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The demonstration of expressed microsatelite proteins (MSH_2) on colorectal carcinoma tissue

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ABSTRACT

This study was carried out to evaluate the expression of microsatellite protein (MSH_2) 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 expression of MSH_2 protein with colorectal cancer samples. Nineteen (59.3%) of colorectal cancer revealed 17(53.1%) showed negative reaction to MSH_2 protein. Five (35.7%) of moderate differentiated adenocarcinoma showed stage II of colorectal cancer with MSH_2 protein and 1(7.1%) showed stage III of colorectal cancer with MSH_2 protein. Four (40%) of well differentiated adenocarcinoma revealed stage II of colorectal cancer while 2(20%) showed stage III of colorectal cancer with MSH_2 protein. In one (12.5%) of poor differentiated adenocarcinoma were stage II of colorectal cancer with MSH_2 protein and 2(25%) showed stage III of colorectal cancer with MSH_2 protein.

Keywords: microsatellite proteins, msh_2, 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 [1]. 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 [2]. 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 [2]. 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 [3]. 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 [4]. Globally more than one million people are presented with colorectal cancer yearly [5] and this resulted to 715,000 deaths in 2010, almost a double of 490,000 in 1990 [6]. In the year 2008, colorectal cancer was rated second most common cancer among women and third most common type of among men globally [7]. 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 [8]. 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.

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 /MSH_2 proteins.

Immunohistochemical Staining Method

Avidin-biotin complex method

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 immunohistochemical 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

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 Evalution

Posh formular was used in grading the work according to World Health Organization criteria [9].

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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)

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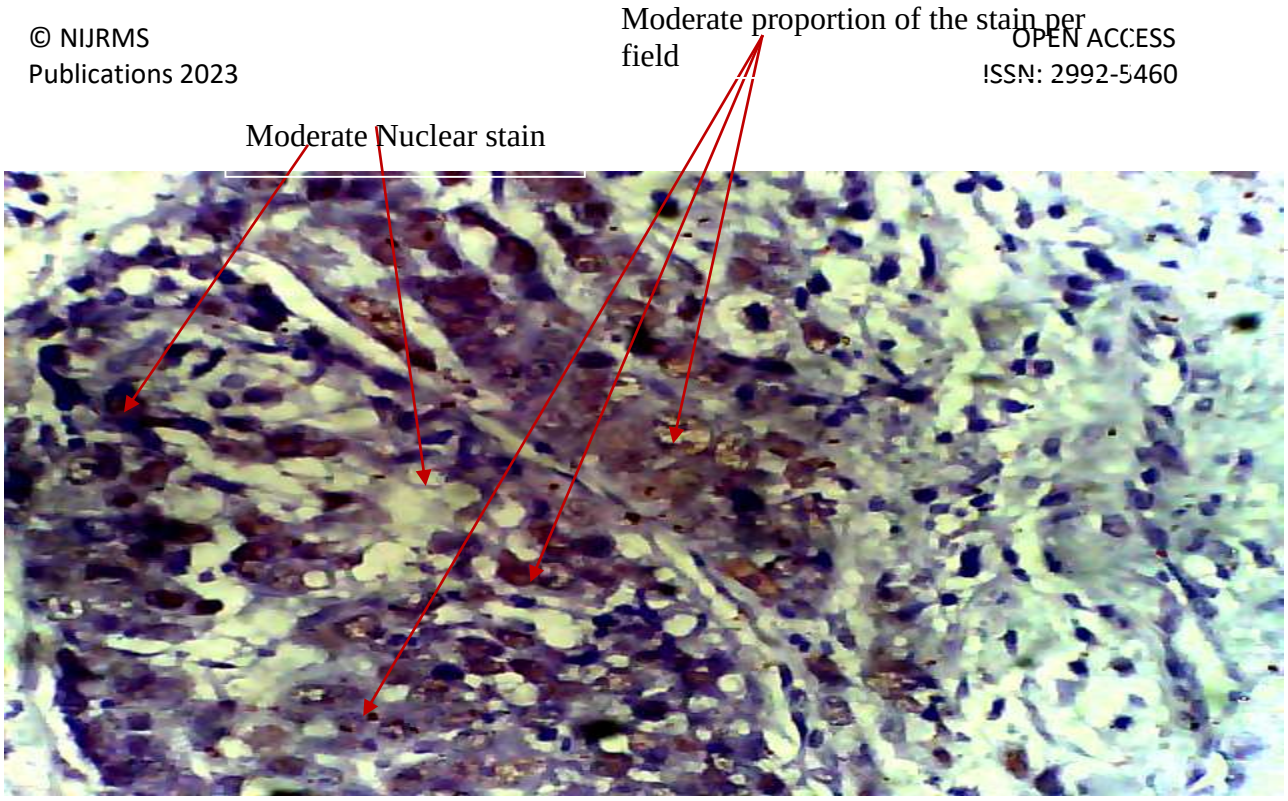

Fig 1: Showing Stage III of Poor Differentiated Adenocarcinoma with MSH_2 protein (Moderate Positive) by x10 magnification.

