# Gene Polymorphisms of Receptors for Advanced Glycation End Products (RAGE) in Association with Incidence of Colorectal Cancer (CRC) among Iraqi Patients

**Omer Salah Al-Doori[1*], Shatha H. Ali[2]**

## Abstract

**Introduction:** Cancer is a well-known public health problem and is a major cause of death worldwide. Colorectal Cancer (CRC), is the most common malignant cancer. Worldwide, (CRC), is the most common cancer in the gastrointestinal tract, and represents 13% of all malignant tumors, affecting men as women, in the same manner, in developed and undeveloped countries.In Iraq, CRC has increased in the last 10 years, and become the leading cause of about 10% of cancer mortality. An increase in the concentration of end products of the advanced glycation is thought to induce a rise in RAGE receptor expression. Their presence causes continual cell stimulation and, as a result, irreparable tissue damage. Diabetic problems, immune reactions, and neoplastic cell proliferation are all examples of such processes in pathological situations.RAGE are presented with several gene polymorphisms, among the commonest gene polymorphism for RAGE are rs1800625, rs1800624, rs2070600, and rs184003, which may contribute to many illness conditions.

**Methods:** One hundred–forty unrelated participants, males, and females, 90 of them are CRC patients, besides 50 apparently healthy subjects, who were age & sex matched that of the patients, to serve as controls.Blood Specimens had been collected from each patient and healthy control subjects, whole blood has been placed into a tube containing EDTA for DNA extraction and genetic study.

**Results:** significant variationswere detectedfrom rs1800624, rs2070600, and rs184003 polymorphisms between patients and controls, but not with rs1800625 gene, yet all studied polymorphisms show no correlation between each other.

**Conclusion:** RAGE polymorphisms (rs1800624), (rs2070600), and (rs184003) have shown to be associated with CRC unlike (rs1800625), which had shown a non-significant differencebetween Iraqipatient and control groups in the current study.

**Recommendation:** Large scale genetic studies including RAGE gene polymorphisms to confirm the association of each gene polymorphismindividually with CRC and other diseases.



**Keywords:**CRC, RAGE gene polymorphisms, rs1800624, rs2070600, rs1800624,rs1800625.

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## Introduction

Cancer is a term that is used to describe a condition where abnormal cells in the human body begin to divide and expand out of control, with the ability to invade other tissues via the lymphatic system and blood, a process referred to as metastasis [1,2].Cancer occurs when cells start to abnormally divide and grow without any control and become capable of invading other tissues through the blood and lymphatic system, through metastasis [3].

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Cancer is a well-known public health problem, and it is a major cause of death worldwide [4].

colorectal cancer (CRC) is a slowly developing cancer type that begins as a tumor or tissue having growth on the inner lining of the rectum or colon. If this abnormal growth, eventually becomes cancerous, it can form a tumor on the wall of the rectum or colon, and subsequently grow into blood vessels or lymph vessels, increasing the chance of metastasis to other sites in the body [5,6].

Worldwide, Colorectal Cancer, is the most common cancer in the gastrointestinal tract, and representing 13% of all malignant tumors, affecting men as women, in the same manner, in developed and undeveloped countries, and it is expected to overcome the mortality rate of heart diseases in the coming years [7,8].

In Iraq, CRC has increased in the last 10 years, and which become the leading cause of about 10% of cancer mortality. It becomes the second and third most common cancer in women and men respectively [9]. Many risk factors have been contributed to CRC including diet with the strong relationship between CRC and high intake of red meat and processed meat [10], a Western diet rich in fat [11], cigarette smoking, and the use of tobacco in all forms [12,13], gastrointestinal inflammation and hyperinsulinemia [14,15]. As well as family history has great relevance to the risk of CRC [16].

