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RESEARCH ARTICLE PUJ

Evaluation of DNA Damage by DNA Fragmentation and Comet Assays in Experimental Toxoplasmosis with Virulent Strain

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ABSTRACT

Background: The outcome of toxoplasmosis is strongly dependent on the virulence of *Toxoplasma gondii* strains. Infection of mice with the high-virulence *T. gondii RH* strain induces inflammatory cytokine over production and causes their rapid death.

Objective: The outcome of toxoplasmosis is strongly dependent on the virulence of *Toxoplasma gondii* strains. Infection of mice with the high-virulence *T. gondii RH* strain induces inflammatory cytokine over production and causes their rapid death.

Material and Methods: *T. gondii* induced apoptosis was studied, and DNA damage in spleen and peripheral blood leukocytes was evaluated by analysis of DNA fragmentation. The level of DNA damage was assessed by the extent of DNA migration in peripheral blood leukocytes using comet assay. This study was carried out on 2 groups (II and III) of mice experimentally infected with *T. gondii* RH tachyzoites strain, sacrificed at 2nd and 7th days post-infection (PI), respectively. In addition, none infected control group (I) was sacrificed at 7th day PI.

Results: Infection with high virulence *T. gondii* strain caused apoptosis and high level of DNA damage especially with prolongation of acute infection. Greater DNA fragmentation and intensity of apoptotic laddering was recorded in splenocytes and blood leukocytes of group III compared to those of group II. In infected groups, there was significant increase in DNA migration in comet tail in peripheral blood compared with the control group. Strongly damaged spots were significantly higher in group III than in group II. Additionally, caspase 3 immunostain showed positive reaction in splenic section of infected groups.

Conclusion: Infection with virulent strains of *T. gondii* caused DNA damage with a genetic hazard to infected blood leukocytes. Apoptosis detected in splenocytes explains the rapid lethality of infected mice during acute infection.

Keywords: *Toxoplasma gondii,* Gel Electrophoresis, Apoptosis, Leukocytes, Caspase 3.

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INTRODUCTION

Toxoplasmosis is a wide spread disease caused by the obligatory intracellular protozoan parasite of Class Apicomplexa, *T. gondii*. It is ubiquitously distributed infecting a wide range of warm blooded hosts, and over a billion individuals constituting more than 20% of the human population worldwide[1].

Human infection occurs through two main routes, ingestion of undercooked meat containing cysts of the parasite and ingestion of oocysts passed into the environment by cats feces[2]. Acute stage of toxoplasmosis is characterized by rapidly multiplying tachyzoites. Within 2-3 weeks, infected individuals develop a potent immune response that effectively controls the growth of the parasite. Some tachyzoites develop into dormant bradyzoites that multiply at a much lower rate than tachyzoites and become progressively enclosed in a dense matrix surrounded by a thick wall, forming a tissue cyst[3-5].Tissue cysts can persist for several years in brain, heart and skeletal muscles of those previously infected with *T. gondii*[6].

Infection of immunocompetent human is most often asymptomatic with lifelong persistence of the parasite[7]. However, *T gondii* may lead to life threatening disease in fetuses or newborns from infected mothers or after reactivation of dormant parasites in immunocopromised patients[4,8,9]. Reactivation of tissue cysts in these individuals leads to the recrudescence of *T. gondii* infection[6].

T. gondii has three distinct strains; high virulence strains (type I) as the RH and low virulence strains (type II and type III) such as ME49. The former are lethal to mice during acute infection, and the latter do not kill the host but establish a long-term chronic infection[10].Type II strains are most prevalent in animals and are most commonly associated with human toxoplasmosis. Type I strains are often associated with severe congenital toxoplasmosis and ocular toxoplasmosis[11].

The host immune response is crucial for controlling *Toxoplasma* growth during acute phase of infection and for preventing the reemergence of acute infection in chronically infected individuals[12]. The initial immune response caused by infection with a pathogen is later down regulated by diverse mechanisms such as the activation-induced cell death by apoptosis and production of modulatory cytokines (e.g., IL-10 and transforming growth factor β), which counteract the pathological effect of excessive pro-inflammatory factors[13]. Acute infection by *T. gondii* is accompanied by Th1 cytokines secretion where IL-12 is released early in infection and potentiates the production of gamma interferon (IFN-γ) by natural killer cells (NK) and T lymphocyts[14]. Over stimulation of the immune response is an important feature of the pathological effect of acute toxoplasmosis[10].

