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RESEARCH ARTICLE

Insights into immunopathology and triggering of apoptosis in chronic cerebral toxoplasmosis using murine models

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ABSTRACT

Background: toxoplasmosis is a cosmopolitan protozoan disease with a wide range of neuropathology. Recent studies identified its potential association with several mental disorders e.g. schizophrenia dependable on apoptosis in their pathogenesis. We investigated value of toxoplasmosis to induce apoptosis of the neuronal cells. Methods: per-orally infected C57BL/6 mice with 15-20 cysts of the avirulent *T. gondii* Beverly strain at 9-11 weeks of age were examined 12 weeks later during parasite establishment. Distributions of the parasite's cysts and the histopathological lesions in the brains were analyzed using Image J software. Relative expression of TNF-α and iNOS of cell-mediated immunity (CMI), Bax (pro-apoptosis) and Bcl-2 (anti-apoptosis) were all assessed using immunohistochemistry. Results: higher parasite burden was seen in the forebrain with p value $\leq$ 0.05. Dramatically increased TNF-α, iNOS, and Bax expressions with Bax/Bcl-2 ratio 2.42:0.52 were reported (p value $\leq$ 0.05). The significant correlation between Bax data and different CMI biomarkers including TNF-α and i-NOS was evaluated. Interestingly, no significant correlation was seen between TNF-α, iNOS, Bax and Bcl-2 expressions and location of the parasite. However, Bax/Bcl-2 ratio was statistically correlated with CMI biomarkers and whole sample mean parasite burden, p value $\leq$ 0.05. Conclusion: Chronic toxoplasmosis exhibits an immense pro-apoptotic signal on the cerebral tissues of experimental mice.

Keywords: Cerebral; toxoplasmosis; TNF-α; Bcl-2; Bax

INTRODUCTION

Toxoplasma gondii (*T. gondii*) is a apicomplexan protozoa that infects warm-blooded vertebrates; Aves and mammals, including human-beings (Dubey *et al*., 2008). Infections with the parasite despite often being asymptomatic, *T. gondii* can cause grave morbidity and death in humans and animals. *T. gondii* infection is so devastating thanks to the three main routes of transmission including placental transmission, ingestion of infected animal tissues containing bradyzoites, and ingestion of the parasite's oocysts due to environmental contamination (Carme *et al*., 2002; Jones *et al*., 2012).

The most infection site is within cerebral cells, wherein the low-virulent strains of the parasite are in the form of cysts containing hundreds of bradyzoites in a sluggish replicating state (Berenreiterová *et al*., 2011). Postnatally acquired toxoplasmosis despite being supposed to be latent in immunocompetent subjects (Mortensen *et al*., 2007; Bouscaren *et al*., 2018; Flegr & Horacek, 2020) several studies related mental diseases to high anti-toxoplasma titers (Torrey *et al*., 2012; Flegr & Escudero, 2016) making the real impact of the parasite on the surrounding cerebral cells a precisely questionable point. For example, *Toxoplasma*-seropositive schizophrenia patients expresses more eminent positive schizophrenia symptomology (Karabulut *et al*., 2015). Typically for this subset of patients there had been documented alterations in cerebral morphology such as reduction in the gray matter of the frontal and the temporal cortices, median cingulate, thalamus, and caudate (Daryani *et al*., 2010; Arias *et al*., 2011; Okusaga *et al*., 2011).

Immune scenario in *T. gondii* is carried out primarily by IL-12 from the stimulated dendritic cells which activates NK

and T cells to synthesize interferon-gamma (IFN-γ) to orchestrate the whole immune matter in concern with the cell-mediated immunity. This sequentially involves stimulation of inducible nitric oxide synthase (i-NOS) enzyme and the production of Tumor necrosing factor-α (TNF-α) (Johnson *et al*., 1992, Lüder *et al*., 2003; Bando *et al*., 2018).

Up to date, way of control exerted by TNF-α and i NOS to hinder the persistence of the toxoplasma still a matter of study. In 2014, a review article published by *Olmos and Lladó* linked high expression of TNF-α to excito-toxicity and neuro-inflammation. TNF-α has been reported to mediate the release of cytochrome c from the mitochondria and induce oligomerization of Bak-Bax into a high molecular mass protein complex in mitochondrial membranes (Degenhardt *et al*., 2002). Also, NO and oxygen spp. had been linked to demyelination in a previous review article (Smith *et al*., 1999). Neuronal apoptosis had been deduced to associate TNF-α dependent i NOS release (Heneka *et al*., 1998; Combs *et al*., 2001).

Bcl-2 family had been documented to play a pivotal role in inducing or suppressing intrinsic apoptotic pathways caused by mitochondrial dysfunction (Chipuk *et al*., 2004). Bax and Bcl-2 are two major members of Bcl-2 family with a crucial role in the progression or inhibition of intrinsic apoptosis generated from mitochondrial dysfunction. Therefore, the refinement between pro- and anti-apoptotic factors in Bcl-2 family can determine the survival fate of the cells.

Triggering of apoptosis by infections despite being distressing to the host, cell death appears to be an imperative way for the eradication of the intracellular pathogens (Williams, 1994). However, it is not surprising to discover that parasites, viruses, and bacteria have evolved tactics and several strategies to overwhelm this programmed cell death (Friedrich *et al*., 2017). During replication and establishment of *T. gondii* infection the parasite induce a media of anti-apoptotic scent in their host cells via targeting pro-apoptotic activity of Bax and Bak to suppress the intrinsic apoptogenic role of mitochondria such as selective degradation of Bcl-2 members, redistribution of cytochrome C from mitochondria to the cytosol, and delaying of caspase activation. Moreover, extrinsic apoptosis induced by Fas-Fas-ligand pathways was also found not to be altered (Carmen *et al*., 2011; Wu *et al*., 2016; Chu *et al*., 2017). Yet, most studies concerted with the parasitized cells while little is known about the bystander host cells.

