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ALL INDIA INSTITUTE OF MEDICAL SCIENCES, JODHPUR
विकृति विज्ञान विभाग
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The Dean (Academics)
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Subject: Submission of M.D thesis.

आदरणीय महोदय,

This is to submit that the M.D thesis by the Academic Junior Resident (July 2020 Batch) of our Department has been duly completed and signed and is ready for submission. Please accept the same.

Details of her thesis are attached herein:

Name of candidate	Thesis topic
Dr. Tanya Garg	EXPRESSION OF SSTR-2 AND HER2/NEU IN MENINGIOMAS AND ITS CORRELATION WITH CLINICO-PATHOLOGICAL PARAMETERS

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EXPRESSION OF SSTR-2 AND HER2/NEU IN MENINGIOMAS AND ITS CORRELATION WITH CLINICO-PATHOLOGICAL PARAMETERS

THESIS

Submitted to

All India Institute of Medical Sciences, Jodhpur

In partial fulfilment of the requirement for the degree of

DOCTOR OF MEDICINE (MD)

PATHOLOGY

JULY, 2020 **DR. TANYA GARG**

AIIMS, JODHPUR

DECLARATION

I hereby declare that the thesis titled **"EXPRESSION OF SSTR-2 AND HER2/NEU IN MENINGIOMAS AND ITS CORRELATION WITH CLINICO-PATHOLOGICAL PARAMETERS"** embodies the original work carried out by the undersigned in All India Institute of Medical Sciences, Jodhpur.

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CERTIFICATE

This is to certify that the thesis titled **"EXPRESSION OF SSTR-2 AND HER2/NEU IN MENINGIOMAS AND ITS CORRELATION WITH CLINICO-PATHOLOGICAL PARAMETERS"** is the bonafide work of **DR. TANYA GARG** carried out under our guidance and supervision, in the Department of Pathology and Lab Medicine, All India Institute of Medical Sciences, Jodhpur.

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- Albert Schweitzer

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INDEX

SYNOPSIS

Meningiomas are one of the most common tumours of the central nervous system. Although commonly benign, atypical and anaplastic meningiomas also constitute part of the spectrum. They are known to have unpredictable outcome. Options for management are limited to surgery and radiotherapy. SSTR-2 has shown great promise as a target for therapy as well as intra-operative imaging of meningiomas. The knowledge of HER2/neu status has been invaluable in various other malignancies. However, it has shown variable results in meningiomas.

The present study was aimed to evaluate the expression of SSTR-2 and HER2/neu in meningiomas and to establish their correlation with various clinico-pathological parameters.

This was an ambispective observational cross-sectional study carried out in a tertiary care hospital in Western Rajasthan. This study included 120 cases of meningioma. On application of immunohistochemistry for SSTR-2 and HER2/neu on these cases, 75.83% cases showed positivity for SSTR-2 and none of the cases were positive for HER2/neu. SSTR-2 expression showed statistically significant association with location of meningioma in brain, histologic subtype and grade of meningioma. No significant association was found with age and gender. No statistically significant association was found between HER2/neu expression and age, gender, location, histologic subtype and grade of meningioma.

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ARB	Antigen retrieval buffer
ASCO	American Society of Clinical Oncology
BAP1	BRCA-1 associated protein 1
BCL2	B-cell leukaemia/lymphoma 2
CAP	College of American Pathologists
cAMP	Cyclic adenosine monophosphate
Ca^{2+}	Calcium ion
CD	Cluster of differentiation
CDKN2A	Cyclin dependent kinase inhibitor 2A
CDKN2B	Cyclin dependent kinase inhibitor 2B
CEA	Carcinoembryonic antigen
cerbB2	Avian erythroblastosis oncogene B
CI	Confidence interval
CNS	Central nervous system
CREBBP	Cyclin adenosine monophosphate response element binding protein binding protein
CT	Computed tomography
DAB	3,3'-Diaminobenzidine
DNA	Deoxyribonucleic acid
DPX	Dibutylphthalate polystyrene xylene
EDTA	Ethylene diamine tetraacetic acid
EGFR	Epidermal growth factor receptor
EMA	Epithelial membrane antigen
ER	Estrogen receptor
FISH	Fluorescence in-situ hybridization
GFAP	Glial fibrillary acidic protein
GLUT1	Glucose transporter 1
H&E	Haematoxylin and Eosin
HCl	Hydrochloric acid
HER2/neu	Human epidermal growth factor receptor2/neu

HMB45	Human melanoma black 45
HPF	High power field
HRP	Horseradish peroxidase
IHC	Immunohistochemistry
IgG	Immunoglobulin G
KLF4	Krueppel like factor 4
MAPK	Mitogen-activated protein kinase
MRI	Magnetic resonance imaging
mTOR	Mammalian target of rapamycin
MVD	Microvessel density
NaOH	Sodium hydroxide
N:C	Nucleo-cytoplasmic ratio
NF	Neurofibromin
PAS	Periodic acid-Schiff
PBRM1	Polybromo-1
PDGF-β	Platelet derived growth factor beta
PI3K	Phosphoinositide 3-kinase
PIK3CA	Phosphatidylinositol-4,5-bisphosphate-3-kinase catalytic subunit alpha
PLL	Poly-L-Lysine
PR	Progesterone receptor
PTCH1	Patched 1
PTEN	Phosphatase and tensin homolog
RR	Relative risk
RT-PCR	Reverse transcription-Polymerase chain reaction
SD	Standard deviation
SMARCB1	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1
SMARCE1	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1
SMO	Smoothened, Frizzled class receptor
SOX10	Sry-related HMg-Box gene 10
SSTR-2	Somatostatin receptor-2

STAT6	Signal transducer and activator of transcription 6
SUFU	Suppressor of fused
TaSC	Target Selection Criteria
TERT	Telomerase reverse transcriptase
TRAF7	Tumor necrosis factor receptor-associated factor 7
V/V	Volume per volume
VEGF	Vascular endothelial growth factor
VHL	Von Hippel-Lindau
WHO	World Health Organization

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INTRODUCTION

Meningioma is a tumour present in the primary central nervous system and forms on the membranes that cover the spinal cord and brain inside the skull. It originates from arachnoid cap cells that form a spider web-like membrane covering the brain and spinal cord. As per the statistics, it is one of the most common central nervous system tumours having an incidence of 8.14 per 1,00,000 population (1). The risk of meningiomas increases with age, especially after 65 years. Children between the age group of 0-14 years are found to be at the lowest risk (2).

The typical and gradual onset of meningioma is found in later decades of life and has generally shown favourable outcomes in comparison to other central nervous system-based tumours. However, there is presence of a great deal of variability in terms of both aggressiveness and outcome. Although, the majority of meningiomas are found to be benign, these tumours can grow slowly until becoming very large, if left undiscovered. Also, based on the presence of its location, an undiscovered meningioma can become severely disabling and life-threatening.

Presently, some of the most common markers used to diagnose meningiomas are, EMA and PR. However, these markers are found to have suboptimal sensitivity that can vary based on grade and type. Newer markers, such as SSTR-2A have helped in providing better performance as compared to the existing ones.

Somatostatin receptor - 2 belongs to a seven transmembrane domain G-protein coupled receptor family, which comprises of five subtypes, that is, SSTR-1-5, which are widely expressed in both normal tissues as well as solid tumours. SSTR-2 is expressed in a variety of tumour tissues, such as pituitary adenomas, melanoma, neuroendocrine tumours, breast cancer and thyroid cancer. However, as per the accumulative evidence, SSTR-2 has been found to be more sensitive diagnostic marker for meningiomas (3).

Human epidermal growth factor receptor 2/neu (HER2/neu) is an oncoprotein belonging to the EGFR/HER family of receptor tyrosine kinase (4). Its overexpression, through either gene amplification or transcriptional dysregulation, is what constitutes its transforming potential. Its expression in breast and gastric

carcinomas is well known, where it acts as a prognostic as well as a predictive marker. With increasing role of targeted therapy in treatment protocols, the potential role of HER2/neu as a target for therapy and as a predictive marker in meningiomas needs investigation (5).

Since the majority of meningiomas are slow-growing, it can become difficult to diagnose them at an early stage. Also, diagnosis becomes challenging when it is present at uncommon locations. Treatment options are limited with the best chance of cure being offered by surgery where meningioma is completely removed including the fibres that are attached to the coverings of the brain and bone. Adjuvant radiotherapy is administered for residual and recurrent lesions (6). However, these treatment modalities come with potential and significant risk, especially when the tumour has invaded brain tissue and surrounding veins.

The study of expression of alternative markers like SSTR-2 and HER2/neu and their correlation with tumour grade and other clinico-pathological parameters will help to develop alternate less invasive treatment options and targeted therapy for meningiomas especially in recurrent cases and cases resistant to radiotherapy. The current study was undertaken with a similar intent.

REVIEW OF LITERATURE

Meningiomas are the most common primary intracranial tumours with their origin from the arachnoid cap cells (3). Antonio Pacchioni was the first to describe arachnoidal granulations in 1705. Rainey, in 1846, suggested meninges as the origin of arachnoidal granulations. John Cleland in 1864 associated meningeal tumours with these pacchionian granulations (7). The term "meningioma" was first introduced by Harvey Cushing in 1922 for tumours arising from the meninges covering the brain and spinal cord. In the same year, Oberling attempted to classify meningioma into subtypes on the basis of meningeal cell structure. In 1930, del Rio Hortega classified them on the basis of structure and architectural features. Bailey and Bucy later classified meningiomas into nine types based on their phenotypic differences. In 1979, WHO classification of tumours of nervous system described seven subtypes which included meningotheliomatous, psammomatous, angiomatous, transitional, haemangioblastic and haemangiopericytic variants with acknowledgement of haemangioblastomas and haemangiopericytomas as distinct entities. John Kepes, in 1982, illustrated many patterns with the current histologic subtypes of meningioma finding their origin in many of those patterns (8).

ANATOMY

The brain and spinal cord are enveloped by three layers of membranes, called meninges. The three layers, from superficial to deep are the dura, the arachnoid and the pia, with the term 'mater' (Latin for mother) following these names. The dura mater comprises of dense connective tissue and is adherent to the inner side of the skull and vertebra. The arachnoid mater is thin and wispy, lying between the dura and inner most pia mater. The pia mater is thin and clear and is adherent to the surface of brain and spinal cord. These three layers form the epidural, subdural and the subarachnoid spaces (9).

Dura mater

It is the outermost meningeal layer composed of 2 layers-

1. The periosteal layer, and

2. The meningeal layer.

The periosteal layer comprises of fibroblasts and osteoblasts with large quantities of extracellular collagen. This collagen gives the dura its strength, fibrous and inelastic nature (9).

Arachnoid mater

It is avascular which bridges the contours of the cortical sulci, but does not follow them. It is thin and lucent and comprises of a superficial layer of mesothelial cells, a middle layer with cells joined by junctional proteins and an innermost layer of loosely placed cells with intercellular collagen fibres. Small protrusions of the arachnoid mater into the dura mater are called the arachnoid granulations (also called the arachnoid villi, the pacchionian granulations or the pacchionian bodies). These arachnoid granulations form a connection between the cerebrospinal fluid and the blood stream (9).

Pia mater

It is the innermost layer and follows the contours of the gyri and sulci. The outer layer is the epipial layer containing collagen fibres and the inner layer is the intima pia containing elastic and reticular fibres. The epipial layer is connected to the arachnoid mater via the arachnoid trabeculae and the intima pia is adherent to the outermost glial membrane of the neural tissue (9).

EMBRYOLOGY

The dura mater, also called the pachymeninx, derives from mesoderm. In contrast, the arachnoid mater and the pia mater, known as leptomeninges, derive from the ectoderm. Meninges begin to develop from the perimedullary mesenchyme containing cells from the ectoderm and mesoderm. The pia and arachnoid form first as a result of differentiation of neural crest cells. The dura differentiates from the sclerotomes a subdivision of mesodermal somites (9)

WHO SUBTYPES OF MENINGIOMAS (10)

1. Meningothelial meningioma

2. Fibrous meningioma

3. Transitional meningioma

4. Psammomatous meningioma

5. Angiomatous meningioma

6. Microcystic meningioma

7. Secretory meningioma

8. Lymphoplasmacyte-rich meningioma

9. Metaplastic meningioma

10. Chordoid meningioma

11. Clear cell meningioma

12. Rhabdoid meningioma

13. Papillary meningioma

14. Atypical meningioma

15. Anaplastic (malignant) meningioma

LOCALIZATION

Meningiomas may be seen in intracranial, intraspinal and orbital locations with the most common sites being the cerebral convexities, sphenoid ridges, petrous ridges, olfactory grooves, tentorium, parasellar/suprasellar regions, posterior fossa and optic nerve sheath (10).

Strong association has been noted between the tumour location and the mutation spectrum (10).

- Convexity meningioma, spinal meningioma (majority) – 22q deletion and/or NF2 mutations
- Skull base meningioma – mutations in AKT1, TRAF7, SMO and/or PIK3CA

CLINICAL FEATURES

Meningiomas are slow growing tumours, with neurological deficits occurring according to the tumour location. The signs and symptoms are produced by pressure effect due to compression of the adjacent structures. The common clinical manifestations include headaches, seizures and weakness. However, these symptoms are not specific for meningiomas (10).

IMAGING

On magnetic resonance imaging, meningiomas appear as dural masses which are isodense with uniform contrast-enhancement. Calcifications are commonly seen, and are better visualized on CT scan. “Dural-tail sign”, which is contrast-enhancing, may be frequently seen at the tumour perimeter and signifies reactive fibrovascular tissue and does not always correspond to dural involvement. Certain histological subtypes, like secretory, lymphoplasmacyte-rich, angiomatous/microcystic and high grade meningiomas may exhibit prominent peritumoural cerebral edema. Few meningiomas may show intratumoural or peritumoural cyst formation. These neuroimaging features are not specific for diagnosis or estimation of prognosis. However, gadolinium-enhanced MRI provides qualitative and quantitative imaging features which may suggest histological grade and likely patient outcomes in meningiomas (10).

EPIDEMIOLOGY

Incidence

Meningiomas are the most common primary brain tumours in adults and least common in children. They account for 37.6% of CNS tumours in the USA occurring at a rate of 8.58 cases per 100,000 population (10). In India, meningiomas account for 23.2% of primary intracranial tumours in the adult population and 4.2% in the paediatric population (11).

Age, sex and race distribution

With age, the risk of meningioma increases with the median age at diagnosis being 66 years (10). Incidence is more than two-fold higher in females, than in males.

However, the atypical and malignant meningiomas have a slight male predominance. Also, the incidence of meningioma is higher in Black Non-Hispanics than in White Non-Hispanics and Hispanics (12).

MOLECULAR ETIO-PATHOGENESIS

Sporadic meningiomas are associated with focal chromosomal deletion while higher grade meningiomas generally have multiple chromosomal copy number alterations (12). The most common cytogenetic change is aberration in chromosome 22 (13,14). The gene located on chromosome 22 is NF2, which encodes for Merlin or Schwannomin, a cell membrane-related protein, that acts as a tumour suppressor. NF2 gene alterations may occur in the form of mutation, splicing alteration, allelic inactivation or chromosome 22 loss. Loss of NF2 gene function is seen in almost all meningiomas associated with neurofibromatosis type II. It is autosomal dominant, and leads to bilateral vestibular schwannomas, intracranial and spinal meningiomas and along with other spinal tumours like ependymoma. Some meningiomas and schwannomas that harbor NF2 alterations show co-occurrence of NF2 and SMARCB1 mutations (14). Atypical meningiomas may show allelic loss in chromosomes 1p, 6q, 9q, 10q, 14q, 17p and 18q. Anaplastic meningiomas may show loss for 6p, 9p21, 10 and 14q (13). A subset of meningiomas are characterized by dysregulation in PI3K-AKT1-mTOR signaling pathway, while some others are characterized by activation of the Hedgehog signaling pathway. Few anterior skull base/olfactory groove meningiomas are associated with hotspot mutations in the *Smoothened, Frizzled Class Receptor (SMO)* and are associated with higher tumour recurrence (14).

RISK FACTORS

Ionizing radiation

Exposure to ionizing radiation is presently the primary environmental risk factor. In a study by Sadetzki et al., a relative risk of almost 10 was observed in a cohort of children who received radiation therapy for Tinea capitis (15). Exposure to high levels

of ionizing radiation as in atomic bomb survivors and therapeutic radiation to the head imparts greater risk (10,12).

Hormones

Association of meningioma risk and hormones is suggested by increased incidence of meningioma in women, increased risk with endogenous/ exogenous hormone use associated with presence of hormone receptors (estrogen/ progesterone/ androgen), association with breast cancer, change in size during pregnancy and luteal phase of menstrual cycle, increased risk associated with body mass index, current smoking and decreased risk with breastfeeding for more than 6 months (10,12).

Head trauma

Results across studies, suggesting the association of head trauma as a risk factor for meningioma, have not been consistent (12). In a study by Inskip et al., of the 228,055 Danish residents which were hospitalized for head injuries like concussion, skull fracture or others, with a follow up period of 8 years, after the first year, the standardized incidence ratio was 1.2 (16). However, this association might be a result of detection bias (12).

Cell phone use

Presently, there is little evidence to suggest an association between cell phone use and meningioma risk. The limitations to the studies conducted include small sample size, short follow-up time since beginning of cell phone use, and crude measurement of cell phone use (12).

Occupation/ diet/ allergy

The studies to associate specific chemicals in occupationally exposed groups have been inconclusive (12). In a study by Terry et al., no association was found between diet and meningioma (17) In a meta-analysis conducted by Linos et al., significant inverse relationship was observed between meningioma and allergy (pooled RR = 0.84, 95% CI 0.72-0.98, P = 0.029) (18). Also, in a study conducted by Schoemaker et al., in 2006, a consistent inverse risk was noted with asthma, eczema and hay fever (19).

Family history

According to a study by Malmer et al., there was a two-fold increased risk of meningiomas in first degree relatives, however, there was no increase in risk in the spouses of these affected individuals (20).

Syndromes like neurofibromatosis type 2 and rarely Gorlin syndrome have been found to be associated with meningiomas. Germline defects in NF1, PTEN, VHL, BAP1, PTCH1, SMARCE1, SUFU and CREBBP have also been reported in association with meningiomas. Single nucleotide pleomorphisms on chromosomes 10 and 11 have recently been detected to have a significant association with meningioma risk (10).

MACROSCOPIC APPEARANCE

Meningiomas are circumscribed masses which are solid and globular having broad based attachment to dura. They may be lobulated or may have a flat, en plaque or carpet like growth pattern. On gross examination, they may appear firm and rubbery, while some may be gelatinous or cystic. Some have gritty texture, as in spinal psammomatous subtype, while some have smooth surface, as in fibrous subtype. Lower grade meningiomas may displace or compress the adjacent brain parenchyma, but they do not show adhesion or invasion. However, higher grade meningiomas may be invasive and broadly adherent with areas of necrosis. Dural sinus invasion may be noted, as in cases of parasagittal meningiomas. Skull invasion may cause reactive hyperostosis of skull bone, sphenoid and bones of the orbit (10).

DIAGNOSTIC CRITERIA (10)

Essential
Classic histomorphological features matching at least one of the meningioma subtypes OR Suggestive histopathological features combined with biallelic inactivation of NF2 or other classic drivers of conventional meningioma (TRAF7, AKT1, KLF4, SMO, PIK3CA), clear cell meningioma (SMARCE1), or rhabdoid meningioma (BAP1) OR Suggestive histopathological features combined with one of the defined DNA methylation classes of meningioma
Desirable
Meningeal localization EMA immunoreactivity Strong and diffuse SSTR2A immunoreactivity Classic copy-number alterations of NF2-mutant meningioma, such as monosomy 22/22q in lower grade meningiomas, with additional losses of 1p, 6, 10q, 14q, and/or 18 in higher grade meningiomas

GRADING

Meningiomas exhibit a wide morphological spectrum with the most common being meningothelial, fibrous and transitional types (10).

According to 2016 WHO classification of CNS tumours, to further assign grades to these tumours, the following criteria is applied (21).

WHO grade II
4 to 19 mitotic figures in 10 consecutive HPF (40x magnification, 0.16 mm^2) OR Brain invasion, with tumour breaching beyond the pia OR Clear cell or Chordoid morphological subtype OR At least three of the following: • Increased cellularity • Small cell with high N:C ratio • Prominent nucleoli • Sheeting (uninterrupted patternless or sheet-like growth) • Foci of spontaneous (non-iatrogenic) necrosis
WHO grade III
20 or more mitotic figures in 10 consecutive HPF (0.16 mm^2) OR Papillary or Rhabdoid morphological subtype OR Frank anaplasia (sarcoma-, carcinoma-, or melanoma-like appearance)

In 2021, with the introduction of the new updated WHO classification of CNS tumours, the criteria for grading of meningiomas was also updated (10).

CNS WHO Grade 2
4 to 19 mitotic figures in 10 consecutive HPF of each 0.16 mm^2 (at least 2.5/mm^2) OR Unequivocal brain invasion (not only perivascular spread or indentation of brain without pial breach) OR Specific morphological subtype (chordoid or clear cell) OR At least three of the following: • Increased cellularity • Small cell with high N:C ratio • Prominent nucleoli • Sheeting (uninterrupted patternless or sheet-like growth) • Foci of spontaneous (non-iatrogenic) necrosis
CNS WHO Grade 3
20 or more mitotic figures in 10 consecutive HPF of each 0.16 mm^2 (at least 12.5/mm^2) OR Frank anaplasia (sarcoma-, carcinoma-, or melanoma-like appearance) OR TERT promoter mutation OR Homozygous deletion of CDKN2A and/or CDKN2B

Meningothelial meningioma

In this subtype, there is formation of syncytia-like lobules by epithelioid cells with nuclei appearing to have holes or pseudoinclusions. The cells resemble arachnoid cap cells in morphology and are predominantly monomorphic, having abundant eosinophilic cytoplasm arranged in lobules separated by collagen septae. Borders between the tumour cells are not fully appreciable on light microscopy giving the

appearance of a syncytium, which on ultrastructual examination shows separate delicate cellular processes, hence the term ‘pseudosyncytium’ (10).

Fibrous meningioma

In this subtype, spindle cells are arranged parallelly, in storiform pattern or in interlacing bundles, in a collagen rich matrix. Fascicular arrangement is noted with intervening variable amounts of collagen. This collagen deposition may be extensive and hence, it is a differential for solitary fibrous tumour. However, solitary fibrous tumour shows STAT6 nuclear positivity with weak or absent EMA expression and strong S100 staining (10).

Transitional meningioma

This subtype contains meningothelial and fibrous pattern along with transitional features. It may show lobular as well as fascicular arrangement with few areas having intermediate pattern. Psammoma bodies and whorl formation are frequent (10).

Psammomatous meningioma

In this subtype, psammoma bodies are predominant over viable tumour cells. Large, confluent calcified masses may be seen due to overlapping of individual psammoma bodies. Viable meningioma cells are minimal conforming to fibrous or transitional subtypes (10).

Angiomatous meningioma

This subtype shows predominance of hyalinized small blood vessels over the meningioma cells. These blood vessels may be thin or thick walled and variably hyalinized. These angiomatous areas may show intermingling with microcystic or metaplastic areas, where the cells may show degenerative nuclear atypia. This subtype is associated with significant peritumoural edema, beyond what is expected for the tumour size (10).

Microcystic meningioma

In this subtype, thin, elongated processes form microcysts, creating a cobweb-like background. Macrocysts may be detectable macroscopically or radiologically. Degenerative nuclear atypia may be seen. Cerebral edema is frequent (10).

Secretory meningioma

Foci of gland-like epithelial differentiation is a characteristic feature with PAS-positive eosinophilic secretions. These eosinophilic secretions, also known as pseudopsammoma bodies, are positive for epithelial and secretory markers like CEA. The surrounding cells may also be positive for CEA and cytokeratins. Blood may show elevated CEA levels, which drop on resection and may rise in a rare recurrence. Peritumoural edema is seen commonly (10).

Lymphoplasmacyte-rich meningioma

It is a rare subtype with predominance of extensive chronic inflammatory infiltrates over the meningothelial component. In spite of its name, macrophages are predominant. Inflammatory disorders with patchy meningothelial hyperplasia are a differential (10).

Metaplastic meningioma

This subtype shows focal or extensive mesenchymal components. These components may include cartilaginous, osseous, myxoid, lipomatous and xanthomatous tissue (10).

Chordoid meningioma

It resembles a chordoma with cords or trabeculae of small epithelioid to spindled cells and variably vacuolated cytoplasm embedded in a mucin rich matrix. These chordoid areas are intermixed with a more typical meningioma. Patchy and sometimes prominent chronic inflammatory infiltrate are seen. They are characteristically large,

supratentorial tumours with average age of presentation being 45 years. Their recurrence rate is similar to atypical meningiomas, and therefore, they have been assigned CNS WHO grade 2. Rare association with haematological conditions like anaemia and Castleman disease may be noted (10).

Clear cell meningioma

This subtype shows a predominant patternless pattern or sheeting architecture comprising of round to oval cells with clear, glycogen rich cytoplasm and prominent perivascular and interstitial collagen. This perivascular and interstitial collagen may sometimes coalesce into large acellular zones of collagen or forms brightly eosinophilic, amianthoid like collagen. The cytoplasmic glycogen is PAS positive and diastase sensitive. This subtype has a preponderance for cerebellopontine angle and spine, especially cauda equina region. They are associated with aggressive behaviour and may show recurrence and occasional cerebrospinal fluid seeding (10).

Papillary meningioma

This subtype is defined by the predominance of perivascular pseudopapillary pattern. In this subtype, the tumour cells surround thin-walled blood vessels in a perivascular, pseudorosette like fashion. Some meningioma cells may show rhabdoid morphology in a papillary architecture. Peritumoural edema, bone hyperostosis or bone destruction. It may show association with brain invasion. Aggressive clinical behaviour in the form of dissemination and metastasis, especially to the lung, may be seen (10).

Rhabdoid meningioma

In this subtype, there is presence of rhabdoid cells. These cells are plump, with eccentric nuclei, open chromatin, macronucleoli and prominent eosinophilic paranuclear inclusions which appear as discernible whorled fibrils and waxy spheres. These features are more prominent on recurrence. These tumours have high proliferative activity. Studies have shown that a large proportion of tumours who have

been diagnosed on the basis of rhabdoid morphology alone, do not fulfil any other criteria for CNS WHO grade 3 tumours. 50% of these tumours have grade 1 features, while the other 50% have features of grade 2.

Some tumours may show rhabdoid morphology with papillary architecture. Consistent with this morphological overlap, same genetic alterations like PBRM1 mutation is predominantly seen in papillary meningiomas, but it is also seen in few cases of rhabdoid meningioma. Germline mutations in BAP1 are predominantly seen in rhabdoid meningioma, but they have also been reported in some cases of papillary meningioma (10).

Atypical meningioma

These meningiomas are intermediate grade and show increased mitotic activity, brain invasion and/or at least three of the following:

- High cellularity
- Small cells with high N:C ratio
- Prominent nucleoli
- Sheeting (uninterrupted patternless or sheet like growth)
- Foci of spontaneous (non-iatrogenic) necrosis

Despite the name, nuclear atypia is not a criterion, as it is often degenerative and not related to prognosis.

Clinical risk factors include-

- Male sex
- Non skull base location
- Prior surgery

There is a high recurrence rate, despite total resection, and bone involvement further increases this risk.

Brain invasion is characterized by irregular, tongue like protrusions of tumour cells into underlying GFAP positive parenchyma, without intervening leptomeninges. It

does not include extension along Virchow Robin spaces, as the pia is not breached (10).

Anaplastic (malignant) meningioma

This meningioma is high grade with overtly malignant cytomorphology that can -

- Resemble carcinoma, high grade sarcoma or melanoma
- Display markedly elevated mitotic activity
- Harbour a TERT promoter mutation
- Have a homozygous CDKN2A and/or CDKN2B deletion

TERT promoter mutation is associated with a high recurrence risk and short progression interval, irrespective of the histomorphological features. Homozygous deletion of CDKN2A and/or CDKN2B is associated with higher grade histological features, increased recurrence risk and shorter progression time. H3 p.K28me3 (K27me3) loss may be seen in 10-20% cases of anaplastic meningioma with shorter overall survival (10).

Other histopathological patterns

Meningiomas may also show oncocytic, sclerosing, whorling-sclerosing, mucinous, GFAP expressing and granulofilamentous inclusion-bearing features. However, these are rare patterns (10).

CYTOLOGY

Intraoperative touch and smear preparations shows typical features with oval, euchromatic nuclei (sometimes with intranuclear pseudoinclusions) and delicate cytoplasm. Touch preparations may show prominent whorling pattern (10).

