

# Alkylresorcinols as an Indicator of Whole-Grain Intake and Its Relation to Colorectal Carcinoma

<sup>1</sup>Shalaby, S.I., <sup>2</sup>Mayssa E-Rzky, <sup>3</sup>Mofeed Shehab, <sup>4</sup>Dina Sabry, <sup>5</sup>Ihab K.Abdel-Aziz, <sup>6</sup>Mohamed AbdAllah, <sup>7</sup>Omar El Shabacy, <sup>8</sup>Devendra Kumar Awasthi

<sup>1,7</sup>Department of Complementary Medicine, Medical Division, National Research Centre, Dokki, Egypt

<sup>2,3,5,6</sup>Department of Endemic Medicine, Faculty of Medicine, Cairo University, Cairo, Egypt

<sup>4</sup>Department of Medical Biochemistry, Faculty of Medicine, Cairo University, Cairo, Egypt

<sup>8</sup>Department of Chemistry, Sri JN MPG College, Lucknow, India

**Abstract - BACKGROUND:** Alkylresorcinols are phenolic lipids that exclusively present in the outer parts of wheat and rye grains. They have been proposed as specific dietary biomarkers of whole-grain wheat and rye intake. Dietary fibers protective role against colorectal cancer has been postulated several decades ago. **AIM:** To verify the relation of dietary fiber intake and incidence of colorectal cancer using plasma total Alkylresorcinols concentrations as an indicator of whole-grain intake. **METHODS:** 148 Subjects were enrolled and categorized into two groups; **Group (A):** 62 patients with colorectal cancer and **Group (B):** 86 subjects with normal colonoscopy “control group”. Blood samples were withdrawn from the patients after overnight fasting for detection of Alkylresorcinols levels. **RESULTS:** Detection of the Alkylresorcinols levels; C17, C21 and Total Cs were significantly higher in the control group in comparison to colorectal cancer group, C19 levels showed no significant difference. **CONCLUSION:** People with high intake of fibers especially cereals probably have a lower risk of developing cancer colon.

**Keywords:** colorectal cancer, Alkylresorcinols, cereals, protective.

## I. INTRODUCTION

Colorectal carcinoma (CRC) is considered to be a major healthcare problem. Worldwide, It is the 3<sup>rd</sup> most common cancer in incidence and the 4<sup>th</sup> common cause of cancer-related mortality (1). Additionally and it represents about 4% of total cancers in Egypt (2).

The highest incidence rates are in Australia and New Zealand, Europe and North America, and the lowest rates are found in Africa and South-Central Asia. These variations may refer to differences in dietary and environmental factors (3).

The beneficial relation between administration of high fiber diet (FD) and low incidence of CRC was noticed in rural Africans ; who are used to eat diet with high fiber content (4), and this relation regained interest when the European

Prospective Investigation into Cancer and Nutrition (EPIC) study reported a linear decrease in the risk of colorectal cancer with increasing fiber intake (5).

Several mechanisms had been postulated about the protective role of FD ; including increased stool bulk, dilution of carcinogens in the colonic lumen, bacterial fermentation of fiber to short chain fatty acids (6). Also , the ability of FD to alter the intestinal flora has a positive impact on the general condition and human health , in addition to enhancing bile acid de-conjugation, production of short chain fatty acids (SCFAs) and modulation of inflammatory bioactive substances (7-9).

DF is a broad category of non-digestible food ingredients that includes non-starch polysaccharides, oligosaccharides, lignin, and analogous polysaccharides (10). DF is classified according to water solubility,. Soluble DFs include pectin, gums, and polysaccharides, whereas insoluble DFs include cellulose, hemicellulose and lignin (11).

More recently, the European Food and Safety Authority (EFSA) defined dietary fiber as non-digestible carbohydrates plus lignin, including non-starch polysaccharides: cellulose; hemicelluloses; pectins; hydrocolloids (12).

