RISK FACTORS FOR K-RAS PROTO-ONCOGENE MUTATION IN COLORECTAL CANCER

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By

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CONTENTS

Abstract

Colorectal cancer (CRC) is now regarded as a prevalent cancer worldwide with many contributing risk factors.

In this study we tried to find out the relation between the K-Ras proto-oncogene mutation and those risk factors, especially the RBC folic acid level.

Eighty CRC patients were examined For RBC folate and mutations codon 12 of K-Ras gene (assayed by enriched RFLP/PCR) and it was found that RBC folic acid (assayed on the immulite 1000 by enzyme chemiluminescence) was significantly deficient in CRC patients with K-Ras (23 patients, mean RBC folate=100.96±51.3 ng/ml) mutation than those without the mutation (57 patients, mean RBC folate=216.6±166.4 ng/ml). Gender also was found to be another predicting risk factor, inspite of being masked by the accentuated folic acid deficiency in females.

It was concluded that folic acid supplementation is mandatory, and those at high-risk for CRC should be screened for the risk of K-Ras mutation using the calculation method depending on sex and RBC folate followed by close monitoring for those at high risk for the mutation.

Key words: CRC- K-Ras – Folic acid – RFLP/PCR.

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List of abbreviations

- **5-MTHF: 5-methyltetrahydrofolate**
- **AFP: Alpha-fetoprotein**
- **AJCC: American Joint Committee on Cancer**
- **APC: Adenomatous Polyposis Coli gene**
- **β-HCG: beta human chorionic gonadotrophin.**
- **BMI: Body mass index**
- **B-Raf: v-raf murine sarcoma viral oncogene homologue B1**
- **cAMP/PKA: Cyclic adenosine monophosphate/ protein kinase activator.**
- **CD: Crohn's disease.**
- **CEA: Carcinoembryonic antigen**
- **COX: Cyclooxygenase.**
- **CPB: Competitive protein binding**
- **CRC: Colorectal cancer**
- **CT: Computed tomography.**
- **DCC: Deleted in colorectal carcinoma;**
- **DFE: Dietary folate equivalents**
- **DNA: Deoxyribonucleic acid.**
- **DPC 4: Deleted in pancreatic cancer.**
- **EGFR: Epidermal growth factor receptor.**
- **ERK: Extracellular signal-regulated kinase**
- **FAP: Familial Adenomatous Polyposis**

- **FOBT: Fecal Occult Blood Testing**
- **FRET: Fluorescence resonance energy transfer.**
- **GAP: GTPase-activating protein.**
- **GDP: Guanosine diphosphate.**
- **GEFs: Guanosine nucleotide exchange factors.**
- **GIT: Gastrointestinal tract**
- **GPx: Glutathione peroxidase**
- **GTP: Guanosine triphosphate.**
- **GTPases: Guanosine triphosphatases.**
- **HNPCC: Hereditary Nonpolyposis Colorectal Cancer.**
- **HPLC: High performance liquid chromatography.**
- **H-Ras: Harvey-Ras**
- **HRT: Hormone Replacement Therapy.**
- **IBD: Inflammatory bowel diseases.**
- **IGF-I: Insulin-like growth factor-I.**
- **IL-6: Interleukin-6.**
- **IUAC: International Union Against Cancer.**
- **KDa: Kilo Dalton.**
- **K-Ras: Kirsten- Ras**
- **LDH: lactate dehydrogenase.**
- **LOH: Loss of heterozygosity.**
- **mCRC: Metastatic CRC.**

- **MEK : Mitogen-activated protein kinase**
- **MRI: Magnetic resonance Imaging**
- **mRNA: Messanger ribonucleic acid.**
- **MSI: Microsatellite instability.**
- **MMR: Mismatch repair genes**
- **MSI-H: Microsatellite instability - high**
- **MSI-L: Microsatellite instability - low**
- **MTHFR: Methylenetetrahydrofolate reductase**
- **NF1: Neurofibromatosis 1 gene.**
- **NHANES: National Health and Nutrition Examination Survey.**
- **N-Ras: Neroblastoma- Ras**
- **Nrf2: Nuclear factor E2- related factor-2**
- **NSAIDs: Nonsteroidal anti-inflammatory drugs.**
- **NTDs: Neural tube defects.**
- **PCR-RFLP: Polymerase chain reaction- restriction fragment length polymorphism.**
- **PI3K: Phosphatidylinositol 3-kinases.**
- **PPAR: Peroxisome proliferator–activating receptor gene**
- **Pte: Pteroic acid**
- **RalGEF: Ral small GTPase guanine nucleotide exchange factors**
- **Ras: Rat sarcoma.**
- **Ras-GRD: Ras GAP-related domain**
- **RDA: Recommended daily allowance**