IMMUNOHISTOCHEMISTRY

The diagnosis of meningiomas may be challenging if they arise in uncommon locations. Immunohistochemistry is a useful tool in such cases. The markers most commonly used currently for meningioma are EMA, Vimentin and PR.

However, EMA staining may be faint, focal or absent, particularly in fibrous meningioma and other higher grade subtypes (10). Vimentin also shows low specificity (10).

Ki67 labelling index can highlight uneven distribution of proliferation and guide mitotic counts assessment. According to some studies, a proliferation index of >4% is associated with a recurrence rate that is similar to CNS WHO grade 2 and an index of >20% is associated with mortality rates similar to that of CNS WHO grade 3 meningiomas (10).

Newer markers like SSTR-2A have been shown to have better performance.

SSTR-2

Somatostatin is a regulatory peptide released by inflammatory cells, immune cells and neuroendocrine cells. It acts through somatostatin receptors, which include five subtypes and belong to a family of G-protein coupled receptors. These receptors block secretion from cells by inhibiting intracellular cAMP and Ca^{2+}. They can also cause inhibition of secretions stimulated by cAMP and Ca^{2+}. The induction of this effect is caused by exocytosis inhibition mediated by somatostatin dependent calcineurin activation. Somatostatin receptors also cause inhibition of secretion of cytokines and growth factors which results in their antiproliferative activity. These effects are mediated either directly by somatostatin receptors on tumour cells, or indirectly by inhibition of hormones and growth factors implicated in promoting tumour growth, inhibition of angiogenesis and promotion of vasoconstriction via somatostatin receptors present on non-tumour cells. Modulation of protein tyrosine phosphatase, cAMP and Ca^{2+} have been shown to play a role (22).

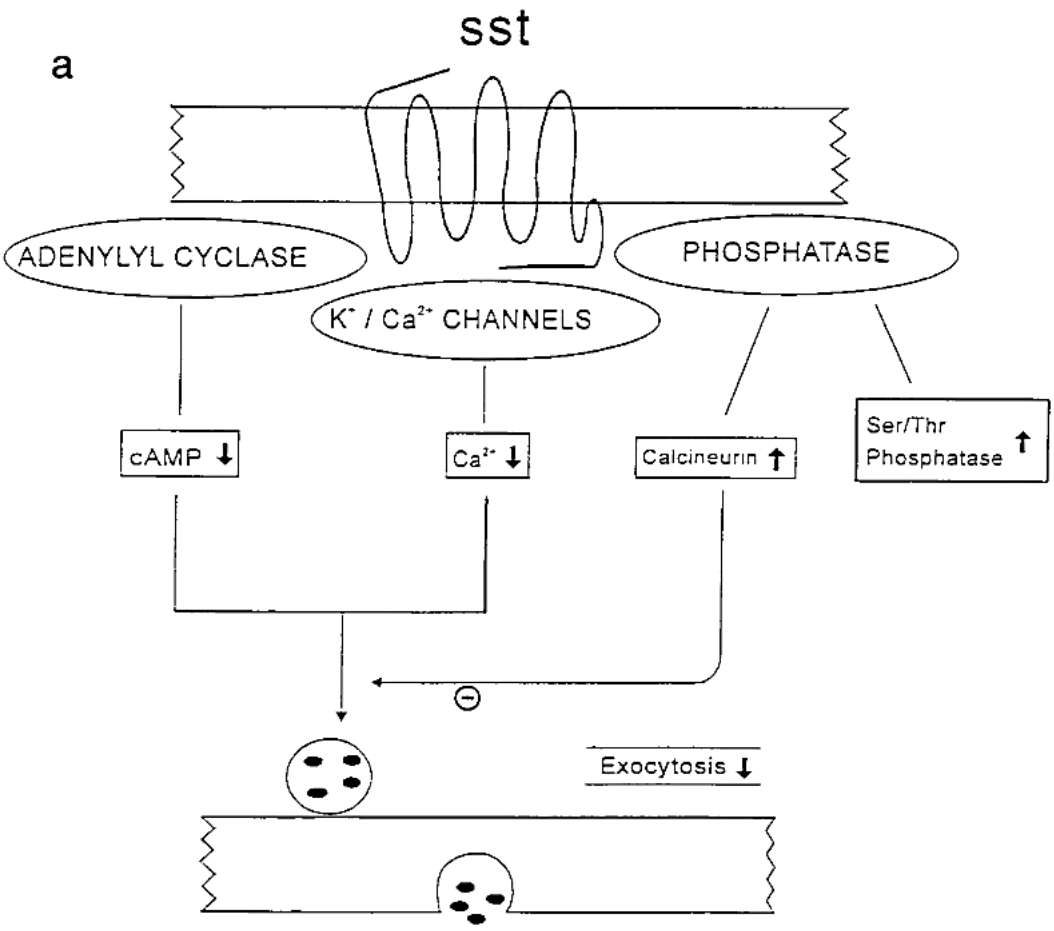


Figure 1 (a). Somatostatin induced inhibition of secretion of cAMP and Ca^{2+} with further inhibition of exocytosis (22)

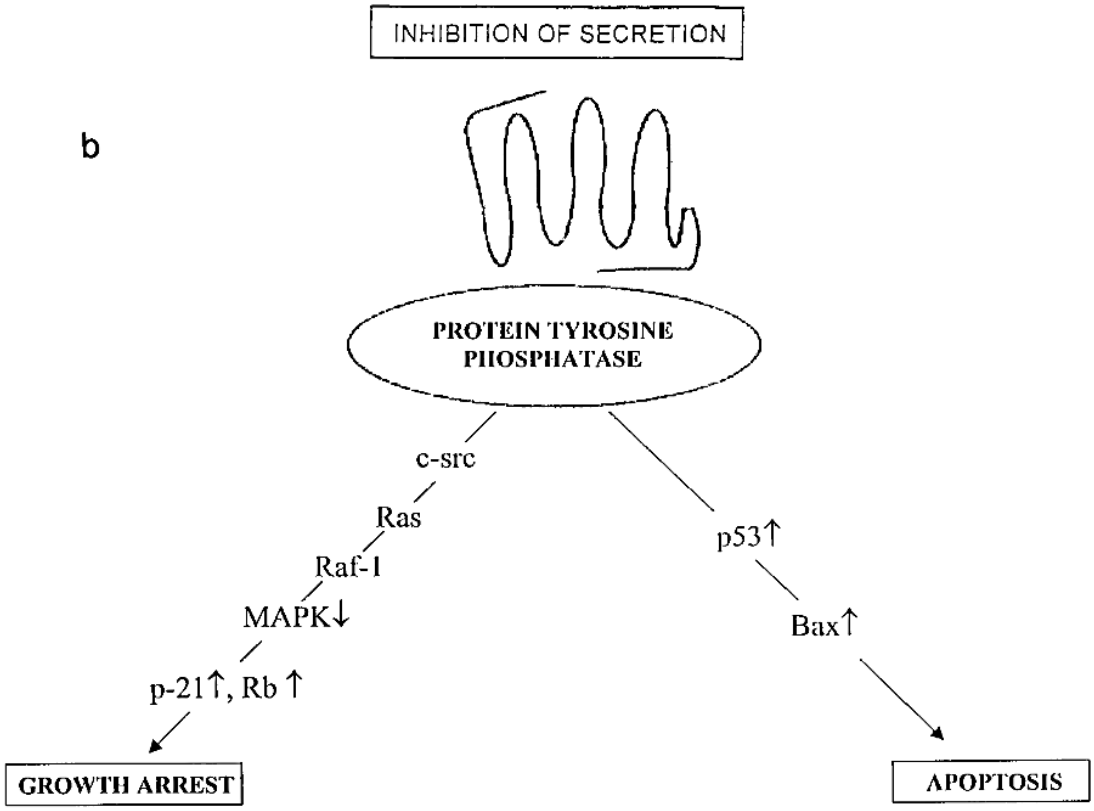


Figure 1 (b). Antiproliferative activity of somatostatin via somatostatin receptors (22)

This antiproliferative and antiangiogenic activity of somatostatin via somatostatin receptors and the expression of these receptors on meningioma may explain the role of SSTR-2 in targeted imaging and therapy of meningiomas (13).

In 2008, Barresi et al. analyzed the expression of SSTR-2A in grade II and grade III meningiomas and compared it to its expression in grade I meningiomas. They included 35 cases of meningioma in their study including 13 grade I, 19 grade II and 3 grade III meningiomas. In the same cohort, they also studied the expression of CD105 and Ki67 labelling index. A total of 26 cases showed immunopositivity for SSTR-2A with frequent expression in higher grade meningiomas. They found significantly higher microvessel density (assessed by CD105) in high grade meningiomas. They also reported a significant correlation between expression of SSTR-2A and high MVD in meningiomas (23).

In 2014, Agaimy et al. did a comparative study of meningiomas and soft tissue perineuriomas using a five marker IHC panel which included SSTR-2, EMA, Claudin-1, GLUT-1 and PR. They included 58 grade I meningiomas, 10 grade II meningiomas and 20 soft tissue perineuriomas in their study. They confirmed the ubiquitous strong expression of SSTR-2 in most meningiomas (87%) and found that it was rarely expressed in soft tissue perineuriomas (24).

In 2015, Silva et al. investigated the expression of SSTR family receptors (SSTR1-SSTR5) in 60 cases of meningiomas. Their study cohort included 47 cases of grade I meningioma, 11 cases of grade II and 2 cases of grade III. In their study, although positivity was observed for all SSTR subtypes, a strong positivity was observed for SSTR2 in 100% of cases with no correlation with age, gender, location and tumour grade (25).

In 2017, Boulagnon-Rombi et al. evaluated different immunohistochemical markers for the differential diagnoses of meningiomas from their morphological mimics. EMA, PR, SSTR2A, CD34, STAT6, S100, SOX10, HMB45, MelanA, GFAP, inhibin and BCL2 antibodies were applied on one hundred and twenty-seven cases of meningioma, twenty-six solitary fibrous tumour/hemangiopericytomas, thirty-nine schwannomas, seventeen hemangioblastomas, twenty-one melanomas, nine gliosarcomas, five neurofibromas, nine peripheral primitive neuroectodermal tumours, seven synovial sarcomas and five malignant peripheral nerve sheath tumours. They concluded that SSTR2A was the most sensitive (95.2%) and specific (92%) marker of meningiomas. Combined with EMA, the sensitivity reached 100% and specificity was 94.8% (26).

In 2018, Dijkstra et al. conducted a study to identify the most promising biomarker for targeted intra-operative fluorescence guided meningioma surgery. In their study, they took one hundred and forty-eight meningioma specimens, representing all meningioma grades and analysed the expression patterns of EMA, PDGF-beta, VEGF and SSTR-2. They found that all the cases were positive for SSTR-2 with 51.4% strong/diffuse, 30.4% moderate/diffuse and 18.2% focal/weak staining patterns. They concluded that SSTR-2 was the most promising receptor for meningioma targeting (27).

In 2021, Tollefsen et al. in their study, assessed the significance of somatostatin receptors as diagnostic and prognostic markers in meningiomas. Both manual and digital assessment of the immunoreactivity of the SSTRs was done in the form of staining index and digital score respectively. They inferred that there was significant correlation between staining index and digital score. A significant correlation was also found between expression of SSTRs 1, 2, 5 and tumour grade and SSTR-2 with time to recurrence (28).

In 2022, Fodi et al. studied the prognostic role of all five known SSTRs in a large cohort of 666 cases of meningioma. The immunohistochemical expression was scored on the basis of an intensity distribution score and a multivariate analysis was done for comprehensive prognostic assessment. A significant correlation was found between tumour recurrence and expression of SSTR-2A, SSTR-3 and SSTR-4. They concluded that SSTR-2A had a negative prognostic impact with a higher SSTR-2A expression associated with unfavourable prognosis (29).

HER2/neu

Human epidermal growth factor receptor-2 (HER2/neu, cerbB2) belongs to a four-member family of receptor tyrosine kinases. The intracellular domains of HER proteins dimerize and transphosphorylase upon binding of ligand to extracellular domain. Due to lack of a ligand, HER2, when expressed at high levels, either undergoes heterodimerization or homodimerization. This causes activation of HER2. In turn, the transcription factors are activated via activated downstream secondary messenger pathways. These transcription factors are involved in the regulation of many genes with involvement in cell survival, differentiation, proliferation and angiogenesis (30).

Overexpression of HER2/neu and its gene amplification has been detected in many tumours including breast, endometrial, gastric and bladder carcinomas (5). Its role in meningiomas is still ambiguous with the existing literature showing variable expression.

In 2004, Andersson et al. analyzed the expression of EGFR family members in 27 astrocytomas, 17 oligodendrogliomas and 26 meningiomas. Immunohistochemistry and quantitative real time RT-PCR were performed. They observed

immunohistochemical expression of EGFR in 58% cases of meningioma. A pronounced expression of EGFR and ErbB2 mRNA was also found by quantitative real time RT-PCR (31).

In 2009, Kandemir et al. aimed to study the correlation of ER, PR and HER2/neu expression with clinico-pathological parameters in 53 cases of grade I meningiomas. They found positivity for PR in 50.9% cases and for HER2/neu in 22.6% cases. None of the cases were immunoreactive for ER. A positive correlation was noted between expression of PR and HER2/neu with more frequent association of HER2/neu overexpression with meningothelial meningiomas. There was no significant correlation with the clinico-pathological parameters (32).

In 2011, Khamis et al. studied the correlation between expression of HER2/neu, PR and Ki67 labelling index in meningiomas and the disease-free survival of the patients, tumour grade and histologic subtypes. They included 38 cases of meningioma in their study and found positivity for HER2/neu in 34.2% cases. Of the grade II/III meningiomas, 57.1% cases showed HER2/neu positivity, while 29% cases were positive of the grade I meningiomas. They found a significant correlation between disease-free survival and HER2/neu expression with greater recurrence and shorter mean survival time in HER2/neu positive cases (33).

In 2012, Mahzouni et al. studied the expression of HER2/neu in meningioma and its correlation with tumour grade by IHC method in a retrospective study. They included 72 cases of meningioma in their study and observed that HER2/neu was expressed in 43% of those cases. The HER2/neu positive cases included 55% of the grade II/III meningiomas and 38.6% of the grade I meningiomas (34).

In 2016, Telugu et al. studied the expression of HER2/neu in meningiomas in 100 cases of meningioma and studied its correlation with different factors including location, gender, tumour grade and histologic subtype. In their study, HER2/neu was expressed in 73% of meningiomas including 75% of grade I, and 65% of grade II/III meningiomas. They did not find any statistically significant relationship with gender, location, tumour grade and histologic subtype (35).

In 2018, Arnli et al. studied the expression and amplification of intracellular and extracellular domains of HER2/neu along with activated receptor levels in 186 cases

of meningioma. They found immunoreactivity (predominantly cytoplasmic) for HER2/neu in all the cases with antibodies against intracellular domain, in 48% cases with antibodies targeting the extracellular domain and in 11% cases with antibodies against the activated receptor. They did not find any significant correlation with tumour grade. None of the cases exhibited HER2 gene amplification on FISH studies (5).

In 2020, Dar et al. studied expression of HER2/neu, p53 and Ki67 labelling index in 17 cases of meningioma to ascertain their prognostic and therapeutic value as biomarkers for meningioma. Their study included 11 primary and 6 recurrent cases. 70.5% of the cases were grade I, 17.5% cases were grade II and 12% cases were grade III. Ki67 labelling index and p53 immunoreactivity was observed in all cases with increasing expression in higher grades. None of the cases showed immunoreactivity for HER2/neu (36).

In 2020, Ramezani et al. evaluated the expression of HER2/neu in 117 cases of meningioma. They found HER2/neu positivity in 65% of the cases with high expression in 7.7% of the cases. They did not find any significant correlation with patient age and gender and tumour grade (37).

AIM AND OBJECTIVES

Aim :

- To study the immunohistochemical expression of SSTR-2 and HER2/neu in meningiomas and its correlation with clinico-pathological parameters.

Objectives :

Primary Objectives :

- To study the immunohistochemical expression of SSTR-2 in meningiomas.
- To study the immunohistochemical expression of HER2/neu in meningiomas.

Secondary Objectives :

- To correlate the immunohistochemical expression of SSTR2 and HER2/neu with tumour grade in meningiomas.

MATERIALS AND METHODS

Type of study

Ambispective observational study

Study setting

Department of Pathology and Lab Medicine and Department of Neurosurgery, All India Institute of Medical Sciences, Jodhpur

Source of data

The study included resection specimens of the cases diagnosed as meningioma received in the Department of Pathology and Lab Medicine at AIIMS Jodhpur from January 2018 to July 2022. Haematoxylin and Eosin-stained slides of the diagnosed cases were retrieved from the departmental archives. Approval from an institutional review board was obtained at the initiation of the study.

Duration of study

January 2018 to July 2022

Ethical clearance

The study was approved by the institutional ethical committee on 12 March 2021, bearing Certificate No. AIIMS/IEC/2021/3352.

Inclusion criteria:

- All cases diagnosed as meningioma during the study period

Exclusion criteria:

- Recurrent cases of meningioma
- Other dural based neoplasms

Method of data collection

- History and radiology findings
- Biopsy or excision specimens received from the Department of Neurosurgery

- Routine processing and microscopic study of H&E stained sections of cases of meningioma with application of relevant immunohistochemistry markers where needed
- Microscopic features of meningioma
- Grading of the tumours as per the WHO 2016 classification of tumours of the central nervous system
- Immunohistochemistry for SSTR-2 and HER2/neu in confirmed cases of meningioma

Experiment design

Descriptive, Observational, Cross-sectional study

Sample processing, staining and immunohistochemistry

After approval from the Institutional ethics committee, the study was started. Informed consent was obtained from the patients. Specimens were received in 10% neutral buffered formalin in the Department of Pathology and Lab Medicine from the Department of Neurosurgery, AIIMS, Jodhpur along with duly filled up consent and case record form. After receiving, grossing was carried out and representative sections submitted and processed as per standard tissue processing protocol. Paraffin-embedded tissue blocks were prepared using routine histopathological techniques. Thin sections (3 µm) were stained with routine Haematoxylin and Eosin stains. Light microscopy results were recorded and histopathological grading as per the WHO 2021 classification was given. An appropriate representative block was subjected to IHC for SSTR-2 and HER2/neu. Immunohistochemical expression of SSTR-2 was evaluated using the scoring system proposed by Volante et al.(38) and of HER2/neu was evaluated as per the ASCO-CAP scoring guidelines (39) described for the carcinoma breast.

Grossing:

The biopsy specimens were examined grossly. The gross descriptions such as size, colour, cut surface, consistency, areas of haemorrhage and necrosis were described. Comment was made on dural attachment and/or presence of adjacent brain parenchyma.

Steps of block preparation and section cutting (40) ***:***

After the representative sections were taken, tissue was processed as follows:

1. Dehydration was carried out by passing the sections through a series of ascending grades of ethyl alcohol, from 50%, 70%, 95% to absolute alcohol.
2. The clearing was done by passing the tissue through two changes of xylene.
3. Impregnation was done in molten paraffin wax which had a melting point of 54-62˚C.
4. Embedding: Embedding station (Leica EG 1150 H) was used through which a small amount of liquid paraffin was layered into aluminium moulds. Properly oriented tissues were placed inside the moulds, which were then filled with liquid paraffin (60-62˚C) and allowed to cool and harden. The lower portion of the cassette with an identification number was used as the final block.
5. Microtomy: Microtome (Leica-RM2255) was used and thin ribbons (3 μm) were cut and floated in warm water (~56˚C) for expansion of the curled sections. These sections were then collected on frosted glass slides and kept for drying.

Staining of sections: (for H&E stain) (41)

1. Deparaffinization – The glass slides containing the tissue sections were kept over the hot plate at 60 ˚C for 10 minutes, followed by two changes in xylene (Xylene I & Xylene II), 10 minutes each.
2. Hydration – Through graded alcohol (100%, 95%, 70%, 50%) to water, 10 minutes respectively.
3. Haematoxylin – The sections were kept in Harris' Haematoxylin for 5 minutes.
4. Washing – The sections were washed well in water for 2 minutes.
5. Differentiation – Done in 1% acid alcohol (1% HCl in 70% alcohol) for 10 seconds.
6. Washing – Done under running tap water (usually for 15 – 20 minutes) until the sections 'blue'.
7. Eosin – Stained in 1% Eosin Y for 10 seconds.
8. Washing – Done in running tap water for 2 minutes.
9. Dehydration – Through graded alcohol (50%, 70%, 95%, 100%), 10 minutes each.
10. Clearing –Through xylene (Xylene II & Xylene I), 2 minutes each.
11. Mounting – The sections were mounted in DPX with a coverslip.

Immunohistochemistry (42) ***:***

Antibodies used -

1. Primary antibodies: Ready to use SSTR-2 antibody (Rabbit polyclonal antobody, Quartett, Germany) and HER2/neu antibody (Rabbit monoclonal antibody, EP3 clone, PathnSitu, USA) were used.
2. Secondary antibody: BOND Polymer Refine Detection System (Leica Biosystems, Newcastle, UK)
3. Peroxide block, 3-4% (v/v-volume per volume)
4. Post Primary, Rabbit anti-mouse IgG in 10% (v/v) animal serum in tris-buffered saline
5. Polymer, Anti-rabbit Poly-HRP-IgG containing 10% (v/v) animal serum in tris-buffered saline
6. DAB Part 1, in stabilizer solution
7. DAB Part B ≤0.1% (V/V) Hydrogen peroxide in stabilizer solution
8. DAB Part B ≤0.1% (V/V) Hydrogen peroxide in stabilizer solution
9. Haematoxylin, 0.1%

*Steps of IHC staining***:**

A. Preparation of Buffer – Two types of buffers were used.

1. Wash Buffer
2. Antigen Retrieval Buffer (ARB)

Wash buffer preparation: 6 gm powdered TRIS buffer salt was dissolved into 1 litre of distilled water and pH was set at 7.4.

ARB preparation: 6.05 gm TRIS salt and 0.744 gm EDTA salt were dissolved in 1 litre of distilled water, pH was set at 9.0.

Note:

- To increase the pH, NaOH solution was added drop by drop and pH was titrated.
- To decrease the pH, HCl was added drop by drop and pH was titrated.

B. Preparation of Poly-L-Lysine Solution (PLL Solution)-

1 mL of PLL was diluted with 9 mL of distilled water (1 in 10 dilutions).

C. Slide Coating Procedure-

Step 1: Diluted PLL solution was taken in a clean container/ Coplin jar

Step 2: Both sides of glass slides were cleaned with tissue paper

Step 3: The clean slides were immersed in the PLL solution for 5 minutes

Step 4: After 5 minutes, the coated slides were removed and kept overnight for air drying. The coated slides were kept at room temperature. Tissue sections of 4 µm thickness were obtained on the PLL coated slides.

Baking: The slides were kept at 60˚C for 1 hour and then cooled to room temperature.

D. IHC staining procedure-

Step 1: Deparaffinization – The slides were kept in Xylene I (10 minutes), followed by Xylene II (10 minutes).

Step 2: Rehydration – The slides were kept in descending grades of alcohol (100%, 70% and 50%) for 5 minutes each followed by running tap water for 5 minutes.

Step 3: Antigen retrieval – (by pressure cooker method) 200 mL of clean tap water was taken in the empty pressure cooker and heated up to steam formation. The slides were placed in a rack. 300 mL of ARB was put in a container and the rack with slides was placed inside the container. This container was then placed inside the pressure cooker and the lid was closed. After two whistles the pressure was released by lifting the air vent and allowed to cool till it reached room temperature.

Step 4: Washing – Slides were washed in Wash Buffer (pH 7.4) thrice at 1-minute intervals.

Step 5: Peroxide blocking – Blocking reagent was added to the sections and incubated for 10 minutes in a humidity chamber at room temperature. This step prevents unwanted, non-specific background staining.

Step 6: The peroxide was decanted and not washed with buffer.

Step 7: Primary antibodies – SSTR-2 and HER2/neu were added to the sections and incubated in the humidity chamber for one hour.

Step 8: Washing – The slides were then washed in Wash Buffer (pH 7.4) thrice at 1-minute intervals.

Step 9: Amplifier – Amplifier was added over the sections and incubated for 30 minutes in the humidity chamber at room temperature.

Step 10: Washing – The slides were washed in Wash Buffer (pH 7.4) thrice at 1-minute intervals.

Step 11: HRP labelling – The HRP was added and incubated for 30 minutes in the humidity chamber at room temperature.

Step 12: Washing – The slides were washed in Wash Buffer (pH 7.4) thrice at 1-minute intervals.

Step 13: DAB – The DAB chromogen was applied to the sections and incubated in the humidity chamber for 10 minutes, avoiding light exposure as much as possible.

Step 14: Washing – The sections were washed in distilled water twice at 1-minute intervals.

Step 15: Counterstaining – Slides were counterstained using Harris' Haematoxylin for 2-3 minutes.

Step 16: Washing – The slides were washed in running tap water for 5 minutes.

Step 17: Dehydration – The slides were then dehydrated by immersing in ascending grades of alcohol (50%, 70%, 95%, 100%), 1 minute each.

Step 18: Mounting – Slides were air-dried, mounted with DPX and examined under the microscope.

Interpretation of IHC :

- Cortical brain parenchyma and epithelial cells of invasive carcinoma of the breast were taken as positive controls for SSTR-2 and HER2/neu respectively to show the reliability of staining.
- SSTR-2 expression was interpreted based on the scoring system proposed by Volante et al. (38). The expression was scored on a scale of 0 to 3, where 0 represented no expression and 3 represented highest level of expression.

Score	Staining pattern
0	Absence of any immunoreactivity
1	Pure cytoplasmic immunoreactivity (focal or diffuse)
2	Membranous reactivity (usually incomplete) in <50% tumour cells, irrespective of the presence of cytoplasmic staining
3	Circumferential membranous reactivity in >50% tumour cells, irrespective of the presence of cytoplasmic staining

- For statistical analysis, the scores of 0 and 1 were considered to be negative, while scores of 2 and 3 were considered to be positive.
- HER2/neu expression was interpreted based on the ASCO CAP 2018 guidelines for carcinoma breast (39). The expression was scored on a scale of 0 to 3+, where 0 represented no expression and 3+ represented highest level of expression.

Score	Staining pattern
0	No staining is observed Or Membrane staining that is incomplete and is faint/barely perceptible and in less than or equal to 10% of tumour cells
1+	Incomplete membrane staining that is faint/barely perceptible in more than 10% of tumour cells
2+	Weak to moderate complete membrane staining in more than 10% of tumour cells
3+	Circumferential membrane staining that is complete, intense in more than 10% of tumour cells

- Scores of 0 and 1+ were interpreted as negative, 2+ as equivocal and 3+ as positive.

Statistical Analysis

- We enrolled 120 cases of meningioma diagnosed at the Department of Pathology and Lab Medicine, All India Institute of Medical Sciences, Jodhpur between January 2018 to July 2022.
- The collected data was entered in the Microsoft Excel spreadsheet. This data was then analyzed using Statistical Package for Social Sciences (SPSS) software, IBM manufacturer, Chicago, USA, ver 25.0.
- The association of variables which were quantitative in nature was analyzed using Independent t test (for two groups) and ANOVA (for more than two groups).
- The association of variables which were qualitative in nature was analyzed using Fisher's exact test and Chi-Square test.
- A p value of less than 0.05 was considered statistically significant.

OBSERVATIONS AND RESULTS

This was an observational ambispective cross-sectional study conducted in the Department of Pathology and Lab Medicine at All India Institute of Medical Sciences, Jodhpur. A total of 120 cases of Meningioma, from January 2018 to July 2022, meeting the inclusion and exclusion criteria, were included in this study.

