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# Study immunological parameters ADA activity and ferritin with Toxoplasmosis in Pregnancy woman in Iraq

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## Abstract

Toxoplasmosis is a protozoan illness that affects both poor and industrialized nations. Human infection occurred by incidental ingestion of oocyst-contaminated food, water, or others Toxoplasma an opportunistic pathogen in which the reactivation of a latent infection can cause death in congenitally infected fetuses, newborns, and immunocompromised patients. this study was to assessthe seropositive rateof T. gondiiand and the possible association with its relation to high body ADA activity and ferritin in pregnancy patients womans and risk factors of toxoplasmosis infection In this study.. Venous blood samples were collected from 20 patients (20 samples of pregnancy womans with toxoplasmosis infected patients and 12 samples of healthy person as control group), their ages were ranged from 20 –41years. The patients attending Baghdad Teaching Hospital and Al- Yarmouk Teaching HospitalThe elevated level of ADA had been observed pregnancy non prgnancy womans in toxoplasmosis patients while the high level of ADA was in the sera of patients (0.0000250 nanomol/minute/µg). significant ($p< 0.05$). difference was observed in the level of ADA activity between infected and healthy control groups. However, there was difference seen among the test groups for both studied parameters. of statistically there were significant ($P\leq0.01$) differences between these groups. , On the other hand ferritin level was ferritin concentration was observed in the pregnancy non pregnancy womans with Toxoplasmosis groups, which was 91.32 ng/ml. Ferritin was non significantly ($P>0.05$) different between the two groups, almost within the normal limits for all groups, and statistically there were no -significant ($P\leq0.01$) differences between them. Other factors parameters did not show significant differences between these two groups. Finally, this study showed that T. gondii played a significant role in changing only ADA level while other parameters Ferritin of were not influenced .The results of this study investigating immunological molecules Since most immunosuppressive patients are exposed to various possible risk factorsincludingToxoplasmaprimary infection or reactivation,, the effect of toxoplasmosis infection on the level such as ADA enzyme, and ferritin as possible parameters, so it is important to diagnose toxoplasmosis In conclusion, these results demonstrated that toxoplasmosis infection plays a pivotal role in increasing cytokines (ADA and ferritin) associated with pregnancy non prgnancy womans with prognosis for Toxoplasmosis disease and treat in

patients to reduce the consequencesof the infection and development

**Keywords**
Toxoplasmosis, ADA activity and ferritin .

Mammals and birds are almost always infected by the intracellular parasite *Toxoplasma* gondii (T. gondii), which has poor host specificity. *Toxoplasmas* is an infectious and inflammatory syndrome most cases symptomatic and may include abortions in addition to intrauterine growth retardation, stillbirths, early deliveries, and fetal abnormalitie (1) It is widespread around the world linked with the poverty, rural areas as well as urban areas Both Centre of Disease Control and prevention (CDC) and National institute of Health (NIH) (2) classified The complex life cycle of *Toxoplasma* gondii encompass every method of transmission that the parasite can employ to spread from particular hosts. Cats (domestic and wild - live cats) are the last host in the intricate "traditional" life cycle of T. gondii, with their prey serving as an intermediate host Three infectious phases of T. gondii exist: an invasive stage known as a tachyzoite that divides quickly, a bradyzoite that divides slowly in tissue cysts, and This parasitic infection includes an acute phase followed by a latent stage inside tissue cysts. Serum (3) immunoglobulin IgM is the markerof the acute phase response of infection,while IgG is a marker of the response for the latent phase an environmental stage known as a sporozoite that isshielded inside an oocyst .The *T. gondii* infected immunocompromised patients could develop severe neurological diseas myocarditis has been involvement in a number of autoimmune troubles including systemic sclerosis, autoimmune thyroid diseases, rheumatoid arthritis, inflammatory bowel disease (4) These variations could be explained by the seroprevalence estimates for human populations, which fluctuate significantly across different geographic regions within a same nation, or by a number of other elements, such as age, cultural level, nutritional habits, or rural and urban areas (5) The T cell is stimulated by the proper co-stimulatory signals after being exposed to pathogen-derived antigens by the APC Strong CD8 T-cell responses provide immune defense against a variety of intracellular pathogens, including viruses, bacteria, and protozoa. In lymphoid tissues, naive CD8 T lymphocytes come into contact with an antigen-presenting cell after infection (APC) (6) Although humoral immunity also contributes to *T. gondii* management, cell-mediated immunity is still the most crucial Compoent in *T. gondii* resistance There are currently few effective therapies for this disease, with the primary aim being reducing parasite reproduction rates in order to prevent further harm to the organs involved.(7) As a result, it is apparent that on going medication treatment is required to ensure the avoidance of serious problems. *Toxoplasmosis* medicines should be effective, easy to acquire, and inexpensive, with no toxicity or hypersensitive responses allowing them to be used in pregnant women for patients who are unable to take the medication. It should also be effective against all strains of *T. gondii*, have a high ocular and cerebral penetration, and be susceptible to killing tachyzoites (8) Different protein types, such as ADA cytokines, Consequently, the ADA level in these patients has been estimated can be used as indicators for their primary functions in catalysis, as structural components in signaling pathways, and as molecular systemic parameters that provide information on the stages of disease development, whether acute or chronic, and new drug discovery Cell mediated immunity is the main immunological response in T. gondii..(9) Numerous people have been found to have elevated serum ADA activity. As a result, there has been a lot of interest in the parameters for

