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Original Article

Insulinoma-Associated Protein 1 (INSM1) as a diagnostic immunohistochemical marker for neuroendocrine differentiation – A tertiary care centre experience

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ABSTRACT

Background: Neuroendocrine neoplasms represent a heterogeneous group of tumours and require accurate diagnosis and grading for appropriate management. Although conventional synaptophysin and chromogranin-A are the conventional immunohistochemical markers, they have variable sensitivity and specificity. Insulinoma-associated protein 1 has recently emerged as a nuclear marker with promising diagnostic utility. This study aims to compare the sensitivity, and specificity of Insulinoma-associated protein 1 with Synaptophysin and Chromogranin A in neuroendocrine neoplasms of different grades and sites.

Materials and Methods: This cross-sectional study included 62 histologically diagnosed cases of neuroendocrine neoplasms and 30 non-neuroendocrine neoplasms retrieved from pathology archives. Expression of Synaptophysin, Chromogranin A, and INSM1 were evaluated using the H-score. Sensitivity, specificity, positive and negative predictive values were calculated and compared.

Results: INSM1 showed nuclear positivity in 95.2% of neuroendocrine neoplasms and was negative in all non-neuroendocrine neoplasms. The mean H-score was 232.9± 93.5. INSM1 was highly sensitive (95.1%) and specific (96.7%) compared to synaptophysin but less specific (66%) compared to chromogranin-A.

Conclusions: INSM1 was found to be highly sensitive and specific nuclear marker for neuroendocrine differentiation. Its high diagnostic accuracy and characteristic nuclear localization justify its inclusion as a valuable marker in routine immunohistochemical panels.

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INTRODUCTION

Neuroendocrine neoplasms (NENs) encompass a heterogeneous group of tumours distinguished by neurosecretory granules having characteristic histology and immunoprofile. The incidence varies according to the anatomic location. They are most commonly seen in the gastrointestinal tract and lungs, but can occur in any organ. They comprise cells that secrete cell-type-specific peptide hormones stored in electron-dense membrane-bound granules.[1]

Identification and accurate grading of tumours with neuroendocrine differentiation is essential for choosing the right treatment protocol. Synaptophysin (SYN) and chromogranin A (CGA) are commonly used immunohistochemical (IHC) markers to confirm the differentiation.[2] Synaptophysin is highly sensitive but not entirely specific, as it is also expressed in some non-neuroendocrine carcinomas.[3] Chromogranin A is highly specific but less sensitive than synaptophysin.[4]

Insulinoma-associated protein 1 (INSM1) is a zinc-finger transcription factor protein that plays a cardinal role in the differentiation of embryonic neuroendocrine cells.[5] It is encoded by the insulinoma-associated-1 (IA-1) gene, the cDNA of which was first identified in a human insulinoma subtraction library by Yasuhiro Goto and colleagues in 1992.[6]

The human INSM1 protein binds both to DNA and to other proteins. It can bind to and compete with CDK4 for binding to cyclin D1. This, in turn, leads to inhibition of phosphorylation of the protein and, thus, to cell cycle arrest. Inhibition of cellular proliferation may lead to differentiation induction.[7] The binding of cyclin D1 also directly mediates the transcriptional effects of INSM1. Thus, INSM1 functionally links transcriptional activity to cell cycle arrest.[8] The INSM1 gene is found to be reactivated in neuroendocrine tumours. This type of dedifferentiation mimics normal embryonic development.[9]

Studies have shown expression of INSM1 in various NENs as well as neuroepithelial tumours like pituitary adenomas, phaeochromocytomas, medulloblastomas, etc. Since its identification, INSM1 has been proposed as part of the diagnosis of neuroendocrine differentiation. Many authors have researched the expression of INSM1 in various NENs; most of them being selective cohorts of NENs. A 100% sensitivity was obtained for pancreatic NETs and also in neuroendocrine carcinomas (NEC) of the GIT.[10,11] Some studies showed a lower sensitivity in pulmonary and urothelial NEC.[12,13] Only a few studies have addressed the expression of INSM1 in both neuroendocrine and non-neuroendocrine neoplasms and have found it to be more specific and sensitive compared to SYN and CGA.[14,15]

INSM1 has emerged as a robust IHC marker of neuroendocrine differentiation. It is currently the only nuclear neuroendocrine marker available for diagnostic immunohistochemistry.[16] To date, not many studies have been conducted on INSM1 expression in neuroendocrine neoplasms in South India. This study aims to establish the utility of INSM1 as a novel specific biomarker of neuroendocrine differentiation.

