Oxygen Free Radicals Released in Placentae of Ewes Naturally Infected with *Toxoplasma Gondii*

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Abstract

This study is a part of research series, carried out in our laboratory at the College of Veterinary Medicine, Mosul University, Iraq, aimed to explore the capability of *Toxoplasma gondii* for induction an oxidative stress status at different hosts and tissues. In present study, we deal with the placentae of ewes naturally infected with *T. gondii* at Mosul area, Iraq. The oxidative status have been estimated via the levels of malondialdehyde (MDA), a major products of lipid peroxidation, glutathione GSH) and, superoxide dismutase (SOD) as an antioxidant, in placental homogenates. In comparison with non infected placentae, results of this study indicate a significant elevation in MDA levels concomitant with a significant reduction in GSH and SOD levels in placentae of infected ewes. Histopathologic alterations of infected placentae have been mentioned too. In conclusion, this study provide further evidence on the capability of *T. gondii* to induce an oxidative stress status in infected host. Moreover, this oxidation status as well as the pathological changes seen in infected placentae may be the primitive cause for the abortion in infected ewes. Our future goal will be on the antioxidant status of *T.gondii* infected ewes.

تحرر جذور الأوكسجين الحرة في أسخاد النعاج الخمجة طبيعيا بالمقوسة الكوندية

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الخلاصة

تعتبر هذه الدراسة جزءا من سلسلة أبحاث علمية أجريت في كلية الطب البيطري/ جامعة الموصل، العراق، والتي جميعها تهدف إلى أستيضاح قابلية المقوسة الكوندية على أحداث حالة الإجهاد التأكسدي في مختلف المضائف الحيوانية والأنسجة المختلفة. في الدراسة الحالية، كان التعامل مع أسخاد النعاج الخمجة طبيعيا بالمقوسة الكوندية في منطقة الموصل– العراق. تم التحقق من حدوثية الإجهاد التأكسدي بقياس مستويات المالوندايلديهايد، وهو الناتج الرئيسي في تزنخ الدهون، والكلوتاثايون والسوبرأوكساید ديسميوتيز، باعتبارهما مضادي أكسدة، في المعلق المتجانس من تلك الأسخاد. عند المقارنة مع أسخاد نعاج غير خمجة، فأن نتائج هذه الدراسة تشير إلى ارتفاع معنوي في مستويات المالوندايلديهايد تزامنا مع انخفاض معنوي في مستويات كل من الكلوتاثايون والسوبرأوكساید ديسميوتيز عند الأسخاد الخمجة. كما تم الإشارة إلى التغيرات المرضية النسجية في جميع الأسخاد المفحوصة. يستنتج من هذه الدراسة بأنها إضافة حقائق أخرى حول قابلية المقوسة الكوندية في أحداث حالة الإجهاد التأكسدي في المضائف الخمجة بتلك المقوسة. كما أن نتائج هذه الدراسة ترجح بكون حالة الإجهاد التأكسدي المحدث مع التغيرات النسجية في الاسخاد الخمجة قد يكونا السبب الأساسي لحالة الإجهاض في النعاج الخمجة. ستكون دراساتنا المستقبلية منصبة على العوامل المضادة للتأكسد في النعاج الخمجة بالمقوسة الكوندية.

Introduction

Toxoplasma gondii is a cyst- forming coccidian parasite of domestic ruminants world- wide (1). It is a major causes of abortion and perinatal mortalities in ovine and caprine (2). Recent experimental studies were investigate the capability of *T. gondii* to induce oxidative stress at different hosts including mice (3), domestic cats (4) and broiler chickens (5). These studies revealed that levels of malondialdehyde (MDA), as TBA reactive substances, in livers, heart and aortas, were significantly elevated among *T. gondii* infected cats and chickens (4, 5). Measurement of lipid peroxidation proposed as the principle indicator of an agent- induced free radicals (6). This study was dimed to investigate the oxidative stress status in placentae of ewes naturally infected with *T. gondii* at Mosul area, Iraq.

