Fluorescence in situ hybridization (FISH) for chromosome 14q deletion in subsets of meningioma segregated by MIB-1 labelling index

A DISSERTATION SUBMITTED IN PART FULFILMENT OF THE REGULATION FOR THE AWARD OF THE DEGREE OF M.D. PATHOLOGY BRANCH III

THE TAMIL NADU DR.M.G.R.MEDICAL UNIVERSITY

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CERTIFICATE

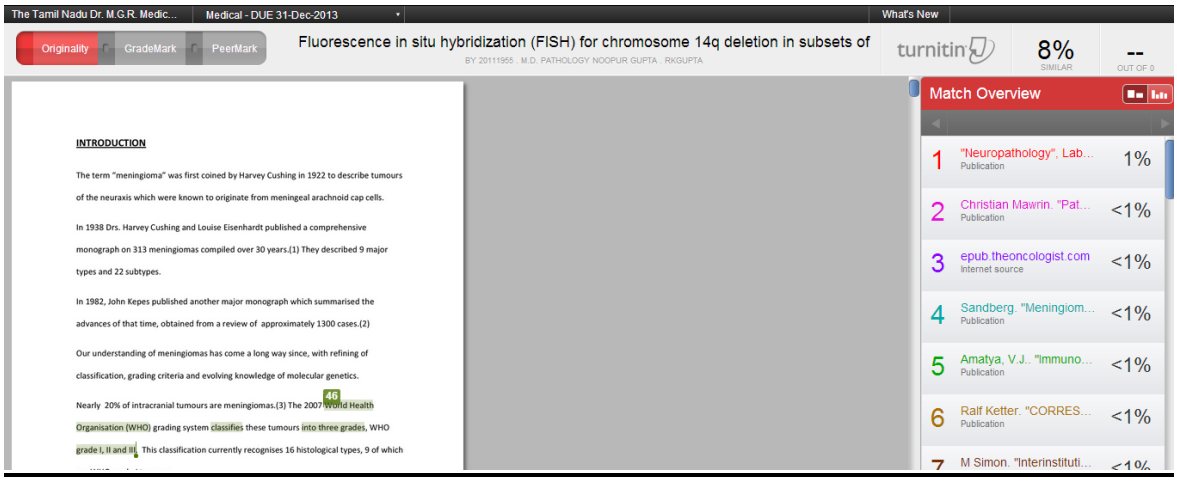
This is to certify that this dissertation titled **"Fluorescence in situ hybridization (FISH) for chromosome 14q deletion in subsets of meningioma segregated by MIB-1 labelling index"** is a bonafide work done by Dr. Noopur Gupta, in part fulfilment of rules and regulations for the M.D. Branch III (Pathology) Degree Examination of the Tamil Nadu Dr. M.G.R. Medical University, to be held in April 2014.

Dr. Banumathi Ramakrishna, MBBS, MD, MAMS
Professor & Head,
Department of Pathology,
Christian Medical College,
Vellore.

CERTIFICATE

This is to certify that the thesis titled **"Fluorescence in situ hybridization (FISH) for chromosome 14q deletion in subsets of meningioma segregated by MIB-1 labelling index"** submitted by Dr. Noopur Gupta, in part fulfilment of the requirement for the M.D. Branch III (Pathology) Degree Examination of the Tamil Nadu Dr. M.G.R. Medical University, Chennai, to be held in April 2014, is a bonafide work done by her under my guidance.

Dr. Geeta Chacko MBBS, MD., Ph. D
Professor of Pathology,
Department of Pathology,
Christian Medical College,
Vellore.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In 1938 Drs. Harvey Cushing and Louise Eisenhardt published a comprehensive monograph on 313 meningiomas compiled over 30 years.(1) They described 9 major types and 22 subtypes.
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Our understanding of meningiomas has come a long way since, with refining of classification, grading criteria and evolving knowledge of molecular genetics.
Nearly 20% of intracranial tumours are meningiomas.(3) The 2007 World Health Organisation (WHO) grading system classifies these tumours into three grades, WHO grade I, II and III. This classification currently recognises 16 histological types, 9 of which
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ABBREVIATIONS

ADTB1	-	Adaptor-related protein complex 1, beta 1 subunit
AgNOR	-	Agyrophilic nucleolar organizer regions
Akt	-	Alpha serine/threonine-protein kinase
ALPL	-	Alkaline phosphatase, liver/ bone/ kidney
APM-1	-	Adaptor-related protein complex 1, mu subunit
CAV	-	Cyclophosphamide, doxorubin, vincristine
CC1 solution	-	Cell Conditioning 1
CdK4/cyclin D	-	Cyclin dependent kinase - 4
CDKN2A	-	Cyclin-dependent kinase inhibitor 2A
CDKN2A/p16INKa	-	Cyclin-dependent kinase inhibitor 2A /p^{16} inhibitor kinase a
CDKN2B	-	Cyclin-dependent kinase inhibitor 2B
CDKN2B/p15ARF	-	Cyclin-dependent kinase inhibitor 2B/ p^{15} alternate reading frame
CDKN2C	-	Cyclin-dependent kinase inhibitor 2C
CEA	-	Carcinoembryonic antigen
CGH	-	Comparative genomic hybridization
CSF	-	Cerebrospinal fluid
CT	-	Computerized Tomography
DAL-1	-	Differentially expressed in adenocarcinoma of the lung
DAPI	-	4',6-diamidino-2-phenylindole
DCC	-	Deleted in colorectal caqrcinoma
DMBT1	-	Deleted in malignant brain tumours 1
DNA	-	Deoxyribonucleic acid
DPX	-	Dibutyl phthalate, m-xylene, p-xylene
EMA	-	Epithelial membrane antigen
EPB41	-	Erythrocyte membrane protein band 4.1
FISH	-	Fluorescence in situ hybridization
GADD45A	-	Growth arrest and DNA damage 45A
GFAP	-	Glial fibrillary acidic protein
hpf	-	high power fields

hTERT	-	Telomerase reverse transcriptase subunit
hTR	-	Telomerase RNA subunit
IGF	-	Insulin-like growth factor
IMRT	-	Intensity modulated radiotherapy
INK4C	-	Inhibitor kinase 4C
LOH	-	Loss of heterozygosity
MADH2	-	Mother Against DPP Homolog 2
MADH4	-	Mothers Against Decapentaplegic Homolog 4
MDM2	-	Mouse double minute 2
MEG3	-	Maternally expressed gene 3
MIB-1 LI	-	MIB-1 labelling index
MN 1	-	Meningioma 1
MRI	-	Magnetic resonance imaging
mRNA	-	messenger ribonucleic acid
MXI1	-	MAX interactor 1, dimerization protein
NDRG2	-	N-Myc Downstream-Regulated Gene 2
NF-2	-	Neurofibromin 2
N-Myc	-	v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)
P14ARF	-	p^{14} alternate reading frame
P15INK4b	-	p^{15} inhibitor kinase 4b
p16INK4a	-	p^{16} inhibitor kinase 4a
p18INK4C	-	p^{18} inhibitor kinase 4C
PAS	-	Periodic acid-Schiff
PCNA	-	Proliferating cell nuclear antigen
PDGFRB	-	Platelet derived growth factor receptor, beta polypeptide
PR	-	Progesterone receptor
pRB	-	Retinoblastoma-binding protein
PTEN	-	Phosphatase and tensin homolog
RFS	-	Recurrence free survival
RNA	-	Ribonucleic acid

RPS6K	-	Ribosomal protein S6 kinase
RPS6KB1	-	Ribosomal protein S6 kinase, 70kDa, polypeptide 1
RRP22	-	Ras-Related Protein on chromosome 22
RTOG	-	Radiation Therapy Oncology Group
SD	-	Standard deviation
SPSS	-	Statistical Package for Social Sciences
SRS	-	Stereotactic Radiosurgery
TGF-β	-	Transforming growth factor, beta
TIMP3	-	Tissue inhibitor of Metalloproteinase 3
TP53	-	Tumour protein p53
TP73	-	Tumour protein p73
WHO	-	World Health Organisation
WNT	-	Wingless-Type

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INTRODUCTION

The term “meningioma” was first coined by Harvey Cushing in 1922 to describe tumours of the neuraxis which were known to originate from meningeal arachnoid cap cells.

In 1938 Drs. Harvey Cushing and Louise Eisenhardt published a comprehensive monograph on 313 meningiomas compiled over 30 years.(1) They described 9 major types and 22 subtypes.

In 1982, John Kepes published another major monograph which summarised the advances of that time, obtained from a review of approximately 1300 cases.(2)

Our understanding of meningiomas has come a long way since, with refining of classification, grading criteria and evolving knowledge of molecular genetics.

Nearly 20% of intracranial tumours are meningiomas.(3) The 2007 World Health Organisation (WHO) grading system classifies these tumours into three grades, WHO grade I, II and III. This classification currently recognises 16 histological types, 9 of which are WHO grade I tumours.

However, the biological behaviour of meningiomas may not always correspond to their histological grading. It is well known that some meningiomas with WHO grade I histology can display more aggressive behaviour in the form of grossly invasive tumour growth, rapid growth of residual tumour or recurrence despite apparently total surgical resection. (3–7)

The proliferative capacity of tumour, measured by the MIB-1 labelling index has emerged as an important predictor of tumour aggressiveness.(8–14) MIB-1 labelling index, the full form of which is E3 Ubiquitin-Protein Ligase Mind-Bomb (MIB) is a reference monoclonal murine antibody which demonstrates Ki-67 antigen, which is a protein present in the nuclei of all multiplying human cells. It has been found in several studies that there is a statistically significant rise in the MIB-1/Ki-67 index from benign, through atypical, to anaplastic meningiomas.(9,12,14,15)

Though the extent of tumour resection and histological grade are two of the strongest predictors of biological behaviour, there is significant variability in their biological behaviour that cannot be accounted for by these parameters alone. This has resulted in a need to understand the underlying basis of initiation and progression of meningiomas to enhance the prognostic power. Researchers have therefore begun to look at genetic alterations within meningiomas to determine correlates to behaviour. Deletion of chromosome 22 is the most frequent cytogenetic alteration seen, which is an early event in tumour formation followed by deletions of 1p and 14q.(16,17) The latter are implicated in tumour progression.(18–29)

In this study we looked for chromosome 14q deletion detected by Fluorescence in situ hybridization, in subgroups of meningiomas segregated by MIB-1 labelling index in order to determine whether any significant correlation existed between these two parameters. We also studied if any correlation existed between specific histopathological features, the MIB-1 labelling index and chromosome 14q deletion.

AIM

To correlate histopathological grading of meningiomas segregated into subgroups based on MIB-1 (E3 Ubiquitin-Protein Ligase Mind-Bomb) labelling index with chromosomal loss of 14q using Fluorescence in situ hybridization (FISH).

REVIEW OF LITERATURE

OVERVIEW

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MENINGIOMAS

1. Definition

Meningiomas are tumours that originate from meningeal arachnoid cap cells.(7)

2. Incidence and Epidemiology

Meningiomas are the commonest primary brain tumours. They are also the most common spinal (intradural) tumours.(30,31) They account for 24-30% of all primary intracranial neoplasms in adults.(32) The reported incidence of meningiomas is 4.4 per 100,000 person years and they are most commonly diagnosed at an average age of 63 years.(7) Incidental asymptomatic meningiomas are found in about 2-3% of the population.(33) Women have more of a predilection for intracranial meningiomas, the female: male ratio being 2:1. This gender disparity together with an association of meningioma with breast cancer suggests that meningioma growth is hormone-dependent.(34) Progesterone receptors are expressed by almost 61% of meningiomas and are reported to have a role in their growth.(35,36) Progesterone receptor expression correlates with low grade histology, low recurrence rate and better prognosis.(37) However the differences of sex hormone expression between males and females cannot entirely explain the greater incidence in females.(34)

Intracranial meningiomas most often occur in the fifth decade of life.(38)

3. World Health Organisation Grading System

According to the WHO grading system meningiomas are classified into Grade I, II and III (Table 1).(4,5,39–41)

Table 1. Grading system for meningiomas (WHO 2007)(41)

WHO grade	Frequency	Pathologic features	Histology	Recurrence rates
I	80%-90%	Pleomorphic; occasional mitotic figures; criteria of anaplastic or atypical meningiomas are missing.	Meningothelial, fibrous, transitional, psammomatous, angiomatous, secretory, lymphoplasmacyte rich, microcystic, metaplastic	7%-20%
II	5%-15%	>4 mitotic figures/ 10 high-power fields; any three of the following: (a) hypercellularity, (b) small cells with high nuclear: cytoplasmic ratio, (c) prominence of nucleoli, (d) sheet-like pattern, (e) necrosis; or invasion of brain parenchyma	Clear cell, chordoid, atypical	30%-40%
III	1%-3%	>20 mitotic figures/ 10 high-power fields or frank anaplastic features	Papillary, rhabdoid, anaplastic	50%-80%

[Saraf S, McCarthy BJ, Villano JL. Update on meningiomas. The oncologist. 2011;16 (11):1604–13.]

Atypical meningiomas (WHO grade II) comprise between 4.7% and 7.2% of meningiomas in some older series,(42,43) however using the current definitions, it has been reported to account for up to 20%, whilst anaplastic meningiomas (WHO grade III) account for between 1.0% and 2.8% of all meningiomas.(4,5,42–44)

4. Etiology

- Idiopathic (most common).(45)
- Cranial irradiation for primary brain tumours, tinea capitis etc.(46–50)
- In association with Neurofibromatosis type 2.(51)
- Following exposure to harmful radiations such as dental X-rays, atomic explosions.(52)

Meningiomas that arise following radiation exposure more often show atypia, multifocality, high proliferation indices and usually occur at a younger age.(53–55)

The genetic disease most commonly associated with meningioma is Type 2 Neurofibromatosis which occurs due to an autosomal dominant chromosomal mutation on 22q12. (51) Type 2 Neurofibromatosis associated meningiomas differ from sporadic ones in that they occur in a younger age group, are usually multiple and are more commonly fibroblastic meningiomas. These tumours do not show an increased frequency of atypia or malignancy.(56–58)

5. Localisation and Clinical features

Meningiomas can occur in any location where arachnoidal cap cells are found. These include the arachnoid granulations for dural based meningiomas and in the stromal base of tela choroidea, in the case of rare intraventricular tumours.

Meningiomas of the intracranial meninges: Meningiomas occur most frequently over the cerebral convexities, with a predilection for the parasaggital region, in association with the falx and venous sinuses. The olfactory grooves, sphenoid ridges, suprasellar regions,

petrous ridges, tentorium and posterior fossa are other common sites. Meningiomas are slowly growing masses and produce symptoms by compression of adjacent structures i.e. focal neurological deficits, increased intracranial pressure and seizures.(59,60)

Meningiomas of the optic nerve sheath: These tumours can present with visual loss, strabismus and/or ptosis.(61)

Intraspinal meningiomas: Most spinal meningiomas occur in the thoracic region and are situated ventrally or laterally near the nerve root exit. Intradural, extramedullary meningiomas produce segmental neurological deficits.(62,63)

Other rare sites include intraventricular, epidural, intraosseous, ear and temporal bone, skin, lungs, sinonasal tract, mediastinum and peripheral nerves.(64–72) Metastasis of malignant meningioma is a rare complication and occurs in around 0.1% of meningiomas.(73) It most commonly involves the lung, pleura, bone and liver.(7)

6. Neuroimaging

Meningiomas are isodense and contrast enhancing dural masses on Magnetic resonance imaging (MRI). A characteristic sign, the “dural tail” is often seen surrounding the dural perimeter of the mass. This may or may not correspond to dural extension of the tumour and may indicate a rim of reactive fibrovascular tissue. Peritumoral cerebral oedema is prominent, especially around atypical and anaplastic meningiomas that are attached to the pia. Oedema appears to be more common in meningiomas with a higher MIB-1 index.(74) Some meningiomas, particularly the microcystic variant, are associated

with large intratumoral or peritumoral cysts.(74,75) CT scan demonstrates calcification better.

7. Gross Findings

Most meningiomas are soft to firm, rounded, smooth surfaced, well demarcated masses with broad based dural attachment. Some meningiomas are gritty, implying the presence of numerous psammoma bodies. Atypical and anaplastic meningiomas tend to be larger than benign ones.(43) The cut surface is white, yellow, or tan. Intraventricular meningiomas are often large, expand and distort the ventricular cavity, often with resultant hydrocephalus.

