Study of Enzymatic Changes in Placentae of Toxoplasmosis Infected Pregnant Women

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***Abstract*—*This* study was concerned with the activity measurement of some enzymes in nucleotides pathway in placentae of infected pregnant women with toxoplasmosis. These enzymes were adenosine deaminase (ADA), cytidine deaminase (CDA), xanthin oxidase (XO), alkaline and acidic deoxyribonuclease (DNase), and ribonuclease (RNase). The study included (24) specimens from placentae of aborted women were infected with toxoplasmosis, and 8 of non-infected as a control group. The infected placentae from (1-3), (3-6), (6-9) months of pregnancy attending AL-Salam Teaching Hospital in Nineveh Governorate. The results showed a significant increase at $P \leq 0.05$ in activities of ADA, CDA, XO, and alkaline DNase during the three period of pregnancy, whereas the activity of acidic DNase decreased in all periods of pregnancy in addition, the result of the activity of RNase during the different months of pregnancy indicate that there is a non-significant change when compared with non-infected placentae as a control group.**

***Index Terms*—Adenosine deaminase, DNase, Infected placenta, Toxoplasmosis, Xanthin oxidase.**

I. Introduction

Toxoplasmosis is a worldwide zoonotic intracellular parasite disease [1], caused by the protozoal parasite *Toxoplasma gondii* [2]. Felids are the definitive hosts for *T. gond*ii, but encysted parasites can survive for very long periods, perhaps lifelong, in the tissues of most or all hosts. Some clinical cases result from new exposures to *T. gondii*; others occur when parasites in tissue cysts become reactivated. Infection with *T. gondii* is common in warm-blooded animals, including humans, and usually causes no illness or mild clinical signs in immunocompetent, non-pregnant individuals [3]. However, infections acquired during pregnancy can result in mild to serious congenital defects in the fetus, and immunocompromised humans or animals can develop severe, life-threatening infections [4]. In a small number of people, a weak immune system, severe symptoms such as seizures and poor coordination may occur [5]. If infected during pregnancy, a condition known as congenital toxoplasmosis may affect the child [6].

Primary maternal infection during pregnancy was frequently associated with transmission of *T. gondii* from mother to fetus [7]; primary infection during gestation may cause serious neurological damage, blindness, and even fetal death [8].

The ability of *T. gondii* tachyzoites to differentiate into latent bradyzoite forms is essential for the pathogenesis of the clinical disease.

The aim of this study was to examined the effects of the causative agent, *T. gondii*, on the activity of some enzymes in nucleotide signaling pathways in placentae of infected pregnant women compared with non-infected women as control group. Because they are important in the stress-induced conversion of *T. gondii*. Furthermore, no available literature has been found which concerns the studying enzymes in placentae of infected pregnant women within the three trimesters of pregnancy.

Adenosine deaminase (ADA) also known as adenosine aminohydrolase, or ADA (E.C.3.5.4.4) is an aminohydrolase which playing a role in the catabolism of nucleotides, [9]. Its primary function in humans is the development and maintenance of the immune system, that is mean it is plays a relevant role in immune responses, and peptidase activity, which may be altered in some autistic patients [10]. It is one of the main enzymes of the purine metabolic pathway, catalyzing the deamination of adenosine to inosine and deoxyadenosine to deoxyinosine. During inflammatory reactions, the enzyme is released into the extracellular fluid, and in serious effusions of differing pathology, the levels of ADA activity increase considerably. This increase has been shown to correlate with the number of nucleated cells, particularly T lymphocytes and macrophages, present in the effusions [11]. ADA activity can be found in lymphocytic cells 10 fold more compared to erythrocytes and also, it is higher in T lymphocytes than to B lymphocytes. ADA is considered as a cellular immunity indicator and its activity can be found in lymphocytic cells 10 fold more compared to erythrocytes as well as it is higher in T lymphocytes than to B lymphocytes [12]. Cytidine Deaminase (CDA)—(E.C. 3.5.4.5) is an enzyme that in humans encoded by the CDA gene. This gene encodes an enzyme involved in pyrimidine

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salvaging. The encoded protein forms a homotetramer that catalyzes the irreversible hydrolytic deamination of cytidine and deoxycytidine to uridine and deoxyuridine, respectively. It is one of several deaminases responsible for maintaining the cellular pyrimidine pool.