Age Distribution

Age (years)	Frequency	Percentage
0-9	1	0.83%
10-19	3	2.50%
20-29	5	4.17%
30-39	13	10.83%
40-49	36	30.00%
50-59	31	25.83%
60-69	21	17.50%
70-79	10	8.33%

Table 1. Age distribution of patients

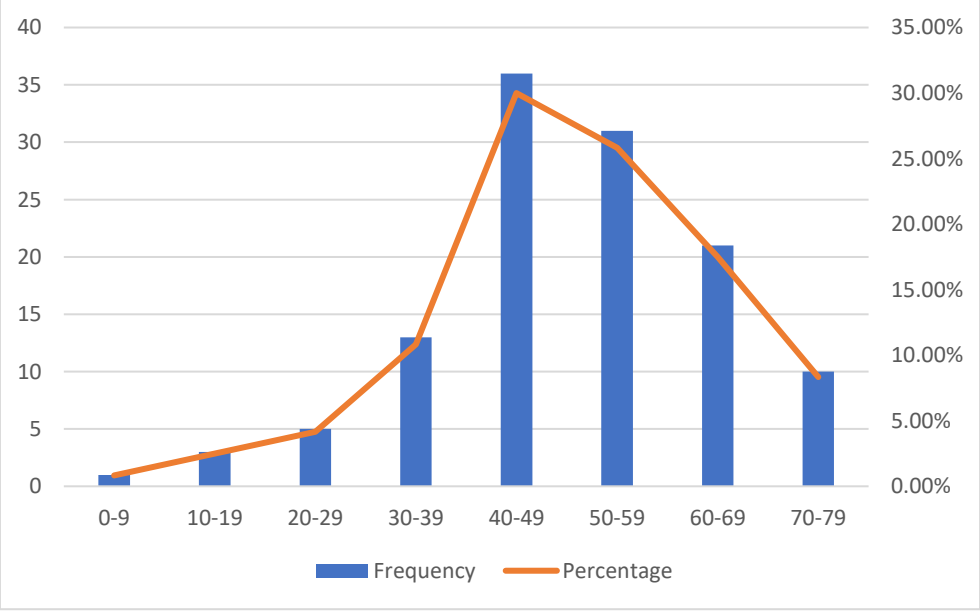


Figure 2. Age distribution of patients

The age of the patients in the study ranged from 9 years to 76 years with a mean age of 50.07 years with a standard deviation of 13.9 and a median age of 50.5 years. Maximum number of patients were in the age range of 40 years to 49 years (36 out of 120, 30.00%).

Gender distribution

Gender	Frequency	Percentage
Female	81	67.50%
Male	39	32.50%

Table 2. Gender distribution of patients

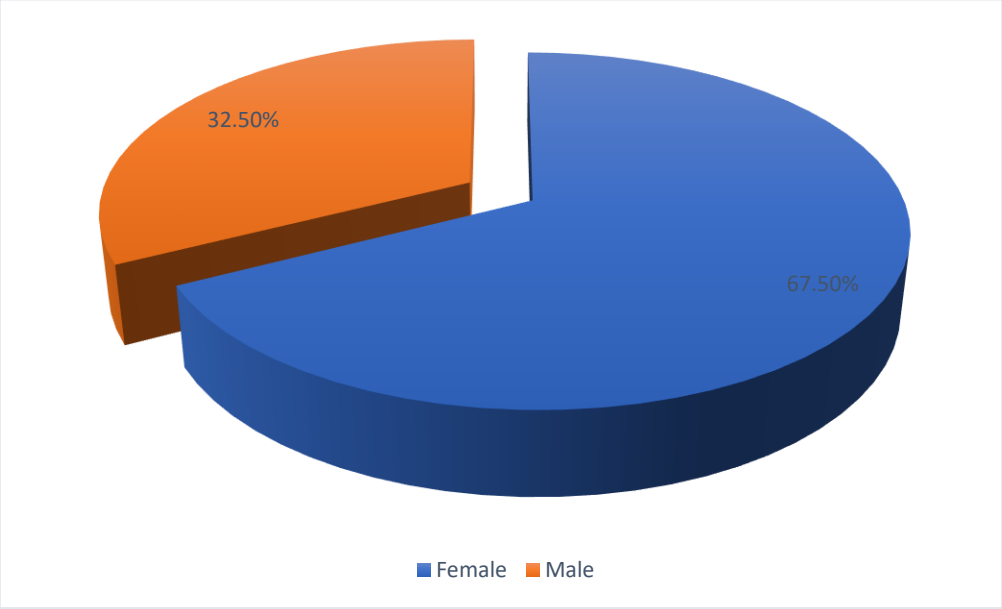


Figure 3. Gender distribution of patients

Out of 120 patients that were included in the study, 81 were females (67.50%) and 39 were males (32.50%) with a female to male ratio of 2.08:1.

Location of meningioma

Location	Frequency	Percentage
Brain	108	90.00%
Spine	12	10.00%

Table 3. Distribution of location of meningioma

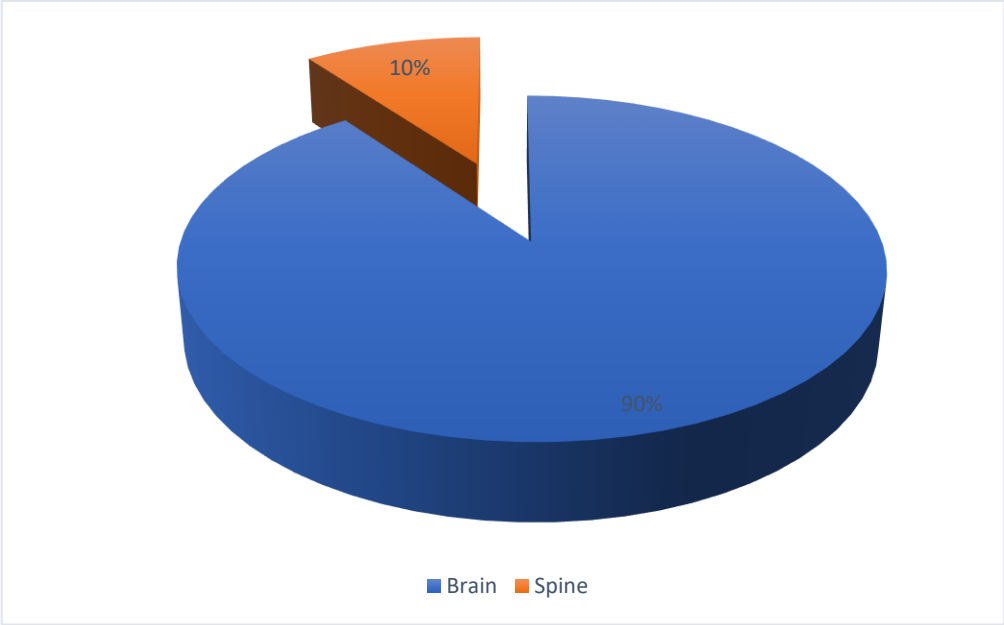


Figure 4. Distribution of location of meningioma

Location in brain	Frequency	Percentage
Anterior cranial fossa	38	35.19%
Middle cranial fossa	40	37.04%
Posterior cranial fossa	30	27.78%

Table 4. Distribution of location of meningioma in brain

Level of spine	Frequency	Percentage
Cervical	2	16.67%
Thoracic	10	83.33%

Table 5. Distribution of location of meningioma in spine

In this study, of the 120 cases included, 90% (108 cases) were located in the brain while the rest (10%; 12 cases) originated from the spine. Of the 108 cases, 40 cases (37.04%) were located in the middle cranial fossa, while 38 (35.19%) and 30 cases (27.78%) were located in the anterior and posterior cranial fossa, respectively. Out of the meningiomas located in spine, majority originated from thoracic spine (10 cases; 83.33%), while the rest originated in cervical spine (2 cases; 16.67%).

Histologic subtypes of meningioma

Histologic subtype	Frequency	Percentage
Meningothelial	45	37.50%
Psammomatous	9	7.50%
Fibrous	12	10.00%
Transitional	19	15.83%
Angiomatous	11	9.17%
Microcystic	2	1.67%
Metaplastic	1	0.83%
Atypical	16	13.33%
Clear cell	1	0.83%
Chordoid	1	0.83%
Anaplastic	1	0.83%
Rhabdoid	2	1.67%

Table 6. Distribution of histologic subtypes of meningioma

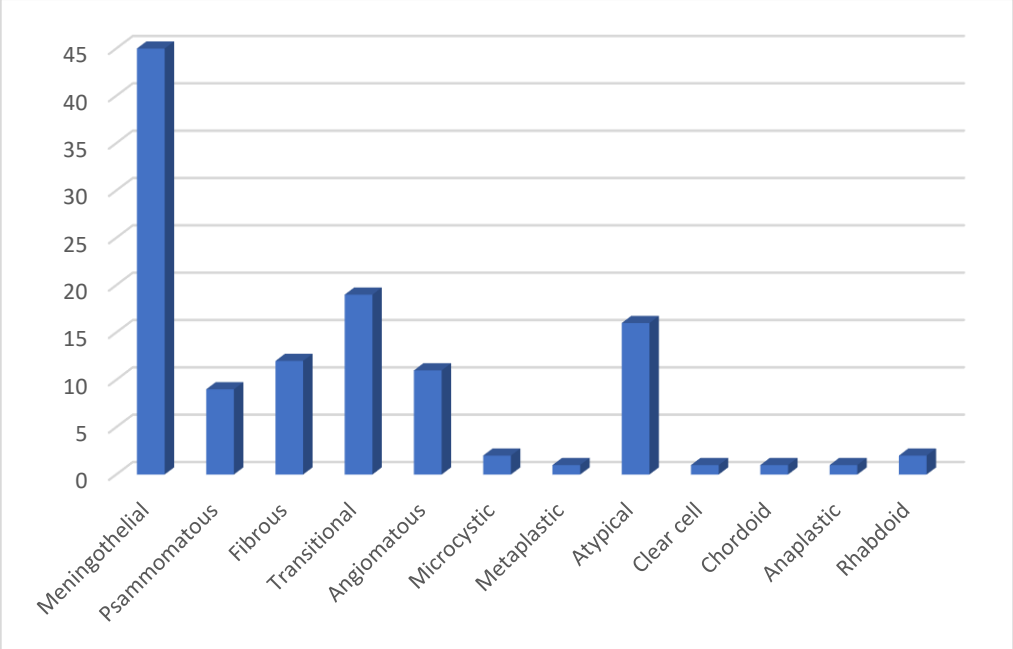


Figure 5. Distribution of histologic subtypes of meningioma

Most of the cases included in this study were meningothelial (45 cases; 37.50%), followed by transitional (19 cases; 15.83%), atypical (16 cases; 13.33%), fibrous (12 cases, 10%), angiomatous (11 cases; 9.17%) and psammomatous (9 cases; 7.50%). There were 2 cases each of rhabdoid and microcystic meningioma (1.67% each) and one case each of metaplastic, clear cell, chordoid and anaplastic meningioma (0.83% each).

Grade of meningioma

Grade	Frequency	Percentage
I	97	80.83%
II	20	16.67%
III	3	2.50%

Table 7. Distribution of grade of meningioma

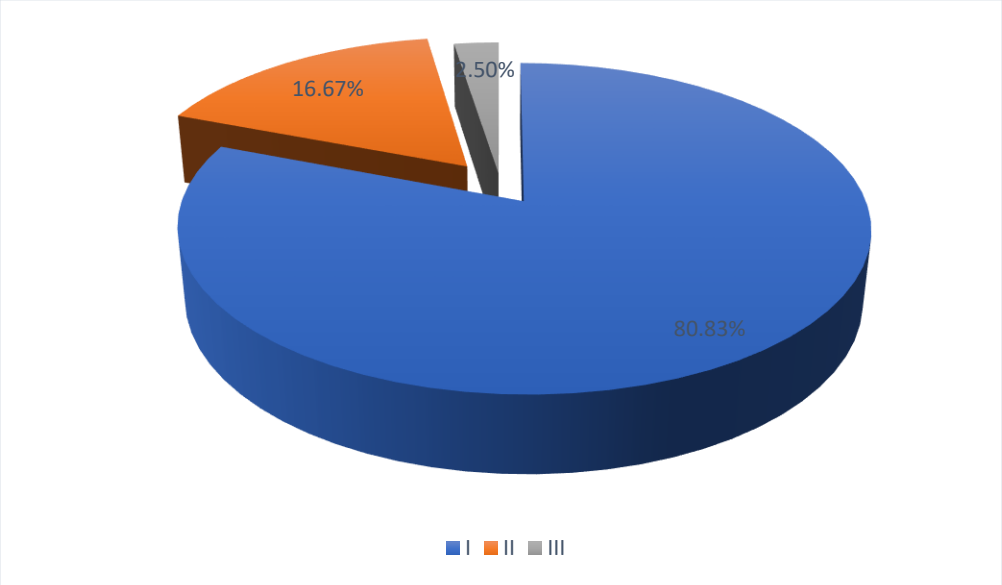


Figure 6. Distribution of grade of meningioma

In this study, 97 out of the 120 cases included were diagnosed as Grade I meningiomas (80.83%), 20 cases as Grade II (16.67%), while only 3 of the cases were diagnosed as Grade III (2.50%).

Number of mitoses

Mitoses (per 10 HPF)	Frequency	Percentage
< 4	110	91.67%
4-19	8	6.67%
≥ 20	2	1.67%

Table 8. Number of mitoses in meningioma

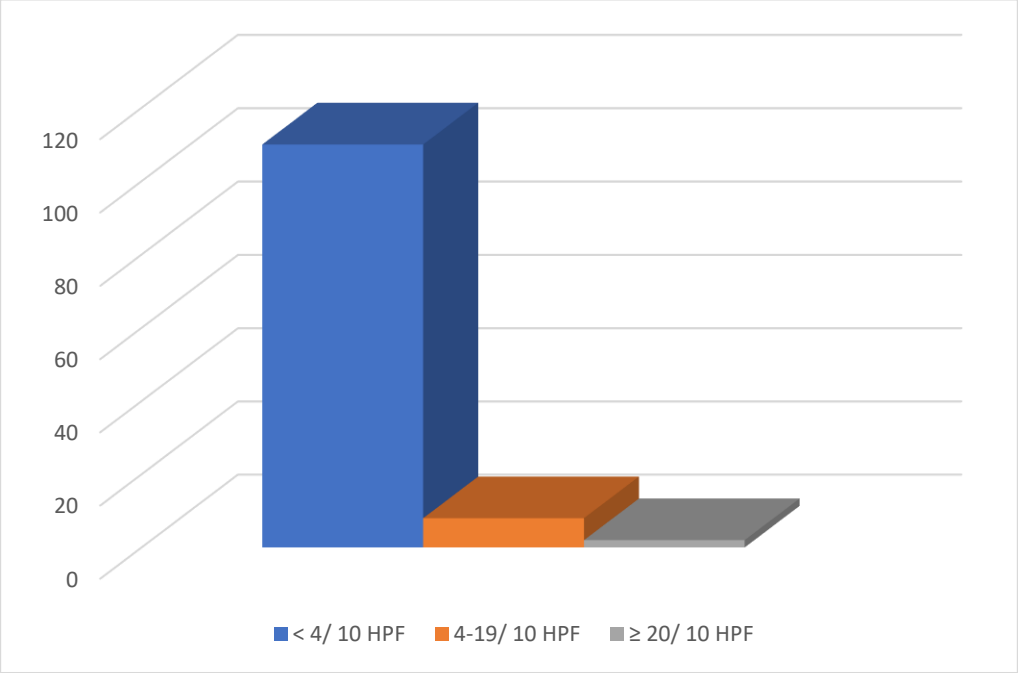


Figure 7. Number of mitoses in meningioma

Of the 120 cases, most of the cases had <4 mitoses per 10 HPF (110 cases; 91.67%). 8 cases had a mitotic count of 4-19 per 10 HPF (6.67%), while only 2 cases had 20 or more mitotic figures per 10 HPF (1.67%).

Brain invasion

Brain invasion	Frequency	Percentage
Present	11	9.17%
Absent	109	90.83%

Table 9. Number of cases with brain invasion in meningioma

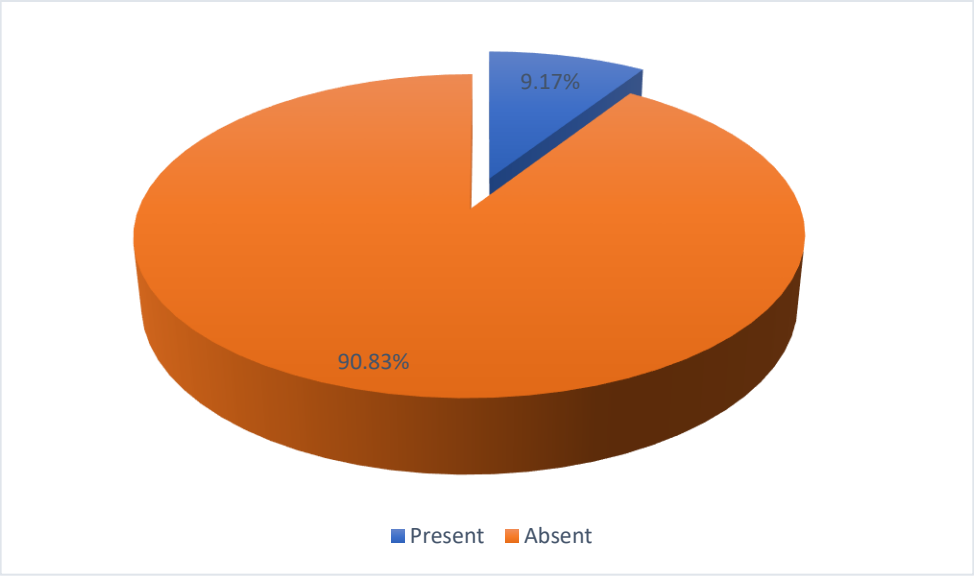


Figure 8. Number of cases with brain invasion in meningioma

Out of the 120 cases included in the study, 11 cases had brain invasion (9.17%), causing them to be classified under Grade II.

Specific histological features

Histologic feature	Present		Absent	
	Frequency	Percentage	Frequency	Percentage
Increased cellularity	4	3.33%	116	96.67%
Small cell change	1	0.83%	119	99.17%
Prominent nucleoli	5	4.17%	115	95.83%
Sheeting	5	4.17%	115	95.83%
Spontaneous necrosis	7	5.83%	113	94.17%
Frank anaplasia	0	0.00%	120	100.00%

Table 10. Specific histological features in meningioma

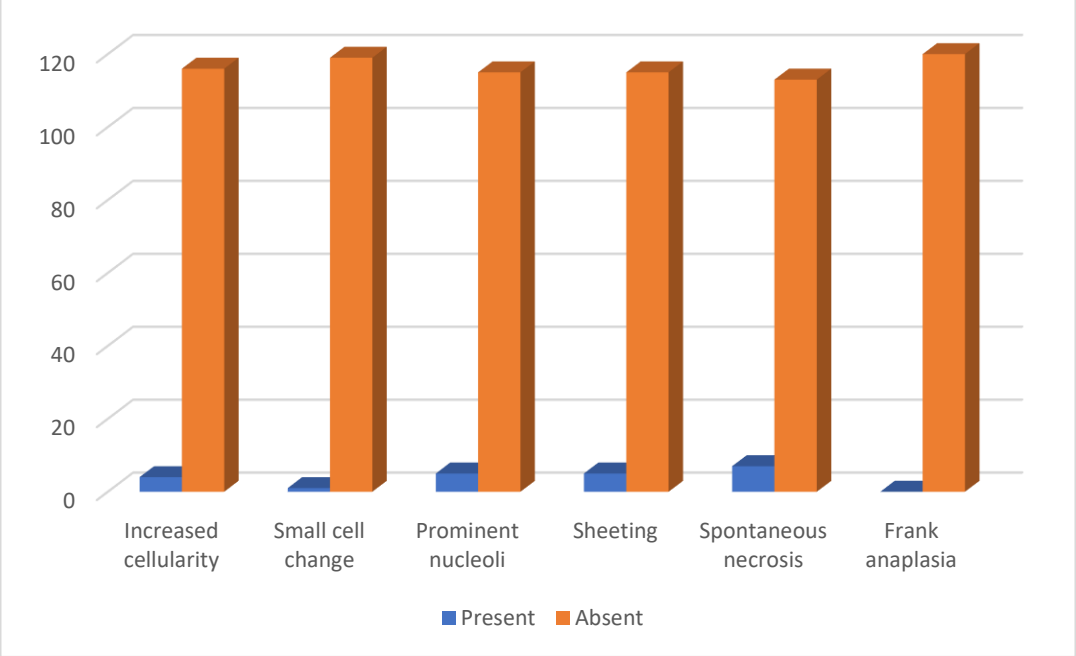


Figure 9. Specific histological features in meningioma

Out of the 120 cases, only 4 cases showed increased cellularity (3.33%), one case showed small cell change (0.83%), 5 cases showed prominent nucleoli (4.67%), 5 cases showed sheeting (4.67%), 7 cases showed spontaneous necrosis (5.83%) and none of the cases showed frank anaplasia.

Ki67 labelling index

Ki67 (%)	Frequency	Percentage
≤ 4	43	59.72%
> 4 to ≤ 20	26	36.11%
> 20	3	4.17%

Table 11. Ki67 labelling index in meningioma

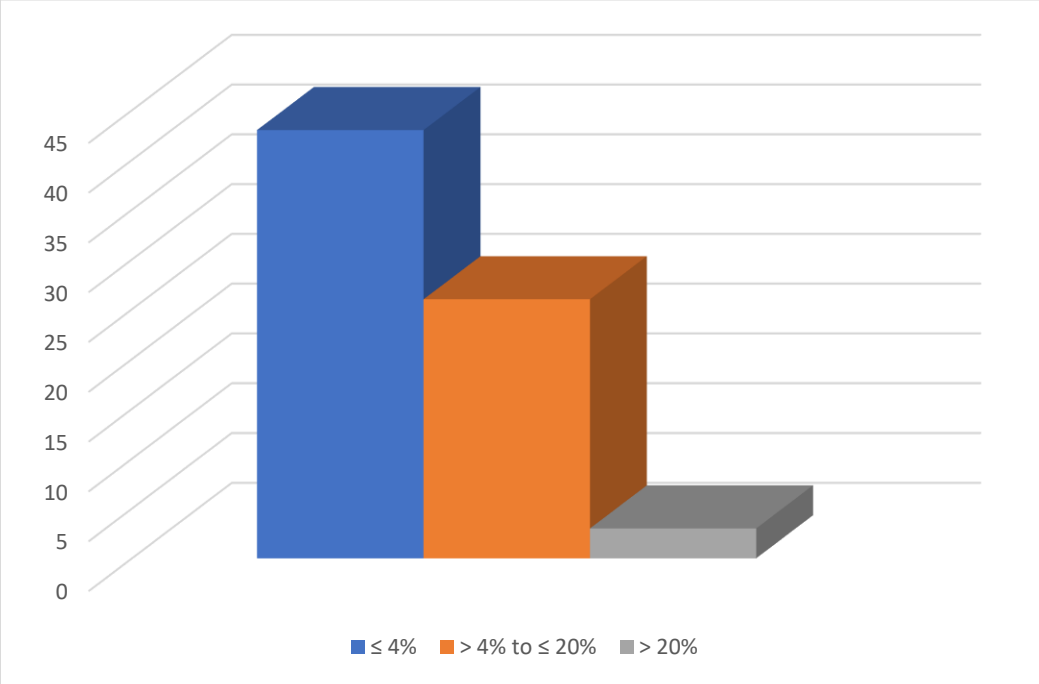


Figure 10. Ki67 labelling index in meningioma

Though Ki67 labelling index does not affect the grade of meningioma, it affects the prognosis of the patient (10). In this study, immunohistochemistry for Ki67 labelling index was available in 72 cases, of which, 43 cases (59.72%) had a low Ki67 labelling index, i.e. ≤ 4%, 26 cases (36.11%) had a Ki67 labelling index of > 4% to ≤ 20%, while 3 cases (4.17%) had a Ki67 labelling index of >20%.

SSTR-2 expression in meningioma

SSTR2	Frequency	Percentage
0	19	15.83%
1	10	8.33%
2	19	15.83%
3	72	60.00%

Table 12. SSTR-2 expression in meningioma

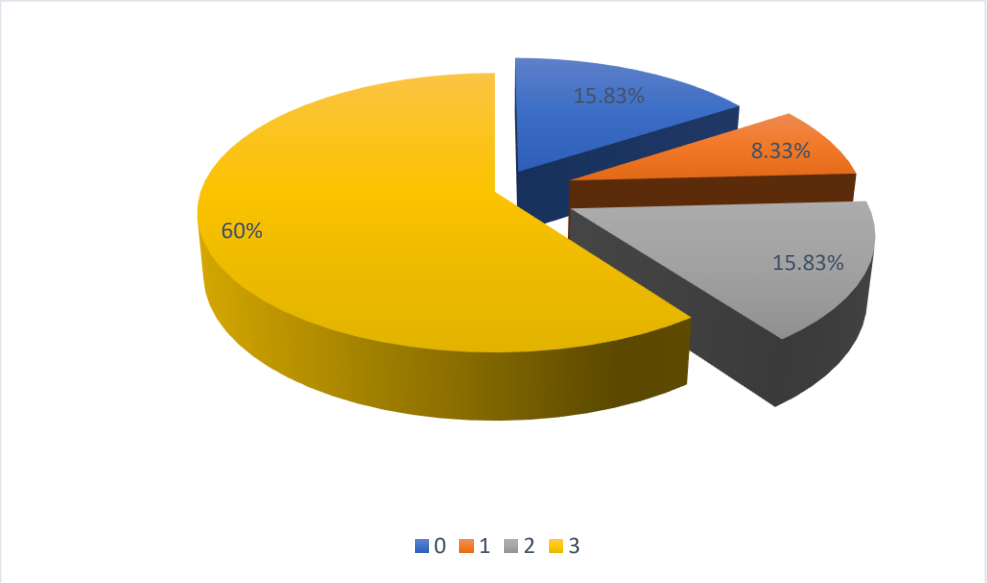


Figure 11. SSTR-2 expression in meningioma

SSTR-2 expression was studied in all the 120 cases included in this study and scored according to the scoring system proposed by Volante et al.(38). 72 cases (60%) showed circumferential membranous positivity in >50% of the tumour cells and were given a score of 3. 19 cases (15.83%) showed incomplete membranous reactivity in <50% of the tumour cells and thus were assigned a score of 2. 10 cases (8.33%) showed pure cytoplasmic positivity and therefore were given a score of 1. 19 cases (15.83%) did not show any immunoreactivity and thus were given a score of 0.

Scores of 2 and 3 were considered positive while 1 and 0 were considered negative. Hence, overall, out of the 120 cases, 91 cases (75.83%) were positive for SSTR-2 and 29 cases (24.17%) were negative.

HER2/neu expression in meningioma

HER2/neu	Frequency	Percentage
0	113	94.17%
1+	7	5.83%
2+	0	0.00%
3+	0	0.00%

Table 13. HER2/neu expression in meningioma

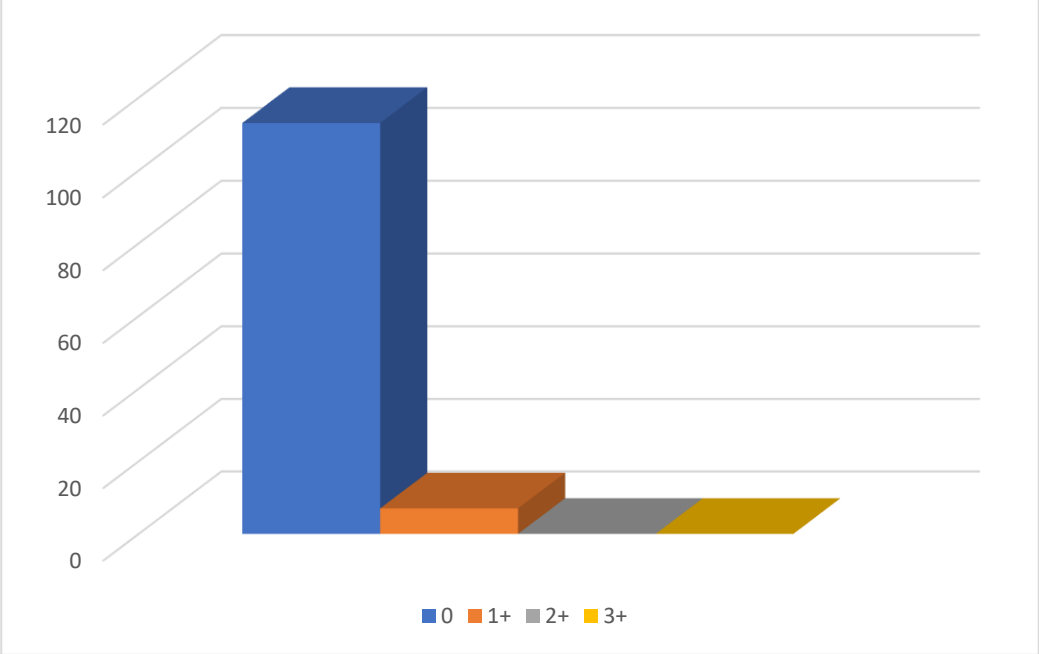


Figure 12. HER2/neu expression in meningioma

HER2/neu expression was also studied in all the 120 cases and scored according to the ASCO CAP 2018 guidelines for carcinoma breast (39). 113 cases (94.17%) did not show any staining and were given a score of 0. 7 cases (5.83%) showed incomplete membranous staining that was faint and barely perceptible in more than 10% of the tumour cells, and thus were given a score of 1+. None of the cases showed complete membranous staining.

According to the guidelines, scores of 0 and 1+ were considered negative, 2+ as equivocal and 3+ as positive. Therefore, all the 120 cases in this study were negative for HER2/neu.

