Correlation of Expression Transforming Growth Factor-β1, E-cadherin, and Ki-67 in Meningiomas

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Abstract

BACKGROUND: Meningioma is the most common primary intracranial tumors in adults, accounts for 36% of total intracranial tumors. Obtaining the clinicopathological characteristics of patients with meningioma and investigating, the association between signaling pathways with disease progression could provide a basis for therapeutic development.

AIM: This study aims to investigate the expression of transforming growth factor-β1 (TGF-β1), E-cadherin, and Ki-67 in meningiomas.

MATERIALS AND METHODS: This study examined the expression levels of E-cadherin, Ki-67, and TGF-β1 with respect to the WHO grade in patients with meningioma. A total of 62 meningioma samples were analyzed. By the WHO criteria, 54 specimens were diagnosed as the WHO Grade 1, 6 as Grade 2, and 2 as Grade 3. Grade 1 was classified as low-grade meningioma, while Grade 2 and Grade 3 were classified as high-grade meningioma (HGM).

RESULTS: In this study, the mean age diagnosis was 41.97 ± 9.79 years old, with female: male ratio of 8:1. There was no association between age, sex, and tumor location with the progression of meningioma. Immune-characterization studies reported that dysregulation or loss of TGF-β contributes to malignant transformation, especially in tumor development and progression. The loss of E-cadherin is regarded as a major molecular event for dysfunction in cell-cell adhesion, particularly during epithelial–mesenchymal transition (EMT) [9].

E-cadherin is a crucial molecule in intercellular adhesion in epithelial tissues. It is localized on the surfaces of epithelial cells in regions of cell-cell contact known as an adherent junction [8]. Besides its role in physiological condition, this highly conserved gene contributes to malignant transformation, especially in tumor development and progression. The loss of E-cadherin is regarded as a major molecular event for dysfunction in cell-cell adhesion, particularly during epithelial–mesenchymal transition (EMT) [9].

CONCLUSION: This study concluded that HGM was highly proliferative (high Ki-67) and invasive (low E-cadherin), with dysregulated TGF-β1 signaling. In addition, younger age at diagnosis and high female: male ratio in our series suggests that Indonesian females might possess specific risk factors for having meningioma.
that E-cadherin was dysregulated in meningioma and might contribute to meningioma agenesis [11],[12].

The association between TGF-β1 and E-cadherin has been reported in several tumors. In gastric cancer, TGF-β1 overexpression contributes to decreased expression of E-cadherin; this phenomenon involves in the progression of gastric cancer[13]. A similar association was observed in colorectal cancer, TGF-β1 expression is inversely correlated with E-cadherin expression [14]. In this study, we aim to investigate TGF-β1 and E-cadherin expression in meningioma, to clarify the association between TGF-β1, E-cadherin, and Ki-67, and to gain better insight regarding those signaling in meningioma genesis. In addition, we also aim to obtain the clinicopathological characteristics of patients with meningioma in Indonesia and to provide a basis for therapeutic development.

Materials and Methods

Patients

This retrospective study included 62 patients at Raden Mattaher Hospital, Jambi, who underwent surgical resection from January 2012 to December 2017, in which the diagnosis of meningioma was established. Clinical data were collected from archival medical records. Surgically resected tumor tissues were fixated in 10% formaldehyde and preserved in paraffin-embedded blocks. Tumors were then classified and graded according to the 2016 WHO classification of CNS tumors [15]. Clinical data of patients are summarized in Table 1. Clinical data of patients are summarized in Table 1.

Immunohistochemistry (IHC)

Four-micron thickness slides from the tumor tissue were deparaffinized and rehydrated. Antigen retrieval was carried out with microwave and antigen retrieval solution (citrate buffer 10 mmol/L, pH 6.0). Sections were then allowed for cooling at room temperature, followed by washing 3×, each for 5 min with phosphate-buffered saline (PBS). Endogenous peroxidase activity was blocked by dipping sections in 3% H2O2 for 3 min, then 0.3% H2O2 in Tris-HCl buffer (pH 7.6). The specificity of staining was confirmed by the absence of staining when the primary antibodies were omitted. The slices were counterstained with hematoxylin and mounted with Entellan (Millipore, USA).

