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Original article

Association of Toxoplasma infection and susceptibility with NLRP1 rs8081261 and rs11652907 gene polymorphism using RFLP among the Egyptian population

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ABSTRACT

Background: Toxoplasmosis is zoonotic protozoal disease that poses a significant risk to human health, with a high incidence rate observed globally. This study aimed to investigate the association between *NLRP1* gene polymorphisms (rs8081261 and rs11652907) and susceptibility to *Toxoplasma gondii* infection by establishment of the groundwork for broader-scale investigations using a novel PCR-RFLP technique. The RFLP findings were confirmed through Sanger sequencing of representative samples, reinforcing the reliability of the RFLP technique. *NALP1 or NLRP1* serves as an inflammatory sensor of the innate immune response to intracellular pathogens, such as *Toxoplasma gondii*. **Methods:** Ninety subjects from Sharkia governorate, Egypt were tested for *Toxoplasma* IgG and genotyped for the rs8081261 and rs11652907 Single nucleotide polymorphisms (SNPs), which have been previously linked to congenital toxoplasmosis. **Results:** The overall seropositivity rate in the study population was 72.2%. The detected genotypes for rs8081261 were *GG* and *AG*, with percentages of 88.89% and 11.11%, respectively, while for rs11652907, the detected genotypes were *TT* and *CT* alleles, with percentages of 85.55% and 14.45%, respectively. The results showed that the (*A*) allele of rs8081261 increased the risk of past infection by approximately 3.64, while the (*C*) allele of rs11652907 increased the risk by 1.31 folds. **Conclusion:** A significant association was found between the level of IgG and both alleles. These findings provide insight into the potential role of these SNPs in different intracellular infections and their mechanisms. Further research is necessary to elucidate the underlying mechanisms associated with the effects of these SNPs.

Introduction

Toxoplasmosis is a cosmopolitan apicomplexan parasitic disease caused by *Toxoplasma gondii* that can infect all warm-blooded animals. Various reports indicate that approximately one third of the world's population has been infected [1, 2]. Among various hosts, the feline particularly cats are the final host where the intestinal tract serves as the specific site for gametogony and sexual reproduction of the protozoan. [3].

The worldwide seropositivity rates of Toxoplasmosis varies significantly, ranging from below 10% to over 90% [4]. African countries have exhibited the highest seroprevalence, ranging from 20.8% to 87.8%, while in Egypt; it ranged from

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2.5% to 67.4% [5]. Other regions have observed lower but still notable seroprevalence rates, such as 38.5% in Oceania, 31.2% in South America, 29.6% in Europe, 17.5% in the USA/Canada, and 16.4% in Asia [6]. Toxoplasmosis in Egypt is considered as a common cause of abortion, congenital abnormalities and complications in pregnant women. The wide spread of the stray cats is one of the main causes of the higher prevalence rate of toxoplasmosis in Egypt [7].

Toxoplasmosis can be transmitted through direct contact with cats or their feces, oocysts in cat faeces contaminating drinking water or unwashed vegetables, consumption of undercooked meat containing tissue cysts, sexual contact, and blood transfusion. Vertical placental transmission can also lead to congenital toxoplasmosis [3], [8], [9]. The disease has serious symptoms ranged from mild to severe encephalitis, hydrocephalus, chorioretinitis, and in some cases, fetal death or abortion [3].

The protozoan has the ability to secrete specific effector proteins that mimic the host immune response as successful adaptation. This mechanism enables it to persist chronically within host cells [10], [11]. *Toxoplasma* infection in host cells' cytosol can trigger the activation of the inflammasome complex. In this complex, Nod-like receptor (NLR) family function as a sensing system for the innate immune system. It detects the presence of pathogen-associated molecular patterns (PAMPs) by surveying the cytosol for foreign invaders. The activation of inflammasomes is crucial for protecting the host and limiting the dissemination of the parasite [12], [13], [14], [15]. Inflammasome activation has also been linked to pyroptosis mediates the heavily secretion of the proinflammatory cytokines and the flow of cellular signaling during *Toxoplasma* infection [10], [16] .

