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EVALUATION OF THE DIAGNOSTIC RELEVANCE OF SOMATOSTATIN RECEPTOR-2A (SSTR-2A), MUCIN 4 (MUC4) AND EPITHELIAL MEMBRANE ANTIGEN (EMA) IN MENINGIOMAS

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Abstract

Meningiomas are common neoplasms that originate from arachnoid cells. They usually attach to the inner surface of the dura mater. Immunohistochemistry (IHC) can help with their definitive diagnosis. Somatostatin receptors-2A (SSTR-2A) was recently assessed as a potential therapeutic target for the treatment of meningioma. Mucin-4 (MUC4) is overexpressed in various carcinomas, and its expression was reported to correlate with higher tumour progression and worse prognosis. Epithelial membrane antigen (EMA) is highly expressed by most adenocarcinomas, associated with poor prognosis. This study aimed to evaluate the diagnostic value of SSTR-2A, MUC4 as well as EMA in meningiomas and to assess expression of these markers in different grades of meningiomas.

Paraffin blocks of 60 selected specimens, diagnosed as different subtypes of meningiomas and 15 paraffin blocks of non-meningioma cases were selected. All specimens underwent immunohistochemical staining for SSTR-2A, MUC4 and EMA expression.

There was a strong significant relation between combination of SSTR-2A, EMA and MUC4 in differentiating meningioma from non-meningioma cases (P value <0.05). There was a significant relation between immunoexpression of both SSTR-2A (scoring and intensity) and EMA and the grade of meningioma (p value <0.05).

Using a panel of the three markers (SSTR-2A, MUC4 and EMA) is diagnostically superior to using each of which alone in differentiation of meningioma from non-meningioma cases. EMA immunophenotyping is considered the most specific and sensitive marker for meningioma while MUC4 is more specific for meningioma than SSTR-2A but it is less sensitive.

Keywords: Meningioma; SSTR-2A; MUC4; EMA.

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Background:

Archnoidal cell-derived meningiomas are relatively prevalent neoplasms that typically metastasize to the inner side of the dura mater. Adults with a typical age of 65 years and women in particular are more likely to develop these tumours, which account for 13%-30% of main intracranial tumours, 25% of intraspinal tumours, and less frequently from orbit [1] .

The World Health Organization (WHO, 2021) defines three distinct categories for meningiomas. Most meningiomas are of the low-grade variety, or Grade I, and are thought to be relatively harmless. While grade I meningiomas are the most prevalent, grade II tumours are uncommon but have a high recurrence rate, and grade III tumours are extremely rare but are linked with a dismal prognosis [2].

Meningothelial, fibrous, and transitory meningiomas are the three WHO categories of meningiomas seen most frequently [3].

In most cases, the diagnosis of a meningioma is uncomplicated; however, some forms of soft tissue sarcoma can develop in intracranial or dural locations and exhibit epithelioid or spindle cytomorphology mimicking meningioma, which can lead to diagnostic confusion [3, 4].

When dealing with the fibrous form of meningioma, schwannomas and other uncommon meningeal tumours like solitary fibrous tumors/hemangiopericytomas are the most frequent alternative diagnoses. Meningiomas, particularly those with microcysts or transparent cells, can have overlapping physical features with hemangioblastomas. Differentiating an anaplastic meningioma from a sarcoma, melanoma, or cancer can be challenging [1].

Primary extradural meningioma and metastasis are two examples of rare types of meningiomas that can make identification difficult. When a prognosis is particularly difficult to make, immunohistochemistry (IHC) can be a lifesaver [1].

One type of somatostatin receptor is called somatostatin receptor-2A (SSTR-2A). Somatostatin receptor type 2A (SSTR-2A) was also evaluated as a possible therapeutic target for somatostatin analogue-based treatments for the treatment of meningioma [5].

It is expressed in cytoplasm or membrane of normal tissues as (brain, pituitary, stomach foveolar cells, duodenal glands, small and large intestine surface epithelial cells, kidney, pancreatic islet, testis, endothelium, lymphocytes, monocytes, macrophages and fibroblasts), it is also expressed in a broad variety of neuroendocrine tumors and also in pituitary adenoma, gastrointestinal stromal tumor, olfactory neuroblastoma, paraganglioma and small cell lung cancer [6].

Mucin-4 (MUC4) is a member of the human mucin family. It is a high molecular weight transmembrane glycoprotein that plays a role in cell growth signaling and is expressed in various epithelia, where it serves protective roles. MUC4 is overexpressed in a variety of carcinomas, and its expression is reported to be correlated with higher tumour progression and worse prognoses [7].

Epithelial membrane antigen (EMA) is expressed normally in apical surface of almost all glandular and ductal epithelial cells including breast, lung, kidney, pancreas, salivary glands & skin. It is also highly expressed by most adenocarcinomas, associated with poor prognosis [8]. Currently, there are some studies eliciting the role of SSTR-2A, MUC4 and EMA in diagnosis of meningiomas [1, 8].

The aim of this work was to evaluate the diagnostic value of SSTR-2A, MUC4 as well as EMA in meningiomas and to assess expression of these markers in different grades of meningiomas.

Methods:

This retrospective study was carried out on paraffin blocks of 60 selected specimens, that were diagnosed as different subtypes of meningiomas and 15 paraffin blocks of selected non-meningioma cases, 6 of them

were diagnosed as schwannoma, 3 cases were hemangiopericytoma, 4 cases were neurofibroma and two cases were hemangioblastoma. Specimens were collected from the files of Pathology Department of Tanta Faculty of Medicine, Tanta Cancer Center and from private laboratories during the period from February 2019 to March 2022. Written informed consent was obtained from the cases' parents or guardians. The study was approved by the Research Ethics Committee (REC), Faculty of Medicine, Tanta University with approval number 33481/11/19. Patients were chosen for the research depending on the quality of the blocks.

Clinic-pathological data

The available clinical data were obtained from patients' medical reports. The paraffin blocks were cut by ordinary microtome to sections 3-5 micron in thickness for Haematoxylin and Eosin staining. All cases were clinicopathologically re-evaluated and classified into histological meningioma subtypes according to the WHO classification 2021 [2] and into the non-meningioma cases.

Immunohistochemical staining:

Immunohistochemical staining for SSTR2A, MUC4 and EMA were performed on all 60 blocks of meningioma cases and on the 15 blocks of the non-meningioma cases by using primary antibodies:

1- SSTR2A antibody: Concentrated rabbit polyclonal antibody (clone A3135; dilution 1:100, ABclonal, China). Positive control was normal gastric tissue.
2- MUC4 antibody: Ready to use rabbit monoclonal antibody. (Clone: EP256, BioSB, United States). Positive control was normal colonic tissue.
3- EMA antibody: Ready to use mouse monoclonal antibody (Clone: E29, IR629, Dako, Glostrup, Denmark). Positive control was membrane of secretory epithelia.

Procedure of immunohistochemical staining: [9]

After mounting tumour pieces (5 µm thick) on positively charged slides, we allowed them to cure at 37 °C for 30 minutes. After deparaffinization, sections were retrieved using high and low PH EnVision FLEX antigen retrieval solutions in a Dako PT link device (at 97o C for 20 minutes). Dako Autostainer Link 48 was used for immunohistochemistry. In summary, a peroxidase blocking solution was used, and then the main antibodies were incubated for 30 minutes. Following a 20-minute incubation with horseradish peroxidase polymer, the chromogen diaminobenzidine (DAB) was added. We used hematoxylin as a counterstain on the slides.