Receptors for Advanced Glycation End Products (RAGE)can be defined as a surface protein with a mass of 45–55 kDa and three distinct fragments with distinct functions in its structure [17]. It consists of a cytosolic tail, a portion exposed to the outside of the cell, and a single hydrophobic transmembrane domain. It is the first transmembrane receptor from the immunoglobulin superfamily to be discovered, and it can bind a wide range of ligands [18].An increase in the concentration of end products of the advanced glycation is thought to induce a rise in RAGE receptor expression. Their presence causes continual cell stimulation and, as a result, irreparable tissue damage. Diabetic problems, immune reactions, and neoplastic cell proliferation are all examples of such process in pathological situations [19].

Various exogenous and endogenous ligands exist for RAGE.The ligand-RAGE axis has emerged as a new route involved in a wide range of illnesses, such as atherothrombosis, diabetes, rheumatoid arthritis,chronic renal failure, cancer, aging and neurodegeneration [20].RAGE are present with several gene polymorphisms, among the commonest gene polymorphism for RAGE are: rs1800625, rs1800624, rs2070600, and rs184003 [21-23]. All of thesepolymorphisms could play crucial roles in several types of cancers [24,25] such as hepatocellular carcinoma [26], breast cancer [27,28], lung cancer [29,30], urothelial cell carcinoma [31] and prostate cancer [32] and others [33].

## Aim of the Research

Study the relationship between RAGE gene polymorphisms and incidence of CRC in Iraqi patients.

## Subjects and Methods

One hundred–forty unrelated participants, males and females, were recruited from the Oncology Hospital at Baghdad Medical City-Complex, Baghdad/ Iraq. during the period from April/ 2021 to February/ 2022, with an age range of (27-78 years) both (males and females). Ninety subjects were diagnosed to have CRC (60 patients with colon cancer and 30 patients with rectal cancer) by a specialized physician, first detected by CT scan, ultrasound scan, colonoscopy, and tumor markers supported by information recorded in the hospital by physical and clinical examination. In addition to fifty apparently healthy subjects, with age & sex matching that of the patients, to serve as controls (Table-1).



**Table1.**Gender and BMI distribution between patients and controls

| Variable | | Patients | Controls | p-value |
|---|---|---|---|---|
| Number | | 90 | 50 | |
| Gender | Male | 52 | 27 | 0.66 |
| | Female | 38 | 23 | |
| BMI | | | | |
| <18.5 | | 20 | 9 | 0.14 |
| 18.5- 24.9 | | 32 | 24 | |
| 25-29.9 | | 24 | 13 | |
| 30 | | 14 | 5 | |

BMI=Body Mass Index; P-value >0-05 non-significant.

Patients enrolled in this research were not diagnosed with other types of cancer, nor been previously on chemotherapy, did not have colorectal cancer surgery, not having chronic diseases like cardiovascular, diabetes mellitus, chronic kidney disease, and not hypertension.

Blood Specimens had been collected, by taking 5-7

ml of venous blood from each patient and healthy control, 3 mlof blood has been placed in a be containing EDTA (Ethylene Diamine Tetracetic Acid) for DNA extraction and genetic study. The remainder of blood was allowed to clot for obtaining serum to analyze some related biomarkers (to be published soon).

Primers used in the present work, along with their sequences are indicated in table (2):

**Table 2.**Primers Utilized in the Work

| Primer | Sequence (5'→3' direction) |
|---|---|
| Sequence (rs1800624 and rs1800625) | |
| F | AACTGGAATGGCAGGCAAAG |
| R | AGTGAGCAAACTGAGGCACA |
| HRM rs2070600 | |
| F | AGTGTGGCTCGTGTCCTTC |
| R | CCTCATCCTGGATCCCGAC |
| HRM rs184003 | |
| F | GCTGGGAGGTAGGGTGAAC |
| R | TTTCCCTCGTTAGCCCTCTG |

For quality analysis regarding the extracted DNA, agarose gel electrophoresis has been used in order to establish integrity and existence of extracted DNA fragments. The existence of standard DNA has been also utilized to establish the presence of amplification of the PCR interaction.

The nucleotide sequence regarding the RAGE gene was known in 140 samples, as 25 microliters of each PCR product with RAGE gene Primers were submitted to Macrogen in Korea, and after receiving the results, all of the results have been directly compared with the nucleotide sequence of RAGE gene. BioEdit Pro. version: 7.0.0 can be defined as a computer application that is available on the website,

(www.mbio.ncsu.edu/bioedit/bioedit.html) and can be found on the internet (http: NCBI Reference Sequence). The results have been compared to the original gene sequence.

