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Prevalence of PIK3CA Mutation Based on Tumour Locations Among Colorectal Cancer Patients in Bali

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ABSTRACT

Colorectal tumour location is important in determining therapeutic strategies because the location of the tumour will affect the histological and molecular characteristics of the cancer. Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) is a gene known to be associated with colorectal cancer location. The prevalence of PIK3CA mutations in colorectal cancer (CRC) ranges from 10-20%. Although the prevalence is small, this gene has a potential as a predictive and prognostic biomarker in the management of CRC. 80% of PIK3CA mutations occur in exon 9 (codon 542 and 545) and exon 20 (codon 1047). This study aimed to determine the prevalence of PIK3CA gene mutations based on tumour location among colorectal cancer patients in Bali, especially in Prof. dr. I G.N.G. Ngoerah Hospital, Denpasar, Bali. DNA was isolated from formalin-fixed-paraffin embedded (FFPE) blocks of colorectal cancer samples which were stored in the Department of Pathology of Prof. dr. I G.N.G. Ngoerah Hospital. Mutation was detected using polymerase chain reaction (PCR) and direct sequencing. Data on age, gender, and tumour location were recorded from patient medical records.

Our findings on 31 colorectal cancer samples showed 26 samples (96.3%) were found with heterozygous mutation in exon 9 with AC genotype, but no mutation was found on exon 20. Based on tumor location, among 26 samples with mutation, 16 samples (61.5%) were left colorectal cancer and 10 samples (38.5%) were right colorectal cancer. Further studies are needed to identify the association of this mutation with colorectal cancer location and the other clinicopathological aspects of CRC.

Keywords: Colorectal cancer, PIK3CA gene mutation, PIK3CA exon 9, PIK3CA exon 20, colorectal cancer location, left-sided colorectal cancer, right-sided colorectal cancer

INTRODUCTION

Aging is a biological process in every living organism characterized by declining in body function at the molecular, cellular, or tissue level, resulting in the occurrence of various degenerative diseases such as cancer that can interfere the quality of life [(1)]. Colorectal cancer is one of the cancers whose incidence increases by age [(2)]. This cancer is still one of the highest causes of death in the world [(2)]. One of the aetiologies of colorectal cancer is gene mutation. The presence of mutations in certain genes can cause the onset of colorectal cancer [(3)]. These mutations can occur in oncogenes, tumour suppressor genes or genes that regulate DNA repair mechanisms [(3)]. One gene that is often mutated in colorectal cancer is Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA). The PIK3CA gene plays an important role in Akt activation and the regulation of various vital cellular activities, such as proliferation, protein metabolism

and synthesis, angiogenesis, apoptosis, and autophagy [(4)]. PIK3CA mutations activate the p110α enzyme, the main catalytic subunit of PI3K, and over-stimulate Akt signalling, which in turn triggers cancer cell proliferation and migration [(5)]. There are two mutation hotspots in the PIK3CA gene, namely exon 9, codons E542K and E545K in the helical domain and exon 20, codon H1047R in the kinase domain [(5)]. Mutations in these two domains indicate an increase in enzymatic function and trigger oncogenic transformation [(6,7)]. Several studies have shown an association of PIK3CA gene mutations with colorectal cancer sites. Based on its anatomical location, colorectal cancer is divided into left sided colorectal cancer (LCRC) and right sided colorectal cancer (RCRC) [(8)]. Knowing the tumour location in CRC is important because it is a crucial factor in determining its progressivity, management and prognosis [(9)]. Several studies abroad have shown a positive association between PIK3CA gene mutations and colorectal cancer location. However, data on prevalence of PIK3CA mutation in colorectal cancer in Bali are limited. Therefore, through this study we sought data on the prevalence of PIK3CA mutations in colorectal cancer especially colorectal cancer sites.

LITERATURE REVIEW

Cancer is a complex disease characterised by mutations in genes that control various hallmarks of the disease, including escape from programmed cell death, promotion of genome instability and mutation, and proliferative signalling [(1)]. Aging is well known as a major risk factor for cancer. One type of cancer whose incidence increases by age is colorectal cancer [(2)]. One of the characteristics of aging is hyperplasia, the most serious type of which are cancerous [(1)]. Dysfunctional epigenetic aging of the normal colon has been associated with an increased risk of colorectal cancer [(8)]. Colorectal cancer is a complex and multifaceted disease involving genetic and environmental factors [(9)].