Most meningiomas push the leptomeninges before them with a margin that serves as a cleavage plane between the tumour and the adjacent brain. Adjacent brain is compressed but rarely shows frank parenchymal invasion. Meningiomas may grow onto or into major venous sinuses. Radical excision of these meningiomas is difficult.(76) Occasionally meningiomas may invade the dura to involve the skull inducing hyperostosis. At some sites, particularly the sphenoid wing, meningiomas may grow as a flat, carpet-like mass, a pattern termed ‘enplaque meningioma’.(77)

8. Histologic subtypes

Meningiomas display a broad range of histological patterns. They possess both epithelial and mesenchymal properties like their normal meningothelial counterparts. Epithelial features include epithelioid morphology, presence of desmosomes, epithelial membrane antigen positivity, formation of gland-like lumina (in secretory variant) and carcinoma-

like histology in anaplastic meningiomas. Mesenchymal features include spindled morphology (fibroblastic variant), ability to produce collagen, mesenchymal metaplasia (metaplastic meningiomas) and sarcoma-like histology in some anaplastic meningiomas. Whilst pure histological variants exist, meningiomas often show histological features of more than one variant. The most common histological subtypes are meningiothelial, fibroblastic and transitional. There are four subtypes which are considered to be more aggressive. Of these the chordoid and clear cell variants are assigned a WHO grade of II and the papillary and rhabdoid variants are WHO grade III tumours.

8.1 Histological subtypes of WHO grade I meningiomas:

8.1a. *Meningothelial meningioma*: The cells are arranged in lobules separated by thin septa which are fibro-collagenous in nature. Tumour cells are polygonal or epithelioid and contain round to oval, uniform nuclei with fine chromatin. Within the lobules the tumour cells appear to form a syncytium. Some cells display intra-nuclear pseudoinclusions which arise from invaginations of the cytoplasm into the nucleus. Whorls and psammoma bodies are uncommon.

8.1b. *Fibrous (fibroblastic) meningioma*: In this variant, cells with elongated nuclei are arranged in parallel, storiform or interlacing bundles set in a collagenous matrix. The cells are spindle in shape. Psammoma bodies and whorl formation are infrequent.

8.1c. *Transitional meningioma*: These, as the name implies have features of both fibrous and meningothelial meningiomas, with prominent lobules, psammoma bodies and

whorls. The centres of the lobules are often syncytial whereas the periphery has elongated fibroblast-like cells.

8.1d. *Psammomatous meningioma*: Meningiomas containing a predominance of psammoma bodies over that of tumour cells are called psammomatous meningiomas. Calcification and sometimes ossification may be so extensive that it may obscure the underlying meningothelial cells. Thoracic spinal region is the site of predilection for psammomatous meningiomas and they occur most frequently in middle aged women. Expression of bone-related proteins, like osteopontin may be responsible for psammoma body formation.(78)

8.1e. *Angiomatous meningioma*: This subtype has a predominance of blood vessels over that of tumour cells. The blood vessels are of varied calibre and often have markedly hyalinised walls.

8.1f. *Microcystic meningioma*: This variant is characterised by cells with thin, elongated processes enclosing microcystic spaces filled with mucinous fluid. Degenerative nuclear atypia is present frequently. These tumours have high vascularity and peritumoral oedema is common.(79)

8.1g. *Secretory meningioma*: These tumours are characterised by intracytoplasmic PAS (periodic acid-Schiff)-positive, diastase resistant, inclusions within gland like spaces. Designated pseudopsammoma bodies, these inclusions, show positive immunohistochemical staining for carcinoembryonic antigen (CEA).(80,81) Elevated CEA

levels have been reported in several cases.(81) Mast cells and peritumoral oedema are significant features of these tumours.(82,83)

8.1h. *Lymphoplasmacyte-rich meningioma*: This rare subtype features an extensive chronic inflammatory response that often overshadows the inconspicuous meningothelial component. Whether it represents a distinct pathological entity is controversial as its course often resembles an inflammatory process.(84) It may be multifocal. The fact that spontaneous regressions as well as multifocal recurrences are known in these meningiomas, raises the possibility that they may represent reactive meningothelial hyperplasia rather than a true neoplastic process.(7)

8.1i. *Metaplastic meningioma*: Metaplastic meningiomas are characterised by focal or widespread mesenchymal differentiation including osseous, lipomatous, cartilaginous, myxoid or xanthomatous. Metaplastic bone must be distinguished from the reactive bone over-run by meningiomas invading the skull.

8.2 Histological subtypes of WHO grade II meningiomas

8.2.a. *Chordoid meningioma*: Chordoid meningioma is an infrequent variant that consists of nests, cords and trabeculae of eosinophilic epithelioid cells, often with clear vacuoles resembling physaliferous cells set in a mucin rich stroma.(85,86) The chordoid histology is usually mingled with transitional or meningothelial pattern. Meningiomas with pure chordoid histology are uncommon. They are typically large supratentorial tumours. Chronic inflammatory infiltrate may be prominent. An association with Castleman's disease has been documented.(86)

8.2b. *Clear cell meningioma*: In this tumour, the cells are polygonal in shape, and have clear cytoplasm due to abundance of glycogen. They show extensive interstitial and perivascular collagen deposition. The cytoplasmic clearing is due to PAS-positive, diastase-digestible glycogen accumulation. The tumour shows a predilection for the cerebellopontine angle and cauda equina region. It tends to affect younger patients. These tumours have high recurrence rates and occasionally seed the CSF.(87,88)

8.2c. *Atypical meningioma*: Any meningioma with high mitotic activity defined as 4 or more mitoses per 10 consecutive high power(40x) fields(defined as 0.16mm^2) or three of the following histologic features: hypercellularity, small cells with high nuclear to cytoplasmic ratio, prominence of nucleoli, uninterrupted patternless or sheet-like growth and foci of 'spontaneous' or geographic necrosis are considered atypical.(4) The recurrence rates of atypical meningiomas are high even after apparently complete surgical excision.(89) Surgeons find that complete resection is more difficult with atypical meningiomas.(90)

8.2d. *Brain invasive meningiomas*: Invasion of the brain is present when the tumour, breaks through the pia to involve the underlying cortex. The relationship of the tumour to the brain can be clearly discerned with the aid of glial fibrillary acidic protein (GFAP) immunostaining, as tumour nests are completely surrounded by GFAP positive brain parenchyma. A pushing margin caused by the tumour with no breach of the pial plane does not qualify for a brain invasive meningioma. The recurrence and mortality rates of brain invasive, histologically benign meningiomas are comparable to that of atypical meningiomas. They are hence considered as WHO grade II tumours.(4)

8.3 Histological subtypes of WHO grade III meningiomas

8.3a. *Papillary meningioma*: Papillary meningiomas are characterised by papillae or pseudopapillae of loosely cohesive cells. Classical meningothelial histology may be seen in foci. The walls of blood vessels forming the vascular cores may be markedly thickened. These tumours tend to occur in young patients.(91) 75% of cases show brain invasion, 55% recur, 20% metastasize (most commonly to lung) and death occurs in roughly 50%.(92,93)

8.3b. *Rhabdoid meningioma*: These rare tumours contain sheets of rhabdoid cells, i.e. plump cells with eccentrically placed nuclei and conspicuous nucleoli. Their cytoplasm frequently contains a brightly eosinophilic inclusion which is paranuclear in location. Ultrastructurally these inclusions are aggregates of intermediate filaments. Malignant features like cytological atypia and high mitotic counts are often present. Cells with rhabdoid histology, even though absent in primary tumour may be first seen with tumour recurrence and may increase in number with time.(94,95) The behaviour of meningiomas with a focal rhabdoid morphology is yet to be determined and such tumours are therefore not classified as WHO grade III neoplasms. In such tumours, WHO recommends the addition of a cautionary note mentioning the percentage of rhabdoid differentiation.

8.3c. *Anaplastic(malignant) meningioma*: These tumours show malignant features defined as either ≥ 20 mitoses per 10 high power fields(0.16mm^2) or frankly anaplastic histology, defined as malignant cytology resembling that of carcinoma, melanoma or sarcoma.(5)

9. Immunohistochemistry

The most diagnostically useful marker of meningioma is a membranous pattern of immunoreactivity for epithelial membrane antigen (EMA).(96–99) Positivity is more marked in meningothelial and transitional meningiomas and subdued in fibrous, clear cell and papillary lesions.

Diffuse immunoreactivity for vimentin, is typical of all forms of meningiomas.

Over 50 percent of meningiomas are reactive for S-100 protein, but the reaction is patchy and less intense than it is in schwannomas.(96)

A cytokeratin reaction is typically present in secretory meningiomas, with staining limited to cells with pseudopsammoma bodies.(98) The pseudopsammoma bodies are positive for CEA.(99,100) Immunoreactivity for progesterone receptor is common in well-differentiated Grade I lesions, but is less prominent as the grade increases. (101)

10. Molecular genetic alterations in meningiomas

This tumour was one of the first solid tumours recognised to have cytogenetic alterations. Chromosomal deletion in meningiomas was first recognised in 1967 and in 1972 it was localised to chromosome 22q.(102,103)

10.1 Meningioma Initiation

10.1a. *NF2 gene*

Somatic mutations involving the NF2 gene, situated at 22q12.2 are present in almost 60% meningiomas that arise sporadically.(104–106) 50-70% of meningiomas display loss of heterozygosity at chromosome 22, which is associated with reduced expression of merlin or schwannomin, the NF2 gene product.(107)

Merlin, a protein of the 4.1 family, plays a role in cytoskeletal functions, regulation of cell growth and motility. Merlin is considered as a negative regulator of tumour growth as evidenced by the significant inhibition of in-vitro proliferation of human meningioma cells (both with and without NF2 gene mutations) that had overexpression of merlin.(108)

Most mutations are small insertions, deletions, nonsense mutations or frameshifts involving the 5' two thirds region of the NF2 gene.(56,109–112) These mutations result in a truncated merlin protein that is non-functional. Aberrantly methylated promoter regions may also lead to inactivation of the NF2 gene in meningiomas.(113) A few meningiomas do not harbour NF2 gene mutation or LOH 22q even though they have reduced levels of merlin protein. This can be explained by increased proteolysis of merlin mediated by calpain.(114)

It has been postulated that LOH 22q occurs early in meningioma development, and is not involved in their progression to higher grades.(115) The fact that NF-2 mutations

occur with almost equal frequency among different tumour grades suggests that they are also associated with meningioma initiation rather than progression.(106)

The frequency of NF2 mutations varies among meningioma subtypes. NF-2 mutations are present in 70-80% of fibroblastic and transitional variants but only in 25% of meningothelial variants.(112) In accordance with the frequency of NF-2 mutations, merlin expression is correspondingly decreased in majority of fibrous and transitional meningiomas, but seldom in meningothelial tumours.(116,117) The low frequency of NF2 alterations in the meningothelial variant suggests that these alterations may be insignificant in the development of this variant.(115) Secretory and microcystic meningiomas also only rarely harbour NF2 mutations, suggesting that their genetic origin is independent of NF2 mutations.(106,112,118) LOH 22q is strongly associated with fibrous histology. NF2 gene mutations are seen in up to 70% of atypical and anaplastic tumours.

10.1b. *Other genes on chromosome 22*

In meningiomas LOH 22 occurs more frequently than NF2 gene mutations suggesting that other tumour suppressor genes may lie outside the NF2 region on 22q.(115) Deletion mapping on 22q revealed interstitial deletions at loci other than NF2.(119) Lomas et al. described a case of multiple meningiomas with monosomy of chromosome 22 but lacking NF2 mutation.(120)

The candidate genes include ADTB1 (b-adaptin, BAM22), RRP22 and GAR22 which map to 22q12.2 region in close proximity to the NF2 gene. Studies suggest that epigenetic

alterations underlie ADTB1 gene inactivation.(121) Another gene, MN1 was found to be disrupted by a translocation in a meningioma, however further studies demonstrated that it acts as an oncogenic transcription coactivator and not as a tumour suppressor gene.(122,115) The LARGE gene which maps to 22q12.3 is another candidate gene.(123)

The tissue inhibitor of metalloproteinase 3 (TIMP3) gene, on 22q12 has also been associated with progression of meningiomas, as suggested by the results of a study by Barski et al.. In their study 67% of anaplastic meningiomas showed hypermethylation of the TIMP3 promoter, while only 17% of benign and 22% of atypical meningiomas had this alteration.(124)

10.1c. *DAL-1 Gene and Other Alterations on Chromosome 18*

The DAL-1 protein shares significant homology with merlin and also belongs to the 4.1 protein family. It functions as a tumour suppressor and maps to chromosomal region 18p11.3. 76% of meningiomas arising sporadically had absent expression of DAL-1 by immunohistochemical studies. This frequency parallels that of absent merlin expression.(125,126)

The frequency of loss of DAL-1 protein is only slightly higher in anaplastic meningiomas (87%) when compared to benign and atypical meningiomas (70%–76%). This difference is statistically insignificant and suggests that even DAL-1 protein loss occurs early in meningioma development.(126,127)

TSLC1 is another protein that interacts with protein 4.1B. Reduced expression levels of TSLC1 are correlated with high grade meningiomas and worse prognosis, while expression of TSLC1 in meningioma cells slows growth.(7)

10.2 Meningioma Progression

Karyotypes of meningiomas with aggressive behaviour are more complex.(128) Malignant progression, thought to follow the theory of clonal evolution, is associated with progressive accumulation of mutations, resulting in more aggressive subclones which have a higher proliferative potential. (16,22,129)

The chromosomal alterations in atypical and anaplastic meningiomas commonly include 1p, 10q and 14q deletions. Deletions of 6q and 18q are seen less often.

Gains on chromosomes 1q, 9q, 12q, 15q, 17q, and 20q are also seen in high grade tumours.(7,128)

In addition to these genetic changes, anaplastic meningiomas exhibit more frequent losses on 6q, 10q, 14q, and 9p, with amplification on 17q23 (Fig. 1).(22,130–133) Epigenetic alterations, including excessive hypermethylation of CpG islands, are also known to occur in progression to malignant meningiomas.(128)

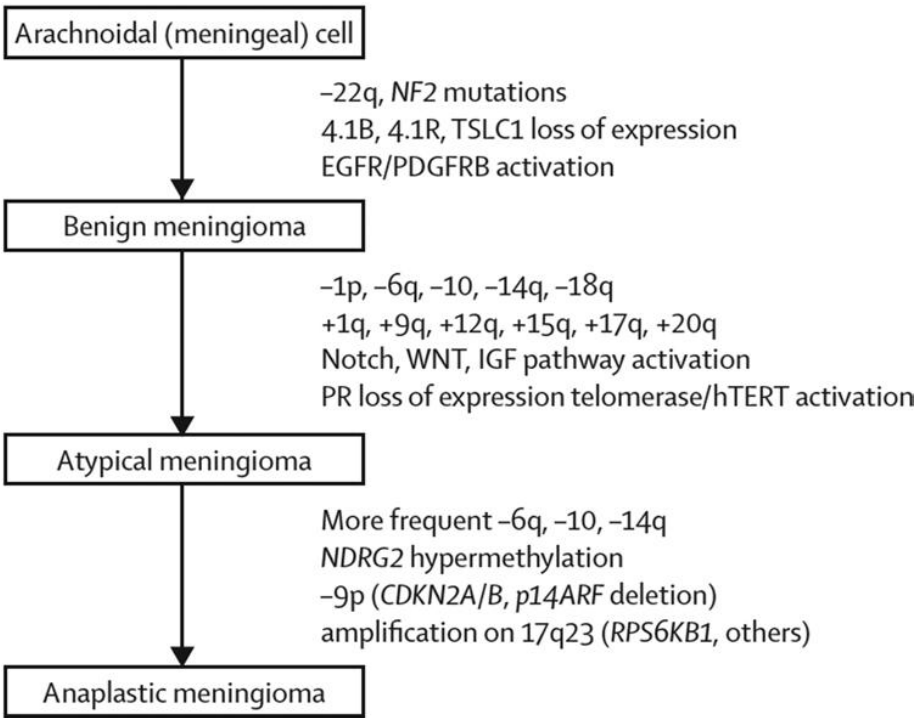


Fig.1. The genetic alterations underlying meningioma formation and progression.(133)

Most of the data from studies aiming to characterize the stepwise progression of meningioma development have been based on cytogenetic analyses of different meningioma grades in various patients. In a cytogenetic analysis of one group of 11 meningioma patients with tumours that exhibited clear progression from benign to higher grades, the authors found a complex karyotype present in the lower grade tumours prior to progression.(134) Contrary to the model of clonal evolution, these findings suggest that this cohort of meningiomas was destined to be malignant.