In addition to the inflammasome-mediated immune response, some genetic factors have been found to play a role in the susceptibility to toxoplasmosis. The nucleotide-binding oligomerization domain-like receptor protein 1 (*NLRP1*) is a member of the NLR family that acts as an inflammatory sensor of the innate defense against intracellular microbes like *T. gondii* [13], [16], [17]. Single nucleotide polymorphisms (SNPs) in the *NLRP1* gene have been associated with increased susceptibility to toxoplasmosis, particularly in the congenital form of the disease[18]. In a study by Witola et al. (2011), two *NALP1* (*NLRP1*) SNPs (rs8081261 and rs11652907) were found to be significantly associated with congenital toxoplasmosis [19]. These findings suggest that genetic factors may play a significant role in determining an individual's susceptibility to toxoplasmosis.

The NLR family consists of 22 members and can be categorized into four subfamilies *(NLRA, NLRB, NLRC, and NLRP*) based on their N-terminal effector domain structures [20]. Each of the NLR is composed of three domains [21], [10]. *NLRP1* is the only member of the *NLR* family that possesses an additional C-terminal caspase activation and recruitment domain (CARD). It exhibits a dichotomous effect in human diseases. For example, it can modulate the gut microbiome for protective purposes. However, it also promotes nervous, cardiac, pulmonary disorders, and cancer by enhancing inflammation [14], [15], [22]. It is believed that *NLRP1* leucine-rich repeats can recognize the danger signal to trigger the inflammasome's assembly [12], [23], [24]. *NLRP1* gene of rat genome is present in a robust orthologous region (*Toxo1*) to a region in the human genome which responsible for susceptibility/resistance against toxoplasmosis [10], [25], [26]. Witola et al. (2011) conducted a study to examine the relationship between *NLRP1* and congenital toxoplasmosis. They analyzed a set of 23 tag-SNPs in the human *NLRP1* gene using the HapMap project (http://www.hapmap.org) and bioinformatics tools. The results revealed that only two SNPs, rs8081261 and rs11652907, were associated with congenital toxoplasmosis [10].

In this study, we developed a Restriction Fragment Length Polymorphism (RFLP) PCR assay to examine the two previously reported SNPs (rs8081261 and rs11652907) identified in the Witola et al. study[10]. The RFLP PCR method is a simple, fast, and cost-effective genotyping technique suitable for laboratories with limited resources. It does not require sophisticated bioinformatics tools and can be easily interpreted using agarose gel electrophoresis. We employed this method to investigate the correlation between anti-*Toxoplasma* IgG levels, which signifies previous exposure or past infection, the inflammasome *NLRP1* gene SNPs (rs8081261 and rs11652907), and the demographic characteristics of the study population. The utilization of the cost-effective and accessible RFLP PCR assay enables researchers in resource-constrained countries to investigate the potential

impact of these SNPs on different infectious diseases. This can contribute to a deeper understanding of the genetic factors that influence disease susceptibility and assist in the development of targeted interventions and treatments.

SUBJECTS AND METHODS:

1. Patients and clinical samples:

This study included a total of 90 subjects who were recruited from Zagazig University Hospitals between January and March 2021. The total population size of *Toxoplasma* infection suspected patients at zagazig university hospital is (145) patients, prevalence of seropositive IgG among toxoplasma patients is (81%) [27] so the calculated sample size is 90 patients using Open Epi program with confidence level 95% and power 80% [28]. The recruited subjects were from both genders, Urban and rural and varying age. Written informed consent was obtained from each participant, and the study was approved by the Zagazig Faculty of Medicine Institutional Review Board (IRB) with approval number 9087/21-1-2021. The research was conducted in compliance with the ethical guidelines outlined in the World Medical Association's Declaration of Helsinki for studies involving human participants. No specific grants were received for this research from public, commercial, or not-for-profit funding agencies.

Aseptically, two milliliters (ml) of venous blood were collected from each subject. One ml of the blood was transferred to a plain vacutainer tube for the purpose of serum separation, which would be used for human anti-*Toxoplasma* IgG ELISA testing. The remaining one ml of blood was collected in an EDTA vacutainer tube to be used for PCR-RFLP analysis. All collected blood samples were stored at a temperature of -20°C for future use.

