Original Research Article

The Egyptian Journal of Immunology

The Egyptian Journal of Immunology,
E-ISSN (2090-2506)
Volume 32 (1), January, 2025
Pages: 92–104.
www.Ejimmunology.org
https://doi.org/10.55133/eji.320109

The Clinical utility of Interleukin-17A rs2275913 polymorphism with colorectal cancer in Egyptian patients

Heba H. A. Osman, Manal M. Abd Al Aziz, Aya M. M. Yehia, and Rawan M. M. Metwaly

Department of Clinical Pathology, Faculty of Medicine Ain Shams University, Cairo, Egypt.

Corresponding author: Rawan M. M. Metwaly, Clinical Pathology, Faculty of Medicine Ain Shams University, Cairo, Egypt.
Email: Drrawandahman@gmail.com.

Abstract

The worldwide incidence of colorectal cancer (CRC) is roughly two million new instances each year throughout the world, according to the World Health Organization 2022. CRC is the third most prevalent disease and the second most common cancer in terms of fatality. People diagnosed with colorectal cancer in the early stages have a five-year survival rate of roughly 95%, but people identified with the disease in the later stages have a survival rate of approximately 12%. There are a number of variables that contribute to the development of CRC, these factors include both hereditary and environmental influences. We aimed to investigate the clinical utility of Interleukin (IL)-17A rs2275913 polymorphism to assess its association with CRC in Egyptian patients and the ability of using it as a non-invasive biomarker to assist in diagnosis of colorectal cancer. This case–control study included 75 subjects. Of these, 35 were CRC cases, 20 inflammatory bowel disease (IBD) patients and 20 normal control persons. Blood was collected and DNA extracted. The rs2275913 of IL-17A was genotyped using the Real-Time polymerase chain reaction (PCR). In CRC patient's group, (5.71%) had the homozygous AA genotype, (40%) the heterozygous GA genotype and (54.29%) the wild GG genotype. While in IBD group (15%) had the homozygous AA genotype, (40%) the heterozygous GA genotype and (45%) the wild GG genotype. In the normal control group, no one had the homozygous AA genotype, but (45%) had the heterozygous GA genotype and (55%) the wild type of GG genotype. However, there was no statistically significant substantial variation amongst the three groups ($p>0.05$). In conclusion, our study demonstrated no association of IL-17A rs2275913 with both CRC and IBD in the Egyptian population.

Keywords: Colorectal Cancer; Interleukin-17A; Polymorphism

Date received: 23 April 2024; **accepted:** 02 January 2025

Introduction

In 2018, the World Health Organization (WHO) reported 1.8 million new instances of colorectal cancer (CRC) and over 861,000 deaths. This makes CRC) the third most prevalent reason for death and a substantial public health burden globally. More than 7% of all CRC instances were detected in the Middle East and North Africa.[1] Cancers of the gastrointestinal tract, including colorectal and gastric cancers, are examples of multifactorial illnesses, which are

brought on by several hereditary and environmental variables interacting in a complicated manner.[2]

In addition to a poor intake of fruits and vegetables, dietary habits, smoking, and alcohol usage are also substantially linked to CRC.[3] In order to establish a more tailored therapeutic strategy for targeted molecular therapy of gastric cancer and CRC, there is a need to understand the pathogenic processes of tumors and to uncover novel molecular biomarkers for early prediction of cancer risk, it is vital to understand the mechanisms that cause tumors.[4]

Both carcinoembryonic antigen and the carbohydrate antigen 19-9 (CA 19-9) are often utilized in clinical settings to help in the diagnosis of colorectal cancer. Nevertheless, the sensitivity of these two tumor markers is not very high.[5] In spite of the fact that digital rectal examination, colonoscopy, and three-dimensional reconstruction of colon images have a high level of sensitivity, the majority of asymptomatic individuals have a poor acceptance for these methods. As a result, there is an immediate need to design a screening method for CRC that is based on noninvasive biomarkers, has the potential to be broadly accepted and used in asymptomatic populations.[6]

Interleukin-17 (IL17), which is also known as CTLA-8, belongs to a family of cytokines that promote inflammation. It has been classified as the member of the IL-17 family that serves as the prototype. The cytokine known as IL-17A is represented by the IL-17A gene, which may be found on chromosome 6p12.[7] IL-17A has been demonstrated to play a substantial part in the development of a number of human illnesses, including colorectal and stomach cancer.[8]

A single nucleotide polymorphism in the promoter region of the IL17A gene (rs2275913) was reported to have the potential to influence the risk of developing colorectal cancer in a number of studies conducted in a variety of nations.[9] Consequently, this study aimed to determine whether the IL-17A rs2275913 polymorphism may be used as a non-invasive biomarker in the diagnosis of colorectal cancer. The study evaluated the clinical utility of this polymorphism in Egyptian patients.

Subjects and Methods

This research included 35 cases with colorectal cancer and 20 cases with IBD recruited from the Colorectal surgery and the Department Tropical Medicine of Ain Shams University hospitals and 20 normal control individuals. All laboratory work was conducted in the Department of Clinical Pathology, Ain Shams University hospitals, according to their standard methods during the period between May 2022 to October 2022.

Subjects with any of the following conditions were excluded from our study, these included patients who had undergone preoperative irradiation or chemotherapy, subjects with family history of cancer or digestive illnesses and vulnerable groups less than 18 years old.

In the course of our research, every participant was investigated for their medical history and comprehensive clinical examination, laboratory data including Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), Complete blood picture (CBC) and tumor markers [carcinoembryonic antigen and carbohydrate antigen (CA) 19.9] were obtained from hospital medical reports. IL-17A rs2275913 single nucleotide polymorphism genotyping was performed by the real time polymerase chain reaction.