Apoptosis is a mechanism of programmed cell death that plays a critical role in regulation of host response during infection with viruses, bacteria and parasites[15]. Increased apoptosis may assist dissemination of intracellular pathogens or induce immunsuppression. However, in many cases, apoptosis may help to eradicate pathogens from the host[16,17]. There are two pathways of apoptosis of cells; one triggered by death signals and another involving the mitochondria. Activation of death receptors triggers the cleavage of caspase-8, whereas release of cytochrome C from mitochondria activates caspase-9; both pathways eventually converge to activate the execution caspase, caspase-3. Effector caspases in turn attack a variety of target molecules essential for cellular structural integrity thus mediating cell death[18]. Modulation of apoptosis during toxoplasmosis was reported by several contrasting views, where several authors reported inhibition of apoptosis in infected host cells, principally in macrophages, which facilitates the intracellular development of *T. gondii*[12,15,19]. However, others described apoptosis of activated T lymphocytes in toxoplasmosis[10,20].

Single cell electrophoresis assay also known as comet assay is a rapid, simple, visual and sensitive technique for measuring and analyzing DNA breakage in mammalian cells[21-23]. The aim of this study is to determine whether toxoplasmosis caused by RH virulent strain would cause DNA damage with assessment of its extent in experimentally infected mice.

MATERIAL AND METHODS

Study type: Case control experimental study.

Animals: Experimental animals, composed of 30 laboratory bred Swiss albino mice (each weighing 25-30g), were housed under specific pathogen-free conditions in the animal house of Toxicology Department in Faculty of Medicine, Menoufiya University. The mice were fed by commercial complete food mixture and tap water for drinking, and maintained under controlled conditions of lighting and temperature.

Parasite and animal infection: Tachyzoites of RH virulent strain *T. gondii* were obtained from the Department of Parasitology, Faculty of Veterinary Medicine, Cairo University, Egypt. Mice were infected with $1x10^3$ *T. gondii* tachyzoites/mouse by intraperitoneal infection[17].

Experimental design: Mice were divided into 3 groups of 10 mice each: Group I included control non-infected mice; group II included infected mice sacrificed on day 2 PI; group III included infected mice sacrificed on day 7 PI. Infected mice were subjected to:

- The peritoneal and thoracic cavities of anaesthetized mice were exposed and the left ventricle was punctured with a fine needle for collecting peripheral blood: RBCs were lysed from heparinized blood by RBC lysing buffer (0.8 g NH_4CL, 0.6 g $NaHCO_3$ and 0.12 g EDTA in 100 ml)[24].
- The remaining leukocytes were washed with RPMI-1640 culture medium (Biochrom KG, Berlin, Germany) supplemented with 10% fetal calf serum and served for detection of DNA damage.
- Spleens were gently removed. A portion of each spleen was fixed in Bouin's solution and processed in paraffin for immunohistochemical examination by caspase-3 stain[25]. The sections were mounted on ploy-L-lysine coated slides. The avidin-biotin-peroxidase method was performed using primary monoclonal antibody against caspase-3 (1:100 dilutions, Neomarkers,

Fremont, USA), on deparaffinized sections in which the endogenous peroxidase activity was blocked by 0.3% solution of hydrogen peroxidase in phosphate buffered saline (PBS) at room temperature for 10 min. After microwave treatment, primary antibody was applied for 30 min at room temperature and washed in PBS. Linking antibody and streptavidin–peroxides complex (Neomarkers, Fremont, USA) were added consecutively for 10 min at room temperature and washed by PBS. The peroxidase activity was visualized with diaminobenzidine (Sigma, St. Louis, USA) applied for 5 min. Appropriate positive and negative controls were also labeled with the primary antibody. Positive cells for caspase-3 immune stain show intracellular brown punctuations. The remaining portion of each spleen was homogenized and RBCs were lysed using RBC lysing buffer. The resulting cell suspension became enriched with T cells that were kept at -80 ˚C for DNA fragmentation assays[12].