IHC analysis

Slices were observed and images were captured with Olympus BX51 (Tokyo, Japan) by a blinded experimenter. The percentage of Ki-67+ cells was counted on five non-overlapping fields at ×40 objective magnification, at least 500 cells. Semi-quantitative scoring was utilized to determine TGF-β1

| No | Sex | Age | Location | Histological type | WHO grade |
|----|-----|-----|----------|------------------|-----------|
| 1  | Female | 46 | Cranial vault | Meningotheliomatous | 1 |
| 2  | Male  | 66 | Cranial vault | Meningotheliomatous | 1 |
| 3  | Female | 43 | Cranial vault | Meningotheliomatous | 1 |
| 4  | Female | 31 | Cranial vault | Meningotheliomatous | 1 |
| 5  | Female | 42 | Cranial vault | Psammomatous | 1 |
| 6  | Female | 55 | Cranial vault | Meningotheliomatous | 1 |
| 7  | Female | 42 | Cranial vault | Psammomatous | 1 |
| 8  | Female | 39 | Cranial vault | Meningotheliomatous | 1 |
| 9  | Male  | 50 | Skull base | Meningotheliomatous | 1 |
| 10 | Female | 43 | Cranial vault | Meningotheliomatous | 1 |
| 11 | Female | 40 | Cranial vault | Meningotheliomatous | 1 |
| 12 | Female | 29 | Cranial vault | Angiomatous | 1 |
| 13 | Female | 38 | Cranial vault | Meningotheliomatous | 1 |
| 14 | Male  | 38 | Cranial vault | Angiomatous | 1 |
| 15 | Female | 28 | Skull base | Meningotheliomatous | 1 |
| 16 | Female | 43 | Skull base | Fibroblastic | 1 |
| 17 | Female | 45 | Cranial vault | Psammomatous | 1 |
| 18 | Female | 28 | Skull base | Meningotheliomatous | 1 |
| 19 | Female | 56 | Cranial vault | Meningotheliomatous | 1 |
| 20 | Female | 42 | Skull base | Meningotheliomatous | 1 |
| 21 | Female | 34 | Cranial vault | Meningotheliomatous | 1 |
| 22 | Female | 43 | Skull base | Meningotheliomatous | 1 |
| 23 | Male  | 35 | Cranial vault | Angiomatous | 1 |
| 24 | Female | 32 | Cranial vault | Psammomatous | 1 |
| 25 | Female | 47 | Skull base | Meningotheliomatous | 1 |
| 26 | Female | 23 | Cranial vault | Fibroblastic | 1 |
| 27 | Male  | 33 | Cranial vault | Angiomatous | 1 |
| 28 | Female | 48 | Cranial vault | Meningotheliomatous | 1 |
| 29 | Female | 45 | Cranial vault | Meningotheliomatous | 1 |
| 30 | Female | 36 | Skull base | Meningotheliomatous | 1 |
| 31 | Female | 46 | Skull base | Meningotheliomatous | 1 |
| 32 | Female | 68 | Cranial vault | Psammomatous | 1 |
| 33 | Male  | 41 | Cranial vault | Meningotheliomatous | 1 |
| 34 | Female | 30 | Cranial vault | Meningotheliomatous | 1 |
| 35 | Female | 24 | Cranial vault | Angiomatous | 1 |
| 36 | Female | 43 | Skull base | Meningotheliomatous | 1 |
| 37 | Female | 46 | Cranial vault | Meningotheliomatous | 1 |
| 38 | Female | 60 | Skull base | Meningotheliomatous | 1 |
| 39 | Female | 56 | Cranial vault | Angiomatous | 1 |
| 40 | Female | 43 | Cranial vault | Meningotheliomatous | 1 |
| 41 | Female | 38 | Cranial vault | Meningotheliomatous | 1 |
| 42 | Female | 44 | Cranial vault | Angiomatous | 1 |
| 43 | Female | 32 | Skull base | Meningotheliomatous | 1 |
| 44 | Female | 35 | Cranial vault | Fibroblastic | 1 |
| 45 | Male  | 44 | Skull base | Angiomatous | 1 |
| 46 | Male  | 48 | Cranial vault | Meningotheliomatous | 1 |
| 47 | Female | 26 | Skull base | Angiomatous | 1 |
| 48 | Female | 39 | Cranial vault | Meningotheliomatous | 1 |
| 49 | Female | 40 | Cranial vault | Psammomatous | 1 |
| 50 | Female | 43 | Cranial vault | Meningotheliomatous | 1 |
| 51 | Female | 38 | Skull base | Meningotheliomatous | 1 |
| 52 | Female | 36 | Skull base | Fibroblastic | 1 |
| 53 | Female | 30 | Cranial vault | Microcytic | 1 |
| 54 | Female | 40 | Cranial vault | Psammomatous | 1 |
| 55 | Female | 44 | Cranial vault | Atypical | 1 |
| 56 | Female | 51 | Skull base | Chordoid | 2 |
| 57 | Female | 51 | Skull base | Angiomyxoid | 1 |
| 58 | Female | 66 | Cranial vault | Chordoid | 2 |
| 59 | Female | 33 | Skull base | Atypical | 2 |
| 60 | Female | 35 | Skull base | Atypical | 2 |
| 61 | Female | 48 | Cranial vault | Anaplastic | 3 |
| 62 | Female | 45 | Cranial vault | Anaplastic | 3 |
and E-cadherin immunoreactivity. The average counts of the five regions were used for the final report.