monitoring individuals who have *Toxoplasmosis*, HIV, or another type of immunodeficiency (10) the activation of CD8+ T cytotoxic cells to become main cytotoxic effector cells for lysing tachyzoite-infected cells, cytokines released by CD4+ Thl cells can prevent the spread of the parasite during the acute infection phase In terms of clinical confirmation and diagnostic prognosis,(11)Adenosine Deaminase enzyme or Adenosine Aminohydrolase (ADA) has also been considered as a parameter in various infectious and hereditary disorders, such as Tuberculosis (TB) and squamous cell carcinoma. ADA Cytokines secreted from CD4+ Thl cells can subsequently activate CD8+ T cytotoxic cells to turn into major cytotoxic effector cells for lysing tachyzoite-infected cells, limiting parasite dissemination during acute infection phase(12)The main mechanism for iron storage is an iron-storing protein called ferritin. It is essential for maintaining iron homeostasis and makes iron available for vital cellular functions while guarding against iron's potentially harmful effects on lipids, DNA, and proteins. In clinical practice, changes in ferritin are frequently observed (13) Its main function is in the internalization and sequestration of iron in the ferritin mineral core, where it acts as a ferroxidase, converting Fe(II) to Fe(III). The parasite's capacity to multiply inside the host's body and alter the organism's physiology was what made it pathogenic (14) Aims the Study of cytokines (ferttein and ADA) and their potential effects on toxoplasmosis in *toxoplasmosis* patients. Patients getting chemotherapy and biological treatments who are also co-infected with *toxoplasmosis* were compared based on immunologic markers,Investigation their levels ADA activity and ferritin as possible parameters in *Toxoplasosis* during infection,

## Materials and methods

### Patient's collection

A total of 20 blood samples were obtained from the investigated groups shown in figure (1-1) between October 2021 and February 2022, from both genders (20), with ages ranging from 18 to 50years. ***the pregnancy non pregnancy womans with Toxoplasmosis groups,*** from Baghdad Teaching Hospital and Al-Yarmouk Teaching Hospital

### Blood samples

Samples of 5 ml venous blood from each patient were collected in a gel tube; in addition, personal information was documented including: age, sex, Blood samples were centrifuged and the serum was divided into an Eppendorf tube each containing at least 500 µl of pure serum and stored at -20°C for later investigation, as well as several outpatient clinics, provided samples. There are several serological tests available for the detection of T. gondii antibodies, such, enzyme linked immunosorbent assay (ELISA).Diagnosis relies on either direct detection of the organism (direct microscopic examination to the isolated parasites from tissue samples) or indirect serological tests (indicating recent or past infection), which are most effective in immunocompetent adults who are able to mount a humoral response to the parasite

### Detection of Anti - Toxoplasma IgG antibodies

Serological tests such as serum ferritin test

Detection the level of ADA activity and ferritin test by ELISA test.

A. Adenosine Deaminase (ADA) Activity Assay Kit (Colorimetric)

This Adenosine Deaminase (ADA) Activity were ordered from Abcam® Company, USA and stored at -20°C. ADA activity is an assay where inosine formed from the breakdown of adenosine is detected via a multi-step reaction, resulting in the formation of an intermediate that reacts with the ADA convertor and developer to generate uric acid that can be easily quantified at OD293 nm. The kit measures total activity of Adenosine

Deaminase with limit of quantification of 1 mU recombinant Adenosine Deaminase.

## Results and Discussion

Parasite infection All suspected Toxoplasmosis patients involved in this study were pre-diagnosed inthe laboratory before processing ADA ELISA detection. Clinical features of Toxoplasmosis were first examined for each patient, in addition, Anti-*Toxoplasma* IgM and IgG antibody rapid test Kit (Immuno- chromatography) was used in the current study for all subjects When the sample to be detected contains the Tox -IgM antibody, it firstly forms complexes with gold monoclonal antibodyif the test line does not appear, that is a negative result. and posative results (+ve IgG) were confirmed by ELISA method.