The PIK3CA gene is one of the genes that is often mutated in colorectal cancer cases [(5)]. Mutations in the PIK3CA gene occur in 10-20% of colorectal cancer cases where 80% of mutations occur in exons 9 and 20 [(10)]. Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) is a 34 kb gene located on chromosome 3q26.3 consisting of 20 exons encoding 1068 amino acids producing a protein size of 124 kDa [(11)]. PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) encodes the p110α subunit of class IA PI3K catalysis, an important component of the lipid kinase, phosphatidylinositol 3-kinase (PI3K) [(4)]. Class I phospatidyl-inositol 3-kinase is a heterodimeric complex consisting of a 110 kDa catalysis subunit (p110), an 85 kDa regulatory subunit and an accessory subunit. Class I phospatidyl-inositol 3-kinase is further subdivided into two sub classes, class IA and IB; class IA PI3K consists of catalysis sub units p110α, p110β and p110δ encoded by PIK3CA, PIK3CB, PIK3CD respectively [(4)].

The p110α subunit consists of five domains: an adaptor-binding domain (ABD) that provides an interaction site with regulatory subunits, a RAS-
binding domain (RBD) that plays a role in the activation of PI3K through the Ras pathway, protein kinase C homolog-2 (C2) that functions as the domain that has an affinity for lipid membranes in forming bonds, a helical domain bridging the 'gap' in the structure and a kinase domain [(4)]. Generally, regulatory subunits contain several modular protein interaction domains: a Src-homology 3 (SH3) domain, two SH2 domains and an inter-SH2 domain (ISH2). The ISH2 domain binds to p110 and suppresses catalysis activity [(4).]

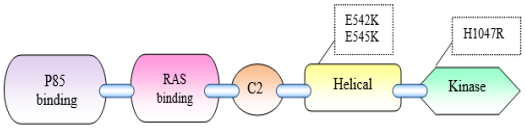

Figure 1. PIK3CA gene (p110a PI3K catalytic subunit) and its functional domain

Once activated through interaction with RTKs or GPCRs, the regulatory subunit will inhibit the catalytic subunit. Class IA PI3Ks facilitate the production of phosphatidylinositol 3-phosphate which ultimately leads to the activation of Akt and the regulation of various vital cellular activities, such as proliferation, protein metabolism and synthesis, angiogenesis, apoptosis, and autophagy [(4)]. Phospatidyl-inositol 3-kinase plays a role in the regulation of various important cellular activities through the production of PIP3. When PI3K is activated by upstream signals such as RTK, it facilitates PIP3 production through phosphorylation of PIP2 and the phosphatase and tensin homologue deleted on chromosome 10 (PTEN) antagonizes PI3K activity through dephosphorylation of PIP3 to PIP2, making it a potent upstream/downstream regulator of PI3K activity. Accumulated PIP3 production is localized at the plasma membrane and recruits phosphatidylinositol-dependent kinase (PDK) and PKB (or Akt). Phosphatidylinositol-dependent kinase activates Akt and triggers the regulation of various important cellular activities such as cell proliferation, metabolism, protein synthesis, angiogenesis, apoptosis and autophagy. In the process of autophagy, it has been previously known that PIP3 produced by class I PI3K inhibits autophagy while PIP3 produced by class III PI3K triggers autophagy. Mechanisms that often trigger the appearance of disturbances in PI3K signaling are genetic or epigenetic changes in the PTEN gene, upregulated activation of constitutive upstream PI3K [(12)]. Mutations in PIK3CA will cause PI3K to lose its upstream signaling ability on growth factors and result in uncontrolled production of PIP3. PTEN inhibition is not enough to overcome the excessive production of PIP3, thus its ability to be a downstream regulator becomes inefficient. Uncontrolled phosphatidylinositol (3,4,5)-trisphosphate will trigger uncontrolled Akt activation resulting in disease progression and proliferation and disruption of the cancer cell cycle [(4)].

