Peripheral blood DNA methylation markers for the early detection of colorectal carcinoma in the Egyptian population: a multicenter study

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LIST OF CONTENT

LIST OF ABBREVIATIONS

AJCC	American Joint Committee on Cancer
APC	Adenomatous Polyposis Coli Syndromes
CD	Crohn's Disease
CDH1	E-cadherin
CDH13	Cadherin 13
CDKN2A/p14	Cyclin-dependent kinase inhibitor 2A
CDKN2A/p16	Cyclin-dependent kinase inhibitor 2A
CEA	Carcino-Embryonic Antigen
CHPRE	Congenital Hypertrophy of the Pigmented Retinal Epithelium
CI	Chromosomal Instability
CIMP	CpG Island Methylator Phenotype
CPG	Cytosine-Phosphate-Guanine
CRC	Colorectal Caner
CTC	CT-Colonography
DCBE	Double Contrast Barium Enema
EGFR	Epidermal Growth Factor Receptor
FAP	Familial Adenomatous Polyposis
FITs	Fecal Immunochemical Tests

FOBTs	Fecal Occult Blood Tests
FS	Flexible Sigmoidoscopy
KRAS	Kirstein Rat Sarcoma
LOH	Loss Of Heterozygosity
HLTF	Helicase-like transcription factor
HNPCC	Hereditary Non Polyposis Colorectal Cancer
IBD	Inflammatory Bowel Disease
MAPK	Mitogen-Activated Potein Kinase
MI	Microsatellite Instability
MGMT	O-6-methylguanine-DNAmethyltransferase
MMR	Mismatch Repair genes
NCI	National Cancer Institute
NHL	Non Hodjken lymphoma
PI3KCA	Phosphatidylinositol 3-kinase catalytic subunit
PJS	Peutz-Jeghers Syndrome
RASSF1A	Ras association domain family 1 (isoform A)
RUNX3	Runt-related transcription factor 3
TGF-β	Transforming Growth factor-β
TSGMP	Tumor Suppressor Gene Methylator Phenotype

UC	Ulcerative Colitis
VEGF	Vascular Endothelial Growth Factor

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

BACKGROUND: DNA methylation is one of the most important epigenetic mechanisms, which denotes the addition of a methyl group to DNA. Aberrant promoter demethylation is usually associated with overexpression of genes that might participate in pathogenesis of many diseases

AIM OF THE WORK: to evaluate serum *ALX4, SEPT9, CDH4* and *CDKN2B/P15* and their candidacy as novel noninvasive markers, in CRC among Egyptian patients.

METHODS: A whole blood sample (10cc) will be collected in an EDTA-containing vacutainer tube and stored at 4C for a maximum of 24 hours. Samples will be delivered within this period to the molecular biochemistry lab for removal of the Buffy coat, DNA extraction and analysis. The molecular biologist will assess all samples in a blinded fashion.

RESULTS: ALX4 shows the highest sensitivity (97.6%) and specificity (99.3%) with cut-off of 0.315 µg/L Followed by CDKN2B/P15 (sensitivity=96.1% and specificity=97.8%) with cut-off of 0.412 µg/L and SEPT9 (sensitivity=87.4% and specificity=98.5%) with cut-off of 0.461 µg/L while CDH4 shows the least sensitivity=75.6% and specificity=70.1% with cut-off of 0.330 µg/L.

CONCLUSON: ALX4, SEPT9, CDH4 and CDKN2B/P15 can be useful in high prediction of colorectal carcinoma with high sensitivity and specificity.

Keywords:

Peripheral blood DNA methylation markers for the early detection of colorectal carcinoma in the Egyptian

INTRODUCTION

Colorectal cancer (CRC), including anal, is the 3rd most common cancer in the world. It is the 4th most common cause of cancer death worldwide (***Ferlay, et al., 2010)***.

Globally, the incidence of CRC varies over 10-fold. 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 geographic differences appear to be attributable to differences in dietary and environmental exposures that are imposed upon a background of genetically determined susceptibility ***(Jemal, et al 2011).***

In Egypt, CRC represents about 4% of total cancers in both sexes. Variation in environmental risk factors particularly the higher content of dietary fibers more physical activity and lower obesity rates can explain different incidence rates between Egypt and the western countries with higher incidence in the western countries ***(Zeeneldin, et al 2012).***

Early diagnosis of CRC potentially reduces the mortality of this disease ***(Walsh & Terdiman, 2003***). Although colonoscopic screening for CRC is currently the most reliable diagnostic tool, its cost and invasive nature limit its use. Thus, there is a pressing need for novel, non-invasive, highly sensitive biomarkers to improve the detection of CRC (***Yang et al., 2013)*** but this is hindered by their cost.

In the last few years, there has been increasing interest in using blood samples to measure DNA methylation in cancer cases and controls ***(Cho et al., 2010)***.

DNA methylation is one of the most important epigenetic mechanisms, which denotes the addition of a methyl group to DNA. It is widespread in the human genome and mainly occurs at cytosine adjacent to guanine (CpG dinucleotides) ***(Cokus et al., 2008)***.

DNA methylation in gene promoter regions often results in long-term silencing of gene expression ***(Jones, 2012).*** Meanwhile, aberrant promoter demethylation is usually associated with overexpression of genes that might participate in pathogenesis of many diseases ***(Qian et al., 2015)***. Previous studies demonstrated that aberrant demethylation in the promoter region of genes occurs in many diseases and may be used as a biomarker ***(Zhang et al., 2015)***.

No solitary biomarker is considered adequately sensitive and specific for CRC screening due to the substantial heterogeneity of colon cancer. A combination of markers spanning multisignaling pathways leading to colon cancer is needed for optimal sensitivity. The "multiple markers," which detect known genetic and epigenetic alterations of colon cancer, will be more effective and beneficial than a single one for early detection and prediction of patient outcome. Identification of "universal methylation markers," if they are highly specific and sensitive for common tumors and in many cases, would be useful for the detection of human cancer ***(Esteller et al., 2001).***

AIM OF THE WORK

The aim of the present study is to evaluate methylation level of serum *ALX4*, *SEPT9*, *CDH4* and *CDKN2B/P15* genes as novel non-invasive markers in CRC among Egyptian patients.