The Association between B-RAF V600E Gene Mutation with Age, Grading and Clinical Response in Colorectal Cancer at the Center General Hospital (RSUP) Prof. Dr. I Goesti Ngoerah Gde Ngoerah in Bali

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Abstract

Background: Colorectal cancer (CRC) is the most common type of cancer found in people over 50 years of age and is a disease associated with aging. CRC is the most preventable and curable cancer due to the pre-clinical phase until it becomes cancer between 10 - 20 years. The B-RAF mutation plays an important role in the prognosis and treatment of CRC patients. The B-RAF V600E mutation is the most common B-RAF mutation. This study aims to analyze the relationship between mutations in the B-RAF V600E gene with age, grading, and clinical response in CRC patients in Bali.
Method: We used FFPE samples that were confirmed as CRC tissues by the pathologist. Identification of BRAF V600E mutation was identified using polymerase chain reaction (PCR) and direct sequencing. Data on age, grade, and clinical response were recorded from the patient's medical record. Data was analized using Fisher-exact test.
Results: The B-RAF V600E mutation samples were identified in 3 out of 74 samples (4.1%). The B-RAF V600E mutation was found in 3/3 of the samples (100%), which aged over 50 years old. All those mutated cases (100%) were low grade. Positive vs. negative clinical response was shown at 2/3 (66.7%) samples vs. 1/3 (33.3%) sample. No significant correlation was found between B-RAF V600E gene mutation and age (p=0.643), grading (p=0.808), and clinical response (p=0.358).
Conclusion: We have identified the prevalence and type of BRAF mutation in our cases, however, there is no significant correlation between mutation B-RAF V600E gene and age, grade and clinical response of CRC patients at the center general hospital (RSUP) Prof. Dr. IGNG Ngoerah, Bali.

Keywords: B-RAF Mutation; Colorectal Cancer; Age; Grading; Clinical Respons; Aging

1. Introduction

Aging is defined as a phenomenon that increases the risk of organ failure with age (Pangkahila, 2019). Aging is a universal biological process that manifests itself as a decrease in functional capacity and an increased risk of morbidity and mortality over time. (Zhang et al., 2017) Cancer is the leading cause of death worldwide due to an aging population and the complex interaction between an individual's genetic makeup and exposure to environmental risk factors. (Gandini et al., 2014). There are several mechanisms of aging associated with cancer, including colorectal cancer (CRC), one of which is a continuous decline in the function of cells and tissues of the body, resulting in an accumulation of damage to cells and tissues of the body, which will eventually result in aging.

Age is the most important risk factor for developing cancer, with the majority of cancer patients being diagnosed over the age of 50. (Gunasekaran et al., 2019; Pickhardt et al., 2013) On histopathological grading assessments, low grade is often found in comparison with high grade. (Jang et al., 2017), while more negative clinical response was found than positive clinical response. (Bendell et al., 2013; Del Rio et al., 2017)

The World Health Organization (WHO) mentions cancer as one of the main causes of death. (Pangribowo, 2019) In 2020, the International Agency for Research on Cancer (IARC) released the Globocan 2020 data update, with an estimated global cancer burden of 19.3 million new cases and 10 million cancer deaths in 2020. (GLOBOCAN, 2020) Worldwide colorectal cancer (CRC) ranks third among cancers the most

with an incident rate of 10.0% and a high mortality rate that ranks second at 9.4%. (Sung et al., 2021) The Global Burden of Cancer Study (Globocan) noted that total cancer cases in Indonesia in 2018 ranked third at 8.6%, while deaths ranked fifth at 7.9%. (WHO, 2020)

Trigger factors for cancer, including CRC, are lifestyle factors such as frequent consumption of red meat, excessive alcohol consumption, abdominal obesity, a lack of physical activity, and smoking. (Aulawi, 2013) Food ingredients such as vegetables and fruits can reduce the risk of colorectal cancer. This is caused by the content of fiber, PUFA, polyphenols, and vitamins, which have a role in preventing colorectal carcinogenesis. (Swari et al., 2019) CRC develops through several genetic and epigenetic pathways. These pathways are defined on the basis of three molecular features: (i) mutations in DNA mismatch repair genes, leading to a DNA phenotype of microsatellite instability (MSI); (ii) mutations in APC and other genes that activate the Wnt pathway, marked by a chromosomal instability (CIN) phenotype, and (iii) global genome hypermethylation, resulting in the turn-off of tumor suppressor genes, which was shown to be the CpG island methylator phenotype (CIMP). (Mojarad et al., 2013)

