Original Article

Single Nucleotide Polymorphism of IL-18 Gene and Resistin Gene in Children Infection with *Entamoeba histolytica*

Aseel Kariem Al-Sultany, Kassim Abdulla Hamza Al-Morshidy[1]

Department of Biology, College of Science, University of Kerbala, Kerbala, [1]Department of Biology, College of Science, University of Babylon, Hillah, Iraq

Abstract

Background: Human amebiasis is caused by *Entamoeba histolytica*, which is found in many tropical countries. *E. histolytica* infections are known to have a wide range of clinical consequences. Most infections are asymptomatic; some cause diarrhea and dysentery, and only a few cause extraintestinal consequences, including liver abscess. Single nucleotide polymorphisms (SNPs) are the most prevalent kind of sequence variation in genomes and are regarded as useful genetic markers for exposing the evolutionary history and common genetic variants that explain the heritable risk for common illnesses and effected in the progression of some infections. **Objective:** Investigation of gene polymorphism of the parameters (interleukin 18 [IL-18], resistin) most susceptible to parasitic infection. **Materials and Methods:** Stool samples were collected from patients who were attending the (Kerbala Teaching Hospital for children in the holy city of Kerbala), between February 2021 and January 2022. In total, 3748 samples of feces from children between the ages of 1 and 15 years from both gender were tested using both direct smear and acid-fast stain tests in addition to rapid test techniques. Five milliliters of blood were obtained from 25 patients infected with *E. histolytica*, as well as 5 mL of blood from 25 healthy youngsters (sample control). **Results:** The result showed the distribution of genotype of IL-18 SNPs in intestinal parasites patient in contrast to the control group; there is an increase in the level of IL-18 in some SNPs, resistin concentration was statistically significantly different between resistin gene polymorphisms. **Conclusion:** The IL-18 and resistin polymorphism can be considered one of the genetic factors responsible for the progression of *E. histolytica* infection.

Keywords: *Entamoeba histolytica*, IL-18, resistin, SNPs

Introduction

Human amebiasis is caused by *Entamoeba histolytica*, which is found in many tropical countries. *E. histolytica* infections are known to have a wide range of clinical consequences. Most infections are asymptomatic; some cause diarrhea and dysentery, and only a few cause extraintestinal consequences, including liver abscess. The parasite is thought to be responsible for millions of episodes of dysentery and liver abscess each year, with up to 100,000 deaths worldwide.[1,2] *E. histolytica* is a parasitic infection that comes second to malaria as a leading cause of death due to parasitic infestation. Diarrhea with cramps, lower abdomen pain, low-grade fever, feces containing blood and mucus, and flask-shaped ulcers are all symptoms.[3,4] Interleukin 18 (IL-18) is associated with the pathology of numerous diseases, produced by intestinal epithelial cells (IECs) is essential for maintaining epithelial integrity during inflammation. It has been shown that IL-18 inhibits parasite reproduction in human intestinal cell lines and that the cytokine increases IEC production of a defensin that inactivates *Cryptosporidium parvum* sporozoites.[5]

Resistin is one of the adipokines, mainly produced by macrophages and detectable in human serum. It is a cysteine-rich peptide with different biological effects and initially proposed to regulate obesity, glucose metabolism, and insulin sensitivity. Some researchers reported that human resistin might also play a major role in regulating inflammation. Moreover, inflammatory

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_70_23

Address for correspondence: Dr. Aseel Kariem Al-Sultany, Department of Biology, College of Science, University of Kerbala, Kerbala, Iraq.
E-mail: aseel.k@uokerbala.edu.iq

Submission: 22-Jan-2023 **Accepted:** 07-Mar-2023 **Published:** 02-Feb-2024

How to cite this article: Al-Sultany AK, Al-Morshidy KAH. Single nucleotide polymorphism of IL-18 gene and resistin gene in children infection with *Entamoeba histolytica*. Med J Babylon 2023;20:697-704.

bowel disease is typically characterized by malnutrition, body composition changes, and mesenteric white adipose tissue hypertrophy.[6,7]

Genome-wide patterns of variation between people are the most potent source of information for elucidating the history of human migration, expansion, and adaptation. With the advent of modern technologies that can type millions of single nucleotide polymorphisms (SNPs) in a single experiment, SNP in genome-wide association test has become a smart endeavor. SNPs are the most prevalent kind of sequence variation in genomes and are regarded as useful genetic markers for exposing the evolutionary history and common genetic variants that explain the heritable risk for common illness.[8]

Materials and Methods

Stool sample collection

Stool samples were collected from patients who were attending the (Kerbala Teaching Hospital for children in the holy city of Kerbala), between February 2021 and January 2022. In total, 3748 samples of feces from children between the ages of 1 and 15 years from both gender were tested using both direct smear and acid-fast stain tests in addition to rapid test techniques, where collecting about 3748 stool samples by sterilized plastic containers with tight lid that are used to retain stool samples to prevent dryness and added moisture, after examination, it was found the most prevalence parasites are *E. histolytica* and after study some immunological and physiological parameter the result showed the IL-18 and resistin more effected in patient infected with this parasite when estimated by using ELISA technique, so selected 25 patients infected with *E. histolytica* and 25 noninfected children to study the SNP.