Association of age with SSTR-2 and HER2/neu expression

Age (years)	SSTR-2 expression				Total	P value
	0 (n=19)	1 (n=10)	2 (n=19)	3 (n=72)		
0-9	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)	0.114 by Fisher's exact test
10-19	0 (0%)	0 (0%)	2 (66.67%)	1 (33.33%)	3 (100%)	
20-29	0 (0%)	1 (20%)	3 (60%)	1 (20%)	5 (100%)	
30-39	2 (15.38%)	1 (7.69%)	2 (15.38%)	8 (61.54%)	13 (100%)	
40-49	9 (25%)	2 (5.56%)	4 (11.11%)	21 (58.33%)	36 (100%)	
50-59	2 (6.45%)	3 (9.68%)	3 (9.68%)	23 (74.19%)	31 (100%)	
60-69	3 (14.29%)	2 (9.52%)	4 (19.05%)	12 (57.14%)	21 (100%)	
70-79	3 (30%)	0 (0%)	1 (10%)	6 (60%)	10 (100%)	
Mean ± SD	52.21 ± 12.9	43.8 ± 16.87	44.32 ± 17.67	51.89 ± 12.08	50.07 ± 13.87	0.07 by ANOVA
Median (25th-75th percentile)	46 (42-67)	48.5 (33.5-56.25)	47 (30-59.5)	51.5 (44-59.25)	50.5 (42-60)	
Range	37-73	9-62	13-74	18-76	9-76	

Table 14. Association of age with SSTR-2 expression

Age (years)	HER2/neu expression		Total	P value
	0 (n=113)	1+ (n=7)		
0-9	1 (100%)	0 (0%)	1 (100%)	0.396 by Fisher's exact test
10-19	3 (100%)	0 (0%)	3 (100%)	
20-29	5 (100%)	0 (0%)	5 (100%)	
30-39	12 (92.31%)	1 (7.69%)	13 (100%)	
40-49	36 (100%)	0 (0%)	36 (100%)	
50-59	28 (90.32%)	3 (9.68%)	31 (100%)	
60-69	19 (90.48%)	2 (9.52%)	21 (100%)	
70-79	9 (90%)	1 (10%)	10 (100%)	
Mean ± SD	49.6 ± 13.82	57.57 ± 13.5	50.07 ± 13.87	0.141 by Independent t test
Median (25th-75th percentile)	49 (42-59)	58 (55.5-64.5)	50.5 (42-60)	
Range	9-76	31-74	9-76	

Table 15. Association of age with HER2/neu expression

Mean age of Score 3 for SSTR-2 was 51.89 years and score 2 was 44.32 years. The mean age of Score 1+ for HER2/neu was 57.57 years. No statistically significant association was found between age and expression of SSTR-2 and HER2/neu (p values = 0.114 and 0.396 respectively).

Association of gender with SSTR-2 and HER2/neu expression

Gender	SSTR-2 expression				Total	P value
	0 (n=19)	1 (n=10)	2 (n=19)	3 (n=72)		
Female	13 (16.05%)	6 (7.41%)	14 (17.28%)	48 (59.26%)	81 (100%)	0.905
Male	6 (15.38%)	4 (10.26%)	5 (12.82%)	24 (61.54%)	39 (100%)	
Total	19 (15.83%)	10 (8.33%)	19 (15.83%)	72 (60%)	120 (100%)	

Table 16. Association of gender with SSTR-2 expression

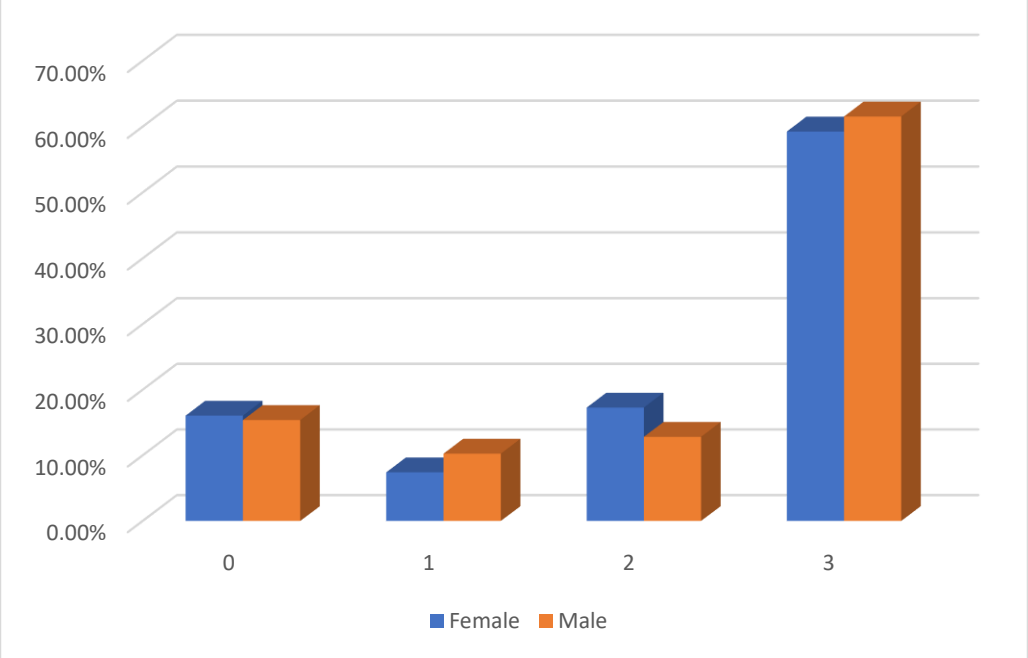


Figure 13. Association of gender with SSTR-2 expression

Gender	HER2/neu expression		Total	P value
	0 (n=113)	1+ (n=7)		
Female	76 (93.83%)	5 (6.17%)	81 (100%)	1
Male	37 (94.87%)	2 (5.13%)	39 (100%)	
Total	113 (94.17%)	7 (5.83%)	120 (100%)	

Table 17. Association of gender with HER2/neu expression

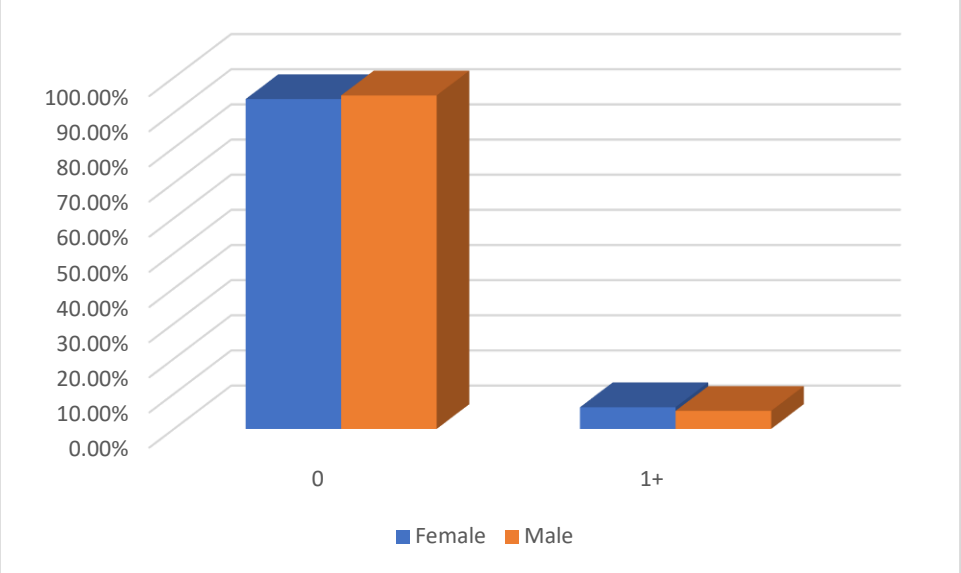


Figure 14. Association of gender with HER2/neu expression

Out of the 72 cases with a score of 3 for SSTR-2, 48 were female and 24 were male, while, of the 7 cases with a score of 1+ for HER2/neu, 5 were female and 2 were male. No statistically significant association was found between gender and expression of SSTR-2 and HER2/neu (p values = 0.905 and 1 respectively; Fisher's exact test).

Association of location of meningioma with SSTR-2 and HER2/neu expression

Location	SSTR-2 expression				Total	P value
	0 (n=19)	1 (n=10)	2 (n=19)	3 (n=72)		
Brain	18 (16.67%)	9 (8.33%)	15 (13.89%)	66 (61.11%)	108 (100%)	0.384
Spine	1 (8.33%)	1 (8.33%)	4 (33.33%)	6 (50%)	12 (100%)	
Total	19 (15.83%)	10 (8.33%)	19 (15.83%)	72 (60%)	120 (100%)	

Table 18. Association of location of meningioma with SSTR-2 expression

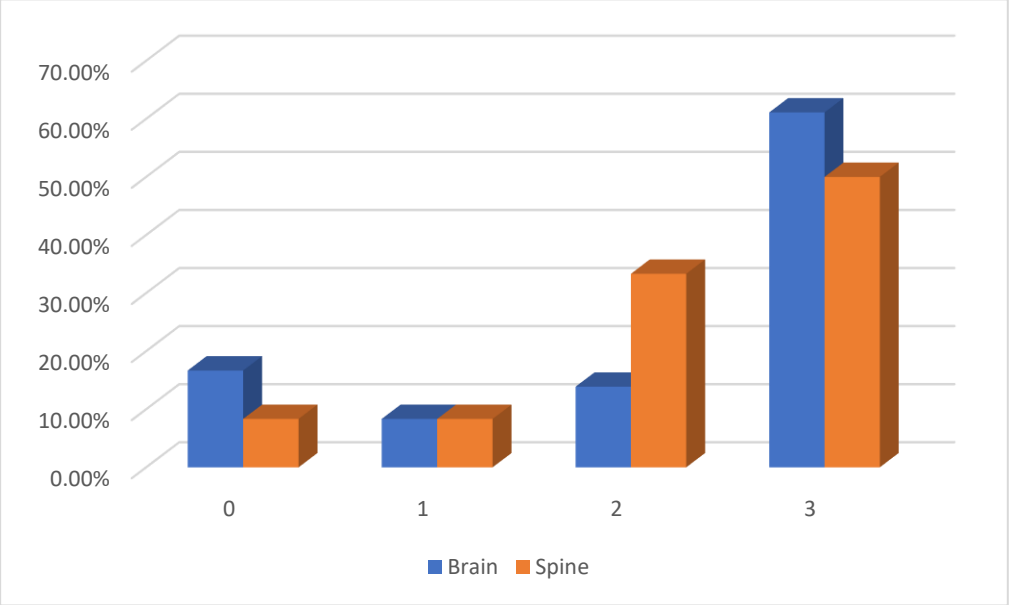


Figure 15. Association of location of meningioma with SSTR-2 expression

Location in brain	SSTR-2 expression				Total	P value
	0 (n=18)	1 (n=9)	2 (n=15)	3 (n=66)		
Anterior cranial fossa	4 (10.53%)	1 (2.63%)	5 (13.16%)	28 (73.68%)	38 (100%)	0.002
Middle cranial fossa	3 (7.50%)	4 (10%)	4 (10%)	29 (72.50%)	40 (100%)	
Posterior cranial fossa	11 (36.67%)	4 (13.33%)	6 (20%)	9 (30%)	30 (100%)	
Total	18 (16.67%)	9 (8.33%)	15 (13.89%)	66 (61.11%)	108 (100%)	

Table 19 (a). Association of location of meningioma in brain with SSTR-2 expression

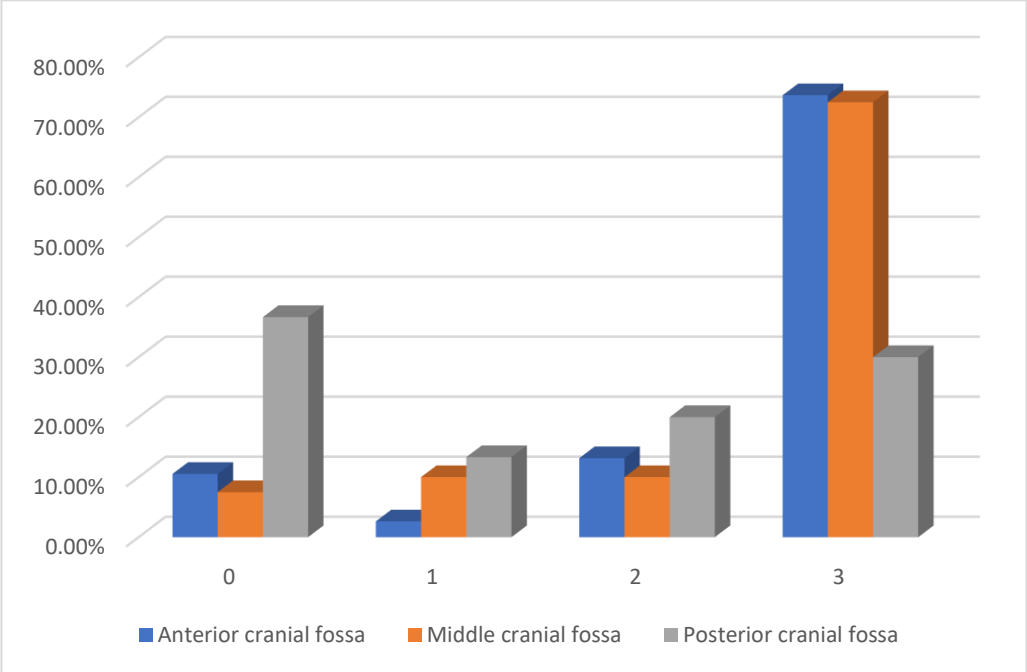


Figure 16 (a). Association of location of meningioma in brain with SSTR-2 expression

Location in brain	SSTR-2 expression		Total	P value
	Positive (n=81)	Negative (n=27)		
Anterior cranial fossa	33 (86.84%)	5 (13.16%)	38 (100%)	0.0009
Middle cranial fossa	33 (82.50%)	7 (17.50%)	40 (100%)	
Posterior cranial fossa	15 (50%)	15 (50%)	30 (100%)	
Total	81 (75%)	27 (25%)	108 (100%)	

Table 19 (b). Association of location of meningioma in brain with SSTR-2 expression

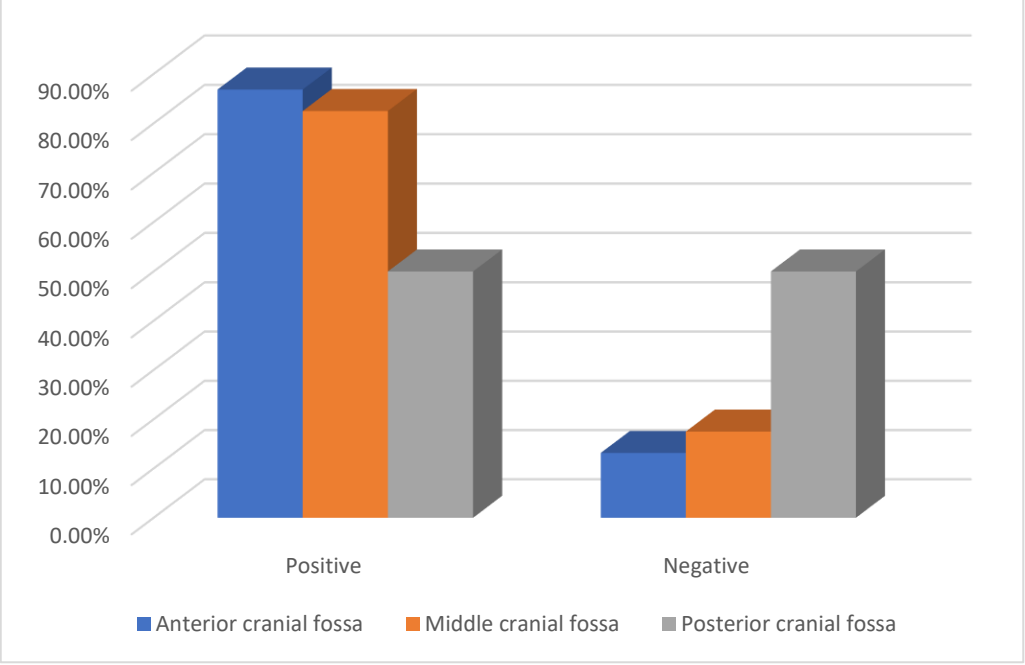


Figure 16 (b). Association of location of meningioma in brain with SSTR-2 expression

Level of spine	SSTR-2 expression				Total	P value
	0 (n=1)	1 (n=1)	2 (n=4)	3 (n=6)		
Cervical	1 (50%)	0 (0%)	0 (0%)	1 (50%)	2 (100%)	0.409
Thoracic	0 (0%)	1 (10%)	4 (40%)	5 (50%)	10 (100%)	
Total	1 (8.33%)	1 (8.33%)	4 (33.33%)	6 (50%)	12 (100%)	

Table 20. Association of location of meningioma in spine with SSTR-2 expression

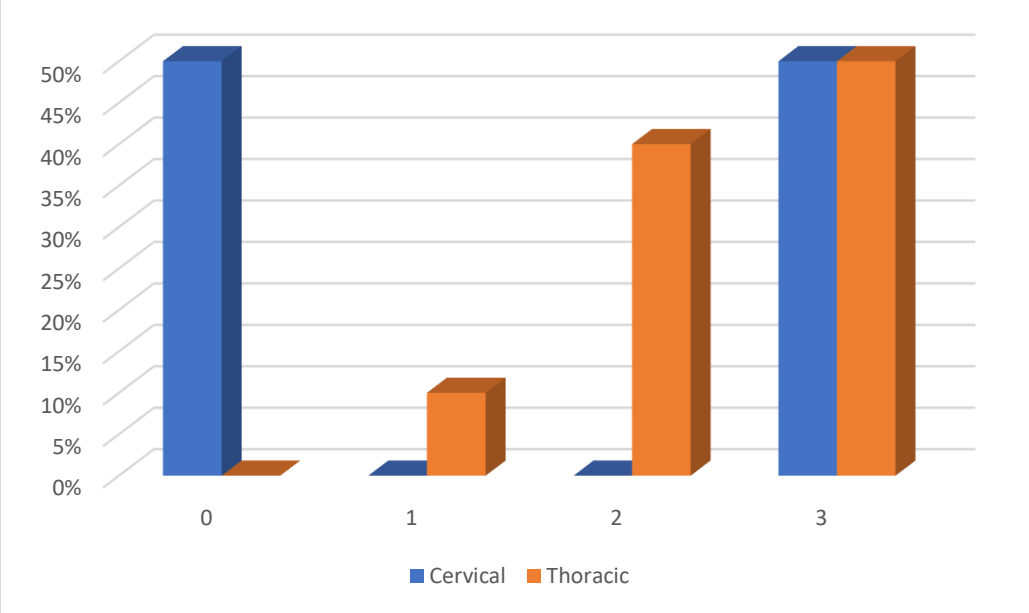


Figure 17. Association of location of meningioma in spine with SSTR-2 expression

Location	HER2/neu expression		Total	P value
	0 (n=113)	1+ (n=7)		
Brain	102 (94.44%)	6 (5.56%)	108 (100%)	0.531
Spine	11 (91.67%)	1 (8.33%)	12 (100%)	
Total	113 (94.17%)	7 (5.83%)	120 (100%)	

Table 21. Association of location of meningioma with HER2/neu expression

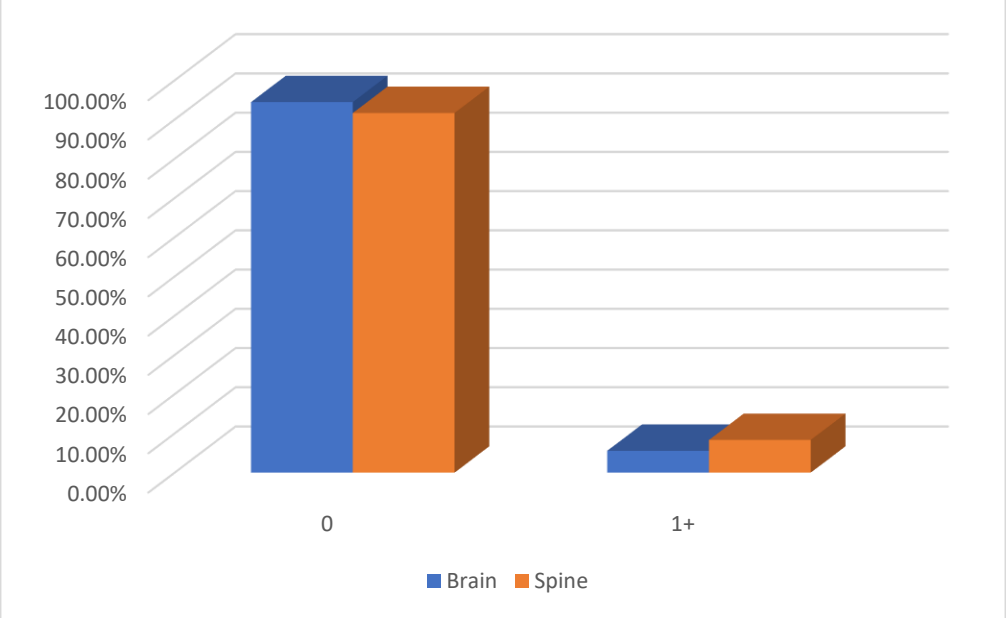


Figure 18. Association of location of meningioma with HER2/neu expression

Location in brain	HER2/neu expression		Total	P value
	0 (n=102)	1+ (n=6)		
Anterior cranial fossa	38 (100%)	0 (0%)	38 (100%)	0.128
Middle cranial fossa	36 (90%)	4 (10%)	40 (100%)	
Posterior cranial fossa	28 (93.33%)	2 (6.67%)	30 (100%)	
Total	102 (94.44%)	6 (5.56%)	108 (100%)	

Table 22. Association of location of meningioma in brain with HER2/neu expression

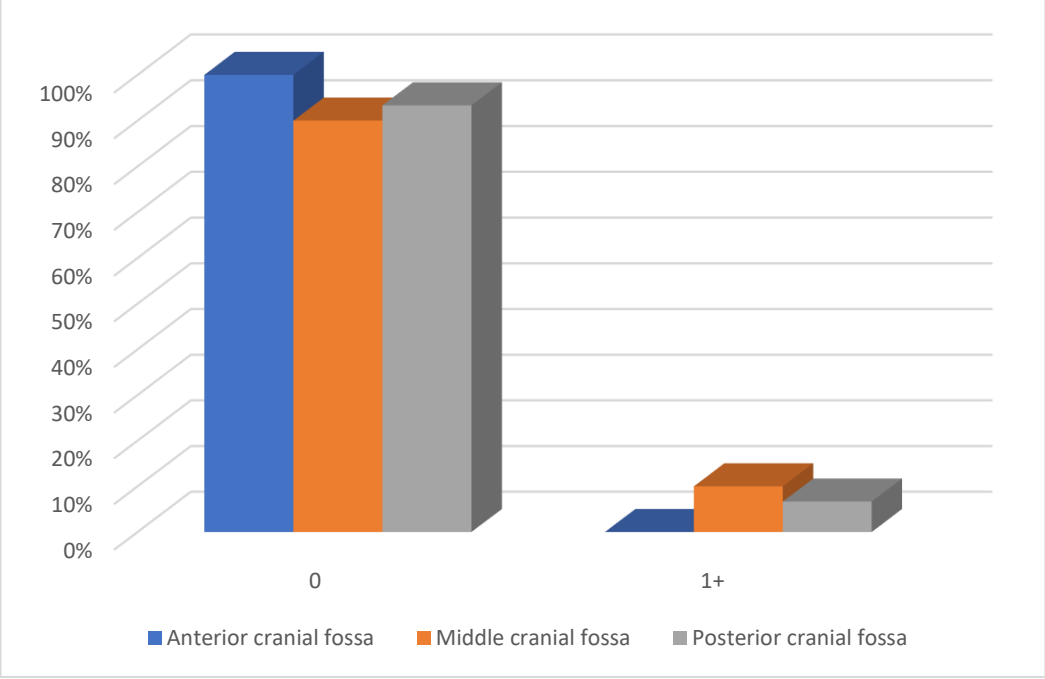


Figure 19. Association of location of meningioma in brain with HER2/neu expression

Level of spine	HER2/neu expression		Total	P value
	0 (n=11)	1+ (n=1)		
Cervical	2 (100%)	0 (0%)	2 (100%)	1
Thoracic	9 (90%)	1 (10%)	10 (100%)	
Total	11 (91.67%)	1 (8.33%)	12 (100%)	

Table 23. Association of location of meningioma in spine with HER2/neu expression

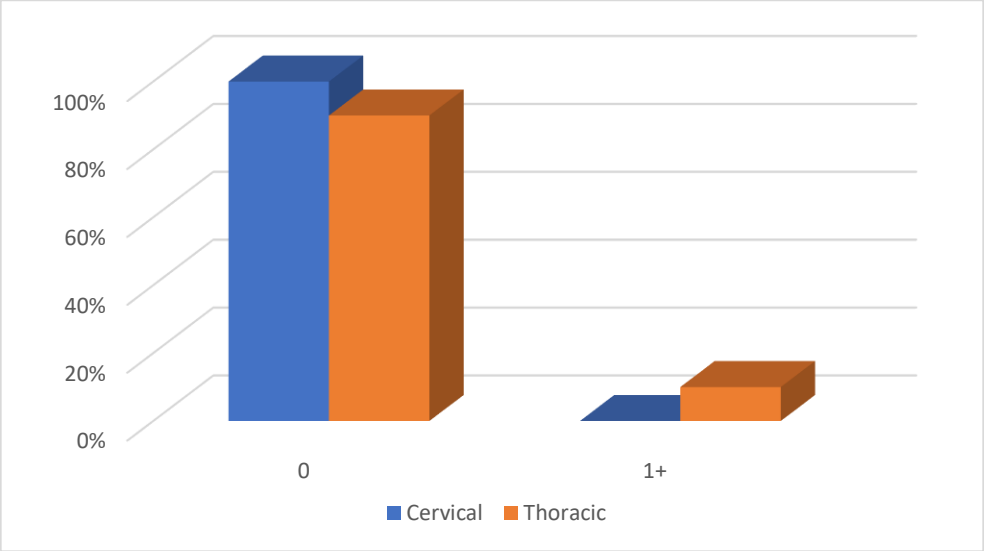


Figure 20. Association of location of meningioma in spine with HER2/neu expression

A statistically significant association was found between the expression of SSTR-2 and location of meningioma in brain (p value = 0.0009; Chi square test). SSTR-2 expression was more commonly seen in meningiomas arising in anterior and middle cranial fossa (86.84% and 82.5% cases respectively) than in posterior cranial fossa (50% cases).

No statistically significant association was found between expression of HER2/neu and location of meningioma in (p value = 0.531; Fisher's exact test), between expression of HER2/neu and location of meningioma in brain and in spine (p values = 0.128 and 1 respectively).