Radiological examination for CRC patients was performed by compound tomography for localization of tumors, lymph node metastasis, tumor size, differentiation and histopathological characteristics. Such data were obtained from the hospital medical records. IBD patients were classified according to type of the disease into either Crohn's disease or Ulcerative colitis.

Assay of IL-17A gene polymorphism (rs2275913) by the Real Time Chain Reaction (PCR)

Genomic DNA was extracted and purified from whole blood using commercial kits (QIAampDNA blood mini kit (QIAGEN, Strasse 1, 40724 Hilden, Germany) according to the

manufacturer's instructions. Detection of the IL-17A polymorphism (rs2275913) was performed by the real time polymerase chain reaction using commercial kits (Thermo Scientific, 168 Third Avenue, Waltham, MA, USA), according to the manufacturer's instructions.

The thermal cycle process included heating at 95°C for 10 minutes, followed by 40 cycles of denaturation of DNA template by heating to 95°C for 15 seconds followed by annealing/extension at 60°C for 60 seconds. The polymerase chain reaction tubes with a total volume of 20 µl contained 2 µl of DNA extract, 10 µl of the TaqMan Universal PCR Master Mix, 7 µl of DNase free water and one µl of the 20x working stock of single nucleotide polymorphism (SNP) Genotyping Assay. The sequence for rs2275913 was [HEX/FAM]: TTCAGAAGGAGAGATT [A/G] CTTCAGAAGAAGA GATT. The genotyping of the two potential variant alleles at the single nucleotide polymorphism site in a DNA target sequence is made feasible by the introduction of two probe pairs into every reaction. On the basis of the alteration in fluorescence of the dyes linked with the probes, the genotyping test is able to detect whether or not a SNP is present.

The analysis revealed that a significant escalation in FAM dye fluorescence exclusively suggested homozygosity for allele 1 (G) (Wild allele); an equivalent increase in HEX dye fluorescence exclusively suggested heterozygosity for allele 2 (A) (Mutant allele); and a substantial increase in both FAM and HEX dye fluorescence indicating heterozygosity for both allele 1 and allele 2 (Muter allele).

Statistical Methods

The analysis of the data was performed using the Statistical Package for Social Science (SPSS, IBM®) version 2. The mean ± standard deviation (±SD) was applied to express quantitative parametric data; the median and inter-quartile range were applied for quantitative nonparametric data and Categorical data are expressed as number (n) and percentage (%). For the purpose of analyzing the correlation amongst two variables (Pearson chi-square) otherwise comparing two independent groups with respect to categorical data, the Chi square test (X^2) was used. Mann-Whitney U-test (Wilcoxon Rank-Sum test) (z) was applied for statistical comparison between two independent sets of data if one or both of them have a skewed distribution, Statistical significance of the variance amongst two research group averages for parametric data was assessed using the Student t-test, abbreviated as t, One way ANOVA (f) test was utilized to compare between more than two independent groups regarding quantitative data in case of parametric distribution as it can be used to compare means of two or more samples (using the F distribution),the Kruskal–Wallis test (H) by ranks, The Kruskal–Wallis H test is an example of a procedure that is not parametric. It is used for comparing two or more independent samples of equal or different sample sizes, In order to compare all of the potential combinations of group means, the post hoc test is used, The Fisher's extract test is utilized to observe the connection among two qualitative variables when the expected count is less than 5 in more than 20% of cells, it tends to be employed instead of chi square test when sample sizes are small.

Results

Our study population included 35 CRC patients, 20 patients with IBD serving as a pathological control group and 20 age and sex matched normal control subjects. Comparative statistics of the demographic and laboratory data between the three studied groups are illustrated in Table (1). The age was higher in the CRC patients compared to the IBD and the control group (p<0.001). In contrast, there was no substantial difference in the gender and smoking among the three groups (p>0.05).

Table 1. Demographic and laboratory data of the study groups.

		Group			*p-value*
		Controls	IBD	Colorectal cancer	
		N (%) Mean ± SD	N (%) Mean ± SD	N (%) Mean ± SD	
Gender	Male	12 (60%)	9 (45%)	15 (42.86%)	NS^{X2}
	Female	8 (40%)	11 (55%)	20 (57.14%)	
Age in years		40.1 ± 14.09	36.45 ± 12.77	52.69 ± 12.68	$<0.001^{(A1)f}$
Smoking	Yes	8 (40%)	9 (45%)	10 (28.57%)	NS^{X2}
	No	12 (60%)	11 (55%)	25 (71.43%)	
Hemoglobin (g/dL)		13.55 ± 0.99	10.91 ± 2.25	10.66 ± 1.54	$<0.001^{(A1)f}$
ESR (mm/hr.)		15 (7.5 - 15)	30 (20 - 55)	40 (28 - 70)	$<0.001^{(K1)H}$
CRP (mg/dL)		2 (1 - 2)	8.5 (3.85 - 35.2)	56 (10.3 - 133)	$<0.001^{(K2)H}$
CEA (ng/mL)		2.25 (1.05 - 2.7)	1.75 (1.25 - 2.35)	4.8 (2.1 - 20.8)	$<0.001^{(K3)H}$
CA 19.9 (U/mL)		9.85 (3.65 - 13.85)	8.2 (4.95 - 16.6)	57.3 (16.9 - 345.8)	$<0.001^{(K3)H}$

ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; CEA: Carcinoembryonic antigen; Carbohydrate antigen (CA) 19.9. (X^2): Chi-Square test of significance; (f): One Way ANOVA test of significance; (H): Kruskal Wallis test of significance; $p > 0.05$ is not significant (NS); Mean ± SD: mean ± standard deviation; IQR: interquartile range; Post-hoc Bonferroni test was significant between: $^{(A1)}$ Controls Vs. (IBD and Colorectal cancer); $^{(K1)}$ Controls Vs. (IBD and Colorectal cancer); $^{(K2)}$ Between all groups; $^{(K3)}$ Colorectal cancer Vs. (Controls and IBD groups).