Whole grains are a major source of dietary fiber and contain germ, endosperm, bran, several vitamins, minerals, and phytochemicals, which have anticancer properties which may decrease the incidence of CRC (13). Administration of 10 grams of FD per day can cause 10% reduction in risk of CRC (14).

On the other hand, Some studies refer the low risk of CRC among people with high DF to other dietary or lifestyle factors such as higher intake of other nutrients such as calcium and folic acid , lower intake of alcohol and tobacco, higher rates of physical effort and decreased incidence of metabolic syndrome components (15).

Whole grain intake measurement may be a challenging processes due to calculation errors and difficulty in determination the whole grain products. Therefore, using biomarkers of whole grain intake could overcome some of these problems. Alkylresorcinols (ARs) are phenolic lipids found exclusively in the bran part of wheat, rye, and, to a very minor extent, barley among commonly consumed foods. Alkylresorcinols homologs, consisting of carbon chain lengths C15:0 - C 25:0 are present in rye, and of these C19:0 is the most abundant (16).

Detection of ARs or their main metabolite concentration in blood plasma, erythrocytes, adipose tissue, and in urine are used to estimate whole cereal intake (17).

## II. MATERIALS AND METHODS

### Patients and study design

This is a cross sectional study which was performed on 148 patients presenting for colonoscopy at GI endoscopy unit (Gieluka), Cairo University and National Cancer Institute from January 2014 to June 2015. All patients were recruited after a written informed consent and the study protocol was approved by the Ethics Review Committee of Kasr al Ainy, Cairo University Hospital.

### Inclusion criteria:

Adult patients (> 18 years old) referred to colonoscopy unit for any indication including:

- Patients with symptoms such as chronic diarrhea, chronic constipation, alternating bowel habits and bleeding per rectum, unexplained weight loss, unexplained anemia.
- Asymptomatic patients presenting for screening colonoscopy (family history of CRC, occult blood positive or Family history of polyposis).

### Exclusion criteria:

- Patients who previously received chemotherapy or hormonal therapy for colorectal malignancy.
- Patients having cancer at any other site at the time of selection
- Incomplete colonoscopic examination due to technical causes e.g. difficult colonoscopy (a neoplastic mass causing lumen stricture was not excluded).

All enrolled subjects will perform colonoscopy with biopsy taking form any susceptible lesion. Upon biopsy results, Participants were divided into:

Group A: 62 patients with histologically proven CRC.

Group B: 86 with normal colonoscopic examination, they served as a control group.

### Chemicals and standards

HPLC reagents were diethylether, ethanol, ethyl acetate, formic acid, hexane and methanol. All were purchased from Sigma (St. Louis, MO, USA). Alkylresorcinol reference (AR) compounds (C17:0, C19:0 and C21:0) and AR internal standard which does not exist naturally (C20:0) were purchased from Thermo Fisher Scientific (Rockford, IL, USA). Syringic acid, used as an internal standard for the determination of AR metabolites in plasma samples was purchased from Sigma Chemicals (St. Louis, MO, USA). Solid phase extraction (SPE) cartridges were obtained from Waters (Milford, MA, USA). Trifluoroacetic anhydride (TFAA) was purchased from Sigma–Aldrich, Sweden.

### Samples collection

Blood samples were collected in the morning; after the patients had fasted overnight. Blood drawn from an antecubital vein into EDTA coated vacuum tubes was immediately centrifuged in a Hettich centrifuge (Bach, Germany) (2000 X g for 10 min at 4 °C) to separate plasma from erythrocytes. Plasma samples were portioned into 2-mL cryotubes and stored at -80 °C until analyzed.

