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VO HONG MINH CONG

STUDY THE CLINICAL CHARACTERISTICS, ENDOSCOPY, HYSTOLOGY, EXPRESSION PROTEIN OF P53, KI67, HER-2/NEU IN COLORECTAL CANCER AND COLORECTAL POLYP WITH SIZE MORE THAN 10 MM

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INTRODUCTION

Colorectal cancer (CRC) is a fairly common malignancy worldwide, more common in European countries, America and increasingly tends to increase , especially in Asia. CRC has become the concern of the public in general and for physicians specializing in Digestive system diseases in particular.

Currently , immunohistochemistry (IHC) is a technique that has been applied in many countries around the world, which not only helps observe histopathological morphology but also determines the presence of antigens in cells and tissues and identifies the origin of malignant cells...The U.S study suggeststhe ability to direct, detect, and early warn of CRC through the testing of protein expressions such as p53 , Ki67 , Her- 2/neu, etc., plays an important role not only in CRC but also in helping diagnosing CRC in patients with large colorectal polyps.

In Vietnam , there has been study of expression of proteins : p53 , Ki67 , Her- 2 / neu on CRC , but the amount is not much, especially researches in patients with colorectal polyps greater than 10 mm in diameter . Therefore , we have conducted the topic: "***Study the clinical characteristics, endoscopy, histopathology, expression of proteins : p53, Ki67 , Her- 2 / neu in colorectal cancer and colorectal polyps greater than or equal to 10 mm***" with two objectives as follows:

1. Study the clinical characteristics, endoscopy, histopathology, expression of proteins : p53, Ki67 , Her- 2 / neu in colorectal cancer and colorectal polyps greater than or equal to 10 mm.

2. Study the relationship between the expression of proteins p53, Ki67 and Her- 2/neu with histopathological characteristics, lymph node metastasis in colorectal cancer and colorectal polyps greater than or equal to 10 mm.

Summary of new main scinetific contribution of the thesis

The thesis is one of the few research projects in Vietnam to determine the rate of protein expression p53, Ki67 and Her-2/neu in patients with colorectal cancer, especially colorectal polyps. The level of protein expression p53, Her-2/neu in patients with benign polyp lesions: negative. While in the group of cancer polyps, colorectal cancer group level high positive expression. The level of protein expression of p53, Ki67 also tends to increase with the degree of invasive colorectal cancer.

Protein expression of p53, Ki67 and Her-2/neu in cancer and colorectal polyp support for histopathological diagnosis is more deeper, helped for chemotherapy colorectal cancer more correct and towards target treatment.

Structure of the thesis

The thesis consists of 140 pages (not including appendices and references) with 4 main chapters: Introduction 2 pages; Chapter 1 - Overview 38 pages; Chapter 2 - Subjects and methodology 19 pages; Chapter 3 - results is 41 pages; Chapter 4 - Discussion is 38 page; Conclusion 2 pages. The thesis has 42 tables, 12 charts, 38 pictures and 1 diagram, 181 reference documents including 45 Vietnamese, 135 English and 1 French.

CHAPTER 1 OVERVIEW

1.2. ROLE AND IMPACT OF GENE IN COLORECTAL CANCER

1.2.1. The basic genes in colorectal cancer

1.2.1.1. Oncogene

An oncogene is a mutated gene. A proto-oncogene is a normal gene, playing the role of controlling cell reproduction and differentiation. When A proto-oncogene is mutated and works abnormally, it causes the cell to excessively grow, escape the control of the body, and create a clone of tumor cell which is the beginning

of cancer. At this time, it is called an oncogene, gene acting in a dominant manner.

1.2.1.2. Tumor Suppressor Genes

Normally, tumor suppressor genes are able to stop the cell cycle even when oncogenes were activated . If tumor suppressor genes fail to repair the damaged DNA , they will start apoptosis, the process of programmed cell death. Tumor suppressor genes were first described in Knudson's study of the epidemiology of childhood retinoblastoma. Those are genes acting in a dominant manner, of which function is only lost when both alleles are inactivated.

Once, a tumor suppressor gene transmits germline mutation, the individual inheriting this mutation just needs a further mutation in the remaining allele to cause the gene's loss of function . When a tumor suppressor gene has two normal alleles, there must be two somatic mutations occuring in both alleles to cause the gene's loss of function. This two-hit hypothesis explains why inherited disease usually manifests at an earlier age than sporadic disease, as well as the concept of tumor suppressor genes operating in a recessive fashion.

P53 gene produces p53 protein, also known as tumor suppressor gene p53,which plays an important role in regulating the cell. When DNA has sustained damage, p53 stops the cell cycle until the damaged DNA is repaired or p53 can force the damaged cells to commit suicide through the programmed cell death pathways (apoptosis) if DNA damage is irreparable.

The reason p53 can stop the progression of cell cycle was that it activates transcription process of creating CKI , p21 to inhibit rotation of CDK activation . Once CDK is activated , it phosphorylates Rb and phosphorylation which loses the effectiveness of Rb - gene of which function is to stop cell cycle progression by binding to E2F1 and preventing transcription of genes required for cell in S phase. Nonfunctional mutations p53 increase genomic instability and reduce programmed cell death.