Research ethics

Ethical approval was obtained from the Faculty of Medicine, Andalas University, and Dr. M. Djamil Hospital, Padang, Committee of Ethics.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 7.03. The data were evaluated for normality distribution before statistical comparison. Fisher Exact test was used to examine the difference between female and cranial vault proportion between low-grade meningioma (LGM, WHO Grade I) and high-grade meningioma (HGM, WHO Grade II-III). T-test was used to examine the difference of age at diagnosis between LGM and HGM. The Mann–Whitney U-test was used to examine the difference between LGM and HGM, in terms of Ki-67, E-cadherin, and TGF-β1 expression. The association between each variable was evaluated by linear regression. The statistically different consider significant when p < 0.05.

Results

Patients characteristics

We enrolled 62 patients diagnosed with cranial meningiomas, which tumor specimens were available. By the WHO criteria [15], 54 of the specimens were diagnosed as Grade I, six as Grade II, and two as Grade III. In the case of Grade I meningioma, meningiotheliomatous was the most common type, about 59.26% (32/54), followed by angiomatous (18.52%), psamommatous (12.96%), fibroblastic (7.41%), and microcystic (1.85%). In the case of Grade II, atypical was the most common type, about 66.67% (4/6), the other type was chordoid (33.33%). All Grade III meningiomas were anaplastic variants (2/2). To ease further analysis, we classified Grade I meningiomas as LGM and Grade II-III meningiomas as HGM.

There was no difference in the mean age at the diagnosis of meningioma between LGM and HGM (41.28 ± 9.61 vs. 46.63 ± 10.32, p = 0.1508). In this study, meningioma was rarely observed in the elderly population (>65 years old, 4.84% [3/62]). The female proportion was not statistically different between LGM and HGM (46/54 vs. 8/8, p = 0.5810). In this study, we observed a high female proportion (±8:1), which higher than literatures [2,16]. There was no difference in cranial vault meningioma proportion between LGM (70.37%, 38/54) and HGM (62.50%, 5/3, p = 0.6917).

Patient characteristics are summarized in Table 1.

IHC results of meningioma patients

To gain insight about TGF-β1 and E-cadherin association in meningioma, we performed IHC to examine: Ki-67, TGF-β1, and E-cadherin. We observed that HGM tumor specimens were more proliferative than LGM (Ki-67+ cells: 11.38 ± 2.15% vs. 3.83 ± 1.05%, p < 0.0001, Mann–Whitney U-test, Figures 1a, d, g and 2a).