The B-RAF gene encodes a serine/threonine protein kinase that participates in the MAPK/ERK signaling pathway and plays an important role in cancer. B-RAF participates in the MAPK signaling pathway. This pathway regulates important cell functions, including cell growth, differentiation, proliferation, senescence, and apoptosis. (Hussain et al., 2015) Most of the B-RAF gene mutations (96%) occur in exon 15 at codon 600, often known as V600E mutations. A mutation of the B-RAF V600E gene is found in 1 out of 10 CRC patients. Patients with the B-RAF V600E mutation have a poor prognosis, with a median survival of less than 12 months. (Luu & Price, 2019)

According to an epidemiological study by Seligman et al., it was found that mutations in the B-RAF gene occur in 5–15% of CRC. (Seligmann et al., 2017) Mutations in the B-RAF gene act as biomarkers to determine the prognosis and treatment of CRC. From a prognostic point of view, B-RAF gene mutations are associated with poor prognosis and survival shorter life/decreased numbers survival overalls (OS) in CRC patients. (Wang et al., 2019) The existence of this mutation has an impact on therapeutic mechanisms such as anti-EGFR drugs, which are ineffective, because the MAPK pathway is continuously active, which makes cells also continuously proliferate. (Rompis & Dewi, 2019)

Prevention of CRC can prevent individuals from suffering from CRC from the start with a healthy lifestyle such as healthy food, physical activity, not smoking, and not drinking excessive alcohol, and this will improve the quality of life in individuals who are not diagnosed with CRC. Colorectal cancer is the most preventable and curable of all cancers in humans, because this disease is characterized by a very long pre-clinical phase until it becomes cancer, lasting 10–20 years. (Miftahussurur & Rezkitha, 2021; Pickhardt et al., 2013)

B-RAF gene mutations are known to play a role in the pathogenesis, prognosis, and treatment of colorectal cancer, but research on this mutation has not been widely carried out and is very limited in Indonesia, especially in Bali. Limited information about B-RAF gene mutations cannot be separated from the lack of epidemiological data about the profile of B-RAF gene mutations in colorectal cancer patients.

The data of this study can be used as basic information about B-RAF gene mutations in colorectal cancer patients and as additional information in other studies about B-RAF gene mutations in colorectal cancer patients that are related to aging at the age of over 50 years. In addition, this research data may be related to age, grading, and clinical response in colorectal cancer patients, which can later become a molecular parameter for colorectal cancer treatment in Indonesia, especially in Bali. This study aims to analyze the relationship between the B-RAF V600E gene mutation and age, grade, and clinical response in colorectal cancer at the central general hospital (RSUP) Prof. Dr. I Goesti Ngoerah Gde Ngoerah, Bali.

2. Materials and method

2.1 Research ethics

This study has been approved by local ethics committee with EC number: 2645/UN.14.2.2.VII.14/LT/2022.

2.2 Samples

Samples used in this study were 74 formalin-fixed paraffin-embedded (FFPE) stored at the Department of Pathology, Prof. Dr. IGNG Ngoerah General Hospital, Denpasar, Bali, which were histologically confirmed as colorectal cancer specimens in 2018-2020**.** The inclusion criteria were all colorectal cancer patients who were diagnosed histopathologically during 2018–2020, complete medical records (clinical data, histopathology) according to the variables assessed in this study, and FFPE samples of colorectal cancer patients who were confirmed to be in good condition.

2.3 DNA isolation

DNA from 74 FFPE samples was isolated using the Black Prep FFPE DNA Kit (Analytic Jena GmbH, Germany). Briefly, 2×10 µm FFPE slices were lysed with 400 µL Lysis Solution MA and 40 µL Proteinase K. After incubation at 65 °C for one hour, samples were then incubated at 90°C for one hour in a thermal mixer at 1,000 rpm. Following incuba tion for 5 min at room temperature, the sample was centrifuged at 13,000 rpm for 2 min. The supernatant was transferred into a 1.5 mL microcentrifuge tube and 400 µL absolute ethanol 99% was added. The sample was transferred into the spin column and centrifuged at 12,000 rpm for 1 min. The sequential washing steps were carried out using 500 µL Washing Solution C and 650 µL Washing Solution BS, each centrifuged at a speed of 12,000 rpm for 1 min. After washing with 650 µL of 99% absolute ethanol and centrifuged at a speed of 12,000 rpm for 1 min, DNA was eluted in 100 µL elution buffer and centrifuged at 12,000 rpm for 1 min. DNA concentration was measured using SimplyNano (Biochrom).