Blood sample collection and analysis

Five milliliters of blood were obtained from 25 patients infected with *E. histolytica*, as well as 5 mL of blood from 25 healthy youngsters (sample control) put 2.5 mL of blood in an EDTA tube with a sterile medical needle (G23) that is usable once disposable and stored for further use in a molecular study.

Polymerase chain reaction

Conventional polymerase chain reaction (PCR) was used to amplify the target DNA using a specific primer for both (IL-18, resitin). It includes three consecutive steps that are repeated for a specific number of cycles to get PCR product (amplicon), which can finally be visualized after agarose gel electrophoresis. The thermal cycling conditions are mentioned in Table 1.

Sequencing of polymerase chain reaction products

Forty microliters of PCR product were sent to Macrogene/ Korea for Sanger sequencing. After trimming each sequence, the results of the trimmed sequence were blasted in NCBI to check the similarities and differences with the database. Mega5 software, korea was used to check the similarities and differences.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol, subject information, and consent form were reviewed and approved by a local ethics committee according to document number 7/18/6138, dated October 26, 2020, to get this approval.

Results

Study the associated factors of genotypes of IL-8 (SNPs) in intestinal parasite patients compared to control group.

The result showed the distribution of genotype of IL-8 single-nucleotide polymorphisms in the intestine of infected patients compared to the control group. Results of the genotype [Table 2 and Figure 1], which were showed that the mutation in the polymorphism of SNP 1 (rs1866694757), SNP 4 (rs 1946518), SNP 6 (rs1946519), and SNP 7 (rs 1215648807) were demonstrated a risk factor of intestinal infection of patients group than in control group (odds ratio [OR] = 1.333, 1.800, 1.200, and 1.750; 95% confidence interval [CI] = [0.321–5.538], [0.264–12.296], [0.396–7.732], and [0.396–7.732], respectively). No significant differences were found. While in the genotype polymorphism of SNP 2 (rs 940255648), SNP 3 (rs 1037707423), SNP 5 (rs1213044637), SNP 8 (rs 1866697972), SNP 9 (rs 1866698066), and SNP 10 (rs1866698286) were showed a protective factor (OR = 0.364, 0.308, 0.955, 0.500, 0.393, 0.073; 95% CI = [0.084–1.583], [0.052–1.829], [0.235–3.878], [0.101–2.477], [0.081–1.909], and [0.008–0.689], respectively).

Table 1: Polymerase chain reactions for genotyping of (IL-18, resistin)

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension	Reference
IL18	95°C	95°C	58.7°C	72°C	72°C	This study by researcher
	2 min	30 s	30 s	80 s	5 min	
	1 Cycle		29 Cycle		1 Cycle	
Resistin	95°C	95°C	60.3°C	72°C	72°C	This study by researcher
	2 min	30 s	30 s	50 s	5 min	
	1 Cycle		29 Cycle		1 Cycle	

Table 2: From IL-18 (single-nucleotide polymorphisms) in *E. histolytica* patients compared to control group

Genotype of IL-18	Odds ratio (OR)	Relative risk (RR)	95% CI (lower–upper)	*P* value	Patient	Control
SNP 1 (rs1866694757) CC (wild type)/CA (mutant heterozygous)	1.333	1.167	0.321–5.58	$P \leq 0.05$ (0.731)	C (0.88) A (0.12)	C (0.88) A (0.12)
SNP 2 (rs 940255648) GG (wild type)/GC (mutant heterozygous)	0.364	0.559	0.084–1.583	$P \leq 0.05$ (0.284)	G (0.92) C (0.08)	G (0.82) C (0.18)
SNP 3 (rs 1037707423) CC (wild type)/CT (mutant heterozygous)	0.308	0.481	0.052–1.829	$P \leq 0.05$ (0.242)	C (0.96) T (0.04)	C (0.88) T (0.12)
SNP 4 (rs 1946518) TT (wild type)/TG (mutant heterozygous)	1.800	1.421	0.264–12.296	$P \leq 0.05$ (0.661)	T (0.66) G (0.34)	T (0.64) G (0.36)
SNP 4 (rs 1946518) TT (wild type)/GG (mutant homozygous)	1.02	1.306	0.564–12.80	$P > 0.05$ (0.91)	T (0.94) G(0.06)	T (0.86) G(0.14)
SNP 5 (rs1213044637) GG (wild type)/GA (mutant heterozygous)	0.955	0.975	0.235–3.87	$P \leq 0.05$ (0.614)	G (0.82) A (0.18)	G (0.78) A (0.22)
SNP 6 (rs1946519) CC (wild type)/AC (mutant heterozygous)	1.200	1.111	0.160–9.01	$P \leq 0.05$ (0.633)	A (0.64) C (0.36)	A (0.6) C (0.4)
SNP 6 (rs1946519) CC (wild type)/AA (mutant homozygous)	1.65	0.25	0.24–0.43	$P \leq 0.05$ (0.15)	A (0.76) T (0.14)	A (0.66) T (0.04)
SNP 7 (rs1215648807) AA (wild type)/AC (mutant heterozygous)	1.750	1.375	0.396–7.73	$P \leq 0.05$ (0.712)	A (0.78) C (0.22)	A (0.78) C (0.22)
SNP 8 (rs1866697972) GG (wild type)/GT (mutant heterozygous)	0.500	0.667	0.101–2.47	$P \leq 0.05$ (0.458)	G (0.94) T (0.06)	G (0.88) T (0.12)
SNP 9 (rs1866698066) GG (wild type)/GT (mutant heterozygous)	0.393	0.575	0.081–1.909	$P \leq 0.05$ (0.283)	G (0.94) T (0.06)	G (0.86) T (0.14)
SNP 10 (rs1866698286) AA (wild type)/AT (mutant heterozygous)	0.073	0.175	0.008–0.68	$P \leq 0.05$ (0.018)*	A (0.86) T (0.14)	A (0.6) T (0.4)
SNP 10 (rs1866698286) AA (wild type)/TT (mutant homozygous)	0.65	0.235	0.264-0.43	$P \leq 0.05$ (0.3)	A (0.56) T (0.24)	A (0.86) T (0.14)