Assessment of DNA damage

1) **Agarose gel electrophoresis for peripheral leukocytes and splenic T cells:** Peripheral blood leukocytic cells and splenic T cells were harvested by centrifugation and lysed by 600 ml lysing buffer (50 mM NaCl, 1mM Na_2 EDTA, 05% SDS, pH 8.3) (Sigma). The cell suspension was shaken gently and kept overnight at 37˚C. DNA was extracted[26] by addition of 200µl saturated NaCl to the samples, then shaken gently and centrifuged at 12000 rpm for 10 min. The supernatant was transferred to a new Eppendorf tube and DNA was precipitated by addition of 700 µl cold isopropanol, followed by centrifugation. The sediment was washed in 500 µ, 70% ethyl alcohol for 8 min at 12000 rpm. The supernatant was decanted and the resulting pellet was gently re-suspended in 50 µl or appropriate volume of TE buffer (10 mM Tris, 1 mM EDTA, pH 8) then supplemented with 5% glycols for 30 min. To get rid of RNA, an appropriate volume of RNase was added and incubated at 37˚C for 1 h. The DNA samples were mixed with 6x loading buffer and analyzed on a 2% agarose gel stained with 1 mg/ml ethidium bromide (Sigma)[24]. Results were quantified by densitometric analysis of bands corresponding to fragmentated DNA on a BioDoc II digital imaging system (Biometra, Gottingen, Germany) at Faculty of Science, Meoufiya University.

2) **Single cell gel electrophoresis (comet assay):** This technique was performed to study DNA damage in peripheral leukocytes. The procedure of comet assay involved the following steps according to Sasaki *et al.*[27] with some modifications[28].

- Encapsulation: A volume of 5 ul peripheral blood was added to 120 ul 0.5% low melting point agarose at 37°C, and then layered onto a pre-coated microscopic slide with 1.5% normal melting agarose and covered with a cover slip. The agarose was gelled at 4°C, and then the cover slip was removed.
- Lysis: The slides were immersed in lysing solution (2.5 mM NaCl, 100 mM EDTA, 10 mM Tris-HCL buffer, pH 10, 1% sodium sarcosinate with 1% Triton X-100 and 10% DMSO) (Sigma, St. Louis ,MO) for ~ 1 h.
- Electrophoresis: The slides were washed in distilled water to remove all salts and then placed on a horizontal gel electrophoresis unit filled with fresh electrophoretic buffer (1mM disodium EDTA and 200 mM NaOH, pH 13). Electrophoresis was conducted for 20 min at 25V and 30 mA. Slides were then stained with ethidium bromide (Sigma). Slides were examined with a Carl Zeiss fluorescent microscope (Jena, Germany) equipped with a 510 nm excitation filter and a barrier filter of 590 nm.

Results interpretation: In damaged cells, breaks appear as fluorescent tails extending from the core towards the anode. The tail length reflects the amount of DNA breakage in the cell[21]. Therefore, the migrated nuclear DNA was considered as a damaged DNA spot. The migration was evaluated by measuring the basal nuclear DNA and migrating DNA in 50 randomly selected cells/ sample. The damaged DNA spots were further classified into two types: the first one was considered a damaged spot when the length of the migrated fragments was less or equal to the diameter of the basal nuclear DNA; the second type was considered a strongly damaged spot when the length of the comet was more than the diameter of the basal nuclear DNA[29].

Statistical analysis: Mean and standard deviation (mean ±SD) were calculated for each parameter studied. The statistical analysis of differences in DNA damage, as measured by the comet assay, was carried out using *t*-test, and multifactor analysis of variance (ANOVA) were used to check the significant differences. The critical level for rejection of the null hypothesis was considered to be a *P*-value of 5%. All analyses were performed with the SPSS 10.0 version software packages.

Ethical consideration: All procedures in the present work were performed in accordance with the regulations and guidelines of experimental animal studies.