2.Quantitative determination human anti-*Toxoplasma* IgG ELISA:

Toxoplasma IgG level was measured for each sample using Indirect immunoenzyme assay solid phase ELISA kit (Human anti-*Toxoplasma* IgG ELISA, Biokit, Spain) following the manufacturer's instructions.

3.Determination of inflammasome *NLRP1 (*rs8081261 and rs11652907*)* gene polymorphism:

3.1 Designing RFLP assay:

The gene sequences of each SNP were retrieved from the GeneBank database using the following URL: https://www.ncbi.nlm.nih.gov/snp/. Primers were designed using the Primer designing tool provided by NCBI (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). The selection of restriction enzymes was performed using Restriction Mapper version III (http://www.restrictionmapper.org/). The final selection was validated using NEB cutter V2.0 software (http://nc2.neb.com/NEBcutter2/).

3.2 Polymerase chain reaction

The amplification of each NLRP1 gene target was carried out using direct blood PCR with the Phusion™ Blood Direct PCR Master Mix from Thermo Scientific™, USA.

3.2.1 Assay procedure

a. A final total volume of 50 µl for the PCR reaction was reached after addition of 2.5 µl of whole blood and 0.5 µM concentration of each specific primer designed for the human NLRP1 (rs8081261) gene and NLRP1 (rs11652907) gene. **Gene 1 (rs8081261) primer sequence was: F-AGAGCCAACAGGTGGATTGC and R-TGGGTGAGATTGATGCTGGA. Gene 2 (rs11652907) primer sequence was: F-TGGCTGCAAGATCCAAGGAG and R-ACGCTTGGATGCCAGACTAC.**

b. PCR amplification was carried out using a thermal cycler (Veriti®96-Well Thermal Cycler, Applied Biosystems, Singapore). The thermal profile employed for both genes consisted of an initial denaturation step at 98°C for 5 minutes, followed by 35 cycles of denaturation at 98°C for 5 seconds, annealing at 60°C for 10 seconds, and extension at 72°C for 40 seconds. Finally, a final extension step at 72°C for 1 minute was performed.

3.2.2 post-amplification analysis using Agarose Gel Electrophoresis

a. In each well of a 1.5% agarose gel, 5 µL of each PCR product and 4 µL of the GeneRuler 100 bp Plus DNA Ladder (Thermo Scientific™, USA) were directly loaded. The loaded samples were then subjected to electrophoresis.

b. After running the samples for approximately 45 minutes, the PCR products were visualized under ultraviolet light **Fig. 1**.

3.2.3 Restriction fragment length polymorphism

In order to investigate the polymorphism of the human NLRP1 gene (rs8081261) in all study participants, the 577 bp PCR product was subjected to digestion using the AflIII restriction enzyme (Thermo Scientific™, USA) following the manufacturer's protocol. The expected outcome of

this digestion was the generation of three distinct banding patterns corresponding to three genotypes:

1. Homozygous G/G genotype, resulting in two bands at 482 bp and 95 bp.
2. Heterozygous G/A genotype, resulting in three bands at 577 bp, 482 bp, and 95 bp.
3. Homozygous A/A genotype, which would generate a single band at 577 bp. (Fig. 2).

Similarly, the polymorphism of the human NLRP1 gene (rs11652907) was investigated by digesting the 607 bp PCR product with the TaiI restriction enzyme (MaeII or HpyCH4IV, Thermo Scientific™, USA) according to the manufacturer's protocol. The expected banding patterns were as follows:

1. Homozygous T/T genotype, resulting in a single band at 607 bp.
2. Heterozygous T/C genotype, resulting in three bands at 607 bp, 501 bp, and 106 bp.
3. Homozygous C/C genotype, resulting in two bands at 501 bp and 106 bp.

A limitation in this study is the very rare G allele of human *NLRP1* (rs11652907), which cannot be detected by the used TaiI (HpyCH4IV) restriction enzyme.

Digested fragments were identified using gel electrophoresis on a 2% Agarose gel.