We aimed in the present study to validate toxoplasmosis as an apoptosis triggering model for mental health studies. Our experiment manipulated the parasite weight and its associated cell mediated immunity versus expression of pro and anti-apoptosis factors and defined Bax/Bcl-2 ratios in the bystander cerebral cell during established cerebral toxoplasmosis.

MATERIALS AND METHODS

Animals and Experimental Design

Ten unisex (male) 9-11 weeks old immune-competent mice of average weight 35-40 gm of C57BL/6 strain were utilized for the experiment as it entails especially i-NOS activity (one of the utilized biomarkers in the study) to control chronic *T. gondii* similarly to humans. It is worthy to mention that the research team avoided using some mice strains such as BALB/c mice as it is resistant to *T. gondii* in a mechanism independent of the iNOS enzyme (Schlüter *et al*., 1999; Mahmoudvand *et al*., 2016). Age and sex matched mice were used as healthy controls in the current study.

The experimental supplements were acquired from the Laboratory Animal House interconnected to the Parasitology Department in Theodor Bilharz Research Institute, Egypt. Outbred mice were put into full terms of proper housing conditions according to the "Guidelines for the Care and Use of Laboratory Animals" and were accepted by the Institutional Animal Care and Use Committee, Cairo University, recorded by CU/III/F/52/19. Animal care involved balanced diet formula, all sanitary conditions, elimination of dead bodies, adjusted breeding temperature (32°C), light pattern (12 h dark/12 h light), and suitable humidity. The serological check-up was periodically achieved by the research unit to assure that the murine models are pure of any other communal pathogens such as viral hepatitis (Szabo & Finney, 2017). A well-trained laboratory technician terminated the experiment on the 12 weeks post-infection (104 days) by cervical dislocation of the sample study.

Parasite Inoculation

Cystogenic strain of *T. gondii*, Beverly strain (type-II) (El-Zawawy *et al*., 2015), was obtained from the National Research Center, Giza, Egypt. Cysts for use in the experiment were obtained from murine model sacrificed by cervical dislocation 8 or above weeks after it had been infected; brains were smashed manually using the tissue grinder, Teflon pestle provided in a sterile Falcon tube. Brains homogenates were then suspended in 2 mL of Hanks' Balanced Salt Solution (HBSS). A 20 µL wet mount sample was to be inspected via the light microscopy, in a magnification power of 40x and the parasite's cysts were counted; in this accordance, one cyst in a 20 µL was considered to be correlated with 100 cysts in the whole 2 mL sample suspension (the detective limit of the infective sample). In this study, the infection was carried out per-oral by administrating a suspension of 0.25 mL (0.9% NaCl) that contained 15–20 cysts of the low virulent Beverly strain. Brains of the infected mice in the two groups were then harvested 12 weeks later. Classical laboratory signs of acute toxoplasmosis in the form of arched posture, coarse rough fur, and lethargy were not documented post-infection as it's a chronic strain (Berenreiterová *et al*., 2011).

Quantification of Histological Sections Containing the Parasite

Using hematoxylin and eosin stained cerebral tissue cut sections and Olympus microscopy (40X) images were taken for all brain areas gathered from the infected murine. Images were introduced into Image J software to be gridded as such to assign and compute the toxoplasms within certain squares with defined and automatically calculated pixel area. Wherever, the host cells' and parasites' nuclei can't be discerned counting was dismissed. The mean cysts number (C/N), and the mean pixel areas (A/N) were attained to calculate the average no. of parasite's cysts per pixel^2 using this equation (Ferguson & Murray, 1971; Moon *et al*., 2013; Russ & Russ, 2017):

$$\frac{C/N}{A/N}$$

C: no. of *Toxoplasma* cysts; N: number of deployed squares; A: area/point.

Immunohistochemistry

Formalin-fixed and paraffin-embedded cerebral murine tissue preparations were adjusted for the process. The study followed the mouse on Mouse (MOM) protocol and the kit instructive brochure for the immune staining; in this regard, the tissue cut sections were incubated in Hydrogen Peroxide

Block stock solution included in the Thermo Scientific Ultra Vision kits for 10-15 min. Hence, the nonspecific background staining was blocked.

We escorted down the following primary monoclonal antibodies of rodent origin to detect the following individual biomarkers: (1) Anti-Murine Tumor Necrosis Factor-alpha (TNF-α cytokine) antibody: monoclonal un-conjugated murine IgM, kappa antibody clone DBM15.28 (#Mob502) of high affinity to 17-26 kDa protein that react with cytoplasmic and secreted extracellular TNF-α (2) Anti-Inducible nitric oxide synthase (iNOS) enzyme antibody: polyclonal IgG antibody (#MBS9406022) of rabbit origin specific to the peptide sequence nearby the phosphorylation site of tyrosine 151 in human iNOS enzyme; (3) Anti-Bax antibody: murine polyclonal IgG antibody (#ab216494) specific to mitochondrial and cytoplasmic BAX aa sequence of murine origin conjugated to keyhole limpet hemocyanin; (4) Anti-Bcl-2 antibody: polyclonal IgG to Bcl-2 (#ab59348) of rabbit origin specific to the total endogenous BCL-2 levels.

