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RECEPTOR FOR THE ADVANCED GLYCATION END PRODUCTS (RAGE) PROMOTER GENE POLYMORPHISMS IN INFLAMMATORY BOWEL DISEASE

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Background: *Inflammatory bowel disease (IBD), consisting of Crohn's disease (CD) and ulcerative colitis (UC), is a chronic immune-mediated disorder, characterised by relapsing and remitting course. As a pattern recognition receptor, RAGE has a known role in intestinal inflammatory responses via the activation of multiple intracellular signalling molecules, such as NF-κB, and mediates neutrophil migration across intestinal epithelial in inflamed intestinal lumen.* ***Aim:*** *We aimed to study the association between RAGE promoter gene polymorphisms (-374T>A & -429 T>C) and risk of disease development, and probable effect on clinical characteristics and management. The study was conducted on 81 control subjects and 90 IBD patients (71 UC & 19 CD), genotyping of -374T>A & -429 T>C SNPs was done using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-REFLP).* ***Results:*** *when CD genotypes compared with UC genotypes, the genotype AA and A allele were significantly associated with CD (P=0.014 & 0.026). No significant association between CD and -429T/C genotype, however, the genotype TC of -429T>C SNP might decrease the risk of UC disease (P=0.049, OR=0.047,CI(0.22-0.98)), in addition, patients with extra intestinal manifestations and carrying C allele (TC or CC) were more than those carrying the TT genotype (P=0.007, OR= 22.08, CI= 3.19-444.38) and also patients receiving to biological treatment were having C allele more than TT genotype (P= 0.023, OR=3.9, CI=1.21-12.95).* ***Conclusion:*** *These observed results suggest that RAGE may have a role in disease development and may influence the disease phenotype and further may affect the choice of medical treatment*

Keywords*: Inflammatory Bowel Disease, Ulcerative Colitis, RAGE, single nucleotide polymorphism*

INTRODUCTION

Inflammatory bowel disease (IBD), consisting of Crohn's disease (CD) and ulcerative colitis (UC), is a chronic gastrointestinal (GI) immune-mediated disorder, characterised by relapsing periods of inflammation and remission, affecting millions of people worldwide, with an increasing incidence[1]. Complications or comorbid conditions caused by IBD, have profound effects, it could lead to lower life satisfaction, reduced social activities, increased work absenteeism and presenteeism, and increased healthcare consumption and costs[2].

RAGE expression level is markedly increased wherever its ligands are produced or released and accumulate at sites of inflammation, not only in several immune cell types such as neutrophils, T and B lymphocytes, monocytes, macrophages, and dendritic cells[3], but also in endothelial and smooth muscle cells within the vasculature. HMGB1(high mobility group box 1 protein**)** and S100A12, recognized RAGE ligands, are abundantly present in inflamed intestinal tissue from IBD patients [4].

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RAGE is involved in intestinal inflammatory responses via the activation of multiple intracellular signaling molecules, such as mitogen-activated protein kinases (MAPKs) and nuclear factor-kappa B (NF-kB)[5]. Additionally, RAGE mediates neutrophil migration across intestinal epithelial in inflamed intestinal lumen[6]. Because RAGE is upregulated in IBD[7], it is tempting to speculate that the −429 T/C and −374 T/A polymorphisms located at the promoter region could alter transcription, leading to an enhanced level of RAGE expression, and RAGE-mediated inflammation pathways [8].

This work aimed to investigate the association of both allelic and genotypic -374T/A and -429T/C polymorphisms and inflammatory bowel disease, and to find if there is an association between the studied SNPs, disease activity and the clinical features of the disease.

MATERIALS AND METHODS

This case-control study was conducted on ninety patients diagnosed as inflammatory bowel disease (71 UC & 19 CD) were recruited from IBD outpatient clinic of El-Rajhey Liver Hospital, Assiut University hospitals, Egypt. Diagnosis was determined according to established European Crohn's and Colitis Organisation (ECCO) Guidelines[8]. Phenotype characteristics of UC and CD was defined according to the Montreal classification[9]. The clinical disease activity was assessed using Mayo scoring system of UC disease activity[10], and Harvey Bradshaw Index (HBI) for CD[11]. Patients with systemic autoimmune diseases as Rheumatoid arthritis (RA), systemic lupus erythematosis (SLE),..were excluded from the study. Eighty one apparently healthy individuals were included in this study as the control group.

- 8 ml venous blood samples were collected from patients and control subjects under aseptic conditions, and divided as following:
  - 4 ml blood was collected into 2 tubes containing anticoagulant Ethylene-Diamine Tetra Acetic Acid (EDTA), one tube used immediately for CBC & ESR and the other one stored at -70 ° C to be used later for DNA extraction.
  - 4 ml blood was collected in plain vacutainer tube and left to clot for 30 min at 37□, then sera were obtained by centrifugation at 3000 rpm for ten minutes and divided into aliquots for immediate routine investigations or stored at -70 ° C for later investigations.