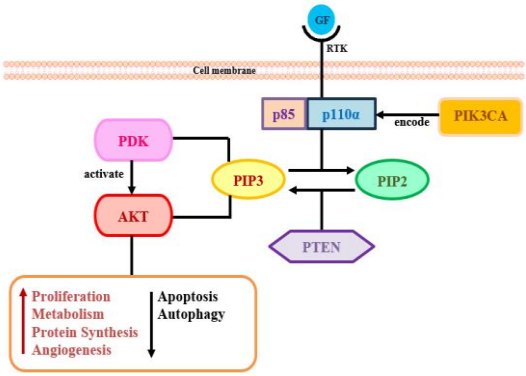

Figure 2. class IA phosphatidylinositol 3-kinase (PI3K) signaling and downstream effects.

Colorectal Cancer Location

Tumor location is a crucial factor in determining tumor progressivity, management and prognosis [(13)]. Based on its anatomical location, colorectal cancer is divided into two namely left sided colorectal cancer (LCRC) and right sided colorectal cancer (RCRC) [(14)]. Right-sided colorectal cancer originates from the ascending colon, cecum and transverse colon, while left-sided colorectal cancer originates from the descending colon, splenic flexure, sigmoid colon and rectum [(15)]. The histologic features of RCLC are different from LCRC. The morphology of RCRC tends to be flatter, sessile serrated adenoma or mucinous adenocarcinoma. Whereas LCRC is more often tubular, villous, and typical adenocarcinoma. Because LCRC has a polypoid morphology, left-sided tumors are more easily detected by colonoscopy at an early stage, whereas right-sided tumors tend to be more difficult to detect at an early stage due to their flat shape [(14)]. Patients with RCRC tend to have larger tumour size, more advanced stage and poorer differentiation [(16)]. LCRC is more common in males and young age, while RCRC is more common in females and the elderly. In terms of metastatic ability, LCRC often spreads to the liver and lungs, while RCRC often spreads to the peritoneal (peritoneal carcinomatosis), this form has a worse

prognosis. Besides the differences, location, characteristics, histology, the left and right tumours also have different molecular pathways. Patients with RCRC tend to have microsatellite instability-high (MSI-H) tumours while patients with LCRC more often have chromosomal instability-high (CIN-high) tumours [(14)]. This difference in side also affects the determination of therapy predicting cancer response to the therapy given [(17)]. Right colorectal cancer responds well to immunotherapy while left colorectal cancer responds well to standard chemotherapy including adjuvant chemotherapy [(14).]

PIK3CA mutations in colorectal cancer

Missense mutations are the most common type of mutation in colorectal cancer. Mutations are often found in two different domains of p110α, namely the helical domain at positions E542K and E545K where the amino acid glutamate changes to lysine and in the kinase domain at position H1047R where the amino acid histidine changes to arginine [(12)]. Gymnopoulus et al (2007) reported fifteen rare mutation variants of p110α that have the ability to induce oncogenic transformation, including; R38H, K111N, N345K, C420R, P539R, E545A, E545G, Q564K, Q546P, H710P, T1025S, M1043I, M1043V, H1047L, and H1047Y [(18)]. Mutations in either domain were shown to enhance enzymatic function through activation of the Akt pathway and trigger oncogenic transformation both in vitro and in vivo [(7)]. Mutation of the helical domain will reduce the inhibitory effect of p85 on p110α. One of the most common mutation hotspots is E545K which results in amino acid substitutions of opposite charge, thought to cause disruption of interactions between catalytic and regulatory proteins resulting in loss of regulatory and constitutive functions to PIK3CA activity which can trigger oncogenesis [(19)]. Mutations in the helical domain do not rely on binding to p85 but require RAS-Guanosine triphosphate (GTP) interactions. Kinase domain mutations will increase the interaction of p110α on lipid membranes [(20)]. Mutations in the kinase domain can be activated without requiring RAS-GTP binding but are strongly influenced by p85 interactions [(21)]. Liao et al have a hypothesis that mutations in exon 9 or 20 affect tumour properties and the presence of mutations in both exons causes tumours to be more aggressive than PIK3CA wild type cancer or single mutations in either exon 9 or exon 20 [(7)]. Somatic PIK3CA mutations that occur in the helical domain of exon 9 and the kinase domain of exon 20 are known as important biomarkers in the assessment of patient survival and response to chemotherapeutic agents [(4)]. According to the results of a meta-analysis study conducted by Juan Jin et al in 2019, it shows that in general PIK3CA mutations have an association with proximal tumour location, mucinous differentiation, KRAS mutations and MSI [(5)]. Patients with cancer located in the proximal area of the colon tend to have larger, poorly differentiated and advanced tumours. Many studies have shown that the prognosis of RCRC is worse than LCRC [(14)].