The primary antibody was diluted in 1:200 applied and incubated with the tissues. In whatever biomarker and relying on the percepts of several previous experiments, the dilution of the primary antibody was adjusted in pH 7.4 (10mmol/L $CaCl_2$, 1% BSA and 0.1% NaN_3) and TBS of 20 mmol/L. Each of the tested tissue cut sections was tested for a single supreme biomarker. Tissue cut sections were then allowed to be incubated with the applied primary antibodies overnight in a temperature adjusted at 37°C.

Thereafter biotinylated goat anti-polyvalent secondary antibody was applied on the tissue cut sections and allowed to be incubated for 10 min. adjusted at 25°C to bind with the primary antibody. The final staining was performed in a solution of diaminobenzidine tetrahydrochloride (DAB) (34 mg imidazole, 49 mL TBS-buffer, 1mL 30% DAB, 17 µL, and 30% hydrogen peroxide) for 10-20 min. EconoTek HRP (DAB) Anti-polyvalent kits (# AEX080) were purchased from ScyTek Laboratories, Logan, Utah, USA. Each step in the whole immunohistochemistry staining process was followed by PBS wash four to six times at 25°C for 5-10 minutes each. The tissue nuclei were stained with Mayer's hematoxylin as a counterstain for 1 min. The extra stain was washed afterward with tap water (El-Aal *et al*., 2015). Control slides were performed where the step of primary antibody was dismissed.

Real-Time Quantitative Morphocytometry

Leica Qwin Analyzer, 500 images (LEICA Imaging System Ltd., Cambridge, England) which is a digital real-time quantitative photocytometry was used in the present study for the pathological analysis and the quantitative real-time morphometric measurement. Immune staining was automatically calculated in ten fields in each tissue cut section at low (10x) and high magnification powers (100x). All recorded values were saved for fairly statistical analysis (El-Aal *et al*., 2015).

Statistical Methods

Data were introduced via Microsoft Excel (2013) and analyzed statistically using version 24 of the Statistical Package for the Social Sciences (SPSS). Simple descriptive statistics in the form of the median interquartile range were used to summarize the skewed quantitative data; in addition to the frequencies for the qualitative data (percentiles). Mann-Whitney and Spearman's rho correlation tests were used to compare the quantitative variables of abnormal distribution. P-values ≤ 0.05 were considered significant. R-value was considered negative or positive according to its sign where the results of <0.5 assures the weak correlation, 0.5-0.7 are of moderate correlation, and > 0.7 refers to the strong correlation (Chan, 2003a, 2003b, 2003c).

RESULTS

T. gondii established several niche-like cystic lesions in the cerebral tissues (Figure 1). The lesions are remarkable for the parasite's chronicity and contain hundreds of bradyzoites encased by cyst wall. No significant histological changes can be noted but for some inflammatory cells. Since the parasite was not isolated out of tissues micrometry was not performed to evade false measurement. Unifying the parasite strain and infection dose contributed to the insignificant variations in the total parasite counts among the experimental mice *p*- value > 0.05.

As much as 80% of the examined brain regions were confined with tissue cysts; however, toxoplasma's burden steadily increased in the forebrain. Ratios of mean counts of the parasite's cysts to the mean pixel area in the forebrain tissue cut-sections were of insignificant differences (*p*-value >0.05) while being of significantly different *p*-values ≤ 0.05 when compared with other cerebral areas e.g. brain-stem in (Figure 2). The study displayed the histological and immune-stained comparisons between two fixed cerebral areas; amygdala in the forebrain and pons in the brain-stem.

- Bioassay of Cell-Mediated Immune Response (CMI) (i-NOS and TNF-α)

Compared with healthy mice, the number of iNOS and TNF-α positive cells were much higher in the examined brains of

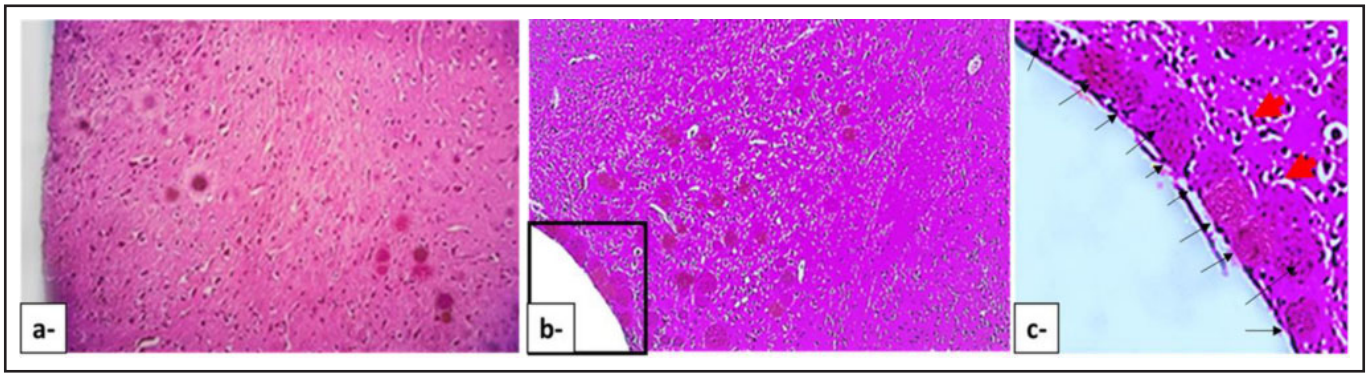

Figure 1. Hematoxylin & eosin stain shows intra-cerebral *Toxoplasma* cysts in Beverly strain (type-II)-infected C57BL/6 mice (40X), 12th weeks post-infection. a: Pontine part of the hind brain with scattered cysts of variable sizes (b). Amygdala region of the forebrain stacked with cysts containing hundreds of bradyzoites. Note the dot-like nuclei at black arrow heads embraced in the parasite's cysts and the infiltrating inflammatory cells (red head arrows) in (c); a cropped magnified figure from b.

