Human epidermal growth factor receptor 2/neu protein expression in meningiomas: An immunohistochemical study

Ramesh Babu Telugu, Amit Kumar Chowhan, Nandyala Rukmangadhna, Rashmi Patnayak, Bobbidi Venkata Phaneendra, Bodapati Chandra Mowliswara Prasad, Mandyam Kumaraswamy Reddy

Department of Pathology, Christian Medical College Hospital, Vellore, Tamil Nadu, Departments of Pathology and Neurosurgery, Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, India

ABSTRACT

Background: Meningiomas are common slow-growing primary central nervous system tumors that arise from the meningothelial cells of the arachnoid and spinal cord. Human epidermal growth factor receptor 2 (HER2) or HER2/neu (also known as c-erbB2) is a 185-kD transmembrane glycoprotein with tyrosine kinase activity expressed in meningiomas and various other tumors. It can be used in targeted therapy for HER2/neu positive meningiomas.

Aim: To correlate the expression of HER2/neu protein in meningiomas with gender, location, histological subtypes, and grade.

Materials and Methods: It was a prospective study from March 2010 to October 2011 and retrospective study from May 2008 to February 2010. Immunohistochemistry for HER2/neu protein was performed along with scoring. Statistical analysis was done using Chi-square test to look for any association of HER2/neu with gender, location, grade, and various histological subtypes of meningiomas at 5% level of significance.

Results: A total of 100 cases of meningiomas were found during the study period. Of which, 80 were Grade I, 18 were Grade II, and 2 were Grade III meningiomas as per the World Health Organization 2007 criteria. The female-male ratio was 1.9:1 and the mean age was 47.8 years. HER2/neu protein was expressed in 75% of Grade I and 72.2% of Grade II meningiomas. About 72.7% brain invasive meningiomas showed HER2/neu immunopositivity.

Conclusion: HER2/neu protein was expressed in 73% of meningiomas. Statistically significant difference of HER2/neu expression was not seen between females and males of Grade I and Grade II/III meningiomas, and various histological subtypes of meningiomas.

Key words: Central nervous system, prognostic marker, receptor, targeted therapy, tumor

Introduction

Meningiomas are common slow-growing primary central nervous system (CNS) tumors that arise from the meningothelial cells of the arachnoid and spinal cord and account for 30% of all primary intracranial tumors.[1] These tumors show more predilection for women than males and often seen in middle-aged or elderly age group.[2] The World Health Organization (WHO) 2007 classification of tumors of CNS described 16 histological subtypes of meningiomas. Table 1 shows meningioma grades and histological subtypes according to the 2007 WHO classification.[2] Meningiomas were graded into three groups - benign (Grade I), atypical (Grade II), and anaplastic or malignant (Grade III).[2] In the study published by Ferry et al.,[3] tumor recurrence was observed in 7–20% of Grade I, 29–40% of Grade II, and 50–78% of Grade III meningiomas, respectively.
following surgery. Since Grade II/III meningiomas were associated with high tumor recurrence, additional treatment protocols with targeted therapy may be required with the available chemotherapeutic drugs.[4]

Human epidermal growth factor receptor 2 (HER-2/neu) or HER2 (also known as c-erbB2), proto-oncogene belonging to the family of tyrosine kinase growth factor receptors, is a 185-kD transmembrane glycoprotein with tyrosine kinase activity mapped on long arm of chromosome 17. It has a significant role in cell proliferation, apoptosis, cell motility, and cell adhesion.[5] HER2/neu protein overexpression and/or gene amplification have been identified in various types of malignancies including breast, gastric, ovarian, lung, pancreatic, prostatic, esophageal, renal, colorectal, endometrial carcinomas, and synovial sarcoma, and some of these tumors were associated with poor prognosis. HER2/neu protein can be a therapeutic target with monoclonal antibodies that interact with HER2/neu receptors.[6,7] In the present study, we aimed to correlate the positivity of HER2/neu expression between gender, location, various histologic subtypes, and grade of meningiomas.

Materials and Methods

This is a prospective (March 2010–October 2011) and retrospective (May 2008–February 2010) study of primary intracranial and intraspinal meningiomas diagnosed in the Department of Pathology. The study was approved by the Institutional Ethics Committee (IEC No. 132). The clinical details of the patients were noted from the computerized hospital information system. The tumor tissue samples fixed in 10% neutral-buffered formalin and paraffin-embedded tissue blocks were cut into 4–5 microns thin sections and stained with routine hematoxylin and eosin stain. Subtyping and grading of meningiomas were done according to the WHO 2007 criteria [Tables 1 and 2].[2] Immunohistochemical (IHC) staining was performed in 100 cases for HER2/neu protein by polymer-horseradish peroxidase (HRP) method.

