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Original article

The relationship of tumor necrosis factor alpha levels in plasma toward the stage and differentiation degree in colorectal cancer☆

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ABSTRACT

Introduction: Colorectal cancer (CRC) is one of the most common malignancies in western countries. Furthermore, the morbidity and mortality are increasing around the world including Indonesia. One indicator used to conduct an examination is TNF-α. Therefore, this research aims to study the correlation between TNF-α level in blood plasma with the incidence of CRC.
Method: This was a cross-sectional analytic observational research. The purpose was to determine the differences in TNF-α level in the plasma of CRC patients. These observations were in July-November 2018 at Wahidin Sudirohusodo Hospital.
Results: Data analysis indicate that there was a significant relationship between the stages in CRC patients and TNF-α level in blood plasma with a p-value of 0.000. Other variables without significant relationship were age $p = 0.681$, sex $p = 0.822$ and differentiation $p = 0.141$.
Conclusion: Examination of TNF-α level in plasma can be used as a diagnostic factor in assessing the development and prognosis of CRC patients, and as preliminary information in the high-risk CRC group before other more invasive tests (CT-Scan, endoscopy, and biopsy).

Introduction

Colorectal cancer (CRC) is one of the most common malignancies in western countries. Furthermore, the morbidity and mortality is increasing around the world including Indonesia. In the United States, this disease was the third most common cause of deaths regardless of sex after lung and prostate cancer in men. More than 130 thousand new CRC cases and 49 thousand deaths were recorded in 2016.[1] Furthermore, in 2000-2013, there were decrease in the mortality of CRCs as well as an increase in 5-year survival rates for sufferers over 50 years, and a decrease for those under 50 years.[2] According to Globocan 2012, the incidence of colorectal cancer in Indonesia is ranked 3rd cause of death supported by 12.8 data per 100,000 adult population, with a mortality rate of 9.5% of all cancer cases.[3]

The prognosis of CRC sufferer is strongly influenced by several factors, such as clinical variable, stage, histopathology and molecular oncogenetic factors. Furthermore, clinical variable factors consist of age, sex, symptoms of obstruction, perforation, primary tumor location, peri-operative blood transfusion, and bleeding per year. The tumor stage variables are based on TNM and Duke's classification, residual tumors, presence of nodes and distant metastases. Histopathological variables consist of pathology stage, tumor histopathology type and grading, presence of limphovascular and perineural invasion, lymphocyte infiltration and the reaction form of lymphatic nodes. Also, molecular oncogenetic variables include ploidy DNA, expression of oncogenes and antioncogenes (K-ras mutations, Deleted in Colon Carcinoma/DCC mutations, p53, nm23 gene and other allele abnormalities), tumor related antigens and other immunological factors (Carcinoma Embryonic Antigen, mucin-associated antigen, HIA-DR, and EGFR).[4–6]

One of the key chemical mediators of inflammation associated with cancer is TNF-α, and its production by tumors has been shown to be associated with a poor prognosis. Normally, TNF-α cannot be detected in serum or plasma of normal individuals but can be detected in individuals with cancer, especially in advanced case. In

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other words, TNF-α can be used as an examination factor to predict the stage of CRC and its prognosis.[7]

Materials and methods

Research methods

This is a cross-sectional analytic observational research. The purpose was to determine the differences in TNF-α level in CRC patient's plasma. Therefore, the observation includes 41 CRC patients from July to November 2018 at Wahidin Sudirohusodo Hospital. Furthermore, this research has received permission from the Health Research Ethics Commission of Medicine Faculty, Hasanuddin University with number 591/H4.8.4.5.31/PP36-KOMETIK/2018.

Population and sample

The population in this research were patients diagnosed with CRC, treated at Wahidin Sudirohusodo Hospital, Makassar. The sample was taken by total sampling.

Data analysis

The data were further processed using SPSS for Windows 21 software (IBM SPSS Statistics for Windows, Version 21.0. IBM Corp., Armonk, NY). Chi-square test was used to determine the relationship between variables.

Result

Characteristics of research subjects

Table 1 shows the distribution and characteristics data of 41 CRC patients. The age-based sample distribution has the highest number in the age category of 49–85 years with a total of 31 samples or 75.6% of the total respondents. Furthermore, male sex dominated the incidence of CRC in Wahidin Sudirohusodo Hospital with a total of 28 people or 68.3% of all samples. From 41 research samples, the majority are Muslims with 56.1%. Also, a total of 73.2 research samples are the Bugis Makassar tribe, and 18 samples (43.9%) were identified at stage III. Meanwhile, the examination of histopathology patients with CRC in Wahidin Sudirohusodo Hospital is 20 samples at medium grade or 48.8%.

Table 1
Characteristic of patients.

Characteristics	Distribution	
	n (*n* = 41)	%
Age (years)		
36–48	10	24.4
49–85	31	75.6
Sex		
Male	28	68.3
Female	13	31.7
Religion		
Islam	23	56.1
Protestant	8	19.5
Catholic	7	17.1
Confucianism	3	7.3
Tribe		
Bugis-Makassar	30	73.2
Java	2	4.9
Chinese	8	19.5
Etc.	1	2.4
Stages		
II	8	19.5
III	18	43.9
IV	15	36.6
Differentiation		
Good	11	26.8
Medium	20	48.8
Bad	10	24.4

Table 2
The relationship of TNF-α with research variables.

