# ASSESSMENT OF HER2 EXPRESSION STATUS, TUMOUR BUDDING AND POORLY DIFFERENTIATED CLUSTERS IN COLORECTAL CANCER

BY

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# ABSTRACT

In Malaysia, colorectal cancer (CRC) is the second most common tumour. Tumour budding (TB) and poorly differentiated clusters (PDC) have been shown to be independent prognostic factors in CRC, that may allow for stratification of patients into more meaningful risk categories than those defined by TNM staging. However, these parameters are not routinely incorporated as part of the histopathological assessment. The role of Human Epidermal Growth Factor Receptor 2 (HER2) as a prognostic biomarker in CRC remains uncertain. Negative prognostic impact of HER2 expression has been proposed with a trend towards worse overall survival. Therefore, we aimed to evaluate the TB, PDC and HER2 expression status in our CRC cases and assess their associations with the established prognostic clinicopathological parameters including lymphovascular invasion, lymph node metastasis, tumour grade and tumour stage (pTNM). For evaluation of TB and PDC, hematoxylin and eosin-stained slides prepared from tissue blocks of 129 CRC cases diagnosed from 1st January 2017 to 31st December 2018, in Hospital Tengku Ampuan Afzan (HTAA) and Sultan Ahmad Shah Medical Centre @ IIUM, Kuantan Pahang. PDC, and TB according to the International Tumour Budding Consensus Conference (ITBCC) criteria were scored. For HER2 expression, IHC and FISH analysis were utilized, following HERACLES diagnostic criteria. Results: For TB, of the 129 cases studied, 63 (48.8%), 35 (27.2%) and 31 (24%) cases were classified as budding 1, budding 2, and budding 3, respectively. As for PDC, 73 (56.6%), 33 (25.6%) and 23 (17.8%) cases were classified as grade 1, grade 2 and grade 3, respectively. High TB and PDC grades were significantly associated with lymphovascular invasion ($p<0.05$), nodal metastasis ($p<0.05$) and high pTNM stage ($p< 0.05$). For HER2, 5 (3.9%) cases were positive for HER2 expression by IHC (scores 3+), equivocal 9 (7.0%) and negative 115 (89.1%). All HER2 2+ and 3+ (total 14 cases) was further evaluated by FISH analysis and *HER2 gene* amplification was seen in 4 cases. There is an association between HER2 protein expression status and pTNM stage ($p<0.005$). Otherwise, there is no correlation between HER2 protein expression with lymphovascular invasion, nodal metastasis, tumour grade, tumour budding or poorly differentiated clusters. Conclusion: There is strong evidence to suggest that tumour budding, and poorly differentiated clusters are an adverse prognostic factor. Our findings indicate that HER2 overexpression occurs in a small population of CRC cases and may benefit with HER2 targeted therapy.

# خلاصة البحث

زيامتلا) TB) عويش ماروﻷا عاونأ رثكأ يناثً تبث دقل. ايزيلام يف ا مرولا ومن نأ) CRC) طرس ربتعى كلت نم قيمهأ رثكأ رطاخم تائف ىلإ بضرملل يقبطلا ،CRC يف ةلقتسم قيؤبنت لماوع يهو) PDC) ةفيعضلا لتكلاو يضرملا يحيرشتلا ميقتلا نم ءزجك .جيردتلا . رود لازى TNM ميسقتلاب حمست دق يتلاو ةددحملا ةطساوب رشؤمك دكؤم ريغ لفطلا قوقح قيقافتا يف صيخشتلل يويح. ريبعتل يبلسلا ريذنلا ريثأتلا حارتقا مت HER2 لا لذكو PDC و) TB) مرولا ومن ميقت ىلإ فدهن انك ، كلذل .ماع لكشب أوسأ ءاقب وحن هجوت عم HER2 ريياعملاب مهتاطابترا ميقتو ثيبخ مرو ، قيومدلا قيعولأاو يوافملا CRC تلاح يف HER2 نع ريبعتلا ةلاح ك يئاعولا وزغلا كلذ يف امب ةخسارلا ريذنلا قيكينيلكلإا مرولا ومن ميقتل .مرولا ةلحرمو قيوافميللا ةدقعلا يف نم CRC نم 129 ةلاح) E&H) هيجيسن حئارش ترضح و نيليسكوتاميهلاب تغبصو نيزويلااب PDC و TB يف ىفشتسم 2018 ربمسيد 31 ىلإ 2017 ، (HTAA) Tengku Ampuan Afzan اهصيخشت مت 1 ريانى قفوً ا .ماروﻷا ومن TB و PDC ، ليجست مت .جناهاب ناتناوك IIUM يبطلا هاش دمحأ ناطلس زكرمو .HERACLES قفو ،ً ريياعمل ا FISH و IHC عامجلإ يلودلا رمتؤملا ريياعمل ليلحت مادختسا مت نيب نم ، 129 تمت ةلاح فينصت مت ، اهتسارد 63 و 35 و 31) TB) قيصيخشتلا ةجيتن: ضرملم قبسنلاب فينصت مت دقف ، PDC 73 ةئشان اهنأ ىلع ةلاح 1 ةئشان ةلاحو 2 ةئشان ةلاحو 3 ىل قبسنلاب اما .يلاوتلا ىلع وزغ عم ريبك PDC و ةعفترملا) TB) و 33 و 23 تاجرد اهنأ ىلع ةلاح 1 و 2 و 3 تاجرد تطبترا .يلاوتلا ىلع HER2 (. ينيتوربلا ريبعتلا نيب ةقلاع دجوت لا ، كلذ فلاخ P 005.0 (ةلحرمو pTNM قيلاع p) . لكشب وزغلاو لكشب ةنيابتم تاعومجم وأ مرولا معربتو ، يدقع ثيبخ مرو ، يوافملا يئاعولا جاتنتسا.ئيس : لماع يه ئيس لكشب ةزيامتملا ديقانعلاو مرولا ومن نأ ىلإ ريشت قيوق ةلدأ كانه ريشت .راض يؤبنت نيتورب نع ريبعتلا يف عم تاساردلا نم ديزم CRC تلاح نم قريغص قبسن يف ثدحى HER2 طارفلإا نأ ىلإ اهيلإ انلصوت يتلا جئاتنلا ىلإ ةجاح كانه نوكتسو ربكأ تانيع ماجحأ

# APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Medical Sciences

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# DECLARATION

I hereby declare that this thesis is the result of my own investigation, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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**DEDICATION**

TO MY PARENTS

TO MY TEACHERS AND

TO MY BELOVED WIFE AND DAUGHTER

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| | |
|---|---|
| % | Percent |
| ≥ | Greater or equal to |
| ≤ | Lesser of equal to |
| µg | Microgram |
| µl | Microliter |
| 5 FU | 5 Fluorouracil |
| ACS | American cancer society |
| AJCC | American Joint Cancer Committee |
| APC | Adenomatous polyposis coli |
| Bd | Budding |
| BRAF | v-raf murine sarcoma viral oncogene homolog B1 |
| BWL | Body weight loss |
| CAP | College of American pathologist |
| CEA | Carcinoembryonic antigen |
| CEP | Centromeric region of chromosome |
| CIBH | Change in bowel habit |
| CIMP | CpG island methylator phenotype |
| CIN | Chromosomal instability |
| CT | Computerized tomography |
| CTG | Computed tomographic colonography |
| DMMR | Deficient Mismatch Repair |
| DNA | Deoxyribonucleic |
| DPX | Dibutylphthalate polystyrene xylene |
| DRE | Direct rectal exam |
| EGFR | Epidermal growth factor receptor |
| FAP | Familial adenomatous polyp |

FDA Food drug administration

FFPE Formalin-fixed paraffin-embedded

FISH Fluorescent in situ hybridization

FIT Fecal immunochemical test

FOBT Fecal occult blood test

G1 Growth phage I

G2 Growth phase II

GDP Guanosine diphosphate

GLOBOCAN Global cancer incidence

GTP Guanosine-5'-triphosphate

H&E Haematoxylin and Eosin.

HERACLES HER2 Amplification for Colorectal Cancer Enhanced Stratification

HIER Heat induced epitope retrieval

HNPCC Hereditary nonpolyposis colorectal cancer

HTAA Hospital Tengku Ampuan Afzan

IDA Iron deficiency anemia

IHC Immunohistochemistry

IREC IIUM Research Ethics Committee

IRL Integrated Research Lab

ITBCC International tumour budding consensus conference

KRAS Kirsten rat sarcoma

KRC Kulliyyah of Medicine Research Committee

LEF Lymphoid Enhancer Factor

LVI Lymphovascular invasion

MLH1 MutL homolog 1

MMR Mismatch repair gene

MREC Medical Research and Ethical Committee

MRI Magnetic resonance imaging

MSI Microsatellite instability

| | |
|---|---|
| NMRR | National Medical Research Registry |
| NRAS | Neuroblastoma RAS viral oncogene homolog |
| OS | Overall survival |
| PALM | Pathology and Laboratory Medicine |
| PCR | Polymerized chain reaction |
| PDC | Poorly differentiated cluster |
| PET scan | Positron emission tomography |
| PIK3CA | phosphatidylinositol 3-kinase catalytic alpha polypeptide |
| pN | Pathological lymph node stage |
| PNI | Perineural invasion |
| RCT | Randomized control trial |
| SASMEC | Sultan Ahmad Shah Medical Centre |
| RFS | Relapse free survival |
| SISH | Silver in situ hybridization |
| SPSS | Statistical Package for the Social Sciences |
| TB | Tumour budding |
| TCF | T cell Factor |
| TP 53 | Tumour protein |
| UICC | Union for International Cancer Control |
| UV | Ultra violet |
| DAPI | 4',6-Diamidino-2-Phenylindole |
| WHO | World health organization |
| Wnt | 'Wingless/Integrated' |

