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Serum carcinoembryonic antigen tends to decrease in poorly-differentiated colorectal cancer

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ABSTRACT

BACKGROUND

Carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19-9 are tumor markers for colorectal cancer (CRC), both having prognostic value. According to the Colorectal Working Group of the American Joint Committee on Cancer, increases in CEA and CA 19-9 levels of >5 µg/l and > 37 U/ml, respectively, are considered abnormal. Increased serum CEA may be encountered in postoperative CRC patients from recurrences or metastases. There are no research data in Indonesia on the characteristics of CEA and CA 19-9 levels according to preoperative CRC cellular differentiation. The objective of this study was to determine differences in serum CEA and CA 19-9 levels according to CRC cellular differentiation.

METHODS

This was a cross-sectional study conducted on 40 CRC subjects from July 2012 until May 2013. Determination of serum CEA and CA 19-9 levels and histopathological (cellular) differentiation grades in CRC biopsies was done in all subjects.

RESULTS

The study involved forty CRC patients, consisting of 22 males and 18 females, with mean age of 51.93 ± 11.63 years, CEA levels of 51.93 ± 84.07 ng/ml and CA 19-9 levels of 33.81 ± 62.39 U/ml. Carcino-embryonic antigen levels tended to decrease with decreasing CRC histopathological grade, while CA 19-9 levels increased in well-differentiated CRC. However, both relationships were statistically not significant (with p=0.314 and p=0.787, respectively).

CONCLUSIONS

Carcinoembryonic antigen (CEA) levels tend to decrease with decreasing histopathological grade of CRC, and CA 19-9 levels tend to increase in well-differentiated CRC.

Key words: Carbohydrate antigen 19-9, carcinoembryonic antigen, cellular differentiation, colorectal cancer

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Karsinoembrionik antigen serum cenderung menurun pada sel kanker kolorektal diferensiasi buruk

ABSTRAK

LATAR BELAKANG

Carcinoembryonic antigen *(CEA) dan* carbohydrate antigen *19-9 (CA 19-9) adalah penanda tumor untuk kanker kolorektal (KKR), keduanya memiliki nilai prognostik. Berdasarkan* Colorectal Working Group of the American Joint Committee on Cancer *dikatakan nilai abnormal bila peningkatan nilai CEA di atas normal yaitu ≥5 µg/l dan peningkatan nilai CA 19-9 ≥37 U/ml. Peningkatan CEA dalam serum bisa dijumpai pada penderita KKR pasca operasi bila terdapat rekurensi atau metastasis. Belum ada data penelitian di Indonesia mengenai karakteristik kadar CEA dan CA 19-9 berdasarkan diferensiasi sel penderita KKR preoperatif. Tujuan penelitian ini untuk membedakan kadar CEA dan CA 19-9 dalam serum berdasarkan diferensiasi sel yang berbeda pada KKR.*

METODE

Penelitian ini merupakan desain potong-silang mengikutsertakan 40 penderita KKR yang dilakukan antara bulan Juli 2012 hingga Mei 2013. Pemeriksaan CEA dan CA 19-9 dalam serum serta diferensiasi sel (histopatologi) di jaringan biopsi tumor dilakukan pada penderita KKR.

HASIL

Hasil penelitian didapatkan 40 penderita KKR 22 pria dan 18 wanita dengan umur rata-rata 51,93 ± 11,63 tahun, kadar CEA 51,93 ± 84,07 ng/ml, dan kadar CA 19-9: 33,81 ± 62,39 U/ml. Kadar CEA cenderung semakin menuurn dengan semakin rendahnya grade *histopatologi dari sel KKR, tetapi secara statistik tidak bermakna (p=0,314). Kadar CA 19-9 semakin meningkat pada sel KKR berdiferensiasi baik, tetapi tidak bermakna secara statistik (p=0,787)*

KESIMPULAN

Kadar CEA cenderung semakin menurun dengan semakin rendahnya grade *histopatologi sel KKR, sedangkan kadar CA 19-9 cenderung semakin meningkat pada sel KKR berdiferensiasi baik.*

Kata kunci: Carbohydrate antigen *19-9,* carcinoembryonic antigen*, diferensiasi sel, kanker kolorektal*

