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Ki67 Expression and Prognostic Aspects of Colorectal Cancer

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Abstract

Colorectal cancer is the growth of malignant cells in the mucosal lining of the large intestine caused by uncontrolled cell growth. Ki67 protein expression found throughout the cell division cycle except in resting cells (G0) can be used as a marker of tumor cell proliferation. Ki67 protein expression was the result of pathological examination with Immuno Histochemistry Staining (IHC) staining from paraffin block of primary tumor biopsy specimens. 38 samples have data on Ki67 level, where the male gender is 28 people (73.7%) and 10 women (26.3%). The age of the patients were 40-64 years, with a mean age of 53 years. Meanwhile, the mean hemoglobin level of the study subjects was 12 g / dl, and the mean CEA level was 77 ng/ml, and a total of 38 samples the median value of Ki67 levels was 80%. There was no correlation between CEA levels and Ki67 expression with p = 0.411. There was no statistical relationship between Ki67 levels with the clinical stage of the tumor (p = 0.316), histopathological grade (p = 0.183) and tumor spread to regional lymph nodes (N) (p = 0.573).

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1 Introduction

Colorectal cancer is the growth of malignant cells in the mucosal lining of the large intestine caused by uncontrolled cell growth. In Indonesia, a report from the National Cancer Registry by the Directorate of Medical Services of the Ministry of Health in collaboration with the Association of Pathologic Anatomy shows that the incidence of colorectal cancer occurs at a younger age when compared to reports in western countries. The prevalence of colorectal cancer in Indonesia in 2013 was around 1.4% or as much as 347,792 cases in all age groups. Based on the estimated number of cancer patients, the provinces of Central Java and East Java are the provinces with the highest number of cases, namely around 68,638 people and 61,230 people, while the prevalence of colorectal cancer in Bali is around 2% or around 8,279 people (Murdani Abdullah: Ministry of Health, 2015).

Various studies have shown that tumors consist of a population of cells with heterogeneous proliferative activity. Cytokinetics in cancer is important because cancer growth, invasion, and metastasis are highly dependent on the reproduction of cancer cells. The growth fraction is the proportion of cells in the tumor that are proliferating. Estimation of the size of the cell growth fraction is important because this fraction shows the number of cells sensitive to cycle-dependent chemotherapy (Kausch & Böhle, 2002; Penault-Llorca & Radosevic-Robin, 2017). The kinetic status of the tumor can be seen from the rate of tumor cell proliferation, where the markers of proliferation describe the intensity of cell proliferation, namely the number of new cells produced per unit time. Some examples of this proliferation marker are Ki67, PCNA (proliferating Cell Nuclear antigen), and TPS (tissue polypeptide specific antigen). The expression of these antigens shows a good correlation with DNA synthesis so that it can be used as an index of cell proliferation and by looking at the rate of cell proliferation it can be used as a guide in the choice of therapy (Duchrow et al., 2003; Buskermolen et al., 2019; Raper and Hung, 2013).

Several factors play a role in determining the prognosis of colorectal cancer, including tumor grading, histological type subtypes, cancer stage, and several molecular markers such as microsatellite instability (MSI), KRAS, NRAS, BRAF, and others (Ashley & Tan, 2015; Nishimukai et al., 2015; Bertucci et al., 2013). Ki67 protein expression found throughout the cell division cycle except in resting cells (G0) (Sysel et al., 2015, Tong et al., 2020) can be used as a marker of tumor cell proliferation. Studies that have looked at Ki67 expression associated with colorectal cancer have yielded very inconsistent results.

Multiple studies have shown that the expression of this gene has implications as a poor prognostic factor for colorectal cancer. Tong et al. (2020) used Ki67 with a cut-off value of <25% as a poor prognostic factor in colorectal cancer. Likewise other studies (Brown & Gattter, 2002; Luo et al., 2019; Heidari et al., 2017; Hayashi et al., 2015). However, several other studies have found high Ki67 exposure as a prognostic factor that prolongs survival. Melling et al. (2016) obtained high Ki67 expression which was significantly associated with good clinical outcomes in patients as well as other studies (Salminen et al., 2015; Weber et al., 2001; Xing et al., 2017; Xi et al., 2011; Ivanesch, 2014). The difference in Ki67 expression obtained in colorectal cancer with other malignancies is still not well known. Arihiro et al. (2016), found a significant relationship between Ki67 expression and clinical outcome of breast cancer, Konstatinous et al. (2013), obtained a significant relationship from high Ki67 expression with high grade in endometrial cancer. Tian et al. (2016), found a significant relationship between high Ki67 expression and progressivity in urinary bladder tumors, Clay et al. (2017) also found a relationship between the expression of Ki67 pulmonary carcinoid tumors. A systematic review and meta-analysis by Berlin et al. (2017) reported Ki67 as a prognostic score for local stage prostate cancer. Based on the background as above, the researchers conducted a study looking for the relationship between Ki67 expression and prognostic factors in colorectal cancer patients at Sanglah General Hospital, Denpasar.