Material and Methods

Placentae were collected from 25 aborted and 12 normal Awassi ewes (local Iraqi breed). Those ewes aborted at 3-4 months of gestation. Aborted normal placentas were inspected grossly for any pathological lesions, and making an impression smears stained with Giemsa in order to investigate tissue cysts and Tachyzoits of *T. gondii* (6). Pieces of placentae were rinsed with isotonic saline for the estimation of MDA levels using thiobarbituric acid (TBA) test (7). GSH and superoxide dismutase (SOD) concentrations were measured according to Morone et al assay (8, 9). Moreover, placental tissues were immediately harvested into slices and fixed with 10% neutral buffered formalin for 48 hours and, tissue specimens were dehydrated, clearing and embedded in paraffin. Histological sections were cut at 4-6 µm thickness and stained with H & E (7). Other part was fixed in 2.5% gluturaldehyde in 0.1 M cacodylate buffer (PH 7.2-7.4) at 4 C for 24 hours. Then, the samples were washed with cacodylate buffer post fixed in 1% osmium tetroxid (freshly prepared) for two hours, dehydrated through a graded series of ethanol (70%, 95% and 100%), then cleared propylene oxide. They were infiltratd with propylene oxide: resin mixture for 12 hours and embedded in epoxy resin medium (Araldite CY 212: 10g; hardener: 10g; accelerator DMP 30: 0.5g and Di-nbutylophthalate plasticizer: 0.6 g polymerization accomplished in oven at 60 C for 48 hours plastic blocks were sectioned by ultramicrotome (Riechert Co.) into 1 Mm) thick section for light microscopy. The sections were stained by 1% toluidine blue in 1% Borax for light microscope (10).

Results

Impression smears of placentae stained with Giemsa illustrated presence of tissue cyst and tachyzoit as pyriform shape. (Table 1) showed that *T.gondii* infective placentae induce positive TBA reactive substances represented by significant elevation in MDA concentrations (362.21±0.02 nmol/ gm wet tissue) as compared with that estimated in control placentas (186.42±0.32 nmol/ gm wet tissue). Infective placentas revealed a significant reduction in GSH (0.91±0.35 µmol/ gm wet tissue) and SOD levels (3.35± 0.41 µmol/ gm wet tissue) in comparison with the normal non-infected controls (1.95± 0.22 and 7.22± 0.62µmol/ gm wet tissue, respectively).

Gross lesions of the aborted *T. gondii* infected placentae characterized by presence of small white necrotic foci ranged 0.5-1.5 cm in diameter, yellowish white color on the tip of cotyledons and intervillous connective tissues, sever congestion associated with presence of hemorrhagic patches and, some cotyledons looks blue- black in color (Fig 1).

Histopathologicaly, sections of infected placentae showed ; coagulative necrosis in placental plate and interplacental stroma, high invasion of Tachyzoites with tissue cysts in placental stroma and around maternal blood vessels. Additionally, microscopic

pictures revealed proliferation and infiltrations of inflammatory cells such as macrophages (hofbaur cells), lymphocytes and neutrophiles (placentitis), desquamation of syncytiotrophoplast as knot. Calcification and fatty changes. (Fig 2,3). Plastic sections of infected placentae revealed presence of tachyzoit and tissue cyst (Fig 4,5).

Table (1) levels of MDA in placental tissues of ewes naturally infected with *T.gondii*

Samples	MDA (nmol/g wet tissue)	GSH (µmol/g wet tissue)	SOD (mmol/g wet tissue)
Control non- infected Placentae	186.42± 0.32	1.95± 0.22	7.22± 0.62
Infected placentae	362.21± 0.22*	0.91± 0.35	3.35± 0.41