Association of histologic subtype with SSTR-2 and HER2/neu expression

Histologic subtype	SSTR-2 expression				Total	P value
	0 (n=19)	1 (n=10)	2 (n=19)	3 (n=72)		
Meningothelial	2 (4.44%)	3 (6.67%)	6 (13.33%)	34 (75.56%)	45 (100%)	<.0001
Psammomatous	1 (11.11%)	0 (0%)	4 (44.44%)	4 (44.44%)	9 (100%)	
Fibrous	7 (58.33%)	2 (16.67%)	2 (16.67%)	1 (8.33%)	12 (100%)	
Transitional	3 (15.79%)	3 (15.79%)	4 (21.05%)	9 (47.37%)	19 (100%)	
Angiomatous	0 (0%)	0 (0%)	0 (0%)	11 (100%)	11 (100%)	
Microcystic	0 (0%)	0 (0%)	0 (0%)	2 (100%)	2 (100%)	
Metaplastic	0 (0%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	
Atypical	3 (18.75%)	1 (6.25%)	1 (6.25%)	11 (68.75%)	16 (100%)	
Clear cell	0 (0%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	
Chordoid	1 (100%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	
Anaplastic	1 (100%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	
Rhabdoid	1 (50%)	1 (50%)	0 (0%)	0 (0%)	2 (100%)	
Total	19 (15.83%)	10 (8.33%)	19 (15.83%)	72 (60%)	120 (100%)	

Table 24 (a). Association of histologic subtype with SSTR-2 expression

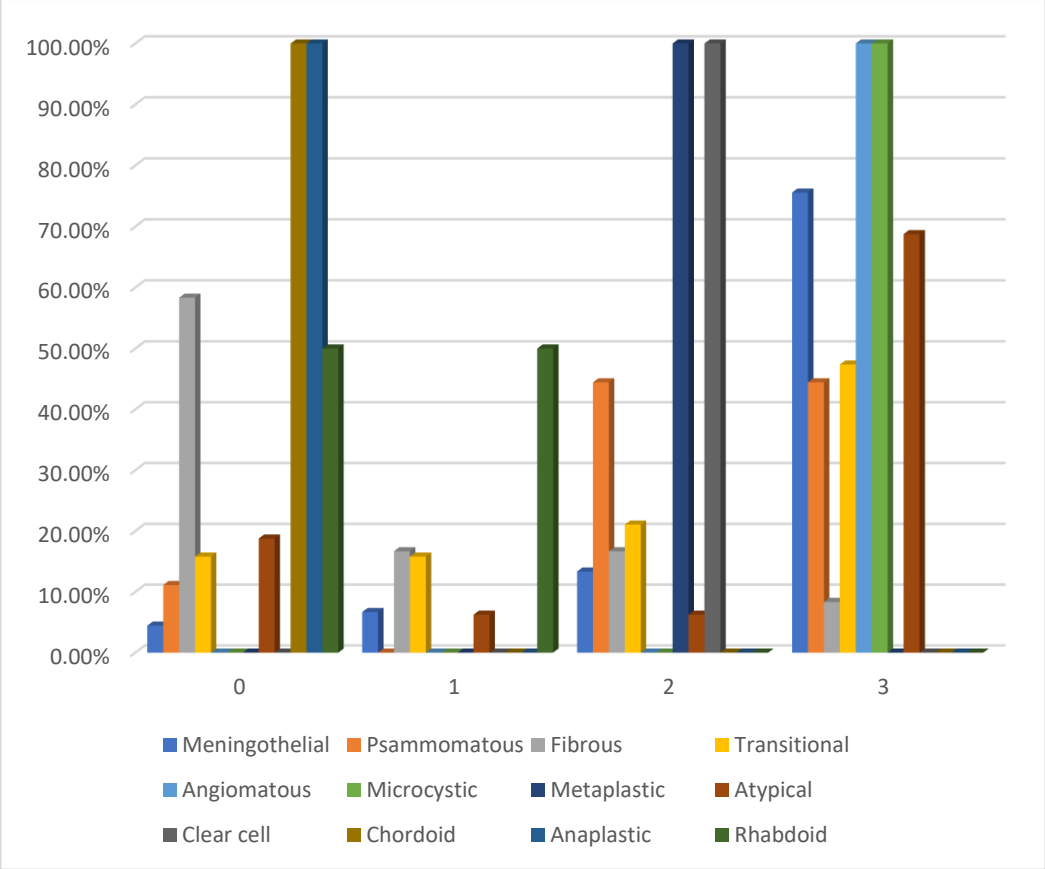


Figure 21 (a). Association of histologic subtype with SSTR-2 expression

Histologic subtype	SSTR-2 expression		Total	P value
	Positive (n=91)	Negative (n=29)		
Meningothelial	40 (88.89%)	5 (11.11%)	45 (100%)	<.0001
Psammomatous	8 (88.89%)	1 (11.11%)	9 (100%)	
Fibrous	3 (25%)	9 (75%)	12 (100%)	
Transitional	13 (68.42%)	6 (31.58%)	19 (100%)	
Angiomatous	11 (100%)	0 (0%)	11 (100%)	
Microcystic	2 (100%)	0 (0%)	2 (100%)	
Metaplastic	1 (100%)	0 (0%)	1 (100%)	
Atypical	12 (75%)	4 (25%)	16 (100%)	
Clear cell	1 (100%)	0 (0%)	1 (100%)	
Chordoid	0 (0%)	1 (100%)	1 (100%)	
Anaplastic	0 (0%)	1 (100%)	1 (100%)	
Rhabdoid	0 (0%)	2 (100%)	2 (100%)	
Total	91 (75.83%)	29 (24.17%)	120 (100%)	

Table 24 (b). Association of histologic subtype with SSTR-2 expression

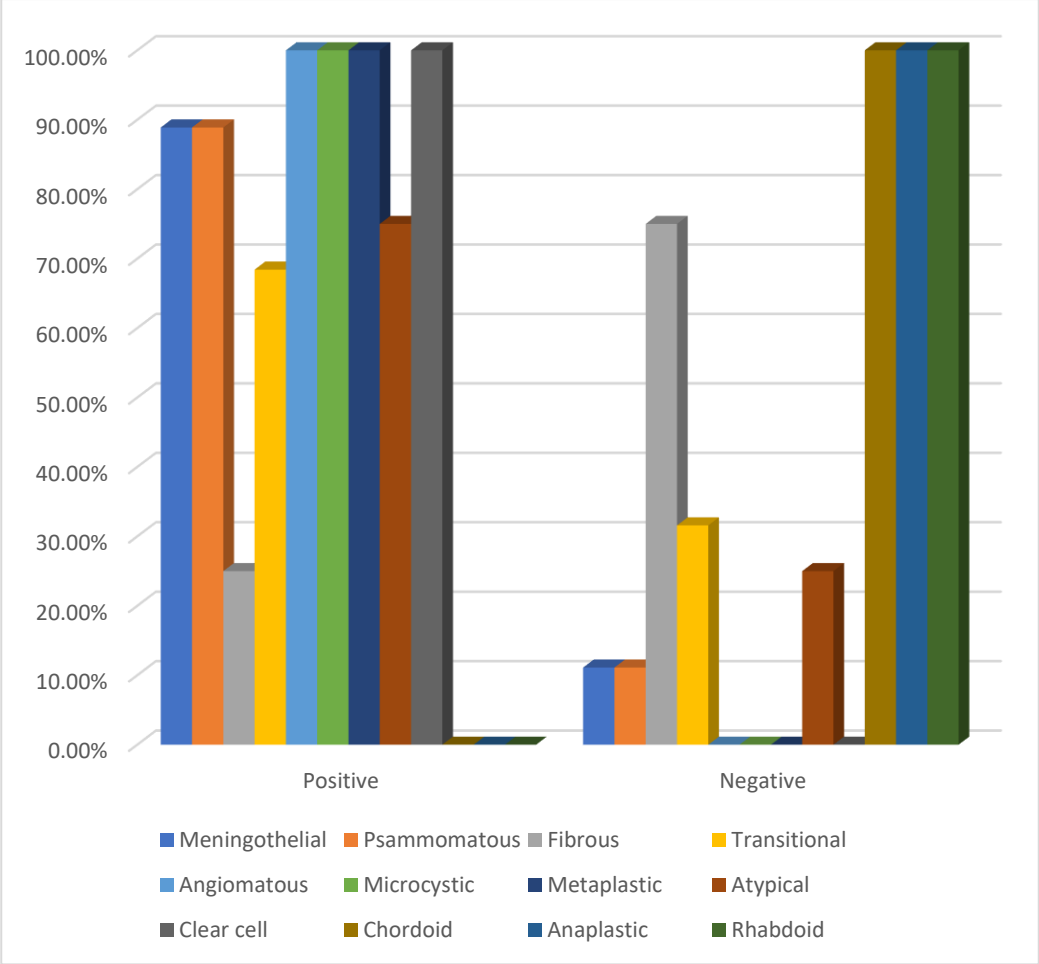


Figure 21 (b). Association of histologic subtype with SSTR-2 expression

Histologic subtype	HER2/neu expression		Total	P value
	0 (n=113)	1+ (n=7)		
Meningothelial	44 (97.78%)	1 (2.22%)	45 (100%)	0.506
Psammomatous	8 (88.89%)	1 (11.11%)	9 (100%)	
Fibrous	11 (91.67%)	1 (8.33%)	12 (100%)	
Transitional	16 (84.21%)	3 (15.79%)	19 (100%)	
Angiomatous	11 (100%)	0 (0%)	11 (100%)	
Microcystic	2 (100%)	0 (0%)	2 (100%)	
Metaplastic	1 (100%)	0 (0%)	1 (100%)	
Atypical	15 (93.75%)	1 (6.25%)	16 (100%)	
Clear cell	1 (100%)	0 (0%)	1 (100%)	
Chordoid	1 (100%)	0 (0%)	1 (100%)	
Anaplastic	1 (100%)	0 (0%)	1 (100%)	
Rhabdoid	2 (100%)	0 (0%)	2 (100%)	
Total	113 (94.17%)	7 (5.83%)	120 (100%)	

Table 25. Association of histologic subtype with HER2/neu expression

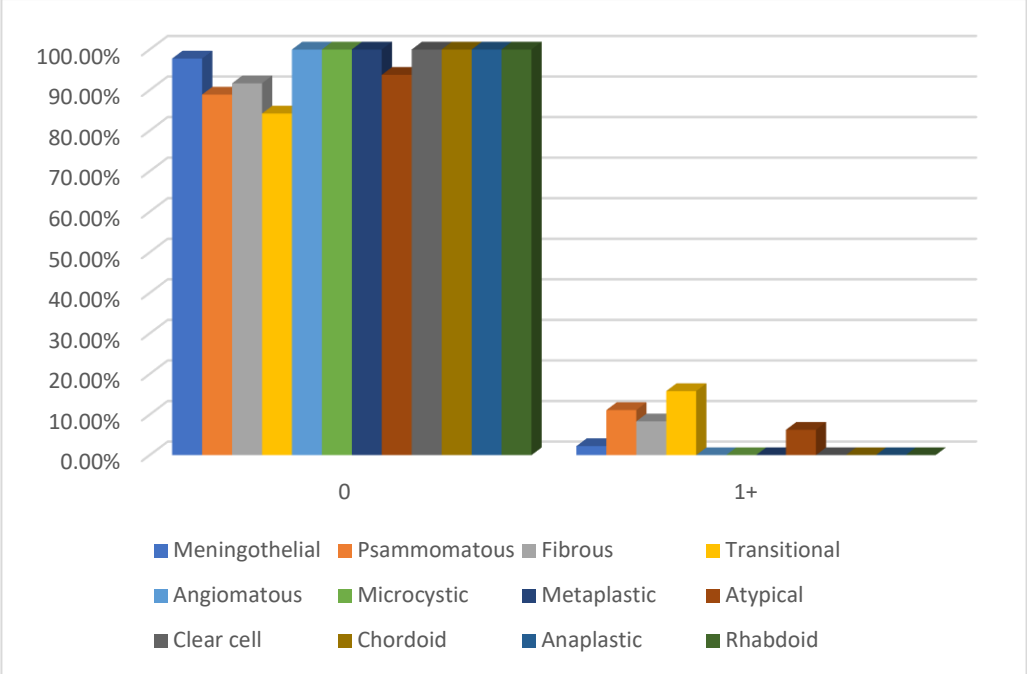


Figure 22. Association of histologic subtype with HER2/neu expression

The 120 cases included in this study were of different histologic subtypes, including 45 cases of meningothelial, 9 of psammomatous, 12 of fibrous, 19 of transitional, 11 of angiomatous, 2 of microcystic, 1 of metaplastic, 16 of atypical, 1 of clear cell, 1 of chordoid, 1 of anaplastic and 2 of rhabdoid.

A statistically significant association was found between histologic subtype and expression of SSTR-2 by Fisher's exact test (p value < 0.0001). SSTR-2 expression was seen in most of the cases of meningothelial meningioma and psammomatous meningioma (88.89% cases each). Furthermore, all cases of angiomatous meningioma (11 cases), microcystic meningioma (2 cases), metaplastic meningioma (1 case) and clear cell meningioma (1 case) showed SSTR-2 expression. However, the sample size for microcystic meningioma, metaplastic meningioma and clear cell meningioma was too less to reach statistical significance. None of the cases of chordoid meningioma, anaplastic meningioma and rhabdoid meningioma showed SSTR-2 expression.

Of the 7 cases with Score 1+ for HER2/neu, 3 were transitional along with one each of meningothelial, psammomatous, fibrous and atypical. No statistically significant association was found between histologic subtype and expression of HER2/neu (p value = 0.506; Fisher's exact test).

Association of grade of meningioma with SSTR-2 and HER2/neu expression

Grade	SSTR-2 expression				Total	P value
	0 (n=19)	1 (n=10)	2 (n=19)	3 (n=72)		
I	13 (13.40%)	8 (8.25%)	17 (17.53%)	59 (60.82%)	97 (100%)	0.106
II	4 (20%)	1 (5%)	2 (10%)	13 (65%)	20 (100%)	
III	2 (66.67%)	1 (33.33%)	0 (0%)	0 (0%)	3 (100%)	
Total	19 (15.83%)	10 (8.33%)	19 (15.83%)	72 (60%)	120 (100%)	

Table 26 (a). Association of grade of meningioma with SSTR-2 expression

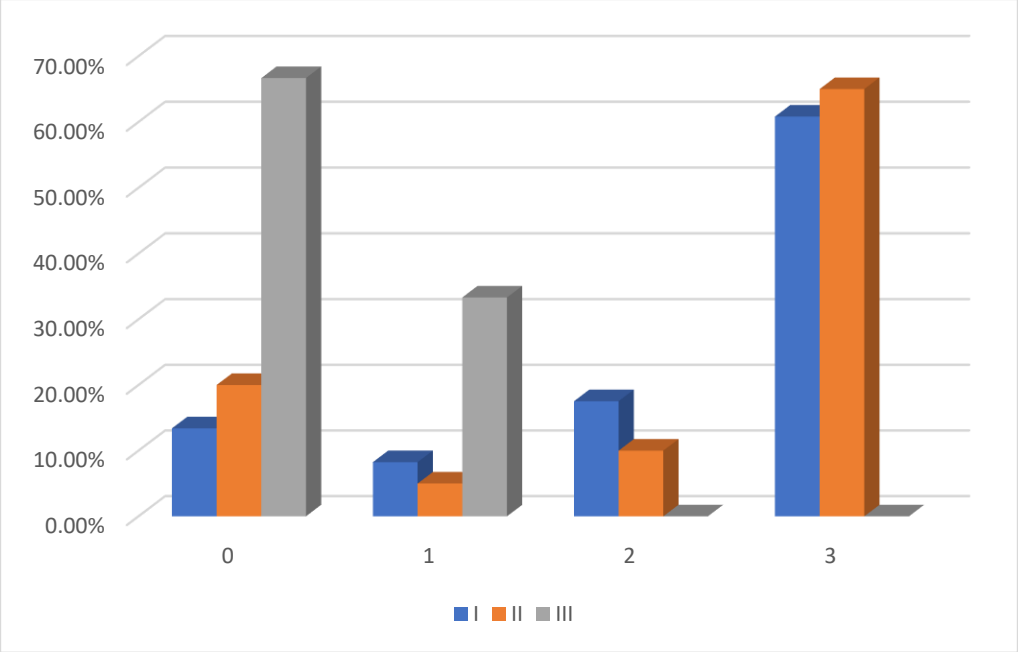


Figure 23 (a). Association of grade of meningioma with SSTR-2 expression

Grade	SSTR-2 expression		Total	P value
	Positive (n=91)	Negative (n=29)		
I	76 (78.35%)	21 (21.65%)	97 (100%)	0.019
II	15 (75%)	5 (25%)	20 (100%)	
III	0 (0%)	3 (100%)	3 (100%)	
Total	91 (75.83%)	29 (24.17%)	120 (100%)	

Table 26 (b). Association of grade of meningioma with SSTR-2 expression

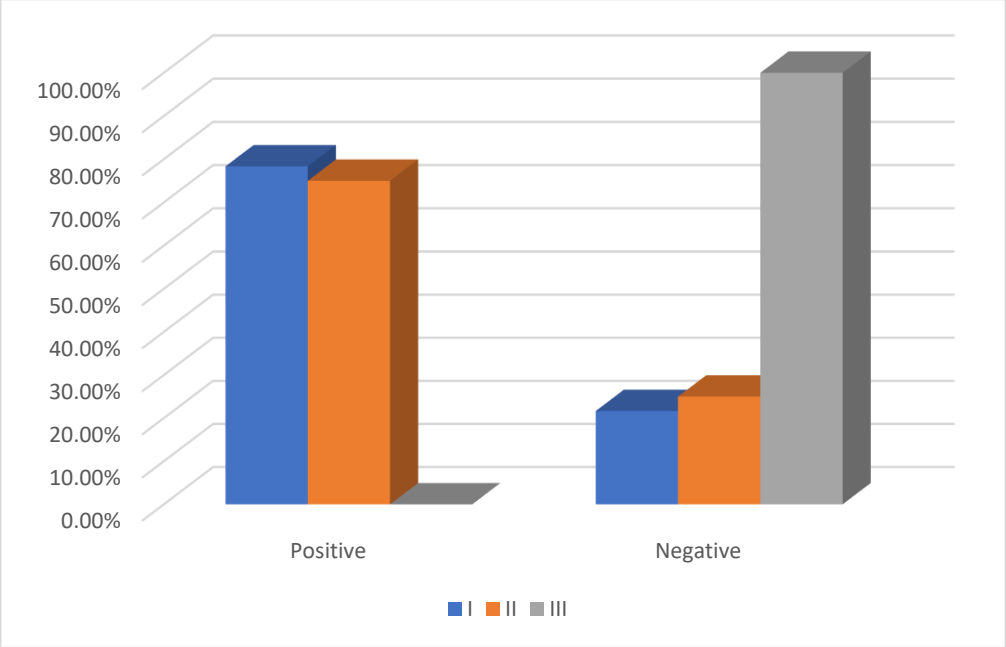


Figure 23 (b). Association of grade of meningioma with SSTR-2 expression

Grade	HER2/neu expression		Total	P value
	0 (n=113)	1+ (n=7)		
I	92 (94.85%)	5 (5.15%)	97 (100%)	0.452
II	18 (90%)	2 (10%)	20 (100%)	
III	3 (100%)	0 (0%)	3 (100%)	
Total	113 (94.17%)	7 (5.83%)	120 (100%)	

Table 27. Association of grade of meningioma with HER2/neu expression

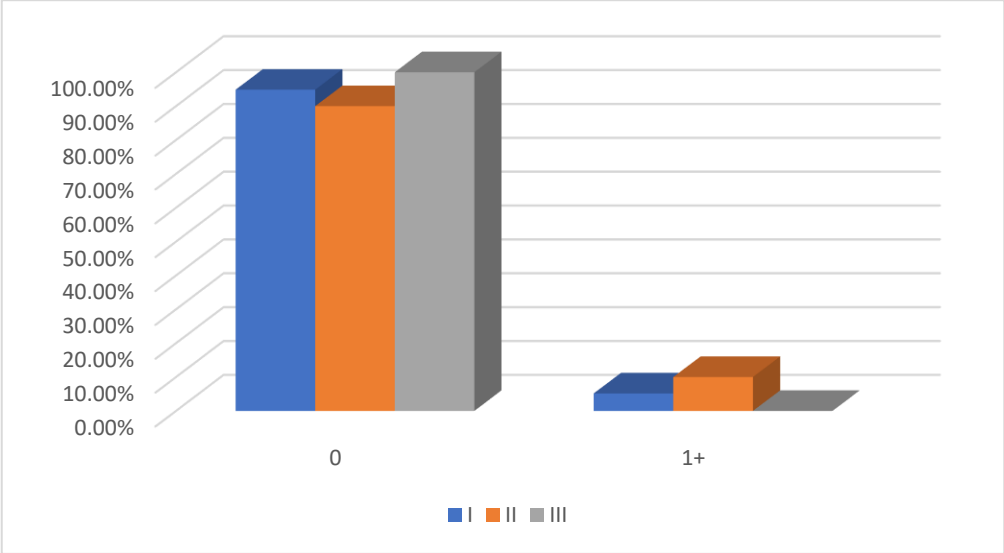


Figure 24. Association of grade of meningioma with HER2/neu expression

The present study included 97 cases of Grade I meningioma, 20 cases of Grade II meningioma and 3 cases of Grade III meningioma.

A statistically significant association was found between grade of meningioma and expression of SSTR-2 (p value = 0.019; Fisher's exact test). SSTR-2 expression was seen more commonly in Grade I and Grade II meningiomas (78.35%

and 75% cases respectively). All the Grade III meningiomas were negative for SSTR-2.

No statistically significant association was found between the grade of meningioma and the expression of HER2/neu (p value = 0.452; Fisher's exact test). Most of the cases, irrespective of the grade, had a score of 0 for HER2/neu. Out of the 7 cases having a score of 1+ for HER2/neu, 5 were Grade I and 2 were Grade II.

Association of histo-morphological features with SSTR-2 and HER2/neu expression

Histological feature	SSTR-2 expression				Total	P value
	0	1	2	3		
Mitoses (per 10 HPF)						
<4	16 (14.55%)	8 (7.27%)	18 (16.36%)	68 (61.82%)	110 (100%)	0.151
4-19	2 (25%)	1 (12.50%)	1 (12.50%)	4 (50%)	8 (100%)	
≥20	1 (50%)	1 (50%)	0 (0%)	0 (0%)	2 (100%)	
Brain invasion						
Present	1 (9.09%)	1 (9.09%)	0 (0%)	9 (81.82%)	11 (100%)	0.418
Absent	18 (16.51%)	9 (8.26%)	19 (17.43%)	63 (57.80%)	109 (100%)	
Increased cellularity						
Present	3 (75%)	0 (0%)	0 (0%)	1 (25%)	4 (100%)	0.057
Absent	16 (13.79%)	10 (8.62%)	19 (16.38%)	71 (61.21%)	116 (100%)	
Small cell change						
Present	1 (100%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0.4
Absent	18 (15.13%)	10 (8.40%)	19 (15.97%)	72 (60.50%)	119 (100%)	
Prominent nucleoli						
Present	3 (60%)	1 (20%)	0 (0%)	1 (20%)	5 (100%)	0.028
Absent	16 (13.91%)	9 (7.83%)	19 (16.52%)	71 (61.74%)	115 (100%)	
Sheeting						
Present	1 (20%)	1 (20%)	0 (0%)	3 (60%)	5 (100%)	0.608
Absent	18 (15.65%)	9 (7.83%)	19 (16.52%)	69 (60%)	115 (100%)	
Spontaneous necrosis						
Present	3 (42.86%)	1 (14.29%)	1 (14.29%)	2 (28.57%)	7 (100%)	0.107
Absent	16 (14.16%)	9 (7.96%)	18 (15.93%)	70 (61.95%)	113 (100%)	

Histological feature	SSTR-2 expression				Total	P value
	0	1	2	3		
Frank anaplasia						
Present	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	NA
Absent	19 (15.83%)	10 (8.33%)	19 (15.83%)	72 (60%)	120 (100%)	

Table 28. Association of histo-morphological features of meningioma with SSTR-2 expression

Histological feature	HER2/neu expression		Total	P value
	0	1+		
Mitoses (per 10 HPF)				
<4	104 (94.55%)	6 (5.45%)	110 (100%)	0.465
4-19	7 (87.50%)	1 (12.50%)	8 (100%)	
≥20	2 (100%)	0 (0%)	2 (100%)	
Brain invasion				
Present	10 (90.91%)	1 (9.09%)	11 (100%)	0.499
Absent	103 (94.50%)	6 (5.50%)	109 (100%)	
Increased cellularity				
Present	3 (75%)	1 (25%)	4 (100%)	0.216
Absent	110 (94.83%)	6 (5.17%)	116 (100%)	
Small cell change				
Present	1 (100%)	0 (0%)	1 (100%)	1
Absent	112 (94.12%)	7 (5.88%)	119 (100%)	
Prominent nucleoli				
Present	5 (100%)	0 (0%)	5 (100%)	1
Absent	108 (93.91%)	7 (6.09%)	115 (100%)	
Sheeting				
Present	3 (60%)	2 (40%)	5 (100%)	0.027
Absent	110 (95.65%)	5 (4.35%)	115 (100%)	
Spontaneous necrosis				
Present	7 (100%)	0 (0%)	7 (100%)	1
Absent	106 (93.81%)	7 (6.19%)	113 (100%)	

Histological feature	HER2/neu expression		Total	P value
	0	1+		
Frank anaplasia				
Present	0 (0.00%)	0 (0.00%)	0 (0.00%)	NA
Absent	113 (94.17%)	7 (5.83%)	120 (100%)	

Table 29. Association of histo-morphological features of meningioma with HER2/neu expression

In this study, statistically significant correlation was found between expression of SSTR-2 and prominent nucleoli (p value = 0.028; Fisher's exact test). However, no statistically significant association was found between the expression of SSTR-2 and HER2/neu with other histo-morphological features like mitoses, brain invasion, increased cellularity, small cell change, spontaneous necrosis and frank anaplasia.

Association of Ki67 labelling index with SSTR-2 and HER2/neu expression

Ki67 (%)	SSTR-2 expression				Total	P value
	0 (n=14)	1 (n=7)	2 (n=8)	3 (n=43)		
≤ 4	6 (13.95%)	3 (6.98%)	6 (13.95%)	28 (65.12%)	43 (100%)	0.517
> 4 to ≤ 20	7 (26.92%)	4 (15.38%)	2 (7.69%)	13 (50%)	26 (100%)	
> 20	1 (33.33%)	0 (0%)	0 (0%)	2 (66.67%)	3 (100%)	
Total	14 (19.44%)	7 (9.72%)	8 (11.11%)	43 (59.72%)	72 (100%)	

Table 30. Association of Ki67 labelling index with SSTR-2 expression

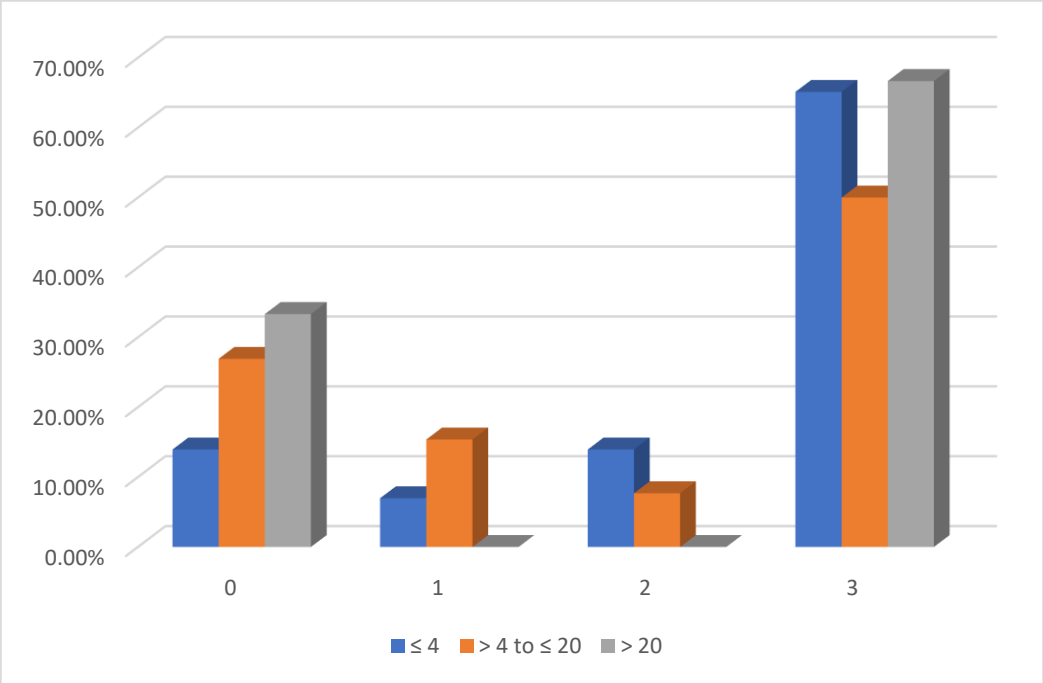


Figure 25. Association of Ki67 labelling index with SSTR-2 expression

Ki67 (%)	HER2/neu expression		Total	P value
	0 (n=67)	1+ (n=5)		
≤ 4	41 (95.35%)	2 (4.65%)	43 (100%)	0.179
> 4 to ≤ 20	24 (92.31%)	2 (7.69%)	26 (100%)	
> 20	2 (66.67%)	1 (33.33%)	3 (100%)	
Total	67 (93.06%)	5 (6.94%)	72 (100%)	

Table 31. Association of Ki67 labelling index with HER2/neu expression

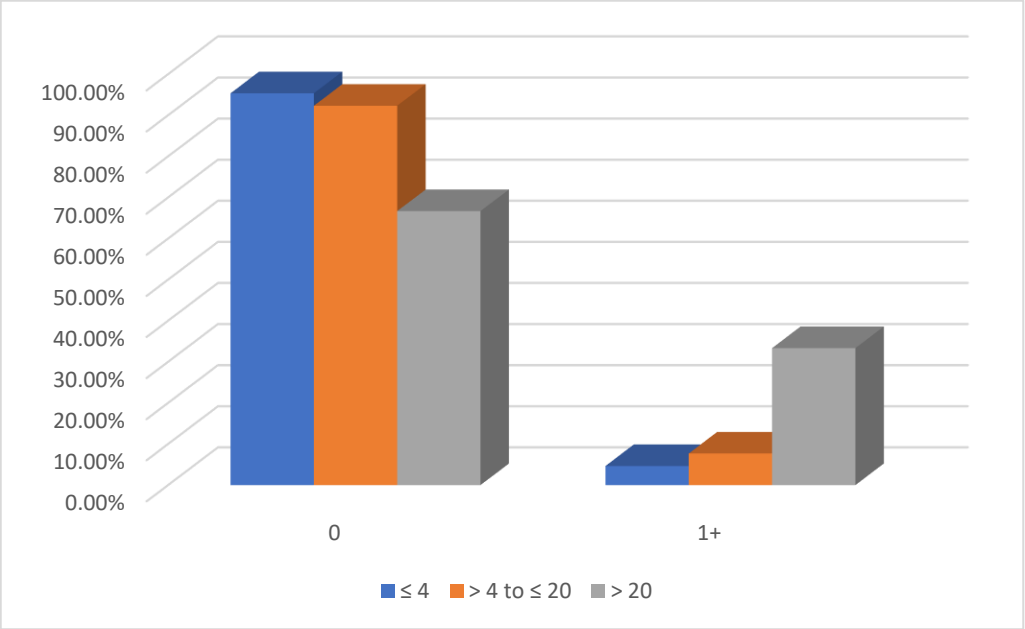


Figure 26. Association of Ki67 labelling index with HER2/neu expression

Ki67 labelling index was applied in 72 of the 120 cases included in the study. Majority of the cases with Score 3 for SSTR-2 had a Ki67 labelling index of less than or equal to 4. No statistically significant association was found between Ki67 labelling index and expression of SSTR-2 and HER2/neu (p values = 0.517 and 0.179 respectively; Fisher's exact test).