This method was used by Al-Deresawi (2012) in the study of genetic mutations and differences in the TPo gene in women with PCOS.

DNA purity and concentration are mostly evaluated semi-quantitatively by the gel electrophoresis technique. However, for more accurate evaluation, it can be measured by UV-Spectrophotometer in which the nucleic acids show maximum absorption at 260 nm wavelength which refers to DNA concentration. Protein contaminants show maximum absorption at 280 nm wavelength to determine DNA purity according to the formula:

DNA purity = OD260/OD280 = ~1.8-2

As a result of using large volumes of DNA moreover to the contamination due to the use of cuvette, the DNA quantity and purity evaluated by using a NanoDrop spectrophotometer (Desjardins and Conklin, 2010). In the current study, an amount of 2µl of each sample was used to evaluate DNA concentration in ng/µl and purity by using NAS99 Nanodrop spectrophotometer with easy computerized software control and data storage preceded by using TE buffer as a blank solution.

## Statistical Analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). The receiver operating characteristic curve (ROC curve) was used to identify the validity of markers as an indicator of the disease. The markers were compared according to the area under the curve. The correlation coefficient also was estimated. The analysis was submitted using MedCalc Software.P < 0.05 is considered statistically significant.

The reference for statistical analysis is SAS.2010.SAS/STAT Users Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C., USA.MedCalc Statistical Software version 16.4.3 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2016)".

SAS.2010.SAS/STAT Users Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C., USA.

The sensitivity (a probability that the test will be positive when the infection is present) was calculated using the following formula:

Sensitivity (Sn)%=TP/(TP+FN) x 100

Where; TP= True positive test, FN=False negative test

The specificity (a probability that the test will be negative when the infection is absent) was calculated using the following formula:

Specificity (Sp)%= TN/(TN+FP) x 100

Where; TN= True negative test, FP= False-positive test.

The cutoff point was determined by using Youden Index. Also, the Chi-square test was applied to test for significant comparison between percentage and least significant difference–LSD test was used for significant comparison between means in this study.

## Results

### *RAGE (rs2070600) Gene Polymorphism*

The current study analyzed the distribution of the RAGEgene polymorphism rs2070600 in CRC patients and healthy control groups, as presented in table (3)

The genotypic frequencies in controls were 70.0%(n=38) wild GG and 14.0%(n=7) heterozygous AG with Mutant homozygous was found in AA 10.0%(n=5). In CRC patients the results were 27.7%(n=25) wild-type GG, 58.8%(n=53) heterozygous AG, and mutant homozygous AA at 18.8%(n=17).

The result of genotype frequencies of CRC patient's analysis shown in Table (3) reveals that the mutant type genotype and mutant type allele were taken as reference. In RAGE gene polymorphism rs2070600, the odds ratio for the GG genotype was 0.19 with (P-value =0.004) indicating that wild genotype GG was at a higher risk of CRC than the Homo-type AA.

The frequency of the G allele in controls and patients was86.6%(n=78) and 90.0%(n=45) respectively. While the frequency of the A allele in controls and patients were 77.0%(n=70) and 24.0% (n=12) respectively.

