CORRELATION BETWEEN COX-2 EXPRESSION WITH HISTOPATHOLOGICAL GRADING IN ASTROCYTOMA

Erliano Sufarnap, M Sajid Darmadipura
Department of Neurosurgery
Faculty of Medicine, Airlangga University
Dr.Soetomo General Hospital Surabaya

ABSTRACT

Cox-2 is an isoform of cyclooxygenase which probably has a role in the pathogenesis of intracranial astrocytoma. Although the specific mechanisms has not elucidated, cox-2 had been suggested involve in tumor angiogenesis. In many cases of human neoplasm including astrocytoma, cox-2 expression increases especially in the high proliferation cell type of tumor (malignant type) for instance GBM, despite there are no data showed the correlation between cox-2 expression and histopathological grading. The objective was to prove the correlation between cox-2 expression and histopathological grading in astrocytoma. It was analytic cross sectional study using immunohistochemistry for cox-2 in 25 cases of astrocytoma during January 2003 until March 2007. Data were analyzed by statistical methods to rule out the correlation between histopathological grading (WHO System), patient age and sex to cox-2 expression. Twenty five pathological materials , exsiced from 25 brain astrocytoma patients who underwent surgery for brain astrocytoma were evaluated . Their age range from 12 to 62 years , with the means of 33,36. The 31-45 age group was the highest (31 %).The sex ratio was 56 % men and 44 % women. Histopathological grading revealed : Diffuse astrocytoma (WHO Grade II ) 40 %, Anaplastic astrocytoma (WHO GradeIII) 28 %, GBM (WHO Grade IV) 24 %, and Pilocytic astrocytoma(WHO Grade I) 8 %. The highest positive cells for cox-2 were showed in GBM with the mean of 5,802 %, and the lowest were on Pilocytic astrocytoma (WHO Grade I) for 0,194 %. Spearmen correlation analysis shows positive correlation between between cox-2 expression and histopathological grading in astrocytomas. There is a correlation between cox-2 expression and histopathological grading according to WHO system. There is also correlation between cox-2 expression and patient age. In addition, patient age has a positive correlations to astrocytoma histopathological grading. This study shows that the higher the cox-2 expression is the higher is the histopathological grading of astrocytoma and the older patient is the higher is histopathological grading of astrocytoma.

Keywords: astrocytoma, cox-2 expression, immunohistochemistry, histopathological grading

Correspondence: Erliano Sufarnap, Department of Neurosurgery, Faculty of Medicine, Airlangga University, Dr.Soetomo General Hospital Surabaya

INTRODUCTION

Astrocytoma an intracranial neoplasm derived from cells of astrocyte neuroepitel. Astrocytoma vary in the degree of histological differentiation, the astrocyte-like normal (well differentiated) to highly anaplastic (not terdefersiasi or malignant). This neoplasm is a primary brain tumor most often found, namely, the incidence of 16 000 cases per year in the United States. Of all intracranial neoplasm is glioma 45-55%, as for glioma is a neoplasm derived from glia cells (microglia, oligodendrogioma, astrocyte). And 30% of glioma is astrocytoma. Neoplasm of the brain itself merupakan15-20% of all human neoplasms. (NF Gregory, Peter CB, 1996; Hao Ding et al, 2000). In Surabaya, from January 2002 to December 2004 showed 405 patients with brain tumors where astrocytoma a majority of primary brain tumors is 29%. (Anab, Wahyuahadi 2005). Death due to primary brain tumors in the United States in 1991 found 11 000 patients from 16 000 patients with primary brain tumors. The overall primary brain tumors accounted for 2% of all deaths due to malignancy. (Graham GG, 1995)

Cyclooxygenase (cox) are enzymes that play a role in the synthesis of prostaglandins derived from arachidonic acid. Cyclooxygenase or prostaglandin H synthase (PGHS) is an enzyme that consists of two isoforms of cox-1 and cox-2. Gene for each isoforum is located on separate chromosomes, cox-1 is located on chromosome 9q, while cox-2 is located on chromosome 1p (Choy, 2003; Shono, 2001; Jockey, 2000; New, 2004). Cox-2 is
concentrated in the walls of the nucleus while cox-1 is located in the endoplasmic reticulum.

Cox-1 is expressed in almost all normal tissues and is responsible for the synthesis of prostaglandins necessary for the physiological function of tissue hemostasis, while the cox-2 is expressed in response to various stimuli such as inflammatory signaling, mitogen, cytokines and growth factors (Shono, 2001; New, 2004).

In some studies found evidence that the isoform cyclooxygenase-2 (cox-2) plays a role in the pathogenesis and growth of malignant processes. Increased expression of cox-2 in cells occurs in the early stages of carcinogenesis and the growth and spread of tumors. On examination immunohistokimia, increased levels of cox-2 is found primarily in epithelial neoplasms, inflammatory cells and vascular system on tumor nests, whereas cox-2 was not detected in the vascular system of normal tissue as well as non-neoplastic tissue. (De Almeida, 2001; Chef, 2002).