*significant results

Interestingly, only the genotype of SNP 10 (rs1866698286) AA (wild type)/AT mutant heterozygous) was shown a statistically significant difference among study groups.

Study the associated factors of genotypes of resistin (single-nucleotide polymorphisms) in *E. histolytica* patients compared to the control group

Figure 2 demonstrates the distribution of the genotype of resistin single-nucleotide polymorphisms in *E. histolytica* patients compared to control group. Results of the genotype of resistin shown in Table 3, added was indicated that the mutation in the polymorphism of SNP 1(rs1862513), SNP 2(rs567367264), and SNP 3(rs536392382) were demonstrated a risk factor of intestinal infection in the patients' group than in control group, (OR = 2.068, 1.360 and 1.360; 95% CI = [0.477–8.973], [0.344–5.379], and [0.344–5.379], respectively). While in the genotype of resistin polymorphism of SNP 4 (rs2032442393) showed a protective factor (OR = 0.952; 95% CI = [0.056–16.279]). No significant differences were found in all genotypes of resistin polymorphisms.

Association between biochemical markers and gene polymorphisms

Association between IL18 and polymorphisms of IL-18 gene

Although IL-18 gens polymorphisms were never shown any statistically significant differences with any of the gene polymorphisms SNPs ($P \leq 0.05$). There is a rising in the level of IL-18 in SNPs (SNP 1/CA, SNP 2/GC, SNP 3/CT, SNP 5/GA, SNP 7/AC, SNP 8/GT, SNP 9/GT) compared with wild type respectively and (SNP 4/TG/GG, SNP 6 AC/CC), compare with wild, and heterozygote type respectively and (SNP 10 TT/AC) compare wild and heterozygote type, respectively [Table 4].

Association between resistin and polymorphisms of resistin gene

Resistin concentration was statistically different between Resistin gene polymorphisms, SNP 1 and SNP 5. There are significant increases from SNP 1 (rs1862513) CC to CG ($P = 0.005$) and from SNP 1 (rs1862513) CG

(mutant heterozygous) to GG (mutant homozygous) ($P = 0.009$).

Moreover, there was a statistically significant difference between the resistin level and SNP 4 (rs2032442393) and the resistin concentration in the SNP 4 (rs2032442393) from CC (wild type) to CT (mutant heterozygous) ($P = 0.019$), while no statistically significant difference with the rest of the resistin gene SNPs [Table 5].

Discussion

Study the associated factors of genotypes of IL-18, and resistin (single-nucleotide polymorphisms) in *E. histolytica* patients compared to the control group

Four IL-18 genetic polymorphisms, SNP 1 (rs1866694757), SNP 4 (rs196518), SNP 6 (rs1946519), and SNP 7 (rs1215648807), showed a high risk of *E. histolytica* infection, as in the current study, suggesting that these polymorphisms play causative roles in the