**Table 3.**Result of DNA Sequencing for rs2070600 Gene

| RAGE polymorphism rs2070600 | Frequencies (%) | | P-value | Odd ratio (95% CI) |
|---|---|---|---|---|
| | Control Group (n=50) | Patients Group (n=90) | | |
| Codominant | | | | |
| AA | 10.0%(n=5) | 18.8%(n=17) | --- | 1.00 (Reference) |
| AG | 14.0%(n=7) | 58.8%(n=53) | 0.21 | 2.22 (0.6-7.9 ) |
| GG | 70.0%(n=38) | 27.7%(n=25) | 0.004 | 0.19 (0.6-5.0 ) |
| | | | | |
| Dominant | | | | |
| AA | 10.0%(n=5) | 18.9%(n=17) | --- | 1.00 (Reference) |
| AG+GG | 90.0%(n=45) | 86.6%(n=78) | 0.2 | 0.5 (0.1- 1.4) |
| Recessive | | | | |
| AA+AG | 24.0%(n=12) | 77.7%(n=70) | --- | 1.00 (Reference) |
| GG | 76.0%(n=38) | 27.7%(n=25) | 0.001 | 1.0 (0.5-2.4 ) |
| Allele | | | | |
| A | 24.0%(n=12) | 77.0%(n=70) | --- | 1.00 (Reference) |
| G | 90.0%(n=45) | 86.6%(n=78) | 0.0001 | 0.2(0.1-0.4) |



### *RAGE (rs184003) Gene Polymorphism*

The analysis of (distribution of the RAGEgene polymorphism rs184003 in CRC patients and healthy control groups.

The distribution of genotype and allele frequencies among patient groups compared with the healthy group for the RAGE gene polymorphism rs1484003 is shown in Table (4).

The genotypic frequencies in controls, were 60.0%(n=30) wild GG and 28.0%(n=14) heterozygous GT with Mutant homozygous was found in TT 12.0%(n=6). In CRC patients the results were 30.0%(n=27) wild type GG, 54.4%(n=49) heterozygous GT and mutant homozygous TT 15.0%(n=14).

The result of genotype frequencies of CRC patient's analysis shown in Table (3-10) reveals that the wild genotype and wild type allele were taken as reference. In RAGE gene polymorphism rs148003, the odds ratio for the GT genotype was 3.8 with (P-value = 0.0007) indicating that hetero genotype GT was at a higher risk of CRC than the Homo-type AA.

The frequency of the G allele in controls and patients was 84.4%(n=76) and 88.0%(n=44) respectively. While the frequency of the T allele in controls and patients was 70.0%(n=63) and 40.0%(n=20) respectively.

**Table 4.**Result of DNA Sequencing for rs184003 Gene

| RAGE polymorphism rs184003 | Frequencies (%) | | P-value | Odd ratio (95% CI) |
|---|---|---|---|---|
| | Control group (n=50) | Patients Group (n=90) | | |
| Codominant | | | | |
| GG | 60.0%(n=30) | 30.0%(n=27) | --- | 1.00 (Reference) |
| GT | 28.0%(n=14) | 54.4%(n=49) | 0.0007 | 3.8 (1.7-8.5) |
| TT | 12.0%(n=6) | 15.0%(n=14) | 0.08 | 2.5 (0.8-7.7) |
| | | | | |
| Dominant | | | | |
| GG | 60.0%(n=30) | 30.0%(n=27) | --- | 1.00 (Reference) |
| GT+TT | 40.0%(n=20) | 70.0%(n=63) | 0.0007 | 3.5 (1.6-7.2) |
| Recessive | | | | |
| GG+GT | 88.0%(n=44) | 84.4%(n=76) | --- | 1.00 (Reference) |
| TT | 12.0%(n=6) | 15.0%(n=14) | 0.5 | 1.3 (0.4-3.7) |
| Allele | | | | |
| G | 88.0%(n=44) | 84.4%(n=76) | --- | 1.00 (Reference) |
| T | 40.0%(n=20) | 70.0%(n=63) | 0.005 | 2.12 (1.2-3.6) |

*RAGE (rs1800624) Gene Polymorphism*

Current analysis of the distribution of genotype and allele frequencies among patient groups compared with the healthy group for the RAGE gene polymorphism rs1800624 is summarized in Table (5).

The genotypic frequencies in controls were 87.7%(n=79) for mutant TT and 30.0%(n=15) heterozygous AT with wild homozygous was found in AA 60.0%(n=30). In CRC patients the results were 21.1%(n=19) mutant type TT, 66.6%(n=60) heterozygous AT and mutant homozygous AA 12.2%(n=11).

