Al-Nahrain Journal of Science
ANJS, Vol. 28(3), September, 2025, pp. 23 – 30

Amylase, Phosphate, Zinc & Magnesium Serum Levels in Women with Toxoplasmosis

Sarra A. Abrahem [1], Suhad A. Ibrahim [1,*], Maryam Y. Hashim[2]

[1] Department of Chemistry, College of Science, Al-Nahrain University, Baghdad, Iraq
[2] Industrial Property and Technical Design Directorate, the Central Organization for Standardization and Quality Control (COSQC), Baghdad, Iraq.

Article's Information

Received: 13.11.2024
Accepted: 23.02.2025
Published: 15.09.2025

Keywords:
Amylase enzyme,
Trace elements,
Toxoplasmosis.

Abstract

Toxoplasmosis has a significant infection rate in several Arab countries, such as Iraq. The role of the amylase enzyme and some trace elements, such as phosphorus (P), zinc, and magnesium, was evaluated with the incidence of toxoplasmosis in 80 Iraqi women (50% fertile and 50% infertile). Samples were collected from eighty blood infertile women (as patients, P group) and fertile women who were apparently healthy (as controls, C group), with an age range of 20-40 years. Each group was subdivided into two groups: with seropositive toxoplasma gondii IgG group (PP, and PC for patient and control group, respectively) and seronegative toxoplasma gondii IgG (NP, and NC for patient and control group, respectively). Amylase enzyme activity, phosphorus (P), zinc, and magnesium were estimated in the sera of women for each collected sample. There was a decrease in amylase level in positive and negative patients compared with healthy patients. The zinc level in fertile patients was significantly higher ($P<0.05$), while it was lower in positive patients compared to the C groups. The magnesium level increased significantly in positive patients ($P<0.05$) compared to the C groups. However, the magnesium level in patients with negative patients compared to healthy controls was non-significantly lower ($P>0.05$). Phosphorus level increased significantly ($P<0.05$) in positive patients compared to C groups. Changes in the level of the Amylase enzyme and the elements such as zinc, phosphorus, and magnesium can be considered indicators of the causes of toxoplasmosis.

http://doi.org/10.22401/ANJS.28.3.04
*Corresponding author: su_aziz2015@yahoo.com

1. Introduction

Toxoplasmosis is a parasitic zoonosis that can be caused by ingesting food or water that is contaminated with the parasite (*Toxoplasma Gondii*). It can be transmitted to the fetus of a pregnant woman who is infected with this parasite and produce systemic, neurological, or even ocular damage [1]. During acquired infections, there are key players involved in controlling T. Gondii, these include human predominantly IgG1, IgG subclasses, as well as their Fc receptors. In infected pregnant women, a specific IgG1 was found to be related to health issues in their infected infants [2]. It can be argued that this might be the result of different eating and cooking habits, in addition to the oocyst's ability to endure various climates [3-4]. During the past 30 years, some scholarly literature has tracked down evidence of an association between autoimmune diseases and infection with T. gondii [5]. Trace elements are key to biological processes. They may act as inhibitors or activators of enzymatic reaction. They can also influence the cell membrane permeability, or its function as a direct anti-oxidant enzyme [6]. The diet of pregnant rats includes up to 5000 mg/kg concentrations of Zinc, causing hypocu-prosis in their fetuses. It induced incidences of fetal resorptions and stillbirths [7- 8] (Ketcheson et al., 1969). In another study, Kinnamon (1963) asserted contrasting results. The findings of his research on rats receiving similar zinc amounts, showed no resorption. However, zinc toxicity in human subjects is still under research. [9] Kumar (1976) reported that three out of four women who took (300 mg) of zinc per day during pregnancy, had premature