I. Routine investigations:

a. CBC (ADVIA 2120i (Siemens heathineers, USA).
b. ESR by westergren tube
c. Liver function tests, serum iron, and CRP (ADVIA 1800 chemistry Auto-Analyzers Siemens Healthineers, USA)

II. PCR-RFLP method used for genotyping of -374T>A SNP & -429T>C SNP as following:

- Genomic DNA was then obtained from EDTA-treated whole blood samples using a DNA extraction kit (**Gene JET Whole Blood Genomic DNA Purification Kit**, (Cat.No. K0781, K0782, Thermo Fisher Scientific, Baltics) according to the manufacturer's instructions. The following PCR mixture (25 µl total volume) was prepared to amplify DNA fragment of 671 bp of the promoter gene using forward and reverse primers[12] as following:

- PCR Master Mix 12.5 µl: using Dream Taq Green PCR Kit (Cat.No. K1071, Thermo Fisher Scientific, Baltics)
- Forward Primer(10umol):1 µl, 5′-CCT GGG TTT AGT TGA GAA TTT TTT -3′
- Reverse primer (10 µmole):1 µl, 5′-GAA AGG CAC TTC CTC GGG TTCT-3
- Extracted DNA: 3µl
- Deionised water (RNase/DNase free): 7.5µL

Then PCR was performed with thermocycling conditions as follows: a denaturing step of 94°C for 2 min, followed by 30 cycles of 94 °C for 30 s, then a cycle of 58 °C for 30 s, followed by 72 °C for 1 min, and a final incubation at 72 °C for 10 min[12].

The amplified PCR fragments then were analyzed by restriction enzyme digestion:

i. -374T>A polymorphism genotyping using *Tsp509 I* enzyme (Cat.No. ER1351, Thermo

Fisher Scientific, Baltics). *Tsp509 I* enzyme recognizes the restriction sequence:

5'... ↓A A T T ...3'
3'... T T A A↑...5'

Digestion was performed by incubation in Veriti 96 well-thermal cycler (Applied Biosystems, USA) reaction of: 10 µl the PCR product with 1µl.TasI (10 unit / ul), 2 µl of 10x Buffer and 18 µl nuclease free water at 65 °C for 16 hours. Then thermal inactivation is done by incubation at 80°C for 20 min. to stop the reaction. Then, 10µl of the digest mixture were loaded into 3% agarose gel followed by electrophoresis to separate the restriction fragments and visualized by UV illumination[12]. The Tsp509 I digestion of the 671-bp fragments were cut into 5 fragments of 284, 217, 110, 44, and 16 bp for the T allele, or 4 fragments 327, 284, 44, 16 bp for A allele. The digestion of **Homozygous wild type (T/T)** results in 5 fragments, whereas digestion of **Homozygous mutant type (A/A)** produce 4 fragments, whereas digestion of **Heterozygous (A/T)** produce 6 fragments with 327, 284, 217, 110, 44, and 16 bp as shown in **Fig (**1**)**.

ii.-429T>C polymorphism using *Alu* enzyme, (Cat.No.ER0012, Thermo Fisher Scientific, Baltics). *Alu* enzyme recognizes the restriction sequence:

5'... A G↓C T …3'
3'...T C↑G A ...5'

Digestion was performed by incubation in Veriti 96 well-thermal cycler (Applied Biosystems, USA) reaction of: 10 µl the PCR product with 1µl *Alu*(10 unit / ul), 2 µl of 10X Buffer Tango and18 µl nuclease-free water at 37 °C for 16 hours. then thermal inactivation is done by incubation at 65°C for 20 min. to stop the reaction, then 10µl of the digest mixture were loaded into 2.5% agarose gel followed by electrophoresis to separate the restriction fragments and visualized by UV illumination[12]. The nucleotide substitution from T to C of the RAGE promoter gene at position -429 results in digestion at restriction site, accordingly the 671 product was cleaved in the mutant C- allele into 2 fragments 590 bp & 81 bp, while it was not cleaved in wild T-allele. Homozygous wild type (T/T) is not cleaved and remains as the original size (671 bp), whereas digestion of Homozygous mutant type (C/C) is cleaved into two fragments (590 bp & 81 bp), whereas digestion of Heterozygous (T/C) produce three fragments with 671 bp, 590 bp and 81 bp **Fig (2).**