1.2.1.3. Mismatch Repair Genes (MMR)

The function of these genes is to correct errors of DNA replication. Six human mismatch repair genes found are MSH2 (on the short arm of chromosome 2-2p16) , hMLH1 (on the short arm of chromosome 3-3p21) , HPMS1 (long arm of chromosome 2-2q31-33) , hPMS2 (long arm of chromosome 7-7q11) , hMSH6 (on the short arm of chromosome 2-2p16) and hMSH3 (on the long arm of chromosome 5-2p11.2 - q13.2) . When both alleles of this gene were inactivated , the mistakes in DNA fail to be repaired and increase , thereby accelerating the process of carcinogenesis (cancer creation process).

1.2.2. The process of formation and development of colorectal cancer

Genetic changes leading to the development of CRC occur earlier and then parallel the transformation of MBH . Genetic changes leading to the development of CRC have been divided into three steps : Alterations in protooncogenes, Loss of tumors suppressor gene activity, Abnormalities in genes involved in DNA mismatch repair . In each phase corresponding to the change mbH , there are many kinds of genes that participate in this program. In the diagram of Fearon E. R. and Vogelstein B. Figure 1 shows that there are many genes involved in the process which transforms normal cells, makes changes in epithelial cells, and ultimately causes cancer . Respectively in each phase of change, there are changes of many different kinds of genes.

This transformation process occurs very early and can last 5-10 years before the formation of CRC . For protein expression patterns of genes such as p53 , Ki67 and Her- 2 / neu is often at an early stage when there is formation of adenomatous polyp and it appeares before the formation of CRC . Thus , the genetic tests may also help make the prediction and diagnosis of diseases better .

1.2.3. Some gens in colorectal cancer and colorectal polyps

Depending on the extend of damage and the source of colon cancer , especially the hereditary colon cancer, there have been many genes identified and rather complex. However , according to

the proportion of colorectal cancer, most of colorectal cancer form adenomatous polyps. Thus , the frequently studied genes include : P53 , Ki67 and Her- 2/neu

1.2.3.1. Gene P53

P53 gene plays an important role in many cellular functions such as : suppressing of tumor growth, encoding p53 nuclear phosphoprotein, regulating programmed cell death and programmed cell survival, preventing DNA mutations . P53 gene mutation is one of the most common genetic changes in human cancer.

Because P53 gene has the role of regulating genome stability and preventing the cell from entering mitosis after DNA damage , when P53 gene is mutated, p53 protein is mutated. The tumor suppressing function will be lost. When cells divide uncontrollably it leads to the formation of tumors ...

Normally , p53 protein has a short half- life and not be detected by IHC . But when these genes are mutated , the half- life of p53 protein lasts longer and can be detected by IHC technique.

Protein p53 is a factor stimulating the transcription of mdm2 and many proteins through which Protein p53 plays the central role of regulating processes:

+ When DNA is damaged, the p53 protein is phosphorylated at two sites Serine - 15 and serine – 20, stopping the cell cycle at checkpoint G1 / S through p21waf1 , Gadd45… at checkpoint G2/M through 14-3-3σ. The transcription of P53 gene is activated to produce an increasing amount of p53 protein from G0 phrase to late phrase of G1. Protein p53 stimulates the transcription of p21 protein.

Protein p21 prevents cell cycle from entering S phase in several ways such as mounting on cyclin-cdk complexes (cyclin dependent kinase) inhibit the activity of C cyclin dependent kinases. Thus, the Rb protein not phosphorylated will be attached to E2F in order to not allow it to stimulates the transcription of genes required for DNA replication .

+ Restart the repair process of DNA damaged through p53R2.

+ Promotes programmed cell death if the damaged DNA is too severe or unrepairable through TP53INP1-HIPK2 (tumor protein 53 - induced nuclear protein 1 - homeodomain - interacting protein kinase - 2) and TP53INP1- PKCδ (tumor protein 53 - induced nuclear protein 1 - δ protein kinase C) , phosphorylated protein 53 at serine - 46 53, causes programmed cell death .

1.2.3.2. Gene Ki67

Ki67 gene known since 1983 and is increasingly popular . Due to showing the fertility of tumors Ki67 provides a means of assessing the extent of tumor growth quite accurate. Ki67 protein is a component in basic substance of the cell nucleus, with a molecular weight of 360 kDa . Ki67 protein coding gene locates on chromosome 10 , which contains 15 exons.

Protein Ki67, nuclear proliferative antigen, presents in all phrases of cell cycle (G1 , S , G2 , and M) , but is absent from resting cells (G0)

Ki67 is closely related to cell proliferation morphology , particularly mitotic index and histologic features. This antigen is related to the growth of the cells . When Ki - 67 is strongly positive , cell proliferation is stronger and vice versa.

1.2.3.3. Gene Her-2/neu

Gene Her- 2 / neu, known as proto-oncogene, is located at chromosome 17 and has a molecular weight of 185 kDa. Today , we see that : Her- 2 / neu can be the target of therapy, especially breast cancer , stomach cancer ...

The process of binding ligand to HER initiates pathways of intracellular signal transduction. When linked with other members called mating pairs. Ligand will be mounted between chain I and III to release chain II . The mating takes place when chains II corresponding to receptors linked together.The HER family members can be mated together (pairing of other type) as EGFR and HER - 2 , EGFR and HER - 3 , HER - 2 and HER - 3 or mated with itself (pairing of the same type) . The mating will cause phosphorylation of the intracellular domain and start a cascade of

intracellular signals , activation of the cell cycle causing tumor growth , cell hyperplasia , programmed cell death , angiogenesis and penetration of blood vessels.