To validate the Restriction Fragment Length Polymorphism (RFLP) method, amplicons from a heterozygous and homozygous sample representing each available genotype were purified using the QIA quick PCR Purification Kit following the manufacturer's protocol.

The purified amplicons were subsequently subjected to sequencing using an ABI 3100 Gene Analyzer (Applied Biosystems, Massachusetts, USA) and a Big Dye Terminator Cycle Sequencing Ready Kit. For each sequencing reaction, one of the primers used for PCR amplification was utilized.

Results

1. Demographic characteristics:

Sex, residence, occupation, age and *Toxoplasma* IgG level of the subjects are shown in ***Table* 1.**

2. Quantitative determination human anti-*Toxoplasma* IgG ELISA:

Anti-*T. gondii* IgG antibodies were detected in 72.2 % of the cases. It was obvious that there was non-significant relation between genotypes of either human *NLRP1* (rs8081261) gene or NLRP1 (rs11652907) gene polymorphisms with either Sex, age, occupation or residence of patients ***Table 2.*** **Error! Reference source not found.**There was a statistically non-significant relation between Genotype *AG* and susceptibility to toxoplasmosis. *AG* indefinitely increased the risk by 3.86 folds. The *A* allele was associated with 3.64-fold increase in susceptibility to toxoplasmosis. The association was less clear in case of *NLRP1* (rs11652907). The genotype *CT* increased risk of susceptibility to toxoplasmosis by 1.15 folds only. There was a statistically non-significant relation between *C* allele and past exposure to toxoplasmosis. *C* increased risk of susceptibility to toxoplasmosis by 1.31 folds, ***Table* 3**. **Figure 1 &2**.

3. Relation between SNP 1,2 and *Toxoplasma* IgG level among the studied patients:

There was a significant relation between *AG and CT* genotypes and level of *Toxoplasma* IgG among the studied patients (IgG was significantly higher among those with AG and CT genotype) ***Table*** .

Table 1. Distribution of patients according to demographic characteristics**:**

		N=90	**%**			**N=90**	**%**			
Sex	**Female**	**62**	**68.9**	**Residence**	**Rural**	**36**	**40**	**Age (years)**	**Mean ± SD**	**30.26 ± 12.123**
	Male	**28**	**31.1**		**Urban**	**54**	**60**			
Occupation	**Farmer/Worker**	**17**	**18.9**	***Toxoplasma* IgG**	**Negative**	**25**	**27.8**		**Range**	**6 – 56**
	Housewife	**23**	**25.6**		**Positive**	**65**	**72.2**			
	Student	**22**	**24.4**		**Mean ± SD**	**80.119 ± 142.989**				
	Semi/professional	**28**	**31.1**		**Median (Range)1**	**18 (8 – 604)**				

Table 2. **Relation between SNP1 & SNP2 and demographic characteristics of the studied patients:**

Genes		SNP1				SNP2			
Alleles		AG	GG	χ2/Z	P	CT	TT	χ2/Z	P
		N=10 (%)	N=80 (%)			N=13(%)	N=77(%)		
Sex	Female	5 (50)	57 (71.2)	1.873	0.171	8 (61.5)	54 (70.1)	0.383	0.536
	Male	5 (50)	23 (28.8)			5 (38.5)	23 (29.9)		
Residenc	Rural	2 (20)	34 (42.5)	Fisher	0.305	2 (15.4)	34 (44.2)	Fisher	0.067
	Urban	8 (80)	46 (57.5)			11 (84.6)	43 (55.8)		
Occupation	Farmer/Worker	0 (0)	17 (21.2)	3.66	0.301	0 (0)	17 (22.1)	6.679	0.083
	Housewife	2 (20)	21 (26.2)			2 (15.4)	21 (27.3)		
	Student	4 (40)	18 (22.5)			6 (46.2)	16 (20.8)		
	Semi/professional	4 (40)	24 (30)			5 (38.5)	23 (29.9)		
Age	Mean ± SD	24.9±15.284	30.93±11.64	-0.663	0.507	21.31±13.738	31.77±11.239	-1.945	0.052
	Median	29	32			19	32		
	Range	8 – 37	6 – 56			6 – 37	14 – 56		

*χ^2 Chi square test Z Mann Whitney test **p≤0.001 is statistically highly significant*

Table 3. Relation between *A/G & C/T* genotypes and toxoplasmosis among the studied patients.