MATERIALS & METHODS

Total sample used in this study were 31 Formalin-Fixed Paraffin-Embedded (FFPE) of colorectal cancer patient's specimen from Prof. dr. I G.N.G. Ngoerah Hospital. Those samples were stored in Department of Pathology, Prof. dr. I G.N.G. Ngoerah Hospital.

Data collection included demographic data and clinical parameters. Demographic data were age and gender. The clinical parameter used was colorectal cancer location. There are two classifications of colorectal cancer: right-sided colorectal cancer (RCRC) and left-sided colorectal cancer (LCRC). RCRC tumours arising from ascending colon, cecum and transverse colon. LCRC tumour arising from descending colon, splenic flexure, sigmoid colon and rectum. This study was approved by ethics committee of Faculty of Medicine Udayana University (EC no 1226/UN14.2.2.VII. 14/LT/ 2023).

DNA extraction from FFPE samples

DNA was extracted using Black Prep FFPE DNA Kit (Analytic Jena GmbH, Germany). FFPE samples were lysed with 400µL of MA solution and 40 µL of Proteinase K. DNA was incubated for one hour at 65°C until completely lysed. Samples were incubated again at 90°C for one hour in a thermal mixer and then centrifuged at 1,000 rpm. Samples were incubated for 5 minutes at room temperature, followed by centrifugation at maximum speed for 2 minutes. The supernatant was transferred into a 1.5 mL microcentrifuge tube and 400 µL absolute ethanol 99% was added. The samples were transferred into a spin filter and then centrifuged at 12,000 rpm for 1 minute. The sequential washing steps used 500 µL of Washing Solution C and 650 µL of 99% absolute ethanol and then centrifuged at 12,000 rpm for 1 minute. Next, DNA was eluted in 100 µL elution buffer then centrifuged at 12,000 rpm for 1 minute. The concentration of isolated DNA was measured using SimpliNano (Bio chrom).

Identification of PIK3CA Mutation

PIK3CA gene exon 9 was amplified using: forward primer 5'-TGGTTCTTTCCTGTCTCTGAAA -3' and reverse primer 5'-TCTCCATTTTAGCACTTACCTGT-3'. Exon 20 was amplified using: forward primer 5'- -GATGCTTGGCTCTGGAATGC -3' and reverse primer 5'-TGCACAATCCATGAACAGCAT -3'. Primers were designed using Primer3 software, PIK3CA Genbank data (Gene ID NM_006218). Amplification was run in a total volume of 10µL, containing 5 µL master mix, 0,2-0,3 µL for each forward and reverse primer of PIK3CA gene, 0-1,6 µL ddH2O and 3-4,6 µL of 10 ng/µL DNA. PCR program was performed at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at a temperature range of 48-54°C for 60 seconds and extension at 72°C for 30 seconds and elongation at 72°C for 5 minutes. The length of amplicon for exon 9 PIK3CA was 322 bp and for exon 20 was 305 bp. PCR product was applied into 0,8% gel dissolved in 1x TBE buffer. Direct sequencing was performed to identify PIK3CA mutation in exon 9 and 20. PCR products were sent to Genetika Science Laboratory, Jakarta. Basic Local Alignment Search Tool (BLAST) was performed to analyzed the base sequence conformity of the sequencing results. The type of mutation was identified by electropherogram using Chromas software.