Candidate Genes Identified Through Chromosomal Losses

10.2a. *Chromosome 1*

The next most frequent chromosomal alteration in meningiomas after 22q deletion is 1p deletion. Their frequency increases with the grade of tumour (13%–26% in Grade I, 40%–76% in Grade II, and 70%–100% in Grade III tumours).(115,135)

The deletion of short arm of chromosome 1 is associated with malignant progression, especially in recurrent meningiomas. The loss of short arm of chromosome 1 is associated with recurrence rate of 30%, in comparison to 4.3% in cases where it is intact. (136)

While a number of candidate targets have been studied on 1p, including *CDKN2C*, *RAD54 L*, *EPB41*, of *CDKN2C*, a cell cycle control gene encoding p18INK4C located at 1p32, found one point mutation and one homozygous deletion at the *INK4C* locus. A study of 29 meningiomas found no mutations in *RAD54 L*, located on 1p32.(115,137)

Loss of heterozygosity and expression analysis failed to find expression losses of *EPB41* and *GADD45A*, located on 1p36.2-p34 and 1p31.2-p31.1 respectively.(128)

ALPL, a gene encoding an alkaline phosphatase, is located on 1p36.1-p34. (115,128,133) *ALPL* has drawn interest as a potential tumour suppressor because 1p loss in meningiomas is strongly associated with loss of alkaline phosphatase activity. However, mutational analysis of *ALPL* is still needed.(115)

Liu et al.(128) found that while many of the candidate genes on 1p lack regular genetic losses, epigenetic changes may have an important role in malignant meningioma progression. Their study found transcriptional silencing via abnormal hypermethylation of various promoter-associated CpG islands of cancer-related genetic regions in atypical and anaplastic meningiomas. For example, *TP73* on 1p26.32 has been examined as a candidate gene. While studies have failed to find significant and consistent *TP73* mutations in meningiomas, one methylation status study found *TP73* methylation-mediated inactivation in 10 of 30 meningiomas with 1p losses, and 3 of 30 meningiomas with intact 1p.(115,138,139) This suggests that assessment of the methylation status of other candidate genes may be a promising avenue of future study.

10.2b. *Chromosome 14*

Similar to 1p losses, deletions on chromosome 14 are important in meningioma progression.(115) These chromosomal abnormalities (1p and 14q) are frequent in anaplastic meningiomas, and are related to poor prognosis. (17)

14q deletions are the next most common chromosomal aberrations seen in meningiomas following losses in chromosome 22 and 1, and have been found in up to 31% of Grade I, 40%–70% of Grade II, and up to 100% of Grade III meningiomas. (18,22,24,25,115,141,142) Studies have also found losses of 14p to be a prognostic indicator of tumour recurrence.(115,141,143)

Genomic analysis conducted by Lusis and Gutmann identified *NDRG2* as a potential tumour suppressor on 14q.(17) The authors found that *NDRG2* is frequently inactivated

in both anaplastic meningiomas and a subset of lower grade yet clinically aggressive atypical meningiomas.

Reduction of *NDRG2* expression was associated with promoter hypermethylation in 40% of atypical and anaplastic meningiomas.(128) Additionally, *NDRG2* mRNA is down-regulated in recurrent meningiomas of all grades relative to primary benign meningiomas.(144) Although the mechanism is unknown, NDRG2 is involved with regulating cell growth, differentiation, and apoptosis.(17,145–148)

Recently, Zhang et al.(149) identified *maternally expressed gene 3 (MEG3)* as a candidate tumour suppressor located at 14q32. Greater loss of *MEG3* expression and allelic loss are associated with higher tumour grades. While MEG3, a noncoding RNA with antiproliferative functions, is robustly expressed in normal arachnoidal cells, it is absent in the IOMM-Lee and CH157-MN meningioma cell lines.

Functional studies suggest that MEG3 mediates its tumour suppressive properties by suppressing DNA synthesis and inhibiting colony formation in the meningioma cell lines. Additionally, MEG3 was found to transactivate p53 (TP53), another tumour suppressor involved in an often dysregulated pathway in anaplastic meningiomas.(7)

Of note, while mutations of *TP53* (17q) are common in many other cancers, direct alterations in *TP53* are rare in meningiomas;(7,50,115,150–152) instead, regulators of the pathway are often mutated.(89,149)

In a study aimed at identifying markers of aggressive behaviour in the Indian population, a loss of heterozygosity (LOH) analysis was carried out at our centre, for tumour

suppressor genes located on 1,10,14,17 and 22. We found that D17S1289 was the most informative locus. LOH was seen most often at D22S417 with mostly equal frequency in WHO grade I and II tumours, suggesting that it is involved in tumour initiation. The majority of high grade meningiomas showed LOH 14/Allelic imbalance, implying that it may be a progression event, however although the LOH 14 did not reach statistical significance, the allelic imbalance for D14S555 was significant, suggesting the presence of a smaller clone carrying a chromosome 14 deletion. The chromosomal deletions in the tumour are therefore more likely to be detected by either laser microdissection of tumour rich foci or by methods such as FISH where the section examined would be representative of the tumour. This would avoid the use of DNA which may have a mixture of normal and tumour tissue causing the detection of only an allelic imbalances and not a LOH.(153)

10.2c. *Chromosome 9*

The frequency of 9p loss is reported to be 5%, 18% and 38% in Grade I, II and III tumours respectively. (50,115) 9p loss is strongly associated with anaplastic, rather than benign or atypical meningiomas.(22,115)

While the actual target genes and tumorigenic mechanisms of many chromosomal losses in meningiomas are still unclear, 9p alterations are associated with specific losses of *CDKN2A/p16INKα* (encoding p16), *p14ARF* (encoding p14), and *CDKN2B/p15ARF* (encoding p15).(7,39) All 3 tumour suppressors are located on 9p21.

p14 is a tumour suppressor involved with regulating cell apoptosis through modulation of the p53 pathway, and p16 and p15 control progression from G1 to S phase of the cell cycle (Fig. 2)(128)

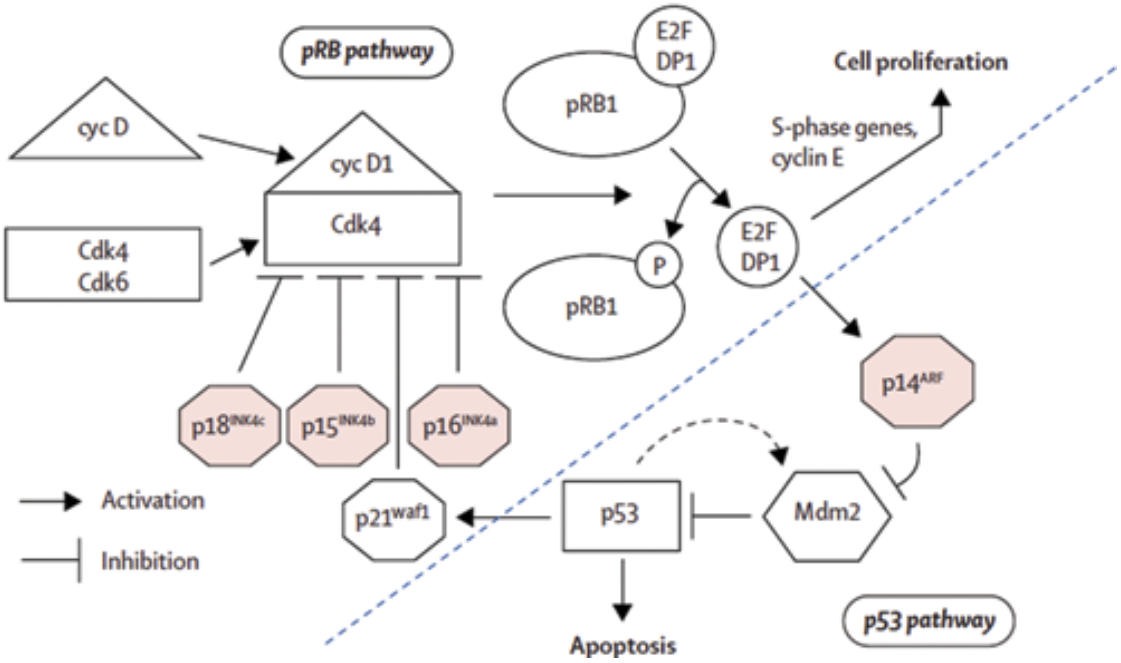


Fig.2. Cell cycle dysregulation in anaplastic meningiomas through interrelated p53/pRB pathways which includes aberrations in *p16INK4a*, *p15INK4b*, and *p14ARF*. *p16INK4a* and *p15INK4b* prevent S-phase entry by inhibiting the Cdk4/cyclin D complex. p14ARF negatively regulates MDM2 and removes MDM2-mediated p53 inhibition and degradation. The shaded proteins are affected in meningioma progression.(133)

Loss of *CDKN2A*, *p14ARF*, and *CDKN2B* has been reported in only 3% of Grade II and 38% of Grade III meningiomas. Grade I meningiomas do not show loss of *CDKN2A*, *p14ARF*, and *CDKN2B.* (115,150) Markedly shorter survival is noted in 70% of anaplastic meningiomas with 9p21 losses. (133,150,154)

Similarly, WHO grade III meningiomas with intact *CDKN2A* have better outcomes than those with *CDKN2A* loss.(115) These findings suggest that dysregulation at G1/S restriction point is associated with clinically aggressive tumours and is a critical component of malignant progression.(128)

10.2d. *Other Chromosomal Alterations*

Deletions on chromosome 10 are associated with meningioma progression.(115) Losses on chromosome 10 are found in 5%–12% of Grade I, 29%–40% of Grade II, and 40%–58% of Grade III tumours;(22,115,142,155,156) however, some studies have suggested that the true frequencies are higher.(115,157,158)

A number of candidate genes have been identified at chromosomal region 10q23-q25, namely *PTEN*, *MXI1*, and *DMBT1*. *PTEN* alterations have been found in Cowden syndrome, but rarely in meningiomas. Studies have also failed to identify mutations of *MXI1* or *DMBT1* in meningiomas.(128)

Similarly, the high frequency of chromosome 17 amplification in malignant meningiomas (42%) compared with lower grade meningiomas (almost 0%) has led to studies of ribosomal protein S6 kinase (*RPS6K*), a proto-oncogene located at 17q23.(22,115) However, *RPS6K* amplifications only occur in a small subset of higher grade meningiomas, despite robust amplification of adjacent loci.(115,130) While *RPS6K* amplification may be important in the progression of a subset of lesions, *RPS6K* does not appear to be the main target of amplification in meningiomas.

Losses in chromosome 18 are hardly seen in Grade I tumours but are frequent in atypical and anaplastic meningiomas. Büschges et al.(159) examined *MADH4*, *MADH2, DCC and APM-1*, which are tumour suppressor genes on chromosome 18q21. However, mutational and LOH analysis of the four genes found only one missense mutation in *APM-1*, suggesting that *MADH4*, *MADH2*, *DCC* and *APM-1* are not the target inactivated genes in 18q losses in meningioma progression.

10.2e. Telomerase/hTERT

Telomeres comprise repeat DNA sequences at the ends of chromosomes and function to prevent chromosomal deterioration. Telomeres shorten during successive DNA replication and mitoses, eventually limiting cell division through signalling senescence. Telomerase, a reverse transcriptase that rebuilds the lost telomere repeat sequences, is often reactivated in malignant cancers to sustain chromosomal integrity during aggressive growth.

Telomerase is made of the telomerase RNA subunit (hTR) and the reverse transcriptase subunit, hTERT. Expression of *hTERT* mRNA, rather than *hTR* in meningiomas is best correlated with telomerase activity.(128)

Telomerase activation is rare in benign meningiomas, found in only 3%–21% of Grade I meningiomas. However, 58%–92% of atypical and 100% of anaplastic meningiomas demonstrate telomerase activity.(115,160–162) In addition to higher grade tumours, telomerase activity is also seen in recurrent and malignant meningiomas, and may act as future prognostic tool.(115,162)

11. Biological behaviour of meningiomas

The clinical outcomes of meningioma cases are extremely variable.(89) Benign meningiomas have higher chance of being cured by surgery alone as compared to higher grade meningiomas. (89)

Most WHO grade I meningiomas grow slowly, while chordoid, clear cell, rhabdoid and papillary variants, brain invasive (Grade II), atypical (Grade II), and anaplastic (Grade III) meningiomas have an aggressive course. (7) However a few Grade I meningiomas have been known to recur even after total surgical resection. Also some Grade I meningiomas may occur at surgically inaccessible anatomical locations.(163)

Even benign meningiomas may show invasive properties of infiltrating bone as well as aggressive behaviour like growth of the unresected part of tumour or recurrence after complete surgical resection.(3–5,133)

WHO grade I meningiomas have slow growth rates with a recurrence rate of 5%, while atypical and anaplastic meningiomas have 40% and 80% recurrence rates after 5 year of surgical resection respectively.(133) In a study by Staffoed et al., 76% of atypical and none of the patients with anaplastic meningiomas had survived after multimodality treatment.(164) The median survival time for anaplastic meningiomas is less than 2 years.(5)

12. The role of proliferation index (MIB-1) in predicting biological behaviour of meningiomas

Several studies have emphasised the value of mitotic activity as a prognostic marker in meningiomas.(7,43,165–167)

Proliferative capacity of meningiomas has been estimated by various methods like numbers of argyrophilic nucleolar organizer regions (AgNOR), the percent of cells in S phase, (168–172) bromodeoxyuridine labelling index, (173–177) and Ki-67 (frozen tissue) or MIB-1 index (paraffin sections). (175,176,178–183)

MIB-1 is an antibody, the full form of which is E3 Ubiquitin-Protein Ligase Mind-Bomb (MIB). The MIB-1 antibody is established as the reference murine antibody which demonstrates Ki-67 antigen, a nuclear protein in human dividing cells. Ki-67 is present during synthetic and replicative phases of the cell cycle (G_1, S, G_2, and mitosis), but not during interphase (G_0).

MIB-1 labelling index rises significantly from benign (average, 3.8), through atypical (average, 7.2), to anaplastic meningiomas (average, 14.7).(9)

Some studies have suggested that meningiomas with indices >4% have increased risk of recurrence similar to atypical meningioma, whereas those with indices >20% are associated with death rates analogous to those associated with anaplastic meningioma.(184)

In a study by Perry et al., a MIB-1 LI of ≥4.2% was highly correlated with lower recurrence free survival.(184)

Matsuno et al.,(10) in a retrospective study of meningiomas from 127 patients, analysed the correlation of proliferative potential of tumour using the anti-Ki-67 monoclonal antibody i.e. MIB-1 LI, histomorphological features, and clinical course. The average MIB-1 labelling index of 50 male cases and 77 female cases was 5.5% and 2.7% respectively. Younger patients had higher MIB-1 labelling indices. These gender and age-related differences in the MIB-1 labelling index were statistically significant. MIB-1 LI of tumours categorised into 3 groups were calculated, namely: meningiomas which did not recur during the follow up period (n = 73), meningiomas that recurred but in which specimen of initial tumour was used (n = 21) and meningiomas which recurred and the specimen of recurrent tumour was used (n = 33). The average MIB-1 labelling index of tumours in these categories was 1.6%, 3.6%, and 8.8%, respectively and this difference was significant on statistical analysis. They found that the tendency for recurrence was significantly higher in meningiomas with a MIB-1 labelling index ≥ 3%, particularly within the first 10-years of follow-up.