- **REMS: Restriction endonuclease-mediated selection.**
- **RT-PCR: Reverse transcriptase polymerase chain reaction**
- **SPSS: Statistical Package for the Social Sciences**
- **THF: Tetrahydrofolate**
- **TNM: Tumor, node, metastasis**
- **TrxR: Thioredoxin reductase**
- **TSGs: Tumor suppressor genes.**
- **UC: Ulcerative colitis.**

INTRODUCTION

There is extensive evidence indicating that the development of colorectal cancer (CRC) is a multifactorial process including familial, environmental, lifestyle and molecular factors. Many variables have shown positive association with colorectal cancer, especially age, family history, folic acid deficiency, smoking and coffee intake ***(Martinez et al, 1999, Diergaarde et al, 2003 and Giovannucci et al, 2005)***.

Reduced dietary intake of folate has been shown to be associated with an increased risk of colon cancer. Furthermore, low dietary or erythrocyte folate levels have also been associated with an increased risk for colorectal adenomas. The precise mechanism by which folate deficiency might be associated with colorectal carcinogenesis is uncertain. Mutations in the Kirsten- Ras (K-Ras) gene may be an important component of this mechanism. Folate is an important coenzyme for DNA methylation and DNA synthesis. It is also possible that folate deficiency impairs DNA repair mechanisms in the colon. Lacking an effective repair mechanism, cells can develop genomic instability and rapidly accumulate somatic mutations or loss of short segments of alleles within oncogenes or tumor suppressor genes. Therefore, as the burden to repair DNA increases in the presence of a folate-deficient environment, this can in turn result in a

higher probability of K-Ras mutations ***(Martinez et al, 1999, Martinez et al, 2004 and Chan et al, 2005).***

Despite the frequency of K-Ras mutations in colorectal neoplasms, data on their etiology are sparse. Undoubtedly, understanding the role of environmental influences on the nature and rate of mutations in colorectal neoplasms, such as mutations in K-Ras, is crucial.

If mutational events play an important role in the colorectal carcinogenesis sequence, one can hypothesize that modification of these events by life-style or other factors would be a useful prevention strategy.

AIM OF THE WORK

This study aims at investigating the association between potential variables, known or suspected to be related to risk of colorectal cancer, and the occurrence of K-Ras mutations, thus, explaining how such variables play a role in CRC tumorigenesis; whether risky or protective role.

Fulfilling this aim would contribute better to our understanding of CRC pathogenesis, elaborate more on the use of K-Ras mutation in CRC diagnostic work-up and underscore protective factors that should be recommended and risk factors that might be avoided.

EPIDEMIOLOGY OF COLORECTAL CANCER

Incidence:

Colorectal cancer (CRC) is a major health problem in industrialized countries. It is the third most common cancer worldwide with only lung and breast cancer having a higher incidence **(*Farley et al, 2000)***. CRC accounts for 98% of malignant tumors of the bowel. Cancer registries have reported relatively large variations in incidence throughout the world. The incidence has been increasing over time & the incidence rates vary approximately 20-fold around the world, with the highest rates seen in the developed world and the lowest in India ***(Dancourt and Faivre, 2004)***.

In Egypt colorectal carcinoma is one of the most common malignant neoplasms. It represents about 6 % of cancers in Egypt (after bladder, breast carcinoma and lymphoma) ***(Zalata et al, 2000).***

Benson (2007) found that every 3.5 minutes, someone is diagnosed with colorectal cancer (CRC); every 9 minutes, someone dies from CRC; and every 5 seconds, someone who should be screened for CRC is not.

Race:

Western nations tend to have a higher incidence than Asian and African countries; however, within the United States little difference in incidence exists among whites, African Americans, and Asian Americans and may be due to differences in attitudes, beliefs and lifestyle factors. Another contributing among different populations that is reflected on the screening for early case finding of CRC among these populations.

The lowest incidence was reported among the Indian population, and this is mostly due to their vegetarian dietary pattern ***(Dancourt and Faivre, 2004, Rajapaksa et al, 2007 and Shokar et al, 2008)***.

Among religious groups, CRC occurs more frequently in the Jewish population. This is because Ashkenazi Jews have a higher incidence of a specific genetic mutation (called I1307K) that increases the risk for colorectal cancer ***(Kelly et al, 2007).***

Sex:

In high-incidence areas such as North America and Australia as well as in Japan and Italy, CRC rates in men exceed those in women by as much as 20%, moreover rectal cancer is up to twice as common in men as in women ***(Fuchs et al, 1999)***. Worldwide, colorectal cancer represents 10.1% of all incident cancer in men and 9.4% in women. For men,