2.4 PCR amplification of BRAF gene

Exon 15 of the B-RAF gene was amplified using the following primers (Macrogen, Korea): forward primer 5'TGCTTGCTCTGATAGGAAAATGA3' dan reverse primer 5'TGGATCCAGACAACTGTTCAAA3'. Amplification was carried out in a total volume of 10 µL containing 5 µL master mix, 0.2-0.3 µL for each forward and reverse primer B-RAF gene (10 µM), 0-1.6 µL ddH2O and 3-4,6 µL of 10 ng/µL DNA. PCR program or KRAS was carried out at 95 °C for 5 min and followed by 40 cycles of denaturation at 95 °C for 15 seconds, annealing at a temperature range of 50-56 °C for 60 seconds and extension at 72 °C for 30 seconds and a final elongation step at 72 °C for 5 min. For the BRAF PCR amplification, the program was carried out at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 30 seconds, annealing at 50 °C for 30 seconds, extension at 72 °C for 40 seconds and a final extension step at 72 °C for 5 min. The length of the amplicon for BRAF was 165 bp. PCR product was applied into 2% gel agarose dissolved in 1X TBE buffer.

2.5 BRAF direct sequencing

BRAF V600E mutation in exon 15 was identified by direct sequencing. PCR products were sent to the Genetika Science Laboratory, Jakarta. Direct sequencing was done using BigDye (Applied Biosystems).

2.6 Sequencing analysis

The sequencing results were checked using the BLAST website (Basic Local Alignment Search Tool): https://blast.ncbi.nlm.nih.gov/Blast.cgi. The purpose of this examination was to match the nucleotide sequence of the B-RAF gene recorded by BLAST with the nucleotide sequence of the samples used in the study. After the sample is confirmed, using computer software Chromas to checks the electropherogram variations of each sample.

2.7 Statistical analysis

Determination of sample size taken by total sampling where the number of samples is equal to the population. Data related to clinicopathological characteristics were recorded on a data collection sheet.

The independent variable in this study was the mutation of the B-RAF V600E gene, where there was a change in nucleotide bases that occurred in the B-RAF gene exon 15 codon 600, which was detected by PCR and sequencing, while the dependent variables were age, grade, and clinical response when a patient was diagnosed with CRC. This data uses secondary data collected from 2018–2020 at the center general hospital (RSUP) Prof. Dr. IGNG Ngoerah, Bali.

In the variable characteristics of the patient's age are categorized into ≥50 years and <50 years. (Gunasekaran et al., 2019; Sahin et al., 2021), while the grading assessment of CRC cancer is classified into: low grade: combination of good and moderate differentiation (grade1 and 2) and high grade: a combination of poorly differentiated and undifferentiated (grades 3 and 4). (Awan et al., 2017; Barresi et al., 2014) The clinical response in this study used the RECIST criteria (Response Evaluation Criteria in Solid Tumors), a recently published rule to define improvement (responses), stability, and progression of cancer. These criteria were published in 2000 by an international collaboration. (Fournier et al., 2022; SMF Ilmu Bedah, 2018)

The data obtained were entered in the master table and analyzed with the SPSS 25.0 computer program, with the following stages of analysis: descriptive analysis, where data analysis begins with conducting variable analysis by describing each variable studied and presenting it in the form of a frequency distribution table. To prove the existence of a association between the B-RAF V600E gene mutation and age, grading, clinical response, and statistical tests were carried out using the Fisher-exact test. The significance limit used

was $p < 0.05$ (95% confidence interval).

3. Results

3.1 Characteristics of samples

Characteristics of CRC samples based on age, grading and clinical responses can be seen in Table 1 and Figure 1. In terms of age, 64 samples (86.5%) were colorectal cancer patients aged over 50 years and 10 samples (13.5%) were patients under 50 years of age. The age range of colorectal cancer patients in this study started from 37 years old to 84 years old. The highest grading was low grade with 69 samples (93.2%), while high grade was 5 samples (6.8%). Data related to the clinical response of the most samples were negative responses as many as 44 samples (59.5%), consisting of clinical stable disease (SD) 1 sample (2.3%) and clinical progressive disease (cPD) 43 samples (97.7%)). The positive response was 30 samples (40.5%) consisting of clinical complete response (cCR) 30 samples (100.0%) and clinical partial response (PR) 0 samples (0%) (Table 1 and Figure 1).

