ABSTRACT

Aim: to compare the expression of VEGF-A and desmoplastic reaction of colorectal cancer between the young aged and old aged patients excluding the stage of tumor.

Methods: our study was a retrospective study on VEGF-A expression and desmoplastic reaction in pathology anatomy specimens of patients with colorectal cancer at young age (less than 40 years of age) and older age (more than equal 60 years of age). The samples had been taken since 2001 until 2008 and the sample size for each group was 42 samples for young age and 36 samples for older age subjects. To carry out the examination of VEGF expression, our study utilized the immunohistochemistry method. In addition, desmoplastic reaction as a preliminary study was evaluated by using routine HE staining. To recognize the difference of VEGF-A expression and the degree of desmoplastic reaction in young aged and older aged patients with colorectal cancer, a Chi-Square test was performed. The statistics was considered as significant when p value < 0.05.

Results: forty-two patients were at younger age (<40 years) and 36 patients were at older age (>60 years), with characteristics of them 37 males and 41 females. Tumor grading was performed based on WHO criteria. Ten subjects with high-grade tumor and 32 subjects with low-grade in the young age group; while in the older age group, there were 9 subjects with high-grade tumor and 27 subjects with low grade tumor.

In young age, there was twice greater likelihood to have pronounced desmoplastic reaction compared to the older age (OR = 2.1).

Conclusion: the VEGF-A expression in younger and older age was nearly the same; however, there was significant difference of desmoplastic reaction in younger aged patients compared to the older aged. There was also comparable correlation between strong positive VEGF-A expression and strong desmoplastic reaction in young patients with colorectal cancer.

Key words: colorectal cancer, young aged, VEGF, desmoplastic reaction.

INTRODUCTION

Developed countries have reported that colorectal cancer is found predominantly in older aged population (60 years of age or more); while American and European literatures have only reported about 2 to 8 percent of such cancer occurs in patients at 40 years of age. A study in Singapore conducted from 1989 until 1992 reported that 5.9 percent of colorectal cancer occurs in patients at or less than 40 years of age. Colorectal cancer in young patients (at or less than 40 years of age) tends to have aggressive tumor behavior and poor prognosis. Data on characteristics include poor differentiated carcinoma, mucinosum adenocarcinomas which is associated with poor prognosis, advanced stage and frequent lymph nodes invasion that statistically significant. Based on such findings, it is common to say that aggressive tumor behavior has become the characteristics of colorectal cancer in young aged patients. Sudoyo AW suggested that colorectal cancer in young aged Indonesian natives, which represented by Javanese, Sundanese, Minangnese and Makassar ethnic, could not be categorized as hereditary colorectal cancer. Neo-angiogenesis is one of the component factors affecting the development of metastases and the most potential cytokines in angiogenesis process includes Vascular Endothelial Growth Factor (VEGF/VEGF-A).

In tumor progression, the “Angiogenic switch” process occurs; it has effect in the form of increased pro-angiogenic factors which allow development, proliferation and metastases of tumor cells. Angiogenesis and lymphangiogenesis capacity as indicated by VEGF over-expression in colorectal cancer is correlated to higher grade of malignancy and poor survival rate. Several studies demonstrated that myofibroblasts might have a supportive or facilitating role...
in tumorigenesis and progression of carcinomas; thus, it has clinical value and flagged as a potentially important marker for diagnosis, treatment, and prognosis of cancer.\textsuperscript{16}

Our study was aimed to compare the expression of VEGF-A and desmoplastic reaction of colorectal cancer between the young aged and old aged patients excluding the stage of tumor. Until now, there have not been any specific data regarding the expression pattern of VEGF-A in young and old age patients with colorectal cancer in Indonesia. Moreover, considering that young patients with colorectal cancer has a more progressive history of disease, a question then emerged, i.e. whether VEGF-A expression is one of potential factors of higher progressive colorectal cancer in young patients compared to the old aged patients.

\textbf{METHODS}

Angiogenesis is essential for both primary tumor growth and metastases. In our study, we compared VEGF-A expression in patients with colorectal cancer aged less than 40 years, who frequently have worse prognosis; and VEGF-A expression in patients with colorectal cancer aged more than 60 years. Moreover, we also compared desmoplastic reaction occurred in the abovementioned age groups. Our study was a retrospective study on VEGF-A expression and desmoplastic reaction in pathology anatomy specimens of patients with colorectal cancer.

Our population of study were tissue specimens derived from biopsy and resection of tumor mass with colorectal adenocarcinomas, which were obtained from young aged patients (<40 years of age) and older age (>60 years of age), who had been treated in Cipto Mangunkusumo Hospital, Jakarta. The samples had been taken since 2001 until 2008 and the sample size for each group was 42 samples for subjects ≤40 years of age and 36 samples for subjects ≥60 years of age.