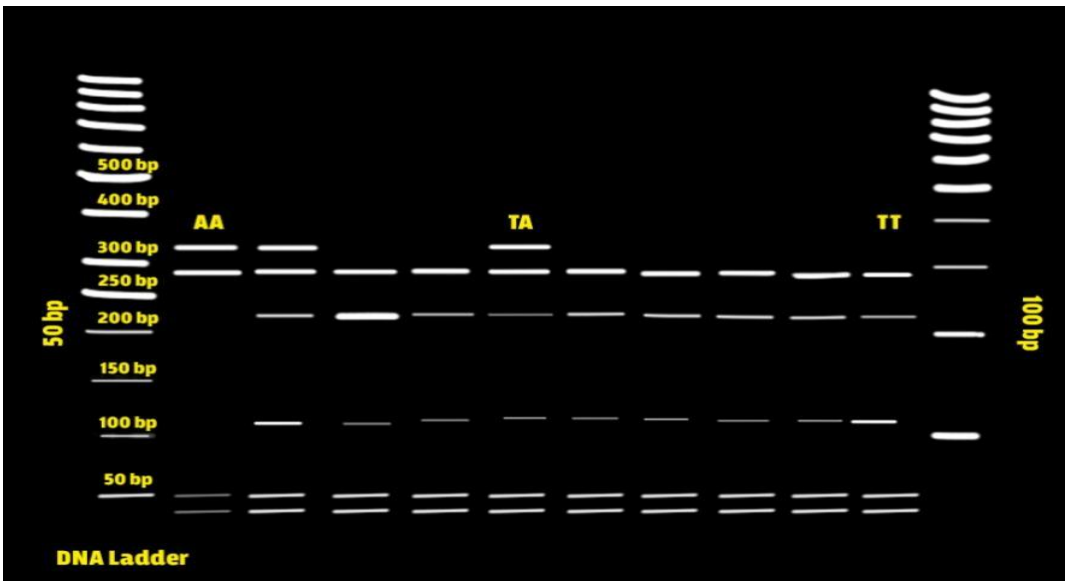

Fig. 1: Digestion of PCR product by Tsp509 I restriction enzyme.

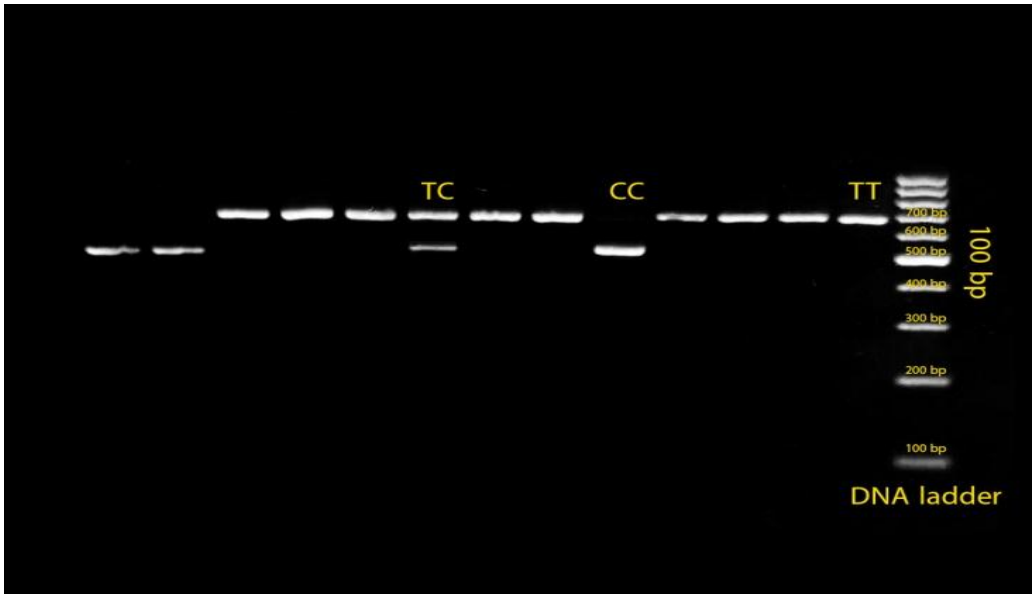

Fig.2 : PCR product digestion by Alu restriction enzyme.

Statistical analyses

The statistical analyses were conducted using RStudio statistical analysis software. Categorical data were expressed as frequencies and percentages, and group comparisons were performed using the Chi-square test or Fisher's exact test. For continuous data, descriptive statistics such as mean ± SD, median (Min-Max) were reported. Normality of continuous data was assessed using the Shapiro-Wilkes test. Student's t-test was employed for group comparisons when data was normally distributed, while the Mann-Whitney test was used for non-normally distributed data. In all statistical tests, significance was set at a p-value < 0.05.

To estimate risk, odds ratios with 95% confidence intervals (CI) were calculated for both genotypic and allelic frequencies. Allele frequency was determined by direct counting followed by division by the number of chromosomes. Genotype frequency was computed by direct counting divided by the number of participants. Hardy–Weinberg equilibrium (HWE) was assessed to evaluate the study sample's quality. The expected frequency for each genotype was calculated (https://wpcalc.com/en/equilibrium-hardy-weinberg/), and a χ^2-test was employed to compare it with the observed value. A χ^2 value greater than or equal to 3.84 indicated a significant deviation of the genotype distribution from HWE.

RESULTS AND DISCUSSION

Results

There was no statistically significant difference between age and sex of the control group and IBD group as shown in **Table (1)**.

Laboratory parameters tested for control subjects, IBD, CD and UC cases are show in **Table (2)**, CBC shows that HB results of IBD (mean 12.26 ± 1.99), CD (mean=12.13±2.53) and UC (mean 12.30 ± 1.84) were significantly lower than control HB results (mean 13.57 ± 1.62), with P =<0.001, 0.021 & <0.001, respectively, while there is no significant difference between Hct of IBD and Control. There is no significant difference between WBCs count between control and IBD, while mean platelet count of IBD (332.28 ± 124.10) is significantly higher than that of the controls (270.83 ± 70.01) with P value=<0.001, and UC mean platelet count (331.49 ± 110.41) is also significantly higher than that of control P=<0.001.

Serum Iron is significantly lower in IBD (mean= 42.10 ± 22.92) and its subtypes (CD mean serum iron =39.63 ± 23.41 and UC =42.82 ± 22.91) than in control samples (65.60 ± 33.26) (P= <0.001, 0.03 & P= 0.001 for IBD group, CD and UC subgroups, respectively).