Table 1 Characteristics of samples based on age, grading and clinical responses

Characteristics of the Sample		Frequency (N)	Percentage (%)
Age (years)			
≥ 50		64	86.5
< 50		10	13.5
	Total	74	100.0
Grading			
Low Grade		69	93.2
High Grade		5	6.8
	Total	74	100.0
Clinical response			
Positive response			
Clinical complete response (cCR)		30	100.0
Clinical partial response (PR)		0	0.0
	Total	30	40.5
Negative response			
Clinical stable disease (SD)		1	2.3
Clinical progressive disease (cPD)		43	97.7
	Total	44	59.5

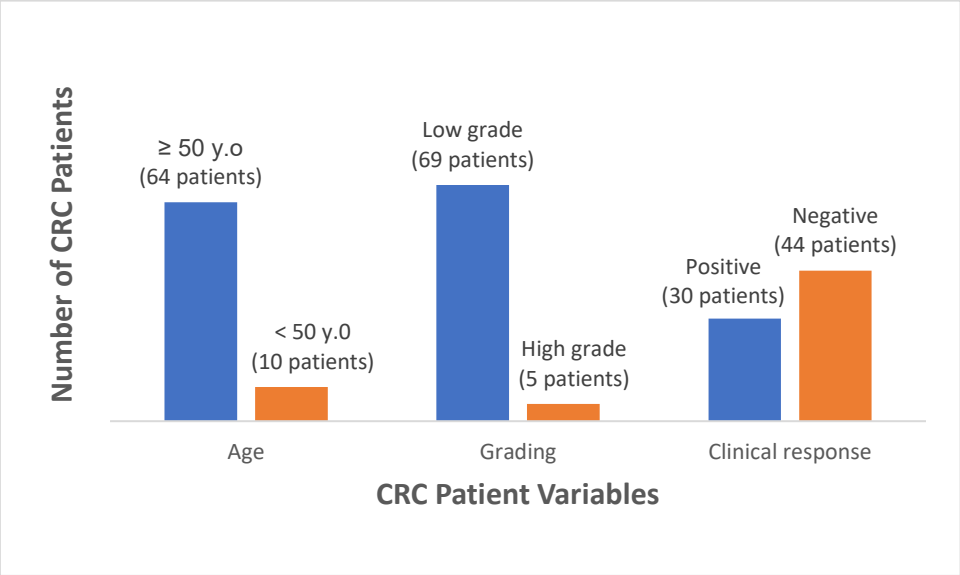

Figure 1. General Characteristics of CRC Patient Samples based on age, grading and clinical response

3.2 Prevalence of B-RAF mutation

The overall B-RAF mutation rate was 4.1% (3/74). From all 3 B-RAF mutated samples, three samples were found to have heterozygote mutations in nucleotide change T→A in codon 600. BRAF V600E mutation marked with alteration of valine (GTG) to glutamic acid (GAG). The frequency and amino acid change of B-RAF mutation are shown in Table 2 and Figure 2.

Table 2 Prevalence of B-RAF mutation in Colorectal Cancer

Mutation types	**Amino acid change Val → Glu**	**Total n = 74 (%)**
B-RAF V600E	Heterozygote mutation	3 (4.1%)
Wild-Type	-	71 (95.9%)