In another study by Pfisterer et al. the number of chromosomal abnormalities had positive linear correlation with MIB-1.(11)

Significant differences were found in the MIB-1 labelling indices between grades of meningiomas by analysis of variance in a study by Hsu et al.(12) The reported values of MIB-1 LI were 0.75 ± 0.21, 3.2 ± 0.57 and 6.04 ± 1.48 for benign, atypical and malignant meningiomas respectively; $P \leq 0.0066$, in their study. MIB-1 labelling index also

correlated with mitotic and PCNA indices (P ≤ 0.0001). MIB-1 labelling index of tumour in male patients was higher than that of females (P ≤ 0.0128). A MIB-1 LI > 3% was a factor predicting worse outcome in meningiomas.

Lanzafame et al.,(13) divided meningiomas into three groups namely those with MIB-1 LI of less than 1%, 1-10% and more than 10%. There were 36, 28 and 5 cases in these groups respectively. Thirty three Grade I (61%) and three Grade II (30%) meningiomas had a MIB-1 labelling index (LI) less than 1%. In contrast, seven Grade II (70%) and all Grade III meningiomas had a MIB-1 LI more than 1%. There was significant correlation between WHO histopathological grade and MIB-1 LI (p value 0.0006). 32 of 42 (76%) of the meningiomas that did not recur on follow-up had a MIB-1 LI <1%. The MIB-1 LI of meningiomas which recurred after surgery was higher than the ones which did not recur. This difference between MIB-1 LI was highly significant (p < 0.001). Moreover benign meningiomas that recurred had higher MIB-1 LI than those which did not recur (p value 0.0006). Hence MIB-1 LI appeared to be of prognostic significance, independent of histology. The authors concluded that MIB-1 labelling index is helpful for diagnostic evaluation, prognostification and therapeutic planning of meningiomas.

Amatya et al.,(14) analysed the expression of MIB-1, p53, p21WAF1 and p27KIP1 antigens in different grades of meningiomas comprising 146 samples. There were 109, 27 and 10 benign, atypical and anaplastic meningiomas respectively. The MIB-1 LI of benign meningiomas was low (mean 1.5%) and very few of them expressed p53 antigen. In comparison, anaplastic meningiomas had higher MIB-1 LI (average, 19.5%) and all expressed p53 antigen. The MIB-1 and p53 LI of atypical meningiomas ranged in between those of benign and anaplastic meningiomas. The expression of these two

proteins was statistically significantly different between the different groups of meningiomas (P <.001). Hence the concluded that immunohistochemistry for these two proteins is of value in differentiating atypical meningiomas from benign/anaplastic counterparts, especially in cases where histological grading is ambiguous.

In a study by Ide et al.,(74) there was significant association between MIB-1 LI and degree of brain oedema($p < 0.0001$).

Carvalho et al.,(15) carried out gene expression profiling of 23 meningiomas using oligonucleotide microarrays. They found that there was a difference in the expression of 28 genes between WHO grade I and WHO grade II meningiomas and 1212 genes between WHO grade I and WHO grade III meningiomas. The genetic profiles of WHO grade II and WHO grade III meningiomas did not differ significantly. The gene expression profiles of meningiomas were further categorised into two major groups, 'low proliferative' and 'high proliferative'. Two molecular mechanisms were used to differentiate these two groups, namely, gain of proliferation markers and loss of transforming growth factor beta (TGF-β) signalling.

All the 8 WHO grade I tumours fell into the 'low proliferative' category and all the 8 WHO grade III tumours fell into the 'high proliferative' category. The 7 WHO grade II tumours however were distributed between both groups. Hence, although atypical meningiomas were a distinct group using the histopathological criteria, their molecular profile was not distinct. It ranged from a spectrum of 'low proliferative' to 'high proliferative' reiterating the fact that genetic alterations are a continuum in tumour progression. In accordance with other studies, the MIB-1 LI increased with histological

grade. The WHO grades I, II and III had MIB-1 LI of 1.4, 7.0 and 14.1 respectively. The MIB-1 labelling index differed in grade II meningiomas belonging to low proliferative and high proliferative groups (higher for high proliferative group as compared to that of low proliferative group), however, the sample size was too small to attain statistical significance. The clinical course of atypical meningiomas was also found to be diverse, with few of them demonstrating growth rates similar to Grade I meningiomas and others showing patterns similar to malignant meningiomas. The authors concluded that the aforementioned molecular profiles may differentiate the slow growing atypical meningiomas from those with aggressive behaviour.

In our own centre a study was done to determine the optimal cut-off of MIB-1 LI in predicting histological atypia in a meningioma.(185) It reiterated the fact that atypical meningiomas have a higher MIB-1 LI than benign meningiomas, and the difference was statistically significant. MIB-1 LI of 7% had the highest diagnostic validity for atypia (sensitivity = 0.86, specificity = 0.93). MIB-1 LI more than 7% was significantly associated with some of the WHO histologic criteria for atypia, namely sheet like growth, hypercellularity and small cells with a high nuclear: cytoplasmic ratio and mitoses more than 4 per 10 high power fields.

However proliferation indices are not yet incorporated in the WHO grading system due to the high inter-laboratory and inter-observer variations, and the fact that reliable cut-offs for the three grades cannot be defined. Moreover studies from our centre have shown that there are cases that have low proliferative potential and yet behave aggressively. (Unpublished data) Also seen are WHO Grade I meningiomas with a high MIB-1 labelling index.(185)

13. Fluorescence in situ hybridization (FISH)

Fluorescence in situ hybridization (FISH) is a potent, morphology based tool which can identify and quantify copy numbers, alterations and rearrangements in targeted genes. It can be used in fresh frozen tissue as well as in paraffin embedded tissues.

In FISH, fluorescent labelled probes hybridize a particular genomic sequence, thus providing numerical as well as cell localizing information. Using locus specific probes, two signals are expected per nucleus and hence four common alterations are detectable namely gene deletions, aneusomy (gain or loss of a chromosome), translocations and amplification.

Role of FISH in meningiomas

It has been reported that while 50-60% of meningiomas have deletion of long arm of chromosome 22, meningothelial hyperplasia does not.(20,186) Hence, it can be diagnostically useful to differentiate these two conditions. But, 22q deletion alone is of no prognostic significance in meningiomas. (187)

However, 22q deletion in conjunction with 1p and 14q deletions can be used to differentiate poorly differentiated meningiomas from dural based tumours, like hemangiopericytomas, superficial glioblastoma multiforme, metastatic carcinomas and gliosarcomas.(188)

It has been found that loss of short arm of chromosome 1 is more commonly seen in high grade meningiomas and may be implicated in tumour progression/recurrence. (11,18,23,28,134,189–192)

Similarly chromosome 14q deletion has been associated with high grade histology and higher recurrence rates. 14q deletion confers higher recurrence risk not only to meningiomas with high grade histology but also to WHO grade I meningiomas.(11,20,134,141) Although paediatric meningiomas are reported to have a higher prevalence of deletions in 1p, 14q or both, the association of these chromosomal abnormalities with histomorphology and prognosis is poor. This is one reason why behaviour of meningiomas in children, is difficult to predict.(187)

Shorter survival periods are reported in anaplastic meningiomas having loss of cyclin-dependent kinase inhibitor 2A (CDKN2A) on chromosome 9p21. These deletions are also found to be more frequent in high grade meningioma. (39)

A study by Schneider et al.,(20) found that when compared to conventional cytogenetics, Fluorescence in situ hybridization was a sensitive method for identifying chromosomal deletions. It also conceded with others that chromosome 14 deletion was associated with progression of meningiomas to higher grades.

Cai et al.(141) studied 180 meningiomas using dual-colour FISH probe targeted to 1p32, 1p36, 14q13, and 14q32. Their study included 77, 74 and 29 benign, atypical, and anaplastic meningiomas respectively. Grade I (benign) and grade II (atypical) meningiomas were categorized into recurrent (following complete surgical removal)

versus non-recurrent (minimum 10 years follow-up) and as brain invasive versus mitotically active subsets. Deletion of 1p and 14q were present in 23% and 31% of Grade I, 56% and 57% of Grade II, and 75% and 67% of Grade III meningiomas ($p < 0.001$ and 0.004 for 1p and 14q respectively). Codeletion of 1p and 14q were present in 7% Grade I, 39% Grade II, and 63% Grade III tumours (p value less than 0.001).The frequency of 14q deletion was lower in non-recurrent Grade I meningiomas when compared to recurrent ones (17% versus 50%, p value 0.013). Though codeletion of 1p and 14q in atypical meningiomas and loss of 14q in anaplastic meningiomas were related to poorer survival, this was not found to be significant statistically. They concluded that loss of 1p and/or 14q had significant association with high grade histology and also contribute to tumour progression.

In a cohort of 124 meningiomas, Taberno et al.(193) analyzed quantitative abnormalities of chromosome 14 by FISH and verified these aberrations by Comparative Genomic Hybridization (CGH). Correlation between chromosome 14 aberrations and clinical findings, histology, factors affecting prognosis was sought. FISH revealed deletion (14.5%) or gain (25.8%) of 14q32 in 40.3% cases (n=50). Many of them were numerical alterations including monosomy (12.9%), trisomy (1.6%) and tetrasomy (24.2%). 14q32 gain or losses were more frequently associated with high grade tumours (p value 0.009). Cases with loss in chromosome 14 belonged more commonly to male gender (p value 0.04), were more likely to recur (p value 0.003) with shorter recurrence free survival (p value 0.03). They concluded that chromosome 14 aberrations seen in meningiomas are mostly numerical changes and amongst these, monosomy 14 is particularly associated with adverse prognosis.

Pfisterer et al.,(11) analysed 111 specimens (96 Grade I and 15 Grade II) of meningiomas for loss of chromosomes 14q, 1p, 22q and trisomy 22q, using FISH. 51% and 93% of WHO grade I and WHO grade II tumours displayed deletions in short arm of chromosome 1 and long arm of chromosomes 14 and 22. The MIB-1 LI (p less than 0.001) and recurrence (p less than 0.01) significantly correlated with the presence of chromosomal aberrations. They suggested that addition of Fluorescence in situ hybridization as an adjunct to routine histology may help to better predict tumour recurrence.

14. Prognostic factors

The major prognostic questions regarding atypical meningiomas involve prediction of recurrence and for malignant tumours the issue is prediction of survival.

14.1 *Role of extent of resection*

In 1957 a classification system based on completeness of tumour excision was proposed by Simpson. This classification identified 5 grades, ranging from complete resection (grade 1) to decompression only (grade 5). (Table 2)

Surgical resection and age at diagnosis were clinical predictors of survival in two large cohorts. In both studies, patients who underwent surgery had longer survival periods. They also reported a negative correlation between age and survival. (194,195)

The Simpson grading system is a useful predictor of meningioma recurrence. The grading based on the extent of tumour resection is shown in Table 2.(196) Tumour resectability

depends a great deal upon its location. Tumours of the convexity are amenable to cure by surgery, whereas tumours of skull base, particularly those arising from petroclival, cavernous sinus region or orbit, have a poorer outcome. (197)

Table 2: Recurrence rates of meningiomas depending on the degree of resection.(41)

Simpson grade	Definition	10-yr Recurrence rate
1	Macroscopic gross total resection with excision of dura, sinus and bone	9%
2	Macroscopic gross total resection with coagulation of dural attachment	19%
3	Macroscopic resection with no resection or coagulation of dural attachment	29%
4	Subtotal resection	40%
5	Biopsy	Not available

[Saraf S, McCarthy BJ, Villano JL. Update on meningiomas. The oncologist. 2011;16(11):1604–13.]

14.2 *Role of Histological Grade*

Histological grade predicts recurrence and mortality. Meningiomas with Grade II and III morphology are more prone to recur with short period of survival when compared to those with Grade I morphology. Recurrence rates as high as 38% and 78% at 5 years have been documented for Grade II and Grade III meningiomas. (197)

The estimated survival at 5 years for benign and malignant meningiomas were 70.1% and 54.6% respectively.(195) A multivariate analysis which included clinical details, extent of surgical excision and histologic factors found that age forty years, male sex,

incomplete resection, involvement of intracranial part of optic nerve, and high mitotic activity were individually linked with shorter duration of progression free survival. (198)

14.3 *Role of Imaging*

Computed tomography scans may provide clues to distinguish benign from malignant meningiomas. Features of benign tumours include calcification and homogeneous enhancement, whereas heterogeneous pattern and “mushrooming” are more often seen in Grade III meningiomas.(199) There is evidence based on data obtained from a cohort of 18 cases showing correlation between tumour grade and recurrence and certain features on single-photon emission tomography.(200)

14.4 *Role of Proliferation Markers*

Higher indices of cell proliferation like Ki-67 and MIB-1 LI are usually associated with higher histological grade and increased risk of recurrence. (14,201,202)

14.5 *Role of Hormone Receptor and other biomarkers*

Benign meningiomas more frequently express progesterone receptors and this is associated with lower recurrence rates and good prognosis.(101,203) Telomerase activity is found more commonly in Grade II and III meningiomas. Its presence denotes poor outcome even in benign meningiomas.(204) Vascular endothelial growth factor levels are higher in atypical and anaplastic meningiomas and it also predicts greater chance of recurrence in Grade I meningiomas.(205)

14.6 *Role of Genetic factors*

Many genetic aberrations such as 1p, 14q, 10q, 6q and 18 q deletions and gains on 1q, 9q, 12q, 15q, 17q, 20q are associated with malignant progression of meningiomas. (133) Of these, 14q deletion has been shown to predict tumour recurrence independently in two studies. (141,143) Gene expression profile study and multivariate interaction analysis may identify aggressive behaviour of tumour but their usefulness needs validation by larger cohorts.(206)

15. Treatment

Meningiomas are treated based on the tumour location, tumour dimension, age, associated symptoms and health status of the patient.

When lesions are small and asymptomatic, close monitoring may suffice. In these cases, though not biopsy proven the tentative diagnosis of meningioma rests on the classical radiologic findings (viz. dural-based mass, dural tail, homogeneous contrast enhancement).

15.1 *Surgery*

Complete resection of the tumour by surgery along with the attached dura is the treatment of choice for symptomatic and progressively enlarging meningiomas.(196) When overlying bone is involved, it need excision too. While gross total excision is feasible in convexity, falcine and spinal meningiomas, it may not be possible for tumours involving the petroclival region, cavernous sinus and optic nerve sheath.(33,207–209)

In cases where complete excision is not feasible, external beam radiotherapy or partial excision with radiotherapy is an option. Studies have shown that long term tumour control obtained from external beam radiotherapy is similar to that with tumour resection and subsequent radiotherapy. (210) The recurrence free survival rates after sub-total removal of tumour was 63%, 45% and 9% at 5, 10 and 15 years respectively.(211) In addition to sub totally resected meningiomas, adjuvant radiotherapy is also advocated for tumours with aggressive histology and recurrent meningiomas.(42,212,213)

15.2 *Radiotherapy*

Ongoing phase II trial by Radiation Therapy Oncology Group (RTOG, NCT00895622) is investigating the role of intensity modulated radiation therapy (IMRT) in treatment of meningiomas. Stereotactic radiotherapy (SRS) is another treatment modality in sub totally resected meningiomas (with residual tumour size ≤35 mm in diameter) and recurrent meningiomas or tumours which are inoperable due to their location or patient comorbidity.(214–217) SRS and Cyberknife surgery (a form of SRS) have demonstrated good tumour control rates, reduction in tumour volumes and cranial nerve deficits. (218,219) Spot-scanning proton beam radiotherapy is an advanced technology which uses focussed proton beams to irradiate tumour tissues sparing the surrounding tissue. Weber et al. treated 16 patients with spot scanning proton beam radiotherapy and documented three year progression and toxicity free survival rates of 91.7% and 75% respectively.(220)

15.3 *Hormonal therapy*

Hormonal therapies that have been tested in meningiomas include mifepristone, tamoxifen and flutamide.(221,222) A prospective multicenter study investigating the therapeutic effects of mifepristone (RU486) in meningioma treatment had disappointing results.(223,224) Nonetheless, a recent small study demonstrated minor responses to mifepristone in 8 of 28 subjects. Seven of the eight subjects who responded were premenopausal women or males. There is a need for further investigating its effects in carefully selected subsets of patients.(194)

15.4 *Chemotherapy*

Chemotherapy has limited role in meningioma treatment. Apoptosis is seen in meningioma cells grown in-vitro on treatment with Hydroxyurea. There is data suggesting that it stabilizes nonresectable and recurrent tumours. (225–228) The cyclophosphamide, doxorubicin, and vincristine (CAV) multi-drug regimen has been studied in 14 patients with WHO grade III meningiomas as adjuvant chemotherapy after surgical resection and radiotherapy. It prolonged the median time to tumour progression and the median survival time modestly than in comparison to historical controls.(229)

15.5 *Other modalities*

Unresected tumours may also be treated by endovascular embolization. In a study, there was marked tumour shrinkage on MRI, especially during the first 6 months of the

20 month mean follow-up period along with symptomatic improvement in six of seven subjects.(230)

Biologic agents like Interferon-α and somatostatin analogs have shown encouraging results in the treatment of recurrent and unresectable meningiomas.(231–235)

Platelet derived growth factor, vascular endothelial growth factor, epidermal growth factor receptor, PI3/Akt and Ras pathways are the molecular targets currently under evaluation for treatment of meningiomas.(236,237)

16. **Conclusion**

The growth rates and probability of meningioma recurrence cannot be predicted adequately by the present criteria. Furthermore, the histological classification does not provide any information about the causal genetic mechanisms.(15) On the contrary, a classification system incorporating genetic alterations may be superior in predicting the tumour prognosis and progression.(238) The information gleaned from underlying molecular mechanisms may lead to development of more effective and less toxic targeted therapies.(238)

The integration of histological grade, biological markers and genetic data may help in the recognition of meningioma subtypes and lead to the development of a prognostic panel of markers that could help identify aggressive tumours, which may aid the development of targeted therapy (7) and predict biological behaviour.