			Total N=90 (%)	Toxoplasmosis		Test		Crude Odds Ratio (95% Confidence Interval)
				Cases	Control	χ^2	p	
				N=65(%)	N=25(%)			
Gene1	Gene	AG	10 (11.1)	9 (13.8)	1 (4)	Fisher	0.273	3.86 (0.46 – 32.15)
		GG	80 (88.9)	56 (86.2)	24 (96)			1 (Reference)
	Allele		N=180	N=130	N=50			
		A	10 (5.6)	9 (6.9)	1 (2)	Fisher	0.288	3.64 (0.45 – 29.54)
		G	170 (94.4)	121 (93.1)	49 (98)			1 (Reference)
Gene 2	Gene	CT	13 (14.4)	10 (15.4)	3 (12.0)	Fisher	>0.999	1.15 (0.38 – 5.99)
		TT	77 (85.6)	55 (84.6)	22 (88.0)			1 (Reference)
	Allele		N=180	N=130	N=50	Fisher	>0.999	1.31 (0.34 – 4.95)
		C	13 (7.2)	10 (7.7)	3 (6)			1 (Reference)
		T	167 (92.8)	120 (92.3)	47 (94.0)			

χ^2 Chi square test p>0.05 is statistically non-significant.

Table 4. Relation between SNP1,2 and *Toxoplasma* IgG level among the studied patients.

Genes	Toxoplasma IgG		Test	
	Mean ± SD	Median (range)	Z	P
	N=7 (%)	N=60 (%)		
AG	442.86 ± 200.98	604 (228 – 604)	-4.35	<0.001**
GG	37.8 ± 36.491	12 (8 – 123)		
CT	353.33 ± 217.084	228 (228 – 604)	-2.681	
TT	67.313 ± 127.288	18 (8 – 604)		0.007*

Z Mann Whitney test **p≤0.001 is statistically highly significant

Figure 1. A: PCR products of the amplified NLRP1 genes. In Lane 1-3, the PCR products of NLRP1 (rs8081261) are observed at 577 bp. In Lane 4-7, the PCR products of NLRP1 (rs11652907) are observed at 607 bp. Lane 4 contains the DNA size marker (100 bp ladder, Thermo) for reference. **B&C:** PCR products of the NLRP1 (rs8081261) Gene after Restriction using AflIII enzyme. **B:** Lane 1 has DNA size marker (100 bp ladder, Thermo) with fragment sizes shown in bp. All lanes 2-15 showed homozygous (*G / G*) sample with fragment sizes of 482 bp and 95 bp. **C:** Lane 1 has DNA size marker (100 bp ladder, Thermo), Lane 2 showed homozygous *(G / G)* while Lane 3 & 4 shows 3 bands at 577 bp, 482bp and 95 bp corresponding to Heterozygous G/A genotype. **D:** PCR products of the NLRP1 (rs11652907) gene after restriction using, TaiI (MaeII enzyme). Lane 1 and Lane 13 contain the DNA size marker (100 bp ladder, Thermo) with fragment sizes indicated in bp. Lane 7 and Lane 9 represent Heterozygous (*C / T*) samples, showing fragment sizes of 607 bp, 501 bp, and 106 bp. Lane 2-6, Lane 8, and Lane 10-12 display a single undigested band with a size of 607 bp, indicating Homozygous (*T/T*) gene alleles.