Increased expression of cox-2 was found in studies with immunohistochemical examinations on various types of malignant tumors such as colon, lung, stomach, esophagus, pancreas, prostate, ovary, cervix, breast, cancer around the head and neck (Chan et al, 1998; Choy, 2003; Jockey, 2000; Koki, 2002; New, 2004; Shono, 2001; Shamma et al, 2000). The role of cox-2 and prostaglandin at the process of malignant process in humans is also supported by the results of the epidemiological studies and retrospective showing that regular use of NSAID drugs reduce the incidence of some cancers in humans (especially in the breast, lung and colon) (Choy, 2003; Koki, 2001; Shono, 2001; Thun 1991). Knowledge of cyclooxygenase, metabolites and inhibitor pada intracranial neoplasms such as glioma, especially astrocytoma not as much data in the process of malignancy on the other limb. Most of the data results of research on the role of cyclooxygenase-2 cox especially in the process of carcinogenesis at the other body is used as a reference for explaining the role of cox-2 in glioma (astrocytoma). (New, 2004)

Studies on the role of cox-2 in glioma / astrocytoma begins with the discovery of elevated levels of prostaglandin pada glioma and the relationship between the increased synthesis of prostaglandin and tumor grading (Castelli, 1989). Based on these results, some researchers conducted a study that found increased expression of cox-2 in glioma / astrocytoma. Deininger et al (1999)

The first report that expression of cox-2, evaluated by immunohistochemistry examination was found less than 20% in glioma cells (Shono, 2001; Deininger, 1999) Prayson et al (2002) examined 47 samples of glioblastoma samples with immunohistochemical examination and found that tumors with high levels of cell proliferation tended to show expression of cox-2 high. (New, 2004; Prayson, 2002). Jockey et al (2000) found the examination immunohistokimia that cox-2 expression in 50 glioma samples showed a higher increase on the High-grade glioma than in low-grade. (Jockey, 200; Shono, 2001; New, 2004). Other researchers also found the same results as mentioned above that the high-grade glioma / astrocytoma showed COX-2 expression is higher. (Shono, 2001; New, 2004; Lee, 2004). In the research studies mentioned above are clearly not related to differences in the relationship cox-2 expression in different gradations astrotoma.

Histopathology studies generally link the expression of cox-2 by the method of therapy (cox-2 inhibitor) and prognosis of glioma. Based on these studies we suspect that there is significant correlation between the expression of cox-2 and gradation astrocytoma (based on the WHO system).

MATERIALS AND METHODS

This study is an observational study with cross sectional study examining the expression of cox-2 on astrocytoma and cox-2 expression correlation with histopathological grading in astrocytoma. Observations were made with a moment on the variables that the event has occurred. In this case is the preparation of patients with a diagnosis of astrocytoma at the Department of Anatomical Pathology, Dr. Soetomo Hospital, Faculty of Medicine, Airlangga University in the period 2001-2006.

Samples were taken from paraffin blocks of astrocytoma tumor specimens in the Department of Anatomical Pathology, Dr. Soetomo Hospital, Faculty of Medicine, Airlangga University in the period 2001-2006 and selected in accordance with the histopathological grading astrocytoma according to WHO, which then sliced and made into preparations on glass objects to be examined by immunohistochemistry.

Analysis of cox-2 expression correlation with histopathological grading is done by using Spearman correlation test. Statistical calculations performed using SPSS.

RESULTS

Cox-2 examination results 3.833%± for all patients obtained an average of 2.687% Cox-2 expression as low as 0% and the highest was 13.674%.
Cox-2 expression differences between patients of male and female

Table 1. Distribution of cox-2 expression by sex

<table>
<thead>
<tr>
<th>Sex</th>
<th>Cox-2</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x</td>
<td>SD</td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>Male</td>
<td>3.140</td>
<td>4.120</td>
<td>0.000</td>
<td>13.674</td>
</tr>
<tr>
<td>Female</td>
<td>2.331</td>
<td>3.709</td>
<td>0.171</td>
<td>13.518</td>
</tr>
<tr>
<td>Total</td>
<td>2.687</td>
<td>3.833</td>
<td>0.000</td>
<td>13.674</td>
</tr>
</tbody>
</table>

Results of analysis with the Wilcoxon - Mann Whitney p = 0.584 obtained results (p> 0.05), which means there is no difference in Cox-2 expression between patients of male and female.

