI's sensitivity for horseshoe extension was comparatively limited in this cohort, underscoring that even with high-quality preoperative imaging, intraoperative assessment remains the definitive diagnostic standard and the final arbiter of surgical strategy. The St. James's University Hospital MRI classification, used alongside the surgical Parks classification, provided a practical and reproducible framework for radiological–surgical communication. Given the morbidity associated with missed secondary tracts and the risk of recurrence or incontinence from inadequately planned surgery, we recommend routine preoperative MRI for fistula-in-ano, particularly for fistulas suspected clinically to be complex or recurrent, while emphasizing that MRI findings should be interpreted as a preoperative road map to be confirmed and, where necessary, revised at the time of surgery.

6 | DECLARATIONS

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: The study was approved by the Institutional Ethics Committee of Pt. Jawaharlal Nehru Memorial Medical College, Raipur. All pro-

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cedures were conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Declaration of Helsinki and its later amendments.

Informed consent: Written informed consent was obtained from all individual participants included in the study.

Data availability: The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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