MATERIALS AND METHODS

A total of 51 tumour samples of meningioma were received in the Norman Institute of Pathology, Christian Medical College and Hospital, Vellore, Tamil Nadu, India, a tertiary referral centre for neurosurgery patients from January 2011 to December 2011. It was a retrospective study. These cases were artificially segregated into five categories based on the MIB-1 labelling indices of <5%, 5-7.5%, 7.5-9.9%, 10% and >10%. From these categories a total of 46 cases were selected randomly (by picking lots) for the study, 9 each with a MIB-1 LI of <5%, 5-7.5%, 7.5-9.9% and >10%, and 10 cases with a MIB-1 LI of 10%.(Table 3) The clinical information was obtained from the CMCH online clinical workstation and the outpatient records from the medical records department.

Table 3. Study cases segregated by MIB-1 labelling index.

MIB category	Frequency	Percent	Cumulative Percent
<5	9	19.6	19.6
5-7.5	9	19.6	39.1
7.6-99	9	19.6	58.7
10	10	21.7	80.4
>10%	9	19.6	100.0
Total	46	100.0	

Inclusion criteria

All the surgical resection cases diagnosed as meningioma (based on the histopathological criteria defined by the WHO 2007 Classification).

Exclusion Criteria

1. Cases of Neurofibromatosis type 2.
2. Psammomatous meningiomas, as they interfere with observations under fluorescent microscope.

Parameters evaluated

1. Clinical information: Age, gender, duration of symptoms, MRI findings, surgical findings like plane of the tumour with the brain, type of resection (gross-total resection versus subtotal resection), adjuvant therapy, clinical follow-up, duration of follow-up, residual/recurrent lesion.

2. Histological subtype of meningioma: According to WHO 2007 Classification. Slides were reviewed and histological features of atypia were noted.

3. MIB-1 labelling index: Immunohistochemical staining for MIB-1 (Dako,Glostrop, Denmark, clone MIB-1) was performed on 'Ventana Benchmark XT Autostainer' (Multimer method with diaminobenzidine followed by haematoxylin counterstaining). The dilution of the primary antibody used was 1:100. Tonsil or lymph node was used as positive control. Omission of the primary antibody served as negative control.

Protocol for automated immunostaining:

a. Paraffin embedded tissue sections were cut at 4µ thickness and floated onto slides coated with poly L-lysine, followed by overnight incubation at 37˚C.
b. These slides were then treated with 4% milk solution for 10 minutes to eliminate the hydrophobic effect and give positive charge to the slides.
c. The slide labels were bar coded and the labeled slides were loaded in 'Ventana Benchmark XT' autostainer (a fully automated immunostainer).

b. Individual protocols have been designed in the software attached to the machine for each marker. Specific protocol for MIB-1 was selected.

c. The steps included in this protocol were as follows:

- Deparaffinization
- Liquid coverslip application.
- Heat induced antigen retrieval by treating with standard CC1 solution (pH patent with the company) for one hour at 90˚C.
- Then the primary antibody was added and incubated for 40 minutes at 37˚C.
- Then the secondary antibody (Multimer) was added and incubated for 8 minutes.
- Finally the slides were counterstained with haematoxylin and incubated for 8 minutes, followed by incubation with the bluing reagent for 4 minutes.

(From antigen retrieval till counterstaining, in between every step the slides were washed with reaction buffer. The whole process is automated).

f. Then the slides were brought to 80% alcohol (2 changes) to remove the liquid coverslip and then dried and mounted in DPX.

The MIB-1 labelling index was assessed by a single observer in the region with the highest concentration of positive nuclei, where the number of positive nuclei in 1000 cells were counted at 400x magnification using a Leitz dialux microscope (LeitzWestlar, Germany) and expressed as a percentage.

For the purpose of statistical analysis a MIB-1 LI of >7% was used as the cut-off, as this level has been validated for the diagnosis of atypia in our institution, by a previous study.(185)

4. Chromosome 14q deletion by FISH:

Procedure for FISH

Commercially available FISH paraffin pre-treatment kit and SpectrumOrange fluorophore labelled probe against telomeric region of 14q (Abbott Molecular: Vysis TelVysion) were used.

a. Paraffin embedded tissue was cut at 3 microns thickness.
b. The slides were coated by sialinization method. (refer annexure 2)
c. Incubation

- The tissue block cut at 3 microns was floated onto a coated slide in a water bath at 40° Celsius.
- Slides were incubated overnight at 37° Celsius.
- Next day morning the slides were transferred to an incubator and kept at 56° Celsius for 2 hours.
- The area of interest was marked with a diamond pencil.

d. Deparaffinizing

- Deparaffinizing was done with xylene. Three changes, each lasting 10 minutes were made.
- The slides were dehydrated with 100% isopropanol by making three changes, each lasting 3 minutes.
- The slides were air dried for 2-5 minutes.

e. Slide pretreatment

Note: In the beginning of the procedure two water baths were switched on, one at 80° Celsius and another at 37° Celsius.

- A coplin jar with pretreatment solution (received as a part of the FISH kit) was kept in the 80° Celsius water bath until its temperature reached 80° Celsius.
- The slides were immersed in pretreatment solution at 80° Celsius for 10 minutes. (A maximum of 6 slides were processed at once by placing 2 slides back to back in the coplin jar slot. The end slides were kept singly with the tissue section facing the centre of the jar)
- The slides were immersed in purified water for 3 minutes following which extra water was blotted off the slide edges using paper towels.
- Protease solution was prepared by thoroughly mixing protease powder (received as a part of the FISH kit and stored in a freezer at -20° Celsius) with protease buffer in a coplin jar. The coplin jar was placed in a water bath and allowed to attain a temperature of 37° Celsius.
- Slides were immersed in the protease containing coplin jar after it had reached a temperature of 37° Celsius for 15 minutes.
- Slides were then immersed in purified water for 3 minutes.

- After removing the slides from the jar of purified water, they were air dried for 2-5 minutes.

f. **Hybridization** was done by codenaturation on the Abbot Molecular ThermoBrite hybridization unit.

- Dehydration was performed by serially immersing the slides in 70%, 85% and 100% ethanol for 1 minute each.
- A strip of paper towel was moistened with water and placed in the channel along the heating surface of the hybridization unit.
- The denaturation temperature was set to 90° Celsius with a melt time of 13 minutes. The hybridization temperature was set to 37° Celsius and hybridization time to 16 hours overnight.
- 10 microlitres of FISH probe (SpectrumOrange fluorophore labelled probe against telomeric region of 14q) was applied to the slide and coverslip was placed immediately.
- The coverslip was sealed with rubber cement and co-hybridization programme was initialized.

g. **Washing**

- After overnight hybridization the rubber cement is removed from the slides.
- 50ml of 2XSSC/0.3% NP40 (commercially available washing solution) was placed at 73° Celsius water bath in a coplin jar.
- The slides are washed in 2XSSC/0.3% NP40 at room temperature and allowed to stand for 2-5 minutes till the coverslips floated off the slides.
- The slides were then immersed in pre-warmed 2XSSC/0.3% NP40 at 73° Celsius and agitated for 1-3 seconds.

- Finally the slides were again agitated in 2XSSC/0.3% NP40 at room temperature for 1-3 seconds and kept in the same solution for 5 seconds to 1 minute.

h. Visualisation

- The slides were air dried in darkness.
- 10 microlitres of DAPI (4',6-diamidino-2-phenylindole) counterstain was applied to the tissue section and coverslip was applied,
- The slides were viewed using Spectrum orange filter under Olympus BX51 fluorescence microscope.
- The observer reporting 14q status was blinded to the MIB-1 labelling index of the cases.
- For each case a total of hundred cells were counted for normal copy, deletions and polysomy. 14q deletion was considered to be present if 80 or more cells out of 100 counted cells displayed a single copy of 14q chromosome.

The cut off value for deletion was determined by calculating the likelihood ratios of the test while considering the cut off for deletions as more than 20, 40, 60 and 80 cells out of 100 cells displaying single copy of chromosome 14.

Table 4. Distribution of meningioma based on number of cells with 14q deletion.

Number of cells /100 cells displaying deletions	WHO grade II & III meningiomas	WHO grade I meningiomas
81-100	17	4
61-80	6	4
41-60	0	1
21-40	1	2
0-20	1	10
Total	25	21

Table 5. Sensitivity, Specificity and Likelihood ratios against cut off points for number of cells with 14q deletion.

Cut off for 14q deletion	Sensitivity	Specificity	Likelihood ratio
>20	0.96	0.48	1.85
>40	0.92	0.57	2.14
>60	0.92	0.62	2.42
>80	0.68	0.81	3.58

As evident by the tables above the likelihood ratio of the test is highest when the cut-off for deletion was taken as 80%.

Now considering a pre-test probability (P) of 50%, the pre-test odds i.e. P/1-P would be 1.
The “post-test odds”, that is the pre-test odds multiplied by the likelihood ratio is, 4 (1x4).
The “post test probability” i.e.post-test odds/1+post-test odds is 80% (4/1+4).

This implies that when the pre-test probability of the diagnosis being WHO grade II/III meningioma, presence of >80 cells with single copy of chromosome 14 out of the 100 counted cells would give us a post-test probability of having a higher grade meningioma as 80%.

80% was therefore used as the cut-off for determining the 14q deletion status of a meningioma.

Statistical Analysis

Data entry was done using Microsoft excel software and the statistical analysis was carried out using the "SPSS software"(Statistical Package for Social Sciences), Windows 16.0 version.

Descriptive statistics such as range, frequency and percentage were used. χ^2 test was used to analyse categorical variables. Mann Whitney U test and the Fisher's exact Test or T-test was used for continuous variables. A p value of <0.05 was considered to be statistically significant. All the continuous variables were expressed as mean ± SD (standard deviation of the mean).

RESULTS AND ANALYSES

Demographic data

There were 46 cases in the study group. Using the WHO 2007 classification, 21 of the meningiomas were classified as Grade I, 22 as Grade II and 1 as Grade III (Table 6, Figure 3). There were two cases in which grading was deferred. Both these cases had a high MIB-1 LI (10%) and a poor surgical plane with the underlying brain, although on histology they appeared as WHO grade I meningiomas. In these two cases a cautionary note was provided to state that these tumours may behave biologically as grade II tumours. For statistical analysis these tumours were considered as WHO grade II tumours. As there was only one WHO grade III meningioma it was clubbed together with the WHO grade II meningiomas, and for statistical analysis these two grades were considered as one group. One of the cases included in the study came to our centre as a consult and some of the clinical details of this case were not available.

Table 6. Histological grades of meningiomas chosen for the study.

WHO Grade	Frequency	Percent
I	21	45.56
II	24	52.18
III	1	2.17
Total	46	100.0

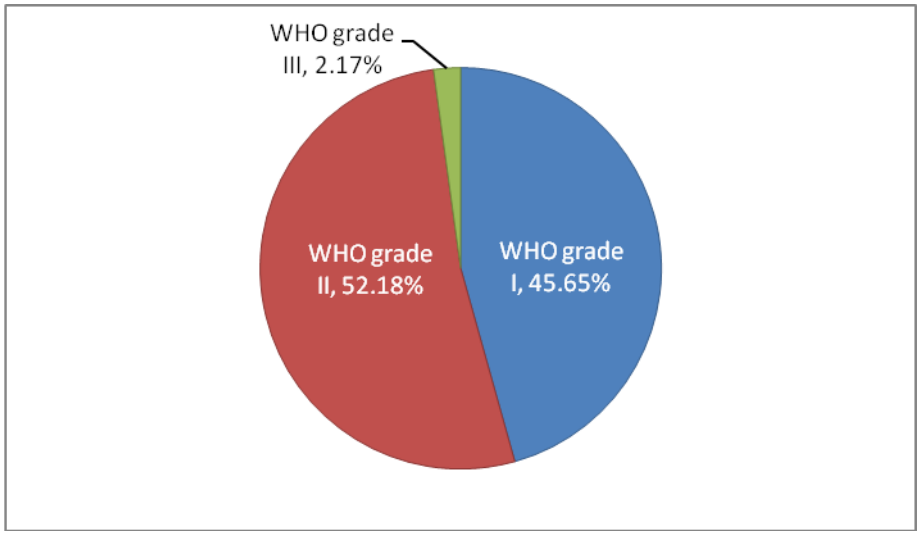


Figure 3. Pie chart depicting the percent of different grades of meningiomas included in the study.

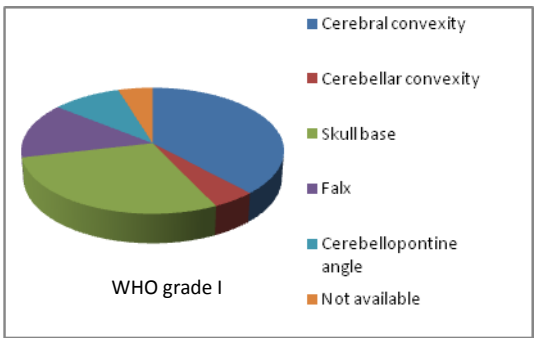
There was equal gender distribution with 23 female and 23 male patients. The mean age at diagnosis was 48.48 years ± 10.23 (range 28-72 years). The mean age for the diagnosis of WHO grade I meningiomas was 47.29 years ± 11.14 (range 29-62 years). The mean age for the diagnosis of WHO grade II meningiomas was 49.48 years± 11.14 (range 36-72 years). The difference between the age at diagnosis of Grade I and Grade II meningiomas was statistically insignificant (p=0.475).

Clinical parameters

There was no difference between Grade I and Grade II, III meningiomas with regard to location (Table7, Figure 4).

Table 7. Location of tumours

Grade	Location	Number	%	Age±SD	Male:female
I	Cerebral convexity	8	38.0	49.63±6.82	3:5
	Cerebellar convexity	1	4.8	62.00	0:1
	Skull base	6	28.6	42.67±11.74	3:3
	Falx	3	14.3	46.67±5.69	0:3
	Cerebellopontine angle	2	9.5	41.00±7.07	1:1
	not available	1	4.8	56.00	1:0
	Total grade I	21			8:13
II	Cerebral convexity	9	37.5	48.11±8.71	6:3
	Cerebellar convexity	1	4.2	59.00	1:0
	Skull base	9	37.5	49.22±13.73	4:5
	Falx	4	16.7	52.75±12.92	2:2
	Cerebellopontine angle	1	4.2	36.00	1:0
	Total grade II	24			14:10
III	Intraventricular	1	100	55.00	1:0
	Total	46			23:23



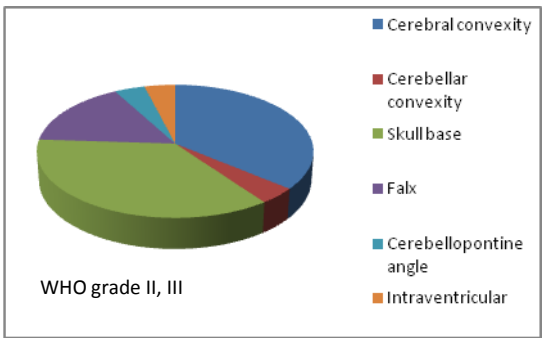


Figure 4. Pie charts depicting the location of Grade I and Grade II, III meningiomas.