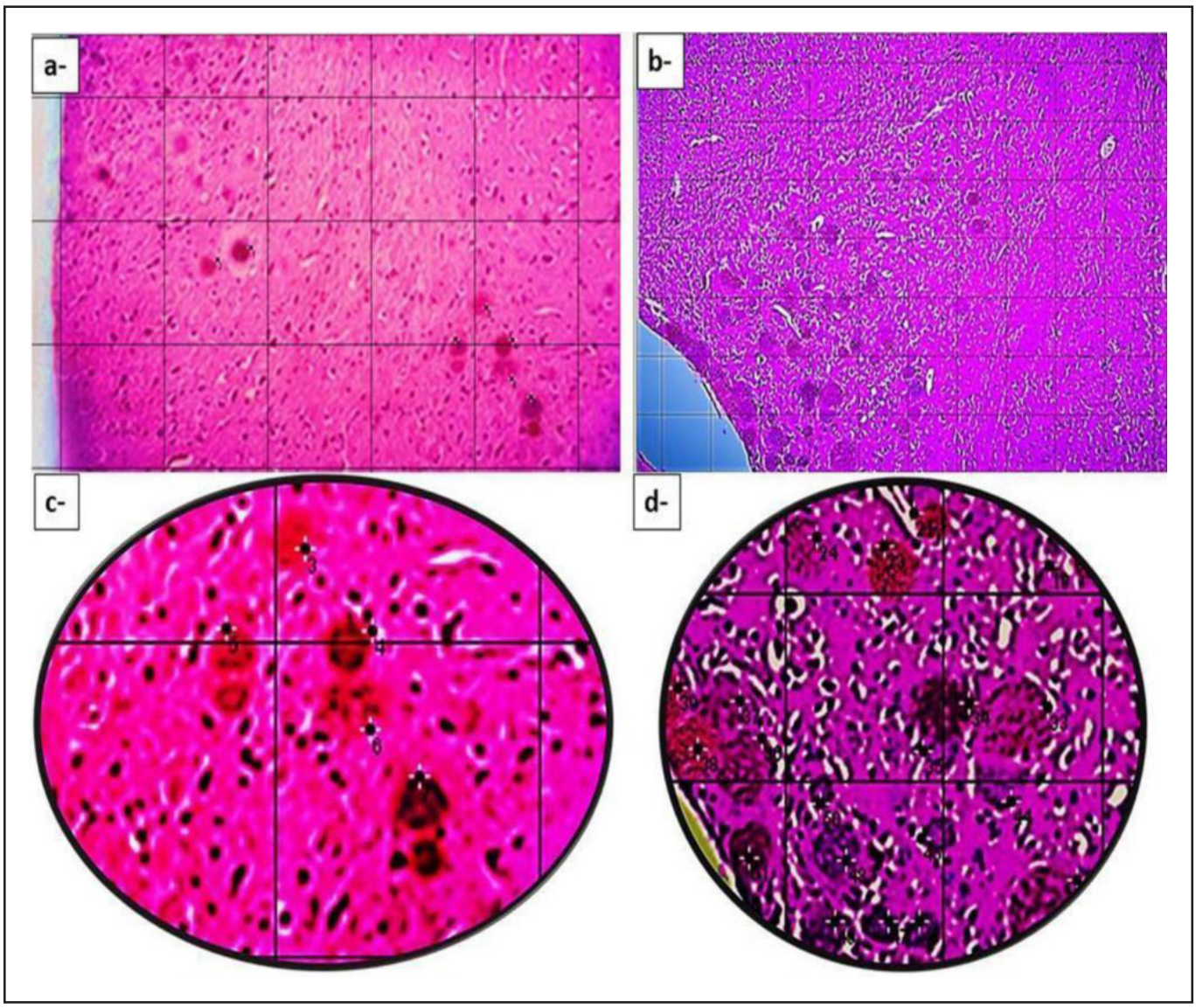

Figure 2. Quantification of intra-cerebral *Toxoplasma* cysts in tissue cut sections (H&E stain, 40X). Image gridding (black in colour) was performed by ImageJ software and the parasites were manually selected (numerated asterisk). (a) Pons region with discrete cysts (7cysts) and (b) amygdala region with gatherings of cysts (48cysts). Note (c,d) are cropped magnified images from a and b successively.

Beverly strain (type-II)-infected C57BL/6 mice (Figure 3) *p*-value ≤0.05. TNF-α immune-reactive cells had a similar distribution iNOS positive cells, with a higher number located peri-vascular. Immune-staining for i-NOS frequently revealed numerous positive immune cells in close vicinity to the parasite's cysts. There were no significant differences between pons and amygdala regions.

i-NOS was statistically of high transmittance expression in established cerebral toxoplasmosis (mean value was 2.66±0.72 for O.D. and 55±7.9 for area %) (*p*-value ≤ 0.05) compared with healthy control (Figure 4-a). TNF-α was of mean O.D 2.1±0.5 and area% 46±9.6 with a statistically significant difference (*p*-value ≤ 0.05) when compared with the healthy control (Figure 4b).

- **Immune- expression assay of apoptotic biomarker**

There was no significant association of Bcl-2 positive cells with established cerebral toxoplasma cysts. Some Bcl-2 positive cells were present perivascular. Both pons and amygdala regions had significantly increased Bax and Bax/Bcl-2 ratio compared to healthy controls *p* value 0.003 and *p* value 0.001 respectively using 2-way ANOVA. There was no significant correlation between Bax expression and parasite location ($p > 0.05$).