Age relationship with the expression of cox-2

Table 2. Distribution of expression of cox-2 by age

<table>
<thead>
<tr>
<th>Age group (y)</th>
<th>Cox-2</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>x</td>
<td>SD</td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>≤ 15</td>
<td>0.287</td>
<td>0.329</td>
<td>0.000</td>
<td>0.760</td>
</tr>
<tr>
<td>16-30</td>
<td>1.814</td>
<td>2.161</td>
<td>0.296</td>
<td>6.316</td>
</tr>
<tr>
<td>31-45</td>
<td>3.129</td>
<td>4.667</td>
<td>0.100</td>
<td>13.674</td>
</tr>
<tr>
<td>46-60</td>
<td>2.956</td>
<td>2.461</td>
<td>0.390</td>
<td>5.700</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>13.518</td>
<td>-</td>
<td>13.518</td>
<td>13.518</td>
</tr>
<tr>
<td>Total</td>
<td>2.687</td>
<td>3.833</td>
<td>0.000</td>
<td>13.674</td>
</tr>
</tbody>
</table>

Results of analysis with Spearman correlation showed the value $r_s = 0.449$ and $p = 0.024$ (p <0.05), which means there is a correlation between age of patients with Cox-2 expression. Rs value is positive, implying that the older the patient, the higher the expression of Cox-2.

The degree of relationship with the expression of Cox-2 Gradation Astrocytoma

Table 3. Relationship between degrees cox-2 expression with grading astrocytoma

<table>
<thead>
<tr>
<th>Astrocytoma Gradation</th>
<th>Cox-2</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x</td>
<td>SD</td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>I</td>
<td>0.194</td>
<td>0.031</td>
<td>0.172</td>
<td>0.216</td>
</tr>
<tr>
<td>II</td>
<td>0.571</td>
<td>0.331</td>
<td>0.000</td>
<td>1.042</td>
</tr>
<tr>
<td>III</td>
<td>3.752</td>
<td>4.686</td>
<td>0.150</td>
<td>13.674</td>
</tr>
<tr>
<td>IV</td>
<td>5.802</td>
<td>4.395</td>
<td>0.100</td>
<td>13.518</td>
</tr>
<tr>
<td>Total</td>
<td>2.687</td>
<td>3.833</td>
<td>0.000</td>
<td>13.674</td>
</tr>
</tbody>
</table>

Results of Spearman correlation analysis by showing the value $r_s = 0.628$ and $p = 0.001$ (p <0.05), which means there is a correlation between the degree of expression of Cox-2 with gradations astrocytoma. Rs value is positive, implying that the higher expression of Cox-2, the higher the grading astrocytoma.

DISCUSSION

From the 25 cases for which we have started in January 2003 until March 2007 at dr Soetomo, female patients (56%) more than male patients (44%). This is in accordance with a retrospective descriptive study was conducted at four hospitals in Surabaya (RSU dr.Soetomo, RS RKZ, Adi Husada Hospital, Ward) patients in the years 2002-2004 astrocytoma most female (56% of cases) (Anab, Wahyuhadi, 2005).

Based on data from the WHO classification of tumors, astrocytoma tumor incidence was higher in males than females (1.8: 1) with an average occurrence astrocytoma tumor in men aged 52.2 years, while the 37.2-year-old woman. The older age of patients with higher grading histopathologinya. (Kleihues, 2000). In this study, the average patient age of 33.36 years, with the highest age group is 31-45 years ie 31% of cases.

Most specimens in this study were in WHO Grade II histopathological grading (Diffuse Astrocytoma) equal to 40% of cases followed by Anaplastic Astrocytoma (WHO Grade III) 28% of cases, glioblastoma multiforme (WHO Grade IV) of 24% and the remaining 8% of cases of Pilocytic Astrocytoma (WHO Grade I). There are differences between the results of research data with the data of Central Brain Tumor Registry of the United States, 2004, in which glioblastoma multiforme is the most case that is 50% (Ashby, 2006). Similarly, research conducted at Shono et al (2001), GBM found 47% of cases, while the results of this study found only 24% of cases.

Differences in epidemiological data are extensive opportunities for further research to determine the nature of the tumor astrocytoma especially in Indonesia and for the development penatalaksanaanmnya.
The correlation between cox-2 expression with patient age and sex

In this study, that the older age of patients was higher expression of cox-2. This is evident from the data analysis with the Spearman correlation statistic indicates the value rs = 0.449 and p = 0.024 (p <0.05). (Graph 5.1). In research conducted Shono et al (2001), the expression of cox-2 is not related to the patients' age. Also obtained additional data that the older age of patients with higher tumor grading astrocytoma histopathology demonstrated by Spearman correlation analysis showed the value rs = 0.612 and p = 0.001 (p <0.05). Similar results were obtained by Flowers et al (2000) who find a relationship between increasing age with histopathological grading astrocytoma which obtained at least 50% of patients with GBM at the age of more than 55 years.