There was a statistical difference between the hemoglobin being higher and ESR being lower in the control group compared to CRC patients' group and IBD patients (p<0.001). Also, there was a statistical difference in CRP amongst the three groups being highest in colorectal cancer patients followed by IBD patients (p<0.001). Carcinoembryonic antigen (CEA) and CA19.9 levels were significantly higher in the CRC group compared to the IBD and control groups (p<0.001).

Statistical comparison of histopathological findings amongst early-stage CRC (stage I and II) and late-stage colorectal cancer (stage III and IV) are shown in Table 2. There was a statistically substantial variance amongst early and late stages (p< 0.001) regarding tumor size being larger in late stages. By utilizing the Chi-square test, a statistically substantial variance in the degree of tumor differentiation (p<0.05) being differentiated in early stages, a statistically substantial variance in lymph nodes metastasis (p< 0.001) where it was more associated with late stages.

Table 2. Comparison of histopathological findings amongst early-stage colorectal cancer (CRC) (stage I and II) and late-stage CRC (stage III and IV).

		Stage		*p*-value
		Early stage (I-II)	Late stage (III-IV)	
		N (%)	N (%)	
Site	Colon	10 (52.63%)	6 (37.5%)	NS^{X2}
	Rectum	9 (47.37%)	10 (62.5%)	
Histological type	Adenocarcinoma	15 (78.95%)	14 (87.5%)	NS^{FE}
	mucinous adenocarcinoma	4 (21.05%)	2 (12.5%)	
Size	< 5cm	15 (78.95%)	2 (12.5%)	$<0.001^{X2}$
	> 5cm	4 (21.05%)	14 (87.5%)	
Differentiation	Low	1 (5.26%) [a]	4 (25%) [a]	0.007^{FE}
	Moderate	11 (57.89%) [a]	12 (75%) [a]	
	High	7 (36.84%) [a]	0 (0%) [b]	

Table 2. Continued.

		Stage		*p*-value
		Early stage (I-II)	Late stage (III-IV)	
		N (%)	N (%)	
T	2	6 (31.58%)	3 (18.75%)	NS[FE]
	3	12 (63.16%)	8 (50%)	
	4	1 (5.26%)	5 (31.25%)	
N	0	18 (94.74%) [a]	0 (0%) [b]	<0.001[FE]
	1	1 (5.26%) [a]	11 (68.75%) [b]	
	2	0 (0%) [a]	5 (31.25%) [b]	
M	0	19 (100%)	14 (87.5%)	NS[FE]
	1	0 (0%)	2 (12.5%)	

TNM: malignant staging system. (X^2): Chi-Square test of significance; (FE): Fisher's Exact test of significance; $p > 0.05$ is not significant (NS).

No statistical difference in the histological type, the extent of the tumor and distant metastasis was found amongst both groups (p>0.05). Also as regards tumor site by using Chi-square test for qualitative data.

Descriptive and comparative statistics of IL-17A (rs2275913) genotype frequency between CRC patients' group, IBD patients and normal control group is shown in Table 3. In CRC patients' group, 2 patients (5.71%) had the homozygous AA genotype, 14 patients (40%) had the heterozygous GA genotype, and 19 patients (54.29%) had the wild GG genotype. While in IBD group, 3 patients (15%) had the homozygous AA genotype, 8 patients (40%) had the heterozygous GA genotype, and 9 patients (45%) had the wild GG genotype. In the normal control group, no one had the homozygous AA genotype, 9 subjects (45%) had the heterozygous GA genotype, and 11 subjects (55%) had the wild type of GG genotype.

Table 3. Comparison of IL-17A (rs2275913) genotype frequency among the control group, inflammatory bowel disease (IBD) and colorectal cancer (CRC) groups.

		Group			*p*-value
		Controls	IBD	Colorectal cancer	
		N (%)	N (%)	N (%)	
IL-17A rs2275913 polymorphism	AA homozygous mutation	0 (0%)	3 (15%)	2 (5.71%)	NS[FE]
	GA heterozygous mutation	9 (45%)	8 (40%)	14 (40%)	
	GG wild type	11 (55%)	9 (45%)	19 (54.29%)	

(FE): Fisher's Exact test of significance; p-value<.05

The comparative statistics using the Fisher's Exact test of the genotype frequency of IL-17A (rs2275913) polymorphism amongst CRC cases group, IBD patients and normal control group showed no difference amongst the three groups (p>0.05).

The comparative statistics of the genotype frequency of IL-17A (rs2275913) polymorphism amongst IBD cases and normal control group showed no difference amongst both groups (p>0.05). The AA genotype was higher in IBD patients' group than normal control, but this did not reach a statistically significant level as shown in Table 4.

Table 4. Comparison of IL-17A (rs2275913) genotype frequency among inflammatory bowel disease (IBD) cases and the normal control group.

		Group		*p-value*
		Controls	IBD	
		N (%)	N (%)	
IL-17A rs2275913 polymorphism	AA homozygous mutation	0 (0%)	3 (15%)	NS[FE]
	GA heterozygous mutation	9 (45%)	8 (40%)	
	GG wild type	11 (55%)	9 (45%)	

(FE): Fisher's Exact test of significance; $p > 0.05$ is not significant (NS).

By utilizing Fisher's Exact test for comparison of genotype frequency of IL-17A (rs2275913) polymorphism amongst CRC patients and the normal control group there was no difference between both groups (p>0.05). The AA genotype was higher in CRC individuals' group than in normal control group, but this did not reach a statistically significant level as shown in Table 5.