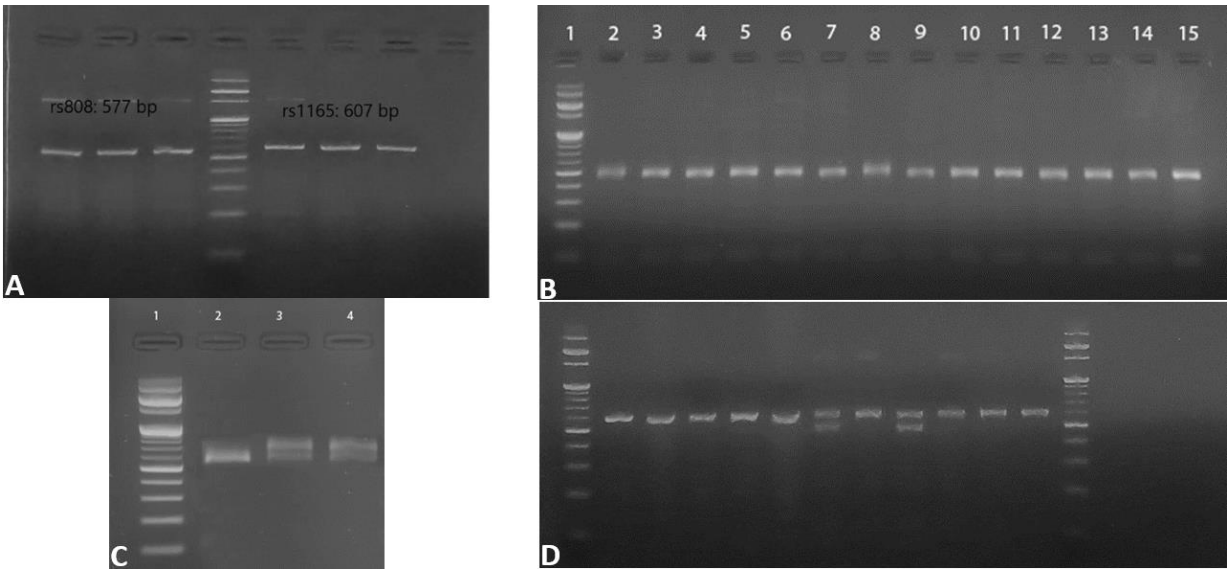
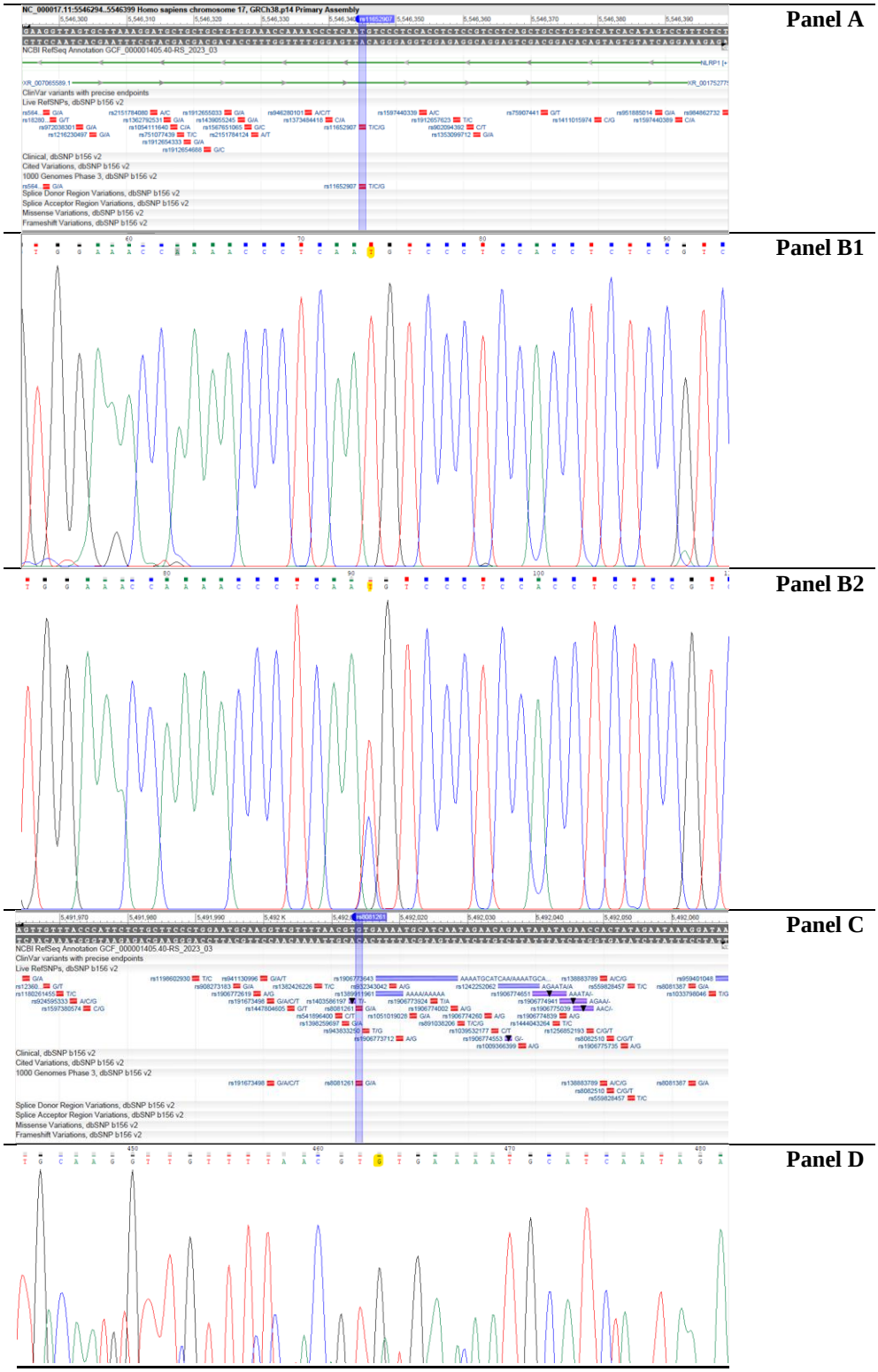