Duration of symptoms

The mean duration of symptoms of WHO grade I meningiomas (n=19) was 10.05±8.69 months and that of WHO grade II meningiomas (n=23) was 5.24±5.65months. This difference was statistically significant (p<0.028), (Figure 5).

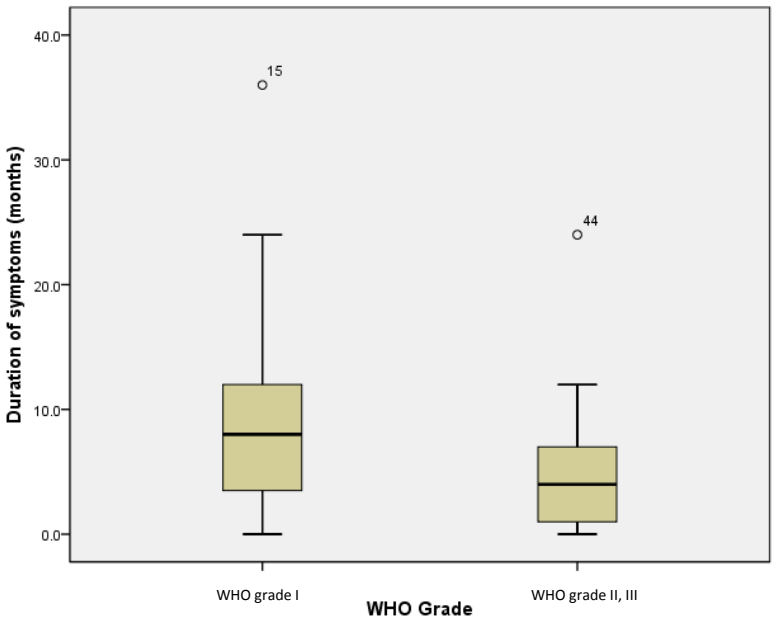


Figure 5. 95% confidence interval of duration of symptoms of Grade I and Grade II, III meningiomas.

Tumour size

Grade II meningiomas were larger than Grade I counterparts. The average maximum dimension of WHO grade I meningiomas (n=20), determined by magnetic resonance imaging at the time of diagnosis was 4.42 ±1.16 centimetres and that of WHO grade II meningiomas (n=23) was 5.60±1.33 centimetres. This difference was highly statistically significant (p<0.003), (Figures 6a, 6b, 8).

Although the difference in the mean maximum dimension of the two grades of tumours was significant, there was a wide variation in size of both WHO grade I (range: 2.40-7.30 centimetres) and WHO grade II tumours (range: 2.50-8.00 centimetres). Using 4.42 centimetres as cut-off, 45% of WHO grade I tumours were less than 4.42 centimetres while only 15% of WHO grade II tumours were less than 4.42 centimetres. This difference was statistically significant (p=0.039), (Figure 7).

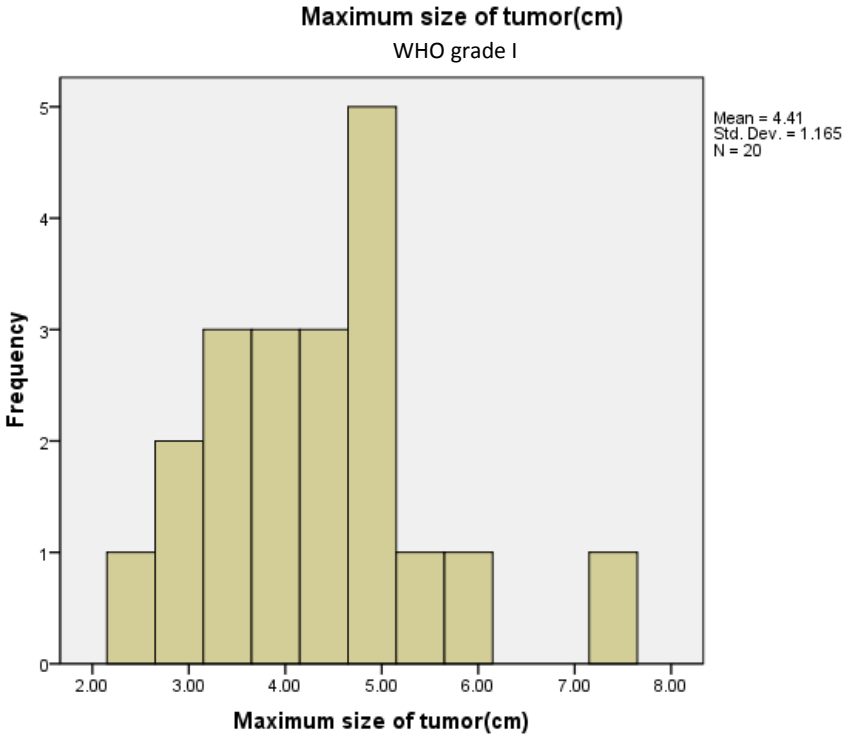


Figure 6a. Distribution of maximum dimension of WHO grade I meningiomas.

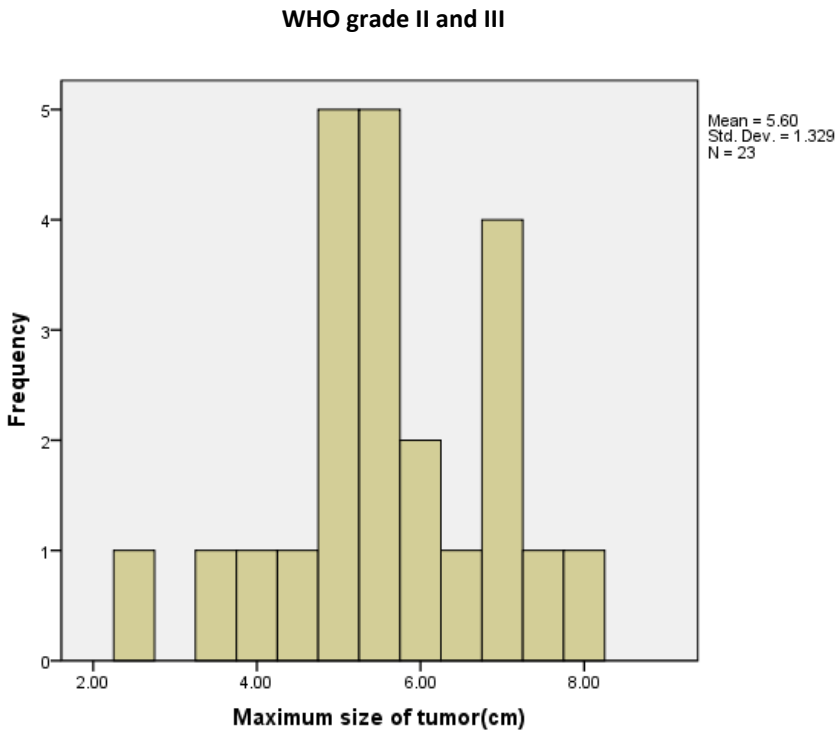


Figure 6b. Distribution of maximum dimension of WHO II/III meningiomas.

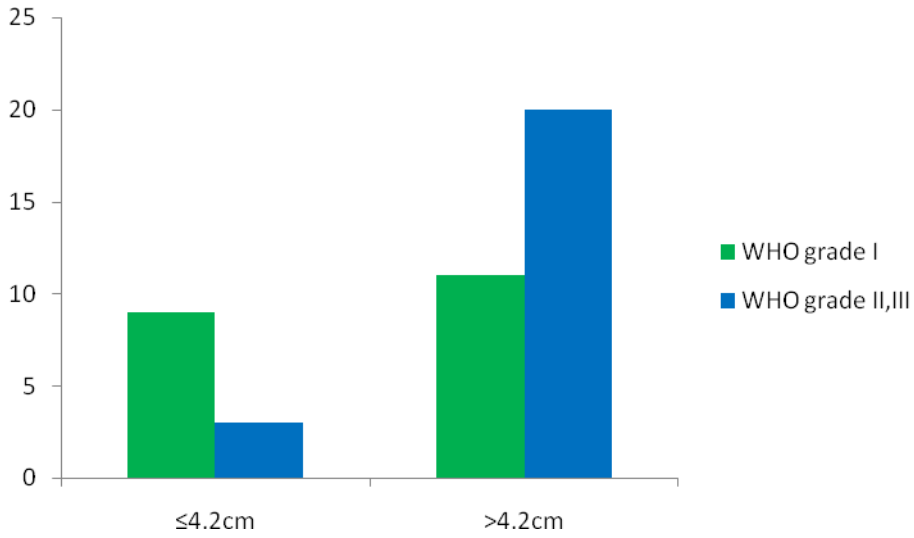


Figure 7.Distribution of Grade I and Grade II,III meningiomas depending on size at a cut-off of 4.2 cm.

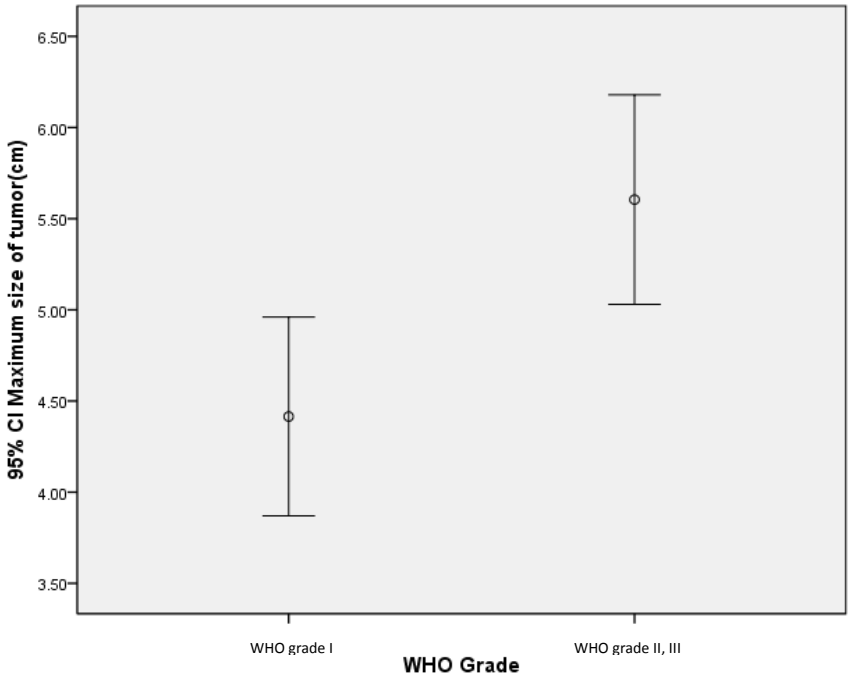


Figure 8. 95% confidence interval of size of tumour of Grade I and Grade II, III meningiomas.

Surgical plane of tumour with brain

20 tumours had good surgical plane and 24 tumours had poor surgical plane with the surrounding brain. 3 of the 11 WHO grade I tumours with poor surgical plane with the brain were skull base tumours. There was no significant association between WHO grade and poor surgical plane (Table 8).

Table 8. Segregation of meningiomas based on surgical plane with the brain and WHO grade.

	Grade I meningiomas	**Grade II, III meningiomas**
Good surgical plane	9	11
Poor surgical plane	11	13

Type of excision

Table 9. Segregation of meningiomas by type of excision and WHO grade.

Type of excision	Grade I meningiomas	Grade II, III meningiomas
Total	8	20
Near total	8	2
Partial	4	3
Not available	1	0
Total	21	25

8/21 (38%) WHO grade I and 20/25 (80%) WHO grade II & III meningiomas were radically excised (Table 9).

13/ 28 patients with total excision, 2/ 10 patients with near total excision and 5/ 7 patients with partial excision received adjuvant radiotherapy.

Follow-up Information

All the tumours were removed between January to December 2011. Follow-up information was available in 24 of 46 cases. 9 of these were WHO grade I tumours and 15 were WHO grade II, III tumours. The mean duration of follow-up was 13.58±6.5 months. 3 WHO grade I and 2 WHO grade II meningiomas had residual lesions; however there was no increase in size of these lesions during the follow-up period. There were no tumour recurrences during the period of follow-up.

Histological Subtypes

Table 10 depicts the distribution of meningiomas by histological subtype. Amongst the Grade II meningiomas 6 were brain invasive. All the atypical meningiomas diagnosed were those that met the WHO histological grading criteria for atypia. There were no clear cell or chordoid meningiomas in this study.

Table 10. Histological subtypes of meningioma.

Grade	Subtype	Number	%	Age±SD
I	Meningothelial, transitional and fibrous	18	85.7	47.94±9.49
	Angiomatous	2	9.5	42.50±9.19
	Microcystic	1	4.8	45.00
II	Atypical	18	75.0	49.72±12.56
	Brain invasive	6	25.0	47.83±7.08
III	Anaplastic	1	100	55.00

MIB-1 labelling index (Figures 9a, 9b)

The mean MIB-1 labelling index of WHO grade I tumours in this study was 5.71±2.74% (range 2 -12%) and that of WHO grade II was 11.04± 4.04% (range 5 -22%). The MIB-1 labelling index was significantly different between WHO grade I (n = 21) and WHO grade II & III meningiomas (n = 25), (p=0.00), (Figure 10).

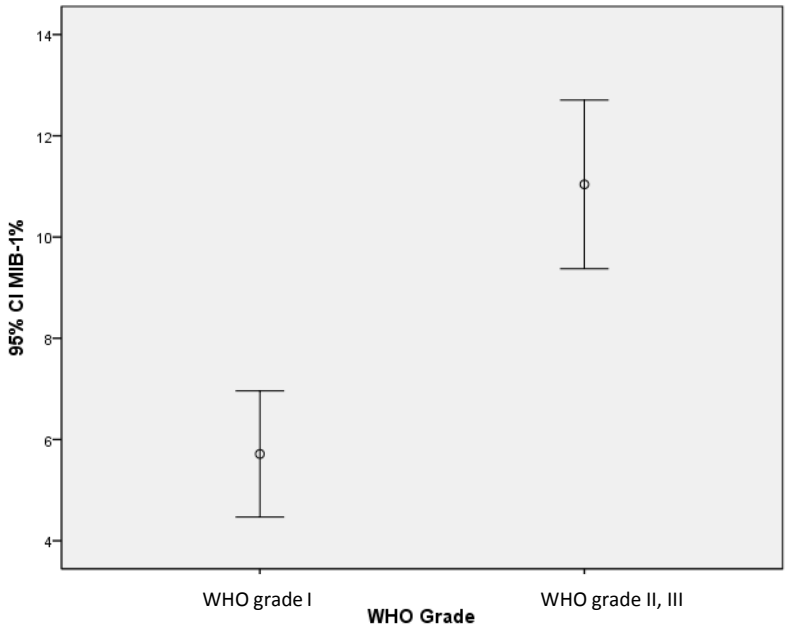


Figure 10. 95% confidence interval for MIB-1 LI of Grade I and Grade II, III meningiomas.

We grouped meningiomas into 5 groups based on their MIB-1 labelling index and found that as the MIB-1 labelling index increased the proportion of cases in WHO grade II,III were statistically higher (p=0.000), (Table 11, Figure 11).

Table 11. MIB-1 LI versus tumour grade.

