ANALYSIS OF MINICHROMOSOME MAINTENANCE-2 (MCM-2) AND CYCLIN D1 EXPRESSION IN MENINGIOMA

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ABSTRACT

World Health Organization histopathological grading of meningioma is associated with recurrence and clinical outcome. Grade II meningioma can be difficult to distinguish with grade I especially in case in which mitosis is not easily identified. MCM-2 and Cyclin D1 play crucial role in cell cycle and have been reported overexpressed in many malignant tumors. The aim of this observational analytic study was to analyze the differences and correlation between MCM-2 and Cyclin D1 in various meningioma grading. Immunohistochemystry with MCM-2 and Cyclin D1 was performed on 25 paraffin blocks of grade I, II and III meningiomas at the Laboratory of Anatomical Pathology, Dr. Soetomo Hospital. The immunoexpression are evaluated using Labelling Index, then analyzed statistically. The results showed significant difference in expression of MCM-2 within various meningioma grading (p=0.000) and significant difference in expression of Cyclin D1 within grade I and II also within grade I and III (p<0.050), but not in grade II and III (p>0.050). There was also positive correlation between MCM-2 and Cyclin D1 expression in various meningioma grading (rs=0.683, p=0.000). MCM-2 may play role in distinguishing various meningioma grading. Cyclin D1 can distinguish grade I and II also grade I and III, but not grade II and III. Overexpression MCM-2 was along with Cyclin D1 in various meningioma gradings.

***Keywords**: Meningioma; MCM-2; Cyclin D1*

ABSTRAK

Derajat histopatologi meningioma menurut WHO berkaitan dengan kekambuhan dan prognosis. Membedakan meningioma derajat II dengan derajat I dapat menjadi sulit karena bergantung pada jumlah mitosis dan pengamatan subjektif. MCM-2 dan Cyclin D1 berperan dalam siklus sel dan telah dilaporkan terekspresi berlebihan pada banyak neoplasma ganas. Penelitian ini bertujuan untuk menganalisis perbedaan dan korelasi antara MCM-2 dan Cyclin D1. Pulasan imunohistokimia dengan antibodi MCM-2 dan Cyclin D1 dilakukan pada 25 sampel meningioma derajat I, II, dan III di Laboratorium Patologi Anatomik, RSUD Dr. Soetomo Surabaya, dihitung menggunakan Labelling Index, kemudian dilakukan uji statistik. Hasil penelitian menunjukkan perbedaan bermakna ekspresi MCM-2 pada meningioma derajat I, II, dan III (p=0.000). Terdapat perbedaan bermakna ekspresi Cyclin D1 antara derajat I dan II serta derajat I dan III (p<0.050), tetapi tidak terdapat perbedaan antara derajat II dan III (p>0.050). Terdapat korelasi antara ekspresi MCM-2 dan Cyclin D1 pada semua derajat meningioma (rs=0.683, p=0.000). MCM-2 dapat digunakan untuk membedakan meningioma derajat I, II, dan III. Cyclin D1 dapat membedakan meningioma derajat I dan II serta derajat I dan III tetapi tidak dapat membedakan derajat II dan III. Peningkatan ekspresi MCM-2 seiring dengan Cyclin D1 pada semua derajat meningioma.

***Kata kunci**: Meningioma; MCM-2; Cyclin D1*

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pISSN:2355-8393 ● eISSN: 2599-056x ● doi: http://dx.doi.org/10.20473/fmi.v54i3.10016
● Fol Med Indones. 2018;54:207-212 ● Received 20 Dec 2017 ● Accepted 7 Jun 2018

INTRODUCTION

Meningioma is one of the most frequent primary brain neoplasms, comprising approximately 30-35% of all central nervous system tumors (Domingues et al 2015). The World Health Organization histological grading of meningiomas is associated with recurrence and clinical outcomes. This histological grading can be particularly difficult and render to subjective assessment. Novel biomarkers are needed to improve diagnostic and prognostic accuracy (Gauchotte et al 2012).

Uncontrolled cellular proliferation is a sign of neoplastic cells. The basic prerequisite is misregulated DNA replication. Normal DNA replication is limited to a single round per cell cycle. Protein that initiates DNA replication is called prereplicative complex (pre-RC) proteins. MCM-2 is a subunit of Minichromosome Maintenance protein heterohexamer complex which form a part of the pre-RC. This protein limits the replication not more than once per cell cycle. MCMs overexpression has been reported in various human malignancies (Gauchotte et al 2012, Giaginis et al 2010).