Currently , HER family receptor targeted therapy has being widely studied , in order to prevent ligand binding (as anti- EGFR antibody) and prevent ligand-independent activation of receptors (such as anti- HER-2 antibody, trastuzumab) . Anti-EGFR antibodies have shown effects in the therapy of a variety of tumor types, including colorectal cancer, non-small cell lung cancer , head and neck cancer, kidney cell cancer.

Anti-EGFR antibodies which affect directly to this receptor have been used clinically as Cetuximab , Panitumumab.

1.3. COLORECTAL CANCER HISTOPATHOLOGY

1.3.2.4. Histopathologic diagnosis of colorectal cancer

This is a method of diagnosing colorectal cancer decision. MBH may allow classification of microscopic type , malignant level, TNM classification, and cancer stage.

Macroscopic images

Colorectal cancer can be divided into four groups: vegetant; ulcers; diffuse infiltrates; ring cell carcinoma

Microscopic images

WHO has divided microscopic images of colorectal cancer as follows.

Carcinoma

Carcinoma includes the following categories:

* Adenocarcinoma
* Mucinous adenocarcinoma
* Sinnet ring cell carcinoma
* Small cell carcinoma
* Adenosquamous carcinoma
* Medullary carcinoma
* Undifferentiated carcinoma

1.4. OVERVIEW OF COLORECTAL POLYPS

1.4.3. World Health Organization classification of Polyps

In year 1976, classification of Morson was applied by many pathologists , oncology, and the World Health Organization (WHO). In year 2000 WHO has adds detailed classification, including the following types of polyps:

* Neoplastic polyps

- Adenomatous polyps:

• Tubular adenoma

• Tubulovillous adenoma

• Villous adenoma

- Polypoid carcinoma

- Carcinoid tumors

- Non-epithelial tumors (lipoma, leiomyoma, hemangioma, lymphangioma...)

* Non-neoplastic polyps

- Polyp Peutz-Jeghers

- Juvenile polyps. This group is divided into three categories: mere juvenile polyp, inflammatory Juvenile polyp and adenomatous Juvenile polyp.

- Hyperplastic polyps

- Inflammatory polyps

- Unclassified polyps: Benign lymphoid polyps

1.4.6. Histopathological characteristics of cancerous polyp

MBH Change of Cancerous Adenomatous Polyps is divided into four stages based on the vulnerability of polyps.

* Level 1: Cancer only appeares in the lining (mucosa). There's no intrusion into the muscle layer membranes (Muscularis) of polyps and it is called carcinoma in situ

* Level 2: Cancer has invaded through the lining into the muscle layer of the polyp, but not penetrate the lymphatic system. The differentiation degree of cancer is at high or medium level.

* Level 3: Cancer has invaded the muscle layer and penetratde the lymphatic. Or if it has not yet penetrated the lymphatic system , but cells are poorly differentiated.

* Level 4: Cancer has invaded the muscle layer , into the lymphatic system and to stem polyp
and penetrate the colon wall

CHAPTER 2 SUBJECT AND METHODOLOGY

2.1. RESEARCH SUBJECT

Patients with colorectal polyps and colorectal cancer are clinical examinated, colorectal endoscopy with soft endoscope and biopsy at Gia Dinh People Hospital and 108 Military Central Hospital. MBH, p53 IHC, Ki 67 , Her- 2 / neu tests are conducted at Department of Surgery - 108 Military Central Hospital.

2.1.1. Criteria for selecting patients

- Patients are clinical examinated, conducted colorectal endoscopy, tested MBH, and diagnosed to confirm diagnosis of colorectal polyps or colorectal cancer.
- Patients without chemotherapy or radiotherapy before surgery
- Patients were divided into 2 groups:

* Group 1: colorectal cancer

Patients are conducted colorectal endoscopy, collected specimens by endoscopy and / or surgery at Gia Dinh People Hospital and 108 Military Central Hospital.

* Group 2: colorectal Polyp.

Patients with colorectal polyps' size $\geq$ 10 mm were cut through endoscopy or surgery (with large-size polyp) at Gia Dinh People Hospital

2.1.2. Exclusion criteria

- Patients under 18 years old.
- Contraindications to colon endoscopy: heart failure , respiratory failure ...
- Patients who don't agree to cooperate in the study.
- Colorectal polyps' size < 10 mm.

2.2. METHODOLOGY

2.2.1. Study Design

Descriptive studies , cross-sectional analysis.

2.2.2. Sample size

- Group of colorectal cancer sample: 117 patients with colorectal cancer

- Group of colorectal polyps' size ≥ 10 mm sample: 55 patients with colorectal polyps

2.2.3. Duration of Study

From 01/2010 to 9/2013

2.2.4. Study sites

- Clinical studies and endoscopy at two hospitals: Nhan Dan Gia Dinh Hospital and Central Military Hospital 108.

2.6. Method of data handling

- Data processing using SPSS 20.0 software.

- Calculate frequency, percentage; compare pairs audited by $\chi 2$.

- The relationship between excessive expression of p53 protein , Ki67 , HEU - 2 / neu with:

- The clinical features of polyps and colorectal cancer.
- General characteristics of polyps and colorectal cancer.
- Relationship with MBH polyps and colorectal cancer.
- Correlation with cell differentiation , with lymph node metastasis.