MATERIALS AND METHODS

This was a cross-sectional study conducted in the Department of Pathology of MES Medical College, Perinthalmanna, Malappuram District, Kerala, India, from January 2023 to March 2025. 62 cases diagnosed as NEN were included in the study. Lymph nodes with metastasis from neuroendocrine neoplasms and cases with inadequate tissue for IHC were excluded.

A predesigned proforma was used to document the particulars of the patient (age and gender) along with location, diagnosis, and immunohistochemical status of the tumour.

Morphologically diagnosed 62 cases of NEN, confirmed with SYN and CGA, were retrieved from the department archives. A set of randomly selected 30 non-neuroendocrine tumours was also retrieved. All the cases were then subjected to immunohistochemical evaluation with INSM1 (A-8; mouse monoclonal antibody; BIOGENEX). Sections of 4µm thickness were cut and mounted on gelatin-coated slides, followed by deparaffinisation and rehydration. Antigen retrieval was carried out using citrate buffer (pH 6.0) at 150˚C in a microwave oven. After blocking endogenous peroxidase activity with hydrogen peroxide, slides were incubated with primary antibodies for 30 minutes. Visualisation was achieved using the Polyexcel HRP-DAB system, followed by counterstaining with hematoxylin. Normal appendiceal tissue served as a positive control, and non-stainable tissue as a negative control.

Any nuclear staining for INSM1 was considered positive and was graded as 1+, 2+, and 3+. (fig.1) In all INSM1-positive cases, the percentage of positive tumour cells was recorded. An H-score was calculated for each marker. The formula for the calculation of the H-score is as follows: 1 × (% of 1+ tumour cells) + 2 × (% of 2+ tumour cells) + 3 × (% of 3+ tumour cells).[12] The H score ranges from 0 to 300.

The data was entered into an Excel Worksheet, and analysis was performed using SPSS 26 and MedCalc software. The categorical variables were expressed as frequency and percentage, and quantitative variables in terms of descriptive statistics (mean and standard deviation). Sensitivity, specificity, positive and negative predictive values were calculated for each IHC stain.

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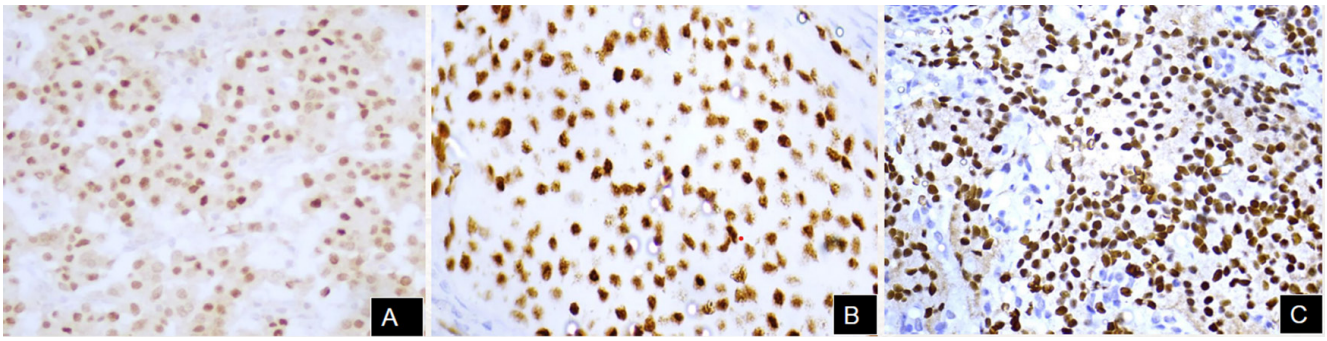

Figure 1: *Intensity of INSM1 staining. (A) showing 1+ intensity; (B) showing 2+ intensity; and (C) showing 3+ intensity in different neuroendocrine neoplasms.*