Table 1: Demographic data in control group vs. and IBD, CD and UC groups.

Characteristic		Control N = 81	IBD N = 90	p-value^
Age (years)		36.70 ± 11.47	34.49 ± 12.11	0.137
Sex	**Female**	43 (53.1%)	55 (61.1%)	0.289
	Male	38 (46.9%)	35 (38.9%)	

Mann-Whitny test and Chi-squared test. N: number, IBD: inflammatory bowel disease

Table 2: Laboratory data of studied groups.

	Control N = 81	IBD N = 90	p-value[1]^	CD N= 19	UC N= 71	p-value[2]^	p-value[3]^
CBC							
Hb F (12-15) g/dl M (13-17) g/dl	13.57± 1.62	12.26 ± 1.99	**<0.001***	12.13 ± 2.53	12.30 ± 1.84	**0.021***	**<0.001***
Hct **F (36-46%) M (40-50%)**	38.83 ± 4.50	38.03 ± 4.95	0.411*	38.29 ± 6.84	37.96 ± 4.36	0.982*	0.305*
WBCs **(4.0-11.0)× 10^9/L**	6.33± 1.78	7.24± 4.37	0.219ˆ	9.16 ± 8.25	6.72 ± 2.34	0.206ˆ	0.387ˆ
Platelets (150-450)× 10^9/L	271.31 ± 68.75	332.28±1 24.1	**<0.001ˆ**	335.2 ± 169.48	331.5 ± 110.41	0.141ˆ	**<0.001ˆ**
Inflammatory markers							
ESR M (3-5) mm/hr F (7-12) mm/hr	4.12 ± 1.3	39.47 ± 27.46	**<0.001***	35.8 ± 32.78	40.4 ± 26.03	**0.001***	**<0.001***
CRP (0-5) mg/l	2.36 ± 1.70	13.68 ± 17.16	**<0.001***	21.8 ± 23.14	11.5 ± 14.63	**<0.001***	**<0.001***
Serum Iron	65.60 ± 33.26	42.10 ± 22.92	**<0.001***	39.6 ± 23.41	42.8 ± 22.91	**0.003***	**<0.001***
Liver Function Tests							
Albumin	42.93 ± 3.77	42.14 ± 7.35	0.405	38.58 ± 8.83	43.58 ± 4.74	**0.036***	0.060
TP	72.44 ± 5.20	70.85 ± 7.36	0.134	66.7 ± 10.81	71.96 ± 5.74	**0.023***	0.408

*P^1 Control vs. IBD, P^2 Control vs. CD, P^3 control vs. UC *Students t- test ^ Mann-Whitny test SD: standard deviation N: number CD: Crohn's UC: ulcerative colitis CBC: complete blood count Hb: hemoglobin WBCs: white blood cells HCT: hematochrit ESR: erythrocyte sedimentation rate CRP: C-reactive protein.*

ESR of Control and that of IBD, CD and UC are significantly different (P= &<0.001, 0.001 &<0.001 respectively), with higher mean results for IBD (35.79 ± 32.78), CD (35.79 ± 32.78) and for UC (40.45 ± 26.03) than that of control (13.59 ± 7.09). CRP is also higher in IBD patients (mean =13.68 ± 17.16), UC patients (mean =11.50 ± 14.63), and CD patients(mean =21.85 ± 23.14) than that of control samples (mean =4.50 ± 6.01), and significantly different (P= <0.001).

There is no significance difference detected between total protein and albumin of control individuals and IBD patients or UC patients, while Albumin of CD (mean =36.79 ± 11.88) is lower than albumin of control (mean= 42.93 ± 3.77) and shows significance difference by P value = **0.036**, and also total Protein of CD (mean= 72.44 ± 5.20) is lower

than that of control (mean= 66.70 ± 10.81) and shows significant difference of P= **0.023.**

Hardy-Weinberg equilibrium analyses were performed for two polymorphisms, -374T/A and -429T/C , within the studied population. The -374T/A polymorphism exhibited a chi-square ($\chi 2$) value of 1.85 with a p-value of 0.167, while the -429T/C polymorphism showed a $\chi 2$ value of 1.78 with a p-value of 0.189. In both cases, there was no significant departure from Hardy-Weinberg equilibrium, indicating that the observed genotype frequencies for -374T/A and -429T/C align with the expected frequencies in the study population.

As shown in **Table (3)** there is no significant difference observed between control individuals and IBD patients regarding both SNP (-374T>A & -429T>C).

There was no statistically significant difference in genotype distribution between control individuals and CD patients or between control individuals and UC patients as shown in **Table (4)**. Otherwise, there is a statistically significant difference between both the AA genotype frequency between CD and UC patients and the A allele frequency of CD and UC patients, (P value=**0.014***, OR =**0.14,** 95% CI **=0.02-0.64)** &*.(P value=**0.026***, OR =**0.42,** 95% CI **=0.20-0.91).**

There was no significant difference between genotype distribution of -374 T/A & -429 T>C gene regarding clinical features of CD cases **Table (5)**, including gender, age at diagnosis location, disease activity and extra intestinal manifestations and type of medical treatment.