2 Materials and Methods

This research is a cross-sectional study to determine the relationship between Ki67 expression and prognostic factors in colorectal cancer patients at Sanglah General Hospital, Denpasar for 2 years from August 2016-September 2018. The sample of this study was taken from the medical records of cancer patients. colorectal and had received standard surgical therapy and chemotherapy that met the inclusion and exclusion criteria. Ki67 protein expression was the result of pathological examination with Immuno Histo Chemistry Staining (IHC) staining from paraffin block of primary tumor biopsy specimens. A semi-quantitative calculation of the Ki67 protein was recorded by IHC (Monoclonal Mouse Anti-Human Ki67 Antigen, DAKO, Denmark) and looked at the cell nucleus in a large microscopic field of view. The results of the interpretation of IHC staining are stated following the interpretation of the Immuno Histo Chemistry Staining (IHC) staining tool which is expressed by the percentage of Ki67 cell nucleus expression recorded by the Pathologist. Consecutive sampling method data collection on patients who have received standard chemotherapy for at least 4 cycles either inpatient or who come to the clinic in Sanglah Hospital and then the sample of biopsy results (paraffin block) will be examined Ki67 expression. The level of significance that was received was $p < 0.05$.

3 Results and Discussions

Of the 41 research subjects, there were 3 samples with uninterpretable results, namely 2 samples due to the preparations no longer immunoreactive with Ki67 antibody and 1 sample unable to be painted because the preparations were damaged or fell out during the painting process. 38 samples can be interpreted as the results of this ki67 level, where the sex of male is 28 people (73.7%) and female is 10 people (26.3%). Patients who participated in this study were aged 40-64 years, with a mean age of 53 years. The mean hemoglobin level of the study subjects was 12 g / dl, and the mean CEA level before surgery or chemotherapy was 77 ng/ml, and a total of 38 samples the median value of Ki67 levels was 80%. There was no correlation between CEA levels and Ki67 expression with $p = 0.411$.

Table 1
Patient characteristics (N=38)

Variable	n (%)
Sex, n (%)	
Man	28 (73,7%)
Woman	10 (26,3%)
Age(year), mean	52,92 + 12,925
Clinical stadium	
1	3 (7,9%)
2	12 (31,6%)
3	8 (21,1%)
4	15 (39,6%)
Tumor grade	
Well differentiated	4 (10,5%)
Moderately differentiated	26 (68,4%)
Poorly differentiated	8 (21,1%)
Nodal)	
N0	22 (48,9%)

N1	18 (40 %)
N2	5 (11,1 %)
Metastasis status	
M0	21 (55,3%)
M1	17 (44,7%)
Treatment responses	
Complete response	17 (44,7%)
Partial response	1 (2,6%)
Stable diseases	5 (13,2%)
Progressive diseases	15 (39,5%)
Hb (g/dl), *mean*	12,0 +1,94
CEA ng/ml	77 ([redacted] 80,0 (10,0-
Ki67 (%) *median expression*	95,0)

Relationship between Ki67 levels and clinical stage in colorectal cancer

To determine the relationship between the Ki67 level and the general clinical stage, the results showed that there was no statistical relationship between the Ki67 level and the tumor clinical stage with a significance level of p = 0.316.

Table 2
Relationship of Ki67 and clinical stage

		N	Ki67	p
Clinical stage	1	3	85 (80-85)	0,316
	2	12	85 (10-95)	
	3	8	82,5 (10-95)	
	4	15	70 (20-90)	

Table 3
Relationship of Ki67 and nodal and metastatic state

Nodal	N	Ki 67 Median (Minimum-Maximum)	p
N0	22	85 (10-95)	0,573
N1-3	23	80 (40-95)	

Metastases	N	Ki 67 Median (Minimum-Maximum)	p
M0	21	85 (10-95)	0,031
M1	17	70 (10-90)	

There was no significant relationship between Ki67 and nodal status, although there was a significant relationship with metastatic status, Ki67 expression was lower in cases with metastasis.

Relationship between Ki67 levels and histopathological grade in colorectal cancer

To determine the relationship between Ki67 levels and the degree of histopathologic grading, we performed the Kruskal-Wilis test, where the results showed that there was no statistical relationship between the Ki67 level and the histopathological grade of colorectal cancer (p = 0.183). We also analyzed by grouping the histopathological degree into 2 groups, namely poorly differentiated and not poorly differentiated with Fisher Exact analysis, there was no relationship between histopathological grade and therapeutic response with p = 0.697.