INTRODUCTION

World-wide, colorectal cancer (CRC) ranks third among cancers in males and second among cancers in females, with more than 1.2 million new cases annually and 608.700 deaths in 2008.[1] In the US a total of 146.970 new CRC cases are found annualy, and an estimated total of 49.920 deaths from CRC.[1] In general the prevalence of CRC shows a sharp increase after the age of 50 years, which is associated with exposure to various carcinogens and lifestyle.[2] In Indonesia, various reports indicate an increase in the number of CRC cases. To date CRC is a public health problem that ranks third among the most frequently occurring cancers in Indonesia. The age-standardized incidence of CRC in Indonesia per 100.000 persons was 19.1 in males and 15.6 in females.[3]

A study on CRC profile at Pirngadi Hospital in Medan found 197 CRC patients among a total of 760 patients examined by colonoscopy (25.9%), while 101 (51.3%) of the CRC patients were female. Around one-third (28.9%) of the CRC patients were in the age range of 51-60 years, 46.1% were ethnic Batak,

and 74.6% of the CRC were located in the rectum.[4]

Carcinoembryonic antigen (CEA) is the most common tumor marker for CRC, and its levels are of value in continuous monitoring and as prognostic markers.[5,6] The American Society of Clinical Oncology (ASCO) recommend the preoperative assessment of CEA levels to assist in CRC staging, operative planning, or in monitoring therapeutic response during treatment.[6,7]

Serial CEA determination to detect recurring CRC has a sensitivity of 80%, a specificity of 70% and may persist for 5 months. One study concluded that assessment of preoperative serum CEA and CA 19-9 levels may be used prognostically for 5-year recurrence free survival (RFS) in CRC.[8] Elevated CEA concentration is associated with grade 1 and 2 tumors, late stages of CRC and metastases to internal organs. Although serum CEA concentration is an independent prognostic factor, serum CEA values may be called significant only upon continuous postoperative monitoring.[9]

In spite of the limited specificity and sensitivity of the CEA test, it is frequently suggested to find early recurrences. Preoperative CEA testing is very useful as a prognostic factor and to determine any connection between elevated CEA and the primary tumor. Elevated preoperative CEA is of benefit for early identification of metastases, since metastatic tumor cells frequently result in elevated CEA.[9] There are many factors influencing the prognosis of CRC, one of them being histopathological grade determined from biopsies. Apart from being a determinant of prognosis, histopathological grade is also an important determinant of CRC etiology and management. Particularly for CRC management, histopathological examination of CRC is mandatory, because histopathological grade is important for determining further management of CRC. Among the histopathological characteristics evaluated are tumor species and degree of differentiation. The histopathological picture may determine the degree of malignancy of a neoplasm. In addition to being a diagnostic determinant, the histopathological picture is also of great influence in determining tumor prognosis and recurrence.[10] The study carried out by Sudoyo et al.[11] concludes that there are significant differences in the histopathological picture comprising CRC grade and stage.

Poorly differentiated CRC is associated with genetic mutations, but this has yet to be proven. Around 20% of CRCs are poorly differentiated, and have a poor prognosis.[12] Histopathological grade significantly affects patient survival in addition to tumor stage. Patients with well-differentiated carcinomas (grades 1 and 2) have better 5-year survival rates than those with poorly differentiated carcinomas (grades 3 and 4).[9] One study showed that well-differentiated CRC have higher CEA levels in comparison with poorly differentiated CRC.[13] On the basis of the abovementioned information, the purpose of the present study was to find differences in serum CEA and CA 19-9 concentrations associated with cellular differentiation (tumor histopathological grade) in subjects with CRC.

METHODS

Design of the study

This study was a comparative analytical study of cross-sectional design. The study was carried out at H. Adam Malik and Pirngadi Medan hospitals between July 2012 and May 2013.

Study subjects

The target population comprised patients with suspected CRC and diagnosed by colonoscopy and biopsy. The study sample consisted of patients with CRC meeting the inclusion criteria, (patients with histopathologically established diagnosis of CRC, both females and males >18 years of age) and the exclusion criteria (CRC patients with

cancer of the head of the pancreas, pancreatitis, or pulmonary, ovarian, or hepatic tumors). The study subjects were selected by non-random consecutive sampling.

Data collection

All study subjects were interviewed by means of a questionnaire whose items included the subject's name, age and gender. The subject's weight was measured using Omron scales and his/her height was determined by a Kenko height measuring instrument.