Table 5. Comparison of IL-17A (rs2275913) genotype frequency among colorectal cancer (CRC) cases and the normal control group using.

		Group		*p*-value
		Controls	Colorectal cancer	
		N (%)	N (%)	
IL-17A rs2275913 polymorphism	AA homozygous mutation	0 (0%)	2 (5.71%)	NS[FE]
	GA heterozygous mutation	9 (45%)	14 (40%)	
	GG wild type	11 (55%)	19 (54.29%)	

(FE): Fisher's Exact test of significance; $p > 0.05$ is not significant (NS).

No statistical difference in the genotype frequency of IL-17A (rs2275913) polymorphism was observed among the IBD and CRC patients' groups by using Fisher's Exact test (p>0.05). The AA genotype was higher in IBD patients' group than in the CRC patients, but this did not reach a statistically significant level as shown in Table 6.

Table 6. Comparison of IL-17A (rs2275913) genotype frequency among inflammatory bowel disease (IBD) and colorectal cancer cases.

		Group		*p*-value
		IBD	Colorectal cancer	
		N (%)	N (%)	
IL-17A rs2275913 polymorphism	AA homozygous mutation	3 (15%)	2 (5.71%)	NS[FE]
	GA heterozygous mutation	8 (40%)	14 (40%)	
	GG wild type	9 (45%)	19 (54.29%)	

(FE): Fisher's Exact test of significance; $p > 0.05$ is not significant (NS).

Comparative statistics of IL-17A (rs2275913) genotype frequency between early stages (I and II) and late stages (III and IV) of CRC showed no statistical difference (p>0.05) utilizing the Fisher's Exact test as shown in Table 7.

Table 7. Comparison of IL-17A (rs2275913) genotype frequency between colorectal cancer (CRC) early- stages (I and II) and late-stages (III and IV).

		Stage		*p*-value
		I-II	III-IV	
		N (%)	N (%)	
IL-17A rs2275913 polymorphism	AA homozygous mutation	1 (5.26%)	1 (6.25%)	NS[FE]
	GA heterozygous mutation	9 (47.37%)	5 (31.25%)	
	GG wild type	9 (47.37%)	10 (62.5%)	

(FE): Fisher's Exact test of significance; $p > 0.05$ is not significant (NS).

Discussion

According to the WHO, in 2018 the worldwide incidence of colorectal cancer was about two million new instances per year throughout the world. CRC is the third most prevalent disease and the second most common cancer in terms of fatality. In individuals with colorectal cancer who are detected in the early stages, the five-year survival rate is roughly 95%, but in those diagnosed in the later stages, the survival rate is approximately 12%.[10]

In addition to being a multifactorial illness, CRC is caused by the intricate interactions of a large number of hereditary and environmental variables.[2] One of the main problems of CRC is the late diagnosis as the presenting symptoms are vague and nonspecific. Currently, the major methods available for screening and diagnosis of colorectal cancer include fecal occult blood test and colonoscopy. The fecal occult blood test is a non-invasive and low-cost method but with limited sensitivity. On the other hand, colonoscopy, which is the gold standard method for CRC diagnosis, is related to high risk of bowel tears and bleeding. These limitations lead to low compliance with colonoscopy screening.[11]

Therefore, there is a need to establish a screening technique for CRC that is accurate and based on noninvasive biomarkers. This strategy should be generally accepted and extensively utilized in asymptomatic populations for early prediction of prostate cancer. Additionally, there is a need to develop a more tailored therapeutic approach for targeted molecular therapy.[4] There are six members of the IL-17 family, and IL-17A is one among them. It is a cytokine that is primarily produced by activated T cells, and its receptor is found everywhere. It acts on a wide variety of cell types and is involved in the regulation of a wide range of immune functions, including the production of antibodies, the expression of a variety of inflammatory cytokines and chemokines, the activation and recruitment of leukocytes, and it plays a critically important role in the development of a number of cancers and autoimmune illnesses.[12]

A single nucleotide polymorphism (SNP) is the dominant kind of genetic variation in humans. As a result of the significant studies that have been carried out over the course of the last ten years about the connection amongst certain SNPs and the risk of CRC, dozens of SNPs have been discovered to have a tight correlation with the incidence of CRC. [(13)].

Individuals who have SNPs in the IL17A gene have been linked to an augmented risk of developing a variety of immune-mediated and inflammatory illnesses. Each polymorphism is named according to the restriction site that was initially used to identify it. There are a number of SNPs that have been found in the IL-17A gene. However, the polymorphisms that are most often studied in genetic association studies include rs2275913 (197G>A), rs1974226 (3'UTR C>T), and rs3748067 (1249C>T). Notably, the IL-17A rs2275913 polymorphism has been linked to an increased likelihood of developing various malignancies. Therefore, it is necessary to provide more clarity since the published data regarding the connection of the IL-17A rs2275913 polymorphism with the risk of CRC is inconsistent.[14]

Our SNP of interest, rs2275913 (A/G) is located at the promoter region of IL-17A gene and contains a single Guanine to Adenine base

transition at position 197 from the start codon of the IL-17A gene.