***Value expressed as means ± SD of 25 placentae ($p \leq 0.05$).**

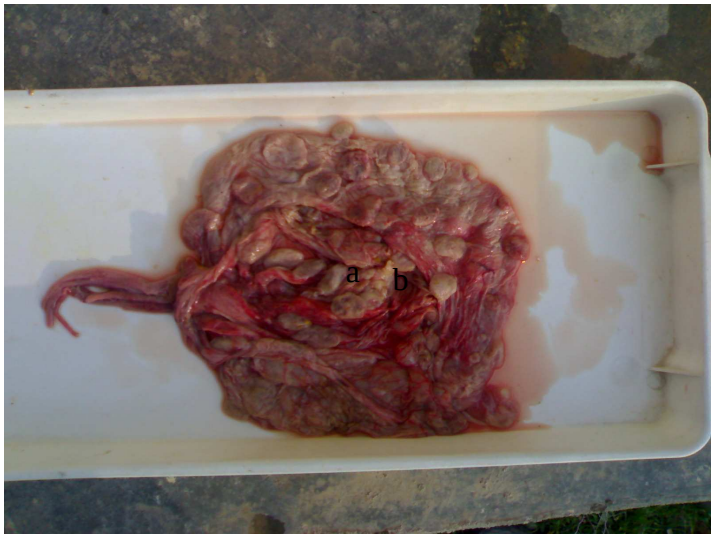


Fig.(1) Placenta of ewes infected with *T.gondii* showed yellowish white color cotyledon (a), small white focal necrotic foci in intervillous connective tissue associated with hemorrhagic patches

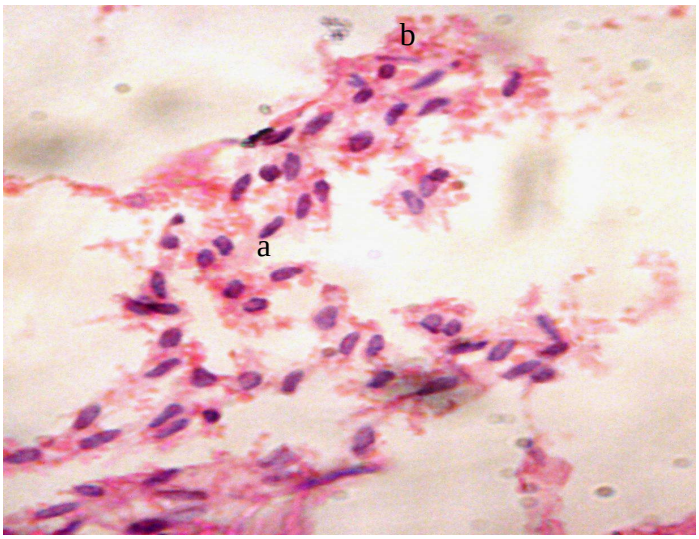
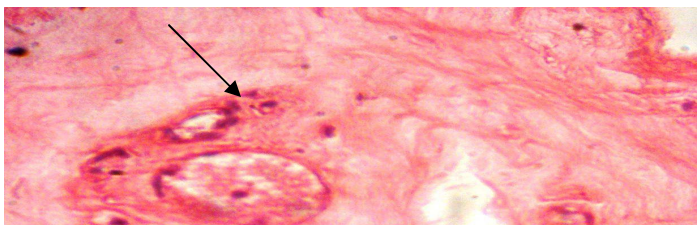


Fig.(2) Photomicrograph of ewes placenta infected with *T.gondii* showed presence of tachyzoite (a) and hemorrhage(b), H& E. 450 X



a

Fig.(3) Photomicrograph of ewes placenta infected with *T.gondii* showed, presence of tachyzoit (arrow) and congestion associated with coagulative necrosis in interplacental stroma (a). H&E. 450 X

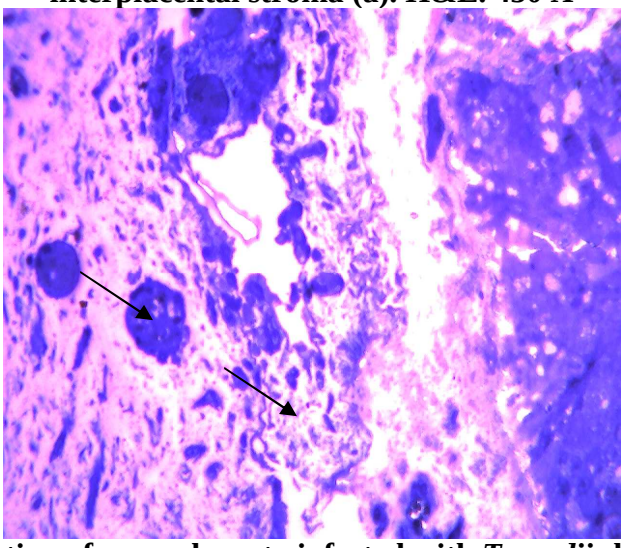


Fig.(4) Plastic section of ewes placenta infected with *T.gondii* showed presence of tachyzoit and tissue cyst (arrow). T.B. 450 X