Mean values of Bax were 2.42±0.5 for O.D. and 41±8.2 for area % in the infected animals while in healthy control mean values were 0.78±0.2 for O.D. and 20±6.5 for area% (*p* value 0.001). Bcl-2 mean values were 0.52±0.3 for O.D. and 15±7.9 for area% post-infection whereas in healthy controls O.D. was 2.57±0.29 and area% was 58.13±11.6 (*p* value 0.001). Relative Bax/Bcl-2 ratio in the infected animals was 2.42:0.52 while in healthy control Bax/Bcl-2 ratio was 0.78:2.57 with *p*-value 0.001.

- **Association Between CMI and Apoptosis Biomarkers**

Pearson Correlation showed a positive relation-ship between CMI biomarkers and Bax, the pro-apoptotic biomarker. The correlation factor between densities of TNF-α and i-NOS versus Bax/Bcl-2 ratio was of *rho* value 0.76 and 0.686 respectively, *p* value ≤ 0.05.

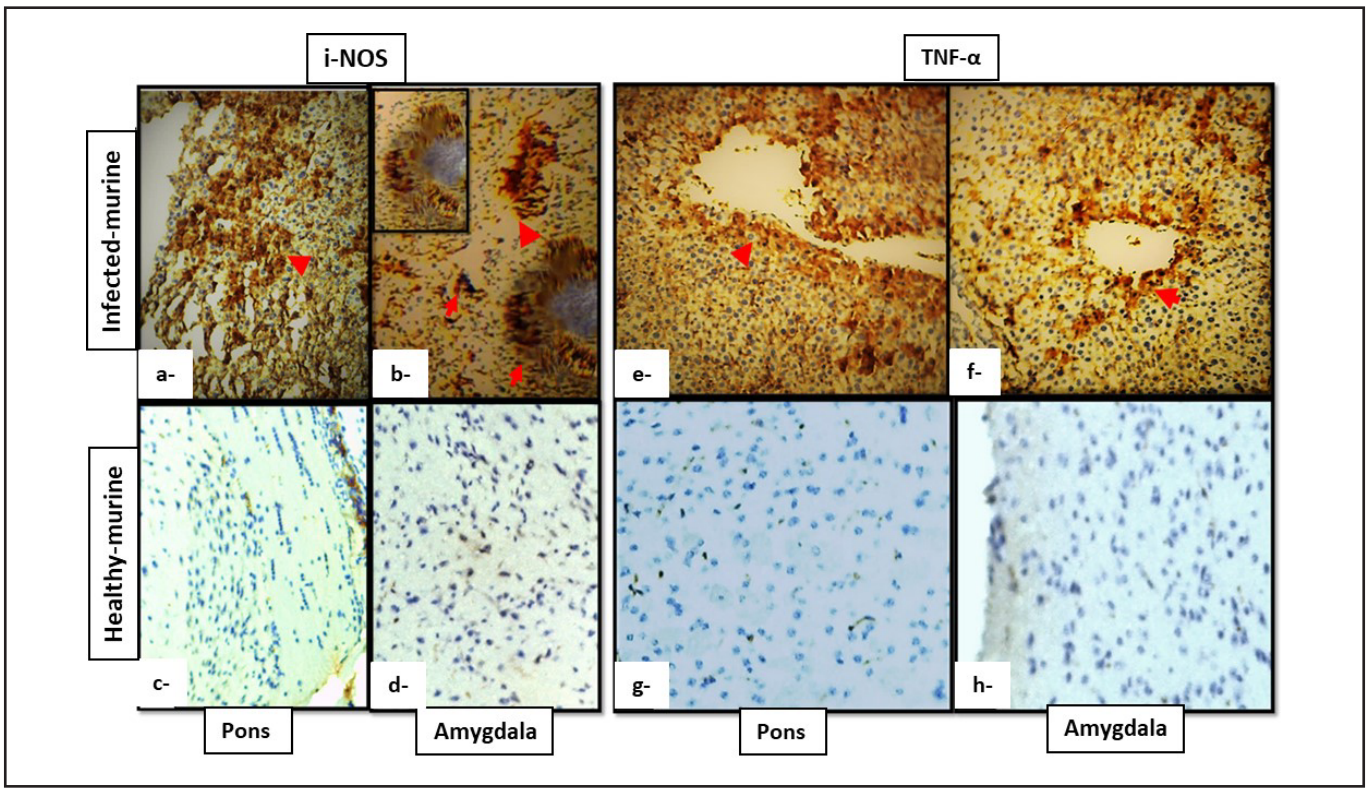

Figure 3. Immunohistochemical assessment of both Beverly strain (type-II)-infected C57BL/6 mice 12th wks. post-infection and healthy mice with anti-iNOS (a,b,c,d) and anti-TNF-α antibodies (e,f,g,h). iNOS expression in infected mice: (a) cerebral cells with cytoplasmic immune-reactivity in pons region. Red arrows point to area of intense perivascular immune staining. (b) Several iNOS immune reactive cells in amygdala region and the upper-left cropped image shows iNOS immune reactive cells surrounding an established cyst. iNOS expression in healthy controls: (c,d) shows cerebral cells along the healthy pons and amygdala regions without immune-reactivity. TNF-α expression in infected-mice: (e,f) cytoplasmic expression of TNF-α cytokine, red arrow points at peri-vascular immune-reactive cerebral cells in pons and amygdala regions; meanwhile (g,h) negative TNF-α expression in healthy cerebral controls. Magnification power (100X).