The level of statistical significance with p values < 0.05.

CHAPTER 3 RESULTS

We have studied the clinical characteristics, endoscopic images, histology and immunohistochemistry were performed in 55 patients with colorectal polyps size larger than 10 mm, 117 colorectal cancer patients in Nhan Dan Gia Dinh Hospital and Central Military Hospital 108, since 01/2010 -04/2013.

3.1. RESULTS COLORECTAL POLYPS SIZE LARGER THAN 10 MM

We have studied the clinical characteristics, endoscopic images, histopathology, immunohistochemistry (IHC) at 55 patients with the 72 colorectal polyps ≥ 10 mm, and 117 colorectal cancer patients. For that reason, we offer the following discussion.

3.1.1 The clinical features of colorectal polyps size larger than 10 mm

3.1.1.1. Distribution incidence of colorectal polyps by age

Table 3.1. Distribution incidence of colorectal polyps by age

Age	n	(%)
≤ 20	2	3.6
21 – 40	6	10.9
41- 60	21	38.2
61- 80	26	47.3
Total	55	100
Mean age	57.3 ± 15.3 (18 – 78)	

Comment: Age polyp disease most common from 41-80 (85.7%).
The average age: 57.3 ± 15.3 (18-78). male / female: 31/24 = 1.29

3.1.3. Histopathological characteristics of polyps ≥ 10 mm size

3.1.3.1. Histopathological classification of colorectal polyps ≥ 10 mm size

Table 3.6. Histopathological classification of colorectal polyps ≥ 10 mm size.

Histology	N0 polyp	(%)
Neoplastic polyps	56	77.8
Non-neoplastic polyps	16	22.2
Tổng	72	100

Comment: + Neoplastic polyps 77,8%
+ Non-neoplastic polyps 22,2%

3.1.3.2. . Histopathological classification of colorectal polyps ≥ 10 mm size.

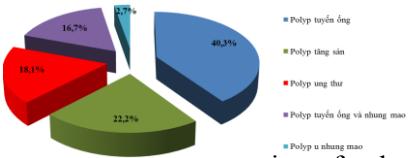

Chart 3:3. Histopathological classification of colorectal polyps ≥ 10 mm size.

Comment: + Adenomatous polyps 40.3%.

+ *Hyperplastic polyps 22.2%.*
+ *Polypoid carcinoma 18.1%.*

3.1.5. The relationship of dysplasia with the characteristics of polyps.

Table 3:12. Percentage of dysplastic polyps, Neoplastic polyps and Non-neoplastic polyps.

Histology	Neoplastic polyps n (%)	Non-neoplastic polyps n(%)	Total n(%)
Dysplasia	55/56 (98.2)	0/16 (0)	55/72 (76.4)
Not dysplasia	1/56 (1.8)	16/16 (100)	17/72 (23.6)
Total n(%)	56 (100)	16 (100)	72 (100)

Comment: dysplastic polyps: 55/72 (76.4%). The rate increased in dysplastic Neoplastic *polyp 98.2%. In contrast,* Non-neoplastic *polyps is not dysplasia.*

3.1.7. P53 protein expression, Ki67, Her-2 / Neu colorectal polyps

3.1.7.1. Protein manifestation of p53, Ki67, Her-2 / neu.

55 patients with colorectal polyps size larger than 10 mm, were tested immunohistochemistry: p53, Ki67, Her-2/neu.

Table 3.15: Percentage of protein manifestation: p53, Ki67, Her-2/neu.

Protein manifestation gen		No polyp	%
p53	Positive	8	11.1
	Negative	64	88.9
Ki67	Positive	47	65.3
	Negative	25	34.7
Her-2/neu	Positive	3	4.2
	Negative	95,8	96.4

Comment: Percentage of protein manifestation: P53 of polyps size larger than 10 mm: 65.3%, protein manifestation Ki67 (+)11.1% and Her-2/neu- (+) 4.2%.

3.1.7.3. Protein manifestation of p53, Ki67, Her-2 / neu in 13 ofcancer polyps.

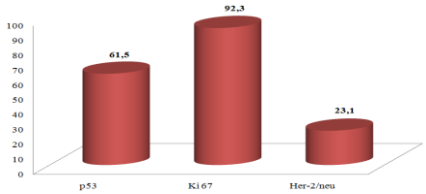

Chart 3:10. Ratio p53, Ki67, Her-2 / neu in in 13 ofcancer polyps.
Comment: Percentage of Ki67-positive (92.3%) 61.5%; p53-positive (92.3%); Percentage of Her-2/neu -positive (92.3%)

3.2. Colorectal Cancer

3.2.1. General characteristics of colorectal cancer (n = 117)

3.2.1.1. Features age and gender

The mean age: 63.68 ± 13.37 (28-89 years old).

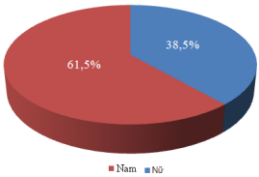

Chart 3:11. male / female.
Comments: Male / Female: 72/45 ~ 1.6

3.2.3. Histopathological characteristics of colorectal cancer

3.2.3.3. The lymph node metastasis in colorectal cancer

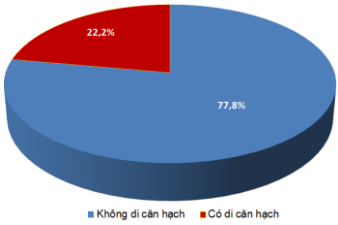

Chart 3:14. The lymph node metastasis in colorectal cancer

Comment:Percentage colorectal cancer metastases in the mesenteric lymph nodes was 22.2%, 77.7% have no mesenteric lymph node metastasis.