There is no significance difference between Genotype distribution of -374T/A gene regarding clinical features of UC (including age, sex, disease duration, age at onset, location and extent, disease activity & type of medical treatment), moreover, there was no association between -429 T>C polymorphism and the clinical features in UC, except for UC cases with extra-intestinal manifestations where the C carrier cases were significantly higher than those with TT genotype **(P=0.007),** and also UC cases carrying the C allele were significantly more than those with TT genotype receiving biological therapy **(P=0.048)** as shown in **Table (6).**

Table 3: Genotype and allele frequencies of studied SNPs; Control vs. IBD.

Characteristic	Control N = 81	IBD N = 90	p-value^	Risk Estimates OR (95% CI)
-374T>A				
Genotypic Frequency:				
TT	43 (53.1%)	54 (60%)	--	Ref.
TA	31 (38.3%)	28 (31.1%)	0.320	0.72 (0.37-1.38)
AA	7 (8.6%)	8 (8.9%)	0.865	0.91 (0.30-2.78)
A carrier (TA + AA)	38 (46.9%)	36 (40.0%)	0.362	0.75 (0.41-1.38)
Allelic Frequency:				
T Allele	117 (72.2%)	136 (75.6%)	--	Ref.
A Allele	45 (27.8%)	44 (24.4%)	0.484	0.841 (0.52-1.36)
-429T>C				
Genotypic Frequency:				
TT	51 (63%)	67 (74.4%)	--	Ref.
TC	28 (34.6%)	17 (18.9%)	0.032	0.46 (0.23-0.93)
CC	2 (2.5%)	6 (6.7%)	0.324	2.28 (0.50-16.03)
C carrier (TC + CC)	30 (37.0%)	23 (25.6%)	0.105	0.58 (0.30-1.12)
Allelic Frequency:				
T Allele	130 (80.3%)	151 (83.9%)	--	Ref.
C Allele	32 (19.7%)	29 (16.1%)	0.380	0.78 (0.45-1.36)

Chi-square test was used OR: odds ratio CI: confidence interval.

Table 4: Genotype and allele frequencies of studied SNPs in control vs. CD and UC.

Characteristic	Control N = 81	CD N = 19	UC N = 71	p-value[1^] Risk Estimates OR (95% CI)	p-value[2^] Risk Estimates OR (95% CI)	p-value[3^] Risk Estimates OR (95% CI)
-374T/A						
Genotypic Frequency:						
TT	43(53.1%)	10 (52.6%)	44 (62.0%)	-- Ref.		
TA	31 (38.3%)	4 (21.1%)	24 (33.8%)	0.355 0.55 (0.14-1.83)	0.421 0.76 (0.38-1.49)	0.630 1.36 (0.41-5.39)
AA	7 (8.6%)	5 (26.3%)	3 (4.2%)	0.100 3.07 (0.77-11.80)	0.228 0.42 (0.09-1.61)	**0.014*** **0.14 (0.02-0.64)**
A carrier (TA+AA)	38 (46.9%)	9 (47.4%)	27 (38.0%)	0.971 1.02 (0.37-2.77)	0.269 0.69 (0.36-1.33)	0.460 0.68 (0.25-1.89)
Allelic Frequency:						
T Allele	117 (72.2%)	24 (63.2%)	112 (78.9%)	-- Ref.		
A Allele	45 (27.8%)	14 (36.8%)	30 (21.1%)	0.271 1.52 (0.72-3.19)	0.180 0.70 (0.41-1.18)	**0.026*** **0.42 (0.20-0.91)**
-429T/C						
Genotypic Frequency:						
TT	51 (63%)	13 (68.4%)	54 (76.1%)	-- Ref.		
TC	28 (34.6%)	3 (15.8%)	14 (19.7%)	0.204 0.42 (0.09-1.44)	**0.049*** **0.47 (0.22-0.98)**	0.869 1.12 (0.31-5.38)
CC	2 (2.5%)	3 (15.8%)	3 (4.2%)	0.066 5.88 (0.89-48.35)	0.709 1.42 (0.23-11.09)	0.103 0.24 (0.04-1.43)
C carrier (TC+CC)	30 (37.0%)	6 (31.6%)	17 (23.9%)	0.656 0.78 (0.27-2.28)	0.081 0.54 (0.26-1.08)	0.557 0.68 (0.22-2.07)
Allelic Frequency:						
T Allele	130 (80.3%)	29 (76.3%)	122 (85.9%)	-- Ref.		
C Allele	32 (19.7%)	9 (23.7%)	20 (14.1%)	0.590 1.26 (0.54-2.93)	0.191 0.67 (0.36-1.23)	0.154 0.53 (0.22-1.28)

P^1 Control vs. CD, P^2 Control vs. UC & P^3 CD vs. UC.

Table 5: Genotype distribution of -374T>A & -429 T/C SNPs according to some characteristics of CD.