RESULTS

DNA damage analysis

DNA fragmentation in peripheral leukocytic cells and splenocyte T cells by agarose gel electrophoresis: The optical density of DNA extracted from peripheral leukocytic cells of infected mice showed fragmented DNA laddering or apoptotic bands with higher intensity of DNA fragmentation in group III, than in group II. Apoptotic bands were also observed in spleen T cells of both groups with higher intensity of fragmentation in group III (Table 1). The results also showed that DNA fragmentation in leukocytes of group II (Lane 6) were more observable than those in splenocyte T cells of the same group (Lane 3) (Figure 1).

Comet assay: *T. gondii* infected groups (II and III) showed significant increase in the mean number of both damaged and strongly damaged spots (43.4±1.17 and 44.2±2.01, respectively) in leukocytes compared with control group (I) (7±1.69) (P<0.001) (Table 2). Moreover, no strongly damaged spots were detected in control group (Data not shown in table).

Groups II and III presented different values of mean tail moment with statistically significant difference between both infected groups as regard DNA migration, the mean numbers of normal (intact nuclear DNA, Figure 1), damaged and strongly damaged spots (Table 3). Damaged spots were significantly higher in group II while strongly damaged DNA spots were significantly higher in group III (Figures 2, 3, 4). In addition, there was a positive significant relation between the extent of DNA damage and duration of infection, where more DNA damage occurred in mice that were killed after 7 days PI (group III) than those killed after 2 days PI (group II).

Immunohistochemical caspase-3 stain: The number of splenic cells showing positive reaction was higher in mice of group III than those of group II indicating extensive apoptosis in spleens of this group (Figures 5-6a,b). Moreover, it was observed that apoptosis involved non invaded cells rather than the infected ones (Figure 7).

Table (1): Maximal optical density (MOD) of DNA fragments in leuekocytes and splenocytes of studied groups

Base pairs	Maximal optical density (MOD) of DNA fragments					
	Splenocytes			Leukocytes		
	Control (L1)	Group II (L3)	Group III (L4)	Control (L2)	Group II (L6)	Group III (L5)
Intact DNA	215.12	186.57	166.58	225.37	167.26	159.48
600 bp	7.341	32.299	59.665	16.503	62.246	88.317
400 bp	6.814	24.671	61.479	7.305	71.123	94.541
200 bp	2.251	21.335	57.533	3.865	60.934	97.886

L: Lane number in figure (1)

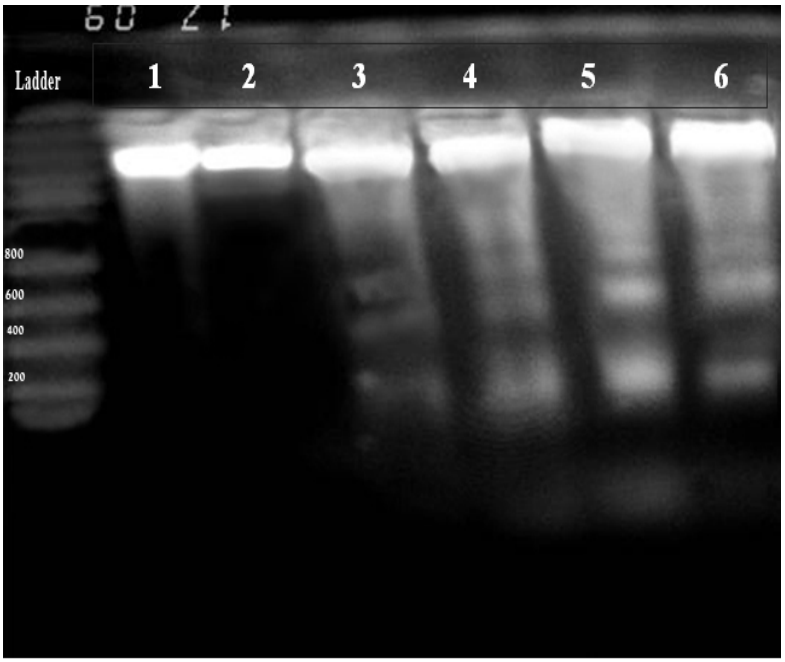


Figure (1): Fragmentation patterns of DNA from mouse spleen and peripheral leukocytes from each group:
First lane: DNA ladder, **Lane 1:** Control splenocytes; **Lane 2:** Control leukocytes; **Lane 3:** Splenocytes (group II), **Lane 4:** Splenocytes (group III), **Lane 5:** Leukocytes (group III) and **lane 6:** Leukocytes (group II). Results were reproducible upon repetition.