Inclusion criteria

All the prospective (March 2010–October 2011) and retrospective (May 2008–February 2010) cases of meningiomas diagnosed in our department.

Exclusion criteria

1. Meningiomas with scanty tissue where immunostain could not be performed

2. Cases in which paraffin blocks could not be retrieved (cases coming from other hospitals where only slides were available).

Immunohistochemistry procedure

Three microns thin, formalin-fixed, paraffin-embedded tissue sections mounted on 3–3aminopropyltriethoxysilane precoated slides were prepared. The slides were incubated overnight at 37°C. For antigen retrieval, heat-induced method using pressure cooker (3 whistles) in Tris ethylenediaminetetraacetic acid pH 9.0 was applied. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide ($H_2O_2$). Sections were washed in TRIS buffer (pH 7.6) and incubated with anti-HER2/neu primary antibody (Rabbit monoclonal primary antibody, clone 4B5, Ventana) for 30 min at room temperature. The slides were treated by Poly-HRP Reagent and diaminobenzidine chromogen with intervening washes in TRIS buffer (pH 7.6). Then, counterstaining, dehydration, and mounting were performed. Section of HER2/neu positive invasive ductal carcinoma of the breast was used as positive control and negative control was by omitting the primary antibody. It was considered to be positive, if the tumor cells showed cytoplasmic membrane staining for HER2/neu antibody.

HER2/neu immunostaining was interpreted using the scoring system recommended for breast cancer [Table 3].[8]

### Table 1: Meningioma grades and histological subtypes according to the World Health Organization 2007 classification

| WHO grade | Histological subtypes |
|-----------|-----------------------|
| I         | Meningothelial, fibroblastic, transitional, psammomatous, angiomatous, microcystic, secretory, lymphoplasmacytic, metaplastic |
| II        | Atypical, chordoid, clear cell, brain invasive |
| III       | Anaplastic, papillary, rhabdoid |

WHO: The World Health Organization

### Table 2: The World Health Organization 2007 criteria for grading of meningioma

| WHO grade | Criteria |
|-----------|----------|
| I         | Mitosis <4/10 HPF |
| II        | Mitosis 4-19/10 HPF or 3 or more of the following five features |
|           | Increased cellularity |
|           | Uninterrupted patternless or sheet-like growth |
|           | Small cells with a high nuclear/cytoplasmic ratio |
|           | Prominent nucleoli |
| III       | Foci of “spontaneous” or “geographic” necrosis |
|           | Mitosis ≥20/10 HPF or exhibiting loss of differentiated features resulting in carcinoma, melanoma, or sarcoma-like appearances |

WHO: The World Health Organization, HPF: High power field
Scores of 0 and 1 + were considered negative, and scores of 2 + and 3 + were considered positive.

**Statistical analysis**

The statistical analysis done in this study was Chi-square test. \( P < 0.05 \) was considered to be statistically significant. Grade II and Grade III meningiomas were combined for statistical analysis since there were only two cases of Grade III meningioma in the study.

**Results**

There were a total of 100 (43 prospective and 57 retrospective) cases of meningiomas during the study period of which 89 were intracranial and 11 intraspinal. There were 66 females and 34 males with female to male ratio of 1.9:1 and the mean age was 47.8 years (range: 5–85 years). Among the 100 cases of meningioma, Grade I were 80%, Grade II 18%, and Grade III 2%. Most common intracranial location was cerebral convexity, and most common intraspinal location was thoracic segment. Of the 16 histological subtypes of meningioma described in the WHO 2007 classification of tumors of CNS, the present study includes 10 histological subtypes. Table 4 shows histological subtypes with grade. Although mentioned under Grade I in the WHO 2007 criteria, 27.3% of meningotheial, 14.3% each of psammomatous and angiomatous, 5.9% of fibroblastic, and 6.2% of transitional meningiomas were found to be of Grade II. Psammomatous meningioma (28%) was most common subtype followed by meningotheial (22%), fibroblastic (17%), and transitional (16%) meningioma. HER2/neu positive cytoplasmic membrane staining score of 2+ [Figure 1] and 3+ [Figure 2] was noted in 73% of meningiomas. Forty-six (63%) cases showed moderate (score, 2+) and 27 (37%) cases showed strong (score, 3+) HER2/neu expression. Highest HER2/neu immunopositivity was noted in meningotheial (81.8%) and metaplastic (100%) subtype followed by psammomatous (78.6%), transitional (75%), and fibroblastic (70.6%) meningiomas. HER2/neu immunopositivity in none of the histological subtypes was statistically significant at 5% level of significance with Chi-square test. HER2/neu was not expressed in anaplastic and papillary meningioma. Positivity of HER2/neu in overall females and males was 72.7% and 73.5%, respectively, which is not statistically significant including those of Grade I and Grade II/III meningiomas. Statistically significant relationship was not found between the positivity of HER2/neu in intracranial (73%) and intraspinal (72.7%) meningiomas. The positivity of HER2/neu within the Grade I (75%) was higher than that in the Grade II/III (65%) meningiomas; this difference did not reach statistical significance with Chi-square test [Table 5]. Brain invasion was noted in 11 cases of meningioma with HER2/neu positivity in 8 (72.7%) cases [Table 5]. All the cases were primary tumors, and no recurrent meningiomas were encountered in the study group.