-374T/A		**TT N = 10**	**A carrier (TA+AA)N = 9**	**p-value**	**Risk Estimates OR (95% CI)**
Age		36.80 ± 12.02	40.33 ± 19.02	0.610	1.02 (0.96-1.09)
Sex	Male	7 (70.0%)	6 (66.7%)	--	Ref.
	Female	3 (30.0%)	3 (33.3%)	0.876	1.17 (0.16-8.59)
Age at onset	Early onset (< 40)	8 (80.0%)	6 (66.7%)	--	Ref.
	Late onset (≥40)	2 (20.0%)	3 (33.3%)	0.513	2.00 (0.25-19.18)
Location and extent	L1- Terminal ileum	2 (20.0%)	2 (22.2%)	--	Ref.
	L2- Colon	4 (40.0%)	2 (22.2%)	0.600	0.50 (0.03-6.96)
	L3- Ileocolon	4 (40.0%)	5 (55.6%)	0.853	1.25 (0.11-14.82)
Disease Activity	Mild	3 (30.0%)	3 (33.3%)	--	Ref.
	Moderate	3 (30.0%)	2 (22.2%)	0.741	0.67 (0.05-7.47)
	Severe	2 (20.0%)	1 (11.1%)	0.638	0.50 (0.02-8.51)
	Remission	2 (20.0%)	3 (33.3%)	0.741	1.50 (0.13-19.11)
Extra intestinal Complications	No	7 (70.0%)	8 (88.9%)	--	Ref.
	Yes	3 (30.0%)	1 (11.1%)	0.330	0.29 (0.01-2.90)
Medical treatment	Conventional treatment	0 (0.0%)	2 (22.2%)	--	Ref.
	Biological treatment	10 (100.0%)	7 (77.8%)	0.995	NA
-429T/C		**TT N = 13**	**C carrier (TC+CC) N = 6**	**P-value**	**Risk Estimates OR (95% CI)**
Age		39.92 ± 17.66	35.33 ± 9.22	0.540	0.98 (0.90-1.04)
Sex	Male	9 (69.2%)	4 (66.7%)	--	Ref.
	Female	4 (30.8%)	2 (33.3%)	0.911	1.12 (0.12-8.78)
Age at onset	Early onset (< 40)	9 (69.2%)	5 (83.3%)	--	Ref.
	Late onset (≥40)	4 (30.8%)	1 (16.7%)	0.523	0.45 (0.02-4.22)
Location and extent	L1- Terminal ileum	4 (30.8%)	0 (0.0%)	--	Ref.
	L2- Colon	5 (38.4%)	1 (16.7%)	0.996	NA
	L3- Ileocolon	4 (30.8%)	5 (83.3%)	0.995	NA
Disease Activity	Mild	5 (38.5%)	1 (16.7%)	--	Ref.
	Moderate	3 (23.1%)	2 (33.3%)	0.398	3.33 (0.22-92.98)
	Severe	3 (23.1%)	0 (0.0%)	0.996	NA
	Remission	2 (15.4%)	3 (50.0%)	0.158	7.50 (0.56-218.09)
Extra intestinal Complications	No	10 (76.9%)	5 (83.3%)	--	Ref.
	Yes	3 (23.1%)	1 (16.7%)	0.751	0.67 (0.03-6.93)
Medical treatment	Conventional treatment	1(7.7%)	1(16.7%)	--	Ref
	Biological treatment	12(92.3%)	5(83.3%)	0.562	0.42(0.01-11.94)

Chi-square test was used OR: odds ratio CI: confidence interval N= number Note: "NA" indicating that the odds ratio (OR) is not applicable due to the presence of zero in one of the cells, preventing the calculation of the OR.

Table 6: Genotype distribution of -374 T/A & -429 T/C SNP according to some characteristics of UC patients.

-374T/A TT N = 44		**TT N = 44**	**A carrier A carrier N = 27**	**p-value**	**Risk Estimates OR (95% CI)**
Age		31.50 ± 10.16	36.56 ± 11.68	0.066	1.04 (1.00-1.10)
Sex	Male	14 (31.8%)	8 (29.6%)	--	Ref.
	Female	30 (68.2%)	19 (70.4%)	0.846	1.11 (0.40-3.24)
Age at onset	Early onset (< 40)	39 (88.6%)	22 (81.5%)	--	Ref.
	Late onset (≥40)	5 (11.4%)	5 (18.5%)	0.404	1.77 (0.45-7.04)
Location and extent	E1- Proctitis	12 (27.3%)	9 (33.3%)	--	Ref.
	E2 - Left-sided	24 (54.6%)	16 (59.3%)	0.829	0.89 (0.30-2.64)
	E3- Extensive Colitis	8 (18.1%)	2 (7.4%)	0.225	0.33 (0.04-1.74)
Disease Activity	Mild	18 (40.9%)	14 (51.9%)	--	Ref.
	Moderate	19 (43.2%)	10 (37.0%)	0.460	0.68 (0.24-1.90)
	Severe	5 (11.4%)	2 (7.4%)	0.465	0.51 (0.07-2.79)
	Remission	2 (4.5%)	1 (3.7%)	0.729	0.64 (0.03-7.39)
Extra intestinal Complications	No	39 (88.6%)	26 (96.3%)	--	Ref.
	Yes	5 (11.4%)	1 (3.7%)	0.284	0.30 (0.02-2.00)
Medical treatment	**Conventional**	30 (68.2%)	23 (85.2%)	--	Ref.
	Biological	14 (31.8%)	4 (14.8%)	0.118	0.37 (0.10-1.20)
-429T/C		**TT N=54**	**C carrier N=17**		
Age		34.74 ± 10.30	29.24 ± 12.26	0.077	0.94 (0.88-1.00)
Sex	Male	15 (27.8%)	7 (41.2%)	--	Ref.
	Female	39 (72.2%)	10 (58.8%)	0.301	0.55 (0.18-1.76)
Age at onset	Early onset (< 40)	45 (83.3%)	16 (94.1%)	--	Ref.
	Late onset (≥40)	9 (16.7%)	1 (5.9%)	0.287	0.31 (0.02-1.86)
Location and extent	E1- Proctitis	17 (31.5%)	4 (23.5%)	--	Ref.
	E2 - Left-sided	31 (57.4%)	9 (53%)	0.755	1.23 (0.34-5.10)
	E3- Extensive Colitis	6 (11.1%)	4 (23.5%)	0.221	2.83 (0.52-15.92)
Disease Activity	Mild	22 (40.7%)	10 (58.8%)	--	Ref.
	Moderate	23 (42.6%)	6 (35.3%)	0.352	0.57 (0.17-1.81)
	Severe	6 (11.1%)	1 (5.9%)	0.381	0.37 (0.02-2.56)
	Remission	3 (5.6%)	0 (0%)	0.991	NA
Extra intestinal Complications	No	53 (98.1%)	12 (70.6%)	--	Ref.
	Yes	1 (1.9%)	5 (29.4%)	**0.007***	22.08 (3.19-444.38)
Medical treatment	**Conventional**	45 (67.2%)	10 (43.5%)	--	Ref.
	Biological	22 (32.8%)	13 (56.5%)	**0.048***	2.66 (1.02-7.17)