3.2.4. Protein expression of p53, Ki67, HER2/neu in colorectal cancer

3.2.4.1. Protein manifestation of p53, Ki67, HER2/ neu in colorectal cancer

All patients were tested immunohistochemistry: p53, Ki67, Her-2/neu.: p53, Ki67, Her-2 / neu:

Table 3.21: Percentage of protein manifestation: p53, Ki67, Her-2/neu in UTDTT.

Protein manifestation gen		No polyp	%
p53	Positive	67	57.3
	Negative	50	42.7
Ki67	Positive	105	89.7
	Negative	12	10.3
Her-2/neu	Positive	34	29.1
	Negative	83	70.9

Comment: protein manifestation p53 positive 57.3%, protein manifestation Ki67 positive 89.7% , protein manifestation Her-2 / neu-positive 29.1%

3.2.5. The relationship protein manifestation P53, Ki67, Her-2 / Neu with histopathological

3.2.5.1. The relationship between protein manifestation patterns with colorectal cancer

Table 3.24: Relationship between Ki67, p53, Her-2/neu with of colorectal cancer.

	p53 (+)		Ki67 (+)		Her-2/neu (+)	
	No pattens	%	No pattens	%	No pattens	%
Polypoid (n=66)	36	54.5	58	87.9	16	24.2
Polypoid + Ulcerative (n=34)	23	67.6	32	94.1	11	32.4
Ulcerative (n=2)	2	100	2	100	1	50.0
Ring (n=4)	3	75.0	4	100	2	50.0
Infiltrating (n=11)	3	27.3	9	81.8	4	36.4
Total (n=117)	67	57.3	105	89.7	34	29.1

Comment: protein manifestation of p53; Ki67 be 100% in ulcer carcinoma, Polypoid and Polypoid + Ulcerative protein manifestation Ki67: 87.9, 94.1%, protein manifestation p53: 54.5, 67.6%.

Bảng 3.35. *: Relationship p53, Ki67 và Her-2/neu vs the lymph node metastasis.*

	p53 (+)		Ki67 (+)		Her-2/neu (+)	
	Neg n(%)	Pos n(%)	Neg n(%)	Pos n(%)	Neg n(%)	Pos n(%)
Lymph node metastasis	8 (30.8%)	18 (69.2%)	0	26 (100%)	15 (57.7%)	11 (42.3%)
No lymph node metastasis	42 (46.2%)	49 (53.8%)	12 (13.2%)	79 (86.8%)	68 (74.7%)	23 (25.3%)
p	p > 0.05		p > 0.05		p > 0.05	

Comment: P53 positive rate. Ki67 and Her-2/neu in the higher group of lymph node metastasis of lymph node metastasis group, but this difference was not statistically significant with p> 0.05.

3.2.5.6. Comparison of protein manifestation with stage colorectal cancer

Table 3:30: Relationship protein manifestation vs Dukes

Dukes	p53		Ki67		Her-2/neu	
	Neg n (%)	Pos (%)	Neg n (%)	Pos (%)	Neg n (%)	Pos (%)
Dukes A	18 (50.0)	18 (50.0)	10 (27.8)	26 (72.2)	28 (77.8)	8 (22.2)
Dukes B	22 (42.3)	30 (57.7)	2 (3.8)	50 (96.2)	38 (73.1)	14 (26.9)
Dukes C	8 (36.4)	14 (63.6)	0 (0.0)	22 (100)	14 (63.6)	8 (36.4)
Dukes D	2 (28.6)	5 (71.4)	0 (0.0)	7 (100)	3 (42.9)	4 (57.1)
Tổng	50 (42.7)	67 (57.3)	12 (10.3)	105 (89.7)	83 (70.9)	34 (29.1)
p	p > 0.05		p < 0.001		p > 0.05	

Comment: The protein manifestation of p53, Her-2/neu increases with Dukes stage, but no differences were statistically significant.

Protein manifestation Ki67 increases with the degree and extent of Dukes Dukes B 96.2%, the level of Dukes C, D 100% (p <0.001).

Table 3:31: Relationship protein manifestation vs TNM.

TNM	p53		Ki67		Her-2/neu	
	Neg n (%)	Pos (%)	Neg n (%)	Neg n (%)	Pos (%)	Neg n (%)
0	1 (100)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	1 (100)
I	17 (48.6)	18 (51.4)	10 (28.6)	25 (71.4)	28 (80.0)	7 (20.0)
II	22 (42.3)	30 (57.7)	2 (3.8)	50 (96.2)	38 (73.1)	14 (26.9)
III	9	17	0	26	16	10

	(34.6)	(65.4)	(0.0)	(100)	(61.5)	(38.5)
IV	1 (33.3)	2 (66.7)	0 (0.0)	3 (100)	1 (33.3)	2 (66.7)
Total	50 (42.7)	67 (57.3)	12 (10.3)	105 (89.7)	83 (70.9)	34 (29.1)
p	p > 0.05		p < 0.001		p > 0.05	

Comment: The p53 protein manifestation increased in phases I 51.4%, 66.7% phases IV. Similarly, Ki67 protein manifestation increased from phases 0 and increase in phase III, IV 100%.