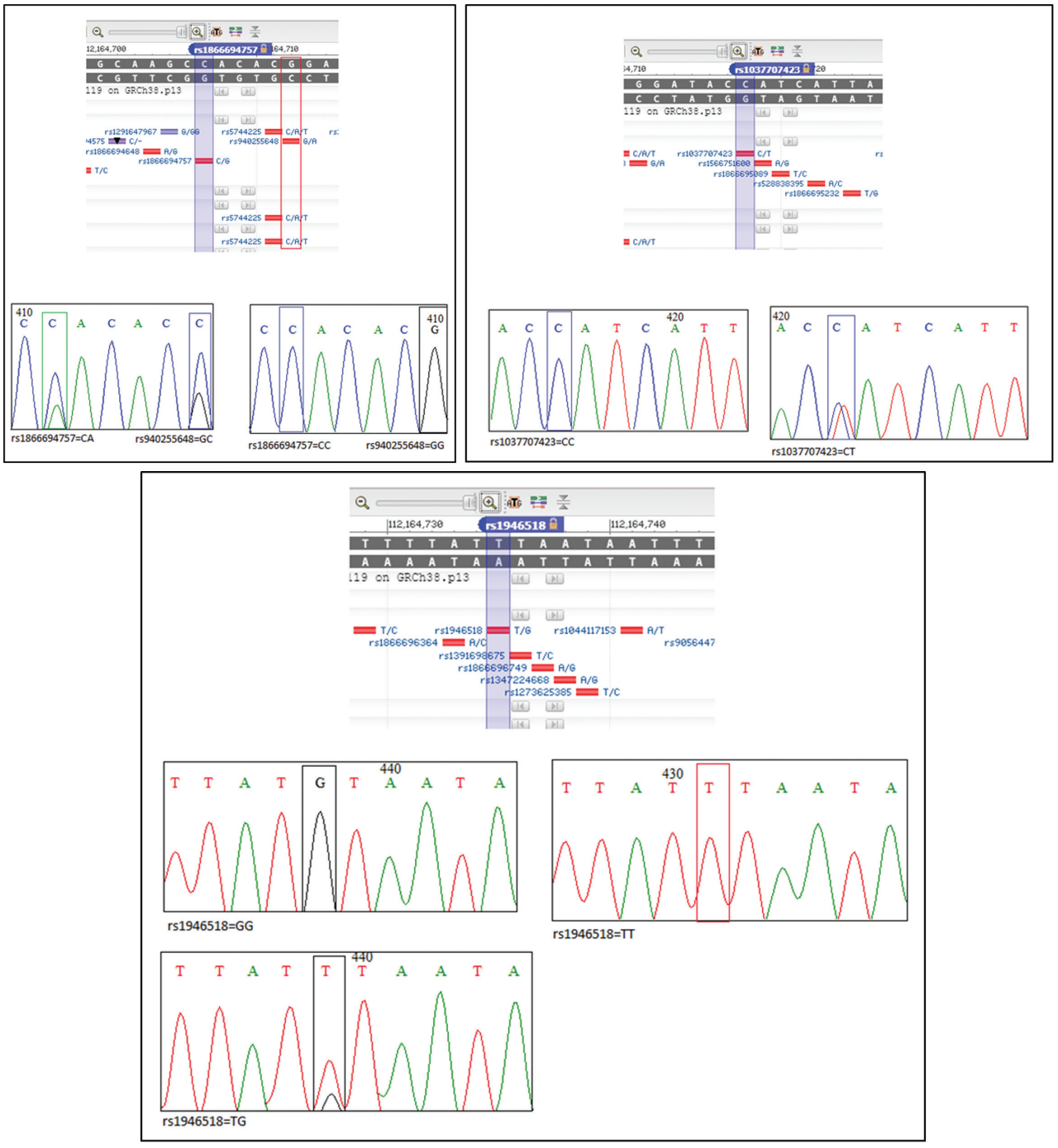

Figure 1: The genotypes of IL-18 (single-nucleotide polymorphism) in *E. histolytica* patients