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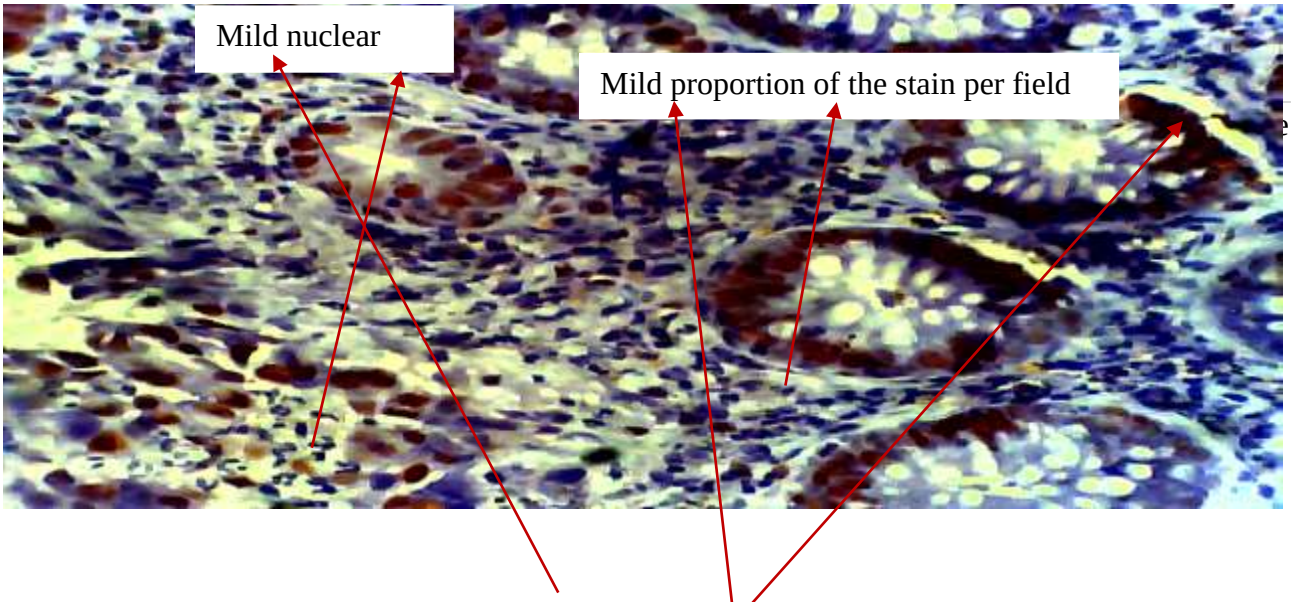

Fig 2: Showing Stage II of Well differentiated adenocarcinoma with MSH_2 (Mild Positive) by x 10 magnification.

RESULTS

Table 1: Expression of MSH_2 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	6	42.8	8	57.1
Well differentiated adenocarcinoma (WDA)	10	6	60	4	40
Poor differentiated adenocarcinoma (PDA)	8	3	37.5	5	62.5
Total	**32**	**15**	**46.8**	**17**	**53.1%**

DISCUSSION

Out of 32 tissue specimens analyzed with immunohistochemical method 15 (46.8%) specimen showed positive expression to MSH_2 protein and 17(53.1%) specimen showed negative expression to MSH_2 protein. This is in agreement with the work of Vasen *et al.* [10-11] which reported that Ninety percent (90%) of hereditary non-polyposis colorectal cancer showed mutation of MLH_1 and MSH_2 protein.

This support the work of Aaltonen *et al.* [12] and Fishel *et al.* [13] which reported that 40%-60% of MSH_2 protein mutation associate with the increase risk that lead to the formation of colorectal cancer and 50%-80% of MLH_1 protein mutation associate with the increase risk that lead to formation of colorectal cancer. Three stages of colorectal cancer were classified in this work using posh formular according to world health organization criteria [9]. While the age group of 51-55 years of 3 specimens showed stage II of colorectal cancer with MSH_2 protein, stage III of colorectal cancer with MSH_2.

CONCLUSION

The results from this study shows that the immunohistochemical test for MSH_2 protein showed a rapid and reliable method for the detection of the larger majority of mismatch repair defect in colorectal carcinoma. Also the results showed that MSH_2 protein expression is useful in grading stages of colorectal cancers from stage II, III and IV in a patient.

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