Laboratory analysis

The laboratory investigations comprised determination of CEA and CA 19-9 levels. A 5 ml venous blood sample was divided equally into 2 tubes of 2.5 ml each. The samples were left to clot, centrifuged at 1500 rpm for 5 minutes, then put into a Cobas 6000 analyzer. The CEA levels were expressed in ng/ml, while the CA 19-9 levels were expressed in U/ml. The cutoff values for CEA and CA 19-9 were 5 ng/ ml and 37 U/ml, respectively.[14]

Histopathologic examination

After colonoscopy, biopsy specimens were obtained from the colon or rectum for histopathologic determination of cellular differentiation. The cellular differentiation of the histopathologic specimens was graded microscopically by a pathologist. Grade 1 (well-differentiated): atypical glands, disorganization with disorganized epithelium, pleiomorphic hyperchromatic nuclei, coarse chromatin, eosinophilic cytoplasm. Grade 2 (moderately differentiated): proliferative glands, disorganization with disorganized epithelium, pleiomorphic hyperchromatic nuclei, irregular nuclear membrane, eosinophilic cytoplasm. Grade 3 (poorly differentiated): proliferative glands, disorganization with disorganized epithelium, pleiomorphic hyperchromatic nuclei, irregular nuclear membrane, eosinophilic cytoplasm.

Data analysis

To compare CEA and CA 19-9 levels for each CRC cellular differentiation grade, one-way ANOVA was used, followed by the chi-square test to find a relationship between CEA and CA19-9 levels on the one hand and CRC cellular differentiation on the other. Statistical analysis was performed using SPSS version 15.0, with the level of significance set at $p<0.05$.

Ethical clearance

The study protocol was approved by the Health Research Ethical Committee of North Sumatra University, Faculty of Medicine. All study subjects signed written informed consent after having been informed about the aims and benefits of the study.

RESULTS

There were in total 40 CRC patients who participated in this study. The clinical characteristics of the CRC patients, CRC location, CRC cellular differentition, and CEA and CA 19-9 levels, are presented in Table 1.

Table 1. Distribution of clinical characteristics of the subjects (n=40)

Variable	n (%)
Age (years)*	51.931 ± 11.63
Gender	
Male	22 (55.0)
Female	18 (45.0)
Location of cancer	
Rectum	30 (75.0)
Sigmoid	4 (10.0)
Descending colon	3 (7.5)
Ascending colon	2 (5.0)
Cecum	1 (2.5)
Histopathological grade of cancer	
Well-differentiated	28 (70.0)
Moderately differentiated	6 (15.0)
Poorly differentiated	6 (15.0)
CEA (ng/ml)*	51.93 ± 84.07
CA 19-9 (U/ml)*	33.81 ± 62.39

CEA= carcinoembryonic antigen; CA= carbohydrate antigen
* Values are mean ± SD

Table 2. Comparison of CEA and CA 19-9 levels by CRC histopathological grade

	CRC histopathological grade			p**
	Well-differentiated (n=28)	Moderately differentiated (n=6)	Poorly differentiated (n=6)	
CEA (ng/ml)*	64.23 ± 92.76	38.04 ± 72.68	8.43 ± 5.42	0.314
CA 19-9 (U/ml)*	38.39 ± 74.21	23.73 ± 12.95	22.55 ± 8.21	0.787

CRC=colorectal cancer; CEA=carcinoembryonic antigen; CA=carbohydrate antigen
* Values are mean ± SD; ** One way Anova test: non significant

Among the 40 subjects, 22 (55.0%) were males and 18 (45%) were females. Mean age of the subjects was 51.931 ± 11.63 years. The colonoscopically diagnosed CRCs according to location consisted of 30 cases (75%) of rectal carcinoma, 4 cases (10%) of sigmoid carcinoma, 3 cases (7.5%) of carcinoma of the descending colon, 2 cases (5%) of carcinoma of the ascending colon, and 1 case (2.5%) of cecal carcinoma. The results of cellular differentiation grading was dominated by 28 samples (70%) of well-differentiated CRC, while moderately and poorly differentiated CRC comprised 6 samples (15%) each.

One-way ANOVA was used to compare CEA and CA 19-9 levels according to cellular differentiation. The ANOVA results showed that CEA levels decreased with decreasing histopathological grade of the CRC cells, but the differences was statistically not significant (p=0.314). The results for CA 19-9 levels did not show a decreasing tendency with decreasing histopathological grade of the CRC cells. There was no significant difference in CA 19-9 levels between the three histopathological grade categories (p=0.787) (Table 2).