Evaluation of SSTR-2A immunostaining:

SSTR-2A expression was scored in cytoplasm of the neoplastic cells of the studied cases modified from the study of Anis et al. [10] as follow: The proportion of tumour cells that were immunostained (less than 5%, 5% to 25%, 26% to 50%, more than 50%), as well as the intensity of the staining (weak, moderate, and strong).

Evaluation of MUC4 immunostaining:

MUC4 expression was scored in cytoplasm of neoplastic cells of the studied cases modified from the study of Matsuyama et al. [3] as follow: Positivity for MUC4 was judged with the percentage of positivity of tumor cells. The expression of 50% or more tumor cells was considered as diffuse staining. Even when only one percent of tumour cells expressed the marker, the staining was deemed positive.

2.5 Evaluation of EMA Immunostaining:

EMA expression was scored in cytoplasm of neoplastic cells of the studied cases modified from the study of Agaimy et al. [11] for EMA expression level as follow: <5% positive cells was considered negative, > 5% positive cells was considered focal positive, > 50% positivity was considered diffuse positive.

Statistical analysis

Statistical analysis was done by SPSS v20 (Armonk, NY: IBM Corp). Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative variables were presented as mean and standard deviation (SD) and were compared by ANOVA test for normally distributed quantitative variables between more than two groups, Kruskal Wallis test for abnormally distributed quantitative variables to compare between more than two groups and Mann Whitney test for abnormally distributed quantitative variables to compare between two groups. Qualitative variables were presented as number and percentage (%) and were compared between different groups by Chi-square test or Fisher's Exact or Monte Carlo correction for cells have expected count less than 5. A two tailed P value < 0.05 was considered significant.

Results

Clinicopathologic and histopathologic characteristics:

This retrospective study included 60 cases of meningiomas with different subtypes divided into: 42 cases of grade I meningioma cases, 13 cases of grade II meningioma cases and 5 cases of grade III meningioma cases. The mean age of the studied cases was 48.08 years (range, 20-75) and more than half of cases 53.3% were females. Most meningioma cases were at intracranial location (85%).

Also, this study included 15 cases of non_meningioma cases as a differential diagnosis for meningioma. The mean age of the studied non-meningioma cases was 39 years (range, 30-50) years. Among them, 7 cases were male, and 8 cases were female. Most of these cases (10) were located at spinal location while 5 cases were at intracranial location. The mean size of non-meningioma cases was 3.62 ± SD 2.13 as illustrated in **table 1and figure 1and 2.**

Statistical analysis of the results revealed statistically significant relation between the gender of the patients and the tumor grade which means that males are at a greater risk for high grade meningiomas (p value < 0.05), while there was no statistically significant relation between the age of the patients, site of the tumor, tumor size and tumor grade (p value > 0.05) as illustrated in **table 2 and figure 6.**

Immunohistochemical results:

SSTR2A immunohistochemical results in meningioma cases (table 3 and figure 3):

Regarding relation between SSTR2A immunoexpression and different grades of meningioma, there was strong negative correlation between scoring, intensity of SSTR2A and grading (p value < 0.05) which mean that SSTR2A positive expression increase with GI meningioma cases than GII and GIII meningioma cases.

EMA immunohistochemical results in meningioma cases (table 4 and figure 4):

Regarding relation between EMA and different grades of meningioma, there was strong negative correlation between EMA immunoexpression and different grades of meningioma (p value <0.05) which mean that EMA positive expression increase with GI meningioma cases than GII and GIII meningioma cases.

MUC4 immunohistochemical results in meningioma cases (table 5 and figure 5):

Regarding relation between MUC4 and different grades of meningioma, Statistical analysis revealed no statistically significant relation was found between MUC4 expression and grading (p value > 0.05)

Correlation between SSTR2A, EMA and MUC4 immunoexpression in different grades of meningioma (Table 6):

Statistical analysis revealed strong statistically significant relation was found between combination of three markers (p value <0.05).

Immunoexpression of SSTR2A, EMA and MUC4 in non-meningioma cases:

Regarding Immunoexpression of SSTR2A, all cases were negative for SSTR2A immunoexpression except one case of

schwannoma which was positive for SSTR2A. Regarding Immunoexpression of EMA and MUC4, All the studied non meningioma cases were negative for EMA and MUC4 immunoexpression as illustrated in **table7 and figure 7**.

On combination of three markers (SSTR2A, EMA and MUC4), Statistical analysis revealed strong significant relation in differentiating meningioma from non-meningioma cases as illustrated in **table 8.**

Validity (AUC, sensitivity and specificity) for SSTR-2A, EMA and MUC4 to discriminate meningioma from non-meningioma (table 9):

SSTR-2A could significantly discriminate meningioma cases from others (P value <0.001) with sensitivity of 85 %, specificity of 93.33 %, PPV of 98.08 % and NPV of 60.87 %. EMA could significantly discriminate meningioma cases from others (P value <0.001) with sensitivity of 91.67 %, specificity of 100 %, PPV of 100 % and NPV of 75 %. MUC4 could significantly discriminate meningioma cases from others (P value = 0.013) with sensitivity of 41.67 %, specificity of 100 %, PPV of 100 % and NPV of 30 %. SSTR-2A + EMA could significantly discriminate meningioma cases from others (P value <0.001) with sensitivity of 96.67 %, specificity of 93.33 %, PPV of 98.31 % and NPV of 87.50 %. SSTR-2A + MUC4 could significantly discriminate meningioma cases from others (P value <0.001) with sensitivity of 95.08 %, specificity of 93.33 %, PPV of 98.31 % and NPV of 82.35 %. EMA + MUC4 could significantly discriminate meningioma cases from others (P value <0.001 with sensitivity of 95.08 %, specificity of 100 %, PPV of 100 % and NPV of 83.33 %. Combination of three markers (SSTR-2A + EMA + MUC4) could significantly discriminate meningioma cases from others (P value <0.001) with sensitivity of 100 %, specificity of 97.62 %, PPV of 98.36 % and NPV of 100 %.

Table 1: Distribution of the meningioma and non-meningioma cases according to subtype, grading, age, gender, site and size (n=60)

Meningioma cases	**Subtype**	**GI**	**Meningothelial**	9 (15 %)
			Fibrous	5 (8.3 %)
			Transitional	8 (13.3 %)
			Psammomatous	6 (10 %)
			Microcystic	3 (5 %)
			Angiomatous	6 (10 %)
			Metaplastic	2 (3.3 %)
			Secretory	3 (5 %)
		GII	**Atypical**	5 (8.3 %)
			Clear	4 (6.7 %)
			Chordoid	4 (6.7 %)
		GIII	**Anaplastic**	3 (5 %)
			Papillary	2 (3.3 %)
	Grading	**I**		42 (70 %)
		II		13 (21.7 %)
		III		5 (8.3 %)
	Age (years)			48.08 ± 11.79
	Gender	**Male**		28 (46.7 %)
		Female		32 (53.3 %)
	Site	**Spinal**		9 (15 %)

		Intracranial	51 (85 %)
Non-meningioma Cases	**Differential diagnosis of non-meningioma**	**Schwannoma**	6 (40 %)
		Hemangiopericytoma	3 (20 %)
		Neurofibroma	4 (26.7 %)
		Hemangioblastoma	2 (13.3 %)
	Age (years)		39.0 ± 6.80
	Gender	**Male**	7 (46.7 %)
		Female	8 (53.3 %)
	Site	**Spinal**	10 (66.7 %)
		Intracranial	5 (33.3 %)
	Size		3.62 ± 2.13

Data are presented as mean ± SD or frequency (%).