### Determination of AR in plasma samples

Plasma samples were analyzed for relative AR homologues composition according to a method slightly modified from Linko et al (2002). Briefly, plasma (500µL) was mixed with the internal standard (40 ng) and incubated with water (0.5 mL) at 37 °C overnight. Samples were extracted with diethyl ether (3 mL × 3), evaporated to dryness, and redissolved in 0.5 mL methanol. The ARs present were separated from nonpolar lipids on a diethyl-amino-ethyl (DEAE)-Sephadex A-25 ion exchange gel (Amersham Biotech, Uppsala, Sweden) in free base form dissolved in methanol. Eluted ARs were analyzed by HPLC. ARs were identified by their molecular ions, and by comparing their retention times with synthetic AR standard (C20:0). A multipoint standard curve was prepared for AR standard (nmol/L). Known concentrations of ARs were set versus the ratio of ARs to internal standard area ratio, and linear regression was applied. Homologues C17:0, C19:0 and C21:0 were quantified (nmol/L) by using the standard curve of C20:0. Plasma samples were analyzed as single samples.

### Statistical analysis

Quantitative variables were expressed by mean and standard deviation (SD) or expressed by median and inter

quartile range (IQR) for non-parametric data. They were compared by student's t test or ANOVA test when appropriate. Qualitative variables were compared by chi-square or Fischer's exact test when appropriate.

In all tests, p value was considered significant if  $< 0.05$ , and highly significant when  $< 0.001$ .

### III. RESULTS

The demographic features of the studied participants are shown in table (I). The studied patients were middle aged with male predominance. The commonest site of CRC affection was the left colon. Moreover, well differentiated CRC were found in 69.3% while poorly differentiated in 30.7% in the studied patients as shown in Table (II).

Total Alkylresorcinols, C17 and C21 and were significantly lower in adenocarcinoma group. C19 showed no significant difference as shown in table (III). However there was no significant relation between level of AR and the site of the lesion (Table IV).

#### Discussion

Screening for CRC – especially in highly susceptible groups- and early-stage diagnosis; potentially improve the outcome of this disease (17). Colonoscopy and histopathology are still the gold standard method for detection of CRC. Nowadays, multiple non-invasive measures for detection are now under evaluation (18).

The mean age of CRC patients was 52 years ; which as well as male predominance in CRC , come in parallels with studies done by El Attar at (2005) and Egyptian national cancer institute (NCI) at 2008 (19-20). While the reported mean age in Western populations is in the range of 63-71 years (21). The difference of age of CRC occurrence between Egypt and Europe may be referred to the wide use pesticides, hormones and fertilizers. These fore mentioned factors are considered high risk factors for CRC (22).

Left sided tumors were more prevalent in our study (77.5%). An Egyptian study was done at 2002 came with same finding (23).

Whole grains are considered as a major source of dietary fiber and contain germ, endosperm, and bran (13). Biomarkers of whole grains (C17, C21 and Total Cs) were significantly higher in control group in comparison with colorectal cancer group, while C19 showed no significant difference; indicating that people with high intake of fibers; specially cereals probably have a lower risk of developing cancer colon. When investigating the association between plasma total alkylresorcinols and colorectal cancer location; our results

showed no significant difference in level of alkylresorcinols between proximal and distal colon.

A meta-analysis done by Aune concluded a similar result. He stated that there is 10% reduction in risk of colorectal cancer for each 10 g/day intake of total dietary fiber and cereal fiber and a about a 20% reduction for each three servings (90 g/day) of whole grain daily (14). Also, Bingham revealed similar findings in his study on 19,978 patients; that depend of dietary data collection via a questionnaire (5). In contrast, Studies of Terry (24) and Mai (25) failed to find a relation between FD and low risk of CRC.

But we need to mention that the contradiction in results of all the fore mentioned studies rely only on dietary data collection via a questionnaire; without assessing the more accurate biomarkers such as ARs that were used in our study.

Similar to our study, Knudsen has assessed the relation of FD and CRC using serum calculation of ARs and they stated a detected drop in the incidence of distal CRC associated with high plasma total ARs concentrations. This study was applied on 522 cases, 562 controls with median follow up period of 4.6 years; which is similar to our findings (26).

### IV. CONCLUSION

Alkylresorcinols (as a marker of whole grain intake) were significantly higher in the non-cancer group suggesting the possibility of a protective role in colorectal cancer.