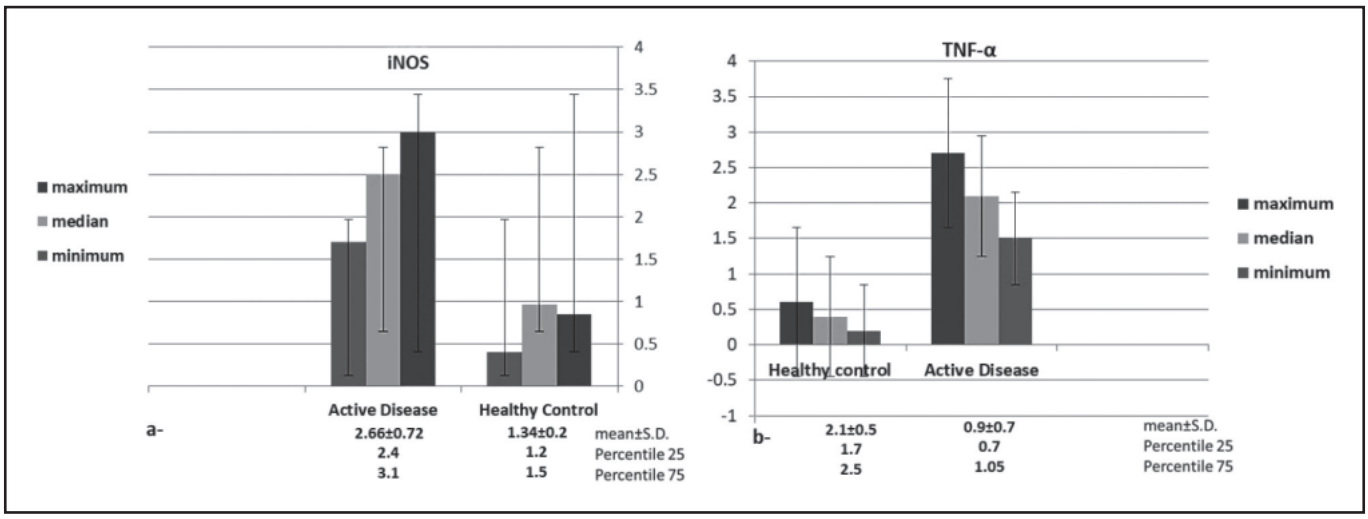

Figure 4. Bar graph represents the median, the minimum and the maximum values of the O.D. in the biomarkers of the CMI in the two independent experiments at the 12th wks. post- *T. gondii* infection. Below each graph mean±S.D, percentile 25, and percentile 75 were demonstrated, *p*-value ≤ 0.05.

- **Pearson correlation analysis of Averages of Parasite burden and Alterations in the Biomarkers' Expressions**

Mean cyst number of the parasite in the whole sample was positively correlated to the cell mediated immunity (TNF-α and iNOS) of *rho* value 0.858 and 0.9 respectively, *p* value ≤ 0.005 and yielded positive correlation with Bax and Bax/Bcl-2 ratio, *rho* value 0.68, *p* value 0.04 and *rho* value 0.72, *p* value 0.002, respectively.