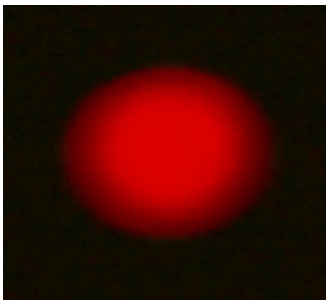


Figure (2): Intact nuclear DNA (×1000).

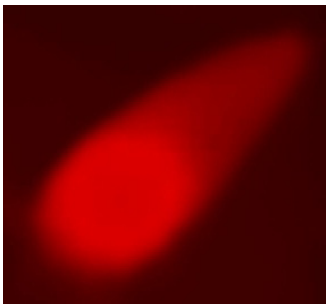


Figure (3): DNA damage expressed by tail moment in a mouse leukocyte cell from group II showing damaged spot (×1000).

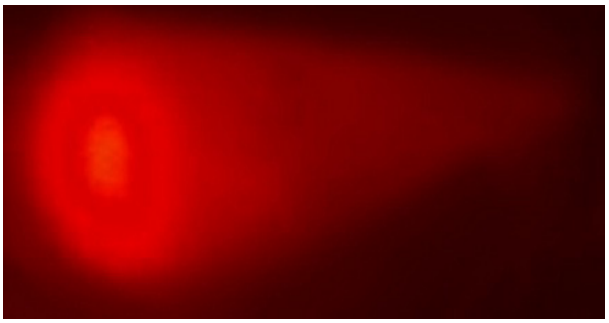


Figure (4): DNA damage expressed by tail moment in a mouse leukocyte cell from group III showing strongly damaged spot (×1000).

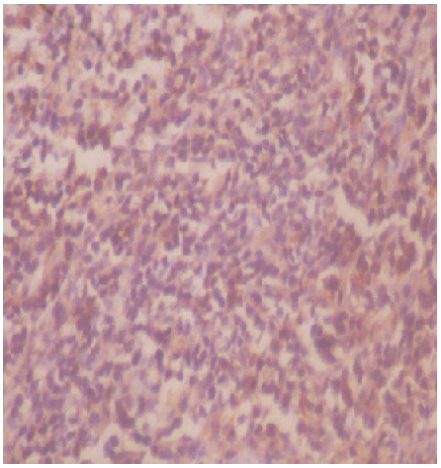
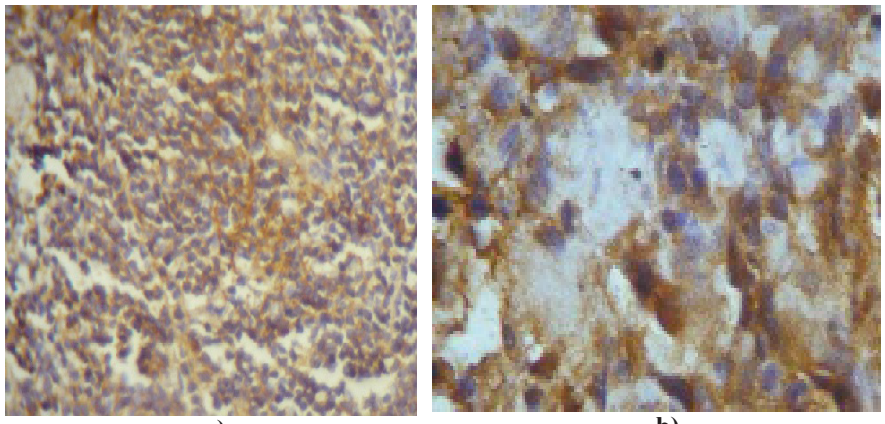


Figure (5): Splenic section in a mouse from group II showing mild positive immune reaction for caspase 3 expression (×200).



a) b)

Figure (6): Splenic section in a mouse from group III showing strong positive immune reaction for caspase 3 expression (a) × 200, (b) ×400.