**Discussion**

HER2/neu receptors in breast and gastric carcinomas has therapeutic implication as they are involved in tumor development, and it is well established in breast carcinoma.\[^6\] Some studies have also noted the HER2/neu expression in astrocytoma, oligodendroglioma, anaplastic astrocytoma, glioblastoma multiforme, medulloblastoma, and pituitary adenoma.\[^10\] According to Waage et al.,\[^13\] HER2 expression in neurons and meningeal cells is variable. However, glial cells are generally HER2 negative, but reactive astrocytes in gliotic tissue may be positive. Hence, HER2 expression is not useful to distinguish reactive gliosis from low-grade astrocytoma. However, there is strong evidence of HER2 involvement in malignant transformation of astrocytoma. The results of HER2 expression on oligodendroglomas and ependymomas are ambiguous. Coexpression of c-erbB2/HER2, c-erbB4, and MIB-1 has impact on cell proliferation. Ependymomas with coexpression of c-erbB2/HER2 and c-erbB4 have worst prognosis, and
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such association was not seen in oligodendrogliomas. Large cell and anaplastic medulloblastomas showed higher HER2 expression with positive correlation with increased proliferative activity, metastases, and worst outcome than classical and desmoplastic subtypes. HER2 expression in meningiomas ranged considerably from 2% to 100% without clear relation between expression and tumor grade. In the present study, HER2 expression was higher in Grade I meningiomas than in Grade II/III meningiomas in contrast to the typically increased expression in higher grade tumor. The results of prognostic significance of HER2 expression in meningiomas are ambiguous, with positive correlation to recurrence in some and others did not. In future strategies, HER2 has a significant role in diagnostic and targeted therapy in intracranial tumors.

Loussouarn et al.,[14] in 2006, showed HER2 immunopositivity in 28.5% of meningiomas with Grade I and Grade II/III meningiomas demonstrated 29.4% and 26.6% HER2 immunostaining. On HER2 positive meningiomas (6 cases with HER2 score 2+ and 4 cases with HER2 score 3+), FISH was performed. FISH revealed 40% meningiomas with increased number of gene copy which were HER2 strong positive. Similarly, increased number of HER2 gene copy was found in another study.[15] In our study, FISH was not performed.

Table 5: Relationship between gender, location, grade, subtype, and human epidermal growth factor receptor 2/neu expression

| Variables         | Total | Her2/neu (%) | P     |
|-------------------|-------|--------------|-------|
|                   | Positive | Negative |       |
| Gender            |         |             |       |
| Female            | 66      | 48 (72.7)   | 18 (27.3) | 0.932 |
| Male              | 34      | 25 (73.5)   | 9 (26.5)  |       |
| I                 |         |             |       |
| Female            | 53      | 38 (71.7)   | 15 (28.3) | 0.226 |
| Male              | 27      | 22 (81.5)   | 5 (18.5)  |       |
| II/III            |         |             |       |
| Female            | 13      | 8 (61.5)    | 5 (38.5)  | 0.658 |
| Male              | 7       | 5 (71.4)    | 2 (28.6)  |       |
| Location          |         |             | 0.983   |
| Intracranial      | 89      | 65 (73.0)   | 24 (26.9) |       |
| Spinal            | 11      | 8 (72.7)    | 3 (27.3)  |       |
| Grade             |         |             | 0.368   |
| I                 | 80      | 60 (75.0)   | 20 (25.0) |       |
| II/III            | 20      | 13 (65.0)   | 7 (35.0)  |       |
| Brain invasion    | 11      | 8 (72.7)    | 3 (27.3)  |       |
| Subtypes          |         |             |         |
| Men               | 22      | 18 (81.8)   | 4 (18.1)  | 0.291 |
| Fb                | 17      | 12 (70.6)   | 5 (29.4)  | 0.806 |
| T                 | 16      | 12 (75.0)   | 4 (25.0)  | 0.844 |
| Psa               | 28      | 22 (78.6)   | 6 (21.4)  | 0.434 |
| Ang               | 7       | 4 (57.1)    | 3 (42.9)  | 0.327 |
| Met               | 3       | 3 (100.0)   | 0 (0)     | 0.285 |
| Aty               | 2       | 1 (50.0)    | 1 (50.0)  | 0.459 |
| Cl                | 3       | 1 (33.3)    | 2 (66.7)  | 0.116 |
| A                 | 1       | 0 (0)       | 1         |       |
| Pap               | 1       | 0 (0)       | 1         |       |