# CHAPTER ONE

# INTRODUCTION

## 1.1 BACKGROUND

Despite expanded access to appropriate healthcare, which has greatly improved cancer detection and treatment, death rates from cancer-related deaths have increased by nearly 40% over the last 40 years (Kuipers et al., 2015). An additional 60% increase is expected in the next 15 years, with 13 million people are estimated to die from cancers in 2030 (Kuipers et al., 2013).

Colorectal cancer (CRC) is one of the most common cancers worldwide (Stewart et al., 2014; Gupta et al., 2018; Siegel et al., 2020). Of the various histologic types and variants of CRC, adenocarcinoma makes up the most CRC cases about 95 % (Rasool et al., 2013; Li et al., 2019). The incidence of CRC across the world showed a wide geographical variation in (Tung et al., 2018). In the United States, CRC is the third most commonly occurring cancer in both men and women and similarly accounts for the second commonest cause of cancer death (Siegel et al., 2020). Approximately 25% of patients have metastatic disease at the time of diagnosis with a 5-year survival rate (Stewart & Wild, 2014; Greally et al., 2018a; Gupta et al., 2018;).

In Malaysia, CRC is the second most common cancer in overall population (Hassan et al., 2016; Veettil et al., 2017b; Arunah et al., 2020). The National Cancer Registry in Malaysia reported that CRC is the most common cancer in males (16.3 %) while in females it is the second most common type of cancer (10.7 %) (Chandran et al., 2020).

Approximately 80 % of these cases in Malaysia are diagnosed in patients older than 50 years of age, with the majority of them presenting as an advanced disease with a poorer prognosis (Saad et al., 2013; Hassan et al., 2016; Schliemann et al., 2020). Most CRC cases in Malaysia are diagnosed in late stages, therefore leads to high mortality rates (Chandran et al., 2020; Schliemann et al., 2020).

In CRC, the treatment and prognosis decisions are based principally on the TNM staging system (Lugli et al., 2017). Unfortunately, a significant number of CRC cases behave poorly even though being classified as low risk based on their TNM stage (Rogers et al.,2016; Konishi et al., 2018; Deb et al., 2019; Demi et al., 2019b). The TNM staging system also seems to be lacking in forecasting the outcomes of patients within the same stage ( Yang et al., 2016; Rogers et al., 2016; Seneviratne, 2018; Deb et al., 2019; Sadek et al., 2020). Thus, the exploration for other prognostic factors of CRC has been a main research attention (Das et al., 2017; Lugli et al., 2017; Poornakala et al., 2019; Ueno et al., 2019; Demir et al., 2019a).

One of the emerging prognostic factors in CRC is Epidermal Growth Factor 2 (HER2) overexpression or amplification by the tumour tissues (Gordian et al., 2019; Wang et al., 2019; Xie et al., 2020; Cuype et al., 2020). The *HER2* oncogene encodes for a transmembrane glycoprotein receptor that functions as an intracellular tyrosine kinase and is a member of the epidermal growth factor receptor (EGFR) family (Greally et al., 2018a; Wang et al., 2019). The percentage of HER2 overexpression or amplification in CRC varies widely, ranging from 0% - 84% (Blok et al., 2013; Shabbir et al., 2016; Liu et al., 2019).

CRC cases with HER2 positive has been suggested to show a negative prognostic impact of HER2 expression as compared to those with HER2 negative (Siena et al., 2018; Wang et al., 2019; Cuyper et al., 2020). HER2 amplification is also instituted to be a

negative prognosticator toward cetuximab (an anti-EGFR treatment) response, which is a targeted therapy of patients with CRC. Studies also have revealed that activation of HER2 signaling confers resistance to cetuximab (Takegawa et al., 2017; Siena et al., 2018; Xie et al., 2020). For identifying HER2 protein expression and gene amplification on formalin-fixed paraffin-embedded tumour samples (FFPE), immunohistochemistry (IHC) and fluorescent or silver in situ hybridization (FISH or SISH) are the present methods practices respectively (Siena et al., 2018).