The single nucleotide polymorphism (SNP) rs2275913, also known as G-197A, is in the upstream region (promoter) of the IL-17A gene, it is a key regulator of IL-17 production. As a result, it is possible that this SNP has an impact on IL-17 transcriptional regulation.[15]

The presence of allele A/G-197 (genotypes GA/ AA) is linked to more efficient IL-17 secretion. It is associated with a broad variety of human disorders such as tumors, illnesses that are contagious, inflammatory, autoimmune and several other disorders and it is significant as target for pharmacogenomics therapy.[16]

The past few decades have seen contrasting perspectives on the dual function of IL-17A, which can paradoxically act as both a promoter and inhibitor of angiogenesis in relation to IL-17's role in cancer pathophysiology. Understanding its inhibitory mechanism in cancer can contribute to the development of future tumor treatments.[17]

A SNP that is positioned in the promoter region of the IL17A gene (rs2275913) has been shown to have the potential to influence an individual's risk of developing CRC, according to the findings of research conducted in various global locations. On the other hand, the data that have been published on the association of the IL-17A rs2275913 polymorphism with CRC are not yet adequate and have been disputed owing to the disparities in ethnicity, country, and experimental methodology.[12] So far, there is limited research carried out to evaluate the clinical value of the IL-17A rs2275913 polymorphism and its connection with CRC in the Egyptian community. Therefore, our research aimed to investigate the clinical utility of the IL-17A gene polymorphism (A/G) (rs2275913) in CRC patients in order to explore its association with CRC disease.

Our study included 75 subjects classified into 35 CRC patients, 20 patients with IBD serving as a pathological control group and 20 normal control subjects. To achieve our goal, genotyping for the polymorphism of the IL-17A gene rs2275913 (A/G) was performed by real time PCR.

By studying the demographic and routine laboratory data of the participants, we noted a substantial increase in age in CRC individuals with mean age (52.69 ± 12.68) compared to IBD patients (36.45 ± 12.77) and normal controls (40.1 ± 14.09). The findings of this research agreed with those of a study by Elhossary et al., 2020,[18] who concluded that the risk of developing CRC rose with increasing age. This was due to the finding that more than 90% of those who were diagnosed with CRC were between the ages of 50 and 60 years.

This data shows some sort of contradiction with the case-control study performed by *Feng et al., 2019*[19] which found no significant difference in the mean age of CRC patients (62.27 years) compared with that of the control group (62.38 years) (p= 0.848).

Our results showed a significant difference in the level of CRP, ESR, carcinoembryonic antigen and CA 19.9 being higher in CRC group compared with both normal and pathological control groups. While hemoglobin (Hb) level is decreased in the CRC group compared to both normal and pathological control groups. This is attributed to the notion that bleeding per rectum is one of the most common signs of colorectal cancer as blood appears in stool of CRC cases. So, the Hb level and red blood cell counts become reduced and lead to anemia.[20] In addition, the elevation in CEA and CA19-9 in CRC cases is attributed to the fact that they are produced from the malignant epithelial cells of CRC and affect tumorigenesis by enhancing tumor cell survival and inducing tumor angiogenesis.[21] These results were in accordance with those reported by Kamel et al., 2021,[22] conducted on Egyptian population and included 30 CRC patients and 30 controls. Their research observed a statistically significant difference in hemoglobin, CEA, CA 19-9, ESR and CRP between the CRC patients and the control group.

Our study showed a statistically significant difference in hemoglobin, ESR and CRP amongst IBD and normal control group. These outcomes were consistent with those reported by Sobhy et al., 2023[23] in a study contained Egyptian population and included 60 cases with CRC, 60 cases with IBD and 30 healthy control subjects.

They noted a substantial reduction in Hb value in IBD group compared to control group as chronic diarrhea affects iron absorption from intestine thus anemia occurs. While CRC and ESR were markedly increased in IBD cases with substantial difference compared to healthy controls. Our findings agree with the notion that CRP level in serum indicates inflammation that is utilized the most often in IBD.[16]

Our study also revealed a statistical difference in the level of CEA and CA 19.9 being higher in CRC group compared with IBD group (p<0.001). These findings agree with those published by Bayo et al., 2022,[2] who carried out cross-sectional research on Spanish population and reported a model effective enough for early detection of CRC by measurement of blood biomarkers. The study included a total of 221 participants. They were separated into four groups: a normal control group of 83 individuals, a benign colonic polyp group of 56 individuals, a CRC group of 45 individuals, and an inflammatory disease group of 37 individuals. Serum levels of CEA and CA 19.9 enabled differentiation amongst the investigated four groups, as levels of CEA and CA 19.9 were greater in individuals who had CRC.

Our results disclosed a significant difference between the early and late stages (p< 0.001). The tumor size (>5 cm) was significantly larger in late stages (p < 0.05). The degree of differentiated tumors was significantly associated with early stages (p<0.001). Lymph nodes metastasis was found between stages of CRC where it is more associated with late stages. Our results agreed with the results of the study by *Shin et al.,* 2019 [25]who conducted a cohort study of 151 CRC patients in Korea and stated that large tumor size (>5 cm) and lymph node metastasis were associated with advanced stages of CRC. Our outcomes also agreed with those reported by a study of Minhajat *et al.,* 2020,[26] who performed research on 268 CRC patients in Indonesia to determine the correlation between CRC histopathological grading and staging. He stated that differentiated CRC tend to be more common in early CRC stages than in late stages.

In the current study, we examined the distributions of genotypes for the IL-17A gene in individuals with CRC, pathological and normal controls. we used homozygous G alleles of rs2275913 as the reference in order to assess the connection of CRC in individuals with AG (heterozygous) and AA (mutant) genotypes. This was done because IL-17A serves as a significant contributor to the growth of CRC.