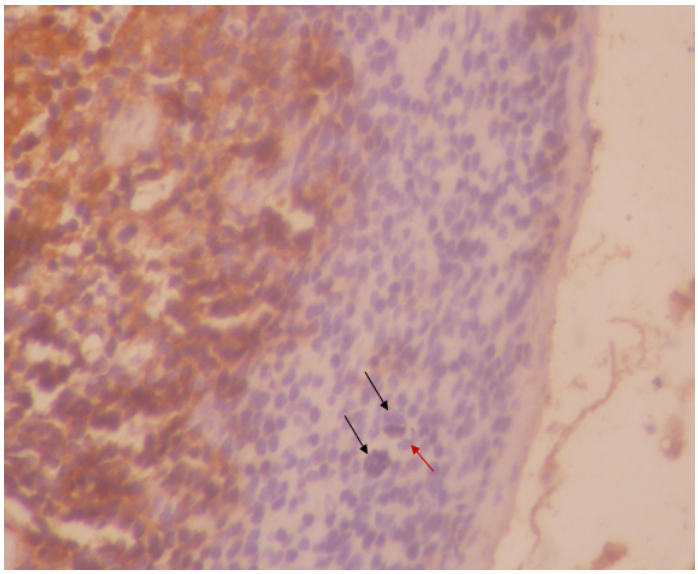


Figure (7): Splenic section in a mouse from group III showing strong positive immune reaction for caspase 3 expression to the left and negative immune reaction to the right. *T. gondii* tachyzoites multiplying in splenic cells (black arrow) and free tachyzoite (red arrow) (×200).

Table (2): Mean number of both damaged and strongly damaged DNA migration in group I (control group) versus groups II and III (*T. gondii* infected groups)

DNA damage	Group I	Group II	Group III	ANOVA test	*P* value
Migration spots	7±1.69	43.4±1.17	44.2±2.01	1639.2	P_1<0.001 P_2<0.001 P_3>0.05

Migration spots in GI were only damaged spots while in GII & GIII, they were both damaged and strongly damaged spots
P_1 = GI & GII, P_2 = GI & GIII, P_3 = GII & GIII.

Table (3): Mean number of damaged and strongly damaged DNA migration in group II versus group III

DNA Migration	GII	GIII	*t* test	*P* value
Normal	6.3±1.16	5.6±2.01	0.69	0.49
Damaged spots	29.4±1.26	13.3±2.06	21.08	<0.001
Strongly damaged spots	14.3±1.83	32.0±2.05	20.35	<0.001

t test = Mann Whitney test

DISCUSSION

The host immune response is crucial for controlling *T. gondii* parasite growth during the acute phase of infection. Type I strains are uniformly lethal to mice, suggesting that they induce inadequate immune control or are endowed with destructive properties that directly cause pathology[10]. In our study, DNA damage triggered during acute infection with high virulent RH strain tachyzoites was analyzed in peripheral blood leukocytes and splenocyte T cells, totally by DNA fragmentation assays using agarose gel electrophoresis and in individual leukocytes by single cell gel electrophoresis.

Previous studies reported that cells of both lymphoid and myeloid lineages were triggered by *T. gondii* parasite to undergo apoptosis during acute infection[13]. In the present study, 7 days PI (group III) splenocyte T cells showed strongly detectable apoptosis in agarose gel electrophoresis, while mild apoptosis was detected after 2 days PI (group II). This suggests that apoptosis of splenocyte T cells is strongly related to the parasite burden in the spleen since the virulent type I RH strain has great ability to disseminate and to replicate in the host[10]. It was previously suggested that apoptosis of T lymphocytes in *T. gondii* is related to virulence and density of parasites in the host[12]. In another study which compared the growth and dissemination of virulent and non virulent (PTG) *T. gondii* strains *in vivo*, it was found that the peak parasite numbers were achieved in 6 days with RH strain infection versus 8 days with PTG infection; and the peak numbers of parasite were achieved in 4 days after high dose challenge with PTG strain parasites[10]. The mentioned study also reported detection of significant DNA damage in liver and lymphoid tissues during lethal RH strain infections, and similar damage induced with a high dose of PTG strain parasite; while two dose challenge with PTG strain parasites induced little tissue pathology during acute infection. Other studies also reported detection of major populations of apoptotic cells, at 8th day[30] and 7th day[13] PI, in spleens of mice infected with the high virulence type I RH strain