### REFERENCES

- [1] Ferlay J., Shin H.R., Bray F., Forman D., Mathers C and Parkin DM: Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*. 2010; 127: 2893–2917.
- [2] Zeeneldin A.A., Saber M.M., El-din I.A.S., and Sara A.F.: Colorectal carcinoma in gharbiah district, Egypt: Comparison between the elderly and non-elderly. *Journal of Solid Tumors*. 2012; 2(3): 13.
- [3] Jemal A., Bray F., Melissa M., Center MM., Ferlay J., Ward E et al.: Global cancer statistics. *Cancer Journal for Clinicians*. 2011; 61(2): 69–90.
- [4] Burkitt D.P.: Epidemiology of cancer of the colon and rectum. *Cancer*. 1971; 28: 3-13.
- [5] Bingham S.A., Day N.E., Luben R., Ferrari P., Slimani N., Norat T., et al.: Dietary fiber in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study. *Lancet*. 2003; 361: 1496-1501.

- [6] Lipkin M., Reddy B., Newmark H., and Lamprecht SA: Dietary factors in human colorectal cancer. *Annu Rev Nutr.* 1999; 19: 545-586.
- [7] Maslowski K.M. and Mackay C.R.: Diet, gut microbiota and immune responses. *Nat Immunol.* 2011; 12: 5-9.
- [8] Noriega L., Cuevas I., Margolles A., and Clara G: Deconjugation and bile salts hydrolase activity by Bifido-bacterium strains with acquired resistance to bile. *International Dairy Journal.* 2006; 6: 850.
- [9] Cummings J.H., Macfarlane G.T. and Englyst H.N.: Prebiotic digestion and fermentation. *Am J Clin Nutr.* 2001; 73: 415-420.
- [10] Raninen K., Lappi J., Mykkanen H., and Poutanen K: Dietary fiber type reflects physiological functionality: comparison of grain fiber, inulin, and polydextrose. *Nutr Rev.* 2011; 69: 9-21.
- [11] Papathanasopoulos A. and Camilleri M.: Dietary fiber supplements: effects in obesity and metabolic syndrome and relationship to gastrointestinal functions. *Gastroenterology.* 2010; 138: 65-72.
- [12] EFSA: Panel on Dietetic Products, Nutrition, Allergies. Scientific opinion on dietary reference values for carbohydrates and dietary fiber. *European Food Safety Authority.* 2010; 8(3): 1462.
- [13] Slavin J.L., Martini M.C., Jacobs D.R. and Marquart L: Plausible mechanisms for the protectiveness of whole grains. *Am J Clin Nutr.* 1999; 70: 459-463.
- [14] Aune D., Chan D.S., Lau R., Vieira R., Greenwood Darren C., Kampman E et al.: Dietary fiber, whole grains, and risk of colorectal cancer: systematic review and dose-response meta-analysis of prospective studies. *BMJ.* 2011; 343: 6617.
- [15] Sun Q., Spiegelman D., van Dam R.M., Holmes MD., Malik VS., Willett WC et al.: White rice, brown rice, and risk of type 2 diabetes in US men and women. *Arch Intern Med.* 2010; 170: 961-969.
- [16] Menzel C., Kamal-Eldin A., Marklund M., Andersson A., Aman P., Landeberg R et al.: Alkylresorcinols in Swedish cereal food products. *J Food Comp Anal.* 2012; 28(2): 119-125.
- [17] Ross A.A., Bourgeois A., Macharia H.N, Kochhar S., Jebb SA., Brownlee IA., et al.: Plasma alkylresorcinols as a biomarker of whole-grain food consumption in a large population: results from the WHOLE heart Intervention Study. *Am J Clin Nutr.* 2012 ; 95: 204-211.
- [18] Yang Y., Gu X., Zhou M., Xiang J., Chen Y. : Serum microRNAs: A new diagnostic method for colorectal cancer (Review). *Biomedical Reports.* 2013; 1(4): 495-498.
- [19] El-Attar I.: Colo-Rectal Cancer: Magnitude of the Problem. *Annual Cancer. Conference of the Egyptian Cancer Society, Danish Cancer Society & Aarhus University Hospital* 2005 (9- 11 February).
- [20] NCI: National Cancer Institute 2008: Administrative and Statistical Report for NCI, 2002 - 2008. <http://www.nci.edu>.
- [21] Lo A.C., Soliman A.S., Khaled H.M., Aboelyazid A., and Greenson JK: Lifestyle, occupational, and reproductive factors and risk of colorectal cancer. *Dis Colon Rectum.* 2010 ; 53: 830-837.
- [22] Lee WJ, Sandler DP, Blair A, Samanic C, Cross AJ, Alavanja MCR. Pesticide use and colorectal cancer risk in the Agricultural Health Study. *International journal of cancer Journal international du cancer.* 2007;121(2):339-346. doi:10.1002/ijc.22635.
- [23] Abou-Zeid A.A., Khafagy W., Marzouk D.M., Alaa A., Mostafa I., Ela MA: Colorectal cancer in Egypt. *Dis Colon Rectum.* 2002; 12: 55-60.
- [24] Terry P, Giovannucci E, Michels KB, Bergkvist L, Hansen H, Holmberg L, et al., "Fruit, vegetables, dietary fiber, and risk of colorectal cancer". *J Natl Cancer Inst.* 2001;93:525-33.
- [25] Mai V, Flood A, Peters U, Lacey JV, Jr, Schairer C, Schatzkin A. Dietary fiber and risk of colorectal cancer in the Breast Cancer Detection Demonstration Project (BCDDP) follow-up cohort. *Int J Epidemiol.* 2003;32:234-9.
- [26] Knudsen MD., Kyrø C., Olsen A., Dragsted LO., Skeie G., Lund E., et al. Self-reported whole-grain intake and plasma alkylresorcinol concentrations in combination in relation to the incidence of colorectal cancer. *Am J Epidemiol.* 2014; 79:1188-1196.