Cyclins, together with Cyclin-Dependent Kinases (CDKs), are proteins responsible for the orderly progression of cells through the cell cycle. Cyclin D is the first elevated cyclin in the cell cycle, arises in the mid G1 phase and no longer detected at the S phase. It activates CDK4, then phosphorylates the retinoblastoma protein (pRB), promoting cell replication after E2F is released. Overexpression of Cyclin D1 has been associated with shorter G1 phase and abnormal cell proliferation. This has been reported in many malignant tumors (Moraes et al 2012, Cheng et al 2015). The aim of this study was to analyze the differences and correlation between MCM-2 and Cyclin D1 in various meningioma gradings.

MATERIALS AND METHODS

This was an analytical observational study with cross-sectional approach conducted in the Laboratory of Anatomical Pathology, Dr. Soetomo Hospital, using paraffin blocks of meningioma between January 2012-December 2016. Grade I and II meningiomas were randomly sampled with 10 samples each, while grade III meningiomas (5 samples) were totally included. Total samples used were 25 samples. This study had been approved by Ethical Committee of Dr. Soetomo Hospital, Surabaya (489/Panke.KKE/VIII/2017) and has no conflict of interest.

Immunohistochemical detection of the biomarkers MCM-2 and Cyclin D1 was performed on paraffin-embedded 4 µm tissue sections, stained with monoclonal mouse antibody MCM2 Ab-1 (Clone CRCT2.1, Thermo Fisher Scientific, UK) diluted in 1:200 and monoclonal rabbit antibody Cyclin D1 (Clone SP4, Diagnostic BioSystems, The Netherlands) diluted in 1:100. Only nuclear staining was considered positive. Tonsil was used as positive control for MCM-2 expression and breast cancer was for Cyclin D1 expression. Both expressions were recorded into Labelling Index (LI). LI was calculated by dividing the number of positive cells by the total number of cells counted in the hot spot area and multiplied by 100%. One thousand cells were counted in five High Power Field (Razavi et al 2015).

MCM-2 expression in grade I, II, and III meningiomas were analyzed with Brown-Forsythe and Games-Howell test. Cyclin D1 expression in grade I, II, and III meningiomas were analyzed with One-way ANOVA and Tukey HSD test. Correlation between MCM-2 and Cyclin D1 expression in various meningioma gradings was analyzed with Spearman correlation test ($p<0.050$ was considered as significant).

RESULTS

Characteristics of samples are summarized in Table 1. Mean age was 44.44 ± 8.25 years old, with largest frequency was in the age group of 41-50 years. There was predominance in female gender. Most of tumors were located in parasagittal, convexity, and sphenoid wing.

Table 1. Clinicopathological data of patients and tumors

Parameters	Frequency	Percentage (%)
Age span in years		
21-30	2	8
31-40	4	16
41-50	15	60
51-60	3	12
>60	1	4
Sex		
Male	7	28
Female	18	72
Tumor location		
Parasagittal	8	32
Convexity	7	28
Sphenoid wing	5	20
Falcine	2	8
Tuberculum sellae	1	4
Cerebellopontine angle	1	4
Retrobulbar	1	4
Histopathological grading		
I	10	40
II	10	40
III	5	20

MCM-2 expression was found in all grade II and III meningiomas. Grade II meningiomas showed lower LI of MCM-2 (range 5%-48%) compared to grade III meningiomas (range 42%-80%). MCM-2 expression in grade I meningiomas was varied from negative to less than 15%. Immunohistochemistry staining of MCM-2 was illustrated in Fig. 1.

Brown-Forsythe test found significant difference in the expression of MCM-2 within grade I, II, and III menin-

gioma (p=0.000). Games-Howell test for multiple comparisons showed significant difference in expression of MCM-2 between all meningioma gradings (Table 2). ROC curve resulted 2 cut-off values for MCM-2 expression. Cut-off 12.0% was obtained to distinguish grade I and II meningioma (sensitivity 80%, specificity 90%). Cut-off 39.5% was obtained to distinguish grade II and III meningioma (sensitivity 100%, specificity 90%). There was positive correlation between MCM-2 expression within all meningioma gradings, analyzed by Spearman correlation test (rs=0.855, p=0.000).

