

Interleukin-10 -1082 A/G Promoter Polymorphism in Newly Diagnosed Gastro-Intestinal Malignancies and Its Relation to the Nutritional and Performance Status

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Abstract

Background: Cancer is associated with a state of systemic inflammation with high levels of circulating inflammatory cytokines. Cytokine gene polymorphisms may affect the inflammatory response in cancer patients and their nutritional and performance status with subsequent effects on their prognosis.

Aim: This study investigated the relationship between IL-10 -1082 A/G promoter polymorphism and anorexia, body mass index (BMI) and performance status in patients with gastrointestinal (GI) malignancies.

Methods 88 newly diagnosed patients with GI malignancies were genotyped for IL-10-1082 A/G promoter polymorphism by real-time PCR allelic discrimination assay. The prevalence of anorexia, BMI and performance status was compared between patients with different genotypes.

Results: In the whole group of patients, anorexia was significantly more prevalent ($p < 0.001$) among patients carrying the variant genotypes (AG and GG) who were also found to have significantly lower BMI ($p = 0.002$) and poorer performance status ($p = 0.027$) than those with the wild genotype (AA). In patients with earlier cancer (stages I and II), a variant genotype was significantly associated with more anorexia and lower BMI.

Conclusion: These results suggest that IL-10-1082 A/G promoter polymorphism may have an effect on the nutritional and performance status of patients with GI malignancies.

Keywords: Anorexia, Body mass index, Cancer, Cytokines, IL-10 polymorphism, Performance status

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Introduction

The inflammatory and nutritional status of patients with gastrointestinal malignancies and their performance status have a significant impact on their short- as well as long-term outcomes ¹⁻⁶.

Nutritional and immune-inflammatory status of patients with gastric cancer are important in recognizing high-risk patients and predicting overall and disease-free survival ¹. Patients with poor nutritional status have a higher risk of cancer and non-cancer related mortality ². Markers of nutritional status like body mass index (BMI),

prognostic nutritional index and preoperative weight loss are important prognostic indicators in patients with gastrointestinal (GI) malignancies ^{3,4,5}. Gastric cancer patients with low BMI (<25) were found to have severe postoperative complications, poor survival and poor prognosis ⁶. Underweight patients with colon cancer have a worse prognosis compared to those with normal BMI at diagnosis ⁷. Involuntary weight loss in patients with GI malignancies is attributed to several factors like systemic inflammation and poor dietary intake ⁸. Exaggerated weight loss due to systemic

inflammatory response to cancer is associated with deterioration of patient's performance status ⁹.

Studies indicated that there is a relation between several pro-inflammatory cytokines like tumour necrosis factor- α , interferon- γ , interleukin (IL)-1, IL-6, and IL-10 with weight loss and anorexia in patients with GI cancer ^{9,10}. Interleukin-10 has a dual effect on the immune response against cancer ¹¹. Its level was found to be significantly high in sera of patients with cancer cachexia ^{12,13}. Since the rate of release of inflammatory cytokines is genetically determined, polymorphisms in cytokine genes may explain the variations among cancer patients in the rate and severity of weight loss and may help in predicting patients susceptible to an exaggerated inflammatory response with subsequent weight loss in response to cancer ¹⁴.

The aim of this work was to study the relationship between IL-10-1082 promoter polymorphism and anorexia, BMI and performance status in newly diagnosed patients with GI malignancies.

Methods

Study population

This was an observational cross-sectional study that included newly diagnosed patients with GI cancer who visited Kasr Al-Ainy Centre of Clinical Oncology and Nuclear Medicine (NEMROC) during the year 2014.

In order to determine the presence of anorexia, we used the appetite item of the European Organization for Research and Treatment of Cancer Core Quality of Life Questionnaire (EORTC QLQ) - C30 version 3.0 ¹⁵. Patients were asked "Have you lacked appetite?" and the response was rated on a 1 to 4-point Likert scale (1= not at all and 4 = very much).

Patients' weight and height were measured and BMI was calculated (BMI=weight in kilogram/height² in meter). Underweight was defined as a BMI<20, a cutoff value used in the definition of cancer cachexia ¹⁶. Patient's performance status was assessed using the Karnofsky performance status (KPS) score ¹⁷.

Genotyping

Detection of IL-10-1082 A/G gene promoter polymorphism (rs1800896) was done by real-time PCR allelic discrimination assay using Taq-Man® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA) as described before ¹⁸. DNA extraction was done using Thermo Scientific GeneJET Whole

Blood Genomic DNA Purification Mini Kit. The sequence-specific forward and reverse primers used to amplify the promoter region of IL-10 gene are the following:

Forward primers:

5'-CCAAGACAACACTACTAAGGCTTCT-3'

Reverse primers:

5'-GCTGGATAGGAGGTCCCTTACTTT-3'

Two TaqMan® minor groove binder (MGB) fluorogenic probes with different fluorescence dyes were used to distinguish the two alleles. One probe labelled with VIC dye to detect the wild allele (A) CCTACTTCCCCTCCCAA-VIC and another probe labelled with FAM dye to detect the variant allele (G) CCTACTTCCCCTCCCAA-FAM.