Association of expression of SSTR-2 with HER2/neu

Score		HER2/neu expression		Total	P value
		0 (n=113)	1+ (n=7)		
SSTR-2 expression	0	19 (100%)	0 (0%)	19 (100%)	0.179
	1	8 (80%)	2 (20%)	10 (100%)	
	2	18 (94.74%)	1 (5.26%)	19 (100%)	
	3	68 (94.44%)	4 (5.56%)	72 (100%)	
Total		113 (94.17%)	7 (5.83%)	120 (100%)	

Table 32. Association of expression of SSTR-2 with HER2/neu

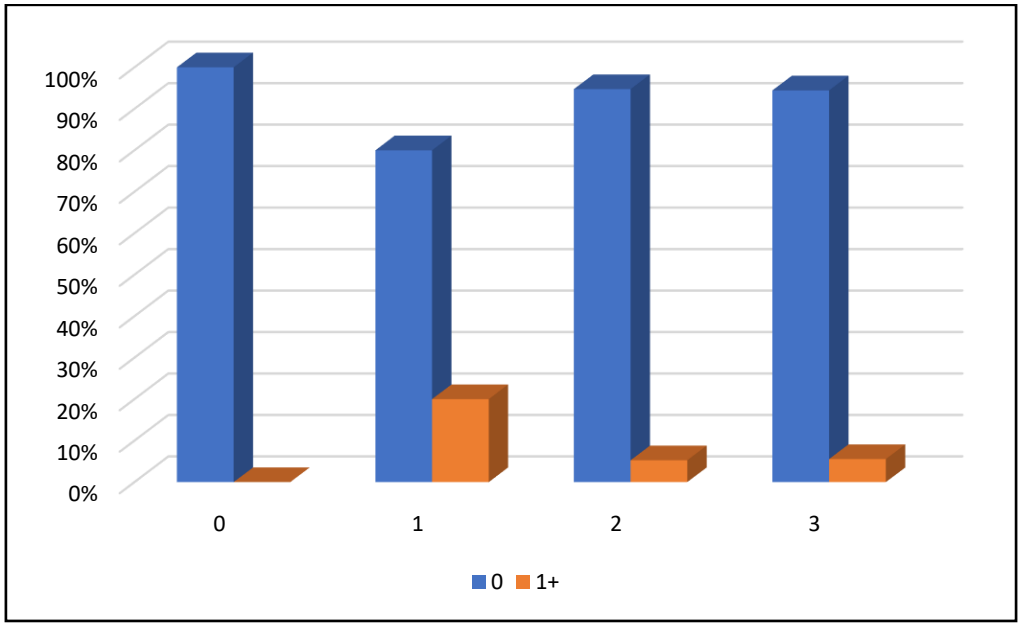
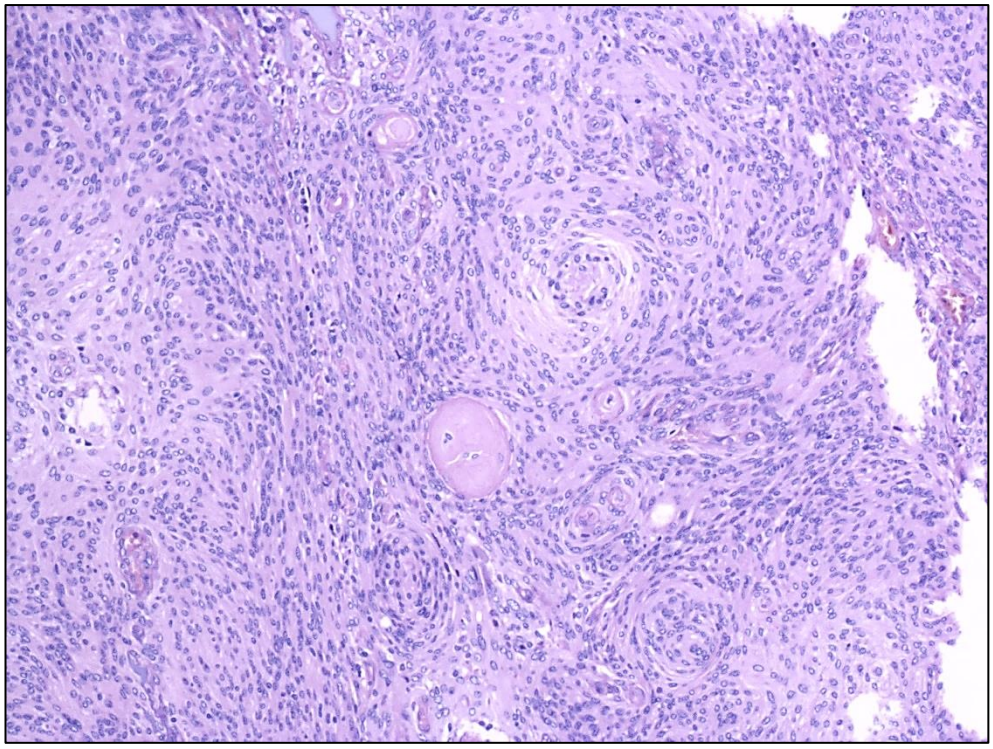
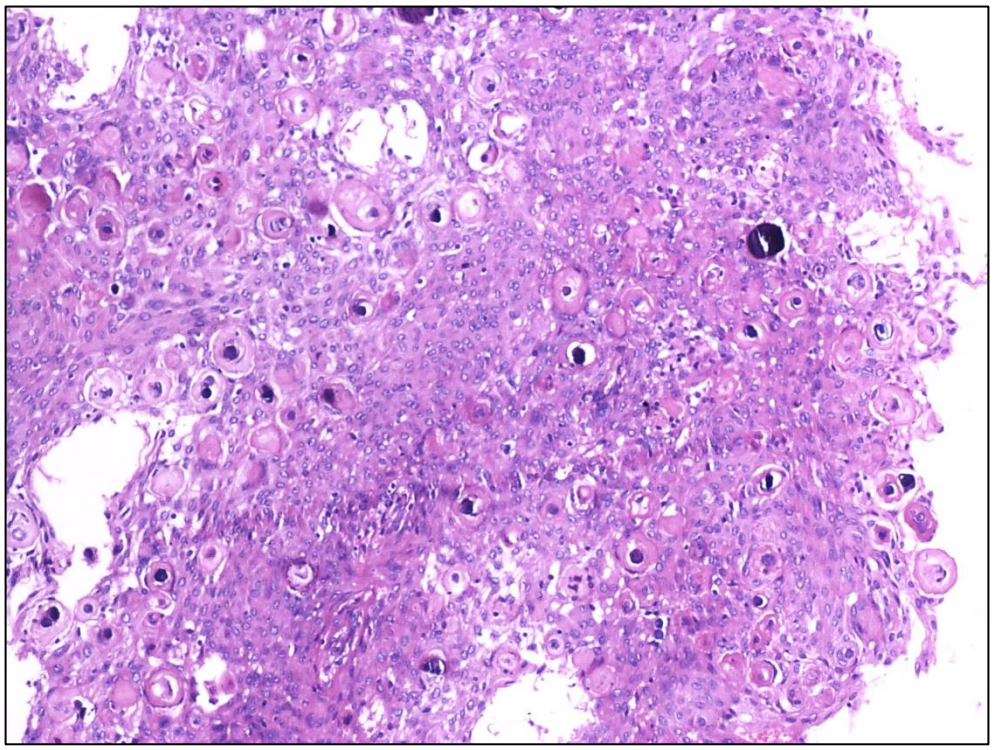


Figure 27. Association of expression of SSTR-2 with HER2/neu

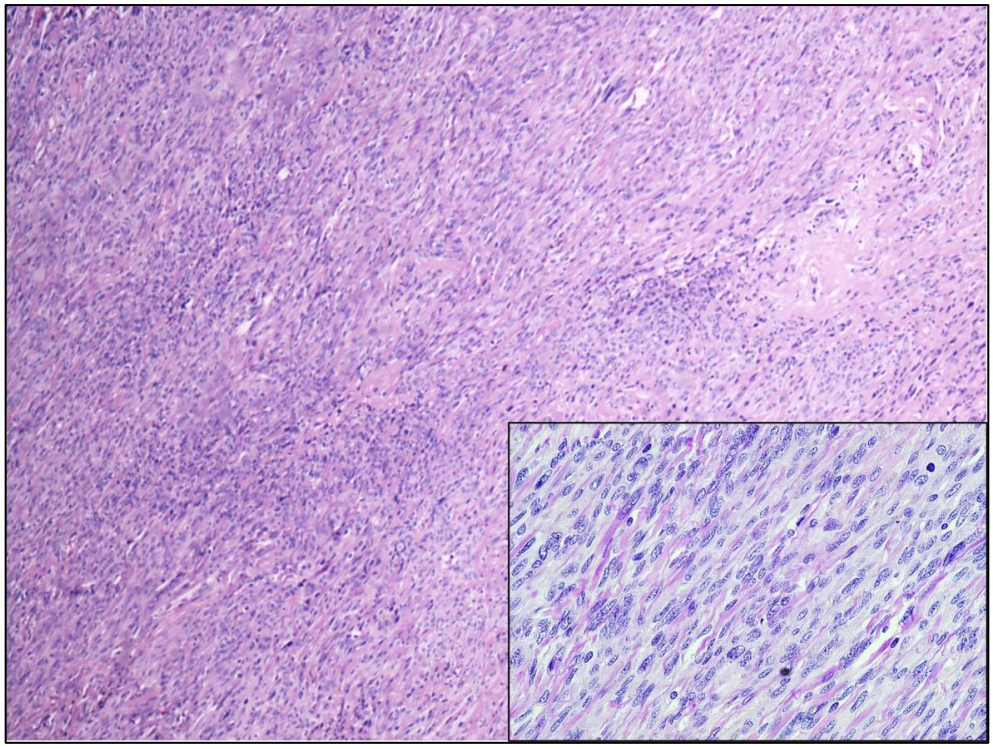
In the present study, no statistically significant association was found between expression of SSTR-2 with HER2/neu (p value = 0.179; Fisher's exact test).



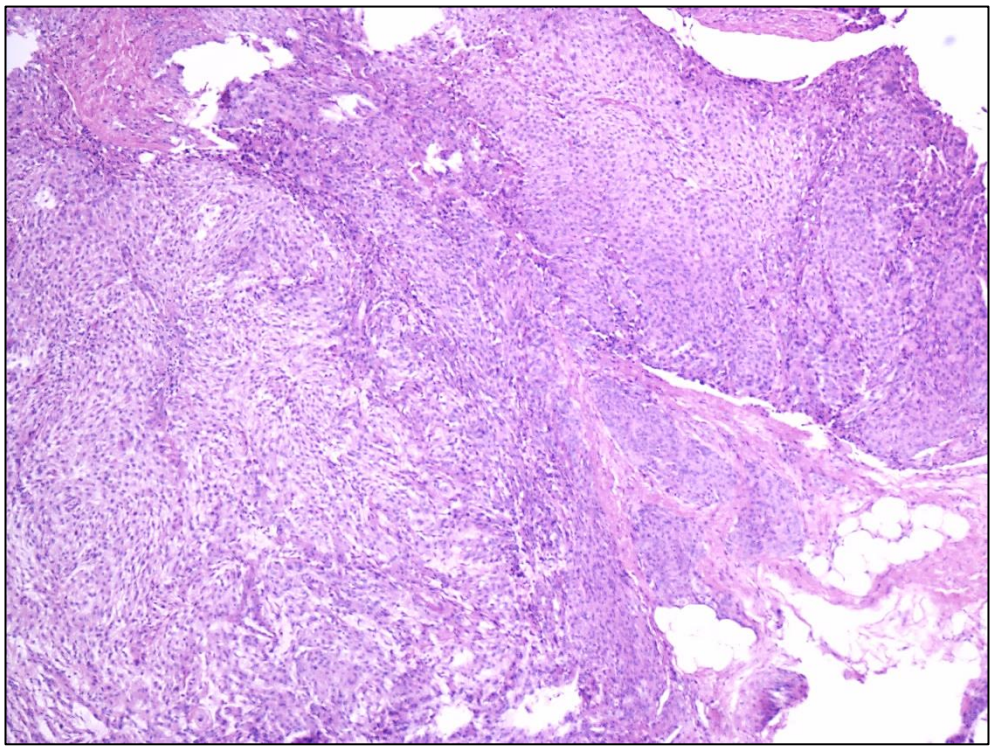
Colour plate 1. Meningothelial meningioma showing whorls of monomorphic meningothelial cells forming ‘pseudosyncytium’ (100x, H&E stain)



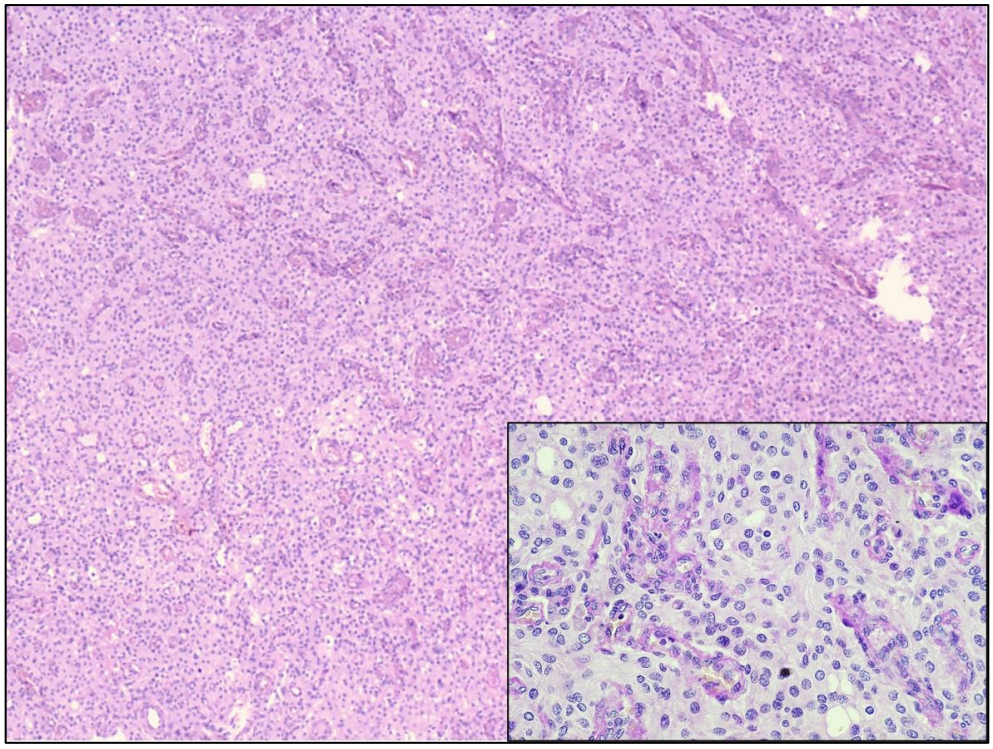
Colour plate 2. Psammomatous meningioma showing predominantly psammoma bodies with intervening meningothelial cells (100x, H&E stain)



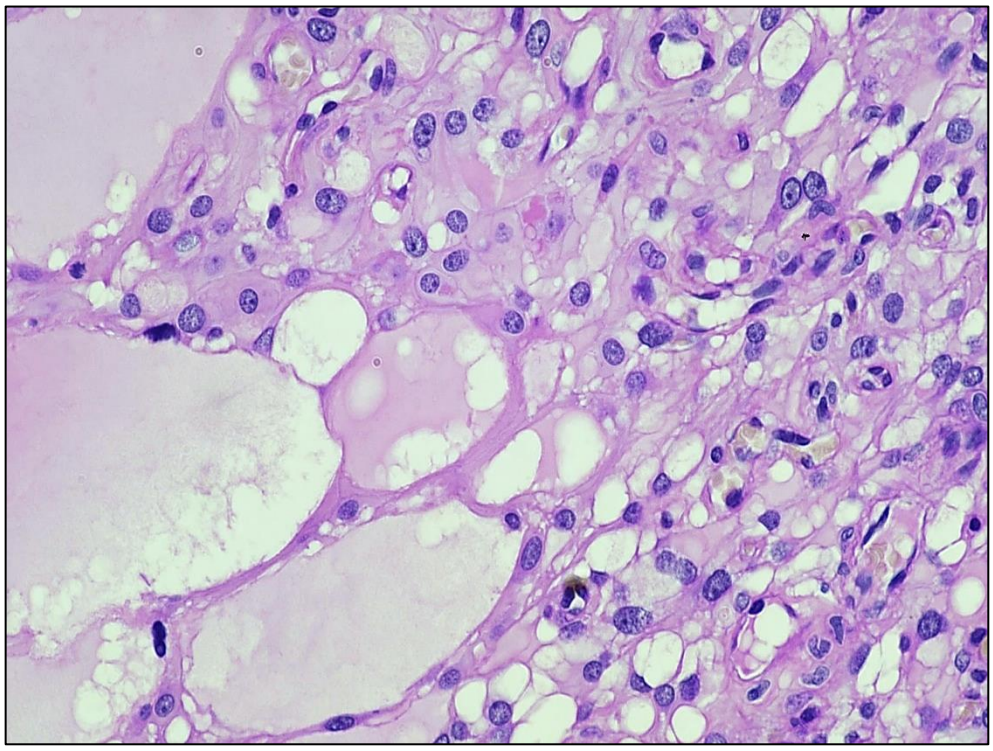
Colour plate 3. Fibrous meningioma showing spindled cells in parallel and interlacing fascicles (40x, H&E stain; Inset: 400x, H&E stain)



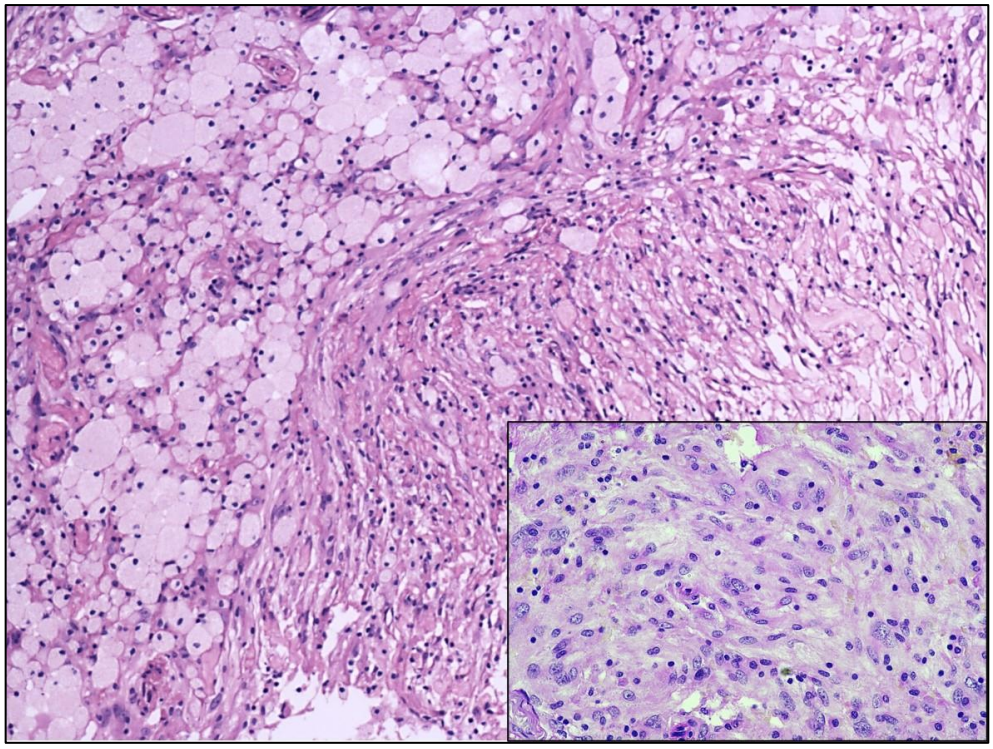
Colour plate 4. Transitional meningioma showing presence of meningothelial as well as spindled areas (40x, H&E stain)



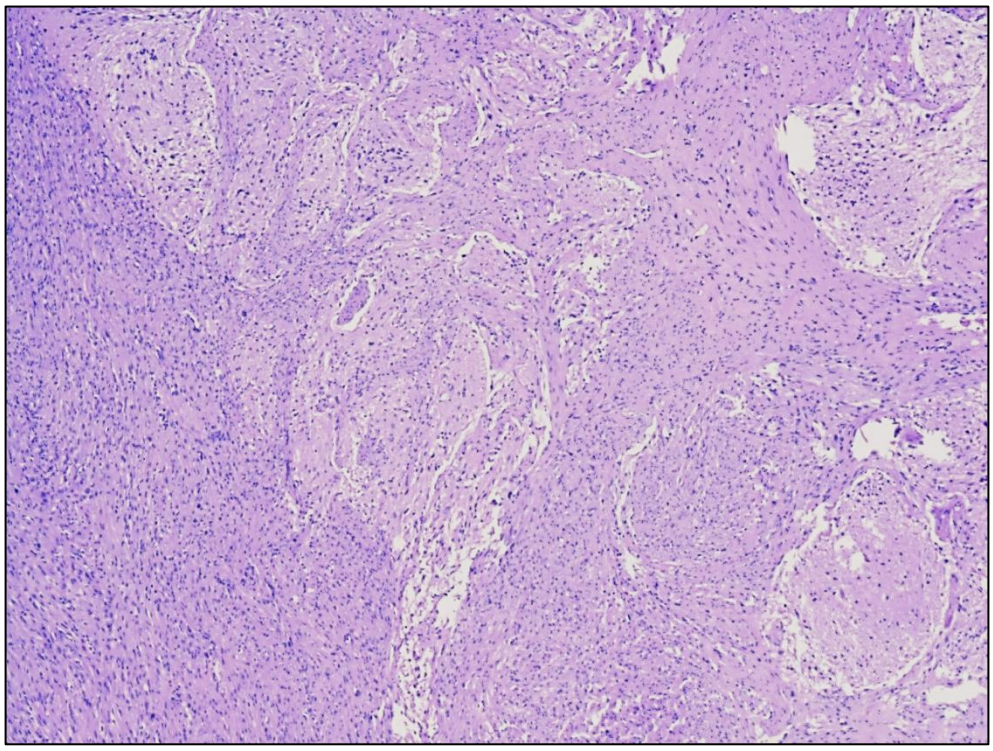
Colour plate 5. Angiomatous meningioma showing numerous small to medium sized variably hyalinized vascular channels (40x, H&E stain; Inset: 400x, H&E stain)



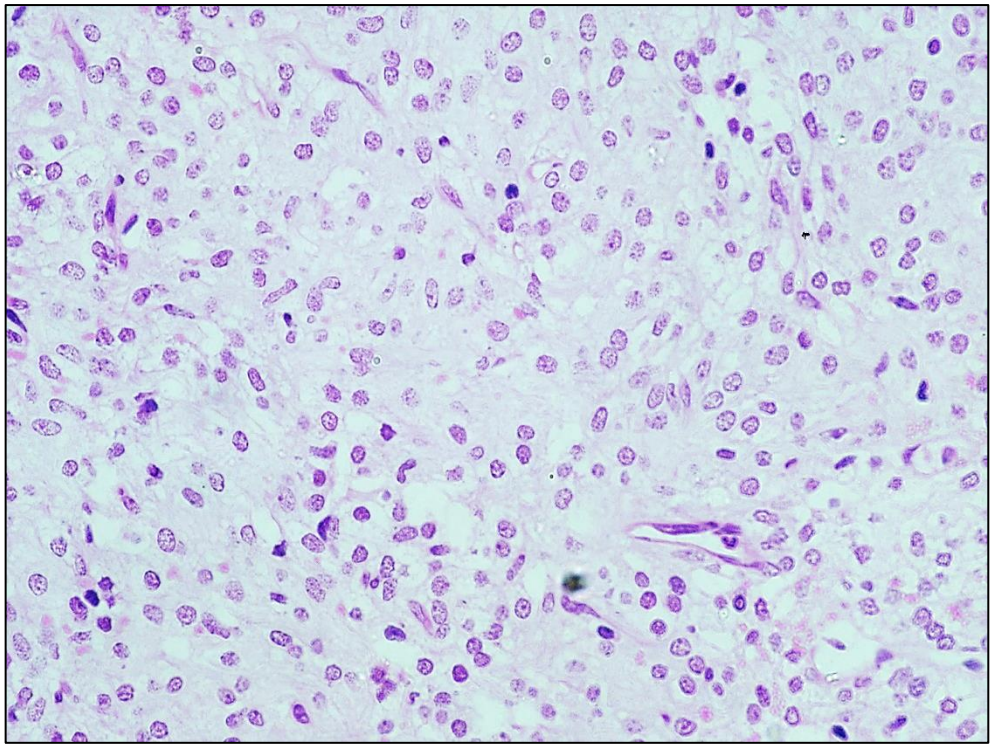
Colour plate 6. Microcystic meningioma showing thin elongated processes of tumour cells forming variably sized microcysts (400x, H&E stain)



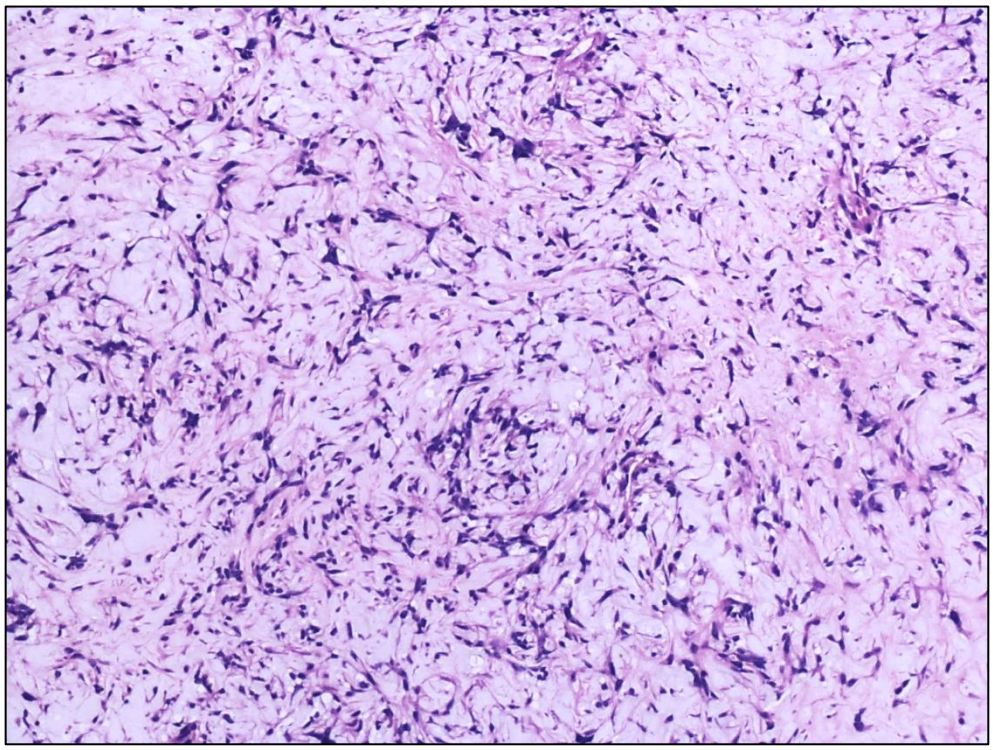
Colour plate 7. Metaplastic meningioma showing foci of xanthomatous change (100x, H&E stain; Inset: 400x, H&E stain)