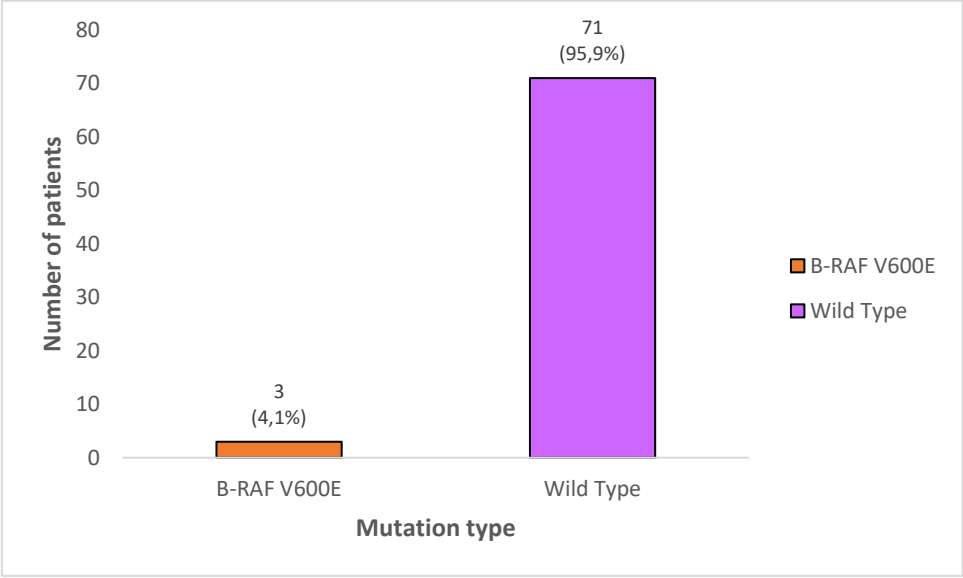

Figure 2. Prevalence of B-RAF mutation in Colorectal Cancer

3.3 BRAF mutation and age, grade, clinical response

Bivariate analysis of the association of the B-RAF V600E gene mutation with age, grading, and the clinical response of CRC can be seen in Tables 3, 4, and 5. From the results of the test analysis, Fisher-exact, found no significant association between mutations in the B-RAF V600E gene and age (p = 0.643), grade (p = 0.808), and clinical response (p = 0.358).

Table 3. The results of the analysis of the association between the B-RAF V600E gene mutation and age in CRC patients at the center general hospital (RSUP) Prof. Dr. IGNG Ngoerah, Bali.

Parameter	Age		Total	P
	≥ 50 yrs	< 50 yrs		
	N (%)	N (%)	N (%)	
Mutation Type				
Wild Type	61 (85.9)	10 (14.1)	71 (100.0)	0,643
B-RAF V600E Mutation	3 (100.0)	0 (0.0)	3 (100.0)	
Total	64 (86.5)	10 (13.5)	74 (100.0)	

Fisher's-exact test, significant if p <0.05

Table 4. The results of the analysis of the association between the B-RAF V600E gene mutation and grading in CRC patients at the center general hospital (RSUP) Prof. Dr. IGNG Ngoerah, Bali.

Parameter	Grading		Total	P
	Low Grade	High Grade		
	N (%)	N (%)	N (%)	
Mutation Type				
Wild Type	66 (92.9)	5 (7.1)	71 (100.0)	0.808
B-RAF V600E Mutation	3 (100.0)	0 (0.0)	3 (100.0)	
Total	69 (93.2)	5 (6.8)	74 (100.0)	

Fisher's-exact test, significant if p <0.05

Table 5. The results of the analysis of the association between the B-RAF V600E gene mutation and clinical response in CRC patients at the center general hospital (RSUP) Prof. Dr. IGNG Ngoerah, Bali.

Parameter	Clinical Response		Total	P
	Positive	Negative		
	N (%)	N (%)	N (%)	
Mutation Type				
Wild Type	28 (39.4)	43 (60. 6)	71 (100.0)	0.358
B-RAF V600E Mutation	2 (66.6)	1 (33.3)	3 (100.0)	
Total	30 (40.5)	44 (59.5)	74 (100.0)	

Fisher's-exact test, significant if p <0.05

4. Discussion

The results obtained from the data on sample characteristics in general are in Table 1, namely that the most common age for CRC patients is ≥ 50 years with 64/74 CRC (86.5%), and <50 years with 10/74 CRC (13.5%). This is in accordance with research conducted by Gunasekaran et.al. in Bali, the most age affected by colorectal cancer is above 50 years, namely 94/121 CRC (77.7%). (6) This research is supported by Sahin et al., which showed that the highest incidence of colorectal cancer was found in patients over 50 years of age, namely 46/60 CRC (76.7%). (24) This is because aging occurs when the function of cells and tissues decreases in maintaining structure and self-repair, resulting in an accumulation of damage to cells. The longer it lasts, the more it will cause a decrease in the body's resistance so that there will be a distortion of metabolism, which accelerates the onset of degenerative diseases and age-related diseases, including CRC. (Darmojo, 2015; Gunasekaran et al., 2019)