Xanthin oxidase (XO)—(E.C. 1.1.3.32) catalyzes the oxidation of hypoxanthine and xanthine to uric acid. During the reoxidation of XO, molecular oxygen acts as an electron acceptor, producing superoxide radical, and hydrogen peroxide [13]. It is the most recognized for its role as the rate-limiting enzyme in nucleic acids degradation through which all purines are channeled for terminal oxidation [14].

Alkaline and acidic deoxyribonuclease (DNase)—(E.C.3.1.22.1) are an endonuclease splitting phosphodiester linkage, preferentially adjacent to a pyrimidine nucleotide yielding 5′-phosphate terminated polynucleotides with a free hydroxyl group on position 3′. Two types of DNase activity have been known to exist in human body fluids, acid and alkaline that differs in pH optimum, affinity for synthetic substrates and an absolute requirement for a divalent cation [15].

Ribonuclease Rnase—Ribonuclease is a type of enzyme which catalyzes the breakdown of bounds in ribonucleic acid (RNA) which is the chemical material in a cell that codes for different proteins. It can be divided into endoribonucleases and exoribonucleases, and comprise several sub-classes within the EC 2.7 (for the phosphorolytic enzymes) and 3.1 (for the hydrolytic enzymes) classes of enzymes, [16]. RNases play a critical role in many biological processes, including angiogenesis and self-incompatibility in flowering plants (angiosperms). Many stress-response toxins of prokaryotic toxin-antitoxin systems have been shown to have RNase activity and homology, [17].

II. Materials and methods

A. Subjects and Samples

The present study included (24) specimens of placentae from abortion women which were attending AL-Salam teaching hospital in Nineveh governorate, (8) of non-infected (control group) and (24) were infected with toxoplasmosis, they have been divided into three groups according to the three trimesters of pregnancy (1-3, 3-6, and 6-9) months.

B. Placentae Extraction

Fresh human placentas were placed in ice immediately after delivery and kept at 0°C until used. Chilled fresh placentas were freed of cord, membranes, and adhering blood by rinsing with cold distilled water, 75 g of soft placental tissue then passed through a meat grinder. The ground tissue was suspended in (2) volumes (w/v) of distilled water and stirred for 1 h at 4°C.

The suspension was refrigerated then centrifuged at ×10,000 g for 20 min. The supernatant was decanted, and the sedimented material was suspended in (2) volumes (v/v) of (20) mM potassium phosphate buffers, pH 6.0 [9], containing (0.25%) of Triton X-100, and (10) mg of sodium taurocholate per mL) (w/v).

The homogenized suspension was refrigerated and then centrifuged at ×10,000 g for 25 min, [18]. The supernatant solution was used for enzymes determination.

C. Methods

1. Determination of ADA according to Giusti method [19].
2. Determination of cytidine deaminase activity (CDA) according to [20].
3. Determination of XO in placentae extracts of pregnant women by the method [21].
4. Determination of Deoxyribonuclease activity (DNase): Acidic DNase activity was determined by the method [22].
5. Determination of alkaline deoxyribonuclease activity by the method [23].
6. Determination of Ribonuclease activity (RNase) according to [24].

D. Statistical Analysis

The results of this study were analyzed using analysis of variance. The results are considered statistically significant ($P \leq 0.05$), [25].

III. Results and discussion

A. ADA

The results showed a significant increased ($P \leq 0.05$) of the activity of ADA in placentae of women with toxoplasmosis infection (Table I) in the three trimesters of pregnancy compared with control. The results indicate that there are no differences between the three trimesters of pregnancy women. The increases of ADA activity may be due to the cells that proliferate in tissues have higher ADA activity, and their ADA percentage is higher in the cytoplasm than nucleus [26]. The exact reason for this finding remains unknown, but it can be speculated that the stage of the pregnancy and the immune response of the host have influence in the release of ADA from the tissue cells [27]. Some studies notice that ADA is the predominant form present in human blood plasma and is increased in many diseases; particularly those associated with the immune system, for example, rheumatoid arthritis, psoriasis, and sarcoidosis. The plasma ADA is also increased in most cancers and lupus erythematosus [28,29].