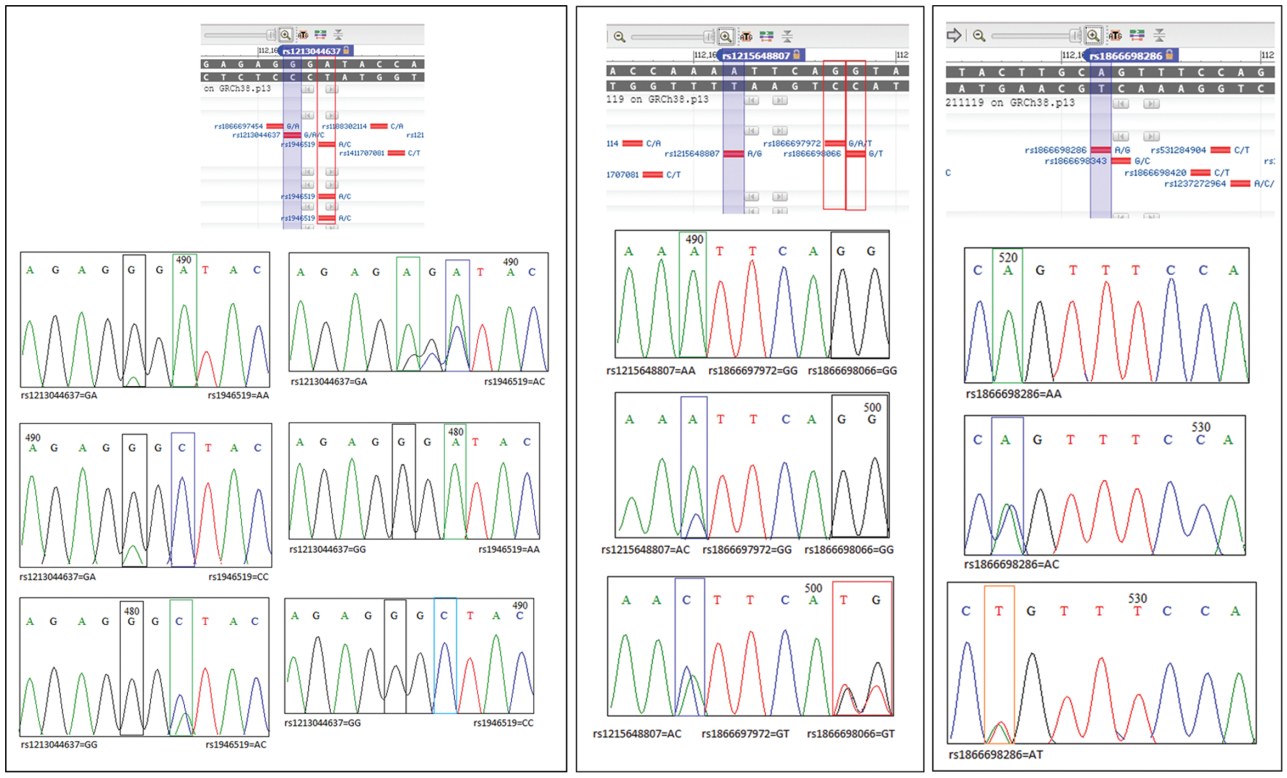

Figure 1: Continued

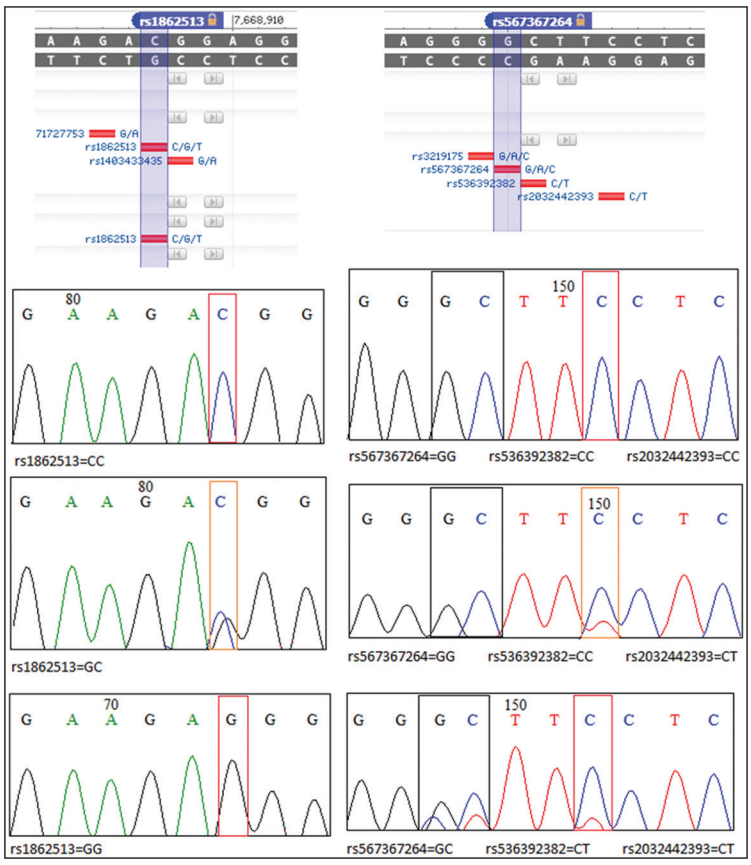

Figure 2: The genotypes of resistin (single-nucleotide polymorphism) in *E. histolytica* patients compared to the control group

Table 3: From resistin (single-nucleotide polymorphisms) in *E. histolytica* patients compared to control group

Genotype of resistin	Odds ratio (OR)	Relative risk (RR)	95% CI (lower–upper)	*P* value	Patient	Control
SNP 1 (rs1862513) CC (wild type)/CG (mutant heterozygous)	2.068	0.720	(0.477–8.973)	$P \leq 0.05$ (0.471)	C (0.58) G (0.42)	C (0.66) G (0.34)
SNP 1 (rs1862513) CC (wild type)/GG (mutant homozygous)	1.750	1.375	(0.275–11.152)	$P \leq 0.05$ (0.658)		
SNP 2 (rs567367264) GG (wild type)/GC (mutant heterozygous)	1.360	1.164	(0.344–5.379)	$P \leq 0.05$ (0.736)	G (0.88) C (0.12)	G (0.9) C (0.1)
SNP 3 (rs536392382) CC (wild type)/CT (mutant heterozygous)	1.360	1.164	(0.344–5.379)	$P \leq 0.05$ (0.736)	C (0.88) T (0.12)	C (0.88) T (0.12)
SNP 4 (rs2032442393) CC (wild type)/CT (mutant heterozygous)	0.952	0.976	(0.056–16.279)	$P \leq 0.05$ (0.774)	C (0.6) T (0.4)	C (0.58) T (0.42)

Table 4: Comparisons of a dependent variable and least significant difference-post hoc test for IL-18 concentration with gene IL-18 polymorphisms