MIB-1	WHO grade II, III	WHO gradeI	Total
<5%	0	9	9
5-7.5%	3	6	9
7.6-9.9%	6	3	9
10%	8	2	10
>10%	8	1	9
Total	25	21	46

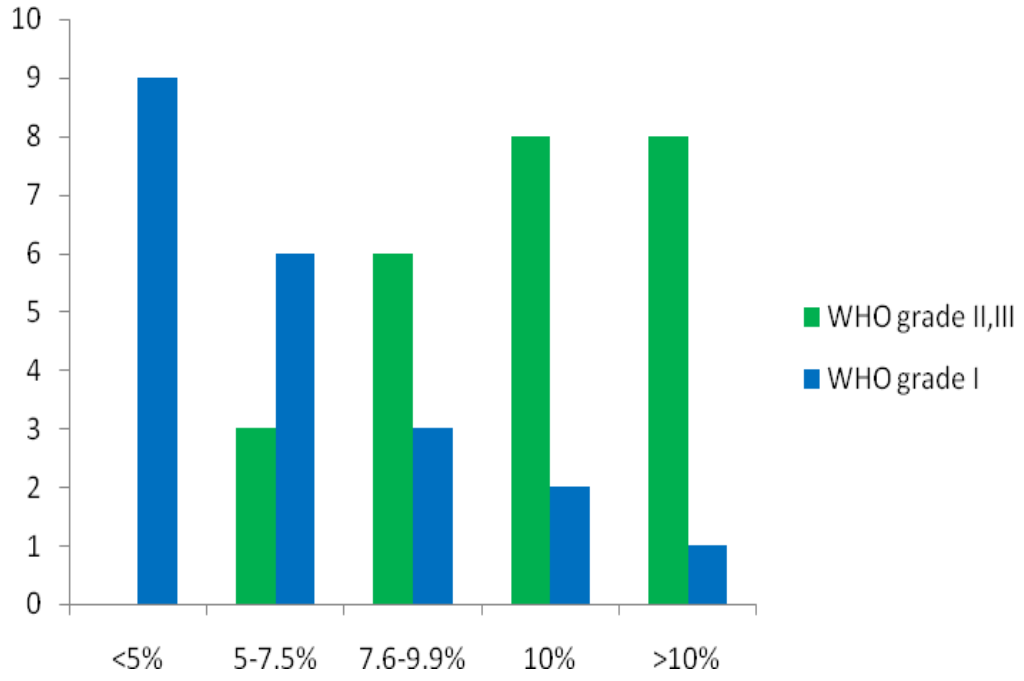


Figure 11. Proportion of cases in categories segregated by MIB-1 LI.

Histological criteria

Of all the histological parameters defined by WHO for diagnosing atypical meningiomas (Table 12, Figures 12a-12j), there was a statistically significant association between a MIB-1 LI >7% and hypercellularity, small cells with a high nuclear: cytoplasmic ratio and sheet like pattern. There was no significant association between prominent nucleoli, necrosis and MIB-1 labelling index.

Table 12. Histological criteria for diagnosis of atypical meningioma versus MIB-1 LI.

	MIB-1 ≤ 7%	**MIB-1 >7%**	
Hypercellularity			
present	3	21	p-value = **0.000**
Absent	15	7	
Small cell change			
present	5	22	p-value = **0.001**
absent	13	6	
Prominent nucleoli			
present	6	7	p-value = 1
absent	12	11	
Sheet-like growth			
present	1	11	p-value = **0.028**
absent	17	17	
Necrosis			
present	9	3	p-value = 0.411
absent	19	15	

There was no significant relationship between age, gender, anatomic location, or completeness of tumour excision and the MIB-1 labelling index.

Chromosome 14q deletion (Figures 13a-13h)

There was no significant difference between the age, gender, duration of symptoms, size of tumour, surgical plane of tumour and the type of surgical excision of cases with the 14q deletion status.

Table13. Histological criteria for diagnosis of atypical meningioma versus 14q deletion.

	14q deletion present	**14q deletion absent**	
Hypercellularity			
present	18	6	p-value = **0.040**
absent	10	12	
Small cell change			p-value = **0.001**
present	22	5	
absent	6	13	
Prominent nucleoli			
present	20	5	p-value = **0.004**
absent	8	13	
Sheet-like growth			
present	15	4	p-value = **0.035**
absent	13	14	
Necrosis			
present	9	3	p-value = 0.411
absent	19	15	

Amongst the histological parameters defined by the WHO for diagnosis of atypical meningiomas, the association between 14q deletion and hypercellularity, small cell change, prominence of nucleoli, sheet-like pattern was statistically significant. There was no significant association between tumour necrosis and 14q deletion (Table 13).

When compared to WHO grade I meningiomas (n = 21), where only 7 cases (33.3%) showed deletion, 21 WHO grade II and III meningiomas (n = 25) (84.0%) had 14q deletion. This difference was found to be significant on statistical analysis, (p=0.000), (Table 14, Figure 14).

All the brain invasive meningiomas (n = 6) had 14q deletion.

Table 14. Meningiomas segregated by 14q deletion.

FISH 14q deletion	Grade II and III meningiomas	Grade I meningiomas
Present	21 (84.0%)	7 (33.3%)
Absent	4 (16.0%)	14 (66.7%)

Figure 14. Chromosome 14q status among Grade I and Grade II, III meningiomas.

X^2 test for trend was applied to assess the relationship between MIB-1% and 14q deletion. When looking at the prevalence of deletions in each sub-group segregated by MIB-1 LI it was found that as the MIB-1% increased the proportion of cases with deletions were significantly higher ($p<0.001$), (Table 15, Figure 15).

Table 15. MIB-1 labelling index versus 14q deletion.

MIB-1	14q deletion present	14q deletion absent	Total
<5%	0	9	9
5-7.5%	3	6	9
7.6-9.9%	9	0	9
10%	10	0	10
>10%	6	3	9
Total	28	18	46

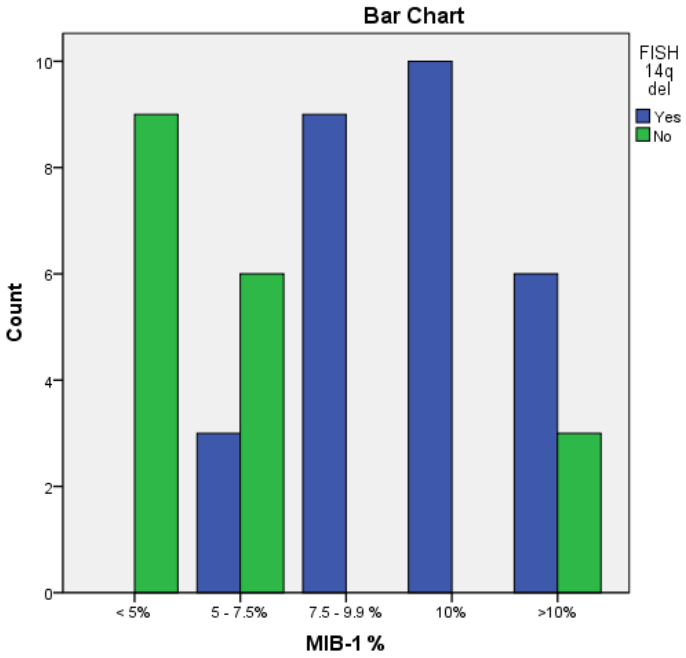


Figure 15. Chromosome 14q status among meningiomas segregated into five groups depending on their MIB-1 LI.

The mean MIB-1 of the WHO grade I meningiomas with 14q deletion (n=7) was 8.86±1.95% when compared to 4.14±1.35% for those without 14q deletions (n=14). This difference was found to be significant on statistical analysis (p=0.000), (Figure 16).

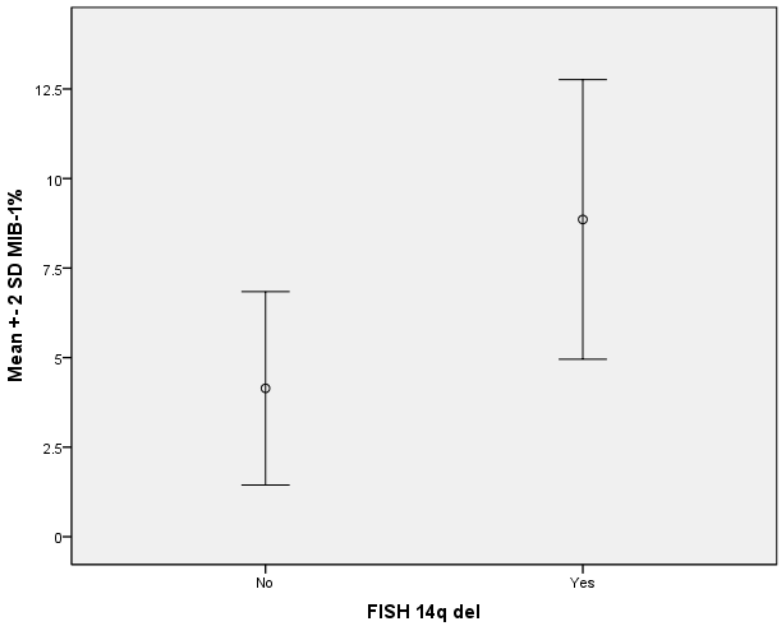


Figure 16. Mean±2SD of MIB-1 labelling index of grade I meningiomas with and without 14q deletion.

On further categorising the cases as MIB-1% ≤7% and >7%, 3 of 18 (16.7%) cases with a MIB-1 of ≤7% had 14q deletion when compared to 25 of 28 (89.3%) cases with a MIB-1 >7 %. This difference was highly statistically significant (p=0.000).

Of the 3 cases with MIB-1 labelling index ≤7% with 14q deletion, one was a WHO grade I meningioma. The only atypical feature in this case was prominence of nucleoli. The other two cases were Grade II meningiomas, one with small cell change, prominent nucleoli and necrosis and the other with brain invasion.

There were only 3 cases with MIB-1 labelling index >7% which did not have 14q deletion. All three were high grade meningiomas. Of these 2 were WHO grade II meningiomas, one with increased cellularity, small cell formation, necrosis, mitotic activity of 9/10 high power fields and a MIB-1 labelling index of 12%, and the other with increased cellularity, small cell formation, sheet-like growth and necrosis with a MIB-1 labelling index of 15%. The third case was that of an anaplastic, WHO grade III meningioma with a MIB-1 labelling index of 22%, (Figure 17).

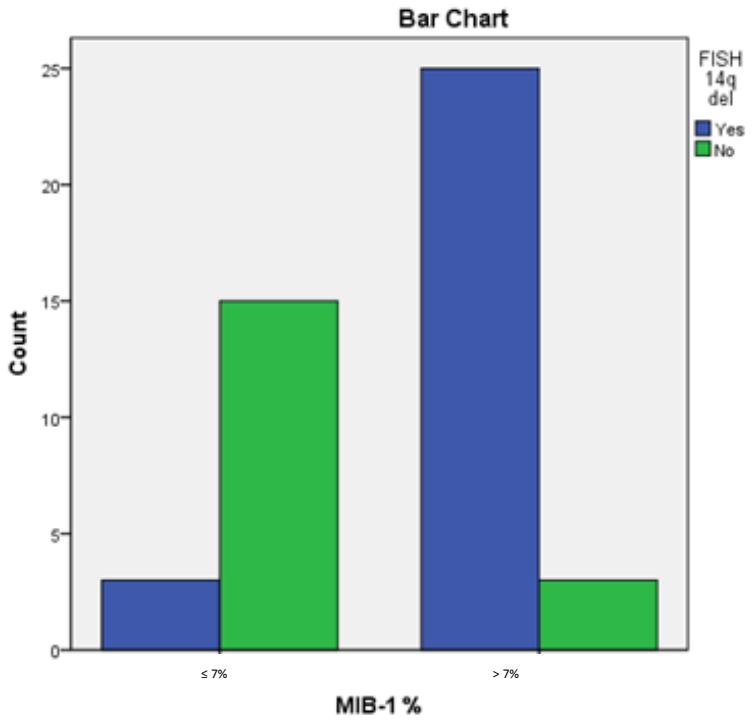


Figure 17. Chromosome 14q status among meningiomas segregated into two groups depending on their MIB-1 LI.

DISCUSSION

Meningiomas, the commonest primary brain tumours, are classified according into three grades based on histological criteria suggested by the WHO (2007).(30)

The incidence of these tumours tends to peak between the sixth and seventh decades with the average age at diagnosis being 63 years.(239,7) In the present study the average age at diagnosis was much lower at 48.48years± 11.14 (range 29-62 years). This discrepancy can be explained by the fact that the cases in this study were selected at random from a cohort of meningiomas seen over a year and therefore are not a true reflection of the average age of meningioma patients seen at our centre. In concordance with another study, the average age of patients with WHO grade I meningiomas was lower than those with WHO grade II tumours, however this difference was statistically insignificant.(14)

Though meningiomas are known to occur twice as frequently in females compared to males, the gender distribution was found to be equal in the present study. (30,239) This again may be explained by the manner of selection of cases. Although the frequency of atypical and anaplastic meningiomas was higher in males when compared to females, and is in concordance with literature(42), it did not reach statistical significance.

Unlike a previous study where atypical/anaplastic meningiomas occurred more frequently over the lateral convexities and falx when compared to benign counterparts, we did not find any significant difference between the anatomic location of these two groups.(43)

The average maximum dimension of WHO grade I and grade II meningiomas was significantly different with grade II tumours being 1.18 centimetres greater than grade I tumours. Also tumours in males were larger than those in females. When compared to WHO grade II meningiomas the mean duration of symptoms was significantly longer for WHO grade I meningiomas. This can be explained by the fact that the WHO grade II tumours owing to their higher rate of proliferation probably cause more secondary effects on neighbouring structures and therefore become symptomatic earlier. Surprisingly, we did not find any meaningful associations between surgical plane of tumour with brain, type of excision, size of tumour, anatomical location of tumour and WHO grade.

The majority of meningiomas (80-90%) are benign WHO grade I tumours, while the WHO grade II and III occur less frequently but display aggressive behaviour with higher recurrence rates.(4,5,41) Though the completeness of tumour excision and histological grading are the chief predictors that determine how a meningioma will behave biologically, tumours belonging to the same grade exhibit varying growth rates after surgery. Despite being completely resected, some WHO grade I tumours may demonstrate overtly aggressive behaviour with bone destruction, rapid growth of residual tumour and recurrence rates approaching 19% by twenty years, following apparently complete resection.(197) (3–5,133) In addition, meningiomas occurring in the skull base, cavernous sinus or invading the sagittal sinus are not amenable to complete resection, often requiring the surgeon to leave behind a significant residue.(163) Variable growth rates of these residues amongst WHO grade I tumours

highlights a need to determine other factors that may predict tumour behaviour and direct the early institution of adjunctive treatment such as radiotherapy.

Despite conflicting reports of the value of MIB-1, this proliferation index has emerged as an important predictor of tumour aggressiveness. Whilst some studies have found the MIB-1 labelling index to be predictive of recurrences. (Hsu et al; Roser 2004; Matsuno 1996) other studies have not found such an association (Abramovich). Interlaboratory variations in staining protocols, and interobserver variations in interpretation have precluded the establishment of a reliable cut-off value for different grades resulting in the exclusion of the MIB-LI from the WHO grading criteria.

In accordance with other studies, in the present study too the difference between the mean MIB-1 LI of WHO grade I and II meningiomas was statistically significant. (9,12,14,15) We determined from a previous study, at our institution, that a MIB-1 LI of 7% had the highest validity in diagnosing atypical meningiomas. The likelihood ratio of the diagnostic test at this value of MIB-1 LI (>7%) was 17, implying that tumours having a MIB-1 labelling index >7% are 17 times more likely to be atypical than benign.(185)

Studies have suggested that differences in MIB-1 labelling index among the three meningioma grades may be useful in borderline atypical cases where mitosis are hard to detect or the histological criteria of atypia are falling short in cases suspected to be of higher grade.(14,240)

In sub totally resected meningiomas when brain invasion cannot be ruled out due to sampling error and the tumour shows aggressive morphological features, insufficient to make a diagnosis of atypical meningiomas by WHO criteria, a high MIB-1 LI may help in assigning the tumour to a higher grade.