Increased angiogenesis was detected by immunohistochemistry test against VEGF-A antigen by using VEGF-A monoclonal (Santa Cruz Biotechnology, Inc., CA, USA), treated with secondary antibody from Trekkie Universal Link, Biocare Medical, USA. Desmoplastic reaction was evaluated by using routine Hematoxylin Eosin staining and interpreted by using light microscope.

\textbf{Immunohistochemistry Staining}

Immunohistochemistry staining was not performed standard protocol, i.e. the sections were not baked over-night and the standard antigen retrieval method was not performed. Such protocol was performed after several trials had been conducted contributing to a decision that the best protocol was without using antigen retrieval method and 1:150 antibody dilution.

\textbf{Statistical Analysis}

To recognize the difference of VEGF-A expression and the degree of desmoplastic reaction in young aged and older aged patients with colorectal cancer, a Chi-Square test was performed. The statistics was considered as significant when p value <0.05.

\textbf{RESULTS}

We found 78 cases which were eligible for analysis in our study. Forty-two patients were at younger age (≤40 years) and 36 patients were at older age (≥60 years), with characteristics of them 37 males and 41 females.

Tumor grading was performed based on WHO criteria. There were 10 subjects with high-grade tumor and 32 subjects with low-grade in the young age group; while in the older age group, there were 9 subjects with high-grade tumor and 27 subjects with low grade tumor. Histopathological data found in both age groups, i.e. the less than 40 years of age and more than equal 60 years of age revealed the adenocarcinoma type as the most frequently found subtype and similar number was found in both groups. In addition, similar number of mucinosum subtype was obtained in both groups, which included 4 subjects. On the contrary, 4 subjects were found with signet ring cells, which only found in the less than 40 years of age group.

Based on the immunohystochemical staining for specimens in both groups, we found that adenocarcinoma type in both groups had similar strength of positivity. No negative result was found on VEGF-A expression. (\textbf{Table 1} and \textbf{Figure 1}) Immunohistochemical staining for VEGF-A in both less than 40 years of age group and more than equal 60 years of age group confirmed no difference on strong positivity. (\textbf{Table 2}) Desmoplastic reaction was evaluated by using specimens stained with routine HE staining. It may show pronounced or weak desmoplastic reaction either in less than 40 years of age or more than equal 60 years of age. (\textbf{Table 3})

Based on such calculation, we found there was significant difference with respect to desmoplastic reaction on the stroma of colorectal cancer between subjects less than 40 years of age and more than equal 60 years of age. In young age, there was twice greater likelihood to have pronounced desmoplastic reaction compared to the older age (OR = 2.1).
Figure 1. The result of immunohistochemical staining. (A) positive control (B) negative control (C) strong positive immunohistochemical staining for VEGF (D) weak positive immunohistochemical staining. (200X)

Figure 2. (A) HE staining – showing pronounced desmoplastic reaction (B) HE staining – showing low-grade desmoplastic reaction. (200X)

DISCUSSION

Our study included patients with colorectal cancer aged less than 40 years and more than 60 years of age groups regardless of their disease stage. We decide to have such samples based on our data at Cipto Mangunkusumo Hospital that generally patients with colorectal cancer who came for their first visit had already had advanced disease stage. There were 78 cases that fulfilled the inclusion criteria. Forty-two subjects (53.8%) were in the less than 40 years of age group and 36 subjects (46.2%) were in the more than 60 years of age group. Determination of age as less than 40 years of age and more than 60 years of age was done in arbitrary fashion and based on previous studies concerning colorectal cancer. Coincidentally, there was almost no difference regarding the number of male and female subjects in our study.

Not only tissues obtained from surgery, but also from biopsy that had been used in our study sample; therefore, there were occasionally small sized tissues with a small number of tumors. However, it still complies with the inclusion criteria.
Histopathological characteristics of our study confirmed that the most frequently found histopathological type was adenocarcinoma. It was found both in the less than 40 years of age group and more than 60 years of age group. Such data are consistent with previous studies. Moreover, the mucinosum adenocarcinoma was also found in similar number for both groups. This is different from several previous studies. Chen et al found that there was greater number of mucinosum adenocarcinoma in younger patients with colorectal cancer than in older patients. Our study also revealed 4 subjects with signet ring cell carcinoma in less than 40 years of age group and such data were not found in the more than 60 years of age group.

Tumor grading in our study did not demonstrate significant difference between both groups with respect to high-grade and low-grade distribution. However, there was greater distribution of low-grade compared to the high-grade in both groups.

Immunohistochemical staining found that all specimens demonstrated VEGF-A (+). There was no negative VEGF-A result. In both groups of less than 40 years of age and more than 60 years of age, adenocarcinoma type were more frequently had strong (+) VEGF-A (78.6% vs 88.9%) compared to other histopathological subtypes. Our study was aimed to provide evidences that there is different VEGF-A expression between younger and older age. Unfortunately, the result of our study could not be processed statistically by Chi Square formula since there was zero value on one of column cells. (Table 2) However, our data did not demonstrate significant difference of VEGF-A (+) expression between younger and older age. In our opinion, it might be caused by specimens derived from biopsy tissue (too small in size); therefore, it is less likely to demonstrate fine VEGF-A expression. In addition, our study did not consider the disease stage. A study conducted by Takahashi et al by using immunohistochemical staining demonstrated that there were higher expression of VEGF protein and receptors in colorectal cancers which already had metastases compared to the non-metastases cancers. Thus, further studies are necessary with respect to factors affecting disease stage.