Men: Meningothelial, Fb: Fibroblastic, T: Transitional, Psa: Psammomatous, Ang: Angiomatous, Met: Metaplastic, Aty: Atypical, Cl: Clear cell, Pap: Papillary, A: Anaplastic meningioma, Her2: Human epidermal growth factor receptor 2

Figure 1: Photomicrograph showing membrane staining (2+) of human epidermal growth factor receptor 2/neu in meningothelial meningioma (IHC, ×200)

Figure 2: Photomicrograph showing membrane staining (3+) of human epidermal growth factor receptor 2/neu in transitional meningioma (IHC, ×200)

Men: Meningothelial, Fb: Fibroblastic, T: Transitional, Psa: Psammomatous, Ang: Angiomatous, Met: Metaplastic, Aty: Atypical, Cl: Clear cell, Pap: Papillary, A: Anaplastic meningioma, Her2: Human epidermal growth factor receptor 2
Torp et al.[16] performed HER2 immunostaining on frozen sections and reported 63.1% HER2 positive meningiomas. In his study, HER2 was positive in benign (11/16) and anaplastic meningiomas (1/3). Potti et al.[17] showed 2.53% of HER2/neu overexpression by immunohistochemistry in meningiomas and stated that HER2 overexpression has no role either as a prognostic factor or biological behavior of meningioma. Loussouarn et al.[14] considered HER2 expression in meningioma was always an overexpression and used a different semi-quantitative four-point scale grading system. His study revealed that tumor recurrence rate was low in HER2 negative meningiomas than in HER2 positive meningiomas. HER2 protein overexpression may play a role as a prognostic factor in meningiomas. In the present study, there are no recurrent cases at all among the HER2/neu immunoreactive meningiomas. HER2/neu positivity was more in Grade I tumors (75%) than in Grade II/III meningiomas (65%), and it was not statistically significant at 5% level of a significance similar to the study by Loussouarn et al.[14] In a study performed by Wang et al.[18] HER2 immunopositivity was higher in recurrent benign, atypical, and anaplastic meningiomas when compared to nonrecurrent benign tumors and was statistically significant (P < 0.05). There was a positive correlation between HER2 with Ki-67. HER2/neu gene amplification increased in 2+ and 3+ immunopositive meningiomas and was not statistically significant. HER2 and Ki-67 may be used objectively to predict the biological nature of meningiomas. In another study also, HER2 expression was statistically significant with Ki-67.[19] Laurendeau et al.[20] studied expression of Erb B1, Erb B2, Erb B3, and Erb B4 receptors and ligand genes by real-time polymerase chain reaction in various grades of meningiomas and found strong erbB1-erbB2 overexpression and erbB3-erbB4 underexpression in meningioma and suggested that these findings might be useful for gene therapy. In another study published in 2011[21] revealed highest HER2 immunopositivity in meningothelial subtype of meningioma. Atypical/anaplastic meningioma types showed highest rate HER2 immunopositivity than benign meningiomas. However, there was no statistically significant difference between tumor grade and HER2/neu. Highest tumor recurrence rate and shorter mean survival rate were observed among HER2 positive meningiomas than in HER2 negative meningiomas.

Complete surgical excision is the treatment of choice for all symptomatic meningiomas, followed by radiotherapy in selected cases. Hydroxyurea, interferon-alpha, mifepristone, tamoxifen alone, or in combination with calcium channel blockers and newer molecular inhibitors could be effective in treatment of recurrent unresectable meningiomas. Among these drugs, hydroxyurea is the most commonly used a chemotherapeutic agent in recurrent meningiomas.[22,23]

**Limitations**

1. There were no recurrent tumors in the present study to evaluate the prognostic significance of HER2/neu in recurrent meningiomas
2. Follow-up data were available only in a few cases. This is required to ascertain true prognostic relevance of HER2/neu expression
3. 100 cases were selected for the study, owing to financial constraints.

**Conclusion**

HER2/neu protein was expressed in 73% of meningiomas including 75% of Grade I and 65% of Grade II/III meningiomas. Slightly higher HER2/neu immunopositivity was seen in males compared to females. Gender, location, and tumor grade showed no statistically significant relationship with HER2/neu protein expression. Highest HER2/neu immunopositivity was noted in meningothelial and metaplastic subtype. About 72.7% brain invasive meningiomas showed HER2/neu immunopositivity. The role of HER2/neu expression as a prognostic factor and in targeted therapy has not yet been established, and larger studies are required to evaluate the possibilities.

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**Conflicts of interest**

There are no conflicts of interest.

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