Based on the grading, the most common cases are low grade of 69/74 CRC (93.2%), meanwhile high grade of 5/74 CRC (6.8%). The results of the same research conducted by Phua et.al where category low grade

of 39/45 CRC (86.7%). (Phua et al., 2015) Research by Pratama and Andrianto with a result of 54/60 TRC (90.0%). (Pratama & Adrianto, 2019) This is because as many as 95% of CRC are adenocarcinomas originating from colonic polyps that develop into cancer, where in general the prognosis is good. (Pramiadi et al., 2016)

Monitoring clinical response can be assessed at least 4 weeks after completing therapy. According to WHO, there are two criteria for assessing clinical response, namely a positive response consisting of clinical complete response (CR) and partial response (PR), as well as a negative response consisting of stable disease (SD) and clinical progressive disease (PD). (28) clinical complete response (CR): Disappearance of all target lesions and all nodes have short axis < 10 mm. Clinical partial response (PR): reduction in the diameter of the largest tumor mass exceeding 30% of the tumor size before chemotherapy. Clinical stable disease (SD): the size of the tumor is fixed or not increasing and no new tumors are found. Clinical progressive disease (PD): a tumor with an increase of more than 20% in the largest diameter or the appearance of new nodules is considered to be a progressive disease. (Fournier et al., 2022; Trimonika et al., 2018) The clinical response in this study was obtained from the results of adjuvant chemotherapy (Folfiri or folfox) with a negative response of 59.5% at 44/74 CRC (SD 2.3% and PD 97.7%) more when compared with a positive response of 40.5% at 30/74 CRC (100.00% CR and 0.00% PR).

The results of the same research conducted by Del Rio et.al where the negative response was 44/79 CRC (55.7%). (10) Research by Bendell et.al with a negative response of 35/47 CRC (74.5%). (9) This was caused by adjuvant chemotherapy (folfiri or folfox) alone. To increase a positive clinical response, a combination of folfiri chemotherapy with other chemotherapy regimens or with radiotherapy can be considered. This combination therapy is expected to kill cancer cells that are sensitive to chemotherapy and change resistant cancer cells to become more sensitive to radiotherapy. (Trimonika et al., 2018)

Characteristics of the sample based on the type of mutation in this study, the B-RAF V600E mutation was found to be 4.1% (3/74 CRC patients). This is almost the same as the research that Yokoto et.al did namely the mutation of B-RAF V600E of 4.7% (15/319 CRC). Another study by that conducted by Modest et.al the prevalence of having the B-RAF V600E mutation was 11.6% (17/146 CRC) and by Clancy et.al the prevalence of B-RAF V600E mutation was 12.0% (56/475 CRC). (Clancy et al., 2013; Modest et al., 2012; Yokota et al., 2011) The number of incidents of BRAF V600E mutations in CRC patients varies, due to different ethnic populations with genetic predisposition and the role of environmental influences such as diet, smoking, and other unknown factors. (Siraj et al., 2014) As stated by Ye et.al (2015), there is a difference in the prevalence of the B-RAF V600E mutation in CRC patients because of geographic and/or racial differences between Eastern/Asian and Western populations. (Ye et al., 2015)

The characteristics of a sample of 74 patients who have been successfully amplified, based on the type of mutation and age, can be seen in Table 3. The results of the analysis of statistical tests in this study concluded that there was no significant correlation between the B-RAF V600E mutation and age (p = 0.643). In terms of age, this study showed that the mutation in the B-RAF V600E gene all occurred at the age of ≥ 50 years, compared to those aged < 50 years, where 3 out of 3 samples (100.00%) had B-RAF V600E mutation. In a study conducted by Siraj et al., (2014) on 757 sample CRC patients who experienced the B-RAF V600E mutation, in terms of age, it was more common in those over 50 years of age, namely 16/511 samples (3.1%) compared to under 50 years of age, namely 3/246 samples (1.2%), had the B-RAF V600E mutation. The results of the statistical test analysis of the research by Siraj et al., (2014), concluded that there was no significant correlation between the B-RAF V600E mutation and age (p=0.093). (Siraj et al., 2014)