In this study, there was no correlation between histopathological grading astrocytoma with gender. Similarly, the relationship between cox-2 expression with gender were analyzed by Wilcoxon test - the results obtained by Mann Whitney p = 0.584 (p> 0.05). Based on the above results it can be concluded that the age factor could be considered as possible prognostic factors of tumor patients astrocytoma.

The correlation between cox-2 expression with histopathological grading according to WHO astrocytoma

The higher the gradation histopathologinya, the higher the expression of cox-2 as evidenced by Spearman correlation analysis showed the value rs = 0.628 and p = 0.001 (p <0.05). We find that the highest expression of cox-2 exist in the GBM (WHO Grade IV) with a mean of 5.802%, while the lowest was pilocytic astrocytoma (WHO Grade I) with a mean of 0.194%. This is consistent with research Jockey et al (2000) who get on the GBM were found as many as 96% of cases with a picture of the expression of COX-2 positive in more than 50% cells. But there are vast differences when compared with the research Jockey et al (2000), namely on the number of GBM cells positive percentage expression of its cox-2. In this research, the number of GBM cells positive expression of cox-2 is not up to 50%, on average only 5.802%. The cause of this difference is still unknown because the specimen preparation procedure and staining of paraffin blocks or similar immunohistochemical examination.

Cox-2 expression increased in the brain can occur in inflammatory conditions (Lacroix, 1998), trauma (Miettinen, 1997) and in areas of necrosis (Deininger, 1999). If the astrocytoma found increased expression of cox-2 in accordance with the gradation histopatologinya then chances are the factors mentioned above play a role in cox-2 in inducing tumor cell necrosis, especially in areas where there is the condition of hypoxia (Jockey, 2000).

Growth factors (eg EGF), tumor promoters, cytokines, and other inflammatory mediators can induce the expression of cox-2. (Smith, 1996; Eberhart, 1995; Jockey, 2000). In the GBM is often met 'loss of heterozygosity' of chromosome 10, which is also in line with the increase of the EGF receptor. Increased EGF receptor is involved in cox-2 enzyme elevations. (Jockey, 2000) In addition, based on previous research, the recognition of cox-2 interaction with p53 gene. Types of wild-type p53 gene can inhibit cox-2 expression. While the p53 gene is mutated, which occurs more frequently with tumors of high gradation, not inhibit cox-2. The interaction is useful for understanding why COX-2 expression increases with tumor grading. Also on astrocytoma, increased p53 gene mutations likely play a role in cox-2 expression increased in accordance with the gradation astrocytoma (Bogler, 1995; von Deimling, 1992; Lang, 1994; Jockey, 2000).

Although the role of cox-2 in the pathogenesis and development of astrocytoma still unclear, but this can be considered in determining the therapeutic target. Increased expression of cox-2 in rat intestinal epithelial cells showed a decrease tendency of apoptotic cells. Tsuji et al (1998) showed that cox-2 stimulates colon cancer cells to release prostaglandins that support the proangiogenic tumor angiogenesis. Although these mechanisms have not been studied specifically in astrocytoma, Jockey et al (2000) showed that NS-398, a cox-2 specific inhibitor, increased apoptosis, decreased proliferation and invasion of cultured human glioma cells (Jockey, 2000). Cox-2 alone by prostaglandins and other factors can affect the aggressiveness of the glioma. Based on the description above, the cox-2 can be considered as a therapeutic target in dealing with glioma. Cox-2 inhibitors can also be considerable as a drug to prevent the development of a low grade astrocytoma Anaplastic astrocytoma or GBM. (Shono, 2001).

This study and other studies related to astrocytoma still needs to be continued and further developed as it is still not yet can be applied directly in the management of patients astrocytoma so beneficial research is still limited in scope only. Expected at a later date this study and other research can be improved so that results can provide direct benefits to patients astrocytoma.
CONCLUSIONS

From a study of 25 specimens from tumor patients graded I to IV astrocytoma according to WHO concluded: (1) there is significant correlation between patients' age to histopathological grading astrocytoma, the older age of patients with higher histopathological grading histopathologi; (2) there is significant correlation between cox-2 expression with histopathological grading astrocytoma, the higher expression of cox-2 histopathologi higher gradation. So the results of this study can be used as reference data and for subsequent research, especially related to prognosis and therapy with drugs cox-2 inhibitors on tumor cases astrocytoma. We suggested for (a) the need for research that includes a larger sample conducted prospectively in order to clarify the role of cox-2 in tumor pathogenesis astrocytoma particularly concerning; (b) the need for continued research on the relationship cox-2 expression with prognosis of patients astrocytoma so it can be used as a prognostic factor; (c) need research to determine the factors influencing the epidemiological data differences astrocytoma tumor patients in Indonesia and other countries; (d) need for research on the use of drugs cox-2 inhibitors on tumor cases astrocytoma in experimental animals and in humans.

REFERENCES