Table 2: Relation between grading and age, gender, site and tumor size (n= 60)

		Grading			**P-value**
		I (n= 42)	**II (n= 13)**	**III (n= 5)**	
Age (years)		48.60 ± 2.71	49.46 ± 7.64	40.20 ± 11.52	0.293
Sex	**Male**	17 (40.5 %)	6 (46.2 %)	5 (100 %)	0.046*
	Female	25 (59.5 %)	7 (53.8 %)	0 (0 %)	
Site	**Spinal**	8 (19 %)	0 (0 %)	1 (20 %)	0.184
	Intracranial	34 (81 %)	13 (100 %)	4 (80 %)	
Tumor size		4.15 ± 2.22	7.65 ± 2.92	4.40 ± 0.55	0.001*

Data are presented as mean ± SD or frequency (%), * significant as $p < 0.05$.

Table 3: Distribution of cases according to SSTR2A and relation between scoring, intensity of SSTR-2A and grading (n= 60)

		Grading			**P-value**
		I	**II**	**III**	
SSTR-2A	**Negative (n= 9)**	4 (44.4 %)	3 (33.3 %)	2 (22.2 %)	-
	Positive (n= 51)	38 (74.5 %)	10 (19.6 %)	3 (5.9 %)	
Scoring of SSTR-2A	**Negative**	4 (9.5 %)	3 (23.1 %)	2 (40 %)	0.033*
	Score 1	6 (14.3 %)	4 (30.8 %)	1 (20 %)	
	Score 2	8 (19 %)	3 (23.1 %)	2 (40 %)	
	Score 3	24 (57.1 %)	3 (23.1 %)	0 (0 %)	
Intensity of SSTR-2A	**Negative**	4 (9.5 %)	3 (23.1 %)	2 (40 %)	0.004*
	Weak	1 (2.4 %)	2 (15.4 %)	0 (0 %)	
	Moderate	19 (45.2 %)	8 (61.5 %)	3 (60 %)	
	Strong	18 (42.9 %)	0 (0 %)	0 (0 %)	

Data are presented as frequency (%), * significant as $p < 0.05$. SSTR-2A: Somatostatin receptors-2A.

Table 4: Relation between EMA and grading (n= 60)

Grading	EMA		P value
	Negative (n= 5)	**Positive+ Diffuse>50% (n= 55)**	
I	2 (40 %)	40 (72.7 %)	0.047*
II	1 (20 %)	12 (21.8 %)	
III	2 (40 %)	3 (5.5 %)	

Data are presented as frequency (%), * significant as $p < 0.05$. EMA: Epithelial membrane antigen.

Table 5: Distribution of cases according to MUC4 and relation between MUC4 % positivity and grading of tumor (n= 60)

		MUC4 %		P value
		Negative (n= 35)	**Positive (n= 25)**	
Grading	**I**	25 (71.4 %)	17 (68 %)	0.913
	II	7 (20 %)	6 (24 %)	
	III	3 (8.6 %)	2 (8 %)	
GI	**Meningothelial (n=5)**	11.0 ± 2.24		0.113
	Fibrous (n=1)	5		
	Transitional (n=4)	36.25 ± 33.51		
	Psammomatous (n=3)	26.67 ± 5.77		
	Microcystic (n=2)	17.50 ± 3.54		
	Metaplastic (n=2)	65.0 ± 21.21		
GII	**Clear (n=3)**	15.0 ± 5.0		0.1
	Chordoid (n=3)	26.67 ± 5.77		
GIII	**Papillary (n=2)**	2.50 ± 0.71		-

Data are presented as mean ± SD or frequency (%). MUC4: Mucin-4.

Table 6: Correlation between SSTR-2A, EMA and MUC4 immunoexpression

	SSTR-2A	**EMA**	**MUC4**	**Q**	**P value**
Negative	9 (15 %)	5 (8.3 %)	35 (58.3 %)	39.0*	<0.001*
Positive	51 (85 %)	55 (91.7 %)	25 (41.7 %)		

Data are presented as frequency (%), * significant as $p < 0.05$. SSTR-2A: Somatostatin receptors-2A, EMA: Epithelial membrane antigen, MUC4: Mucin-4.

Table 7: Distribution of the studied non meningioma cases according to SSTR2A, EMA and MUC4 immunoexpression (n=15)

SSTR2A	**Negative**	**14 (93.3 %)**
	Weak	**0 (0 %)**
	Moderate	**1(6.7 %)**
	Strong	**0 (0 %)**
EMA	**Negative**	**15 (100 %)**
	Positive	**0 (0 %)**
	Diffuse>50%	**0 (0 %)**
MUC4	**Negative**	**15 (100 %)**
	Positive	**0 (0 %)**

Data are presented as frequency (%). SSTR-2A: Somatostatin receptors-2A, EMA: Epithelial membrane antigen, MUC4: Mucin-4.

Table 8: Comparison between meningioma and other non-meningioma cases by immunohistochemical expression of SSTR-2A, EMA and MUC4

	Meningioma (n= 60)	**Non-Meningioma cases (n= 15)**	**P value**
SSTR-2A positive	51 (85 %)	1 (6.7 %)	<0.001*
EMA positive	55 (91.7 %)	0 (0 %)	<0.001*
MUC4 positive	25 (41.7 %)	0 (0 %)	<0.001*

Data are presented as frequency (%), * significant as p < 0.05. SSTR-2A: Somatostatin receptors-2A, EMA: Epithelial membrane antigen, MUC4: Mucin-4.

Table 9: Validity (AUC, sensitivity, specificity) for SSTR-2A, EMA and MUC4 to discriminate meningioma (n= 60) from non meningioma (n= 15)

	AUC	**p**	**95% C.I**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
SSTR-2A	0.892	<0.001*	0.800 – 0.983	85.0	93.33	98.08	60.87
EMA	0.958	<0.001*	0.915 – 1.0	91.67	100.0	100.0	75.0
MUC4	0.708	0.013*	0.588 – 0.829	41.67	100.0	100.0	30.0
SSTR-2A + EMA	0.980	<0.001*	0.951 – 1.0	96.67	93.33	98.31	87.50
SSTR-2A + MUC4	0.960	<0.001*	0.913 – 1.0	95.08	93.33	98.31	82.35
EMA + MUC4	0.956	<0.001*	0.901 – 1.0	95.08	100.0	100.0	83.33
SSTR-2A+ EMA + Muc4	0.998	<0.001*	0.993 – 1.0	100.0	97.62	98.36	100.0

* significant as p < 0.05. SSTR-2A: Somatostatin receptors-2A, EMA: Epithelial membrane antigen, MUC4: Mucin-4.

