Normal and Modified Urinary Nucleosides as Novel Biomarkers for Colorectal Cancer

Thesis

Submitted for Partial Fulfillment of M.D. Degree
in Clinical and Chemical Pathology

By

Hoda Ahmed Abd El-Sattar
M.B., B.Ch. & M. Sc. Clinical and Chemical Pathology
Ain Shams University

Under Supervision of

Professor/ Ola Hamdy El-Demerdash
Professor of Clinical and Chemical Pathology
Faculty of Medicine – Ain Shams University

Professor/ Ahmed Abdel Aziz Abou-Zeid
Professor of General Surgery
Faculty of Medicine – Ain Shams University

Professor/ Ghada Mohamed Sadek Sabbour
Professor of Clinical and Chemical Pathology
Faculty of Medicine – Ain Shams University

Doctor/ Amira Ibrahim Hamed
Lecturer of Clinical and Chemical Pathology
Faculty of Medicine – Ain Shams University

Doctor/ Hala Abdel Al Ahmed
Lecturer of Clinical and Chemical Pathology
Faculty of Medicine – Ain Shams University

Faculty of Medicine
Ain Shams University
2011

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide, with an annual incidence of approximately 1 million cases and an annual mortality of more than 500,000 **(Parkin et al., 2005)**. It contributes for 8.9% of all cancers in the world, and 6.5% of all cancers in Egypt **(El-Bolkainy et al., 2006)**.

Unfortunately, it was found that 20% of colorectal cancer patients have stage IV disease with distant metastasis at the time of first presentation **(Kaiser et al., 2007)**. Even after the introduction of effective chemotherapeutic agents for CRC treatment, the 5-year survival difference between early and late stages is still considerable, being higher than 60% and up to 95% for Dukes' stages A–B and less than 50% for those with more advanced stages **(Hassan et al., 2008)**. Therefore, screening of the population is mandatory to shift the detection of the disease to an earlier stage, where intervention can reduce the risk of death. According to the Advisory Committee on Cancer Prevention in the European Union, screening should be offered to men and women over the age of 50 years, and should be repeated every 2 years **(Kim et al., 2008)**.

The most widely used screening procedures are fecal occult blood test and flexible sigmoidoscopy **(Winawer, 2007)**. However, both are limited by poor compliance and are less sensitive than colonoscopy **(Duffy et al., 2007)**. Although an

invasive procedure, colonoscopy is considered till now the gold standard for cancer colon diagnosis **(Parente et al., 2009)**.

Tumour markers as carcinoembryonic antigen (CEA), carbohydrate antigen CA 19-9 and α-fetoprotein (AFP) have long been applied in gastrointestinal cancer for: screening, diagnosis, determining prognosis, surveillance for recurrence, and assessment of response to therapy **(Ishida et al., 2004)**. However, these markers are neither sensitive nor specific for colorectal cancer diagnosis. The sensitivity of CEA in early stages is only 30-40% while the specificity is 87%. There is still no ideal tumour marker for the early diagnosis and for the effective monitoring of the disease after surgical resection; therefore, there is a great need for new effective biomarkers for colorectal cancer **(Kim et al., 2008)**.

Nucleosides are the basic building blocks of nucleic acids: ribonucleic acid (RNA) and deoxyriboneculeic acid (DNA). They are formed by the loss of water from a sugar plus a purine or pyrimidine, OH from the anomeric position of the sugar, and H from nitrogen of the base. RNA contains four normal nucleosides: adenosine (A), guanosine (G), cytidine (C), and uridine (U). Moreover, a number of modified nucleosides such as 8-bromoguanosine, pseudouridine, 1-methyladenosine, 5-methyluridine, 1-methylinosine, 1-methylguanosine, N^4-acetylcytidine, N^2-methylguanosine and N^6-methyladenosine, are formed post-transcriptionally within the polynucleotide molecule by numerous modification enzymes, in particular methyltransferases and ligases. The major task of these

modifications is thought to be the general improvement of biological activity, integrity and efficiency of RNA in various biochemical processes **(Liebech et al., 2005)**.

Because there are no specific enzyme systems to incorporate the modified nucleosides into macromolecular nucleic acids, these nucleosides, once released in the process of transfer RNA (tRNA) turnover, cannot be reused, but are either metabolized or excreted intact in urine. Consequently, urine contains levels of modified nucleosides, which reflect RNA degradation in the individual **(Feng et al., 2005).**

In all tissues with increased t-RNA turnover, the formation of nucleosides can be expected to increase, especially in cancer patients because of the up-regulated metabolism and cell growth in tumour tissue (**Li et al., 2008)**. Recently, nucleosides have been shown to be elevated in urine of cancer patients and have been proposed to be potential biomarkers for various malignant diseases including renal cancer **(Liebech et al., 1997),** uterine myoma and cervical cancer **(Kim et al., 2001),** thyroid cancer **(La et al., 2003),** head and neck cancer **(Dudley et al., 2003),** breast cancer **(Zheng et al., 2005a),** bladder cancer **(Wang et al., 2007)**, leukaemia and lymphoma **(Hsu et al., 2009).**

Since the presently applied biomedical markers are not recommended for early diagnosis and therapy surveillance of colorectal cancer, the intensified search for more reliable diagnostic markers focuses on the metabolite class of

nucleosides. These observations have formed the basis for investigation of nucleosides as potential tumour markers in colorectal cancer screening and diagnosis **(Li et al., 2008).** The combination of a quantitative determination of selected ribonucleosides with subsequent bioinformatic pattern recognition represents a promising approach for utilizing the phenomenon of an altered nucleoside excretion in the clinical practice for colorectal cancer diagnosis **(Hsu et al., 2009)**.

AIM OF THE WORK

The aim of the present study is to evaluate the clinical utility of normal and modified urinary nucleosides as diagnostic biomarkers to be used for the purpose of screening and surgery monitoring of colorectal cancer, in addition to assessment of the correlation between their preoperative levels, tumour size and Dukes' staging.

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