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The demonstration of expressed microsatelite protein (MLH_1) on colorectal carcinoma tissue

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Abstract

This study was carried out to evaluate the expression of microsatellite protein (MLH_1) on colorectal cancer specimen. A total of 32 tissue specimens of colorectal carcinoma from Meena Histopathological and Pap smear Screening Centre Jos and Federal staff Hospital Abuja were reviewed and analyzed Immunohistochemcially for the expressionof MLH_1 and MSH_2 protein with colorectal cancer samples. Nineteen (59.3%) of colorectal cancer revealed negative reaction to MLH_1 protein and 17(53.1%) showed negative reaction to MSH_2 protein, while 14(43.7%) showed negative reaction to MLH_1 and MSH_2 protein (mismatch repair protein negative) indicating absence of DNA mismatch repair protein. In 10 (31.2%) were positive reaction to MLH_1protein (mismatch repair protein positive). Fourteen (43.7%) showed moderate differentiated adenocarcinoma with haematoxylin and eosin technique while in 10(31.2%) were observed well differentiated adenocarcinoma as 8(25%) revealed poor differentiated adenocarcinoma with the same technique. Four (28.5%) tissue specimens of moderate differentiated colorectal cancer showed stage II of colorectal cancer with MLH_1 protein and 1(7.1%) revealed stage III of colorectal cancer with MLH_1 protein. In 3(30%) of well differentiated colorectal cancer were observed stage II of colorectal cancer with MLH_1 protein and 2(20%) of same showed stage IV of colorectal cancer with MLH_1 protein. Two (25%) poor differentiated adenocarcinoma were stage II of colorectal cancer with MLH_1 protein and 1(12.5%) showed stage IV of colorectal cancer with MLH_1 protein.

Keywords: cancer, biomarker, microsatellite protein, MLH1, colorectal carcinoma

Introduction

Colorectal cancer are those growths that principally originate on the colon, rectum and appendix, and are capable of spreading to other parts of the body (National Cancer Institute, 2014).

This occurs due to mutations along the Wnt signaling pathway. Adenomatous polyposis coli gene (APC) is the common gene that is affected in colorectal cancer. Adenomatous polyposis coli gene produces adenomatous polyposis coli protein that regulates the activity of β-catenin protein in the body (Behrens, 2005). β-catenin if not controlled by APC protein will bind to DNA and activate more proteins that will lead to the production of many abnormal cells (Behrens, 2005). There are some other mutations that cause cells to grow abnormally and they include- those of TP^{53} gene that produce P^{53} protein which monitors division of cell and cells that have defects in Wnt pathway (Markowitz, 2007). Most times, these genes are not mutated, but other protective proteins like BAX and SMAC are not activated. Some genes such as RAS, RAP and P13K which help during normal cell division are over expressed in colorectal cancer due to mutations (Dimitriadis et al., 2001).

Most colorectal cancer occur as a result of so many factors like lifestyle, increase in age, genetic disorders, diet and lack of physical exercise (National Cancer Institute, 2014).

Familial adenomatous polyposis and the hereditary non-polyposis colorectal cancer are inherited conditions that cause colorectal cancer which starts as a benign tumor and then grows to cancerous cells (National Cancer Institute, 2014).

Globally more than one million people are presented with colorectal cancer yearly (Cunningham et al., 2010) and this resulted to 715,000 deaths in 2010, almost a double of 490,000 in 1990 (Lozano, 2012). In the year 2008, colorectal cancer was rated second most common cancer among women and third most common type of among men globally (Jemal *et al.*, 2011). Colorectal cancer is the fourth most common cause of cancer deaths after cancer of the lungs, cancer of the stomach and cancer of the liver (WHO Cancer, 2011). Colorectal cancer is more prevalent in developed countries than in the developing ones. However, adequate statistics are not available in developing countries like Nigeria, because not all cancer-related deaths are documented and reported(Aizaz *et al.*, 2023; Obeagu *et al.*, 2021; Obeagu and Obeagu, 2023; Obeagu *et al.*, 2021; Ahiara *et al.*, 2022; Obeagu *et al.*, 2022; Obeagu and Babar, 2021).