Lower tumor grading has expressed more strong (+) VEGF-A in both groups without any significant difference. Such data are different from previous study conducted by Kekec et al, which demonstrates that lower grade tumor was likely did not express VEGF-A (negative).

Indeed, the association of tumor grading with VEGF-A is still vague, but several previous studies indicate that there is likely higher VEGF-A expression in higher grade of tumor despite insignificant statistic results. Overall, Sun XF, et al concluded in their study that high VEGF expression is associated with tumor size, non-mucinous carcinoma subtypes, advanced stage, vascular invasion, metastases to liver and recurrence.

Considering that there was no difference of VEGF expression in younger and older age, the factor of tumor stroma should be taken into account since it has been well-known that stroma also produces VEGF growth factors. A cancer consists of not only tumor cells but also stromal infrastructure, including tumor blood vessels assembled by host cells.

Stromal factors arrange tumor growth and differentiation, which both are important determinant factors of tumor behavior. Sun XF and Zhang H explain that there are various stromal variables in colorectal cancer that should be noticed as they have important value with respect to treatment. Desmoplastic reaction in our study is considered as an observation of a preliminary study since we only evaluated desmoplastic reaction by routine HE staining and not by immunohistochemical staining. It was evaluated to answer our hypothesis whether there is a difference regarding the degree of desmoplastic reaction between younger age group and the older one as well as to evaluate whether desmoplastic reaction occurring in tumor stroma also has any correlation with VEGF expression.

Our study result demonstrates that there was pronounced desmoplastic reaction (57.1%) in less than 40 years of age group that expressed strong (+) VEGF-A. This is very different when we compare it to the
more than 60 years of age group, which predominantly showed low-grade desmoplastic reaction (61.1%) but expressed strong (+) VEGF-A. The statistical calculation (Table 4) provided a significant difference on the degree of desmoplastic reaction in younger and older age (p = 0.01). Moreover, we found that the odds ratio was 2.1 indicating that younger age has 2.1 greater likelihood to have pronounced desmoplastic reaction compared to the older age. Such a fact is very important considering that desmoplastic reaction is a reaction that takes place in stroma which may support tumorigenesis and it is also an indicator of disease recurrence and progressivity.

The occurrence of pronounced desmoplastic reaction associated with strong (+) VEGF-A is appropriate to theory, which explains that desmoplastic reaction has been implicated as a response of host cells against stimulation induced by tumor cells. Such process resembles to wound-healing process involving myofibroblast, a component of tumor stroma, which also has the role in tissue remodeling through production of growth factors including VEGF, i.e. to activate the angiogenic program. Based on the abovementioned explanation, it suggests that there is a correlation between the degree of desmoplastic reaction in tumor stroma and VEGF-A expression. However, low-grade desmoplastic reaction that predominantly found in the more than 60 years of age group had expressed strong (+) VEGF-A. This does not seem consistent with the theory. It might be caused by other factors that may also have role affecting the degree of desmoplastic reaction. Further studies would be necessary and it should be more specific and focused on VEGF in tumor stroma.

Our study has also demonstrated that of all samples, female subjects have more expression of pronounced desmoplastic reaction than male. This fact is interesting and it shall call for further studies on contributed factors of why female subjects exhibit greater amount of pronounced desmoplastic reaction. Furthermore, the factors may include diet, hormone, immune system and others.

In order to have further knowledge on VEGF-A expression, further studies may be performed involving the tumor stage and stromal VEGF. Probably, those studies will provide greater information about VEGF as one of prognostic factors. We suggest further studies on the degree of desmoplastic reaction by using immunohistochemical method, which utilize specific antibody of alpha-smooth muscle actin. Additionally, we also need further studies about the correlation between immune factors and desmoplastic reaction to reveal why low-grade desmoplastic reaction but strong (+) VEGF-A expression is predominantly found in patients aged more than 60 years old.

CONCLUSION

There is no significant difference found in both age groups. On the contrary, there was significant difference on stromal tissue desmoplastic reaction of colorectal cancer in younger age and older age patients. In addition, there is a likelihood of two fold greater to have pronounced desmoplastic reaction in younger age than in older one. Moreover, we also found a correlation between VEGF-A expression and the degree of desmoplastic reaction in stromal tissue of colorectal cancer in younger age. While lower grade desmoplastic reaction which had strong (+) VEGF-A expression was found in the more than 60 years of age group. However, it does not confirm a direct correlation and needs further studies on possible factors that may affect them.

REFERENCES