MIB-1 labelling index and histological features of atypia

The current study found that MIB-1 labelling index >7% correlated significantly with increased cellularity, sheet like growth and small cells with a high nuclear: cytoplasmic ratio. These findings were similar to those in a previous study,(185) at our institution, which included 21 benign meningiomas, 16 atypical meningiomas and 3 grade III meningiomas, where they found significant association between MIB-1 labelling index >7% and mitotic activity >4/10 high power fields and above three histological criteria. In this study too the MIB-I LI of benign meningiomas was significantly lower (mean, 5.7±2.7%) than that of atypical meningiomas (mean; 11.0 ± 4.0%). Devaprasath et al. suggested that integrating MIB-1 LI with histological grading may improve prediction of meningioma behaviour. They also suggested that tumours that did not show any histologic features of atypia but had high MIB-1 LI must be followed up closely. (185)

Four out of five morphological criteria, used for diagnosing atypia in meningiomas, including sheet-like growth, small cells with increased nuclear: cytoplasmic ratio, hypercellularity and prominent nucleoli have been considered to reflect loss of cellular differentiation.(240)

The fact that a mitotic activity of ≥4mitoses/ 10 high power fields is by itself enough to make a diagnosis of atypical meningioma underlines the importance of proliferative activity of a tumour in determining its grade. However, the significant association of MIB-1 labelling index with three of the morphological criteria of atypia namely, hypercellularity, sheet like growth and small cells with increased nuclear: cytoplasmic ratio, in our study, suggests that these histological features are indicators of high proliferation and may serve as surrogate markers in situations where either it is not possible to ascertain the MIB-1 or where mitotic activity is not evident.

Several studies have been carried out to determine if the MIB-1 LI has a role as a prognostic variable in meningiomas by itself. In a study by Ho et al., (202) MIB-1 labelling index was an independent prognostic variable for meningiomas and the presence of merely 2 of 3 criteria namely pattern-less growth, mitosis ≥ 1.5/mm^2 and necrosis were associated with atypical meningiomas and shorter recurrence free survival ($p<0.001$). Also MIB-1 LI was the sole independent predictor of recurrence and overall survival in a study by Bruna et al. (241)

Progression of meningiomas and 14q deletion

Several studies have delved into the prognostic potential of genetic abnormalities detected in meningiomas for predicting recurrence risk. (25,28,141,191,242,243) In a study carried out by Carvalho et al.,(15) the gene expression profiling of 23 meningiomas using oligonucleotide microarrays there was a difference in the expression of 28 genes between WHO grade I and WHO grade II meningiomas and 1212 genes between WHO grade I and WHO grade III meningiomas respectively. There genetic profiles of WHO

grade II and WHO grade III meningiomas did not differ significantly. The gene expression profiles of meningiomas were further categorised into two major groups namely 'low proliferative' and 'high proliferative'. To differentiate the above mentioned groups two molecular mechanisms were used. These were gain of proliferation markers and loss of transforming growth factor beta (TGF-β) signalling. All the 8 WHO grade I tumours fell into the 'low proliferative' category and all the 8 WHO grade III tumours fell into the 'high proliferative' category. The 7 WHO grade II tumours however were distributed between both groups. Hence, although atypical meningiomas were a distinct group using the histopathological criteria, their molecular profile was not distinct. However the molecular profile of these atypical tumours showed a spectrum from 'low proliferative' to 'high proliferative' suggesting that genetic alterations are a continuum in tumour progression. In accordance with other studies, the MIB-1 LI increased with histological grade. The WHO grades I, II and III had MIB-1 LI of 1.4, 7.0 and 14.1 respectively. The MIB-1 labelling index differed in grade II meningiomas belonging to low proliferative and high proliferative groups (higher for high proliferative group as compared to that of low proliferative group), however, the sample size was too small to attain statistical significance. The clinical course of atypical meningiomas is also diverse, with few of them demonstrating growth rates similar to Grade I meningiomas and others showing clinical patterns similar to malignant meningiomas. This study amongst others supported the view that ancilliary genetic testing may help to better prognosticate meningiomas.

As mentioned earlier, the chromosomal alterations in atypical and anaplastic meningiomas commonly include 1p, 10q and 14q deletions. Gains on chromosomes 1q, 9q, 12q, 15q, 17q, and 20q are also seen in high grade tumours.(7,128)

Epigenetic alterations, including excessive hypermethylation of CpG islands, are also known to occur in progression to malignant meningiomas.(187)

14q deletion, follow the losses in chromosome 22 and 1, as the next most common chromosomal aberration seen in meningiomas. 14 q deletions have been found in up to 31% of Grade I, 40%–70% of Grade II, and up to 100% of Grade III meningiomas. (18,22,24,25,115,141,142) Studies have also found losses of 14q to be a prognostic indicator of tumour recurrence.(115,141,143)

Lusis et al. (244) using gene expression profiling found that N-Myc downstream-regulated gene 2 (NDRG2) on chromosome 14q11.2 acts as a tumour suppressor with a possible role in meningioma progression. Functions of NDRG2 include cell differentiation and p-53 mediated apoptosis.(245,246) Partial or complete loss of chromosome 14 has also been associated with early relapse and poor clinical course.(18,132,247–249) Skiriute et al.(144) found that mRNA expression of N-Myc downstream-regulated gene 2 was reduced in recurrent meningiomas (of all grades) in comparison to primary benign Grade I tumours. Moreover atypical meningiomas (both primary and recurrent) had significant reduction of NDRG2 gene expression in contrast to primary benign counterparts. This study provides evidence supporting the role of N-Myc downstream-regulated gene 2 as a tumour suppressor gene involved in malignant progression and recurrence of meningiomas.

Cai et al. (141) found that the frequency of 14q deletion in benign non-recurring and recurring meningiomas were significantly different on statistical analysis. The frequency of 14q deletion in benign recurring meningiomas was almost as high as those in atypical meningiomas.

Maillo et al.(143) did a multivariate analysis and developed a scoring system combining WHO grade ($p<0.0001$), 14q status ($p=0.03$) and age ($p=0.002$) which stratified patients with meningiomas into three categories with significantly different recurrence free survival rates. They scored these three variables as follows:

Table 16. Scoring of independent variables used by Maillo et al. in their study.

	Score 0	**Score 1**
Histologic grade	WHO grade I	WHO grade II & III
Age	≥ 45 years	< 45 years
Numerical abnormalities of chromosome 14	Absent (normal copy of chromosome 14)	Present

Table 17. The prognostic scores (0-3) with median recurrence free survival rates of the meningioma cases in the study by Maillo et al.

Prognostic score	**0**	**1**	**2,3**
Median recurrence free survival (RFS)	10 year RFS = 100%	10 year RFS = 82% ± 7 5 year RFS = 70% ± 10	5 year RFS = 0%

The findings of this study strengthen the proposition that inclusion of molecular alterations (especially chromosome 14 aberrations) in meningiomas may help in predicting clinical course of disease.

In our own centre a study on the loss of heterozygosity (LOH) analysis for meningiomas was carried out.(153) This study aimed at identifying markers of aggressive behaviour in the Indian population using loss of heterozygosity (LOH) analysis for tumour suppressor genes located on 1,10,14,17 and 22. We found that D17S1289 was the most informative locus and that LOH was seen most often at D22S417.

It was also found that the majority of high grade meningiomas showed LOH 14/Allelic imbalance implying that it may be a progression event, however the LOH 14 did not reach statistical significance, while the allelic imbalance was significant, suggesting the presence of a smaller clone carrying a chromosome 14 deletion. DNA for LOH may have a mixture of normal and tumour tissue making the detection of LOH difficult and this could explain the finding of allelic imbalances instead of LOH in our study.

Schneider et al.(20) found that when compared to conventional cytogenetics, Fluorescence in situ hybridization was a sensitive method for identifying chromosomal deletion.

The present study was therefore carried out using FISH for 14q to determine if the findings of our previous study were corroborated when examining representative sections of the tumour.

Only 33.3% of WHO grade I meningiomas were found to harbour 14q deletion when compared to 84.0% WHO grade II meningiomas. This difference was highly statistically significant and is in accordance with other studies(24,25,141,241). This finding does

support our hypothesis that 14q deletion has a significant role in meningioma progression and the presence of tumour suppressor genes on this chromosome.

All the brain invasive meningiomas in our study had 14q deletion. There have been no studies, to the best of our knowledge that have looked at correlations between 14q deletion and brain invasion. Amongst the histological parameters defined by the WHO for diagnosis of atypia, there was a statistically significant association between the presence of 14q deletion and hypercellularity, small cells with increased nuclear: cytoplasmic ratio, prominent nucleoli and sheet like growth.

MIB-1 LI and 14q deletion

We found that the MIB-1 LI correlated significantly with 14q deletion. Only16.7% of cases with a MIB-1 of ≤7% had 14q deletion when compared to 89.3% cases with a MIB-1 >7%. Additionally both higher MIB-1 LI and 14q deletion were seen with WHO grade II tumours.

This again suggests that 14q deletion can serve as a surrogate marker for atypia.

Moreover, six of the seven WHO grade I meningiomas with 14q deletion additionally had MIB-1 labelling index of >7%. The MIB-1 labelling index of WHO grade I meningiomas harbouring 14q deletions was significantly higher than those without deletion. This implies that within the meningiomas with a WHO grade I histology, there exists a subgroup stratified by the MIB-1 labelling index, which consistently show 14q deletions. A previous study has shown that within WHO Grade I meningiomas, tumours with a high MIB-1 have a higher chance of recurrence.(15) It is possible that these tumours

harbouring 14q deletions may have a higher chance of recurrence. In the present study however, our follow-up information was limited as the cases in the cohort were operated on only 24 months ago, and hence this aspect could not be studied at the present time.

Of the 28 cases with a MIB-1 LI >7%, 3 did not show 14q deletion. These included a WHO grade III/anaplastic meningioma with a MIB-1 LI of 22% and 70% of the tumour cells having a single copy of 14q and two WHO grade II meningiomas one with a MIB-1 LI of 15% and 79% cells with single copy of 14q and the other with a MIB-1 LI of 12% with 38% cells having single copy of 14q. Despite the fact that a high percentage of tumour cells had single copy of 14q in 2/3 tumours, they were not considered as harbouring 14q deletions as they failed to meet the stringent cut-off value of 80% of tumour cells with single copy of 14q used for determining 14q status of a tumour in the present study. Chromosomal alterations apart from chromosome 14 are also implicated in tumour progression and this may explain the reason for third case with WHO Grade II histology and a high MIB not showing 14 deletions.

There was one WHO grade I meningioma with a MIB-1 LI ≤7% that had 14q deletion suggesting that chromosomal abnormalities may precede histological evidence of tumour progression. There were two cases with WHO Grade II histology and 14 q deletion that had MIB-1 LI of ≤7%. This makes a case for screening for chromosomal abnormalities as an ancillary to other tools for prognostication in meningiomas.

In conclusion, the results of this study showed that a strong association existed between histologic grade, MIB-1 LI and the presence of chromosome 14q deletion. Association of high MIB-1 LI with 14q deletions even in meningiomas with a Grade I histology defines a distinct subset of benign meningiomas. The biological behaviour of this subset needs further exploration and follow-up for recurrence and aggressive behaviour.

SUMMARY

Predicting the biological behaviour of meningiomas is challenging and the prognosis expressed in terms of recurrence is difficult to determine in an individual case especially those with borderline histological features. The histologic grade and extent of surgical resection predict the recurrence risk to some extent but several exceptions exist.

In an attempt to identify other correlates to the histologic grade we studied the association between MIB-1 LI, 14q deletion and the 5 histological criteria enumerated by the WHO for diagnosis of atypical meningiomas.

The results of this study showed that a significant correlation existed between histologic grade, MIB-1 LI and the presence of 14q deletion.

The MIB-1 LI showed a statistically significant difference between WHO grade I and II meningiomas. A MIB-1 LI >7% correlated significantly with 3 of 5 histological criteria of the WHO grading system, suggesting that these parameters are related to proliferative capacity of the tumour to some extent and supporting the role of MIB-1 LI as the single best predictor of atypical meningioma analogous to the mitotic activity.

The presence of 14q deletions also showed a statistically significant difference between WHO grade I and II meningiomas and with 4 of 5 histological criteria of the WHO grading system. Moreover MIB-1 LI correlated significantly with 14q deletions.

All the brain invasive meningiomas in our study had 14q deletion. To the best of our knowledge, there have been no studies that have looked at correlations between 14q deletion and brain invasion. However the number of brain invasive meningiomas was small.

We also found that within the meningiomas with a WHO grade I histology, there exists a subgroup stratified by the MIB-1 labelling index, which consistently show 14q deletions. In these and other subtotally resected meningiomas where brain invasion cannot be ruled out due to sampling error and the tumour shows aggressive morphological features, insufficient to make a diagnosis of atypical meningiomas by WHO criteria, a high MIB-1 LI or the presence of 14q deletion may help in assigning the tumour to a higher grade.

The biological behaviour of this subset needs further exploration and follow-up for recurrence are underway.

CONCLUSIONS

1. There was a significant difference between the MIB-1 labelling indices of WHO grade I and II meningiomas, (p=0.00).

2. WHO grade I tumours with a high MIB-1 LI consistently showed 14q deletions.

3. There was a significant difference between the 14q deletion status of WHO grade I and grade II meningiomas, (p=0.0002).

4. All brain invasive meningiomas had 14q deletions.

5. 14q deletion can serve as a surrogate marker for atypia since it correlates with both MIB-1 LI and histologic grade.

LIMITATIONS

1. The findings of this study were limited by the fact that follow-up duration was short and there was no data regarding tumour recurrence. Positive correlations of high MIB-1 LI and 14q deletion with tumour recurrence would add strength to their prognostic implication.

2. We studied only one chromosome deletion associated with tumour progression due to economic constraints. There are several other chromosomal abnormalities implicated in tumour progression which also need to be considered.

3. Fluorescence in situ hybridization is an expensive tool, as a result of which our sample size was small. Our findings need to be corroborated in larger studies.

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APPENDIX I

PROFORMA

Fluorescence in situ hybridization (FISH) for chromosome 14q deletion in subsets of meningioma segregated by MIB-1 labelling index

Biopsy number:

Name:

Age:

Gender:

Duration of symptoms:

MRI:

Intraoperative findings:

Type of excision:

Site:

Histological subtype of meningioma:

WHO Grade: I II III

MIB-1 labelling index: <5% 5-7.5% 7.6-9.9% 10% >10%

Mitosis: <4/10 hpf / ≥4/10 hpf

Increased cellularity: Yes/No

Small cell change: Yes/No

Prominent nucleoli: Yes/No

Sheet-like growth: Yes / No

Spontaneous necrosis: Yes No

Geographic necrosis: Yes / No

Brain invasion: Yes / No

Chordoid %

Clear cell %

Rhabdoid %

Papillary %

Follow up: Yes / No

Duration of follow up: months

Adjuvant therapy: Yes/No

Recurrence: Yes/No

Time to Recurrence:

FISH 14q: Deletion Yes/No

Normal copy:

Deletion:

Polysomy:

APPENDIX II

Procedure for sialinization of slides

1. Preparation of 2% (v/v) sialinized solution.

- Add 2ml of 3-aminopropyltriethoxysilane to 98ml acetone.

2. Procedure

- Dip the slides in sialinized solution for 20 seconds.
- Wash the slides in 2 changes of acetone, each lasting 15 seconds.
- Air dry the slides.

Preparation of 20X SSC stock solution

- Dissolve 8.1 gram of 20XSSC in 31.25 ml of milli-Q water.
- Adjust the pH to 5.3.
- Makeup the final volume to 50ml.

Preparation of working solution of 2XSSC/0.1% NP40

- Take 5ml of 20XSSC from the stock solution.
- Add 40ml of milli- Q water.
- Add 150 microlitre of NP 40
- Adjust the pH to 7.2-7.5.
- Make the final volume to 50 ml.

Preparation of working solution of 2XSSC/0.3% NP 40 used for 3 washes

- Take 15 ml of 20XSSC from the stock.
- Add 120 ml of milli-Q water.
- Add 450 microlitre of NP40
- Adjust the pH to 7.2-7.5.
- Make the final volume to 150ml.

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Ref: Res/2/2012

September 17, 2012

Dr. Noopur Gupta
PG Registrar
Department of Pathology
Christian Medical College
Vellore 632 002

Dear Dr. Noopur Gupta,

Sub: **FLUID Research grant project NEW PROPOSAL:**
Chromosome 14q FISH analysis in subsets of meningiomas segregated by MIB-1 labelling index.
Dr. Noopur Gupta, PG Registrar, Pathology, Dr. Geeta Chacko, Pathology.

Ref: IRB Min. No. 7928 dated 02.08.2012

I enclose the following documents:-

1. Institutional Review Board approval
2. Agreement

Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

CC: Dr. Geeta Chacko, Professor, Department of Pathology, CMC