STATISTICAL ANALYSIS

Descriptive statistics to determine the prevalence of PIK3CA gene mutations in the study sample. Results were presented with frequency tables

RESULT

Table 1. Characteristic of samples based on age, gender and tumour location

Characteristic	Mean	N=31 n (%)
Age	67,42	
<50		2 (6,5)
>50		29 (93,5)
Gender		
Men		13 (41,9)
Women		18 (58,1)
Tumour location		
RCRC		13 (41,9)
LCRC		18 (58,1)

Thirteen (41,9) patients were male and 18 (58,1%) were female and most of them (29 out of 31 or 93,5%) were more than 50 years old. Based on colorectal cancer location, we identified 18 (58,1%) samples classified as left colorectal cancer (LCRC) and 13 (41,9%) were identified as right colorectal cancer (RCRC).

Table 2 Characteristics of samples based on PIK3CA mutation

Mutation Status	N (%)
Exon 9	
Mutant	26 (96,3)
Wildtype	1 (3,7)
Exon 20	
Mutant	0 (0)
Wildtype	27 (100)

Twenty six out of 27 samples (96,3%) identified as mutant PIK3CA. One sample in exon 9 was identified as wild type. No mutation were found in exon 20, all samples are identified as wildtype.

Table 3 Characteristic of mutant samples based on tumour location

Tumour Location	N (%)
RCRC	10 (38,5)
LCRC	16 (61,5)

Based on the colorectal cancer location, 16 out of 26 (61,5%) are sample classified as LCRC while the remaining 10 samples classified as RCRC.
DNA fragments were analyzed by gel electrophoresis (figure 3).

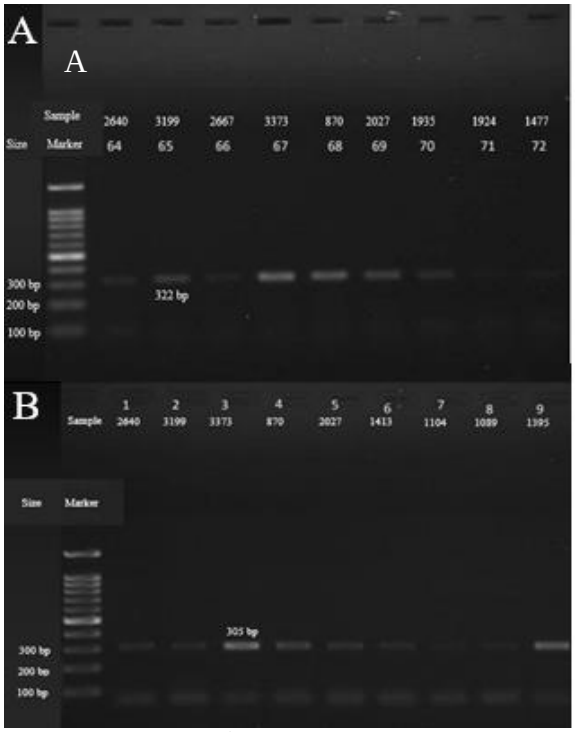

Figure 3 PCR product of PIK3CA gene on 0,8% agarose gel. (A) The size of PCR product of exon 9 is 322 bp and (B) exon 20 is 305 bp PIK3CA gene on 0,8% agarose gel

Furthermore, PCR products were sent to PT Genetika Science for sequencing. After the sequencing results came out, it was continued with the DNA base matching process to ensure the suitability of the sequencing sequence with the PIK3CA exon 9 and 20 genes. The sequence matching process was carried out using the basic local alignment search tool (BLAST) online through the National Centre for Biotechnology Information (NCBI) website access (figure 4).

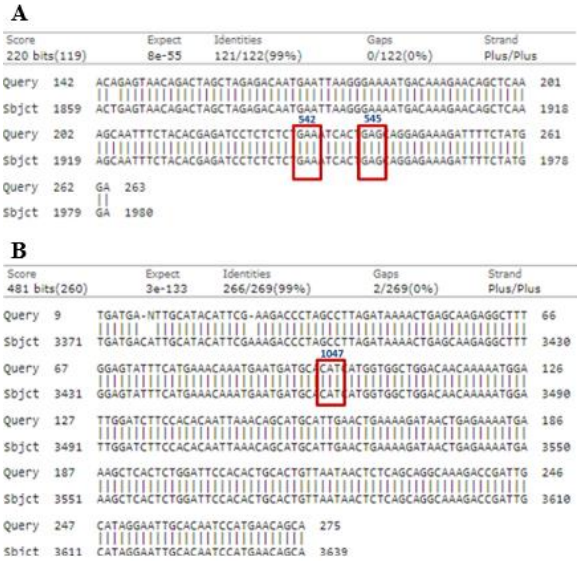

Figure 4 BLAST alignment result of PIK3CA gene (A) exon 9 codon 542,545; (B) exon 20 codon 1047