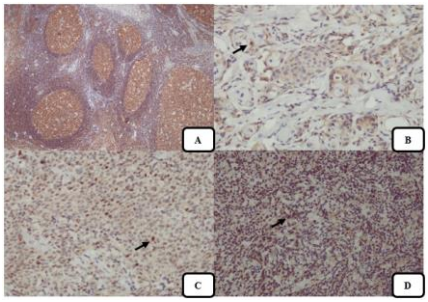

Fig. 1. MCM-2 expression (immunohistochemistry staining) A. Control tissue (100x magnification) B. MCM-2 expression in Grade I meningioma (400x magnification) C. MCM-2 expression in Grade II meningioma (400x magnification) D. MCM-2 expression in Grade III meningioma (400x magnification).

Cyclin D1 expression was found in all grade meningiomas, varied from 10% in grade I meningioma up to 86% in grade III meningioma. The lower mean of LI was found in grade I meningioma (26.80±12.39) compared to grade III meningioma (63.80±24.51). Immunohistochemistry staining of Cyclin D1 was illustrated in Fig. 2.

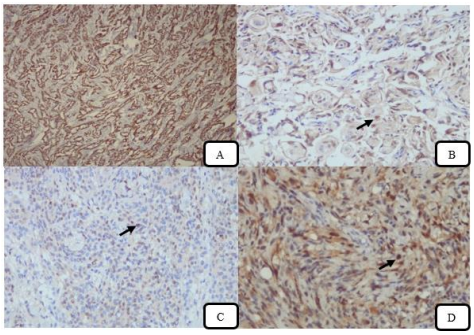

Fig. 2. Cyclin D1 expression (immunohistochemistry staining) A. Control tissue (100x magnification) B. Cyclin D1 expression in Grade I meningioma (400x magnification) C. Cyclin D1 expression in Grade II meningioma (400x magnification) D. Cyclin D1 expression in Grade III meningioma (400x magnification).

One-way Anova test found significant difference in the expression of Cyclin D1 at least between one pair grade (p=0.000). Tukey-HSD test for multiple comparisons showed significant difference in the expression of Cyclin D1 within grade I and II meningioma, also grade I and III, but no difference within grade II and III (Table 3). ROC curve resulted 2 cut-off value for Cyclin D1 expression. Cut off 39.5% was obtained to distinguish grade I and II meningioma (sensitivity 100%, specificity 90%). Cut off 55.5% was obtained to distinguish grade II and III meningioma (sensitivity 80%, specificity 50%). There was positive correlation between Cyclin D1 expression within all meningioma grading, analyzed by Spearman correlation test (rs=0.731, p=0.000).

Table 2. MCM-2 expression in various meningioma gradings

Grade	n	MCM-2 Expression (%)				p
		Mean	SD	Min	Max	
I	10	5.10[a]	4.93	0	14	
II	10	21.60[b]	13.42	5	48	0.000*
III	5	63.40[c]	16.52	42	80	

Notes : * significant in α=0.05 (Brown-Forsythe)
a,b,c different superscript showed difference between groups (Games-Howell)

Table 3. Cyclin D1 expression in various meningioma grading

Grade	n	Cyclin D1 Expression (%)				p
		Mean	SD	Min	Max	
I	10	26.80[a]	12.39	10	50	
II	10	55.70[b]	8.64	41	70	0.000*
III	5	63.80[b]	24.51	25	86	

Notes: * significant in α=0.05 (Oneway Anova)
[a,b] different superscript showed difference between groups (Tukey HSD)

Correlation between MCM-2 and Cyclin D1 expression in meningioma was analyzed with Spearman correlation test (Fig. 3). The test showed positive correlation between MCM-2 and Cyclin D1 expression within all meningioma gradings (rs=0.683; p=0.000).

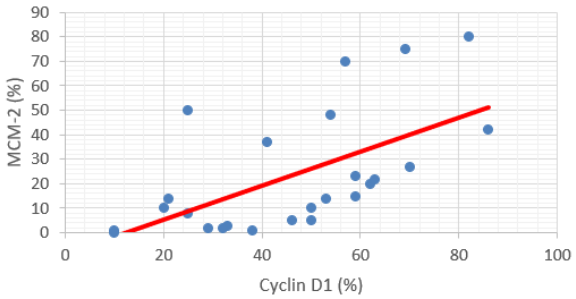

Fig. 3. MCM-2 and Cyclin D1 expression in meningioma.