Figure 2. **(A)** It shows the *NLRP1* gene and (rs11652907) position on chromosome (*GRCh38.p14*). **(B)** The direct DNA sequencing patterns of Genotype (*TT) TT* and (*TC) TC*, point mutations. **In panel (B1),** a Genotype (*TT*) is depicted. **Panel (B2)** represents the (*TC*) genotype. These mutations were identified and confirmed to align with the results obtained from the Restriction Fragment Length Polymorphism (RFLP) technique for the patients. **Panel (C)** displays the NLRP1 gene and the position of rs8081261 on chromosome *GRCh38.p14.* **Panel (D)** presents the direct DNA sequencing patterns of the Heterozygous Genotype *(AG)* point mutation. This mutation was identified and confirmed to match the results obtained from the Restriction Fragment Length Polymorphism (RFLP) technique for the same subject.



Discussion

Toxoplasmosis is globally significant infectious disease[3] . Several studies conducted in Egypt have reported the seroprevalence rate of toxoplasmosis ranging from 23.8% in Dakahlia[29] , 52.4% in Tanta among abattoir workers [30], to 59.6% in blood donors at Mansoura University Hospital and identified associated risk factors [31]. However, this study found a higher prevalence rate in Sharkia Province. The seropositivity rate of toxoplasmosis among the studied population was found to be 72.2% which is close to 73% prevalence rate in Alexandria[32]. The notable variation in the seroprevalence among different governorates in Egypt can be attributed to several factors such as the health status of the recruited subjects, the testing methods used, the sample size and the level of exposure to cat feces [33]. It has been reported that 95% to 97% of cats in Egypt are seropositive for toxoplasmosis, and their continuous contamination of the soil with sporulated infective oocysts contributes to the spread of the infection[5], [34]. The socio-economic conditions, particularly occupation, can also influence the infection rate. Sharkia province, being an agricultural governorate, has a long history of contact between human and farm as well as stray animals. This continuous contact acts as a crucial factor in the spread of contamination and the high rate of *Toxoplasma* infection in the area. Host-parasite interactions play a crucial role in determining susceptibility to infectious diseases. During early acute infection, the host's native and acquired immunity are stimulated to control the rapid replication of the parasite's tachyzoites. Among the host's native immune reactions, Nod-like receptors (*NLRs*) are well-characterized and consist of numerous members[12]. One important member is the *NLRP1* inflammasome; located on chromosome 17 and has several single nucleotide polymorphisms (SNPs), that can either activate or suppress the inflammasome during the pathogenesis of the disease [12], [14], [15]