Chi-square test was used OR: odds ratio CI: confidence interval
N= number Note: "NA" indicating that the odds ratio (OR) is not applicable due to the presence of zero in one of the cells, preventing the calculation of the OR.

Discussion

In this study routine laboratory investigations done showed a significant decrease in Hb level in IBD patients (UC, CD) compared to the control group. The associated iron deficiency anemia with both CD and UC cases was expected because of the bloody diarrhea the patient complained of, the state of chronic inflammation, impaired iron uptake through inflamed mucosa or medicines used[14]. The most common forms of anaemia in IBD are iron deficiency anaemia [IDA], anaemia of chronic disease, and a combination of both[15].Vitamin B12 or folate deficiency, haemolytic anaemia, and drug-induced anaemia are less prevalent forms, but should also be considered[16].

CRP and ESR levels of UC and CD patients were significantly elevated when compared to that of control in our study. CRP & ESR measurment is effective in assessing patients in the active phase of IBD and monitoring the efficacy of treatment, however, it should be noted that the value change was slower than the change in disease activity[17].

Hypoproteinemia and hypoalbuminemia were significantly detected CD patients than in control in our study. Low albumin levels may reflect the malnutrition state resulting from suppressed appetite due to undergoing inflammation and released cytokines, or from excessive loss of nutrients with diarrhea. Also, the malabsorption syndrome can result from the loss of intestinal villi due to inflammation, the presence of fistulas or small bowel resection particularly in CD[18].

RAGE expression level is markedly increased wherever its ligands are produced or released and accumulate at sites of inflammation as in inflamed intestinal tissue from IBD patients[4]. Also, it has been demonstrated that the RAGE promoter region's SNPs -374A/T and -429T/C enhance protein synthesis by around three folds and two folds, respectively, and thus in consequence the NF-κB- dependent inflammation is increased[19], or in adverse, the increase of sRAGE serum could represent an inflammation neutralizer because of its blocking effect on RAGE ligands[20]. In this regard, our study investigated the possible relationship between the two promoter gene polymorphisms and IBD development. Interestingly, the use of RAGE blocking agents as laquinimod (which is an oral small molecule inhibiting the interaction of S100A9 with both toll like receptor-4 and RAGE, prevents receptor downstream signalling which further reduces the release of cytokines), has been successfully tested in management of number of diseases as in Crohns disease, multiple sclerosis and systemic lupus[21].

Regarding the -374T>A gene polymorphism in the current study, we found that there was no statistically significant difference in genotype distribution between control subjects and CD cases and UC cases. Correspondent to our results **Ciccocioppo et al., (2019)** who observed no association of -374T>A with CD cases in Italy, but in contrast, they found that A allele was higher in UC cases ($p = 0.043$)[12]. No significant association was observed of -374T>A genotype with UC in two Chinese studies conducted by **Wang et al., (2015**) and **Wang et al.,(2016**)[13,22].

When comparing the -374T/A genotyping of CD with that of UC, we observed that both the AA genotype of -374 T>A and the A allele were significantly associated with CD when compared to UC (P=0.014) & (P=0.026), respectively. while, according to results of a Chinese study conducted by **Wang et al., (2014)**, the risk for CD associated with the mutant A allele, is decreased by 36% in comparison with control subjects (95%CI: 0.47-0.88, $P = 0.005$)[23].

There was no observed significant difference of the -429T>C genotype distribution between control group and CD in our study or its clinical features, the results are the same as that of a study carried by **Wang et al., (2015)** in China[13]. However, C allele of the -429T>C polymorphism was related to increased risk of CD ($p < 0.001$) as reported by **Ciccocioppo et al., (2019),** but with no significant association between -429 T>C polymorphism and the clinical features included in this study in CD[12].