To identify the type of mutation, the electropherogram was examined with Chromas software (figure 5). In this software, the red graph shows thymine nucleotides (T), green is adenine (A), black is guanine (G), blue is cytosine (C). From the results of electropherogram analysis, there is a double peak graph in 26 samples of exon 9 codon 545 which indicates a heterozygous mutation with AC genotype where there is a substitution in the amino acid glutamate to alanine (E→A). One sample of exon 9 codon 545 was wildtype. No mutations were found in exon 9 codon 542 or exon 20.

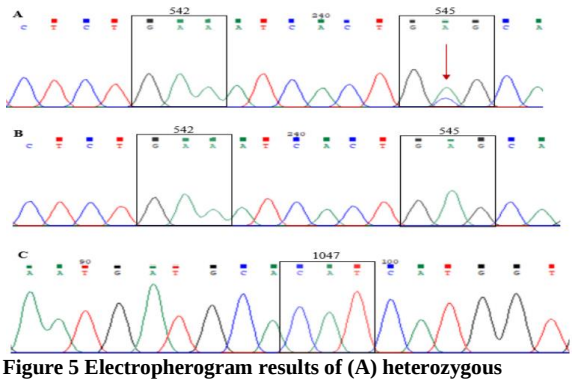

Figure 5 Electropherogram results of (A) heterozygous mutation AC genotype of exon 9 codon 545 of PIK3CA gene; (B) wildtype of exon 9 codon 542 and 545; (C) wildtype of exon 20 codon 1047

DISCUSSION

Based on the results of the study, 29/31 samples (93.5%) were over 50 years old and the remaining 2 samples (6.5%) were under 50 years old. This finding is in line with the research of Alzahrani et al (2021) which states that 90% of colorectal cancer cases occur at the age of ≥50 years [(22)]. Even according to Day & Velayos (2015), the risk of colorectal cancer doubles in the age range of 40 to 80 years [(23)]. The same thing was also found in Gunasekaran's research (2019) where out of 121 samples, 94 samples were over 50 years old [(24)]. The major manifestation of aging is the gradual loss of function or degeneration at the molecular, cellular, tissue and body levels. Aging is an essential risk factor for cancer. Hyperplasia is one of the characteristics of aging which can lead to malignancy [(1)]. The accumulation of cellular damage due to molecular, cellular and tissue degeneration with aging and exposure to carcinogens can trigger cell transformation, increasing the risk of malignancies such as colorectal cancer [(1)].

The results of this study showed that the prevalence of gender in samples of colorectal cancer patients at the Prof. dr. I G.N.G. Ngoerah Hospital was dominated by women, namely 18 samples (58%) and for the male sex as many as 13 samples (42%). This data contradicts Rawla's research (2019) which shows that men have a 1.5 times higher risk of colorectal cancer than women [(25)]. In addition, a study in the UK by White et al (2018) also states that the overall incidence rate of colorectal cancer is higher in men than women with the number of cases at that time being 22,844 and 18,421 cases [(26)]. According to Lin et al (2013) in the United States, the incidence of colorectal cancer is higher in men than in women of the same age. In families with a history of non-polyposis colorectal cancer, the risk of developing cancer is much lower in women (30%) compared to men (74%). Several observational studies have suggested that the increase in female hormones due to pregnancy, the use of exogenous hormones such as oral contraceptives and hormone replacement therapy are associated with a reduced risk of colorectal cancer in women [(27)]. In addition to hormonal factors, White et a l (2018) in their study mentioned several reasons for men's higher susceptibility to colorectal cancer such as biological factors and habits, where men tend to consume a diet high in red meat, processed meat, consume alcohol more often and have a higher tendency to smoke than women, besides that men also tend to experience visceral fat accumulation which can increase the risk of colorectal cancer [(28)].