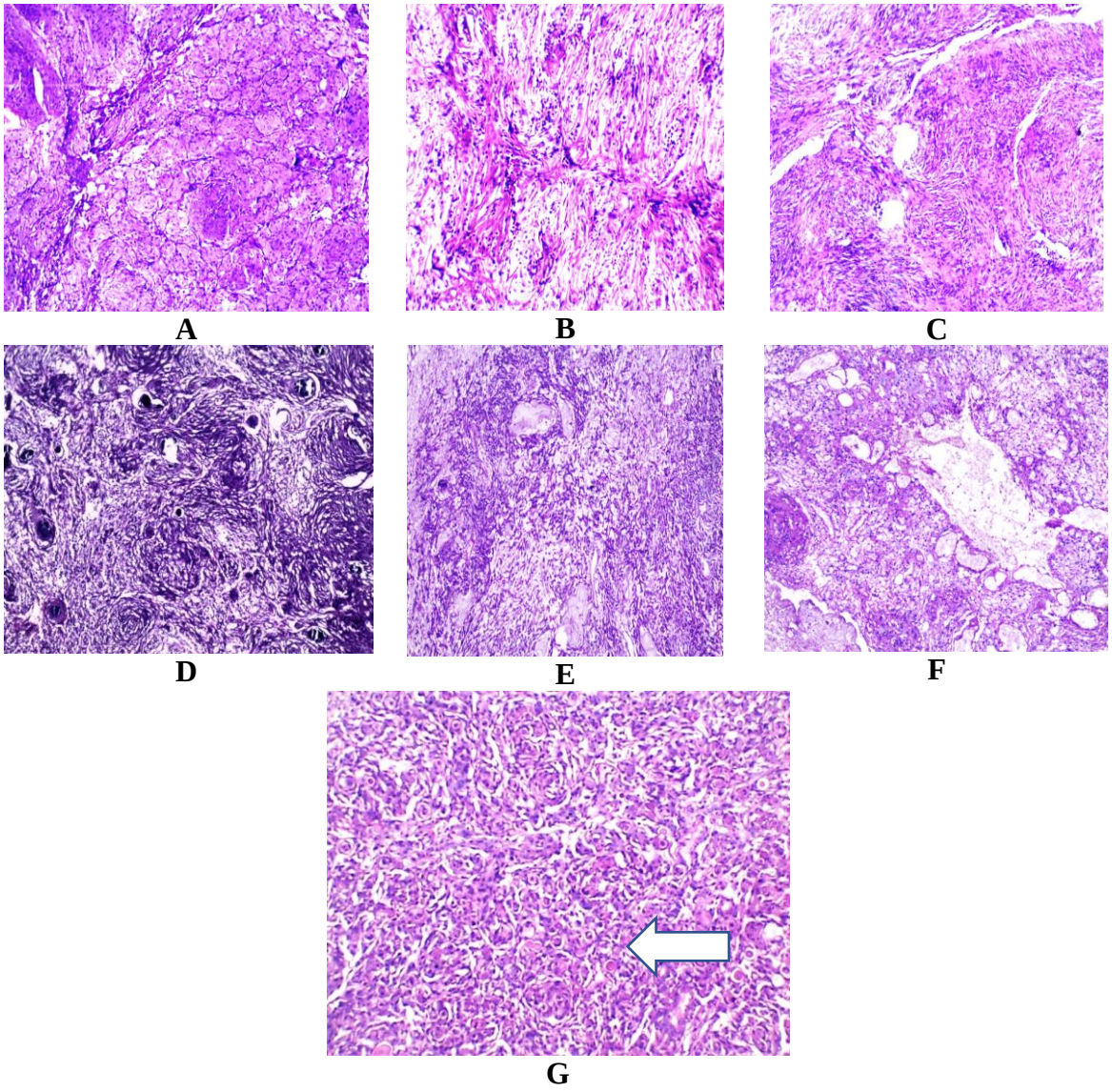


Figure 1: Grade I meningioma cases: (A) meningothelial meningioma showing whorls of meningothelial cells (H&Ex100), (B) fibrous meningioma showing interlacing bundles of spindle cells in collagen rich stroma(H&Ex200), (C) transitional meningioma showing whorls of meningothelial cells admixed with fascicles of spindle cells in collagenized stroma (H&Ex100), (D) psammomatous meningioma showing predominance of psammoma bodies forming calcified mass with intervening