However, there are very few studies investigated the association between NLRP1 SNPs, rs8081261 and rs11652907, and congenital toxoplasmosis, these studies supported the genetic factors' role in determining an individual's susceptibility to toxoplasmosis. In rodents, *T. gondii* acts as a stimulus for NLRP1, triggering an immune response and regulating parasite-induced cell death. Knockdown of NLRP1 has been found to attenuate the production of interleukins 1β, 18, and 12 induced by *T. gondii* 10,13,25,35,36.These findings highlight the role of *NLRP1* and its SNPs in modulating the immune response to *T. gondii* infection. Limited number of studies have been conducted to investigate the role of these mutations in susceptibility to infectious diseases. The development of a simple and cost-effective method would facilitate the study of the effects of these mutations on susceptibility to *Toxoplasma* and other infectious diseases. The PCR-RFLP technique serves as an excellent example of a molecular tool that can be utilized in non-advanced laboratories in low socioeconomic countries with limited resources.

In our study, we have developed a PCR-RFLP assay that provides an easy method for detecting the association between *NLRP1* gene polymorphisms (rs8081261 and rs11652907) and susceptibility to *Toxoplasma gondii* infection. Through the utilization of bioinformatics tools, we have designed new primers and selected appropriate restriction enzymes that can effectively distinguish between the different alleles.

Our findings provide strong support for the association between the mutant alleles rs8081261 and rs11652907 of *NLRP1* and *Toxoplasma* infection. Previous research has also explored the relationship between these alleles and susceptibility to congenital toxoplasmosis. Witola et al. 2011, conducted genotyping of 23 selected tag-SNPs of the *NLRP1* gene in 124 congenitally infected children and found that rs8081261 and rs11652907 were linked to congenital toxoplasmosis. This finding emphasized the role of *NLRP1* in modulating the innate immune response against *Toxoplasma* infection, leading to cell death and disintegration.

In this study, *Toxoplasma* IgG was chosen as a simple serological marker and practical method serves as a convenient and reliable method to evaluate past exposure to *Toxoplasma* infection in this particular study. Compared to more complex techniques such as PCR or IgM detection, measuring IgG antibodies provides a relatively straightforward and accessible approach.

Although our study did not show statistically significant results, it aligned well with previous findings, demonstrating a strong association between past toxoplasmosis infection, as

indicated by IgG levels, and the studied SNPs. The presence of the *A* allele of SNP1 and the C allele of SNP2 increased the risk of susceptibility to toxoplasmosis. Additionally, there was a significant association between IgG levels and the *A and C* alleles of NLRP1 rs8081261 and rs11652907, respectively.

We validated the RFLP method used in this study by performing Sanger sequencing on a representative sample of both heterozygous and homozygous samples for each SNP. The results demonstrated a complete match between the developed PCR-RFLP assay and the sequencing data. This validation process confirms the accuracy and reliability of our developed method in detecting and distinguishing the different genotypes of the studied SNPs.

The limitation of this study is the inability to detect the very rare *G* allele of human *NLRP1* (rs11652907) using the TaiI (HpyCH4IV) restriction enzyme that was used. This allele would require a different enzyme, such as AsuI or AvaII, for digestion and detection. Therefore, the presence of the *G* allele could not be evaluated in our analysis.

The current approach offers a valuable opportunity to investigate the role of the two SNPs in infectious diseases, particularly which characterized by an innate immune response against the intracellular pathogens. It is particularly relevant in regions with limited resources, where access to advanced molecular techniques may be limited. Additionally, it is important to investigate the effects of these mutations on the protein structure or gene expression, particularly in relation to interleukins 1β, 18, and 12 induced by *Toxoplasma gondii* and affected by *NLRP1* knockdown. By elucidating these aspects, we can gain valuable insights into the mechanisms underlying disease pathogenesis and potentially identify novel therapeutic targets.

Declaration of interest

The authors declare no competing interests.

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Data availability

All data generated or analyzed in this research work are available in this manuscript.

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