The study was conducted after getting clearance from the institutional research committee and the institutional ethics committee (IEC/MES/76/2022)

RESULTS

This study included 62 cases of NENs and 30 non-neuroendocrine neoplasms. The mean age of the study population was 55.06 (± 20.65) years, ranging from 7 to 90 years. A male predominance was noted, with a male-to-female ratio of 1.4:1. GIT was the most commonly affected site, and the majority of the cases were grade 1 NET, mainly involving appendix and duodenum (12 and 13 cases, respectively). The non-neuroendocrine tumours included 20 adenocarcinomas of the GIT, 9 non-small cell lung carcinomas, and one infiltrating ductal carcinoma of the breast.

INSM1 expression in NENs:

INSM1 showed nuclear positivity in 59 out of 62 NENs (95.2%), with 14 cases located in the lung and 37 cases in the GIT. Eight INSM1-positive cases were found in other sites, including the urachus, cervix, liver, and breast. INSM1 was negative in three cases of NEC located in the duodenum, gastroesophageal junction, and lung, which were also negative for CGA.

Of the 59 INSM1-positive NENs, 56.5% cases were NETs of different grades, 4.8% were carcinoid tumours, and 33.9% were NECs [Table1]. All the pulmonary small cell carcinomas were positive for INSM1.

Though INSM1 expression was noted in nearly all NENs, no significant difference in INSM1 expression was noted across tumor grades (p >0.05).

Expression of SYN and CGA:

SYN was positive in NETs of all grades except for one case of appendiceal grade 1 NET, which showed diffuse positivity for CGA and INSM1. All carcinoid tumours and NECs were positive for SYN [Table 1]. CGA was positive in 42 out of 62 NENs (67.7%), which included 30 cases of G1 and G2 NETs and carcinoid tumours, along with 12 cases of NECs. 2 cases of G3 NETs were negative for CGA [Table 1]. At least one of the neuroendocrine markers was positive in each case.

Table 1: Expression of INSM1, Synaptophysin and Chromogranin in different neuroendocrine neoplasms

Diagnosis	Insulinoma-associated protein 1 (INSM1)	Synaptophysin	Chromogranin
Neuroendocrine tumor - Grade 1	27/27 (100%)	26/27 (96.3%)	23/27 (85.2%)
Neuroendocrine tumor - Grade 2	6/6 (100%)	6/6 (100%)	6/6 (100%)
Neuroendocrine tumor - Grade 3	2/2 (100%)	2/2 (100%)	0/2 (0%)
Neuroendocrine carcinoma	10/12 (83.3%)	12/12 (100%)	3/12 (25%)
Pulmonary Small cell carcinoma	11/12 (91.7%)	12/12 (100%)	7/12 (58.3%)
Pulmonary typical carcinoid	2/2 (100%)	2/2 (100%)	1/2 (50%)
Pulmonary atypical carcinoid	1/1 (100%)	1/1 (100%)	1/1 (100%)

Comparison of H score:

An H-score was used to address the heterogeneity of INSM1 expression in tumour cells. It will minimize interobserver variability and offer a more objective assessment. The mean H-score was found to be 232.9 (± 93.5) for INSM1. SYN and CGA showed a mean H-score of 272.2 (±64.4) and 153.6 (±130.4), respectively. 42 cases showed 3+ staining of INSM1 in the major proportion of tumour cells. In most cases majority of tumour cells were positive for INSM1. The p-value of H score was calculated using the Chi-square testand was found to be statistically insignificant (p >0.05).

INSM1 expression in non-neuroendocrine neoplasms:

All 30 non-neuroendocrine neoplasms were negative for INSM1.

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Sensitivity and specificity of INSM1 compared to SYN and CGA:

The sensitivity of INSM1 was calculated to be 95.1% and 100% compared to SYN and CGA, respectively. Overall specificity was higher compared to the traditional neuroendocrine markers (96.7% and 66%). The positive predictive value was 98.3% compared to SYN, and the negative predictive value was 100% compared to CGA (Table 2).