B. CDA

The results revealed a significant increased ($P \leq 0.05$) in the activity of CDA in placentae of women with toxoplasmosis infection compared with the control group as shown in Table I, but there are no differences at the three trimester of pregnancy women. The increased activity of CDA in the tissue of toxoplasmosis women may be attributed to several possibilities, one of the serum factors such as autoantibodies, immune complexes, or specific antigen that stimulates the release of CDA or attributed to the damaged B or T cells

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TABLE I
THE RELATIONSHIP BETWEEN TOXOPLASMOSIS INFECTION PLACENTAE NUCLEOTIDE ENZYMES μMOLE/L COMPARED WITH NON-INFECTED PLACENTAE

Specimens	No.	Mean ± SD μmole/L					
		ADA	CDA	XO	Alkaline DNase	Acidic DNase	RNase
Non-infected placentae	8	9.00±2.58 A	0.0004±0.001 A	14.85±1.76 C	10.00±1.05 D	38.85±7.65 D	431.13±79.70 A
Infected placentae from 1 to 3 months of pregnancy	8	10.00±1.23 B	0.0005±0.001 B	38.75±8.09 A	12.40±3.87 C	28.93±6.63 B	335.81±60.51 A
Infected placentae from 3 to 6 months of pregnancy	8	10.00±1.66 B	0.0005±0.001 B	23.57±7.95 B	21.49±7.54 A	14.05±2.21 A	399.26±62.40 A
Infected placentae from 6 to 9 months of pregnancy	8	20.00±2.87 B	0.0007±0.002 B	27.13±5.35 B	14.05±2.51 B	19.01±3.54 C	468.41±67.42 A

Within in columns, means with same letters vertically and horizontally represent no significant difference, while different letters mean significant difference at $P \leq 0.05$

which mainly increased in this disease [30]. In addition to the above suggestions, the release of CDA from cells in which plasma membrane is damaged by reactive oxygen species may be due to oxidative stress associated with parasite toxoplasmosis infection. It means that the membranes of polymorphonuclear neutrophils may be damaged, and CDA may be released into serum [31,32].

C. XO

The results in Table I showed a significant increased ($P \leq 0.05$) in XO activity placentae of women with toxoplasmosis infection in three trimesters period of pregnancy compared to control uninfected pregnant women, and when compared between the trimester period. The increment of XO activity may be due to the function of the enzyme which catalyzes the breakdown of nucleotides to form uric acid, which contributes to the antioxidants capacity of the blood [33]. XO is most recognized for its role as the rate-limiting enzyme in nucleic acids degradation through which all purines are channeled for terminal oxidation. The XO serves as a source of oxygen-derived free radicals which induce cellular injury and edema as well as changes in vascular permeability, during the damaging cell membranes by reacting with membrane fatty acids which lead to release of the enzyme into the blood [34].

D. Alkaline and Acidic Deoxyribonuclease

Results shown in Table I reveal a highly significant increase in the activity of alkaline DNase in placentae of women infected with Toxoplasmosis when compared with placentae of uninfected women, this result is may be a sign of tissue damage which will lead to the changes in cell membrane permeability and the enzyme released into the serum, or may be due to the high turnover of DNA in proliferating cells than in normal cells [35]. The results of this study were in agreement with several studies. It has been well established that the host macrophages and neutrophils release extracellular traps (NEts) to confine invading pathogens on activation. Some of the pathogens release DNase to counteract the trap. The trap structure has been observed in skin blood vessels of children with uncomplicated malaria. Furthermore, *T. gondii*, another apicomplexan, can induce neutrophils to release NETs directly [36,37]. Thus, the data support the conclusion that plasmodial parasites use TatD to degrade ETs structures to escape host immune clearance. whereas acidic DNase activity was significantly decreased in placentae of women infected with toxoplasmosis in three trimesters of pregnancy when compared to control as shown in Table I this result is probably due to drug demonstrated from infected women for treatment, it has been indicated that acidic DNase activity is inhibit, or the decrease in the activity may reflect diminished output of the enzyme from one or more tissues of the body or reflect the increased production of an inhibitor of the enzyme [38].

E. Ribonuclease

The results in Table I showed non-significant changes in RNase activity in placentae of women infected with toxoplasmosis in all period of pregnancy compared to placentae of uninfected women. This result is probably due to drug demonstrated from infected women for treatment caused inhibited the enzyme activity.

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