The PCR conditions consisted of an initial step of 95°C for 10 min for AmpliTaq Gold activation, followed by 50 cycles of 92°C for 15 seconds and 60°C for 1 min.

Analysis was performed using Sequence Detection System (SDS), version 2.0, software. The plotted fluorescence signals indicate the types of alleles in each sample. Detection of VIC dye fluorescence only indicates a wild genotype, of FAM dye fluorescence only indicates a homozygous variant genotype and of both indicates a heterozygous genotype.

Statistical analysis

Numerical data were tested for normality using the Shapiro-Wilk test. The significance of differences in abnormally-distributed numerical data between two independent samples was tested using the Mann-Whitney test.

Qualitative data were expressed as frequency and percentage and were compared using Chi square (χ^2) test. Fisher's exact test was used instead when the expected frequency is less than 5. P values less than 0.05 was considered statistically significant.

Data were analysed using Statistical Package for Social Science (SPSS) (SPSS Inc., Chicago, IL, USA) version 15.

Ethical considerations

The study was approved by the Ethical Committee of Kasr Al-Ainy School of Medicine, Cairo University and patients provided informed consent.

Results

The study included 88 patients with newly diagnosed gastrointestinal malignancies. Their

characteristics are shown in Table 1. The most common primary cancer was colorectal. Sixty-two (70.5%) patients had anorexia (a loss of appetite score of 2 to 4). Genotyping of IL-10 -1082 polymorphism revealed that 30 (34%) patients had the wild (AA) genotype and 58 (66%) had the heterozygous (AG) / homozygous (GG) variant genotype. The prevalence of wild-allele (A) was 83% and that of variant allele (G) 66%.

Table 1: Patients' characteristics (n=88)

Variable	Description	
Age (years)	Median (IQR)	47.5 (39-59.5)
Gender		
Female	n (%)	49 (55.7)
Male	n (%)	39 (44.3)
Primary cancer		
Colorectal	n (%)	68 (77.3)
Stomach	n (%)	11 (12.5)
Pancreas	n (%)	7 (8)
Esophagus	n (%)	2 (2.3)
Cancer stage		
I	n (%)	10 (11.4)
II	n (%)	23 (26.1)
III	n (%)	35 (39.8)
IV	n (%)	20 (22.7)
EORTC QLQ-C30 appetite item score		
1	n (%)	26 (29.5)
2-4	n (%)	62 (70.5)
BMI (kg/m²)	Median (IQR)	22.5 (19.6-25)
KPS score	Median (IQR)	70 (70-80)
IL10 -1082 genotype		
Wild (AA)	n (%)	30 (34.1)
Heterozygous variant (AG)	n (%)	43 (48.9)
Homozygous variant (GG)	n (%)	15 (17)

IQR: Interquartile range, **EORTC QLQ:** European Organization for Research and Treatment of Cancer Core Quality of Life Questionnaire, **BMI:** Body mass index, **KPS:** Karnofsky performance status

Anorexia (EORTC QLQ-C30 appetite score 2 to 4) was significantly more prevalent among patients with the variant genotypes who had a significantly lower performance status score as well (Table 2). Patients with the variant genotype had a significantly lower BMI and were more likely to be underweight.

Twenty-three (26.1%) of patients had both anorexia of any grade combined with underweight, 39 (44.3%) had anorexia without underweight and 26 (29.5%) had no anorexia nor underweight. The majority (20/23, 87%) of patients presenting with anorexia combined with underweight carried a variant genotype. On the other hand, a minority (10/16, 38.5%) of patients without anorexia or underweight carried it. The difference in prevalence between the two groups was highly significant ($p < 0.001$).

A subgroup analysis according to the stage of the disease was performed (Table 3). In patients with earlier stages (I and II) cancer, the prevalence of a variant genotype was significantly higher among patients with anorexia. Also, in earlier cancer patients, the BMI was significantly lower in those with a variant genotype.

Discussion

This study investigated the possible association between IL-10 -1082 polymorphism and anorexia, BMI, and performance status in newly diagnosed patients with GI malignancies.