Mutation in the mut S homologs 2 gene accounts for high percent of genetic defects that are associated with the hereditary non polyposis colorectal cancer. Mutations that associate with hereditary non polyposis colorectal cancer are widely spread in all domains of mut S homologs 2 gene and the functions of the mutations rely on the crystal structure of the mut sα which involve protein-to-protein interaction stability, allosteric regulation, MSH_2 to MSH_6 interface, and the DNA binding (de Wind et al., 1995). Mut S homologs 2 gene which is a member of mismatch repair gene is assessed for its viability through microsatellite instability biomarker test that analyses the short sequence repeats that are very hard for cells to produce without a normal functioning mismatch repair system(Ofor*et al.*, 2016; Obeagu and Obeagu, 2016; Obeagu *et al.*, 2016; Obeagu, 2018; Obeagu, 2018; Obeagu, 2018). Seventy one percent of hereditary non-polyposis colorectal cancer patients show microsatellite instability (Pitsikas *et al.*, 2007). Mut L homologs 1 protein produced from MLH1 gene, is a member of mismatch repair protein that plays an important role in DNA repair and also in correcting mistakes that are made during DNA replication in preparation for cell division (Andersen *et al.*, 2012).

The repairs are done by removing the sections of DNA which contains mistake and replacing it with the corrected DNA sequence (Wang *et al.*, 2001).

This work intends to determine the stages of colorectal carcinoma through the expression of microsatellite proteins (MLH1 and MSH2) on colorectal cancer tissue.

Materials and Methods

Ethical Clearance

Before conducting the research, ethical clearance was obtained from National Hospital ethical committee. Ethical consideration were applied during sampling and analysis stages of the project.

Methods

All the methods used in this work were done following the protocols as prescribed by immunohistochemcial staining procedures.

Sample Collection

The record books of histopathology laboratories of Meena Histopathology Laboratory and Pap Smear Screening Centre Jos and those of Federal Staff Hospital Abuja, were reviewed and thirty two (32) tissue blocks, (20 and 12 samples respectively) diagnosed of colorectal carcinoma were selected and reprocessed, sectioned on microtome and collected on microscopic slides and stained with haematoxylin and eosin and MLH_1 proteins

Immunohistochemical Staining Method

Avidin-biotin complex method (Hsu *et al.*, 1981)

A cut sections of about 2 microns thick each from the 32 tissue blocks were made. The sections were fixed in hot plate for one hour and further dewaxed in xylene and then taken through descending grades of alcohols (100%, 90% and 70%) to hydrate it and finally to water. The sections were treated in antigen retrieval buffer (citrate acid P^H 6.0) and boiled in pressure cooker at 100^{0C} for 25 minutes. The sections were dipped into deionized water and blocked with endogenous peroxidase using peroxidase block for 15 minutes, and rinsed in phosphate buffer saline and block with protein using protein block (Avidin) for 15 minutes. The sections were rinsed in phosphate buffer saline and blocked with biotin using biotin block for 15 minutes and rinsed in phosphate buffer saline, and incubated with MLH_1 and MSH_2 protein (primary antibody) for 60 minutes.

The sections were rinsed in phosphate buffer saline and incubated with post primary antibody for 15 minutes. After rinsing the sections with phosphate buffer saline, the sections were incubated with polymer for 15 minutes and rinsed in phosphate buffer saline, and incubated with diaminobenzidine (DAB) for 4 minutes and the colour changes were observed. The sections were rinsed in deionized water and counter-stained with haematoxylin for 2 minutes, Blued in scoth's tap water, dehydrated in ascending grades of alcohol and allowed to dry. DPX was used to mount the section and covered with coverslip and examined microscopically. The immunohisto chemical method demonstrated showed different levels of positive reaction in the nucleus of the cancer cells, which corresponds to the various levels of brown colour.