Regarding DNA damage of peripheral blood leukocytes triggered by *T. gondii* infection, our study demonstrated higher intensity of DNA laddering on agarose gel electrophoresis in group (III) on 7th day PI than in group (II) on the 2nd day PI. DNA laddering of splenocyte T cells of group II was of lower intensity when compared with that of peripheral leukocytes of the same group. Moreover, it was observed that DNA damage appeared earlier in peripheral blood than splenocyte T cells, probably due to high initial replication of RH strain parasite in leukocytes and there after dissemination of the parasite to spleen and other tissues. It was previously suggested that the early interaction of the parasite with the innate immune response is critical to trigger the lethal cascade of stimulating cytokines[10].

The extent of DNA damage in peripheral leukocytes at the level of the individual leukocytic cell was assessed by single cell gel electrophoresis. This was expressed by tail moment in leukocytic cells of infected mice with significant values in group II and group III when compared to the control group (Table 2). Group III also showed a significant increase in DNA migration and higher number of strongly damaged spots than group II (Table 3).

In a previous study that assessed DNA damage in peripheral blood, liver and brain cells by *T. gondii* infection, using the comet assay, it was reported that DNA damage occurred exclusively in leukocytic cells and not in liver and brain cells[28]. Another study employing comet assay reported similar DNA damage induced by *Leishmania chagasi* in peripheral blood and spleen cells[31]. The oxidative DNA damage in *Plasmodium* infected RBCs was also monitored by the same assay[32].

The host response against *T. gondii* infection including the cellular-immune host-response is implicated in early infection. Studies have shown that macrophages, neutrophils and dendritic cells respond directly to the parasite and produce IFN-γ, TNF-α, nitric oxide and reactive oxygen species[28]. Although, these help in eradication of *T. gondii*, they expose the organs to certain endogenous genotoxic agents which induce DNA damage in peripheral blood cells. Other studies added that infection with high virulence *T. gondii* is associated with excessive levels of pro inflammatory cytokines such as IFN- γ and extensive splenic lymphocyte death, and hypothesized that splenic apoptosis may be induced by the high levels of pro inflammatory cytokines[10,13,30].

In the present work, immunohistochemical caspase 3 stain was used to assess apoptosis in spleens of *T. gondii* infected groups. Apoptosis was detected in stained sections obtained from group III mice involving-different cell types in the spleens. Moreover, apoptosis wasn't detected in infected cells but was rather detected primarily at sites distal from parasite resident cells (Figure 7). Other reports advocated that the extent of tissue inflammation in acute toxoplasmosis with virulent RH strain, is often dispropionate to the presence of parasites, suggesting that the resulting pathology is partially immune mediated. Moreover, it was concluded that infection by *T. gondii* protects the resident cells from apoptosis but cell death is efficiently activated in non infected cells[10,33]. Another study found also that the proportion of infected cells didn't rise above 2% regarding the recorded massive splenic apoptosis which strongly argues against the direct effect of *Toxoplasma* as an inducing agent for apoptosis[13]. The findings of Nishikawa *et al.*[17] indicated that nitric oxide (NO) and other additional soluble factors derived from parasitized host cells play a critical role in the induction of nearby host-cell apoptosis.