meningothelial cells (H&E x100), (E) microcystic meningioma showing meningothelial cells with thin elongated process forming microcysts appearance (H&Ex100), (F) angiomatous meningioma showing increase vascular component, meningothelial cells are wrapped around blood vessels (H&Ex200), (G) secretory meningioma showing sheets of meningothelial cells with eosinophilic intracytoplasmic inclusions "pseudopsammoma bodies" (H&E x200)

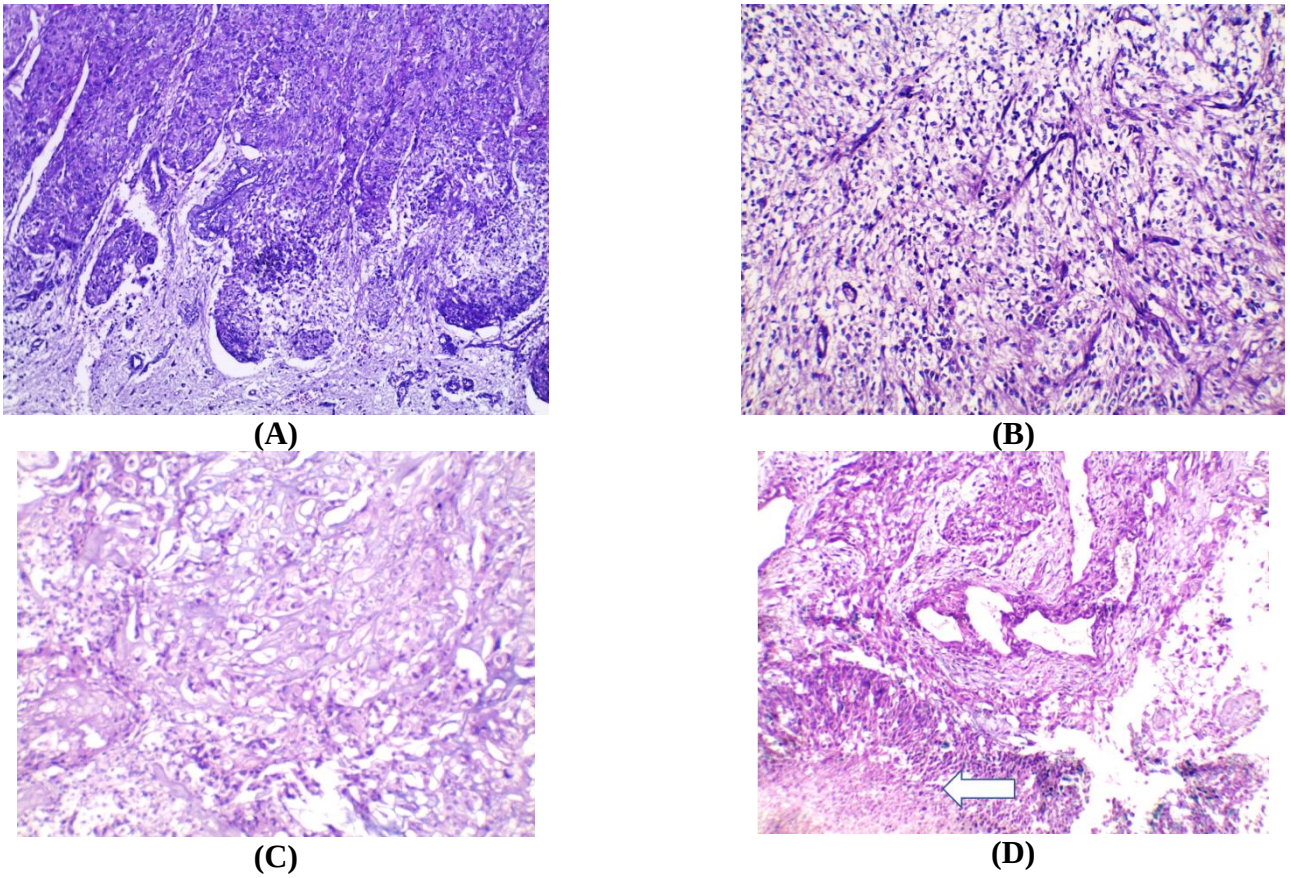
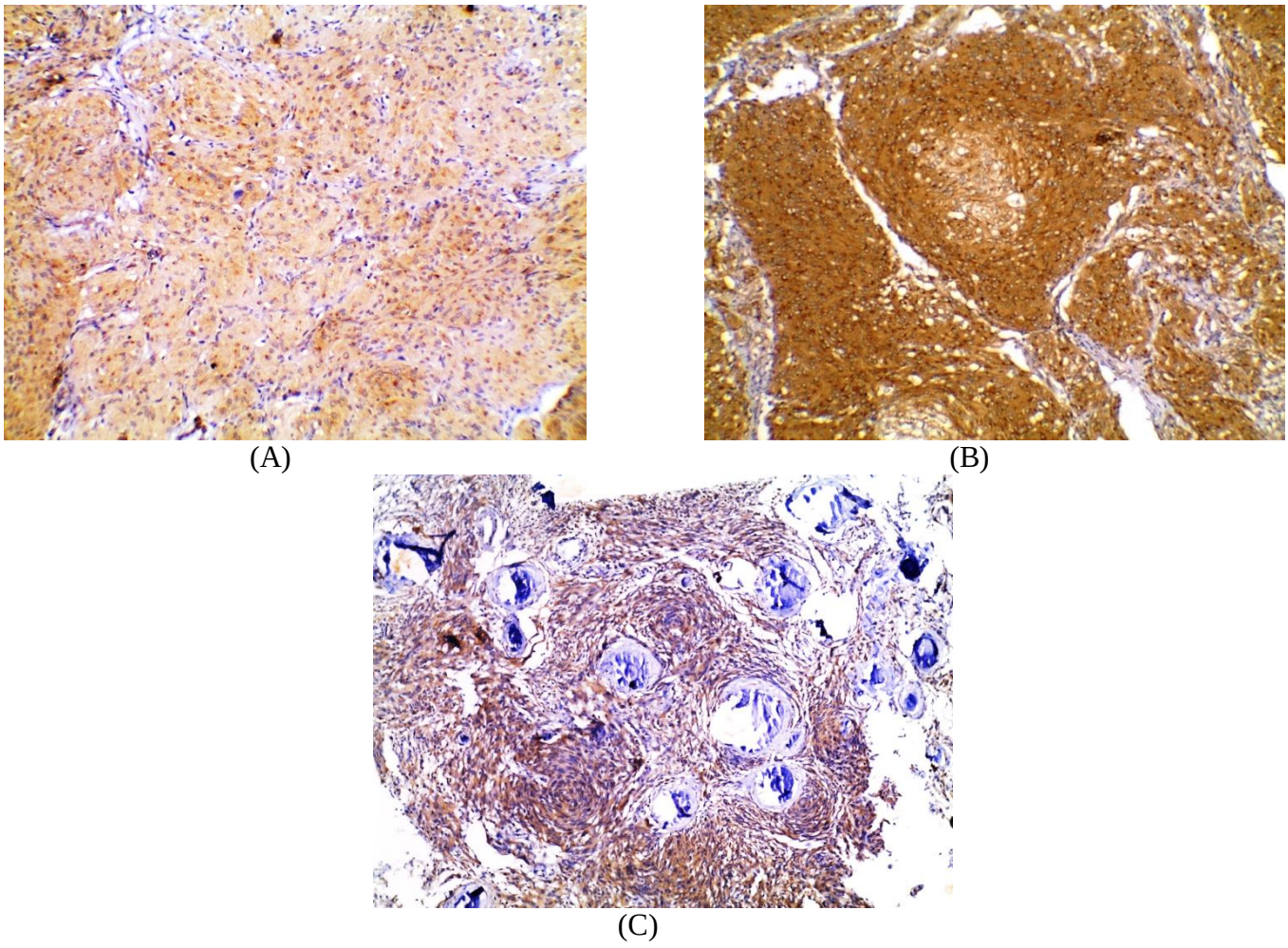