The result of genotype frequencies of CRC patient's analysis shown in Table (3-11) reveals that the mutant genotype and mutant type allele were taken as reference. In RAGE gene polymorphism rs1800624, the odds ratio for the AA genotype was 1.0 with (P-value = 0.001) indicating that wild genotype AA was at a higher risk of CRC than the hetero-type AT.

The frequency of the T allele in controls and patients was 87.7%(n=79) and 40.0%(n=20) respectively. While the frequency of the A allele in controls and patients was 78.8%(n=71) and 90.0%(n=45) respectively.



**Table 5.**Result of DNA Sequencing for rs1800624 Gene

| RAGE polymorphism rs1800624 | Frequencies (%) | | P-value | Odd ratio (95% CI) |
|---|---|---|---|---|
| | Control Group (n=50) | Patients Group (n=90) | | |
| **Codominant** | | | | |
| TT | 10.0%(n=5) | 21.1%(n=19) | --- | 1.00 (Reference) |
| AT | 30.0%(n=15) | 66.6%(n=60) | 0.9 | 1.0 (0.3-3.2) |
| AA | 60.0%(n=30) | 12.2%(n=11) | 0.001 | 0.1 (0.2-3.0) |
| | | | | |
| Dominant | | | | |
| TT | 10.0%(n=5) | 21.1%(n=19) | --- | 1.00 (Reference) |
| AT+AA | 90.0%(n=45) | 78.8%(n=71) | 0.1 | 0.4 (0.1-1.1) |
| Recessive | | | | |
| AT+TT | 40.0%(n=20) | 87.7%(n=79) | --- | 1.00 (Reference) |
| AA | 60.0%(n=30) | 12.2%(n=11) | 0.001 | 0.1 (0.3-2.1) |
| Allele | | | | |
| T | 40.0%(n=20) | 87.7%(n=79) | --- | 1.00 (Reference) |
| A | 90.0%(n=45) | 78.8%(n=71) | 0.001 | 0.2 (0.1-0.4) |

*RAGE (rs1800625) Gene Polymorphism*

The distribution of genotype and allele frequencies among patient groups compared with the healthy

group for the RAGE gene polymorphism rs1800625 is shown in Table (6).

The genotypic frequencies in controls were 20.0%(n=10) for hetero TC and 80.0%(n=40) for wild TT with mutant which was found in CC 0. In CRC patients the results were 6.6% (n=6) heterotype TC, 93.3%(n=84) wild TT and mutant CC0.

The result of genotype frequencies of CRC patient's analysis shown in Table (3-12) reveals that the hetero genotype and hetero type allele were taken as reference. In RAGE gene polymorphism rs1800625, the odds ratio for the TC genotype was 3.5 with (P-value = 0.02) indicating that there are no significant differences that may be considered as risk of CRC than the hetero-type AT.

T allele frequency in controls and patients was 93.3%(n=84) and 100%(n=50) respectively. While C allele frequency in controls and patients was 6.6%(n=6) and 20.0%(n=10) respectively.

**Table 6.**Result of DNA Sequencing for rs1800625 Gene

| **RAGE polymorphism rs1800625** | **Frequencies (%)** | | **P-value** | **Odd ratio (95% CI)** |
|---|---|---|---|---|
| | **Control group (n=50)** | **Patients Group (n=90)** | | |
| Codominant | | | | |
| TC | 20.0%(n=10) | 6.6%(n=6) | --- | 1.00 (Reference) |
| TT | 80.0%(n=40) | 93.3%(n=84) | 0.02 | 3.5 (1.2-10.5) |
| CC | 0 | 0 | 0.2 | 2.7 (0.4-16.0) |
| | | | | |
| Dominant | | | | |
| TT | 80.0%(n=40) | 93.3%(n=84) | --- | 1.00 (Reference) |
| TC+CC | 20.0%(n=10) | 6.6%(n=6) | 0.02 | 3.5 (1.1-10.3) |
| Recessive | | | | |
| TT+TC | 100%(n=50) | 100%(n=90) | --- | 1.00 (Reference) |
| CC | 0 | 0 | 0.9 | 1.0 (0.2-4.0) |
| Allele | | | | |
| T | 100%(n=50) | 93.3%(n=84) | --- | 1.00 (Reference) |
| C | 20.0%(n=10) | 6.6%(n=6) | 0.3 | 1.2 (0.7-2.1) |



## *Linkage Disequilibrium Tests*

The current study shows a linkage between all 4 polymorphisms as shown in figure (1), also table (7) represents the disequilibrium test result.