Haematoxylin and Eosin Staining Method (Bohmer and Fischer in 1876).

Cut sections of 2 micron thick from the tissue blocks were made, dewaxed and taken through descending grades of alcohol. The sections were stained with haematoxylin for 15 minutes and rinsed in water. The sections were further differentiated in 1% acid alcohol, rinsed in water and blued in tap water for 5 minutes. The sections were stained with eosin for 2 minutes and rinsed in water, dehydrated by passing through 90% alcohol for 15 seconds, absolute I for 15 seconds and absolute II for 15 seconds. The sections were cleared in xylene, mounted with DPX and examined microscopically.

Histopathologic Evaluation

Posh formular was used in grading the work according to World Health Organization criteria (Jass and Sobin, 1989).

The formula is stated thus:

$\frac{0}{3} + \frac{0}{5} = \frac{0}{8}$ = Negative control

$\frac{1}{3} + \frac{0}{5} = \frac{1}{8}$

$\frac{1}{3} + \frac{1}{5} = \frac{2}{8}$

$\frac{2}{3} + \frac{1}{5} = \frac{3}{8}$

$\frac{1}{3} + \frac{3}{5} = \frac{4}{8}$

Mild positive control (Stage II)

$\frac{2}{3} + \frac{3}{5} = \frac{5}{8}$

$\frac{3}{3} + \frac{3}{5} = \frac{6}{8}$

Moderate positive control (Stage III)

$\frac{3}{3} + \frac{4}{5} = \frac{7}{8}$

$\frac{3}{3} + \frac{5}{5} = \frac{8}{8}$

Strong or high positive control (Stage IV)

Results

Table 1: Expression of MLH_1 protein in colorectal cancer

Haematoxylin and eosin features	Number of tissue sections	Number positive	Percentage positive (%)	Number negative	Percentage negative (%)
Moderate differentiated adenocarcinoma (MDA)	14	5	35.7	9	64.2
Well differentiated adenocarcinoma (WDA).	10	5	50	5	50
Poor differentiated adenocarcinoma (PDA)	8	3	37.5	5	62.5
Total	**32**	**13**	**40.6**	**19**	**59.3**

Discussion

Colorectal cancer is an autosomal dominant condition due to germ line mutations in DNA mismatch repair protein in particular MLH_1 protein. MLH_1protein is a DNA mismatch repair protein that helps to trace and fix mistakes made during DNA replication (Anderson *et al.*, 2012).

From this work immunohistochemical analysis were used to expressed MLH_1 protein and MSH_2 protein in colorectal cancer specimen.

Out of 32 tissue specimens analyzed with immunohistochemical method 13(40.6%) specimens showed positive expression to MLH_1 protein and 19(59.3%) specimen showed negative expression to MLH_1 protein This is in agreement with the work of Vasen *et al.*,(2007) which reported that Ninety percent (90%) of hereditary non-polyposis colorectal cancer showed mutation of MLH_1protein.

This result is not accordance with the report stipulated by Lynch et al., (2008), which said that colorectal cancer characterized with mismatch repair deficiency are only associated with older age.

This work, reviewed that immunohistochemical analysis of MLH_1 protein is accurate and suitable for examination of DNA mismatch repair status, suitable for large-scale investigation and it provides useful prognostic information for the management of stage II, III and stage IV of colorectal cancer patient (Benson *et al.*, 2004; Zaniboni and Labianca, 2004).

Conclusion

The findings that different molecular pathways are involved in colorectal cancer development have helped researchers build different models and understand how colorectal cancer initiates and progresses. However, the application of molecular marker (MLH_1) on diagnosis is now facilitating the understanding on colorectal cancer behaviour, prognosis and response to treatment. The results from this study shows that the immunohistochemical test for MLH_1 protein showed a rapid and reliable method for the detection of the larger majority of mismatch repair defect in colorectal carcinoma.

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