Table 4
Relationship of Ki67 and pathologic findings

Grade		N	Ki67	p
Grading histopathology	*Well differentiated*	4	87,5 (80-90)	0,183
	Moderately differentiated	26	82,5 (10-95)	
	Poorly differentiated	8	72,5 (20-90)	

Here we found treatment response was significantly related with metastatic state although in reversed rate where high Ki67 strongly related with good response.

Table 5
Treatment responses and Ki67 expression

Treatment response	N	Median (Minimum-Maximum)	p
Good	18	85 (10-95)	0,357
Bad	20	77,5 (10-95)	

Note: Good (CR+PR) CR: *complete response*, PR: *partial response*, Bad (SD+PD)SD: *stable disease*, PD: *progressive disease*

Discussion

This study found quite controversial results, not only unrelated but it can be seen that the high percentage of Ki67 expression was found in cancer cells, which theoretically reflects a low proliferation rate. From the data, it can be seen that well-differentiated cancer cells have a higher Ki67 expression than poorly differentiated cells. Nodal involvement, presence of metastases, and poor response to therapy show lower expression of Ki67 when compared to the reverse condition. In this study, we conducted an analysis test of the Ki67 proliferation index with the clinical stage of the tumor, histopathological grade, and the presence of tumor spread to regional lymph nodes and metastases to other organs. Our study showed that there was no statistical relationship between Ki67 levels with tumor clinical stage (p = 0.316), histopathological grade (p = 0.183) and tumor spread to regional lymph nodes (N) (p = 0.573). However, there was a significant relationship between Ki67 levels and the involvement of metastases to other organs (p = 0.031) even though patients with metastatic status had lower Ki67 expression compared to patients who did not experience metastases.

The kinetic status of the tumor can be seen from the rate of tumor cell proliferation, where the markers of proliferation describe the intensity of cell proliferation, namely the number of new cells produced per unit time. Ki67 protein is an example of a marker that indicates cell growth activity. From these data, this is quite

interesting to follow up considering that the Ki67 protein is a marker of the level of cell proliferation. Gerdes et al. (1983) found that the high expression of the Ki-67 gene was found in cells that were proliferating and not in cells that were not differentiated. Ki-67 gene was detected in the G1, S, G2, and M phases in the cell cycle and disappeared in the G0 phase. Ki67 protein expression can be used as a marker of tumor proliferation. Although the expression of the Ki-67 gene is not associated as a causative factor for cancer, several studies have shown that the expression of this gene has implications as a prognostic factor for tumors. The expression of these antigens shows a good correlation with DNA synthesis, so it can be used as an index for cell proliferation.

Studies conducted to look at Ki67 expression associated with colorectal cancer have varied widely, but it should be noted that the true cell proliferation rate in cases of neoplasms cannot be measured solely based on immunohistochemical staining alone because cell proliferation is a function of growth. Fraction and the time it takes to complete the cell division cycle. The growth fraction calculated by Ki67 only describes the number of dividing cells, not the overall rate of cell growth including the time it takes for each cell to complete the cycle. Therefore, the measurement of Ki67 expression only reflects the status, not the proliferation rate (Duchrow et al., 2003).

Research by Li P et al. (2016), reported that there was no statistically significant difference in prognosis and OS in stage I and stage IV colorectal cancer groups between high and low levels of Ki67 expression ($p > 0.05$). Research by Yan MY et al. (2010), got the same thing, namely that Ki67 plays an important role in the progression of colorectal cancer, especially the incidence of tumor metastasis so that it can be used as a new biomarker to evaluate prognostics and the selection of more appropriate therapies and improve the quality of life of colorectal cancer sufferers. There was a significant relationship with Ki67 and a strong correlation with the clinical stage (UICC) and histopathological grade ($p < 0.05$), high Ki67 expression was associated with low survival, and Ki67 did not correlate with the location of the tumor in patients with colorectal cancer. In contrast, Melling et al. (2016) found that high Ki67 expression was significantly associated with good clinical outcomes in colorectal cancer patients. In our study, the expression level of Ki67 was higher in cases with good response to therapy than in cases with poor response to therapy.

This may be explained as a result that the pathogenesis of colorectal cancer is very complex and complicated, which includes various risk factors and the involvement of gene mutations, and the occurrence of molecular changes that affect the rate of proliferation, differentiation, angiogenesis, and invasion or metastasis. For example, colorectal cancer patients with stages 1-3 with Microsatellite instability (MSI) disorder have a better prognosis than those with chromosomal instability disorders (CIN) and if associated with response to therapy, especially adjuvant chemotherapy, it is thought to have a different therapeutic response. among colorectal cancer patients with MSI and CIN abnormalities (Roper & Hung, 2013; Gonzalez-Moles et al., 2010; Marino et al., 2014).