In 2011, *Liu et al.*[27] studied the variant A-allele with respect to IL17A-197A/G promoter single nucleotide polymorphism. A-allele has been reported to affect gene transcription, transcribes higher levels of mRNA and secretes significantly higher levels of IL-17 than the G-allele. These findings can be explained by that the A-allele has a greater affinity for the transcription factor known as nuclear factor of activated T-cells (NFAT), which is an essential regulator of the production of the IL17A gene. Thus, the A/A genotype may make a person more susceptible to CRC, which could lead to an inflammatory milieu because of increased IL-17A levels in the tumor microenvironment. Additionally, this may increase the risk of cancer by promoting angiogenesis through the stimulation of vascular endothelial growth factor (VEGF) production from cancerous cells and the production of factors that aid in tumor invasion, such as prostaglandin E2 (PGE2), chemokine receptor 6 (CCR6), matrix metalloproteinase-9, and matrix metalloproteinase-13, which are involved in the migration of colorectal cancer cells. Furthermore, immunological cells like neutrophils, which mediate the generation of proinflammatory cytokines like IL-6 and tumor necrosis factor, may be drawn in by the A/A genotype. These cytokines are crucial for giving the recruited myeloid cells a suppressive character, which can produce an immunosuppressive environment and impair the immune response against tumors.[28]

Our results showed no significant difference in the genotypic frequencies of IL-17A gene polymorphism rs2275913 between CRC and the normal control group (p>0.05). Our study findings agreed with those of the study by *Zaid et al., 2022,*[29] who studied the association between IL17A gene polymorphism and CRC on

Moroccan population including 69 CRC patients and reported that SNP in IL-17A (rs2275913) did not reveal a role in the progress of CRC, no allele or genotype differences were noted amongst study individuals and controls.

Our results also agreed with those of the retrospective case-control study by Bedoui et al., 2018[30] who considered the association between IL17-A gene (rs2275913) and CRC in Tunisian population which involved 293 CRC cases and 268 healthy controls. There was lack of association between IL17-A gene (rs2275913) and CRC development, being present in 36.5% (107/293) in CRC patients versus 28.7% (77/268) in the normal control group.

On the contrary to our study findings, a meta-analysis study by *Li et al.,* 2022,[31] that included 10 different studies (involving 2599 CRC patients and 2845 healthy controls) involved Caucasian and Asian population. The findings of this comprehensive study demonstrated considerable positive connections between the IL-17A rs2275913 polymorphism and CRC. This mutation has the potential to be an exceptional genetic risk factor in the development of CRC. Specifically, carriers of the GA or AA genotype had a significantly augmented risk for the development of CRC in comparison to carriers of the GG genotype. It was found that the IL17A rs2275913 genetic variant was located in the 5'-UTR, which plays a role in the regulation of gene transcription. This variant has an effect on the mRNA transcription of the gene, which in turn causes an alteration in the function of the protein. Furthermore, it contributes to CRC by increasing the levels of IL-17A in the microenvironment of the tumor.

In addition, *Zhang et al.,* 2020[12] conducted case-control research of Chinese population. The investigation involved 208 individuals who had CRC and 312 healthy controls. The results of the investigation showed that the genotype and allele distributions for the IL-17A rs2275913 polymorphism were significantly different among individuals with CRC and healthy controls. Specifically, the researchers found that individuals with the GA or AA genotype had a significantly augmented risk for the development of CRC in comparison to those who carried the GG genotype.

In a case-control study by Aleksandrova et al., 2022,[9] involved individuals from the Bulgarian population, included 136 individuals who had CRC and 116 healthy controls. They reported that the GA genotype was found to be protective against the development of CRC. For example, carriers of the heterozygous A/G-genotype had a 2.39-fold lower risk for CRC compared to carriers of the G/G-genotype (odds ratio = 0.418, p = 0.006). This could be explained by the findings of the study by Lin et al., 2015,[32] who demonstrated that IL-17A expression is related to early-stage CRC and better prognosis among individuals with CRC. He found that the number of tumors infiltrating neutrophils is correlated with IL-17A expression in CRC. These neutrophils release myeloperoxidase and hydroperoxide, both of which have anti-tumor activity. This finding agreed with the findings of some studies reported that IL-17A demonstrated anti-tumor activity by lowering the level of IL-10 and IL-13 and increasing the level of IL-12 and interferon-γ. Furthermore, it has the ability to stimulate the activity of natural killer cells, tumor-specific T lymphocytes, and dendritic cells and it can attract these cells into the microenvironment of the tumor, which ultimately results in the inhibition of tumor development.[33]

Variances in patient demographics, nationalities, genetic backgrounds, diverse environmental exposures, sample selection, and sample size might be the possible explanations for these inconsistencies. Selection criteria of the patients, the differences in methodology and lack of standardization in these methodologies, all of these factors are known to have an impact on association studies.

In our study the genotype frequency of IL-17A (rs2275913) polymorphism amongst IBD cases and normal control group showed no statistical difference (p>0.05). The AA genotype is higher in IBD patients' group than normal control but did not reach a statistically significant level. A meta-analysis undertaken by Eskandari et al., 2017,[34] to investigate the link among the IL-17A rs2275913 G/A variation and susceptibility to IBD. The meta-analysis comprised four studies that included 964 cases with IBD and 1270 controls. Our findings are in

accordance with their findings. Under the codominant, dominant, and recessive models, the pooled evidence demonstrated that none of the IL-17A rs2275913 comparisons were related to the risk of inflammatory bowel disease (irritable bowel disease) ($p > 0.05$).

In 2021, AL-Obaidy et al.[35] conducted a case-control investigation in Iraqi population with the aim of identifying IL-17 polymorphism in individuals suffering from inflammatory bowel illness. In contrast to our research findings, the genotyping of rs2275913 in IL-17 A revealed a substantial increase in the AA genotype in IBD patients compared to the control individuals ($p \leq 0.01$). This could be explained by various studies that reported elevated production of IL-17A and upregulation of RNA transcripts of IL17A gene in the inflamed mucosa of IBD patients. IL-17A has been proposed to have a key pathogenic role in etiology and pathogenesis of IBD. IL-17A is a proinflammatory cytokine that stimulates signal transducer and activator of transcription which is involved in triggering strong immune responses during chronic inflammation and results in damage of gut tissue.[36]

Our study revealed a statistically non-significant distribution of the different IL-17A gene G>A genotypes amongst early stages (I and II) and late stages (III and IV) of colorectal cancer. Our results agreed with those reported by Samiei et al., 2018,[37] who conducted case control research involving 70 CRC individuals and 80 healthy controls for the study of the association of IL-17A G197A polymorphism with colorectal cancer risk in Malaysian population and found no association of IL-17A G197A polymorphisms with the tumor stages.