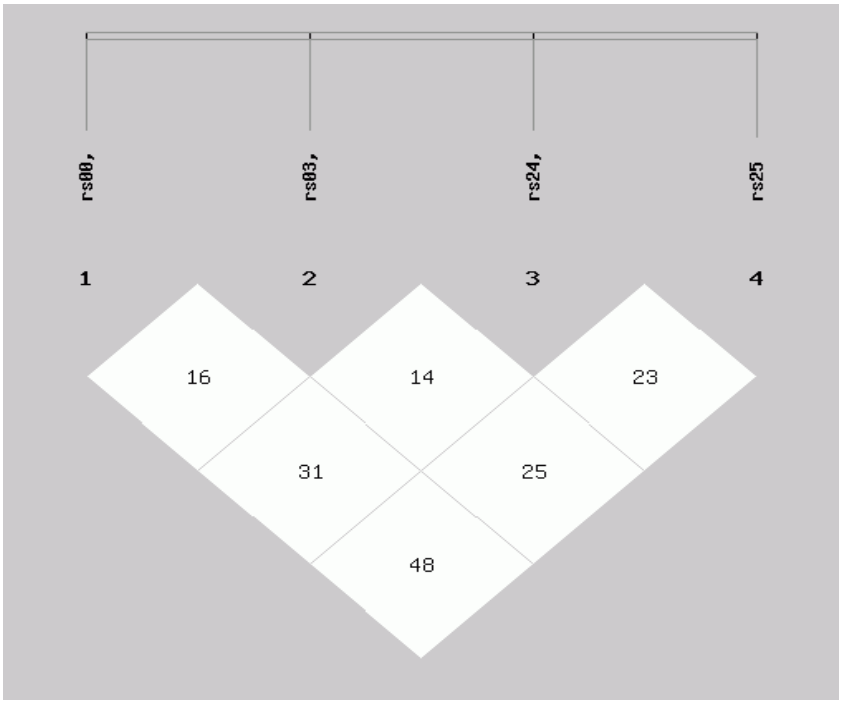


**Figure 1.**Linkage Disequilibrium of Polymorphisms

**Table 7.**Linkage disequilibrium for RAGE polymorphisms

| Linkage disequilibriu | rs2070600 | rs184003 | rs1800624 | rs1800625 |
|---|---|---|---|---|
| m | | | | |
| rs2070600 | | D' | 0.161 | D' | 0.314 | D' | 0.487 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | $r^2$ | 0.025 | $r^2$ | 0.070 | $r^2$ | 0.017 |
| rs184003 | D′ | 0.161 | | | D′ | 0.147 | D′ | 0.256 |
| | $r^2$ | 0.025 | | | $r^2$ | 0.016 | $r^2$ | 0.005 |
| rs1800624 | D′ | 0.314 | D′ | 0.147 | | | D′ | 0.235 |
| | $r^2$ | 0.070 | $r^2$ | 0.016 | | | $r^2$ | 0.006 |
| rs1800625 | D′ | 0.487 | D′ | 0.256 | D′ | 0.235 | | |
| | $r^2$ | 0.017 | $r^2$ | 0.005 | $r^2$ | 0.006 | | |

D': scaled D value [-1,1]
D value represents the Linkage disequilibrium for each pair of SNPs.
$r^2$: Correlation coefficient between any pair of SNPs [0-1].