Table 2: Sensitivity, specificity and predictive values of INSM1 in comparison to Synaptophysin and Chromogranin

	INSM1 with Synaptophysin	INSM1 with Chromogranin
Sensitivity	95.1%	100 %
Specificity	96.7%	66%
PPV	98.3%	71.1%
NPV	90.9%	100%

PPV: Positive predictive value; NPV: Negative predictive value

DISCUSSION

The accurate identification of NENs is crucial owing to their prognostic and therapeutic implications. The well-established markers for neuroendocrine differentiation are SYN and CGA, which show cytoplasmic expression. INSM1 is a relatively new IHC marker localised in the nuclear compartment.

In a successful analysis of 92 cases (including 30 non-neuroendocrine tumours), INSM1 showed positivity in 59 cases, confirming the neuroendocrine differentiation. Three cases diagnosed as NEC were negative for INSM1; one case showed cytoplasmic staining.

INSM1 immunostaining showed a 100% positivity rate in NETs across all three grades. SYN also showed a similar positivity rate in grade 2 and 3 NETs, with a slightly lower rate in grade 1 tumours. In NECs, SYN was found to be superior to INSM1. CGA showed a lower positivity rate than INSM1 and SYN, irrespective of the grade.

15 cases were located in the respiratory tract, constituting 12 small cell carcinomas, 2 typical carcinoid and 1 atypical carcinoid tumour. All except one case of small cell carcinoma showed nuclear INSM1 staining, resulting in an 80% positivity rate. Carcinoid tumours were all positive for INSM1. SYN showed positivity in all of them (15/15), whereas CGA showed only 60% positivity. Similar findings were observed by Mukhopadhyay et al.[16] They obtained a 98% sensitivity for INSM1 and 100% for SYN for small cell carcinoma of lung. Sakakibara et al.[17] studied 141 pulmonary NENs with 92% sensitivity in small cell carcinomas and 95% in carcinoids. They also observed INSM1 expression in cases which showed no expression for other NE markers, and also in a small number of non-small cell carcinomas.

Among GIT tumours, the majority were grade 1 NETs. All cases (25/25) were positive for INSM1, in contrast to 96% and 84% positivity for SYN and CGA, respectively. Out of 8 NECs, two were negative for INSM1 and CGA but positive for SYN. Additionally, in the current study, duodenum was the most affected site in the gastrointestinal tract and were predominantly grade 1 NET. One case of duodenal NEC was negative for INSM1 and positive for the other two markers. McHugh et al.[18] demonstrated INSM1 positivity in 82.9% well differentiated NETs and 85% poorly differentiated NECs of the GIT. The study by Kim et al.[19] observed INSM1 positivity in all gastric NETs and 92% of NECs, including Mixed Adenoneuroendocrine carcinomas.

Kim et al.[19] also noted a decrease in expression of INSM1 in neuroendocrine carcinomas, which was also observed in our study. This was assessed using the H-score, which ranged from 0 to 300. Other researchers also noted a similar decreasing trend in H-score with increasing grade.[20-22]

The current study revealed a sensitivity of 95.1% and 100% for INSM1 when compared with SYN and CGA, respectively. Overall specificity was higher compared to the traditional neuroendocrine markers (96.7% and 66%). El-kareem et al.[23] obtained a sensitivity of 91.1% and 92.3% for SYN and CGA, respectively. The sensitivity of INSM1 in various studies is shown in Table 3.

Table 3: Sensitivity of INSM1 in various studies

Studies	Sensitivity of INSM1
Mc Hugh et al.[18]	82.9%
Mukhopadhyay et al.[16]	95%
Rosenbaum et al.[14]	90%
Rooper et al.[15]	96.1%

INSM1: Insulinoma-associated protein 1

CONCLUSIONS

INSM1 has emerged as a highly sensitive and specific marker for neuroendocrine differentiation. Its exclusive nuclear expression minimizes interpretative errors and enhances diagnostic accuracy. Since it is expressed in all neuroendocrine tumours irrespective of the site, INSM1 can serve as a reliable adjunct or even a primary immunohistochemical marker. Incorporating INSM1 into routine diagnostic panels may significantly improve the diagnostic accuracy, especially in challenging or poorly differentiated cases.

Conflict of interest: None

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