Dependent variable: IL18 LSD		Mean difference	*P* value
SNP 1/(rs1866694757)			
CC	CA↑	-128.51900	0.138
	AA	/	/
SNP 2/(rs940255648)			
GG	GC↑	-107.79806	0.258
	CC	/	/
SNP 3/(rs1037707423)			
CC	CT↑	-14.01779	0.815
	TT	/	/
SNP 4/(rs1946518)			
TT	TG↑	-71.72067	0.317
	GG↑	-119.06442	0.135
TG	TT	71.72067	0.317
	GG↑	-47.34375	0.595
SNP 5/(rs1213044637)			
GG	GA↑	-7.66439	0.911
	AA	/	/
SNP 6/(rs1946519)			
AA	AC↑	-71.72067	0.317
	CC↑	-119.06442	0.135
AC	AA	71.72067	0.317
	CC↑	-47.34375	0.595
SNP 7/(rs1215648807)			
AA	AC↑	-88.45599	0.153
	CC	/	/
SNP 8/(rs1866697972)			
GG	GT↑	-91.05894	0.378
	TT	/	/
SNP 9/(rs1866698066)			
GG	GT↑	-120.23421	0.110
	TT	/	/
SNP 10/(rs1866698286)			
AA	AT	44.20238	0.501
	TT↑	-69.30383	0.295
AT	AA↑	-44.20238	0.501
	TT↑	-113.50621	0.199

Table 5: Comparisons of a dependent variable and least significant difference-post hoc test for resistin level with resistin gene polymorphisms

Reference group	Comparison group	Mean difference	*P* value
SNP 1/(rs1862513)			
CC	CG↑	-432.88830*	0.005* [S]
	GG	196.97970	0.298
CG	CC	432.88830*	0.005* [S]
	GG↑	629.86800*	0.009 * [S]
SNP 2/(rs567367264)			
GG	GC↑	-136.79157	0.293
	CC	/	/
SNP 3/(rs536392382)			
CC	CT↑	-128.36423	0.322
	TT	/	/
SNP 4/(rs2032442393)			
CC	CT	427.77945*	0.019* [S]
	TT	/	/

*significant results

development and progression of *E. histolytica* infection. SNP 2 (rs 940255648), SNP 3 (rs 1037707423), SNP 5 (rs 1213044637), SNP 8 (rs 1866697972), SNP 9 (rs 1866698066), and SNP 10 (rs 1866698286) were found to be protective factors against *E. histolytica* infection progression. Results of the genotype of resistin shown in Table 2 indicated that the mutation in the polymorphism of SNP 1 (rs1862513), SNP 2 (rs567367264), and SNP 3 (rs536392382) demonstrated a risk factor for an intestinal infection in the patient group than in the control group. In contrast, the genotype of resistin polymorphism of SNP 4 (rs2032442393) was shown to be a protective factor.

Association between biochemical markers and gene polymorphisms

Association between IL18 and polymorphisms of IL-18 gene

The result in Table 3 showed that IL-18 gens polymorphisms were never shown any statistical differences with any of the SNPs. The results showed an increase in the level of

IL18 in SNP 1/(rs1866694757) (heterozygous/CA); SNP 2/(rs940255648) (heterozygous/GC); SNP 3/(rs1037707423) (heterozygous/CT); SNP 4/(rs1946518) (heterozygous, homozygous/TG, GG); SNP 5/(rs1213044637) (heterozygous/GA); SNP 6/(rs1946519) (heterozygous, homozygous/AC, CC); SNP 7/(rs1215648807) (SNP 8/(rs1866697972)/AC);/(rs1866698066) (heterozygous/GT); SNP 10/(rs1866698286) (homozygous/TT).

The current study's findings agree with many other studies that discovered relationships between SNPs of IL-18 and susceptibility to different diseases.[9] Qilu Hospital of Shandong University determined that IL-18 (rs1946518) polymorphisms have been shown to have a role in the susceptibility and severity of acute myeloid leukemia (AML) by showing that the bone marrow blasts of individuals having IL-18 polymorphisms are more likely to develop into leukemia (rs1946518). It was shown that patients with the GG or GT genotypes fared better than those with the TT genotype. Individuals carrying the IL-18 (rs1946518) GT or TT genotype had greater plasma IL-18 levels compared to those carrying the GG genotype. Additionally, AML-specific survival was significantly worse in those with the GT genotype of IL-18 (rs1946518).[9]

SNP (rs1946519) IL-18 single-nucleotide polymorphisms were also found to be associated with recurrent spontaneous miscarriage in additive, dominant, and recessive models.[10] Lower serum IL-18 levels were seen between patients and controls and were more pronounced in rs1946519 heterozygous and homozygous genotypes.