## Discussion

Several recent studies have been suggesting the relationship between RAGE polymorphism types and cancer tissue, Sheng-ChunHung*et.al.*[34] report that the four polymorphisms of the RAGE gene, (rs1800625), (rs1800624), (rs2070600), and (rs184003) are associated with a higher risk of developing urothelial cell carcinoma. Meanwhile, Tapan K Mukherjee *et.al.*[35] founds that these polymorphisms are associated with non-small cell lung cancer development and complications.
RAGE and its polymorphic types may be useful diagnostic or prognostic markers of this type of cancer. Shuang Wu *et.al*, [36] founds that the A allele of rs2070600 may decrease the expression of the tumor suppressor gene RAGE, leading to increasing lung cancer risk.Sinda A.Bedoui*et.al,*[37]indicate the association between CRC and the presence of rs2070600, rs1800624, and rs1800625 and rs184003 AGER types had proposed that rs1800625 specifically were significantly low in CRC patients.Moreover, Ying-Erh Chou *et.al*[38] founds those specific patients with rs1800625 'TC + CC' genotypic were at higher risk of prostatic cancer. for all 343 patients who carried the above maintain gene was correlated with a higher risk of biochemical recurrence.
On other hand, Dan Hu, *et.al,*[39] suggest rs1800625 was significantly associated with gastric cancer risk, and both rs1800625 and rs184003 were related to tumor clinical stage, indicating that the RAGE gene may be a gastric cancer-susceptibility gene.
The current study showsthat wild genotype GG was at a higher risk of CRC than the Homo-type AA. Chih-Yang Huang *et.al,*[40] suggested that patients with RAGE-rs2070600 may have a high affinity for the ligandwhich promotes chemoresistance and tumor regrowth, leading to poor survival outcomes for CRC.
Elom K. Aglago*et.al.* [41] founds that rs1800625 was associated with an increased risk of colorectal cancer, this rs1800625 is located in the promoter region of AGER and is involved in the initiation of the production of the RAGE or its isomers so, the recessive model of rs1800625 was associated with an increase of overall cancer risk.
Although Elom K. Aglago*et al.* did not observe a significant association between rs2070600 (AGER) and colorectal cancer, albeit the study showed that the major allele (C allele) of this polymorphism associates with higher sRAGE levels.
Yuzhong Xu *et. al*, [42] founds that The RAGE rs1800625 polymorphism was associated with increased overall cancer risk in Asians in a recessive genetic model. Although the current study did not find any significant differences between patients and controls regarding rs1800625.

### *Linkage Disequilibrium Tests Results*

Although, many studies (34-42) indicate an association between the 4 polymorphisms, but not correlated together.
A recent study by SoudehGhafouri-Fard*et,al.* [43] were studied the association between (rs184003 and rs1800625) in breast cancer, and the results were non-significant.This agreed with the current study result which showed in figure (1) and table (7).
Both showed the four SNPs were not in Linkage Disequilibrium (LD) since the main parameters were not meeting the required conditions due to (D′ less than 1). Were the D value representing the Linkage disequilibrium for each pair of SNPs. while $r^2$:represent the Correlation Coefficient between any pair of SNPs [0-1].
White blocks in figure (1) represent linkage disequilibrium coefficient D′ < 1.0 (this is meaning that SNPs of the current study were not in linkage disequilibrium).
Linkage disequilibrium, which is the nonrandom association of alleles from different loci, actually can provide valuable information on the structure of haplotypes in the human genome map and is often the basis for evaluating the association of genomic variation with human traits among those unrelated subjects.

Negative linkage disequilibrium implies that in the initial population, compared to a similar population, there is an excess of intermediate genotypes and scarcity or absence of extreme (good and bad) genotypes.

An $r^2$=0.025 means a weak correlation between the SNPs which explainsthe 2.5% of variation within the data. While $r^2$=0.016 means a weak correlation between the SNPs which explainsthe 1.6 % of variation within the data.

## Conclusion

RAGE gene polymorphisms (rs1800624), (rs2070600), and (rs184003) have shown to be associated with CRC unlike (rs1800625), which has been shown with no significant differences between patient and control groups in the current study.

## Recommendation

Large-scale genetic studies including RAGE gene polymorphisms to confirm the association of each gene individually with CRC and other diseases.

## Author Contributions

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## Conflicts of Interest

The authors declare no conflict of interest.

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