Regarding -429 T>C genotype frequency in UC patients, TC genotype frequency is found to be significantly higher in control subjects than in UC cases in our study, and may possibly have a protective effect (P value = 0.049, OR= 0.47, CI= 0.22-0.98), while the studies performed by, **Wang et al., (2015)**, **Wang et al., (2016)**, and **Ciccocioppo et al., (2019)** showed no correlation between this

SNP and UC[12,13,22]. We observed that there was no significant association in genotype distribution or allelic frequency of -429 T>C in CD when compared to UC.

To our knowledge this the first study to be conducted on Egyptian IBD patients, the observed discrepancies between the current study and the previous studies, that were performed in different communities and cultures, may be attributed to the complex indeterminate pathophysiology of IBD, exposure to variable risk factors and different genetic background.

In order to evaluate the possible effect of the studied polymorphisms on clinical characteristics, which are usually assessed during disease management, we investigated the studied polymorphisms genotyping in relation with the patients' clinical characteristics. We found that there was no association between -374 T>A polymorphism and age at onset in our study, in contrast to **Ciccocioppo et al., (2019)** who found that homozygous AA genotype that may be associated with late onset of CD, and TT genotype with early onset (p = 0.049)[12], while other demographic data, clinical features included in our study (age, sex, location and extent, disease activity, extra-intestinal complications and response to medical treatment) in either CD or UC revealed no significant difference. Demographic and clinical features included in their study showed insignificant association as well[12] .

Moreover, there were no association between -429 T>C polymorphism and the clinical features included in this study in UC, except for UC cases with extra-intestinal manifestations and UC cases receiving biological therapy, where the C carriers were significantly higher than those with TT genotype (P=0.007) & (P= 0.048), respectively. This may suggest that the presence of the C allele of -429T/C may accelerate the progression and the incidence of complications and may offer an indication for the early use of biological treatment.

The overall conclusion provided by this study supports that the RAGE promoter gene genotype of the two polymorphisms have a role in IBD development and showed differences between CD and UC, in addition it can predict development of disease complications. However, genetic studies may not consider the phenotypic heterogeneity of complex diseases as IBD, resulting in phenotypic missmatch and reduction of precision of the study[24]. It is recommended to confirm these observed data considering larger sample size calculated respecting the numerous and variable disease clinical features. Also, further multi-centre studies have to be conducted allover Egyptian countries to be representative of the Egyptian population.

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مستقبلات المنتجات النهائية للجليكيشن المتقدمة (RAGE) الأشكال الجينية المختلفة في مرض التهاب الأمعاء

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الخلفية: مرض التهاب الأمعاء (IBD)، الذي يتكون من مرض كرون (CD) والتهاب القولون التقرحي (UC)، هو اضطراب مناعي مزمن، يتميز بالانتكاس والتحويل.و للتعرف على الأنماط، يلعب RAGE دورًا معروفًا في الاستجابات الالتهابية المعوية عبر تنشيط جزيئات الإشارة المتعددة داخل الخلايا، مثل NF-κB، ويتوسط هجرة العدلات عبر الظهارية المعوية في تجويف الأمعاء الملتهب.

الهدف:يهدف البحث إلى دراسة العلاقة بين تعدد أشكال الجينات المروجة لـــ RAGE (-374T A>T& – ٤٢٩ C>T) وخطر تطور المرض، والتأثير المحتمل على الخصـــائص الســـريرية وإدارتـــه.أجريت الدراسة على ٨١ شخصًا مراقب و٩٠ مريضًـــا بمـــرض التهـــاب الأمعـــاء (٧١ UC و١٩ قرصًـــا مضغوطًا)، وتم إجراء التنميط الجيني لـــ -٣٧٤T>A& -٤٢٩ T>C SNPs باستخدام تعدد الأشـــكال لطول جزء تقييد تفاعل البوليميراز (PCR-REFLP).

النتائج: عند مقارنة الأنماط الجينية CD مع الأنماط الجينية UC، فإن النمط الجينـــي AA والأليـــل A ارتبطا بشكل ملحوظ مع CD& (P=0.014 ٠.٠٢٦).وأثبتت الدراسة أنه لا يوجد ارتباط كبير بين النمط الجيني CD و -٤٢٩T/C، ومع ذلك، فإن النمط الجيني TC لـــ -٤٢٩T>C SNP قد يقلل من خطـــر الإصابة بمرض UC (P = 0.049، OR = 0.047، CI (0.22-0.98) بالإضافة إلى ذلك، كـــان المرضـــى الذين يعانون من مظاهر معوية إضافية ويحملون أليل C (TC أو CC) أكثر من أولئك الذين يحملـــون النمط الجيني TT (P = 0.007، OR = 22.08، CI = 3.19-444.38) وكذلك المرضى الذين يتلقون العلاج البيولوجي كانوا لديهم أليل C أكثر من النمط الجيني TT (P = 0.023، أو = ٣.٩، CI = 1.21-12.95).

الاستنتاج: تشير هذه النتائج المرصودة إلى أن RAGE قد يكون له دور في تطور المرض وقد يـــؤثر على النمط الظاهري للمرض وقد يؤثر أيضًا على اختيار العلاج الطبي.