On the other hand, a case-control study carried out by Feng et al., 2019,[19] investigated the association among IL-17 gene variants and the risk of CRC in the Chinese population and found that the AA genotype of the rs2275913 polymorphism is more prevalent among individuals in the late stages of the disease (III+IV). Through the stimulation of VEGF synthesis, CRC has the ability to promote angiogenesis and facilitates tumor progression by stimulating tumor invasion factors such as matrix metalloproteinase-13, matrix metalloproteinase-9, CCR6, and PGE2, which lead to migration of CRC cells. The AA genotype has an effect on genomic stability, with higher levels of mRNA being transcribed and significantly higher levels of IL-17A being secreted. IL-17A is involved in angiogenesis, which plays an important role in the progression and metastasis of CRC.

In conclusion, our study demonstrated the presence of IL-17A rs2275913 (AA) genotype in both CRC and IBD patients. However, it did not confirm any association between IL-17A rs2275913 (A/G) polymorphism and colorectal cancer in Egyptian population.

Acknowledgements

This work was carried out at the Clinical Pathology Department, Ain Shams University .We would like to thank the patients and the controls for participating in this study.

Author Contributions

MMA; designed and approved the whole research protocol and amended the final paper version to be published. HHA; Contributed to the protocol design, revised the laboratory work and revised the manuscript draft version to be published. RMM; Monitored data collection process, supervised on the laboratory work, interpreted the data and drafted the manuscript. AMM; Collected the samples and the patient`s clinical data, carried out the laboratory work and statistical analysis of the results and participated in e manuscript draft.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

Self- Funded, This research did not receive any external funding.

Ethical approval

The approval of this study was taken from Institutional Ethics Committee of the Faculty of Medicine, Ain Shams University (FWA 00017585) (Ethical Committees' reference number FMASU MS296/2022).

Informed consent

Written informed consent was taken from all patients who were invited to participate in the research.

References

1. Baidoun, F., Elshiwy, K., Elkeraie, Y., et al. (2021). Colorectal cancer epidemiology: recent trends and impact on outcomes. *Current drug targets*, 22(9), 998-1009.

2. Koustas, E., Trifylli, E. M., Sarantis, P., et al. (2022). Implication of gut microbiome in immunotherapy for colorectal cancer. *World Journal of Gastrointestinal Oncology*, 14(9), 1665.

3. Youssef, A. S. E. D., Abdel-Fattah, M. A., Lotfy, M. M., et al. (2022). Multigene panel sequencing reveals Cancer-specific and common somatic mutations in Colorectal Cancer Patients: an Egyptian experience. *Current Issues in Molecular Biology*, 44(3), 1332-1352.

4. Ho, V., Chung, L., Wilkinson, K., et al. (2023). Prognostic Significance of MRE11 Overexpression in Colorectal Cancer Patients. *Cancers*, 15(9), 2438.

5. Rao, H., Wu, H., Huang, Q., et al. (2021). Clinical Value of Serum CEA, CA24-2 and CA19-9 in Patients with Colorectal Cancer. *Clinical Laboratory*, 67(4).

6. Marcuello, M., Vymetalkova, V., Neves, R. P., et al. (2019). Circulating biomarkers for early detection and clinical management of colorectal cancer. *Molecular aspects of medicine*, 69, 107-122.

7. Berry, S. D. G., Dossou, C., Kashif, A., et al. (2022). The role of IL-17 and anti-IL-17 agents in the immunopathogenesis and management of autoimmune and inflammatory diseases. *International immunopharmacology*, 102, 108402.

8. Song, M., Liang, J., Wang, L., et al. (2023). IL-17A functions and the therapeutic use of IL-17A and IL-17RA targeted antibodies for cancer treatment. *International Immunopharmacology*, 123, 110757.

9. Aleksandrova, E., Vlaykova, T., Ananiev, J., et al. (2022). Protective role of IL7A-197 A/G heterozygosity in the development and severity of colorectal cancer in the Bulgarian population. *Medicina*, 58(11), 1632.

10. Nogueira, M. S., Maryam, S., Amissah, M., et al. (2021). Evaluation of wavelength ranges and tissue depth probed by diffuse reflectance spectroscopy for colorectal cancer detection. *Scientific reports*, 11(1), 798.

11. Chan, S. C. H., and Liang, J. Q. (2022). Advances in tests for colorectal cancer screening and diagnosis. *Expert review of molecular diagnostics*, 22(4), 449-460

12. Zhang, S., and Wang, X. (2020). The IL-17A rs2275913 polymorphism is associated with colorectal cancer risk. *Journal of International Medical Research*, 48(12), 0300060520979117.

13. Law, P. J., Timofeeva, M., Fernandez-Rozadilla, C., et al. (2019). Association analyses identify 31 new risk loci for colorectal cancer susceptibility. *Nature communications*, 10(1), 2154

14. Lang, X., Liu, W., Hou, Y., et al. (2021). IL-17A polymorphism (rs2275913) and levels are associated with preeclampsia pathogenesis in Chinese patients. *BMC medical genomics*, 14(1), 1-8.