**LIST OF TABLES:**

**Table (I): Demographic features of the studied patients**

		CRC (n= 62)	Control (n= 86)	P value
Age (years) Mean± SD		51.8 ±12.6	43.1 ±14.5	<0.001
Sex N (%)	Male Female	32 (51.2%)/ 30(48.8%)	46(54%) 40(46%)	0.65

**Table (II): Characteristics of the lesions**

	N	Proximal (right sided)	Distal (transverse and left)
Total	62	14(22.5%)	48(77.5%)

**Table (III): Results of Alkylresorcinols in CRC Vs. normal**

	CRC (n=62)		Normal (n=86)		P-value
	Mean	St. Deviation	Mean	St. Deviation	
C17	3.90	0.52	4.17	0.50	0.01
C19	11.40	1.24	11.96	2.26	0.22
C21	15.24	3.83	17.54	6.28	0.02
Total Cs	30.55	3.92	33.67	6.61	<0.01

**Table (IV): Alkylresorcinols levels according to tumor location**

	Site	N	Mean	Std. Deviation	P value
C17	Proximal	16	3.63	0.42	0.20
	Distal	59	3.80	0.50	
C19	Proximal	16	11.13	1.15	0.89
	Distal	59	11.18	1.46	
C21	Proximal	16	16.69	4.36	0.78
	Distal	59	16.30	5.07	
total Cs	Proximal	16	31.44	4.49	0.91
	Distal	59	31.28	5.38	

**Citation of this Article:**

Shalaby, S.I., Mayssa E-Rzky, Mofeed Shehab, Dina Sabry, Ihab K.Abdel-Aziz, Mohamed AbdAllah, Omar El Shabacy, Devendra Kumar Awasthi, “Alkylresorcinols as an Indicator of Whole-Grain Intake and Its Relation to Colorectal Carcinoma” Published in *International Research Journal of Innovations in Engineering and Technology - IRJIET*, Volume 6, Issue 4, pp 39-43, April 2022. Article DOI <https://doi.org/10.47001/IRJIET/2022.604006>

\*\*\*\*\*