The chi-square test results showed that elevated CEA levels were more frequently found in well-differentiated CRCs, in comparison with those in poorly differentiated CRCs, but the relationship was statistically not significant (p=0.475). Increased CA 19-9 levels were found in all well-differentiated CRCs, but this relationship was also statistically not significant (p=0.247) (Table 3).

DISCUSSION

CEA and CA 19-9 are the most frequently used tumor markers for CRC, both of which have prognostic value. The use of CEA and CA 19-9 as tumor markers and for prognostic evaluation in various tumor stages is according to the Colorectal Working Group of The American Joint Committee on Cancer (AJCC). Serial CEA determination to detect recurring CRC has a sensitivity of 80%, a specificity of 70% and may persist for 5 months. Serial CA 19-9

Table 3. Comparison of CEA and CA 19-9 groups by CRC histopathological grade

	CRC histopathological grade			p#
	Well-differentiated (n,%)	Moderately differentiated (n,%)	Poorly differentiated (n,%)	
CEA group*				
Normal	8 (61.5)	2 (15.4)	3 (23.1)	0.475
High	20 (74.1)	4 (14.8)	3 (11.1)	
CA 19-9 group**				
Normal	19 (61.2)	6 (19.4)	6 (19.4)	0.247
High	9 (100.0)	0	0	

CRC=colorectal cancer; * Normal CEA (carcinoembryonic antigen) ≤ 5 ng/ml, high > 5 ng/ml; ** Normal CA (carbohydrate antigen) 19-9 ≤ 37 U/ml, high CA 19-9 > 37 U/ml; # Chi-square test: non significant

determination to detect recurring CRC has a sensitivity of 70-80% and a specificity of 80-90%. According to AJCC, a CEA level is considered to be elevated if it is >5 µg/L, while a CA 19-9 level is elevated if it is >37 U/ml.[15]

Our study showed decreasing CEA levels with decreasing histopathological differentiation of the CRC cells, but the relationship was statistically not significant. Similar but statistically significant results were found by Duffy, showing that well-differentiated CRC produced significantly higher CEA levels than poorly differentiated CRC. This may be due to the much larger sample size used in Duffy's study.[13] In addition, in our study there were several samples of well-differentiated histopathology with normal CEA levels (<5 ng/ml).

In a study by Cerna et al.[16] on CEA levels in tumor tissues, the most significant finding was that CEA was present in practically all tissue samples. This is in contrast with the current belief that serum CEA is not elevated in all primary CRCs or even in the metastases. Therefore, to obtain significant results in studies such as ours, in addition to determination of serum CEA levels, the CEA levels in tumor tissues should presumably be assessed. In our study, the tendency for well-differentiated CRCs to result in higher CA 19-9 levels in comparison with poorly differentiated CRCs, is to be considered as not proven, because we found that the above relationship was statistically not significant. Similarly, the study by Chen et al.[5] on the relationship between CEA and CA 19-9 levels on the one hand and histopathologically determined cellular differentiation on the other, also did not find a significant relationship.

With regard to CRC location, our study found that the most frequent location was the rectum (75%) as compared with other sites, and that the least frequent location was the cecum (2.5%). This agrees with the results of a previous study by Effendi et al.[4] who stated that the most frequent location of CRC was the rectum (74.6%). The study conducted by Sudoyo et al.[11] also found the most frequent CRC location to be the rectum (72.7%).

In connection with CRC histopathological differentiation, the most frequently found was well-differentiated CRC (70%), with moderately and poorly differentiated CRCs each accounting for 15%. However, these results differ from those found in the study by Stewart et al.[10] conducted in the US from 1998 to 2001, where adenocarcinomas were more frequently moderately differentiated. Therefore, our findings cannot as yet be used as a prognostic measure for comparing preoperative and postoperative status.

The present study still has a number of limitations. Firstly, the small size of the study population, being restricted to 2 central hospitals, resulting in the lack of significant differences in CEA and CA 19-9 levels in relation to histopathological cellular differentiation. Secondly, in this study no tumor staging was done, so that CEA and CA 19-9 cannot be used as prognostic indicators of CRC. Therefore multicenter studies are needed with larger samples and TNM staging based on Duke's classification.[17]

CONCLUSIONS

CEA levels tend to decrease with decreasing histopathological grade of CRC, and CA 19-9 levels tend to increase in histopathologically well-differentiated CRC.

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