Variable	TNF-α		Total	*p*
	High	Low		
Age				
No risk	3 (25%)	4 (13.8%)	7 (17.1%)	0.681
Risk	9 (75%)	25 (86.2%)	34 (82.9%)	
Sex				
Male	9 (75%)	19 (65.5%)	28 (68.3%)	0.822
Female	3 (25%)	10 (34.5%)	13 (31.7%)	
Stage				
II	0 (0%)	8 (27.6%)	8 (19.5%)	0.000
III	2 (16.7%)	16 (55.2%)	18 (43.9%)	
IV	10 (83.3%)	5 (17.2%)	15 (36.6%)	
Differentiation				
Good	5 (41.7%)	6 (20.7%)	11 (26.8%)	0.141
Medium	3 (25%)	17 (58.6%)	20 (48.8%)	
Bad	4 (33.3%)	6 (20.7%)	10 (24.4%)	
Total	12 (100%)	29 (100%)	41 (100%)	

The relationship of TNF-α with research variables

The cross-tabulation between the TNF-α values and the variables is presented in Table 2. The table shows that although 75% of samples with a high TNF-α category in the variable age are at risk, TNF-V levels in the blood do not have a significant relationship with the respondent's age with *p* value of 0.681.

Furthermore, in the sex variable, 75% respondents with the highest TNF-α levels category were found in male sex and statistical tests showed that there was a *p*-value of 0.822. Therefore, it can be concluded that there is no significant relationship between sex and TNF-α in the blood plasma of CRC patients.

The largest percentage of CRC stages in the TNF-α category is at stage IV. After conducting the statistical test, the *p*-value = 0.000 was obtained. Because the *p* value >0.05, it can be concluded that there is a significant relationship between the CRC stages and TNF-α in blood plasma.

Discussion

In the research subject, it was found that the most sex was male (68.3%), of which 31 people (75.6%) from a total sample of 41 were ≥50 years. This is in line with Kuipers in 2015 and Brenner in 2014, which stated that the most frequent incidence of CRCs occurred in male above 50 years. This might be caused by differences in dietary patterns and lifestyle.[8,9]

Also, most of the subjects were men ≥50 years, and the aging process is closely related to increased inflammatory activity in the blood, including elevated levels of TNF-, IL-6, cytokine antagonists, and neopterin. Previous studies have shown that plasma cytokine levels are strongly influenced by age and other factors such as the presence of chronic disease.[10,11] However, in this research no relationship was found between age and TNF-levels in plasma.

Furthermore, this research found a significant relationship between the CRC stage and TNF-levels in blood plasma ($p<0.001$), where the highest TNF-levels were found at stage IV (443.4ng/L), and the lowest was found at stage II (89.1ng/L), this is in line

with the research conducted by Natkaniec in 2018.[12] This shows that the higher the CRC stage, the higher the TNF-levels in blood plasma. This is in line with some previous studies even with different examination techniques.[13–16] Based on the results, it can be proven that there is a relationship between chronic inflammation and the process of cancer, where TNF- is one of the cytokines that play a role in the inflammatory response. According to previous researches, it is reported that TNF- is highly related to all stages in the tumorigenesis process, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis.[17,18]

Furthermore, the researchers found that no significant relationship between the degrees of differentiation and TNF-levels in plasma (p>0.05). However, the highest level of TNF- is shown in the CRC with bad differentiation (328.1ng/L). These results are not in agreement with others that stated there is a relationship between elevated levels of TNF- in the blood and the differentiation degree of CRC.[12,19,20] This might be due to the small number of samples and the unequal degree of differentiation in the subjects, where the highest number of CRC patients with moderate differentiation was found.

Based on the results, it can be stated that there is a relationship between inflammation and CRC, where TNF- plays an important role in it. Furthermore, TNF- not only functions as a pro-inflammatory cytokine but also contributes to the development of CRC. Therefore, TNF-inhibitors are widely used as cancer therapies today.[21]

At present, the use of anti-inflammatory drugs will not provide good therapeutic effect if used as monotherapy in patients with CRC, but it would be better if given in conjunction with chemotherapy or radiotherapy. In preventing the occurrence of CRC, anti-inflammatory therapy can be used alone or combined with other therapeutic strategies. In addition, nonsteroidal anti-inflammatory drugs (NSAIDs), such as Sulindac which inhibits COX1, COX2, and NF-κβ activity, can be used to prevent or be combined with other therapies to treat CRC. Other NSAIDs, for example Aspirin, can reduce the risk of CRC and are often considered a chemo preventive agent. Specific COX2 inhibitors, such as Celecoxib and Rofecoxib, can reduce the risk of CRC and reduce the colorectal adenomatous polyp progression to carcinoma. Furthermore, TNF-inhibitors such as Infliximab, Etanercept, and Anakinra, have been used as therapy in patients with autoimmune diseases. It is important to know which therapies can prevent IBD (as a major risk factor for CRC), and tumor-promoting inflammation, without reducing the effect of anti-tumor immunity.[7,14,17]

Conclusions

There is a significant relationship between TNF-α levels in plasma and CRC stage, where the higher TNF-α levels in plasma, the higher the CRC stage. Furthermore, the examination of TNF-α level in plasma can be used as a diagnostic factor in assessing the development and prognosis of patients with CRC. It can also be used as preliminary information in a high-risk CRC group before other more invasive tests (CT-Scan, endoscopy and biopsy). Therefore, the use of anti-inflammatory drugs and TNF-α inhibitors can be considered as the therapy of patients with CRC to complement the standard therapy.

Ethical approval

All procedure has been approved by Ethics Commission Faculty of Medicine, Hasanuddin University Number: 591/H4.8.4.5.31/PP36-KOMETIK/2018

Consent

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The patients have given their written informed consent on admission to use their prospective data base and files for research work.

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Authors' contributions

WS, FL, IL, and RL wrote the manuscript and participated in the study design. WS, MP and FL drafted and revised the manuscript. WS, IL, RL and MP performed the treatment and surgery. FL, and MF performed bioinformatics analyses and revised the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no conflict of interests.

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