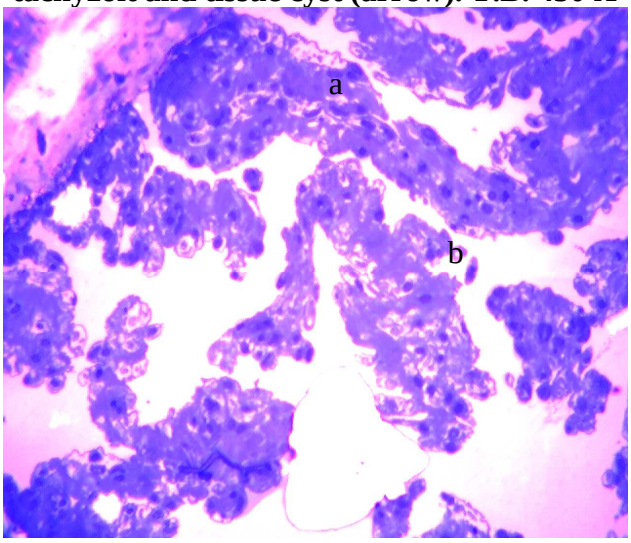


Fig.(5) Plastic section of placental ewes infected with *T.gondii* showed, coagulative necrosis (a) and presence of tachyzoit (b). T.B. 450 X

Discussion

The result of this study showed that isolation and identification of *T.gondii* from placental ewes tissues agreed with previous studies reported by other (11). In the present study , the results mentioned in (Table 1) revealed marked elevation of MDA level in placental tissues and examined. The results suggest that these changes reflect ability of *T.gondii* to induce oxidative stress (5,12).

This study though that the source of lipid peroxidation was proposed to be the end products of lipoprotein metabolism from membranes of the tissues, unsaturated lipids which occur in abundance as constituent of biological membranes have been showed to be particularly susceptible to the actions of reactive oxygen species , forming lipid peroxides autocatalytic reaction in the presence of oxygen (H) the activation as lipid peroxide is probably due to H_20_2 that produces from phagocytosis which causes increase oxygen tension in the tissue leading to increase oxidative metabolism and increased production of other reactive species. The combined data from the present study revealed marked reduction in GSH and SOD content in placental tissue examined. It is also possible that activation of lipid peroxide maybe attributed to H_20_2 induced direct inhibitory effect on the activity of the various endogenous antioxidative system responsible for elimination of these reactive oxygen species product from *T. gondii* infection. This study agreed the suggestion by (13) who suggested that apicomplexan parasites such as *T. gondii* need efficient antioxidant system in order to maintain the crucial balance between antioxidants and pro-oxidants to ensure the survival in their host cells. This result give more evidences for supporting of T.gondii have ability to produce oxidative stress. These observations were in accordance with those seen in mice, cats and chickens infected by *T. gondii* (3, 4, 5). The gross and histopathological lesions of placenta is identical for *T. gondii* infections that reported previously in their tissues (14,15) which confirm presence of placentitis. This study suggested that placentitis due to proliferation and invasion of Tachyzoit in host cells such as binucleated syncyciotrophoplast cells and macrophages to induce releasing some chemical mediators like TNF-x, IL-1, IL-8 and interferon which lead to further attraction and attachment of immune cells (16, 17, 18), which in turn cause more damage in placental tissue particularly in central area infected with parasite. that start to release reactive oxygen species. Finally, further studies are required to examine the degree and extent of placental damage in presence of reactive oxygen species by *T.gondii*.

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