Based on the location of colorectal cancer, the majority of samples were LCRC with 18 out of 31 samples (58%). One of the reasons behind the higher frequency of LCRC is the morphology and histology of LCRC which tends to be polypoid so it is easier to be detected while RCRC often shows a flatter shape so it is more difficult to be detected in the early phase [(14)]. Similar results were also mentioned in the study of Nawa et al (2006) where early-stage polypoid type cancers were dominantly found in the distal colon compared to the proximal (distal 59%; proximal 40%) ($p < 0.01$) and flat-shaped cancers were significantly more often found in the proximal colon (distal 25%; proximal 44%) ($p < 0,01$) [(29)]. Based on gender, the LCRC sample was dominated by women with 11 samples (61.1%) and 7 samples (38.9%) were men, this contradicts the study conducted by Baran et al (2018) where it was stated that LCRC was dominated by men [(14)]. In this study, the majority of LCRCs were located in the rectum with a total of 8 samples (44.4%), sigmoid and recto-sigmoid junction with 4 samples each (22.2%) and the descending colon with 2 samples (11.2%). Similar findings were also obtained in a study conducted by Anthonysamy et al (2020) at the Prof. dr. I G.N.G. Ngoerah Hospital where out of 275 samples of colorectal cancer patients 170 of them (61.8%) had tumours in the rectal area [(30)].

In our study, 13 out of 31 samples (42%) were identified as RCRC with areas covering the cecum, ascending colon, and transverse colon including the hepatic flexure. Based on gender, RCRC samples were dominated by women with 7 out of 13 samples (53.8%) and 6 other samples were men (46.2%), this is in line with the study of Phipps et al (2013) and Baran et al (2018) which states that the diagnosis of RCRC is more often found in women [(14,31)]. In a study conducted by Carethers (2018), it was stated that differences in the embryological origin of the proximal and distal colon, variations in the concentration of bile salts and other compounds, different levels of oxygenation, and differences in the microbial environment in each colonic area play a role in variations in the distribution of colorectal cancer, including its relationship with gender [(32)]. Based on the results of several cohort studies, RCRC is more common in women and old age, while LCRC is more common in men and young age [(14)]. According to Mik et al (2017), the difference in age prevalence may occur because patients with tumours in the proximal area of the colon tend to experience delays in diagnosis due to their flatter morphology and less distinctive symptoms in the early stages. The study found a significant association between right colon cancer and old age (P=0.0087) [(33)]. The results of our study are in line with that study where the mean age of patients with right colorectal cancer is greater than the mean age of patients with left colorectal cancer (66.2±8.81 years vs 64.3±11.8 years).

Mutation of PIK3CA Gene Exon 9 and 20

Missense mutations are a frequent type of mutation in the PIK3CA gene. Mutations often occur in three main hotspots including the helical domain (E542K, E545K) and kinase domain (H1047R) [(12)]. In our study, the PIK3CA gene mutation was examined in those three hotspots. Our findings found PIK3CA gene mutation in exon 9 codon 545, 26 samples (96.3%) out of 27 samples had heterozygous mutations with AC genotype and one sample (3.7%) was wildtype. Heterozygous mutations that occur in exon 9 codon E545A cause changes in adenine (A) nucleotides to cytosine (C) resulting in changes in the amino acid glutamate to alanine. According to Gymnopoulus et al (2007), the E545A mutation is included in fifteen rare mutation variants of p110α that also have the ability to induce oncogenic transformation although the frequency is not as much as mutations in the three main hotspots [(18)]**.** A number of studies have shown that the most common mutation of the PIK3CA gene in colorectal cancer is located in the E545K hotspot [(34)]. A study by Ranjbar (2019) in Iranian population showed the frequency of PIK3CA E545K mutation in colorectal cancer was 10.7%, while in the study of Ranjbar (2019) Prenen et al only found PIK3CA E545K mutation in colorectal cancer at 5.5% and De Roock et al (2010) found PIK3CA E545K mutation at 9.9% [(34)]. Different results were shown by a study of colorectal cancer in Shanghai and breast cancer in Singapore where it was reported that the highest frequency of PIK3CA mutations in exon 9 was found in E545A where there was a change in the amino acid glutamate to alanine. Similar results were also found in other continents, a study in Peruvian women showed the same trend as in these countries, where PIK3CA E545A had the highest incidence found in PIK3CA exon 9 mutations [(35)]. This difference in results may be due to different detection methods and populations [(34)].