The protein manifestation Her-2/neu also increased the level of invasion, but not compared with the lower protein manifestation of stage I-III and phases IV Ki67-positive rate of 66.7%.

Chapter 4: DISCUSSION

We have studied the clinical characteristics, endoscopic images, histopathology, immunohistochemistry (IHC) at 55 patients with the 72 colorectal polyps ≥ 10 mm, and 117 colorectal cancer patients with the surgical treatment in the Gia Dinh People's Hospital and the Army Central Hospital 108. For that reason, we offer the following discussion.

4.1. Group of colorectal polyps ≥ 10 mm in size

4.1.1. The clinical features of colorectal polyps

4.1.1.1. Age and gender characteristics of colorectal polyps

The averageage of patients is 57.3 ± 15.3 (the lowest age is 18, the highest age is 78) has showed polyps regularly distributed for all ages.The rate of men who has polyps is 1.29 times more than the rate of women in accordance with the authors Dinh Duc Anh, Le Quang Thuan, QuachTrong Duc, Do Nguyet Anh turn 1.9; 1.7; 1.3.

4.1.3. Results of histopathology

4.1.3.1. Histopathological characteristics of colorectal polyps and cancer rate of polyp

According to the table 3.6, the rates of adenomatous polyps and nonpolypoid adenomas of colorectal appropriate to 77.8% and 22.2%.

Some domestic and foreign research studies showed that the rates of adenomatous polyp are more than the rates of nonpolypoid adenomas such as Do Nguyet Anh (2011) 96.5%. Le Quang Thuan (2008) 51.47%. QuachTrong Duc (2007) 87.2%. According to the authors Church 54 %. Nusko G (1997) 71%.

The Chart 3.3 showed the percentage of histopathology tubular adenomas with the high rate - 40.3%, 22.2% hyperplastic polyps, tubulovillous adenomas 16.7%, and 18.1% of polypsbecomes cancerous. Hence, following to our study, there are 18.1% of polyps become cancerous.

The adenomatous polyps are considered separately in table 3.7 showed that there are 51.8% of tubular adenomas, 21.4% of tubulovillous adenomas, 3.6% of villous adenomas, 23.2% of polyps become cancerous.

Likewise, the study of author Le QuangThuan at 68 patients with colorectal polyps, endoscopic stained a solution indigocarmine combined with biopsy, is comparable to our study, in which: Proportion adenomatous polyps accounted for 51.5% at the most, followed by 25% hyperplastic polyp, polyps becomes cancerous 17.6% of the polyps ≥ 20 mm in size, whereas the study of author Dinh Duc Anh showed the adenomatous type is common more than 50%, which accounted for 52.6% of tubular adenomas, tubulovillous adenomas 8.5%, 5.4% of villous adenomas, 7.6% of polyps becomecancerous. Hyperplastic polyps of 1.8% is lower than the rate in our study because the core object of our study is adults who aged 18 and older.

4.1.4. Cancerous polyps

4.1.4.1. The role of protein manifestation p53, Ki67 và Her-2/neu and colorectal polyps ≥ 10 mm in size

In our research, we proceed imunohistochemistry for 72 polyps ≥ 10 mm in size and studying the protein manifestation of three genes including p53, Ki67 and Her-2/neu. We combine histopathology and imunohistochemistry.

The result of protein manifestation in table 3.15 shows the positive rates of p53, Ki67 and Her-2/neu were 11.1%, 65.3% and 4.2% respectively. Currently, there is no national study on protein manifestation of gene in colorectal polyp, the study in foreign country on protein manifestation of genes p53, Ki67 và Her-2/neu with large colorectal polyp is not much. However, in the 80-90 decade of the last century has also studied early on this issue.

When exploring the proportion of protein manifestation of genes p53, Ki67 và Her-2/neu in cancerous polyp, the positive rates of p53, Ki67 và Her-2/neu in chart 3.8 were 61.5%, 92.3% and 23.1% respectively. When comparing the two groups of polyp are not cancerous and cancerous polyp, we realize that protein manifestation in cancerous polyp group in table 3.17 express of protein p53 8/13 (61.5%) polyps 0/59 (0%), Her-2/neu 3/13 (23.1%) polyps 0/59 (0%), Ki67 12/13 (92.3%) polyps 35/59 (59.3%) and the difference was statistically significant. This enables our physians that in case of unclear histopathology colorectal polyp we should test imunohistochemistry if p53 (+) and/or Her-2/neu (+). So the treatments and monitoring of cancer is such as polyps.

4.2. Colorectal cancer

4.2.1. The clinical features of colorectal cancer

4.2.1.1. Age and gender characteristics of colorectal cancer

The average age in our study is 63.68 ± 13.37, minimum age is 28, the oldest age is 89. The results of our study are match with studies of Fuszek, P 65.2 ± 12.5; Doubeni, C. A 71.7; Yesil, A 60.08 ± 12.42. Most studies have found that male have colorectal cancer are more than female.

4.2.5. Protein p53, Ki67 and Her-2/neu manifestation

We conducted immunohistochemistry by using three markers: p53, Ki67, Her-2/neu for 117 colorectal cancer patients. The result of study is illustrated in table 3.25 showed: there was a 57.3% of patients was positive with p53, the rate of patients who was positive with Ki67 was 29.1% and the percentage of patients suffering Her-2 was 29.1%.