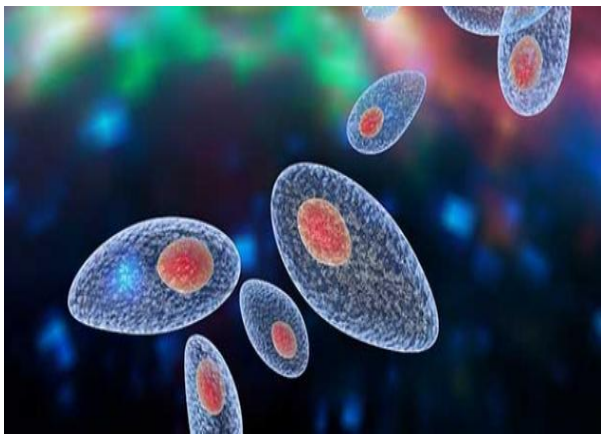

**Figure1: this figure showing intra-cellular of Toxoplasmosis, light microscope 100X.**

Adenosine deaminase enzyme activity and ferritin *Toxoplasmosis* result:

Serum concentration of adenosine deaminase enzyme (ADA) activity was investigated in the studied groups of *Toxoplasmosis* where significant patients with comparison to control group. The findings revealed a Non-significant and healthy serum ferritin in the *Toxoplasmosis* which was treatment stage when compared to the stage controls. However, following the successful treatment of *Toxoplasmosis* subjects, the increased ADA activity in serum was lowered. However, we found that active *Toxoplasmosis* cases had much higher serum ferritin than ADA activity cases.

### ADA activity in new Toxoplasmosis infections.

Results of patients with new infection (no treatment trials) shows that the average of ADA concentration activity level was lower than that of the control group, which were 0.0000250 nanomol/minute/µg and 0.0000488 nanomol/minute/ug, respectively, figure (1-2). However, statistical analysis did not record a significant difference,

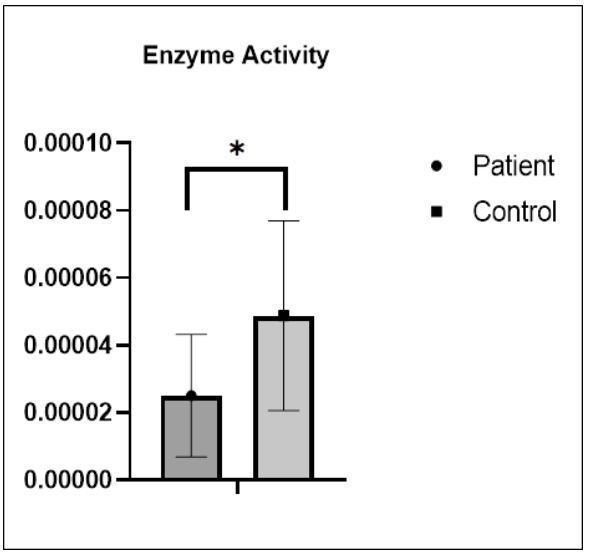


**Figure 2 ADA activity level in the New Toxoplasmoiasis treatment patients, there was significance difference between the test and control group (p value < 0.05).**

Ferritin rapid test in trials treatment infection

The result of both groups received two doses also revealed an elevated mean concentrations of Ferritin rapid test (91.32) respectively, when correlated to the mean of control group, which was equal to 41.9. However, the statistical analysis reported non a significant difference only in the Ferritin group, figure

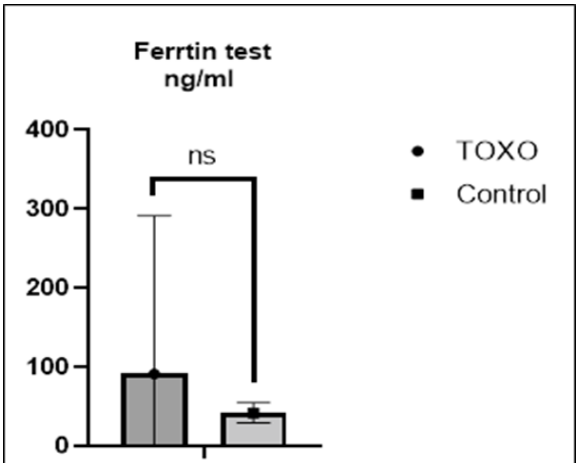


**Figure. 3 .Ferritin rapid test level in the trail treatment patients was seen non significance difference between the test and control group (p value >0.05)**