The prevalence of weight loss among patients with upper GI cancer at the time of diagnosis was found to be 80%¹⁹. Weight loss in patients with GI cancers is attributed not only to dysphagia, nausea and vomiting associated with such tumours but also other factors are involved like the co-existing state of systemic inflammation⁸. The level of acute phase proteins correlates with the rate of weight loss in patients with GI cancer⁸. Cytokine gene polymorphisms can influence weight loss through changing levels of cytokine production. In the present study, anorexia, low BMI and poor performance status were more prevalent among patients with GI malignancies who carry the variant genotypes (AG and GG) of IL-10 -1082. In concordance with our study, IL-10 -1082G allele has been found to be significantly associated with weight loss in three previous studies that included patients with gastroesophageal and gastric cancers^{12, 20, 21}. Anorexia, underweight and poorer performance status may be explained by an exaggerated systemic inflammatory response associated with the presence of the variant genotypes of IL-10 -1082 polymorphism. IL-10 polymorphism at position -1082 is present within the promoter region of the gene and the variant-genotypes have been shown to be associated with

Table 2: Comparing variables according to IL-10 polymorphism

Variable		Wild genotype, AA (n=30)	Variant genotypes, AG/GG (n=58)	p value
Age (years)	Median (IQR)	49.5 (39-58)	47 (39-60)	0.712
Cancer stage				
I-II	n (%)	13 (39.4)	20 (60.6)	0.416
III-IV	n (%)	17 (30.9)	38 (69.1)	
EORTC-QLQ - C30 appetite item score				
1	n (%)	16 (61.5)	10 (38.5)	< 0.001
2 - 4	n (%)	14 (22.6)	48 (77.4)	
BMI (kg/m ²)	Median (IQR)	24 (21.7-27)	21 (19.5-24)	0.002
Underweight (BMI <20)				
No	n (%)	27 (90)	38 (65.5)	0.02
Yes	n (%)	3 (10)	20 (34.5)	
KPS score	Median (IQR)	80 (70-80)	70 (70-80)	0.027

IQR: Interquartile range, **EORTC QLQ:** European Organization for Research and Treatment of Cancer Core Quality of Life Questionnaire, **BMI:** Body mass index, **KPS:** Karnofsky performance status

Table 3: Subgroup analysis according to cancer stage comparing patients with wild genotype to those with a variant genotype

Variable		Wild genotype, AA	Variant genotypes, AG/GG	p value
Cancer stage I-II group (n = 33)				
EORTC QLQ - C30 appetite item score				
1	n (%)	11 (64.7)	6 (35.3)	0.004
2 - 4	n (%)	2 (12.5)	14 (87.5)	
BMI (kg/m ²)	Median (IQR)	25.9 (23.3-27.4)	22.7 (19.8-25)	0.039
Underweight (BMI <20)				
No	n (%)	12 (44.4)	15 (55.6)	0.364
Yes	n (%)	1 (16.7)	5 (83.3)	
KPS score	Median (IQR)	80 (80-80)	80 (70-80)	0.094
Cancer stage III-IV group (n=55)				
EORTC QLQ - C30 appetite item score				
1	n (%)	5 (55.6)	4 (44.4)	0.116
2-4	n (%)	12 (26.1)	34 (73.9)	
BMI (kg/m ²)	Median (IQR)	24 (20-26.7)	20.65 (18.7-23)	0.029
Underweight (BMI <20)				
No	n (%)	15 (39.5)	23 (60.5)	0.058
Yes	n (%)	2 (11.8)	15 (88.2)	
KPS score	Median (IQR)	70 (70-80)	70 (70-80)	0.182

IQR: Interquartile range, **EORTC QLQ:** European Organization for Research and Treatment of Cancer Core Quality of Life Questionnaire, **BMI:** Body mass index, **KPS:** Karnofsky performance status

increased IL-10 production that was attributed to a higher affinity of the G allele for Sp1 transcription factor with subsequent higher expression and IL-10 secretion²². Moreover, IL-10 -1082GG genotype was

found to be associated with elevated markers of systemic inflammation like CRP²³.

A limitation to this study is that the relationship between IL-10 -1082 A/G promoter polymorphism and the degree of weight loss was not assessed. This

is because of the absence of accurate patient weight measurements before presenting with cancer. Other limitations included the conduction of the study in a single center, the inclusion of a heterogeneous group of malignancies without equal representation and the relatively small sample size.

Conclusion

IL-10-1082 variant genotypes (AG and GG) may have an effect on the nutritional and performance status of patients with GI cancer.

Recognizing the cause and effect of systemic inflammation on cancer patients' nutritional and performance status can help to develop proper therapeutic strategies to prevent unnecessary morbidities and to improve patients' response to therapy.

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None.

Authors' contribution

Conception or design: All authors; Acquisition, analysis or interpretation of data: SMM & RAM; Drafting the manuscript: SMM & RAM; Revising the manuscript: WAE; Approval of the manuscript version to be published: SMM, RAM & WAE; Agreement to be accountable for all aspects of the work: SMM, RAM & WAE.

Conflict of interest

The authors declare that they have no conflict of interest to disclose.

Data availability

Deidentified individual participant data used to produce the results of this study are available from the corresponding author (SMM) upon request.

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Study registration

None.

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