15. Seleit, I. A., Mohamed, O. A. B., El-Hefnawy, S. M., et al. (2021). Interleukin 17A gene polymorphism in alopecia areata. *Menoufia Medical Journal*, 34(1), 23.

16. Chen, P., Zhou, G., Lin, J., et al. (2020). Serum biomarkers for inflammatory bowel disease. *Frontiers in Medicine*, *7*, 123.

17. Huangfu, L., Li, R., Huang, Y., and Wang, S. (2023). The IL-17 family in diseases: From bench to bedside. *Signal Transduction and Targeted Therapy*, 8(1), 402.

18. Elhossary, M., Hawash, N., Badawi, R., et al. (2020). Comparative Evaluation of Colon Cancer Specific Antigen-2 Test and Chromocolonoscopy for Early Detection of Egyptian Patients with Colorectal Cancer. *Anti-Inflammatory and Anti-Allergy Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Inflammatory and Anti-Allergy Agents)*, 19(3), 302-312.

19. Feng, H., Ying, R., Chai, T., et al. (2019). The association between IL-17 gene variants and risk of colorectal cancer in a Chinese population: A case–control study. *Bioscience Reports*, 39(11), BSR20190013.

20. Kwon, H. Y., Kim, B. R., and Kim, Y. W. (2019). Association of preoperative anemia and perioperative allogenic red blood cell transfusion with oncologic outcomes in patients with nonmetastatic colorectal cancer. *Current Oncology*, 26(3), 357-366.

21. Pericleous, S., Bhogal, R. H., and Mavroeidis, V. K. (2022). The role of circulating biomarkers in the early detection of recurrent colorectal cancer following resection of liver metastases. *Frontiers in Bioscience-Landmark*, 27(6), 189.

22. Kamel, I. F., Elsadek, H. M., Ahmad, A. M., et al. (2021). Value of Serum Growth Differentiation Factor 15 in diagnosis of Colorectal Cancer. *The Egyptian Journal of Hospital Medicine*, 85(2), 3639-3644.

23. Sobhy, R., Shaker, O., Khairy, A., et al. (2023). Expression of long noncoding RNA H19and miR-675 in colorectal cancer and ulcerative colitis patients.

24. Bayo Calero J, Castaño López MA, Casado Monge PG, et al. (2022). Analysis of blood markers for early colorectal cancer diagnosis. J Gastrointest Oncol, 13(5), 2259-2268.

25. Shin, N., Son, G. M., Shin, D. H., et al. (2019). Cancer-associated fibroblasts and desmoplastic reactions related to cancer invasiveness in patients with colorectal cancer. *Annals of coloproctology*, 35(1), 36.

26. Minhajat, R., Benyamin, A. F., and Miskad, U. A. (2020). The Relationship Between Histopathological Grading and Metastasis in Colorectal Carcinoma Patients. *Nusantara Medical Science Journal*, 51-60.

27. Liu, J., Duan, Y., Cheng, X., et al. (2011). IL-17 is associated with poor prognosis and promotes angiogenesis via stimulating VEGF production of cancer cells in colorectal carcinoma *Biochemical and biophysical research communications*, 407(2), 348-354

28. Ruiz de Morales, J. M. G., Puig, L., Daudén, E., et al. (2020). Critical role of interleukin (IL)-17 in inflammatory and immune disorders: An updated review of the evidence focusing on controversies. *Autoimmunity reviews*, 19(1), 102429.

29. Zaid, Y., Hassikou, R., Oudghiri, M., et al. (2022). Association Study of IL-17A Polymorphisms (rs8193036; rs3819024; rs2275913) with Lung, Breast and colorectal Cancer Risk in a Moroccan cohort International Journal of Chemical and Biochemical Sciences, 22 (2),48-53

30. Bedoui, S. A., Barbirou, M., Stayoussef, M., et al. (2018). Association of interleukin-17A polymorphisms with the risk of colorectal cancer: A case-control study. *Cytokine*, 110, 18-23.

31. Li, G., Ma, J., Zhang, N., et al. (2022). The associations between interleukin-17 single-nucleotide polymorphism and colorectal cancer susceptibility: a systematic review and meta-analysis. *World Journal of Surgical Oncology*, 20(1), 1-13.

32. Lin, Y., Xu, J., Su, H., et al. (2015). Interleukin-17 is a favorable prognostic marker for colorectal cancer. *Clinical and Translational Oncology*, 17, 50-56.

33. Razi, S., Noveiry, B. B., Keshavarz-Fathi, M., et al. (2019). IL-17 and colorectal cancer: From carcinogenesis to treatment. *Cytokine*, 116, 7-12

34. Eskandari-Nasab, E., Moghadampour, M., and Tahmasebi, A. (2017). Meta-analysis of risk association between interleukin-17A and F gene polymorphisms and inflammatory diseases. *Journal of Interferon and Cytokine Research*, *37*(4), 165-174.

35. AL-Obaidy, T. M. M., Mutar, H. A., and Saleh, T. A. Q. (2021). Interlukine-17 Polymorphism in Patients with Inflammatory Bowel Disease (IBD) in Al-Anbar Province. *Indian Journal of Forensic Medicine and Toxicology*, *15*(2), 3053-3060.

36. Abdul-Hussein, S. S., Ali, E. N., Alkhalidi, N. M., et al. (2021). Roles of IL-17A and IL-23 in the Pathogenesis of Ulcerative Colitis and Crohn's Disease. *Iraqi Journal of Science*, 2526-2535.

37. Samiei, G., Yip, W. K., Leong, P. P., et al. (2018). Association between polymorphisms of interleukin-17A G197A and interleukin-17F A7488G and risk of colorectal cancer. *Journal of Cancer Research and Therapeutics*, 14(Suppl 2), S299-S305.