DISCUSSION

Characteristics of samples

This study used 25 paraffin blocks of meningiomas. The largest frequency was in the age group of 41-50 years (60%), in accordance with previous study that meningioma mostly occurred in middle ages, fourth decade, age group of 41-50 years (Gangadhar et al 2013, Bhat et al 2014, Desai & Patel 2015). As stated on epidemiology data by Perry et al (2016) and Park (2017), the incidence of meningioma is higher in females (72%) than males. Most tumor in this study were located in parasagittal (32%), convexity (28%), sphenoid wing (20%), falcine (8%), and tuberculum sellae, cerebellopontine angle, and retrobulbar (each 4%), in accordance with the literatures fact that meningioma mostly occurred in supratentorial (Perry et al 2016).

MCM-2 Expression

MCM-2 is a subunit of Minichromosome Maintenance protein heterohexamer complex (MCM2-7). The MCM protein complex is associated with the origins of DNA replication to form part of the pre-replicative complex (pre-RC). MCM proteins are essential for the process of DNA replication, functioning as license components for the S-phase of cell cycle initiation and further exerting weak helicase activity to unwind DNA from its supercoiled state at replication forks (Giaginis et al 2010). Immunohistochemistry was done in this study to analyze the expression of MCM-2 in each group of meningioma.

In this study, the highest mean of MCM-2 expression was found in grade III with 63.40 ± 16.52, meanwhile the lowest was found in grade I with 5.10 ± 4.93. Statistic analysis showed significant difference in expression of MCM-2 within all meningioma grading (p=0.000).

Study of MCM-2 expression has not been much done. Previous research showed there was an increased of MCM-2 expression significantly in recurrent meningioma, although the WHO histological grading has not been stated. These results suggest that analysis of MCM-2 expression may facilitate identification of patients with a high risk of recurrence, which the most were high grade meningioma (Grade II and III) (Giaginis et al 2010, Hunt et al 2002).

ROC curve analysis resulted 2 cut off value for MCM-2 expression. Cut off 12.0% was obtained to distinguish grade I and II meningioma (sensitivity 80%, specificity 90%). Cut off 39.5% was obtained to distinguish grade II and III meningioma (sensitivity 100%, specificity 90%). Therefore, MCM-2 expression can be used to classify meningioma histopathological grading into grade I, II, and III.

Statistical analyses with Spearman correlation test revealed that MCM-2 expression was positively correlated with meningioma grading (rs=0.855, p=0.000). It showed that the increased of MCM-2 expression was along with meningioma grading. Previous study about the correlation between MCM-6 expression and meningioma histopathological grading demonstrated a strong correlation (Gauchotte et al 2012).

The regulation of MCM-2 expression depends on p53 pathway. The p53 gene mutation play a role in meningioma tumorigenicity. The p53 gene as tumor supressor gene induces the expression of various genes including p21. The p53 mutation results in inactivation of p21, leading to CDK binding with cyclin. The pRB phosphorylation activates E2F, leading to synthesis of CDC-7p, Dbf-4p, CDC-6p, enzym, and MCM-p. The latter has role in the early G phase of the cell cycle to form the origin complex called the pre-replication complex (pre-RC). The hexameric MCM component of the pre-RC shows helicase activity that may provide DNA unwinding services during replication. Overexpressed MCM-p leads to overreplication and induce high cell proliferations (Sudiana 2011).

Cyclin D1 Expression

Previous studies have suggested that Cyclin D1 is highly expressed in cells derived from breast, gastric,

esophageal and colon cancer, as well as glioblastomas, but it still unclear whether and how this multifunctional protein cobtributes to tumorigenicity in meningioma. Cyclin-CDK complex are proteins responsible for the orderly progression of cells through the cell cycle. Gene mutation and protein overexpression leads to uncontrolled cell proliferation and tumor development. Cyclins are synthesized during specific phases of the cell cycle. Each type will appear in sequence and its function is to activate the CDKs that are in inactive form. The first cyclin to increase in the cell cycle is cyclin D. It arises in the mid G1 phase and is no longer detected at the S stage. During the G1 phase it activates CDK4 and this complex has a vital role in the cell cycle, as it phosphorylates pRB, promoting cell replication, after E2F is released (Moraes et al 2012, Cheng et al 2015).

In this study, the mean of Cyclin D1 expression in Grade I meningioma was 26.80 ± 12.39; Grade II was 55.70 ± 8.64; and Grade III 63.80 ± 24.51. Statistical analyses showed significant difference in expression of Cyclin D1 in grade I and II also grade I and III ($p<0.050$), but not in grade II and III ($p>0.050$). Previous immunohistochemistry study in 64 meningioma samples demonstrated Cyclin D1 as a very useful proliferative marker in meningiomas. The expression of Cyclin D1 was elevated in grade II and III meningioma in comparison with grade I ($p<0.050$) (Milenkovic et al 2008).