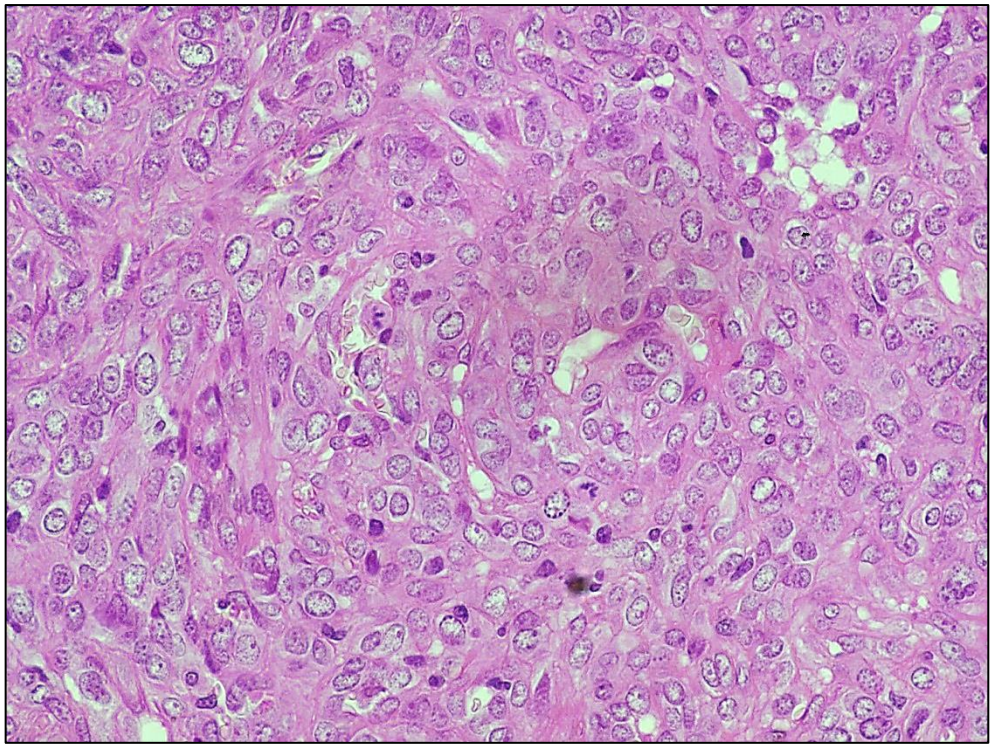
Colour plate 8. Atypical meningioma showing invasion of adjacent brain parenchyma by tumour cells (40x, H&E stain)



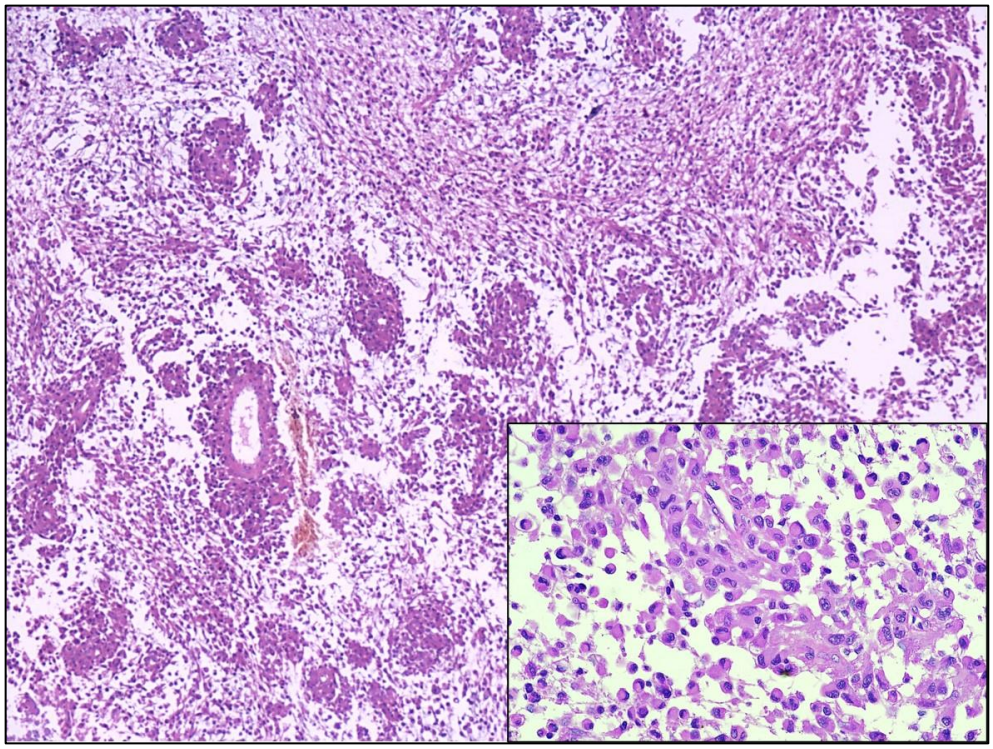
Colour plate 9. Clear cell meningioma showing sheets of round to polygonal cells with clear cytoplasm (400x, H&E stain)



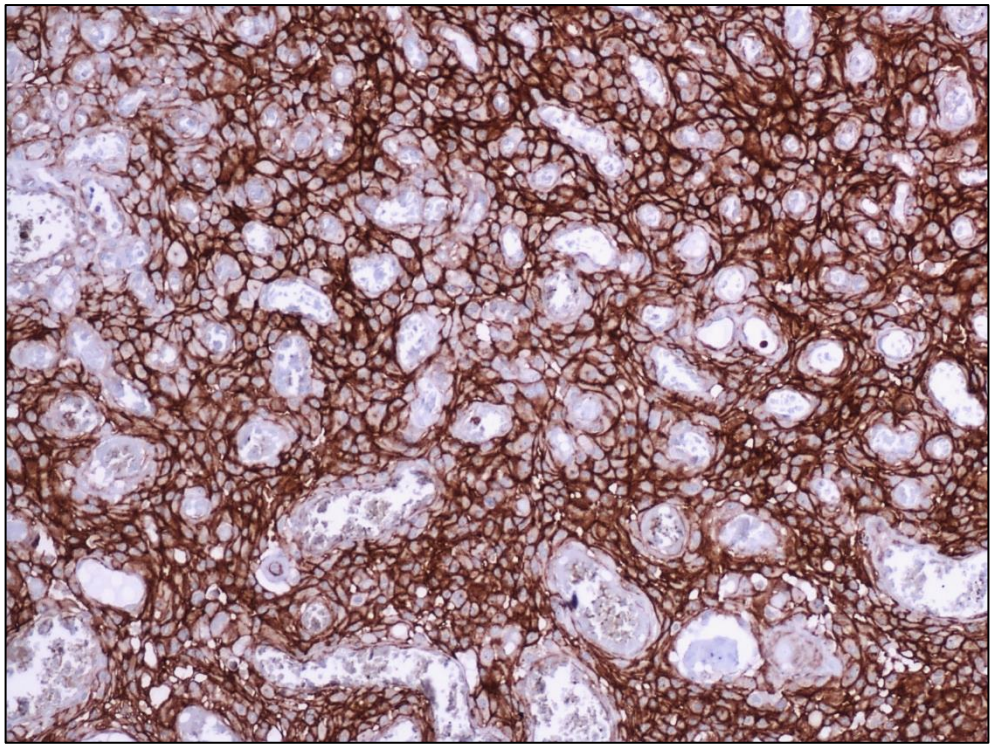
Colour plate 10. Chordoid meningioma showing cords and trabeculae of tumour cells in a mucoid background (100x, H&E stain)



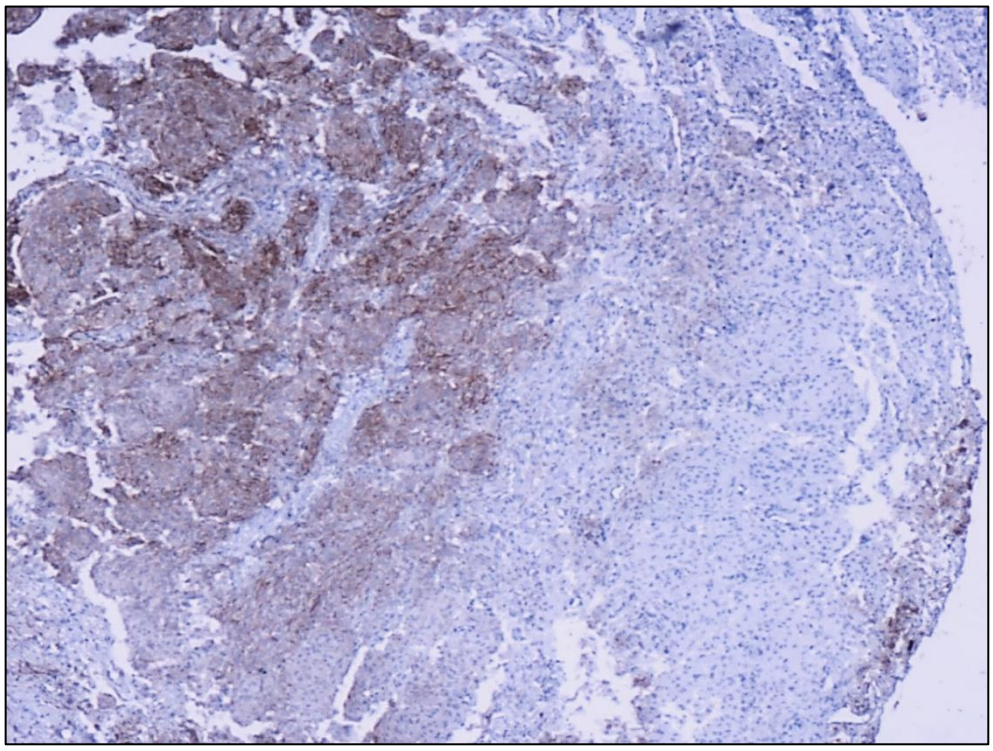
Colour plate 11. Anaplastic meningioma showing moderately pleomorphic cells, multinucleated cells and atypical mitotic figures (400x, H&E stain)



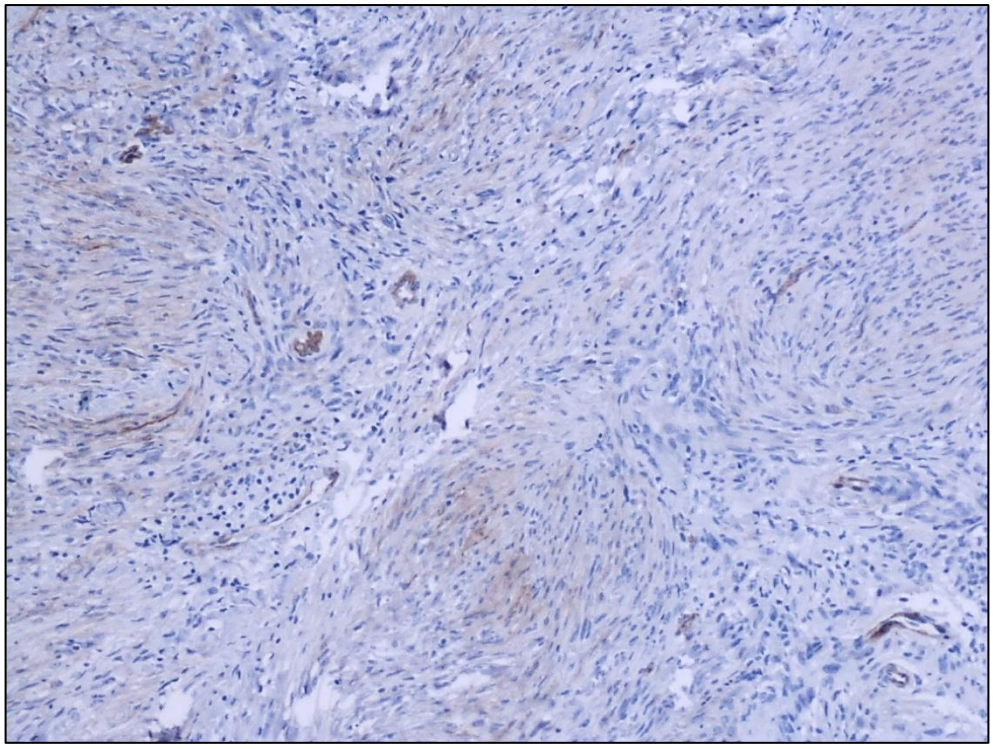
Colour plate 12. Rhabdoid meningioma showing perivascular pattern of rhabdoid cells which are plump, with eccentric nuclei and moderate eosinophilic cytoplasm (40x, H&E stain; Inset: 400x, H&E stain)



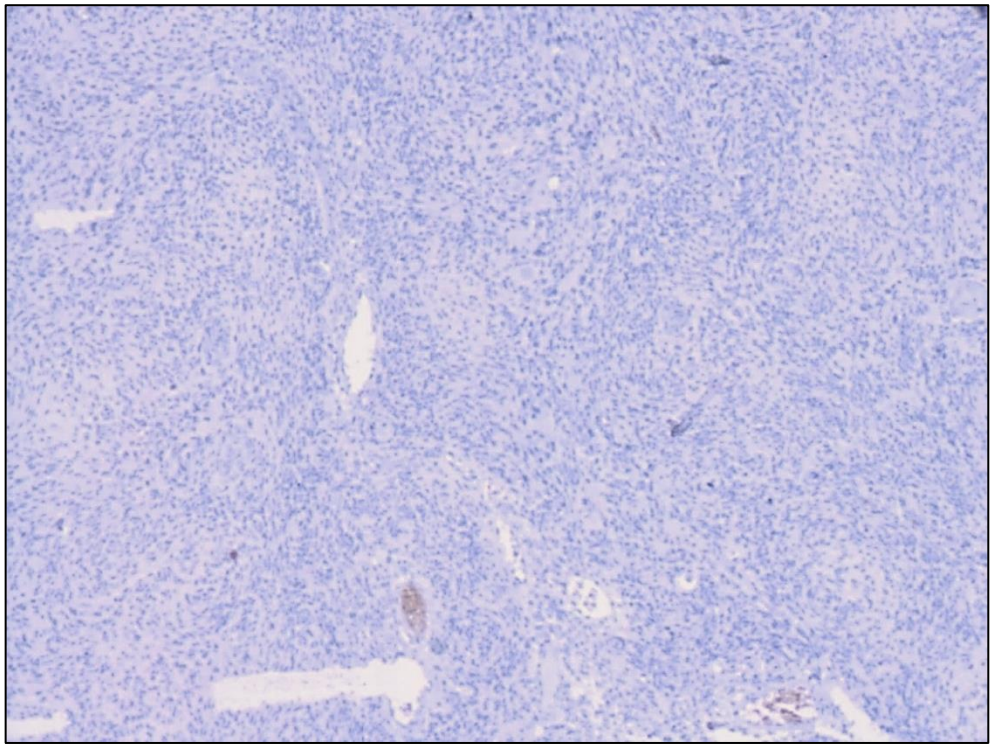
Colour plate 13. Diffuse strong circumferential membranous positivity in >50% of the tumour cells (Score 3) with SSTR-2 antibody (100x, IHC)



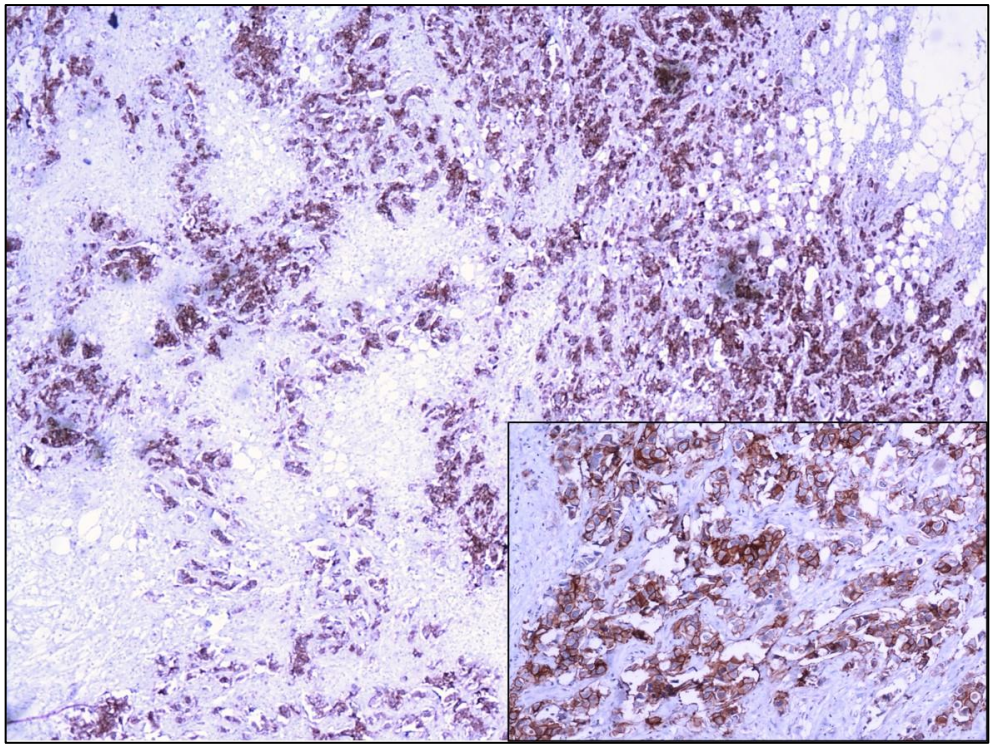
Colour plate 14. Incomplete membranous positivity in <50% of the tumour cells (Score 2) with SSTR-2 antibody (40x, IHC)



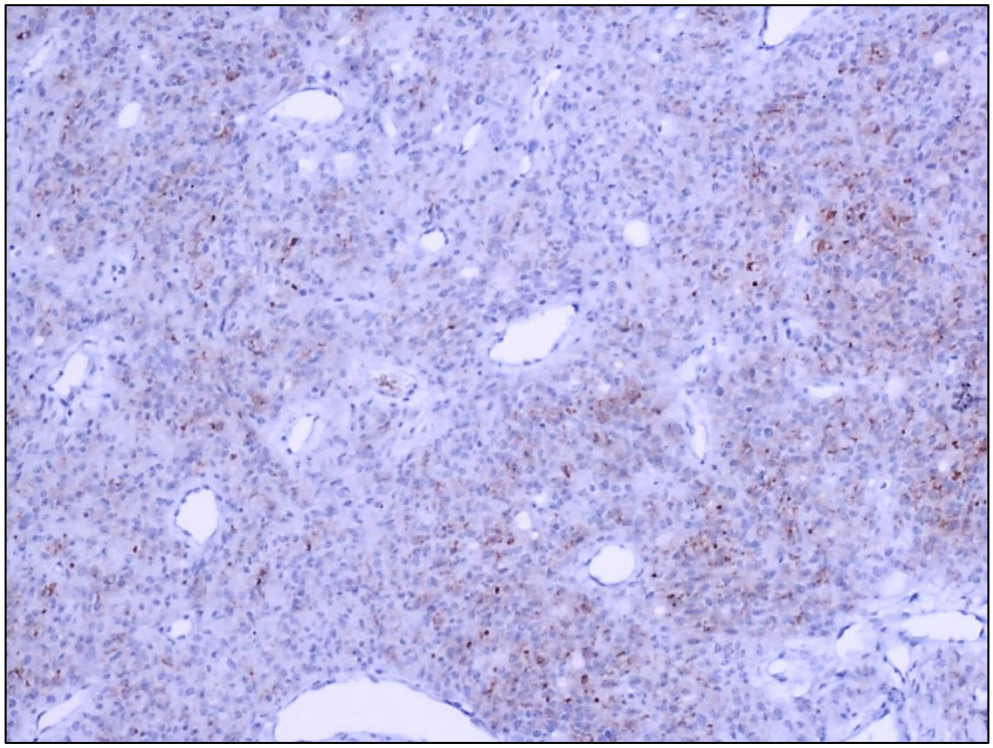
Colour plate 15. Focal pure cytoplasmic reactivity in tumour cells (Score 1) with SSTR-2 antibody (100x, IHC)



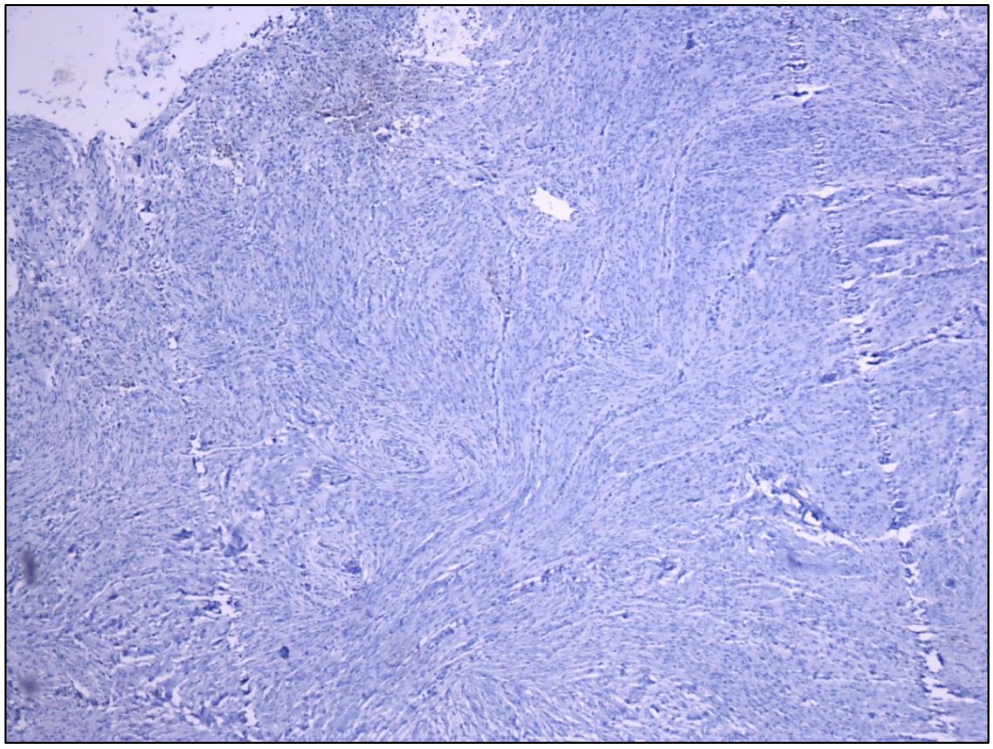
Colour plate 16. No immunoreactivity (Score 0) with SSTR-2 antibody (40x, IHC)



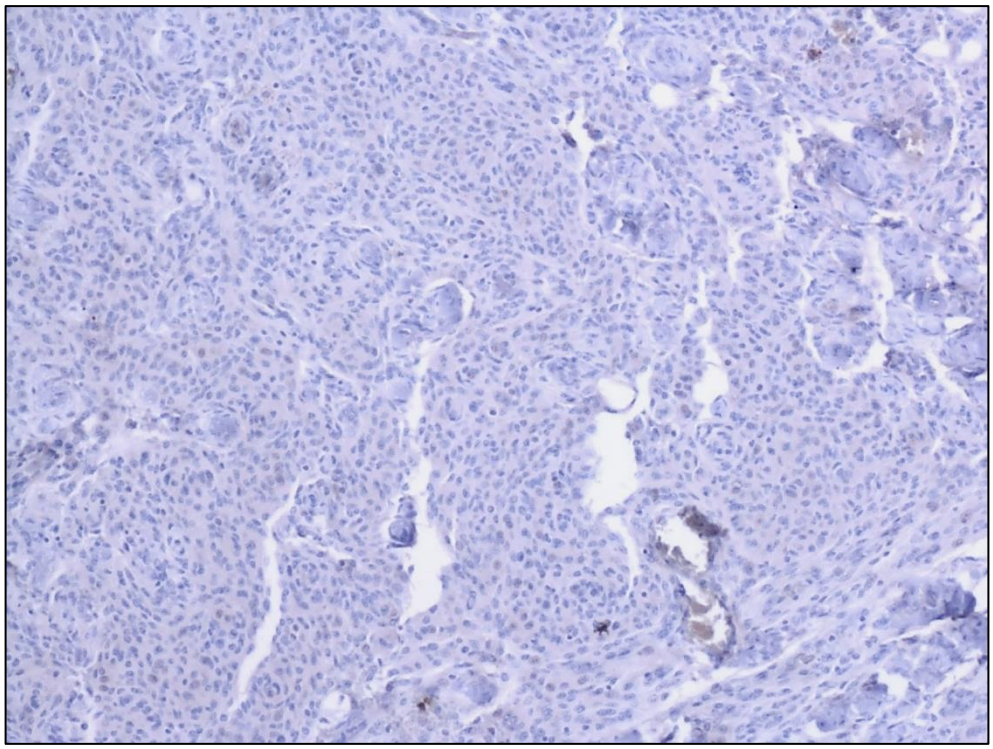
Colour plate 17. Invasive breast carcinoma as positive control for HER2/neu antibody (20x, IHC; Inset: 100x, IHC)



Colour plate 18. Faint incomplete membranous staining in >10% of the tumour cells (Score 1+) with HER2/neu antibody (100x, IHC)



Colour plate 19. No immunoreactivity (Score 0) with HER2/neu antibody (40x, IHC)



Colour plate 20. No immunoreactivity (Score 0) with HER2/neu antibody (100x, IHC)

DISCUSSION

Somatostatin receptor-2 (SSTR-2) is one of five type of somatostatin receptors, belonging to a family of transmembrane G-protein coupled receptors. They bind to somatostatin, which is a regulatory peptide, and its analogues, causing regulation of cell growth, inhibition of proliferation and angiogenesis and promotion of apoptosis (28). The gene encoding SSTR-2 can be localized at chromosome 17q25.1(3).

HER2/neu belongs to the EGFR/HER family which is a receptor tyrosine kinase family. It consists of HER1 to HER4. HER2 lies at chromosome 17q21 and encodes a 185kD transmembranous protein found in many tissues. HER2/neu does not have ligand binding activity and so the signalling occurs with the help of its heterodimeric partners (4).

The assessment of SSTR-2 and HER2/neu expression in meningiomas has been done by many authors. But many of the previous studies lacked a large sample size, especially of higher grade meningiomas. Previous studies also showed highly variable HER2/neu expression in meningiomas.

The current study was conducted in a tertiary care centre in Western Rajasthan and included 120 cases of meningioma diagnosed between January 2018 and July 2022. Expression of SSTR-2 and HER2/neu was assessed in these cases.

Out of the 120 cases of meningioma evaluated for expression of SSTR-2, 19 cases were given a Score of 0 (15.83%), 10 cases were assigned Score 1 (8.33%), 19 cases were given Score 2 (15.83%) and 72 cases were assigned Score 3 (60%). Hence, 75.83% cases (91 cases) were positive for SSTR-2 (Scores 2 and 3) and 24.17% cases (29 cases) were negative for SSTR-2 (Scores 0 and 1).

In the previous studies, similar high expression of SSTR-2 was noted in cases of meningioma. In the study by Barresi et al., 74% of the 35 cases of meningioma showed SSTR-2 expression (23). Agaimy et al., in their study, showed SSTR-2 expression in 87% of the 68 cases of meningioma (24). 100% SSTR-2 expression was seen in the study cohorts of 60 and 176 cases in the studies by Silva et al. and Menke et al. respectively (25,43).

In our study, on evaluation of expression of HER2/neu, 7 cases were given a Score of 1+ (5.83%), while 113 cases were assigned Score 0 (94.17%). Hence, all the 120 cases were deemed negative for HER2/neu (Scores 0 and 1+).

This result was in concordance with the results obtained by Dar et al. in their study in 2020, in which all the 17 cases of meningioma were negative for HER2/neu (36).

In the other studies, however, expression of HER2/neu was highly variable. In the study by Kandemir et al., 22.6% of the 53 cases of meningioma showed expression of HER2/neu (32). Khamis et al., in 2011, showed positivity for HER2/neu in 34.2% of the 38 cases of meningioma, while 73% cases showed positivity in the study conducted by Telugu et al. (33,35).

One reason for this highly variable expression could be the different targets of the HER2/neu antibodies used, with some targeting intracellular domain, while others targeting extracellular domain, as noted in the study by Arnli et al. (5). A difference in demographics of the study cohort might have also played a role (33).