In our study the stained splenic sections obtained from group II showed mild positive reaction of caspase 3 indicating mild apoptosis of splenocytes. Similar results were recorded in a previous study employing TUNEL assay where no positive cells were detected in spleen of 5 days post *T. gondii* infection[13]. The mild apoptosis detected in group II may be attributed to lower production of IFN-γ due to challenge by lower numbers of parasites in early infection. Also it could be attributed to the required time for caspase activation to be maximal. It can be postulated that the high virulence of RH strain, with its great ability to disseminate and replicate[10], and its association with induction of extremely high levels of proinflammatory cylokines such IL-12, TNF-α, IFN- γ and IL-18[17], caused the high level of apoptosis detected in group III of this study. The positive caspase 3 immunostain in splenic sections demonstrated partly the pathway of apoptosis that occurred with *T. gandii* infection with RH strain. It is worthwhile to note that caspase activation is followed by chromatin fragmentation and mitochondal alteration with resulting cytoplasmic acidification and cell death[34].

Several studies included different explanations of apoptosis that occurred in acute toxoplasmosis with RH strain, and which pathway is involved in this process. Suggestions were that there are multiple pathways of apoptosis induced by parasitic infection[10,13,19]. The high level of proinflammatory cytokines associated with RH strain infection were found to induce expression of death effectors molecules such as Fas ligand (FasL) and TNF receptor1 (TNFR1) where TNF- TNFR and Fas-FasL interaction result in activation of Caspase-8[35]. It was also shown that Casp-9 was activated during acute infection suggesting the role of a mitochondrial induced apoptosis pathway[36]. It was noted that this pathway may be activated as a response to stress with NO as candidate mediator[37]. Moreover, NO produced at high level by activated macrophages can be metabolized by auto-oxidation to form peroxynitrite which is potentially toxic to DNA, and may dissociate to form hydroxyl radical leading to DNA oxidation or/and strand breaks. NO can also induce modulation of enzymes involved in the maintenance of genomic integrity[38,39]. Although human infection with type I virulant strains of *T. gondii* is not lethal, there were few reports about the relationship between *T. gondii* and tumorigenesis including primary ocular tumor, meningioma, leukemia and lymphoma[40].

Regarding the findings of this study it may be concluded that *T. gondii* strains of high virulence could cause DNA damage and could induce genotoxicity in infected mice peripheral blood with high level of apoptosis in splenocytes explaining the rapid lethality of infected mice during acute infection.

Author contribution: NM Harba proposed the research idea and wrote the manuscript. **AF Afifi** proposed the study design and reviewed the manuscript. Both authors shared in performing the laboratory work and interpretation of the results.

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تقييم مدى تلف الحمض النووى الناتج عن عدوى الفئران بالسلالة القوية لطفيل التوكسوبلازما جوندى

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الهدف من البحث: دراسة حدوث الموت المبرمج للخلية ومدى تلف الحمض النووى فى خلايا الطحال وكرات الدم البيضاء نتيجة الإصابة الحادة لطفيل التوكسوبلازما (سلالة RH) فى فئران التجارب.

خطوات البحث: تم عدوى الفئران بسلالة (RH) القوية لطفيل التوكسوبلازما جوندى حيث قسمت الفئران إلى مجموعتين بالإضافة إلى مجموعة ضابطة (كل مجموعة 10 فئران). تم أخذ عينات من الدم و الطحال من المجموعة الأولى بعد يومين من العدوى وبعد 7 أيام من العدوى فى المجموعة الثانية ودراستها بالفصل الكهربى على (Agarose gel)،وكذلك على مستوى الخلية الواحدة فى كرات الدم البيضاء بواسطة الفصل الكهربى للخلية الواحدة على جيل (Comet assay) أيضاً تم دراسة تفاعل كاسباس 3 على الطحال.

النتائج: أثبتت الدراسة تأثيراً على الحمض النووى وكذلك موت مبرمج للخلايا نتيجة الإصابة بالسلالة القوية لطفيل التوكسوبلازما فى الطحال وخلايا الدم البيضاء، وأن هذا التأثير يكون أكبر مع زيادة فترة الإصابة. كذلك على مستوى الخلية الواحدة فى كرات الدم البيضاء ثبت أن هناك تأثيراً مدمراً على الحمض النووى.

الاستنتاج: سلالة (RH) لها تأثير مدمر على الحمض النووى فى الجهاز المناعى للمصاب والتى قد تكون السبب فى الموت السريع عند الفئران المصابة مع وجود مخاوف جينية فى كرات الدم البيضاء.