In this study, based on 27 exon 9 samples, 17 samples (63%) were found to be LCRC and 10 samples (37%) were RCRC. Similar results were found in the study of Ranjbar et al (2019) where out of 187 samples, 137 samples (73.3%) were found to be LCRC and the remaining 50 samples (26.7%) were RCRC but in this study there was no significant association between PIK3CA exon 9 mutations and cancer location (p=0.45) [(34)]. Contrary to these studies, a meta-analysis study by Jin et al (2019)

showed that PIK3CA mutations were positively seen in 431 (17.7%) of 2442 patients with tumours in the proximal colon, compared to 421 (10.9%) of 3875 patients in the distal colon or rectum [(5)]. A positive association was found between PIK3CA mutations and proximal tumour location (OR ¼ 1.79; 95% CI: 1.39-2.29). In a subgroup analysis, positive associations were also seen in PIK3CA exon 9 (OR ¼ 1.78; 95% CI: 1.18-2.68) and 20 (OR ¼ 1.61; 95% CI: 1.05-2.45) mutations [(5)]. Based on gender, mutations in exon 9 codon 545 were dominated by women with a total of 15 samples (57.7%) and 11 samples (42.3%) were men. A similar study conducted in Iran found 11/65 (55%) samples were female and 9/102 (45%) samples were male, but no significant relationship was found between exon 9 codon 545 mutations and age (p=0.083), gender (p=0.22) [(34)]. Similar results were shown by a meta-analysis study conducted by Juan Jin et al (2019), 33 studies (14,976 patients) were analyzed to see the association between overall PIK3CA mutations and gender. No association was found between overall PIK3CA mutations and male gender (OR ¼ 0.93; 95% CI: 0.84-1.02); PIK3CA exon 9 and 20 mutations also showed no association with gender [(5)].

This study did not find any mutation in exon 20 of the PIK3CA gene. All samples identified as wildtype. This finding may be due to several factors such as the relatively small number of samples, the low mutation rate of exon 20 so that the possibility of detecting exon 20 mutations is also small. The prevalence of PIK3CA gene mutations in exon 20 tends to be lower than exon 9. This difference was shown in the study of Liao et al (2017) where exon 20 mutations only amounted to 6.2% (73/1170), while exon 9 mutations reached 9.3% (109/1170) [(7)]. A study in China by Xinhui Fu (2021) showed the mutation rate of CRC patients in exon 9 was around 6.2 (511/5733), while in exon 20 it was around 4.7% (270/5733) [(36)]. A previous study by Zhu et al (2012) showed that PIK3CA gene mutations were more common in exon 9 [(37)]. Based on age, a study by Fu et al (2021) also found that mutations in exon 20 were more likely to be found at the age before 50 years with a p value of <0.001, while in this study, the majority of samples were more than 50 years old with an average of 67 years [(36)]. The results of the Phipps et al (2015) study showed that the PIK3CA exon 20 gene mutation was associated with the location of the tumour in the proximal area which was more related to genetic factors [(31)]. This study did not provide genetic-related data such as hereditary history and race/ethnicity.

CONCLUSION

In conclusion, our result showed that colorectal cases in Bali, were more common in females and increased by age. PIK3CA mutations have been identified in exon 9 codon 545 as heterozygous mutation E545A. No mutation was found either in exon 9 codon 542 or exon 20 codon 1047. Although the sample size used in this study is relatively small and can not yet be generalized into a larger population, these findings contribute as a basic data on prevalence and characteristic of PIK3CA mutation in colorectal cancer patients in Bali where the data of PIK3CA mutation still limited. Further studies with a sufficient sample size are needed to obtain conclusive data of the association between PIK3CA mutation and colorectal cancer location.

Declaration by Authors

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