Figure 2: Grade II, III meningioma cases. (A) atypical meningioma showing brain invasion in form of tongue like protrusions of tumor cells infiltrating underlying parenchyma (H&E x100), (B) clear cell meningioma showing sheets of round to polygonal cells with clear , glycogen –rich cytoplasm (H&E x200), (C) chordoid meningioma showing cords of clear epithelioid cells (resembling physaliferous cells) within a myxoid stroma (H&E x200), (D) malignant (anaplastic) meningioma showing foci of necrosis (H&E x200)



(A) (B)

(C)

Figure 3: Cases of (A) meningothelial meningioma showing moderate cytoplasmic positivity for SSTR2A (score 3) (immunoperoxidase for SSTR2A, x200), (B) meningothelial meningioma showing strong cytoplasmic staining for SSTR2A (score 3) (immunoperoxidase for SSTR2A, x200), (C) psammomatous showing strong positive staining for SSTR2A (score 3) (immunoperoxidase for SSTR2A, x100)

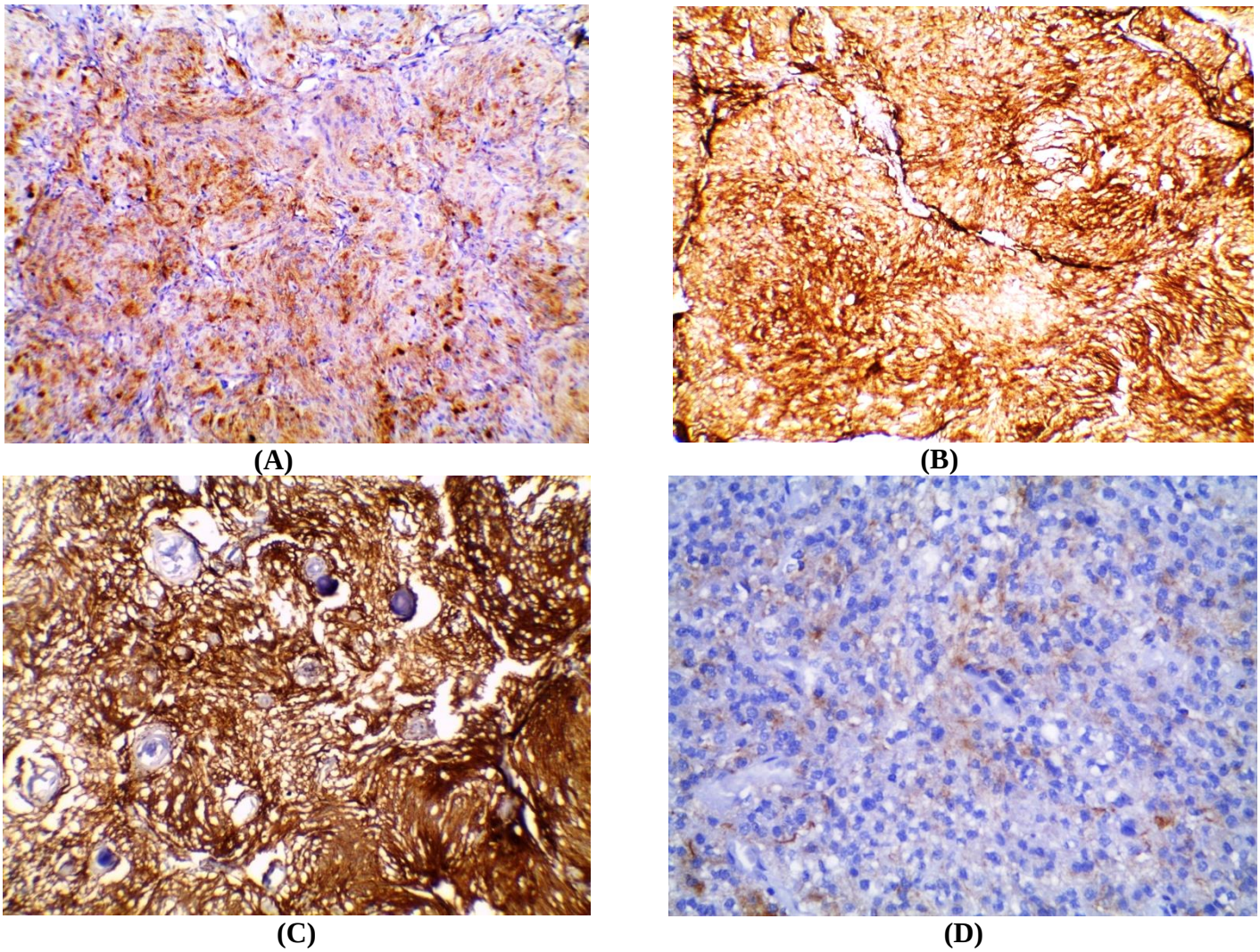


Figure 4: Cases of (A) meningothelial meningioma showing cytoplasmic diffuse positive >50% staining for EMA (immunoperoxidase for EMA, x200), (B) transitional meningioma showing diffuse positive >50% staining for EMA (immunoperoxidase for EMA, x200), (C) psammomatous meningioma showing diffuse positive >50% for EMA (Immunoperoxidase for EMA, x200), (D) atypical meningioma showing focal positive cytoplasmic staining for EMA (immunoperoxidase for EMA, x400)

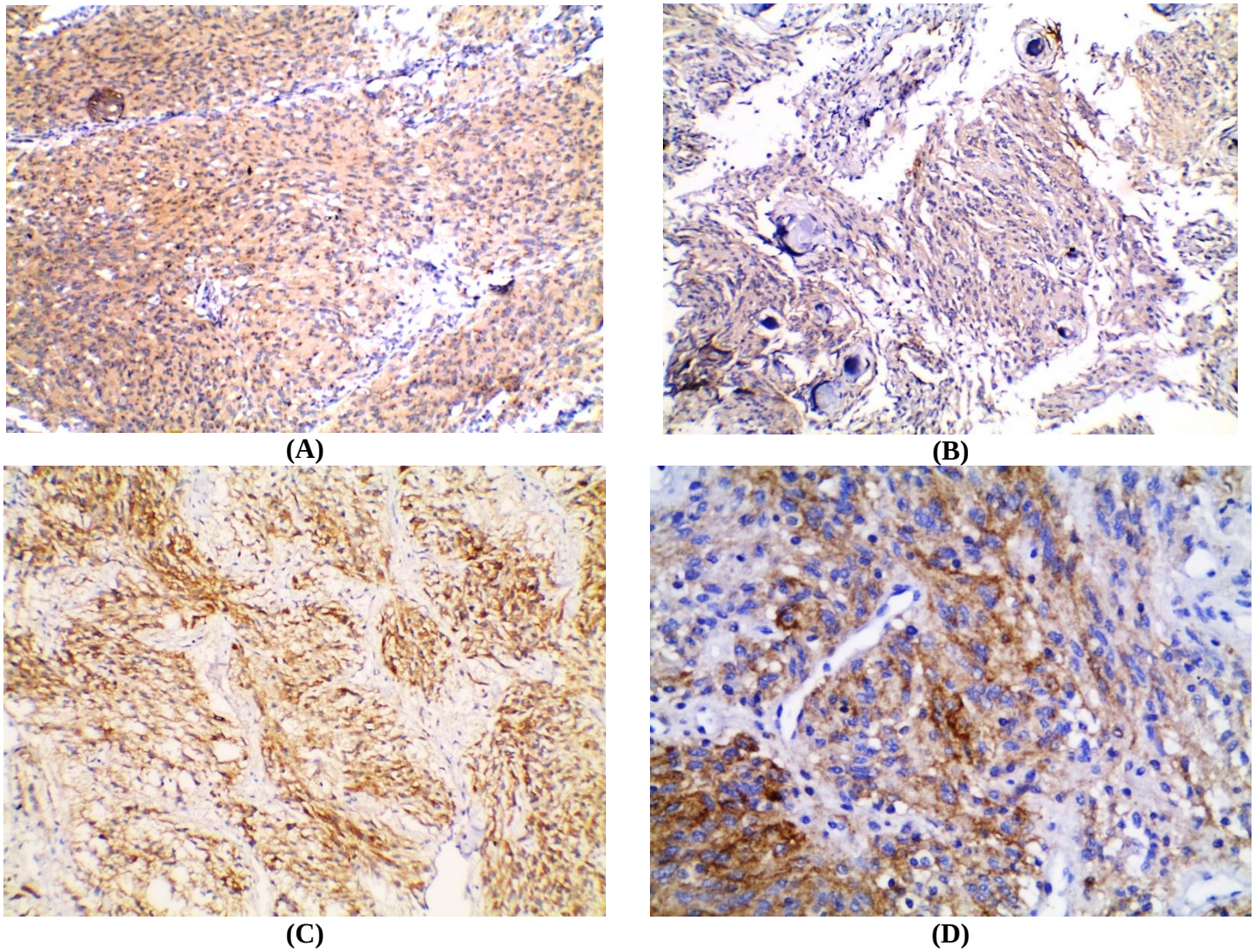


Figure 5: Cases of (A) transitional meningioma showing overall 70% positivity for MUC4 (immunoperoxidase for MUC4, x 200), (B) psammomatous meningioma showing overall 30% cytoplasmic positivity of tumor cells for MUC4 (immunoperoxidase for MUC4, x200), (C) metaplastic meningioma showing overall 60% cytoplasmic positive staining for MUC4 (immunoperoxidase for MUC4, x200), (D) chordoid meningioma showing positive staining for MUC4 with overall 30% positivity(immunoperoxidase for MUC4, x400)