Additionally, other factors which have been shown recently to carry prognostic values in CRC include histological parameters designated as "Tumour Budding" (TB) and "Poorly Differentiated Cluster" (PDC) (Demir et al., 2019; Lee et al., 2018; Nearchou et al., 2019). These two parameters are yet not widely adopted by pathologists in their reporting (Nearchou et al 2019). Data on this potential benefit are limited in the literature, as few studies assess both tumour budding and PDC in the same cohort of CRC cases (Barresi, Reggiani Bonetti et al. 2016). TB is defined as presence of de-differentiated single cells or small clusters of up to 4 cells at the invasive front of the cancer, while PDCs are the presence of cancer clusters in the stroma composed of ≥5 cancer cells. For tumour budding assessment, International Tumour Budding Consensus Conference (ITBCC) has paved the way for standardization of TB reporting and strongly recommends including the parameter as adjunct to TNM reporting system (Lugli et al., 2017; Ozer et al., 2019; Ueno et al., 2019).

## 1.2 JUSTIFICATION OF THE STUDY

The incidence of colorectal cancer (CRC) is fast rising in many Asian countries (Veettil et al., 2017b; Kulkarni et al., 2018;Wong et al., 2019; Siegel et al., 2020). This trend is also

expected to be documented in Malaysia in view of an increased prevalence of risk factors such as high fat content diet and diet rich in red meat, obesity, and smoking as well as sedentary lifestyle and a drift towards an aging population (Naing et al., 2017; Veettil et al., 2017b; Arunah Chandran et al., 2020). According to GLOBOCAN 2018, cancer incidence in Malaysia is expected to be double by 2040 (from 43,837 to 84,158 cases) (Schlieman et al., 2020). This condition is associated with high morbidity and mortality hence incurring significant health and financial burden (Ali et al., 2018; Schliemann et al., 2020; Lim et al., 2020).

Multimodality therapy has given rise to improve in the survival of CRC patients although prognoses of some patients are still poor (Pang et al., 2020; Arunah Chandran et al., 2020). Newer targeted therapies are being evaluated and as such it is necessary to evaluate and establish new prognostic and predictive factors other than the conventional ones in order to risk-stratified patients for management purposes ( Sayadnejad et al., 2017; Liu et al., 2019; Xie et al., 2020). In this respect HER2 overexpression or amplification has the potential of being a prognostic factor which may impact choice of therapy in CRC (Greally et al., 2018a; Siena et al., 2018; Cuyper et al., 2020). In addition, tumour budding (TB) and poorly differentiated cluster (PDC) parameters may be a promising histologic prognostic factors for assessing the aggressiveness of CRC cases (Lee et al., 2018; Wyk et al., 2019; Ammendola et al., 2020; Chandran et al., 2020).

To the best of our knowledge, there is no published data on the status of HER2 overexpression or amplification, TB and PDC among CRC cases in Kuantan, Pahang, Malaysia. Therefore, there is a strong need for this study to be carried out and it will provide surgeons /oncologists information on the HER2 status in order to subclassify patients

prognostically. It also predicts response to anti- EGFR therapy in CRC cases. Lastly, it will help to identify patients for novel Anti-HER2 targeted treatment.

In this study, we aimed to investigate *HER2* expression status and TB and PDC grades in patients diagnosed with CRC and their associations with tumour grading, staging, lymph node metastasis and lympho-vascular invasion.

## 1.3 RESEARCH QUESTIONS

1. What is the HER2 expression status in colorectal cancer cases in Kuantan Pahang?
2. What is the status of tumour budding and poorly differentiated cluster in colorectal cancer cases in Kuantan Pahang?
3. What is the association between HER2 status, tumour budding and poorly differentiated cluster in colorectal cancer cases in Kuantan Pahang?
4. What is the association between HER2 status and tumour grade, stage, lymph node metastasis and lymphovascular invasion?
5. What is the association between tumour budding and tumour grade, stage, lymph node metastasis and lymphovascular invasion?
6. What is the association between poorly differentiated cluster and tumour grade, stage, lymph node metastasis and lymphovascular invasion?

## 1.4 HYPOTHESIS

1. A proportion of colorectal cancer cases have overexpression or amplification of HER2.
2. Higher grade of tumour budding and poorly differentiated cluster in advanced colorectal cancer cases.
3. There is association between HER2 expression status with tumour budding and poorly differentiated cluster in colorectal cancer.
4. There is association between HER2 expression and colorectal cancer grades, stages, lymph node metastasis and lymphovascular invasion.
5. There is association between tumour budding and colorectal cancer stages, grades, lymph node metastasis and lymphovascular invasion.
6. There is association between poorly differentiated cluster and colorectal cancer stages, grades, lymph node metastasis and lymphovascular invasion.