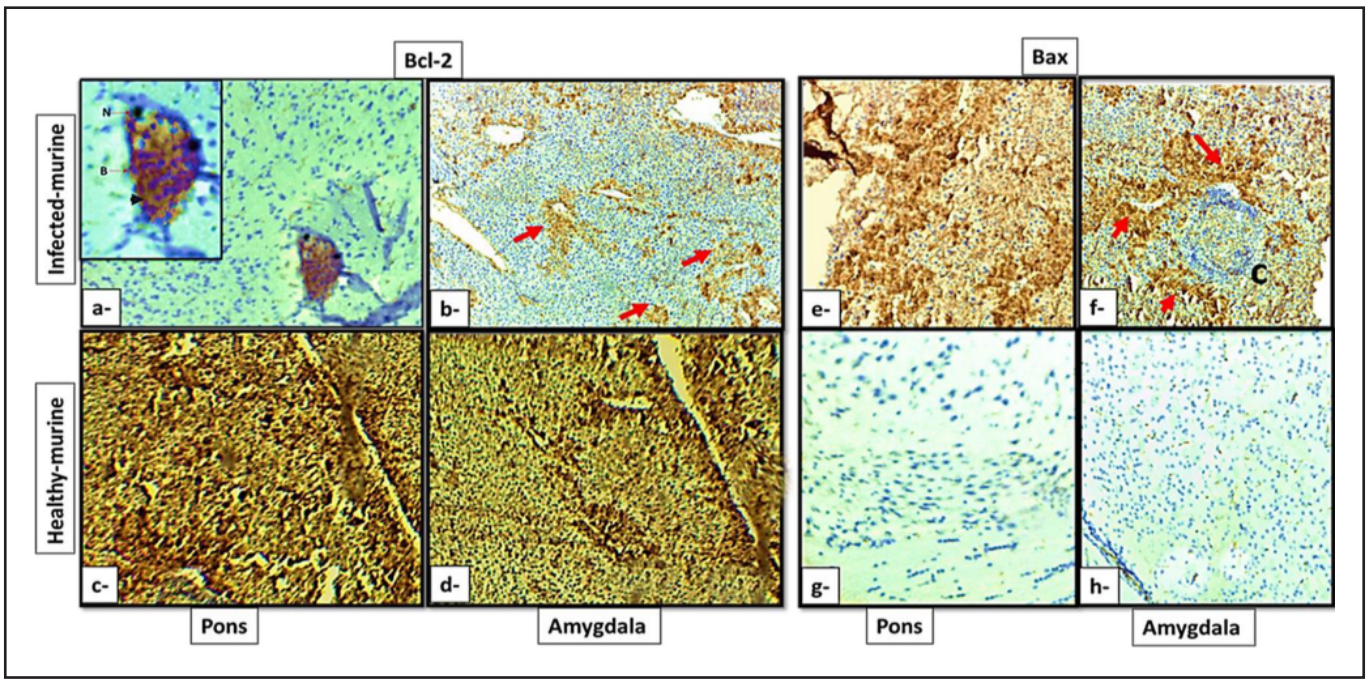

Figure 5. Immunohistochemical localization of both Beverly strain (type-II)-infected C57BL/6 mice and healthy mice with anti-Bcl-2 (a,b,c,d) and anti-Bax antibodies (e,f,g,h). Bcl-2 expression in infected mice: (a) a cropped magnified image in the upper-left showing *Toxoplasma* cyst, black head arrow points to Bcl-2 cytoplasmic expression, (N) refers to host eccentric nucleus, (B) is the basophilic dot-like nuclei of the parasites; meanwhile neighboring cerebral cells are without immune-reactivity. (b) Red arrows points at occasional perivascular Bcl-2 immune reactive cells in amygdala region. Bcl-2 expression in healthy controls: (c,d) Bcl-2 was confined chiefly in the cytoplasm of the positive cerebral cells along the healthy pons and amygdala regions. Bax expression in infected-mice: (e,f) nuclear expression of Bax, red arrow points at positive peri-vascular cerebral cells and in (f) around the parasite's cyst (C); meanwhile (g,h) negative Bax expression in healthy cerebral controls. Magnification power (100X).

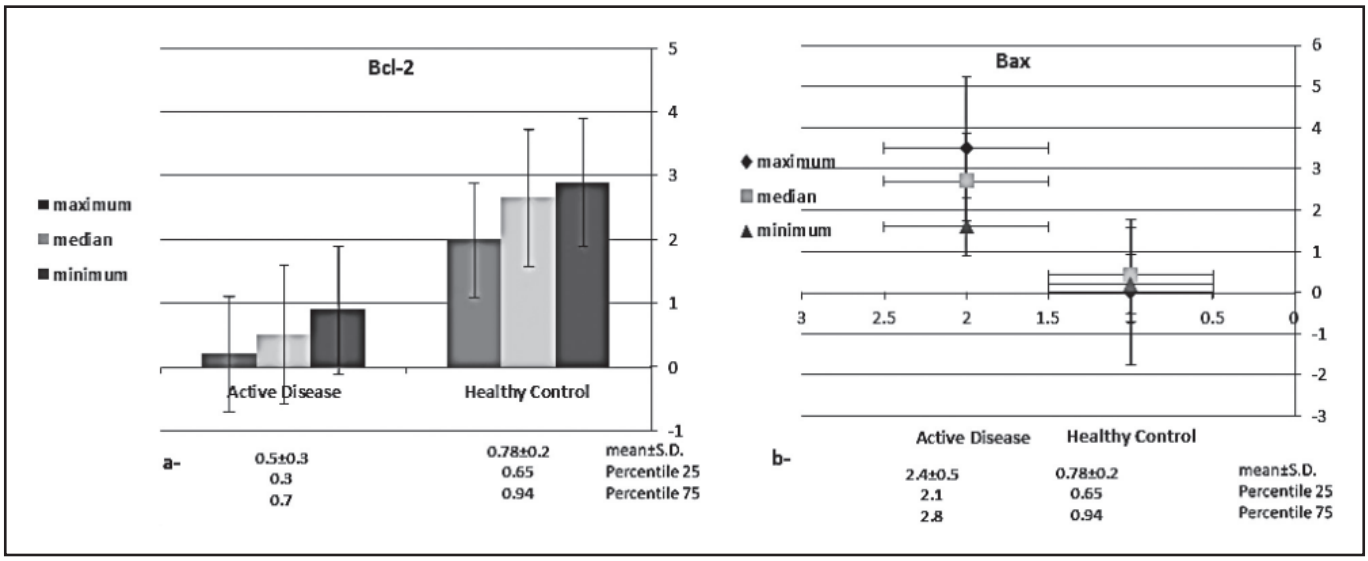

Figure 6. Scattered points and bar graph are representative for O.D. of the apoptotic biomarkers in the two-independent experiments, 12th wks. post-infection in the form of median, minimum, and maximum values. Below each paragraph mean±S.D, percentile 25, and percentile 75 are also demonstrated**,** *p* -value 0.001.

DISCUSSION

Factors beyond *T. gondii* cerebral dissemination despite being vague parasite cysts were of un even distributive pattern in all brain regions but similar to prior studies higher cyst densities were detected in forebrain (amygdala) (Berenreiterová *et al*., 2011).

Remarkable stimulation of cell-mediated immunity represented by high expression iNOS and TNF-α was in association to the parasite establishment. Lüder *et al*. (2003) reported the mandatory role of nitric oxide (NO) production during toxoplasmosis noting that its partial suppression by the parasite may modify the parasite-host balance. However, Schlüter *et al*. (1999) related real impact of i-NOS during the

parasite infection to the mouse strain as suppression of iNOS exacerbates chronic cerebral toxoplasmosis in C57BL/6 mice while being irrelevant in BALB/c murine models. It is noteworthy to mention that using i-NOS$^{-/-}$ mice, the experimental animals survived the acute infection of the parasitic disease. iNOS was also reported to be essential to control the persistent intracellular infection, which is a tissue-specific effect rather than systemic (Scharton-Kersten *et al.*, 1997).