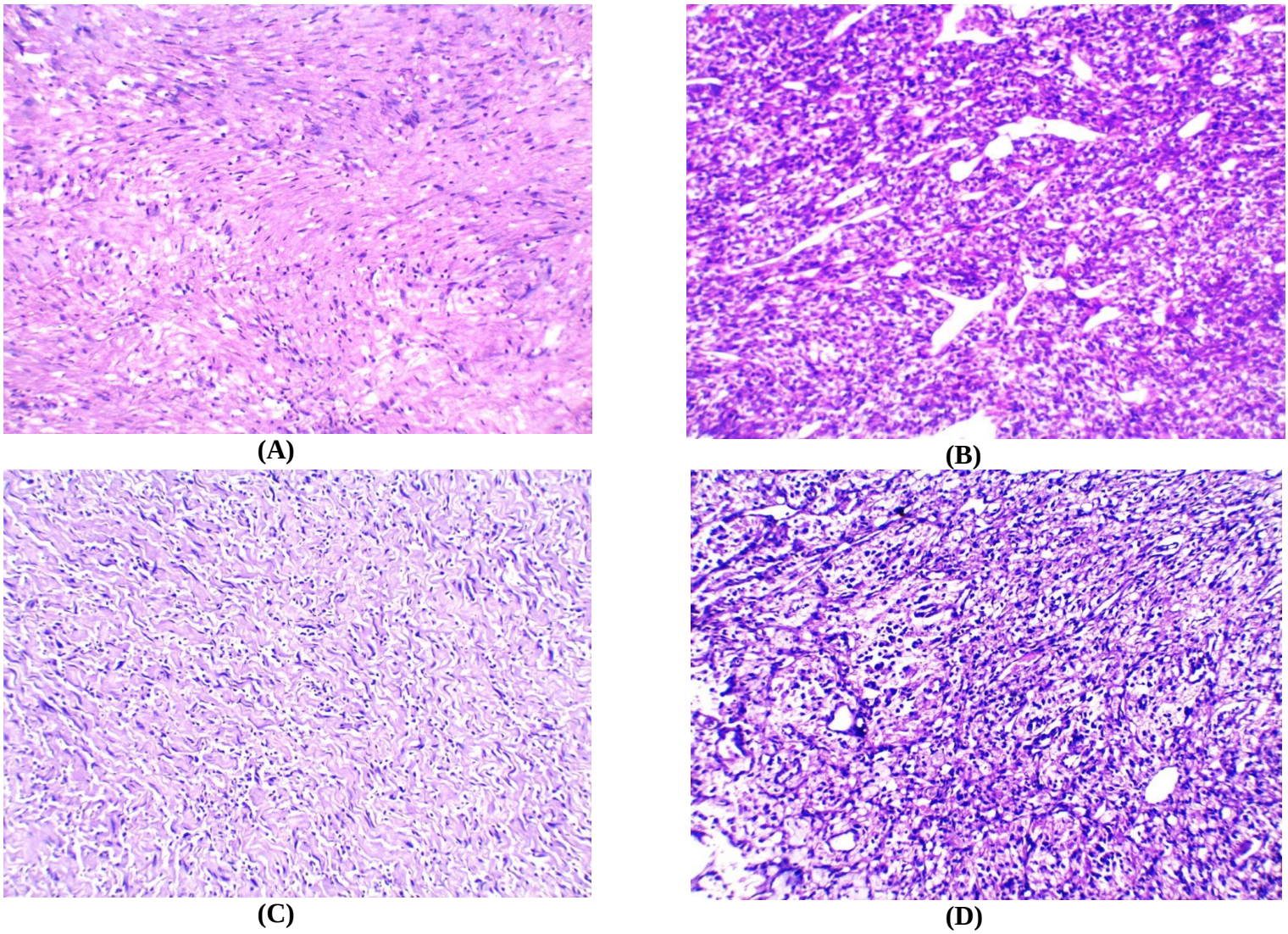


Figure 6: Cases of (A) schwannoma showing (Antoni A) with nuclear palisading (verocay body) alternating with hypocellular areas of fibrillary processes (Antoni B) (H&E X200), (B) hemangiopericytoma showing branching blood vessels (staghorn pattern) surrounded by monotonous tumor cells with high cellularity (H&E x200), (C) A case of neurofibroma showing sheets of spindle cells having wavy nuclei in background of shredded carrot appearance of collagen (H&E x200), (D) hemangioblastoma showing neoplastic cells arranged between numerous small vessels having clear vacuolated cytoplasm (H&E x200)

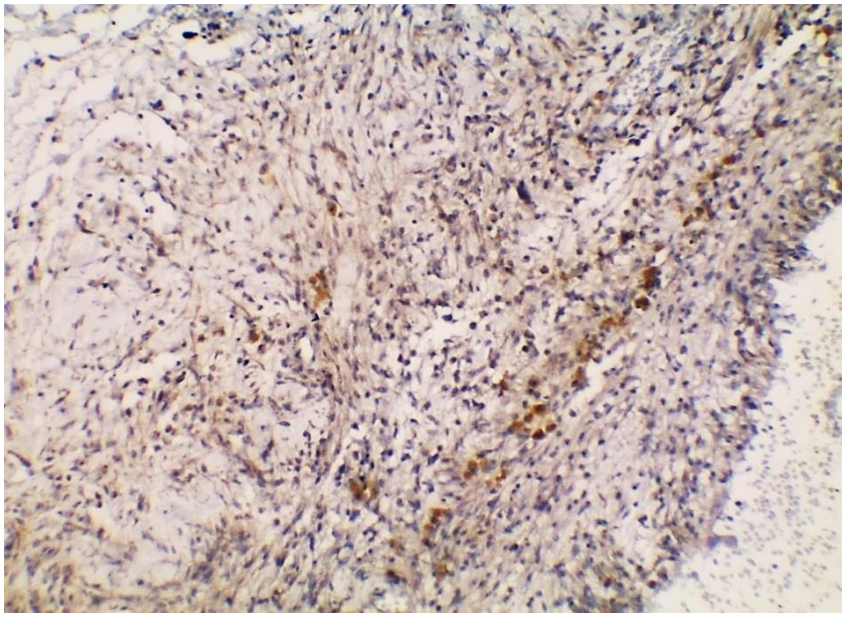


Figure 7: Case of schwannoma showing moderate positive staining for SSTR-2A (score 1) (immunoperoxidase for SSTR-2A, x200)

Discussion

In the present study, the mean age of meningioma cases was 48 years. These results were close to the results of Anis et al. [10] who stated that the mean age incidence of meningioma cases was 42 years which may be due to similar genetic and environmental condition since both studies were carried out on Egyptian population.

But these results were lower than the results of Barresi et al. [12] and Matsuyama et al. [3] who found that the mean age incidence of meningioma cases were 64 years and 65.5 years respectively, this difference in age incidence may be attributed to different genetic and/or environmental factors.

In the current work, the incidence of meningioma in female was (53.3%) of the studied cases which was lower than the studies of Barresi et al. [12] and Mansour et al. [13] who found that the incidence of meningioma in female were 62.9% and 64% respectively. This may be attributed to the large scale of cases in both studies, but it indicates that meningioma is more common in females.

Regarding tumour site, 85% of meningioma cases had cranial location. These results were very close to the studies of Agaimy et al. [11] and Matsuyama et al. [3] who stated that 89.7% and 87% of meningioma cases respectively were located intracranial. Also, this study included 15% of meningioma cases were at spinal location. These results were higher than the results of Agaimy et al. [11] and Matsuyama et al. [3] who stated that 1.5% and 6.4% of meningioma cases respectively were at spinal location. This difference may be due to the higher number of cases in these studies than our study.

In our study, grade I meningioma cases were more common than grade II and grade III. The incidence of grade I meningioma cases was 70% which was closer to the study of Mansour et al. [13] who documented the incidence of grade I cases 80%.

In the present study, the mean size of tumour was 4.9 cm, this result was higher than the result of Matsuyama et al. [3] who recorded mean tumour size 3.6 cm and lower than the study of Mansour et al. [13] who noted that the mean tumour size was 5.4 cm.

Although the diagnosis of meningioma can be based on routine examination of hematoxylin and eosin staining, it can mimic other tumours of CNS. So, using of IHC is mandatory for differential diagnosis. In the

present study, we compared the immunohistochemical expression of SSTR-2A, EMA, MUC4 in meningioma versus their expression in schwannoma, neurofibroma, hemangiopericytoma and hemangioblastoma.

In the current study, positivity of SSTR-2A immunoexpression was detected in 85% of different meningioma cases which was so close to the studies of Agaimy et al. [11] and Anis et al. [10] who stated that incidence of positivity for SSTR-2A was 87% and 89% respectively.

In this study, the expression of SSTR-2A in different grades of meningiomas showed the highest expression in grade I and grade II meningioma cases and the lowest expression in grade III meningioma, this was in agreement of the study of Anis et al. [10]. This meant that the expression of SSTR-2A decreases in grade III meningioma cases, so we can suggest that the detection of strong immunohistochemical staining of SSTR2-A may predict a better outcome.