ROC curve resulted 2 cut off value for Cyclin D1 expression. Cut off 39.5% was obtained to distinguish grade I and II meningioma (sensitivity 100%, specificity 90%). Cut off 55.5% was obtained to distinguish grade II and III meningioma (sensitivity 80%, specificity 50%). Therefore, Cyclin D1 expression can be used to classify meningioma histopathological grading into grade I and II/III. This was concordant with the comparative analysis which there was no difference of Cyclin D1 expression between Grade II and III. Combining grade II and III into one group resulted in higher value of sensitivity (93.3%) and specificity (90%) with cut off value 39.5%. This means to distinguish grade I with grade II and III, cut off value is 39.5%.

Statistical analyses with Spearman correlation test revealed that Cyclin D1 expression was positively correlated with meningioma grading ($rs=0.731$, $p=0.000$). It showed that the increased of Cyclin D1 expression was along with meningioma grading. Previous studies also found positive correlation between Cyclin D1 expression and meningioma grading, in which the higher expression increase the risk of recurrences. High grade meningioma (Grade II and III) showed higher Cyclin D1 immunoreactivity than in low grade meningioma (Grade I) (Gauchotte et al 2012, Cheng et al 2015, Milenkovic et al 2008).

Increased expression of Cyclin D1 is significantly associated with higher proliferative activity, as assessed by PCNA levels and Ki67 percentage (Milenkovic et al., 2008). Other literatures also stated the correlation between Cyclin D1 overexpression and tumor differentiation in gastric cancer, breast, oesophagus, bladder, lung, ovarium, mantle cell lymphoma, and pituitary gland (Shan et al 2017, Hewedi et al 2011, Alao, 2007).

Correlation between MCM-2 and Cyclin D1 expression in various meningioma grading

Spearman correlation test in this study showed positive significant correlation between MCM-2 and Cyclin D1 expression within all meningioma grading ($rs=0.683$, $p=0.000$). This result means the higher MCM-2 expression, the higher also Cyclin D1 expression. Previous study showed there was significant difference between MCM-6 and Cyclin D1 expression in meningioma ($p<0.001$) (Gauchotte et al 2012).

Carcinogenesis results from abnormal cell behavior in proliferation and differentiation. The behavioral changes occur because cell express abnormal proteins due to gene mutations, especially those that encode proteins, which play a major role in regulating cell dividing cycles. Failure in controlling cell dividing cycles result in continuous and fast dividing. The Cyclin-CDK complexes are component in the regulation of cell cycles that result in continuous cell cycles (Sudiana 2011).

Cyclin D1 overexpression leads to RB phosphorylation. The latter induce release of the active E2F and synthesize some proteins like CDC-6p, CDC-7p, Dbf-4p, MCM-p and some enzyme, such as polymerase, primase, helikase, ligase, also topoisomerase (gyrase) (Sudiana 2011).

Prior to DNA replication and during late M and G1 phases of the cell cycle, MCM2-7 form the pre-RC by being loaded on to the origin recognition complex (ORC) at the origin of replication. Abnormal or overactivation of pre-RC protein leads to abnormal DNA replication which induces genomic instability and tumorigenicity. The requirement for MCM proteins in cycling cells but their absence in quiescent cells has led to their potential clinical application as tumor markers. Role of MCM protein in DNA replication and its correlation with clinical characteristic, pathological, and prognosis suggest that MCM may becoming specific proliferative marker (Hua et al 2014).

CONCLUSION

The results showed significant difference in expression of MCM-2 in various meningioma grading, hence MCM-2 can be a useful marker for distinguishing meningioma grading (cut off value 12.0% to distinguish grade I and II meningioma; and 39.5% to distinguish grade II and III meningioma). There was also significant difference in expression of Cyclin D1 in grade I and II also grade I and III, but not in grade II and III, hence Cyclin D1 also can be a useful marker to distinguish grade I and II/III meningioma (cut off value 39.5%). There was also positive correlation between MCM-2 and Cyclin D1 expression in various meningioma grading. The higher MCM-2 expression, the higher also Cyclin D1 expression.

ACKNOWLEDGMENT

We thank Dr. Hari Basuki Notobroto, dr., M.Kes., for his contribution in stastitical analysis.

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