Correlation between protein p53, Ki67, Her-2/neu manifestation and the level of penetration of colorectal cancer

Correlation between the level of penetration of the tumor and the over expression of protein p53, Ki67 and Her-2/neu in 117 colorectal cancer patients, which was shown in table 3.34 illustrated the rate of p53 was 0%, the percentage of T1, T2, T3, T4 were 75%, 47.2%, 56.1% and 78.9%, respectively in the depth of penetrating of T in-situ increased but the difference was not significant. According to Hoang Kim Ngan et al, p53-positive rate increased with the level of invasion, but no statistically significant, Nehls et al found that p53-positive rate in T2 was 52.6%, T3-4 was 47.7%, but still no statistically significant.

The deeper the level of penetrating of tumor was, the higher Ki67 positive rate was, T in-situ 100%, T1 75%, T2 75%, T3 96,5%, T4 100% and the increase was statistically significant, Tsai, WC et al found that Ki67 rose with the level of invasion. Theoretically, proliferation antigen Ki67 nucleus related to the growth of cells, when the tumor developed, its cells proliferated dramatically leading to the higher positive rate of Ki67 antigen. The result of our study also reflected partly this opinion and Ki67 supported that theory. However, the positive rate increased with the level of invasion, but no statistically significant according to Hoang Kim Ngan and Phan Dang Anh Thu. This field needs more studies with larger population.

The positive rate of Her-2/neu in our study was not high, thus the positive rate in T3, T4 was 24.6% and 52.6%, respectively, the result of our study was the same with the study of Hoang Kim Ngan and Phan Dang Anh Thu. The positive rate of Her-2/neu was 8%, 6%, 49% and 2%, respectively based on Pappas, DI et al. Pappas, A and Kavanagh, DO et al found that the positive rate of Her-2/neu did not correlate with the stage and the survival rate of the patient

Correlation between protein p53, Ki67, Ner-2/neu expression with mesenteric lymph node metastasis.

Mesenteric lymph node metastasis is one prognostic indicator. The expression of protein p53, Ki67 and Her-2/neu (table 3.35)

showed that p53 positive rate was 69.2 % in lymph nodes metastasis group and 53.8% in lymph nodes non-metastasis group (p=0.16), Nguyen Thanh Nam et al found that p53 positive rate in lymph nodes metastasis group was lower than that of in the other group and the difference was not statistically significant. Galizia and Tornillo also stated that there was no relation between the accumulation of protein p53 and lymph node metastasis.

The Ki67 positive rate in our study was 100% in lymph nodes metastasis group and 86.8% in lymph nodes non-metastasis group (p=0.05). Salminen E. Tsai WC. Kawazoe N. et al stated that the expression of Ki67 was significant in lymph nodes metastasis group and the difference was statistically significant. Phan Dang Anh Thu convinced that there was no difference in Ki 67 expression between two groups.

Her-2/neu positive rate in lymph nodes metastasis group and the other was 42.3% and 25.3% (p = 0,09), respectively, which was similar to the study of Pappas A. Park DI, the positive rate was low and did not correlate with lymph nodes metastasis and prognosis.

The relation between the protein manifestation of p53, Ki67, Her-2/neu and Duke's stage and TNM.

Table 3.36 indicates that p53 is positive in Duke A 50%, Duke B 57,7% Duke C 63,3% and Duke D 71,4%, in our research is similar to author Jahantigh, M and his colleagues the high positive of p53 is in stage Duke B and C with 56,8% and 32,4%, and author Kressner U p53 is positive in stage Duke A, B, C and D in turn 52%, 43%, 54%, some authors assume that the ratio of p53 is extremely positive in stage C and D.

Ki 67 indicating the proliferation of cells and prognosis of patients in research is realized that positive ratio increase with level of invasion in stage Duke A, B, C, D in turn 72,2%, 96,2%, 100% và 100% (p < 0,001) our research is similar to author Salminen, Nabi and Kubota conclude that there is a relation between Ki 67 and stage Dukes, according to Salminen the extremely high ratio Ki 67 is in stage Dukes B.

In our research, her-2/neu has the low negative ratio is similar to author Pappas, Kavanagh when they research on the patient with Colorectal cancer show that thete is not the relation between Her-2/neu and stages Dukes. Her-2/neu is not the factor to prognosis. However, patients having over manifestation of Her-2/neu response to the treatment Herceptin (target therapy).

The classification of cancer with TMN delivery more information than stage Dukes, give and divide some factors to prognosis into little parts, this classification base on penetration of tumor into colon wall, invading on nearby organ and system around the tumor, number of lymph nodes and whether metastasis has. Thus, we consider the relation between the protein manifestation gene p53, Ki 67, Her-2/neu with stage Dukes.