This study also noted that most of SSTR-2A positive cases in different grades of meningioma showed moderate intensity staining which was in contrast with the study of Anis et al. [10] which noted that most of the grade I and grade II meningioma cases showed strong intensity staining while weak intensity staining in grade III meningioma cases. This may be due to the different clone of marker.

In the current study, the sensitivity of immunohistochemical expression of SSTR2A in meningiomas was 85% , that was close to the study of Agaimy et al. [11] and Anis et al. [10] which was 87% and 89% respectively.

The results of the present study were lower than those documented by Bacchi et al. [14] and Menke et al. [15] as they found that the sensitivity of SSTR-2A in detecting of meningioma was 100% in each study and also lower than the study of Boulagnon-Rombi et al. [1] who reported that the sensitivity of SSTR-2A for meningioma was 95.5%. This may be due to different clone of marker.

The specificity of SSTR-2A in detection of meningioma cases in this study was 93.3% which was very close to the study of Boulagnon-Rombi et al. [1] who found that the specificity of SSTR-2A for meningioma was 92%.

Regarding the non-meningioma cases, the current study found one case of schwannoma was positive for SSTR-2A expression, this was disagreed with Anis et al. [10] who reported that all cases of schwannoma were negative for SSTR-2A expression. This may be due to using of polyclonal antibody in our study. While our findings agreed with the results of Bacchi et al. [14] and Menke et al. [15] who noted the low specificity of SSTR2A as 90% and 88% respectively. Thus, SSTR-2A is not useful marker to distinguish meningioma from schwannoma.

In the current study, all cases of neurofibroma, hemangiopericytoma were completely negative for SSTR-2A expression which agreed with the studies of Boulagnon-Rombi et al. [1] and Anis et al. [10] .

In the present study, the sensitivity of EMA was 91.7%, that was very close to the study of Boulagnon-Rombi et al. [1] who found that the sensitivity of EMA immunoexpression was 89.6%.

In this study, the expression of EMA in different grades of meningiomas showed the highest expression in grade I and grade II meningioma cases and the lowest expression in grade III meningioma, this was in concordance with the study of Boulagnon-Rombi et al. [1]. This is highly suggestive that the expression of EMA decreases in grade III meningioma cases.

At the current work, EMA expression was diffuse positive in 61.7% of meningioma cases which was lower than the study of Agaimy et al. [11] who noted that EMA immunoexpression was diffuse positive in 88.5% of meningioma cases.

The specificity of EMA for meningioma diagnosis in the current study was 100% which was higher than the study of Boulagnon-Rombi et al. [1] who stated that

the specificity of EMA immunoexpression was 87.5%, this may be due to the higher number of cases than our study.

Regarding to the non-meningioma cases, all cases of schwannoma were negative for EMA immunoexpreesion which was disagreed with the study of Boulagnon-Rombi et al. [1] who reported 2 positive cases of schwannoma. This may be due to the different subtypes of schwannoma in their studies than this study.

The other non-meningioma cases (neurofibroma and hemangioblastoma) in this study were negative for EMA immunoexpression this was agreed with the study of Boulagnon-Rombi et al. [1] who found that all cases of neurofibroma and hemangioblastoma were negative for EMA immunoexpression.

Regarding MUC4, the current study noted lower MUC4 positivity for meningothelial and angiomatous subtypes of meningioma cases which was in contrast of the studies of Matsuyama et al. [3] and Kong et al. [16] who found the incidence of MUC4 positivity in meningothelial subtype 100% in each study and in angiomatous subtype with incidence of positivity 100% and 97.8% respectively. A possible explanation for this could be the small number of angiomatous and meningothelial meningioma cases in this study than their studies.

Among the positive cases for MUC4, this study found that all of them showed MUC4 positivity in 10-80% of tumour cells which was disagreed with the study of Matsuyama et al. [3] who noted that 71.5 % of cases were positive for MUC4 expression in 10-100% of tumour cells.

Regarding expression of MUC4 in different grades of meningioma, our study noted that MUC4 immunoexpression was higher in grade I meningioma cases than grade II and grade III cases which was in agreement with the study of Matsuyama et al. [3] and Mansour et al. [13]. This can suggest that MUC4 immunoexpression decreases with grade II and grade III meningiomas.

All atypical meningioma cases (without special pattern) in this study were negative for MUC4 immunoexpression which was in contrast with the study of Mansour et al. [13] who noted that all atypical meningioma cases were positive for MUC4 immunoexpression. This may be explained by different sample size.

Regarding MUC4 sensitivity in detection of meningioma, we detected immunohistochemical expression of MUC4 with sensitivity 41.67% which was lower than the study of Matsuyama et al. [3] who found that the sensitivity of MUC4 immunoexpression was 92.9%. This may be due to higher number of cases than our study.

The specificity for MUC4 immunoexpression in meningioma cases of this study was 100% which was higher than the study of Boulagnon-Rombi et al. [1] who stated that the specificity of MUC4 immunoexpression was 65.7%.

Regarding to the non-meningioma cases in our study, all were negative for MUC4 immunoexpression with 100% specificity, this was in concordance with the study of Matsuyama et al. [3] and Kong et al. [16], but in the study of Mansour et al. [13], they noted that two cases of non-meningioma cases (one case of schwannoma and one case of hemangiopericytoma) were positive for MUC4 immunoexpression which decrease the specificity of MUC4 to 93.3% .

The coexpression of SSTR-2A and EMA in diagnosis of meningioma in the current study showed 96.67% sensitivity which was higher than the study of Boulagnon-Rombi et al. [1] who noted that the sensitivity of coexpression of both markers was 84.9% while, the specificity of coexpression of SSTR2A and EMA in this study was 93.3% which was very close to the study of Boulagnon-Rombi et al. [1] who stated that the specificity of coexpression was 94.8%. So, we can suggest that the combination of two markers have strong diagnostic value.

In comparison between SSTR-2A and MUC4 immunoexpression, all cases of meningioma which were negative for SSTR-2A showed positivity for MUC4 immunoexpression, so MUC4 is a useful

diagnostic marker for meningioma and using it in combination with SSTR-2A is better for diagnosis. This was in agreement with the study of Matsuyama et al. [3].

All negative cases for SSTR-2A in our study were positive for EMA immunoexpression except papillary subtype which was negative for both SSTR-2A and EMA

Using a panel of the three markers (SSTR-2A, MUC4 and EMA) is diagnostically superior to using each of which alone. EMA immunophenotyping is considered the most specific and sensitive marker for meningioma while MUC4 is more specific for meningioma than SSTR-2A but less sensitive. Coexpression of SSTR-2A and EMA showed the highest sensitivity and the highest NPV for meningioma. Co expression of EMA and MUC4 showed the highest specificity and the highest PPV for meningioma. Negative correlation between immunoexpression but positive for MUC4 immunoexpression. This was disagreed with the study of Boulagnon-Rombi et al. [1] who stated that all SSTR-2A negative cases were positive for EMA immunoexpression.

Conclusions

SSTR-2A and EMA with lower grade of meningioma may indicate that they are not only diagnostic but also, they have prognostic value. Rare positivity of SSTR-2A and complete negativity of both EMA and MUC4 in non-meningioma cases refers to their significant diagnostic importance in the differential diagnosis between them and meningioma.

List of abbreviations

IHC	Immunohistochemistry
SSTR-2A	Somatostatin receptors-2A
EMA	Epithelial membrane antigen
MUC4	Mucin-4

Conflicts of interest

The authors report no conflict of interest.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Eman Ahmed Abu-Elenain and Hend Salah Abo Safi and Rania El-Sayed Wasfy. The first draft of the manuscript was written by Mohamed Mostafa Shareef and Maha Mostafa Shamloula, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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