The characteristics of a sample of 74 patients who have been successfully amplified, based on the type of mutation and grading, can be seen in Table 4. The results of the statistical test analysis of this study concluded that there was no significant correlation between the B-RAF V600E mutation and grading (p = 0.808). In terms of grading, the samples that had the B-RAF V600E mutation, this study showed that the mutation in the B-RAF V600E gene all occurred in low grade compared with high grade, where 3 out of 3 samples (100.00%) had the B-RAF V600E mutation. In a study conducted by Siraj et al., (2014) on 757 CRC patients, BRAF V600E mutations were more common in the low grade group, where 16 out of 664 samples (2.41%) had B-RAF V600E mutations, while the high grade of 3/93 samples (3.2%). The results of the statistical test analysis of the study by Siraj et al., (2014), concluded that there was no correlation between the B-RAF V600E mutation and grading (p=0.888). (Siraj et al., 2014)

The characteristics of a sample of 74 patients who have been successfully amplified, based on the type of mutation and clinical response, can be seen in Table 5. The results of the statistical analysis of this study concluded that there was no significant correlation between the B-RAF V600E mutation and clinical response (p = 0.358). Samples that have mutation of B-RAF V600E in terms of clinical response, this study showed that mutation in the B-RAF V600E gene occurred more frequently in positive clinical response, 2 out of 3 samples (66.7%), compared to negative clinical response. The results of a study conducted by Stintzing et al., (2017) on 43 CRC patients, found positive clinical responses of 22/43 samples of B-RAF V600 E (51.2%) more than negative clinical responses of 21/43 samples (48.8%). The results of the statistical test analysis of this study concluded that there was no correlation between the B-RAF V600E mutation and clinical response (p=0.560).

(Stintzing et al., 2017)

The role of the B-RAF mutation in age, grading and clinical response is where the B-RAF V600E mutation has a poor prognosis, this is because, in the B-RAF mutation, the protein in the cell membrane is always in an active state, always sending chemical signals from the cell membrane to the nucleus and continuously activate/reactivate the MAPK signaling pathway. In the nucleus, cell growth and cell proliferation occur continuously and uncontrolled, causing poor cell differentiation (high grade), which worsens the prognosis and resistance to anti-EGFR therapy (negative response). Whereas in old age, there will be an accumulation of damage to cells and tissues continuously with age, the older (≥ 50 years) the worse the prognosis. In addition, there are also mutations in other genes besides B-RAF that play a role in the pathogenesis of CRC, for example APC, KRAS, B-RAF, p53, MMR and hypermethylation in the DNA promoter region. (Sanz-Garcia et al., 2017; Yamagishi et al., 2016)

In this study, we found that B-RAF V600E mutation occurred in all low grade group samples while positive clinical responses were found more commonly as compared to the negative clinical response. This can be used as one of the parameters to prevent the worsening of KKR which has the B-RAF V600E mutation with a healthy lifestyle, so that it is in accordance with the anti-aging medicine concept which is expected to improve quality of life and life expectancy. The results of this study may be due to the proportion of samples in the low grade group is higher (66/74 samples) than the high grade group (5/74 samples). While there were more positive clinical responses, this may be due to differences in terms of geography, genetics, environment and race (Siraj et al., 2014; Ye et al., 2015). Nevertheless, our study is providing scientific data on the prevalence of BRAF gene mutations in CRC patients in Bali and determines the relationship between the B-RAF V600E gene mutation and age, grading, and clinical response in colorectal cancer, so that these data can be used as a basis for adding to the literature on the relationship between B-RAF gene mutations V600E in colorectal cancer patients and colorectal cancer treatment in Indonesia, especially in Bali.

5.Conclusion

Taken together, it was concluded that there was no association between mutation in the B-RAF V600E gene and age, grading, and clinical response in CRC patients at the center general hospital (RSUP) Prof. Dr. IGNG Ngoerah, Bali, period 2018-2020. This may be due to the very small number of B-RAF V600E mutation samples and the population in this study did not find many CRC patients who had B-RAF V600E gene mutations. But from the prevalence of general characteristics of colorectal cancer patients, it shows that most of them are over 50 years old and the low grade group, as well as negative clinical response. This is consistent with the general characteristic clinicopathologic prevalence of CRC patients.

Although the sample size in this study is small and cannot yet be generalized to the Indonesian population, our finding contributes to the data on the prevalence and characteristics of BRAF mutation in colorectal cancer patients in Bali, whose data is very restricted. Further research is needed with a larger sample size, data on lifestyle, as well as family history and personal history so that the data obtained is sufficient for proper statistical test analysis.

Competing Interests

The authors declare no competing interests.

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