Also, Maroufi *et al.*[11] found no significant differences in the genotype frequencies of the rs1946518 C/A polymorphism between breast cancer (BC) patients and healthy controls under the codominant (P = 0.333; OR = 0.95; 95% CI = 0.88–1.51), dominant (P = 0.677; OR = 1.18; 95% CI = 0.69–1.31), recessive (P = 0.522; OR = 0.99). IL-18 rs1946518 and rs1946519 polymorphisms were found to be significantly associated with a predisposition to coronary artery disease in overall combined East Asians.[12]

Also, Kadi *et al.*[13] refer to the change of homozygote wild type to homozygote mutant for (rs1946519) significantly affected the serum IL-18 level, with a median sIL-18 level higher in infants with acute kidney injury. IL-18 is a member of the IL-1 cytokine superfamily and functions as a pro-inflammatory cytokine by activating innate immunity and inflammatory pathways as well as polarizing T cells into T helper 1 (Th1) or T helper 2 (Th2).[14] Macrophages, dendritic cells, keratinocytes, osteoblasts, and IECs mainly produce IL-18.[15] IL-18 was first found to promote interferon—production by anti-CD3-stimulated Th1 cells, especially in conjunction with IL-12. IL-12 is a cytokine that promotes the maturation of Th1 cells.

To add insult to injury, IL-18 suppresses the production of IL-10 and enhances the proinflammatory cytokine tumor necrosis factor-alpha (TNF-α) and T-cell differentiation toward the proinflammatory Th1 phenotype, respectively.[16] Epidemiological evidence suggests that IL-18 may have a role in the etiology of chronic inflammatory illnesses associated with Th1 and Th2 responses.[17]

The IL-18 and IL-1 contribute to host defense against infections by boosting the antimicrobial properties of phagocytes and kicking off Th1 and Th17 adaptive immune responses, all of which may be related to the effects (SNPs) on progression in *E. histolytica* infections.[18] Multiple prior epidemiological studies have shown that IL-18 has a role in the etiology of autoimmune and inflammatory illnesses because of its ability to regulate both innate and acquired immune responses. It is important to highlight that IL-18 may promote T-cell development to the pro-inflammatory Th phenotype by stimulating the production of TNF- and IL-1.[19]

Association between resistin and polymorphisms of resistin gene

The result in Table 4 showed resistin gens polymorphisms were significantly different SNP 1/(rs1862513)(CG/GG)with wild type (CC) and polymorphisms SNP 2/(rs567367264)(GC) with wild type (GG) and SNP 4/(rs2032442393) (CT) with wild type (CC). The results showed an increase in resistin level, while the SNP 3/(rs536392382) (CT), although not show significant differences with wild type (CC), the level of resistin was high too. Also, they have been reported to relate with resistin concentration in some studies.[20,21]

The result of the present study agrees with Hashemi *et al.*[22] Stratification analysis by cancer type revealed that the rs1862513 variant significantly increased the risk of colorectal and BC. Type 2 diabetes susceptibility in the general Japanese population was shown to be linked with the G/G genotype of an SNP (rs1862513) in the promoter region of human resistin.[23] The plasma resistin levels were greatest in those who had the G/G genotype of rs1862513, followed by those who carried the C/G and C/C genotypes. A higher risk of developing type 2 diabetes is associated with the G/G genotype of rs1862513 in RETN, likely because this variant enhances the promoter activity of the gene. While the result disagrees with[24] they showed RETN promoter polymorphism rs34861192 was associated with elevated circulating resistin levels, but rs1862513 was not associated with resistin level.

Allele and genotype frequencies of rs1862513 were not significantly different between rheumatoid arthritis patients and healthy controls (all $P \geq 0.05$) in a Chinese population study.[19] No significant connection was found (all $P > 0.05$) when we looked at the impact of genotypes in dominant and recessive models. Polymorphisms in the resistin gene may influence the production and activity of inflammatory cytokines, which may play a role in the

onset of autoimmune disorders. However, our research has a number of caveats that might potentially affect the reliability of the findings. These include the diversity of our patient population, the severity of their condition, how long it has been treated, and their ethnicity. Therefore, further research is required to replicate the findings, ideally with bigger samples across a variety of populations.[25]