Another point, Dincel and Atmaca in (2015) indicated the role of increased iNOS and NO levels to contribute to neuropathology related to toxoplasmosis encephalitis. Also, a former study published by Mahmoudvand *et al*. (2015) (46) identified the possible relation-ship between iNOS in *T. gondii* infection and increased risk of anxiety and mental disorders together with other cytokines such as IL-1β, TNF-α, IL-6 experimentally in BALB/c mice. An experimental study using rats reported that aminoguanidine, an i-NOS inhibitor, can counteract schizophrenia-like behavioral changes induced by ketamine and apomorphine (Lafioniatis *et al*., 2016).

Interestingly, one human study deduced that i-NOS promotes the growth of *Toxoplasma*; as i-NOS appeared to reduce levels of indole 2,3- dioxygenase 1 (IDO1) protein which is critical to induce IFN-ö in toxoplasmosis (Bando *et al*., 2018). In 2012, Tobin and Knoll recognized a patatin-like protein which protects *T. gondii* from degradation by NO using an un-known mechanism.

Schlüter *et al*. (2003) recorded that TNF$^{-/-}$ mice failed to control intracerebral toxoplamosis and yielded to acute necrotizing encephalitis. A challenging study revealed that murine models lethally infected with C56 strain of *T. gondii* and treated with purified recombinant TNF (1 mg/day for 8 days) survived the infection (Chang *et al*., 1990). *In vitro* studies revealed that exogenous TNF-α could stimulate egress of *T. gondii* from fibroblast cells of human foreskin at a dose of 10 ng/mL in a time-dependent manner (Yao *et al*., 2017). TNF-α in former study was shown to regulate production of IFNö by NK cells (Hunter *et al*., 1994).

However, Halonen *et al*. (1998) demonstrated that collaboration of TNF-α with other cytokines involving IL-1 and IL-6 is required for proper inhibition of the parasite growth. Also, in 2002 an *in vitro* study on human cells documented the capability of *T. gondii* parasite to regulate the expression of TNF-α receptors (Derouich-Guergour *et al*., 2002). In a human study, patients with symptomatic cerebral toxoplasmosis were shown to exhibit higher sera levels of TNF-α (*Meira et al*., 2014).

Similar to our results, Dincel and Atmaca (2016) suggested *Toxoplasma*-mediated apoptosis as an essential and different neuro-degenerative and neuro-pathological type involved in toxoplasmic encephalitis. Interestingly, Kim *et al*. (2019) demonstrated that a *Toxoplasma*-specific protein called GRA-16 can induce p53-dependent apoptosis in a referral to its probable anti-cancer effect. Another *in vitro* study reported induction of apoptosis in human leukaemia (K562)-cell after being treated with *Toxoplasma* tachyzoites (Zhang *et al*., 2007). In contrast to our results, Takahashi *et al*. (2001) (60) suggested that in congenital-murine toxoplasmosis there is no obvious relation-ship between cortical dysplasia and Bax-induced apoptosis. Raisova *et al*. (2001) assumed higher Bax/Bcl-2 ratio to the susceptibility of cells to apoptosis.

Cell mediated immunity positively correlated with Bax/Bcl-2 ratio. Similarly, Nishikawa *et al*. (2007) reported apoptosis in non-infected bystander macrophages in a process related to NO released by infected-host cells lines. However, Khan *et al.* (1996) reported formerly induction of apoptosis in CD4$^+$ T cells during *Toxoplasma* infection. Also, Liesenfeld *et al*. (1997) conveyed that per-orally murine infection with *T. gondii* induce apoptosis of CD4$^+$ and CD8$^+$ in Peyer's patches associating INF-ö up regulation and increased Fas expression.

CMI biomarkers were heavily sensitized to the parasite burden in the whole sample. Strikingly, parasite burden despite being of non-homogenous pattern in all brain areas it seemed to render cerebral cells susceptible to apoptotic signals being relatable to Bax/Bcl-2 ratio (Raisova *et al*., 2001). We are then asking if boosting such pro-apoptotic signals had a substantial advantage on *Toxoplasma* -infected host cell survival. It has been previously reported that *Toxoplasma*-infected cerebral cells can resist apoptosis. This stimulus that is not physiologically relevant in the resting brain tissues. Although, it is interesting to illustrate the signaling in the infected cerebral tissues as strictly pro-apoptotic or pro-survival, the present data here propose a more multifaceted relationship between the burden of *Toxoplasma* parasite and its impact on the entire micro-environment of infected brain tissues than previously esteemed.

CONCLUSION

This is a comprehensive analysis of the apoptotic signal induced by chronic cerebral toxoplasmosis infection of murine brain model. Our study indicated higher parasite weight in the forebrain (presented by amygdala) compared to other brain regions (presented by pontine region). Activation of cell-mediated immunity biomarkers (i-NOS and TNF-α) was observed in all infected brains. Expressions of i-NOS and TNF-α were positively correlated to the density of apoptotic factor and Bax/Bcl-2. Also, the average quantity of the parasites in the whole sample study positively correlated with the expression of the cell-mediated biomarkers (i-NOS and TNF-α), Bax protein, and Bax/Bcl-2 ratio. The current model presented the toxoplasms latent infections as an apoptotic triggering factor in cerebral cells. In this accordance we are presenting our results as an experimental diagnostic tool for further studies on the role of toxoplasmosis in mental and physical health disorders and we recommend further studies correlating Bax/Bcl-2 ratio to the behavioral changes in established toxoplasmosis infection. Another point, speedily replicating parasites like *T. gondii* have been found to be competitive for combating, due to continual evolution and frequent development of drug resistance. Yet, little attention has been driven to improve drugs for selective provoking of apoptosis in parasite-infected cells while shielding the surrounding cellular microenvironment.

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Disclosures

The authors have no financial conflicts of interest.

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