Several other things also support the inconsistency of findings of Ki67 expression as conveyed by Volgestein et al. in 1983, that the transformation of adenomas to become malignant is not only influenced by the rate of cell proliferation but many factors that play a role such as the degree of differentiation, the rate of apoptosis, the presence of metastases and the result of genetic changes and epigenetics, which is a very complex relationship. Thus the relationship between proliferative parameters and therapeutic response is very complex and it is not surprising to date that no single biomarker parameter can represent its use in assessing the response to therapy in colorectal cancer patients.

Besides that, what is no less important can explain the differences in Ki67 expression, especially in colorectal cancer, are the constraints due to examination techniques such as the thickness of the preparation (section), sampling size, painting technique, problems in determining the Ki67 index on the intratumor and intratumor, the difference in the painting method, as well as differences in the calculation method in determining the percentage of the Ki67 index expression. The Ki67 index calculation technique with CCPI (camera capture, printed image) is the best method, although it takes about 10-15 minutes, when compared to other techniques such as Eye Ball count, manual eye counting, Maschine counting, the results are said to be less accurate. and invalid, although it takes less time. Until now, there is no international agreement on the measurement of the standard Ki67 expression.

In our study, the technique for determining the percentage of Ki67 expression was the manual eye counting technique. Several other problems, such as the preparation of pre-treatment or pre-analysis, the difference in processing and fixation of preparations, painting methods, taking samples between intratumor

and intratumor, loss of sample antigenicity due to a long time so that it does not react with Ki67 antibodies and different interpretations of the pathologist, especially in the area of dubious painting/gray area. Strong or high staining of Ki67 expression reflects only the number of dividing cells, does not indicate the time required for cell division, so that high Ki67 expression can be obtained in cells with slow cell division and cell proliferation, and also Ki67 expression can be obtained low in cells with rapid division and proliferation with fast cell cycles. This could explain the cause of the lack of correlation between Ki67 expression with the clinicopathology and prognosis variables in colorectal cancer from previous studies.

Other causes as reported by the study of Duchrow et al. (2003) that about a third (30%) of colorectal cancer patients express a high Ki67 index, but the expression of Ki67 in their mRNA is low and as an implication, this patient group has a better prognosis when compared to the group of high Ki67 expression both on DNA and expression. Ki67 on mRNA. As it is known that Ki67 protein expression causes the translation process to mRNA, which results in 2 variants of mRNA, namely; long type and short type mRNA (Konstantinos et al., 2014). The protein from the Ki-67 marker consists of 2 isoforms, namely; heavy isoform (antigen Ki-67 isoform 1) produced from long-type mRNA, which consists of 3256 amino acids with a molecular weight of 395 kDa. And Light isoform protein (Ki-67 isoform 2 antigen) is produced by short type mRNA, consisting of 2896 amino acids with a molecular weight of 345 kDa. The different types or variants of the Ki67 expression may explain the difference in the results of Ki67 expression from several previous studies, and in our study, the Ki67 expression did not come from the Ki67 expression in mRNA.

The high and low expression of Ki67 can also be a result of the pre-operative procedure, wherein colorectal cancer the procedure performed before the operative procedure for sampling is done by cleansing the intestine by giving a laxative or enema where this action will induce the proliferative activity of the intestinal mucosa and may be due to the presence of laxative or enema ulceration of the intestinal mucosa which induces the proliferative activity of the intestinal mucosa (luminal border), which results in high expression of Ki67 (Salminen et al., 2005; Denkert et al., 2015; Gil & Vagnarelli, 2018).

Nevertheless, regarding the high expression Ki67 related to a better outcome in colorectal cancer as found in this study another situation may be applied. As explained by Fluge et al. (2009) in their meta-analysis stated that positive Ki67 expression in colorectal cancer shows a good prognosis in a patient who received surgical treatment and adjuvant radio-chemotherapy, but not in a patient who only received surgical treatment. The reasonable explanation is due to the more responsiveness of cells that have high Ki67 from radio-chemotherapy because of its higher proliferation. Goals of radio-chemotherapy in cancer therapy are to kill cancer cells, especially quick proliferating cells. And evaluation of Ki67 expression in cancerous tissue often shows that cell is in a high proliferative activity and the probability of cells getting killed is also higher. Weaknesses in our study include research using secondary data that is retrospective and material that is stored for a long time and then staining to assess the expression of Ki67 levels is done by manual eye counting technique in hot spot areas where this area is an area with high tumor proliferation activity and Ki67 mRNA examination was not carried out due to the unavailability of facilities.

4 Conclusion

Ki67 was not significantly related to clinical stage, histologic grade, the nodal status of colorectal cancer patients, although significantly with treatment response but in a reversed relationship where high Ki67 strongly related with good treatment response. Need further study to determine whether Ki67 was true as prognostic factors in patients with colorectal cancer, with the proper testing device to assess protein Ki67 as well as Ki67 mRNA.

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Biography of Author

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