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Ali IKM, Solaymani-Mohammadi S, Akhter J, Roy S, Gorrini C, Calderaro A, *et al*. Tissue invasion by *Entamoeba histolytica*: Evidence of genetic selection and/or DNA reorganization events in organ tropism. PLoS Negl Trop Dis 2008;2:1-6.
2. Abdullah AM. Contamination of fresh vegetables with protozoan parasites, in Duhok City, Kurdistan Region of Iraq. Med J Babylon 2021;18:416-20.
3. Chowdhury RA, Esiobu N, Meeroff DE, Bloetscher F. Different detection and treatment methods for *Entamoeba histolytica* and *Entamoeba dispar* in water/wastewater: A review. J Environ Prot 2022;13:126-49.
4. Tuky HSH, Semender BA. Assessing risk factors and causative organisms of acute diarrhea in children under 5 years in AL-Hindiya, Karbala, Iraq Haider. Med J Babylon 2019;16:117-21.
5. Choudhry N, Petry F, Van Rooijen N, McDonald V. A protective role for interleukin 18 in interferon γ-mediated innate immunity to *Cryptosporidium parvum* that is independent of natural killer cells. J Infect Dis 2012;206:117-24.
6. Deng J, Chen Y, X.u S, Pan F. Elevated serum levels of resistin in inflammatory bowel disease: A meta-analysis. Authorea Prepr 2020:1-9.
7. Ali R, Saud H, Ahmed N, Abed N. The role of resistance and some biochemical parameters in the blood of polycystic ovary syndrome patients. Biochem Cell Arch 2021;21:3525-9.
8. Fareed M, Afzal M. Single nucleotide polymorphism in genome-wide association of human population: A tool for broad spectrum service. Egypt J Med Hum Genet 2013;14:123–34
9. Wang H, Hua M, Wang S, Y.u J, Chen C, Zhao X, *et al*. Genetic polymorphisms of IL-18 rs1946518 and IL-1β rs16944 are associated with prognosis and survival of acute myeloid leukemia. Inflamm Res 2017;66:249-58.
10. Al-Khateeb GM, Sater MS, Finan RR, Mustafa FE, Al-Busaidi AS, Al-Sulaiti MA, *et al*. Analysis of interleukin-18 promoter polymorphisms and changes in interleukin-18 serum levels underscores the involvement of interleukin-18 in recurrent spontaneous miscarriage. Fertil Steril 2011;96:921-6.
11. Maroufi NF, Aghayi E, Garshasbi H, Matin MG, Bedoustani AB, Amoudizaj FF, *et al*. Association of rs1946518 C/A polymorphism in promoter region of interleukin 18 gene and breast cancer risk in Iranian women: A case–control Study. Iran J Allergy Asthma Immunol 2019;18:671-8.
12. Zheng J, Chen T, Lin H. IL-10, IL-18 gene polymorphisms might influence predisposition to coronary artery disease in East Asians: A meta-analysis. Immunol Invest 2020;00:1-10.
13. Kadi FA, Yuniati T, Sribudiani Y, Rachmadi D. The association of rs187238, rs19465518 and rs1946519 IL-8 polymorphisms with acute kidney injury in preterm infants. Biomed 2021;11:43-50.
14. Shaheen IA, Botros SKA, Morgan DS. Detection of expression of IL-18 and its binding protein in Egyptian pediatric immune thrombocytopenic purpura. Platelets 2014;25:193-6.
15. Naik RS, Wala MS. Inflammation, allergy and asthma, complex immune origin diseases: Mechanisms and therapeutic agents. Recent Pat Inflamm Allergy Drug Discov 2013;7:62-95.
16. Veenbergen S, Smeets RL, Bennink MB, Arntz OJ, Joosten LAB, Van Den Berg WB, *et al*. The natural soluble form of IL-18 receptor β exacerbates collagen-induced arthritis via modulation of T-cell immune responses. Ann Rheum Dis 2010;69:276-83.
17. Pastorelli L, Garg RR, Hoang SB, Spina L, Mattioli B, Scarpa M, *et al*. Epithelial-derived IL-33 and its receptor ST2 are dysregulated in ulcerative colitis and in experimental Th1/Th2 driven enteritis. Proc Natl Acad Sci U S A 2010;107:8017-22.
18. van de Veerdonk FL, Netea MG, Dinarello CA, Joosten LAB. Inflammasome activation and IL-1β and IL-18 processing during infection. Trends Immunol 2011;32:110-6.
19. Gao SJ, Zhang L, Lu W, Wang L, Chen L, Zhu Z, *et al*. Interleukin-18 genetic polymorphisms contribute differentially to the susceptibility to Crohn's disease. World J Gastroenterol 2015;21:8711-22.
20. Menzaghi C, Coco A, Salvemini L, Thompson R, Cosmo SD, Doria A, *et al*. Heritability of serum resistin and its genetic correlation with insulin resistance-related features in nondiabetic Caucasians. J Clin Endocrinol Metab 2015;91:2792-5.
21. Asano H, Izawa H, Nagata K, Nakatochi M, Kobayashi M, Hirashiki A. Plasma resistin concentration determined by common variants in the resistin gene and associated with metabolic traits in an aged Japanese population. Diabetol J 2010;53:234-46.
22. Hashemi M, Bahari G, Tabasi F, Moazeni-Roodi A, Ghavami S. Association between rs1862513 and rs3745367 genetic polymorphisms of resistin and risk of cancer: A meta-analysis. Asian Pacific J Cancer Prev 2018;19:2709-16.
23. Onuma H, Tabara Y, Kawamura R, Ohashi J, Nishida W, Takata Y, *et al*. Plasma resistin is associated with single nucleotide polymorphisms of a possible resistin receptor, the decorin gene, in the general Japanese population. Diabetes 2013;62:649-52.
24. Chen BH, Song Y, Ding EL, Manson JE, Roberts CK, Rifai N, *et al*. Association of resistin promoter polymorphisms with plasma resistin levels and type 2 diabetes in women and men. Int J Mol Epidemiol Genet 2010;1:167-74.
25. Li MH, Zhang TP, Li XM, Pan HF, Ma DC. Association of single nucleotide polymorphisms in resistin gene with rheumatoid arthritis in a Chinese population. J Clin Lab Anal 2018;32:1-6.