Figure 1: Representative immunostaining on meningioma tissues. Ki-67 was nuclear staining (left panel). E-cadherin (middle panel) was cytoplasmic staining. TGF-β1 was cytoplasmic and extracellular matrix staining (right panel). Low Ki-67+ meningiomas were associated with high expression of E-cadherin and TGF-β1 (upper panel). Moderate Ki-67+ meningiomas were associated with moderate expression of E-Cadherin and TGF-β1 (middle panel). High Ki-67+ meningiomas were associated with low expression of E-cadherin and TGF-β1 (lower panel). Scale Bar = 100 μm. TGF-β1: Transforming growth factor-β1

In this study, we observed that E-cadherin expression was lower in HGM than LGM (7.32 ± 2.17 vs. 4.25 ± 1.17, p < 0.0001, Mann–Whitney U-test, Figures 1b, e, h and 2b). TGF-β1 expression was also observed to be lower in HGM than LGM (6.39 ± 2.03 vs. 3.75 ± 1.58, p = 0.0002, Mann–Whitney U-test, Figures 1c, f, i and 2c). Further analysis revealed that the number of Ki-67+ cells was inversely correlated with E-cadherin and TGF-β1 expression (Figure 2d and e). We also confirmed that E-cadherin expression was associated with TGF-β1 expression (Figure 2f).

Discussion

In this series, the majority of meningiomas were LGM (87.10%, 54/62), this finding is consistent with reported literatures, LGM accounts for 70–90% of total meningiomas [17],[18] suggesting that the proportion of aggressive meningiomas (HGM) in Indonesia is similar with other countries. The majority of meningiomas
were located in the cranial vault (convexity, falx, and parasagittal), accounts for 69.35% (43/62) of total patients; this finding resembles the reported proportion in India [19]. Additionally, during 2012–2018, in Dr Hasan Sadikin Hospital (Tertiary Neurosurgery Center, Bandung, Indonesia), cranial vault meningioma only accounts for ±25% from a total of 717 meningiomas (Hermanto and Faried, unpublished data). This discrepancy might be attributed to genetic background as well as the tendency for referring difficult cases (skull base meningioma) to the tertiary care hospital.

In this series, meningiomas were commonly observed in middle-aged (30–55 years old) population with a female predominance (8:1); meanwhile, the reported incidence of meningioma is higher in elderly, with the female:male ratio of 3.5:1 [2],[19],[20]. The previous study indicated that male and convexity meningiomas are the risk factors for having HGM [2],[21]; however, we did not those associations in our series. Together, our findings suggest that Indonesian females possess specific risk factors that might increase the likelihood of having meningioma.

We observed a unique association between WHO Grade, Ki-67, E-cadherin, and TGF-β1, suggesting HGM was highly proliferative (high Ki-67+) and invasive (low E-cadherin), with dysregulated TGF-β1 signaling. The role of TGF-β signaling in cancer is intriguing, exhibiting context-dependent effects. It may act as a tumor suppressing factor or tumor promoting factor. In normal cells and early-stage cancers, TGF-β inhibits cell growth and promotes apoptosis [22]. However, its activation in late-stage cancers promotes cell growth, immune evasion, angiogenesis, chemoresistance, and invasion [22]. Several reports indicated that the upregulation of TGF-β in cancers promotes downregulation of E-cadherin and contributes to the EMT [13],[14],[23]. Interestingly, we found that TGF-β1 is downregulated in HGM, although HGM is more proliferative and invasive. Previous studies demonstrated that TGF-β addition inhibits meningioma cell proliferation [6],[24]. Gene expression profile also indicated that there is a loss of TGF-β signaling in HGM [7]. Hence, it seems likely that TGF-β exerts an inhibitory effect on LGM and that loss of TGF-β signaling results in progression to malignancy.

This study is limited by a small number of HGM, the malignant tumor itself is known for its intra- and inter-tumoral heterogeneity. Of note, several reports indicated that activation of TGF-β signaling promotes proliferation and meningioma cells [5],[25], therefore further in vitro and in vivo investigations are needed to clarify the context-dependent of TGF-β signaling in meningioma. Younger age at diagnosis and high female: male ratio in our series suggests that Indonesian females might possess specific risk factors for having meningioma; it also needs for further investigation.

In this study, we found that HGM was highly proliferative (high Ki-67+) and invasive (low E-cadherin), with dysregulated TGF-β1 signaling. TGF-β1 is downregulated in HGM; hence, it seems likely that TGF-β exerts an inhibitory effect on LGM and that loss of TGF-β signaling results in progression to malignancy. In addition, younger age at diagnosis and high female: male ratio in our series suggest that Indonesian females might possess specific risk factors for having meningioma.
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