The two ADA isozymes, ADA1 and ADA2, have been found; ADA1 is the predominant component of human tissue extracts, whereas ADA2 is the main component of total serum ADA. Serum from patients with hepatic problems, hematological malignancies, and other viral diseases, as well as acute leukemias, chronic myeloid blast crisis leukemia, and acute liver injury, has been reported to have considerably increased ADA activity. Patients with adult T-cell leukemia, multiple myeloma (B-J type), infectious mononucleosis, rubella, acquired immunodeficiency syndrome, and tuberculosis all exhibited elevated ADA1 levels in their blood (15) the immunological response to *T gondii* infection complex.The extreme genetic heterogeneity can be used to explain this individual variation. Additionally, *Toxoplasma* can infect all tissues and can cause a unique immune reaction in each tissue, especially in the central nervous system and the placenta. *Toxoplasma* can also spread across all tissues. Due to the potential for recurring infection with *Toxoplasma* strains with varied virulence, complexity is increased to a new level (16) Th1-type cytokines can also lead to apoptosis, which can weaken the trophoblast walls that keep the semi-allogenic fetus away from the mother's immune system and lead to fetal rejection or abortion. Natural killer cells, lymphokine activated killer cells, and cytotoxic T lymphocyte (CTL) cells are all produced as a result of these cytokines, and they can all kill trophoblasts and result in fetal death (17) It is the essential cytokine for resistance to both acute and chronic toxoplasmosis infections, according to a report. It has been established that cytokines play a significant part in the pathogenesis of toxoplasmosis. The establishment of T. gondii immunity requires the activation of a type 1 inflammatory cytokine response (IL-12, IFN-, ADA activity (18) Accordingly to be acute or chronic, affects. Parasites the host endocrine system,which is responsible for altering the host behaviors. Host modifications due to T. gondii infection is significantly related to the gender.(19) Concerning the another study's results, T. gondii infection was associated with vitamin D deficiency.More studies are suggestedto be conductedfor understandingthe relationship between VtD and parasitic infections. The purpose of the study was to demonstrate the impact of parasites at the level of the two ferritin levels on the status of iron, particularly hemoglobin and anemia, which is one of them. The causes of fetal malformations and death were associated with changes in the disease-related proteins' stage that have been shown another studies ferritin levels. Iron insufficiency, hypothyroidism, and ascorbate deficiency are three disorders to diminish serum ferritin levels. it is increased in end-stage kidney failure and chronic disease anemia (20) As a result, whenever cell mediated immunity is increased, the level of ADA rises, reflecting the activity of stimulated T cells. Its activity has been demonstrated to be increased under conditions where T cells proliferate and become activated (21).Similar investigations demonstrated that the ADA level changes over time, revealing a deeper understanding of the pathophysiology and physiology of the internal system.infection. There is mounting evidence that ADA's function in cell-mediated immunity causes an increase in serum

and lymphocyte ADA levels during toxoplasmosis infection (22)Another intraceullar parasite, Plasmodium vivax, was the subject of a concurrent investigation that revealed higher levels of ADA activity in serum samples, erythrocytes, leukocytes, and plasma hemoglobin concentrations when compared to the control group (23) In addition, similar studies on other infectious agents proved significant high serum ADA levels in multibacillary leprosy and in patients of leprosy which may due to increased lymphoreticular activity during the reactional phases Additionally, Oral Squamous Cell Carcinoma (OSCC) showed a significant increase in serum ADA level, suggesting an elevation in serum ADA activity that aids in the identification and follow-up of head and neck cancers (24)The present study showed that Toxoplasma infection in breast cancer patients does not seem to increase progressively with age, which is similar to that shown with a previous study.to clarify the time course, and this was supported by research findings Research has also suggested that the impact of blood-borne illness but it is in disagreement with other studies demonstrated that the seroprevalence rate of toxoplasmosis increases with age and the peak level was seen in cases older than 50 years (25)

Conclusion The present results suggest that host factors, virulence of T. gondii strains, and duration of infection, Thus T. gondii should be diagnosed and treated to decrease the burden on the immune system and reduce the consequences which cause altered cytokine profiles, must be considered, which determine the type of cellular, protective or regulatory response prevalent., these results demonstrated that toxoplasmosis infection plays significant role in altering only ADA level than Frettin while parameters different groups other Clinicians should be more careful with this patients group to prevent the possibility of severe toxoplasmosis considered as risk factors for *Toxoplasmosis*

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