Clinico-pathological parameters

Age

According to WHO, the median age of diagnosis of meningioma is 66 years (10). Silva et al., in their study, included a total of 60 patients in their study with the mean age of diagnosis being 53.18 years (25). Dijkstra et al. conducted their study on 148 cases of meningioma and their mean age of diagnosis was 47.2 years in males and 52.4 years in females (27). Telugu et al., included 100 meningioma cases in their study and their mean age of diagnosis was 47.8 years (35). Mahzouni et al., in their study, included 72 cases, with the mean age at diagnosis being 52.8 years (34). No statistically significant correlation was found between expression of SSTR-2 and age in the studies by Barresi et al. and Silva et al. and between expression of HER2/neu and age in the study by Ramezani et al. (23,25,37).

The present study also showed similar distribution with the mean age of diagnosis being 50.07 years with cases ranging from 9-76 years. No significant association was

found between age and expression of SSTR-2 and HER2/neu (p values = 0.114 and 0.396 respectively).

Gender

According to WHO, incidence of meningioma is higher in females than in males (2.32 times) (10). In the study by Silva et al., the female to male ratio was 3:1 with 44 cases being females and 16 cases being males (25). In the study by Dijkstra et al., 96 cases were females and 52 cases were males with the female to male ratio being 1.84:1 (27). In the study by Telugu et al., out of the 100 cases, 66 were females while 34 were males with the female to male ratio being 1.94:1 (35). In the study by Mahzouni et al., 49 cases were females while 23 cases were males, with the ratio being 2.13:1 (34). In a study by Tollefsen et al., of the 162 meningioma cases included in the study, 119 were females and 43 were males with the female to male ratio being 2.77:1 (28). No significant correlation was found between gender and expression of SSTR-2 in the studies by Barresi et al. and Silva et al. and between gender and expression of HER2/neu in the studies by Kandemir et al., Mahzouni et al. and Telugu et al. (23,25,32,34,35).

In the current study, we recorded similar female predilection with 81 cases being female (67.5%) and 39 cases being male (32.5%). The female to male ratio was 2.08:1. No statistically significant association was found between gender and expression of SSTR-2 and HER2/neu (p values = 0.905 and 1 respectively; Fisher's exact test).

Location

Arnli et al. studied 186 cases of meningioma, all of which were located in brain, most commonly at the convexities and the basis cranii (5). In the study by Telugu et al., in the 100 cases studied, most common location was cerebral convexity in the brain and thoracic segment in the spine (35). Mahzouni et al., in their study, included cases that were most commonly localized at cerebral convexity and parasagittal areas (34). In the study by Barresi et al., the meningioma localization was predominantly in the brain with the most common sites being convexities, parasagittal areas and basal areas (23). In the study by Dar et al., most common location was the parietal region of brain (36). In the study by Fodi et al., of the 666 cases studied, 91% cases of meningioma

were located in the brain at the convexities and skull base while 9% cases were located in the spine (29).

No statistically significant correlation was found between expression of SSTR-2 and location of meningioma in studies by Barresi et al. and Silva et al. (23,25). Also, no significant association was found between expression of HER2/neu and location of meningioma in the study by Telugu et al. (35).

The present study showed similar results as majority of the cases were localized in brain (108 cases; 90%) with predilection for middle cranial fossa and only 10% cases were localized in spine (12 cases), predominantly thoracic level (10 cases).

There was a statistically significant association between the location of meningioma in brain with expression of SSTR-2 (p value = 0.0009, Chi square test) with most of the cases arising from anterior and middle cranial fossa showing SSTR-2 positivity.

There was no statistically significant association between location of meningioma (brain or spine) and location of meningioma in spine with expression of SSTR-2 and HER2/neu by Fisher's exact test.

Histologic subtype

Though there is a wide morphological spectrum of meningioma, according to WHO, the most common subtypes of meningioma include meningothelial, fibrous and transitional (10). In the study by Agaimy et al., of the 68 cases of meningioma studied, the most common subtype encountered was meningothelial (29 cases), followed by fibrous (16 cases) and transitional (10 cases) (24). Of the 60 cases of meningioma studied by Silva et al., majority were meningothelial (41.5%) followed by atypical (18.3%) and transitional (15%) (25). In the study by Boulagnon-Rombi et al., out of 127 cases, most common were meningothelial (22 cases) followed by transitional and fibrous (26). In the study by Mahzouni et al., transitional was the most common subtype (34). In the study by Telugu et al., of the 100 cases studied, the most common subtype was psammomatous (28 cases) followed by meningothelial (22 cases) (35). Meningothelial meningioma was the most common subtype followed by transitional and psammomatous in the study conducted by Dar et al. (36). In the study by Kandemir et al., of the 53 cases of meningioma, 52.8% were meningothelial and

22.6% were transitional (32). The study by Khamis et al. also showed similar distribution with majority of the cases being meningothelial followed by transitional (33).

No statistically significant correlation was found between histologic subtype and expression of SSTRs in the study by Silva et al. (25). However, in the study by Tollefsen et al., there was a significant correlation between SSTR2 expression and histologic subtypes (p value < 0.001) (28).

No statistically significant correlation was found between histologic subtype and HER2/neu expression in the studies by Arnli et al., Khamis et al. and Telugu et al. (5,33,35). In the study by Kandemir et al., frequent overexpression of HER2/neu was seen in meningothelial meningiomas (p value = 0.03) (32).

In the present study, the majority of the cases were meningothelial (45 cases; 37.50%), followed by transitional (19 cases; 15.83%) and atypical (16 cases, 13.33%).

There was a statistically significant association between histological subtype of meningioma and expression of SSTR-2 by Fisher's exact test (p value < 0.0001) with majority of the cases of meningothelial meningioma and psammomatous meningioma showing SSTR-2 positivity along with all the cases of angiomatous meningioma, microcystic meningioma, metaplastic meningioma and clear cell meningioma. None of the cases of chordoid meningioma, anaplastic meningioma and rhabdoid meningioma were positive for SSTR-2.

There was no significant association between histologic subtype of meningioma with expression of HER2/neu (p value = 0.506, Fisher's exact test).

Grade

In the present study, out of the 120 cases included, 97 cases were Grade I (80.83%), 20 cases were Grade II (16.67%) and 3 cases were Grade III (2.50%).

A statistically significant association was found between grade of meningioma and expression of SSTR-2 (p value = 0.019; Fisher's exact test) with SSTR-2 being positive (scores 2 and 3) in 78.35% of Grade I cases and 75% of Grade II cases. None of the Grade III cases were positive for SSTR-2.

HER2/neu was negative in all of the cases, irrespective of grade. Association between grade of meningioma and expression of HER2/neu did not reach statistical significance (p value = 0.452, Fisher's exact test).

In the study by Silva et al., of all the cases included, 78.3% cases were Grade I (47 cases), 18.3% were Grade II (11 cases) and 3.3% cases were Grade III (2 cases). SSTR-2 was expressed in 100% of the cases. No statistically significant correlation was found between tumour grade and expression of SSTR-2 (25).

In the study by Boulagnon-Rombi et al., of the 127 cases of meningioma studied, 81 cases were Grade I, 38 were Grade II and 8 were Grade III. SSTR-2 was positive in 95% of all meningiomas. However, there was no significant correlation between tumour grade and expression of SSTR-2 (p value = 0.23, Chi-square test) (26).

In the study by Dijkstra et al., 148 cases of meningioma were studied, of which 124 cases were Grade I (83.8%), 22 cases were Grade II (14.9%) and 2 cases were Grade III (1.4%). SSTR-2 was variably expressed in all the cases with a high score in 81.8% cases. No statistically significant association was found between tumour grade and expression of SSTR-2 (p value = 0.647, Spearman correlation) (27).

In the study by Agaimy et al., of the 68 cases of meningioma, 58 cases were Grade I and 10 cases were Grade II. No Grade III cases were included in the study. Statistical correlation between tumour grade and expression of SSTR-2 was not studied (24).

In the study by Tollefsen et al., 67.3% cases were Grade I and 32.7% cases were Grade II. No Grade III cases were included. There was a significant correlation between tumour grade and expression of SSTR-2 by Mann-Whitney U test (p value = 0.009) (28).

In the study by Barresi et al., 35 cases of meningioma were included, of which 13 were Grade I, 19 were Grade II and 3 were Grade III. An association was noted between expression of SSTR-2A and high histologic grade, though it did not reach statistical significance (p value = 0.005). However, with exclusion of microcystic subtype cases, this correlation reached statistical significance (p value = 0.033) (23).

Mahzouni et al., in their study included 52 Grade I meningiomas (72.2%) and 20 Grade II/III meningiomas (27.8%). HER2/neu expression was seen in 55% of the Grade II/III meningiomas and 38.5% of the Grade I meningiomas. There was no statistically significant correlation between tumour grade and expression of HER2/neu (34).

In the study by Telugu et al., 80% of the cases were Grade I, 18% were Grade II and 2 % were Grade III. 75% of the Grade I cases were positive for HER2/neu while 65% of the Grade II/III cases showed HER2/neu positivity. However, this association was not statistically significant (35).

Author	Year	Study	Results
Barresi et al. (23)	2008	35 cases of meningioma were evaluated to study the expression of SSTR-2A and its correlation with histologic grade, Ki67 labelling index and MVD	• 74% of the cases showed SSTR-2A expression • An association was noted between expression of SSTR-2A with histologic grade and Ki67 labelling index, however, it did not reach statistical significance (p value = 0.05) • However, when the microcystic meningiomas were excluded, there was a significant correlation between positive SSTR-2A expression and high histologic grade and Ki67 labelling index (p value = 0.033) • There was a statistically significant correlation between SSTR-2A expression and MVD
Agaimy et al. (24)	2014	Immunohistochemical expression of EMA, Claudin-1, GLUT-1, SSTR-2 and PR was compared in 68 cases meningioma and 20 cases of soft tissue perineurioma	• SSTR-2 was expressed in 87% of all cases of meningioma and 5% perineuriomas while EMA was expressed in 97% of meningiomas and 100% of perineuriomas
Silva et al. (25)	2015	Expression of all 5 SSTR subtypes and their	• SSTR-2 expression present in 100% cases

		correlation with age, sex, histology, location and recurrence was studied in 60 cases of meningioma	• Statistically significant association between SSTR subtypes • No statistically significant association between SSTR expression and patient age, sex, tumour location, histologic subtype, tumour grade, and recurrence
Dijkstra et al. (27)	2018	Expression patterns of EMA, PDGF-β, VEGF-α and SSTR-2 were studied using IHC and tissue microarray in 148 cases of meningioma and the most promising biomarker was selected using TArget Selection Criteria (TASC)	• All markers were positive in all cases • SSTR-2 expression, however, had high score in 81.8% cases • No significant association was noted between SSTR-2 and WHO grade (p value = 0.647) • On applying TASC, SSTR-2 was found to be the most promising biomarker for intra-operative application
Tollefsen et al. (28)	2021	Immunohistochemical expression of SSTRs 1-5 was assessed manually as well as digitally in 162 cases of Grade I and II meningiomas and its correlation with histopathology, tumour grade and prognosis	• Strong correlation between digital scoring and manual staining index for all SSTRs • Significant correlation between tumour grade and expression of SSTR-1, 2, 5 (p values = 0.036, 0.009, 0.029 respectively) • SSTR-2 expression showed significant correlation with necrosis, macronucleoli and

			lack of psammoma bodies (p values = 0.010, 0.019, 0.017 respectively) • Significant correlation between SSTR-2 and SSTR-5 expression and histologic subtype (p values <0.001 and = 0.003 respectively) • Significant association of SSTR-2 with time to recurrence (p value = 0.027)
Fodi et al. (29)	2022	Prognostic role of SSTR expression and its impact on tumour recurrence was evaluated in 666 cases of meningioma	• Statistically significant correlation between SSTR-2A expression and tumour recurrence (p value = 0.0312) • A higher SSTR-1 expression and a lower SSTR-2A expression was related to a more favourable progression-free survival
Present study	2022	Immunohistochemical expression of SSTR2 and HER2/neu and its correlation with clinico-pathological parameters was studied in 120 cases of meningioma	• Out of the 120 cases, 91 cases (75.83%) showed positivity for SSTR-2 • There was a statistically significant association between expression of SSTR-2 and location of meningioma in brain • A statistically significant association was found between expression of SSTR-2 and histological

			subtypes and between expression of SSTR-2 and grade of meningioma • No significant association was found with age and gender

Table 33. Summary of the studies on SSTR-2 expression in meningiomas and their conclusions

Author	Year	Study	Results
Andersson et al. (31)	2004	26 meningiomas, 27 astrocytomas and 17 oligodendrogliomas were evaluated for expression of EGFR family members and their correlation with various parameters was studied	• 58% of the cases of meningioma showed immunohistochemical expression of EGFR, with high ErbB2 expression in the capillary endothelium • Pronounced EGFR and ErbB2 mRNA expression was found by quantitative real-time RT-PCR in majority of meningiomas • No significant correlation was found with various clinical parameters
Kandemir et al. (32)	2009	Expression if ER, PR and HER2/neu was evaluated in 53 cases of WHO Grade I meningiomas and its correlation with clinico-pathological parameters was studied	• 50.9% of the cases were positive for PR, 22.6% cases showed overexpression of HER2/neu while all cases were negative for ER • Significant positive correlation between HER2/neu and PR was observed (p value = 0.015) • HER2/neu overexpression was frequent in meningothelial meningioma (p value = 0.03) • No significant correlation of ER, PR and HER2/neu with any other clinic-

			pathological parameters was noted
Khamis et al. (33)	2011	Correlation between HER2/neu, PR and Ki67 expression with disease-free survival and other histopathological parameters was evaluated in 38 cases of meningioma	• 34.2% cases were positive for membranous staining of HER2/neu, with 57.1% expression in Grade II/III and 29% in Grade I • Statistically significant correlation was noted between Ki67 and HER2/neu expression and disease-free survival • No statistically significant correlation between HER2/neu expression and tumour subtype
Mahzouni et al. (34)	2012	Immunohistochemical expression of HER2 and its correlation with tumour grade was evaluated in 72 cases of meningioma	• 43% of the cases were positive for HER2 with 55% of Grade II/III and 38.5% of Grade I meningiomas • HER2 was positive in 39% of males and 44.9% of females • No statistically significant correlation between HER2 expression and tumour grade was noted
Telugu et al. (35)	2016	Immunohistochemical expression of HER2/neu was studied in 100 cases of meningioma and its correlation with gender,	• 73% cases were positive for HER2/neu, including 75% of Grade I meningiomas and 65% of Grade II/III meningiomas

		location, grade and histologic subtype was evaluated	• No statistically significant correlation was found between expression of HER2/neu and gender, location, grade and subtype of meningioma
Arnli et al. (5)	2018	Expression and amplification of intra- and extra-cellular domains of HER2 was evaluated and correlation with prognosis was studied in 186 cases of meningioma	• All cases showed immunoreactivity (predominantly cytoplasmic) when intracellular domain was targeted, while 48% positivity was seen with antibodies against extracellular domain and 11% positivity against activated receptor • No significant correlation with grade was noted • There was significant correlation between activated receptor and risk of recurrence or death (p value = 0.004) • None of the cases showed HER2 gene amplification on FISH
Dar et al. (36)	2020	The immunohistochemical expression of Ki67, p53 and HER2/neu and their correlation with tumour grade and histologic	• Ki67 and p53 expression was seen in all the cases with higher expression in higher grades • All cases were negative for HER2/neu

		subtype was analyzed in 17 cases of meningioma	
Ramezani et al. (37)	2020	Expression of HER2 and its correlation with age, gender and grade was studied in 117 cases of meningioma	• 65% cases showed positivity for HER2 with high expression in 7.7% cases • No significant correlation was seen between expression of HER2 and mean age, gender and grade (p values = 0.672, 0.574 and 0.093 respectively)
Present study	2022	Immunohistochemical expression of SSTR2 and HER2/neu and its correlation with clinico-pathological parameters was studied in 120 cases of meningioma	• All the 120 cases studied were negative for HER2/neu, with Score 0 in 113 cases and Score 1+ in only 7 cases • No statistically significant association was found between expression of HER2/neu and age, gender, location, histological subtypes and grade of meningioma

Table 34. Summary of the studies on HER2/neu expression in meningiomas and their conclusions

Limitations of the study

- The number of cases of Grade II and Grade III meningiomas were limited.
- Recurrent cases were excluded from the study. Therefore, the retention of expression of SSTR-2 and its role in the therapy for recurrent cases could not be evaluated.
- This study did not include follow-up of the patients and survival analysis.

Strengths of the study

- The sample size was substantial (120 cases) and included both retrospective and prospective cases.
- All the cases were worked-up extensively with clinical and radiological details as well as histo-morphological and immunohistochemical features.
- This study aimed to study the expression of SSTR-2 and HER2/neu, any of which, if expressed, have a potential role in target-based therapy of meningiomas.

Recommendations for further work

- Further studies including follow-up and survival analysis of the patients are needed to establish correlation with prognosis.
- Role of SSTR-2 analogues in intra-operative imaging confirmation and targeted therapy of meningiomas needs to studied.
- To establish the role of HER2/neu in meningiomas, more expansive studies are needed.

SUMMARY AND CONCLUSION

The current study was an ambispective, cross-sectional study conducted at the All India Institute of Medical Sciences, Jodhpur in which expression of SSTR-2 and HER2/neu was assessed on 120 cases of meningioma diagnosed at the Department of Pathology and Lab Medicine between January 2018 and July 2022.

- The mean age of patients was 50.07 years, with an age range of 9 years to 76 years.
- A female predilection was noted, with 81 cases being females and 39 cases being males. The female to male ratio was 2.08:1.
- Most of the cases included were located in the brain (108 cases) while the others originated from spine (12 cases).
- The most common histological diagnosis was meningothelial meningioma, followed by transitional meningioma and fibrous meningioma.
- Majority of the cases were Grade I (80.83%) while the rest belonged to Grade II (16.67%) and Grade III (2.50%).
- Out of the 120 cases, majority of the cases were positive for SSTR-2 (91/120; 75.83%) with 72 cases with Score 3 (60%) and 19 cases with Score 2 (15.83%). Remaining 29 cases were negative for SSTR-2 with 19 cases with Score 0 and 10 cases with Score 1.
- A statistically significant association was found between SSTR-2 expression and location of meningioma in brain (p value = 0.0009; Chi square test) with SSTR-2 expression more commonly seen in meningiomas arising in anterior cranial fossa and middle cranial fossa as compared to posterior cranial fossa.
- A statistically significant association was found between SSTR-2 expression and histologic subtype of meningioma (p value < 0.0001; Fisher's exact test). SSTR-2 expression was seen in majority of the cases of meningothelial meningioma and psammomatous meningioma and all of the cases of angiomatous meningioma, microcystic meningioma, metaplastic meningioma and clear cell meningioma. None of the cases of chordoid meningioma, anaplastic meningioma and rhabdoid meningioma showed SSTR-2 positivity.

- A statistically significant association was found between SSTR-2 expression and grade of meningioma (p value = 0.019; Fisher's exact test). SSTR-2 expression was more commonly seen in Grade I and Grade II meningiomas. All cases of Grade III meningiomas were negative for SSTR-2.
- No statistically significant association was found between SSTR-2 expression and age and gender of the patients.
- All the cases were negative for HER2/neu expression. Only 7 cases (5.83%) were given Score 1+, while 113 cases (94.17%) were given Score 0.

Conclusion:

Expression of SSTR-2 has been recommended by the 2021 WHO classification of CNS tumours as a desirable criteria for diagnosis of meningioma (10). In the present study, 75.83% cases were positive for SSTR-2. Most of the Grade I and Grade II meningiomas showed SSTR-2 expression. Also, SSTR-2 expression was most commonly seen in anterior and middle cranial fossa meningiomas. Since half of the cases of posterior fossa meningiomas and none of the cases of Grade III meningiomas, included in the study, showed SSTR-2 expression, we could not reaffirm the diagnostic role of SSTR-2 in these cases. Therefore, to establish a significant role of SSTR-2 in targeted therapy-based management of such cases, further studies on larger sample size are needed.

None of the cases in this study were positive for HER2/neu. Hence, our study does not support the use of HER2/neu as a diagnostic marker. However, further studies may be done on a larger sample size for confirmation of these results.

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ANNEXURES

ETHICAL JUSTIFICATION

- Informed written consent was taken from all study subjects. No pressure or coercion was exerted on subjects for participation in the study.
- Confidentiality and privacy was maintained at all stages.
- Enrolment in the study did not pose any additional risk to the patient and did not increase the cost of the treatment.

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2021/ 3517 **Date: 12/03/2021**

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2021/3352**

Project title: **"Expression of SSTR-2 and HER2/neu in meningiomas and its correlation with clinico-pathological parameters"**

Nature of Project:	**Research Project Submitted for Expedited Review**
Submitted as:	**M.D. Dissertation**
Student Name:	**Dr. Tanya Garg**
Guide:	**Dr. Sudeep Khera**
Co-Guide:	**Dr. Poonam Abhay Elhence, Dr. Jyotsna Naresh Bharti & Dr. Mayank Garg**

Institutional Ethics Committee after thorough consideration accorded its approval on above project.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

Please Note that this approval will be rectified whenever it is possible to hold a meeting in person of the Institutional Ethics Committee. It is possible that the PI may be asked to give more clarifications or the Institutional Ethics Committee may withhold the project. The Institutional Ethics Committee is adopting this procedure due to COVID-19 (Corona Virus) situation.

If the Institutional Ethics Committee does not get back to you, this means your project has been cleared by the IEC.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma
Member Secretary

Member secretary
Institutional Ethics Committee
AIIMS,Jodhpur

Basni Phase-2, Jodhpur, Rajasthan-342005; **Website:** www.aiimsjodhpur.edu.in; **Phone:** 0291-2740741 Extn. 3109
E-mail : ethicscommittee@aiimsjodhpur.edu.in; ethicscommitteeaiimsjdh@gmail.com

All India Institute of Medical Sciences (AIIMS)
Jodhpur, Rajasthan

Informed Consent form

Title of the project: **Expression of SSTR-2 and HER2/neu in meningiomas and its correlation with clinico-pathological parameters**

Name of the Principal Investigator: DR. TANYA GARG Tel. No. :8764162970

Patient/Volunteer Identification No.: _____

I, _____ S/o or D/o _____

R/o_____

give my full, free, voluntary consent to be a part of the study "**Expression of SSTR-2 and HER2/neu in meningiomas and its correlation with clinico-pathological parameters**", the procedure and nature of which has been explained to me in my own language to my full satisfaction. I confirm that I have had the opportunity to ask questions.

I understand that my participation is voluntary and am aware of my right to opt out of the study at any time without giving any reason. I understand that the information collected about me and any of my medical records may be looked at by Doctors from AIIMS, Jodhpur. I give permission for these individuals to have access to my records.

Date:_____ _____

Place: _____ Signature/Left thumb impression

This to certify that the above consent has been obtained in my presence.

Date:_____ _____

Place: _____ Signature of Principal Investigator

Witness 1 Witness 2

_____ _____

Signature/ Left thumb impression Signature/ Left thumb impression

Name: _____ Name: _____

Address: _____ Address: _____

अखिल भारतीय आयुर्विज्ञान संस्थान
जोधपुर, राजस्थान

सूचित सहमति प्रपत्र

थीसिस / निबंध का शीर्षक: मेनिनजियोमा में एसएसटीआर-2 और हर 2 न्यू की अभिव्यक्ति और उसके नैदानिक विकृति मापदंडों के साथ सहसंबंध

पीजी छात्र का नाम : **डॉ तान्या गर्ग** टेल. न.: 8764162970

रोगी / स्वयंसेवक पहचान संख्या : _____

मैं, _____ पुत्र / पुत्री _____

पता_____अध्ययन "**मेनिनजियोमा में एसएसटीआर-2 और हर 2 न्यू की अभिव्यक्ति और उसके नैदानिक विकृति मापदंडों के साथ सहसंबंध**" का एक भाग बनने के लिए पूर्ण, स्वतंत्र, स्वैच्छिक सहमति देता हूँ, जिसकी प्रक्रिया और प्रकृति मुझे अपनी पूरी संतुष्टि के लिए अपनी भाषा में समझाई गई है। मैं पुष्टि करता हूं कि मुझे प्रश्न पूछने का अवसर मिला है। मैं समझता हूं कि मेरी भागीदारी स्वैच्छिक है और मुझे किसी भी कारण दिए बिना किसी भी समय अध्ययन से बाहर निकलने के मेरे अधिकार की जानकारी है। मैं समझता हूं कि मेरे और मेरे मेडिकल रिकॉर्ड के बारे में एकत्रित की गई जानकारी को एम्स जोधपुर के डॉक्टरों द्वारा देखा जा सकता है। मैं इन व्यक्तियों को अपने अभिलेखों तक पहुंच के लिए अनुमति देता हूँ।

तारीख: _____

जगह: _____ हस्ताक्षर / बाएं अंगूठे का छापा _____

यह प्रमाणित करने के लिए कि मेरी उपस्थिति में उपरोक्त सहमति प्राप्त की गई है।

तारीख: _____

जगह: _____ पीजी छात्र के हस्ताक्षर _____

गवाह1: _____ हस्ताक्षर: _____ तारीख : _____	गवाह 2: : _____ हस्ताक्षर: _____ तारीख : _____

PATIENT INFORMATION SHEET

1. Risks to the patients: No interventions or life-threatening procedure will be done.
2. Confidentiality: Your participation will be kept confidential. Your medical records will be treated with confidentiality and will be revealed only to doctors/scientists involved in this study. The results of this study may be published in a scientific journal, but you will not be identified by name.
3. Provision of free treatment for research related injury: Not applicable.
4. Compensation of subjects for disability or death resulting from such injury: Not Applicable
5. You have complete freedom to participate and to withdraw from research at any time without penalty or loss of benefits to which you would otherwise be entitled.
6. Your participation in the study is optional and voluntary.
7. The copy of the results of the investigations performed will be provided to you for your record.
8. You can withdraw from the project at any time, and this will not affect your subsequent medical treatment or relationship with the treating physician.
9. Any additional expense for the project, other than your regular expenses, will not be charged from you.

रोगी सूचना पत्रक

1. रोगियों लिए जोखिम : कोई जीवन को जोखिम में डालने वाली प्रक्रिया नहीं की जाएगी।
2. गोपनीयता : आपकी भागीदारी को गोपनीय रखा जाएगा। आपके मेडिकल रिकॉर्ड को गोपनीयता के साथ रखा जाएगा और केवल इस अध्ययन में शामिल डॉक्टरों / वैज्ञानिकों को ही पता चलेगा। इस अध्ययन के परिणाम एक वैज्ञानिक पत्रिका में प्रकाशित हो सकते हैं, लेकिन आपको नाम से पहचाना नहीं जाएगा।
3. अनुसंधान संबंधी चोट के लिए नि:शुल्क उपचार की व्यवस्था : लागू नहीं।
4. ऐसी चोट से उत्पन्न विकलांगता या मृत्यु के लिए विषयों का मुआवजा : लागू नहीं।
5. आपको जुर्माना या लाभ के नुकसान के बिना किसी भी समय भाग लेने और अनुसंधान से वापस लेने की पूरी आजादी है।
6. अध्ययन में आपकी भागीदारी वैकल्पिक और स्वैच्छिक है।
7. प्रदर्शन की जांच के परिणामों की प्रति आपके रिकॉर्ड के लिए आपको उपलब्ध कराई जाएगी।
8. आप किसी भी समय परियोजना से वापस ले सकते हैं, और यह आपके बाद के चिकित्सा उपचार या उपचार चिकित्सक के साथ संबंधों को प्रभावित नहीं करेगा।
9. परियोजना के लिए कोई भी अतिरिक्त व्यय, आपके नियमित खर्चों के अलावा, आपसे नहीं लिया जाएगा।

All India Institute of Medical Sciences (AIIMS)

Jodhpur, Rajasthan

Department of Pathology

PROFORMA

Date:

Name:

Age: Sex: I.D:

Address:

Relevant clinical History:

Family history of malignancy:

Histological diagnosis:

Requested information for optimal patient care:

(1) Known/Previous malignancy:

(2) History of radiation exposure:

Immunohistochemistry:

SSTR-2 -

Positive □ Negative □

Positive cell percentage

0: Absence of any immunoreactivity ☐

1: Pure cytoplasmic immunoreactivity (0-100% cells) ☐

2: Membranous reactivity in <50% tumour cells ☐

3: Membranous reactivity in >50% tumour cells ☐

HER2/neu -

Positive □ Negative □

Positive cell percentage

0: ≤10% cells ☐

1: Incomplete faint membranous staining in >10% cells ☐

2: Weak to moderate complete membranous staining in >10% cells ☐

3: Intense circumferential membranous staining in >10% cells ☐