Table 3.37, we realize that the deeper tumor invade colon wall, the more increased the manifestation of p53 and Her-2/neu, but this increasing is not statistical significant, while Ki 67 in stage I: 71,4%, stage II: 96,2%, stage III: 100%, and T4 100 (p=0,001). When author Tsai, WC research 117 colorectal cancer patients indicate that there is a relation between p53 and Ki 67 and clinic, the invasion of tumor and stage of AJCC, Hashimoto, Y and his colleague have a research on 131 patients with colorectal cancer show that Ki 67 relate to stage TMN. The over protein manifestation Her-2/neu is confirmed to play a role in breast cancer, with colorectal cancer Her-2/neu has low detected cut off 3,9% without relation to clinic and prognosis, similarly research of author Schuell and his colleague has result of the Her-2/neu protein manifestation: 1-3%, Nathason and colleague research 139 colonrectal cases having over Her-2/neu protein manifestation: 3,6% like author Kavanagh and his colleague, they indicate that the ratio of Her-2/neu protein manifestation is low and there is no relation to stage Dukes and TNM.

CONCLUSION

Researching on 55 patients with colonrectal polyp > 10 mm in size and 117 colorectal cancer patients detected through clinical

examination, colonoscope, histopathological, immunohistochemistry test and treated at Nhan Dan Gia Dinh Hospital and Military Central Hospital 108, we make some conclusions as follows:

1. Characteristic of colonrectal polyp > 10 mm in size and colorectal adenomatous cancer through clinical examination, colonoscope, histopathology and immunohistochemistry

Colonrectal polyp ≥ 10 mm in size

*** Clinical:**

- Ratio: male : female = 1,29; Frequent age: 41-80 years-old, make up 85,5%.
- Frequent symptoms: blood in stool 52,8%, stomachache 49,1% sticky mucus in stool 47,3%.

* Colonoscope:

- Average quantity of polyp per patient: 1,3

Số lượng polyp trung bình ở một bệnh nhân là 1,3.

- Mono polyps make up 74,6%
- Polyp position: 34,7% at sigma colon, 31,9% at anus-rectum
- Peduncular Polyp: 29,2%; sessile polyp: 45,8%; semi-sessile polyp: 25,0%.
- Polyp 10-15mm in size: 58,3%, trên 15-20mm: 19,4%, trên 20mm: 22,3%

*** Histopathology:**

- Adenomatous polyps: 77,8%; Non-neoplastic polyps: 22,2%.
- Tubular adenoma: 40,3%; Tubulovillous adenoma: 16,7%; Villous adenoma: 2,7%.
- Dysphasia Polyp: 76,4%; Cancerous Polyp: 18,1 %.

*** Immunohistochemistry:**

- Correlative proportion of p53, Ki67 and Her-2/neu positive at 72 polyps ≥ 10 mm in size is: 11,1%; 65,3% and 4,2%. Correlative proportion of p53, Ki67 and Her-2/neu positive of 13 cancerous polyps is: 61,5%; 92,3% and 23,1%.

Colonrectal adenomatous cancer

*** Clinical:** colonrectal cancer frequently occurs at age 50-69; average age 63,7 ± 13,4. Ratio male / female: 1,6/1.

- Stomachache and blood in stool are frequent symptoms of colonrectal cancer (made up 76,9% and 58,2%).

* Colonoscope: Tumours at anus-rectal (32,6%) and at sigma colon (25,6%). Frequent types of macroscopic are vegetant and vegetant + ulcers, with correlative ratio 56,4% and 29,1%.

*** Histopathology:** Colonrectal adenomatour cancer makes up 99,1%;

Ung thư biểu mô tuyến chiếm 99,1%; in there, high differentiation: 30,7%; medium differentiation: 49,6%; low differentiation: 3,4%.

*** Immunohistochemistry:** protein manifestation of genes p53, Ki67 and Her-2/neu:

- Correlative pos differentiationionthe polyp 99. cancer scopy, lab-tests itive ratio of p53, Ki67 and Her-2/neu in colonrectal cancer are 57,3%; 89,7% and 29,1%. Ratio of ulcers with positive p53 and Ki67 is 100% and positive Her-2/neu is 50%. For vegetant and vegetant + ulcers, the correlative ratio of positive p53 are 54,5% and 67,6%; positive Ki67 are 87,9% và 94,1%; positive Her-2/neu are 24,2% và 32,4%.

2. Correlation between manifestation of immunohistochemistry marks and histopathological characteristics and lymph node metastasis

- High ratio of positive p53 and Ki 67 in cancerous polyps is 61,5% và 92,3%.
- p53 và Her-2/neu are often negative in benign polyps.
- The ratio of positive p53 and Her-2/neu has a trend of increasingly according to the degree of penetration of tumor following Dukes stage, T and TNM. However, the above correlation is not clear enough ($p > 0,05$).
- The higher positive of Ki67 when the deeper degree of penetration of tumor, the correlation is statistical meaning, with $p < 0,05$.
- Ki 67 is positive in lymph node metastasis group (100%). However, There is no clear correlation between the lymph node metastasis group and no lymph node metastasis.

LIST OF WORKS OF RESEARCH HAS PUBLISHED AUTHOR RELATED TO THE THESIS

1. **Vo Hong Minh Cong, Trinh Tuan Dung, Vu Van Khien (2014)** Histopathology study and the expression of p53, ki67 and her-2/neu in colorectal cancer. *Journal of 108-clinical medicine and pharmacy*, 9(Special Issue), pp 167-173.

2. **Vo Hong Minh Cong, Trinh Tuan Dung, Vu Van Khien (2014)** The role of endoscopy, hystology and immunohistochemistry in diagnosis of colorectal polyp with size more than 10 mm. *Journal of 108-clinical medicine and pharmacy,* 9(Special Issue), pp 174-180.