births, whilst, the child of a fourth was dead at birth [10-14]. α-amylase (α-AMY) enzymes can be responsible for starch digestion [15]. It is considered as a significant industrial enzyme. It is produced by microbial sources, plants, and animals. The enzymes produced from microbial source have stability and economic viability, and thus, they have potential applications. A large number of α-amylases obtained from various sources have been addressed in the literature [16]. Several enzymatic systems activated by element concentrations, and minute minerals can impact the activities of human organs and tissues because they act as an enzyme biocatalyst. For instance, magnesium (Mg) usually integrates into anatomic structures, such as membrane proteins, bone elements' nucleic acids, and enzymes. It is also involved in the ionized active form and is therefore regarded as an essential element, especially in voltage-gated ionic channels [17]. Additionally, inorganic ions e.g. phosphate, are essential supplements for an expansive range of cell capabilities. During contamination, microorganisms should acquire inorganic phosphate (Pi) from the host, yet it is still unclear how the intracellular parasite T. gondii gets Pi from the host cell for development [20]. It has been accounted for that the parasite amasses and stores a lot of phosphorus as polyphosphate (polyP), pyrophosphate (PPi), and phosphate (Pi). Acidocalcisomes specific carriers for PPi and Pi are in all probability accessible on the restricting film of acidocalcisomes yet have not been distinguished at this point in Toxoplasma [21]. For more exhaustive view on toxoplasmosis sickness this exploration endeavored to assess the connections between the amylase compound and the degree of minor components in the blood of women who test positive for toxoplasmosis. The results were compared to those obtained from uninfected women.

2. Materials and Methods

In this study, 80 Iraqi women participated. It is important to mention that informed approval was gained from each participant, and that this research was approved by the human research ethics committee of the hospital, and from the Training and Human Development Center in Rusafa Health Department, Baghdad, Iraq, the approval number is (164727). They were divided into 4 groups as follows: (PP group): 20 infertile women whose T. gondii was positive (NP group): 20 infertile women whose T. gondii was negative (PC group): 20 fertile women whose T. gondii was positive, and (NC group): 20 fertile women whose T. gondii was negative. All groups were matched for ages (20 to 40) years of age and a body mass index (BMI) using the Quetelet formula (18.5-29.9kg/m^2). The infertile participants had been married for at least 2 years, they had a normal sexual life, and were being treated for primary infertility; however, the fertile women were normally ovulating and had at least one baby. Patients with other causes of infertility, such as infections, or ovarian, tubal, and hormonal issues, abortion, galactorrhea, and those taking hormonal medication were excluded from this work. Venous blood samples were collected from selected patients before taking any medications. Sera were separated and stored at (-20°C) to be used later in the analysis. Serum metals using an atomic absorption spectrophotometer (Shimadzu AA-646). SPSS (for Windows, version 23.0) was used for the statistical analysis.

3. Results and Discussion

Two main groups were enrolled in the present work, patients and controls; P (n=40) and C (n=40), which refer to infertile and fertile women. Each group was sub-grouped into 2 groups: women with positive T. gondii (PC and PP groups, respectively) and women with negative T. gondii (NC and NP groups, respectively). Table (1) illustrates age and BMI level; some of the participants were of normal weight, while the others were overweight. A variation in the BMI ratio was observed.

Table 1: The characteristics of the four studied groups (infertile and fertile women).

Group	Age	BMI (%)	
		Normal % (18.5-24.9) Kg/m^2	Overweight % (25-29.9) Kg/m^2
NC	21 - 40	38.24	63.58
NP	20- 39	58.05	41.02
PC	20 - 40	45.10	57.01
PP	20 - 40	39.32	62.88

NC: Negative *T.gondii* Control, **NP:** Negative *T. gondii* Patients, **PC**: Positive *T. gondii* Control, **PP:** Positive *T. gondii* Patients.

The difference was non-significant at ($p > 0.05$) for NC group when compared with NP group, while a highly significant increase ($p < 0.001$) was found for PP in comparison with PC group. Toxoplasmosis caused by the *Toxoplasma gondii* parasite is a prevalent disease; its severity could be an asymptomatic self-limiting infection that can be complicated to a serious, destructive inflammatory [15-16]. Table (2) illustrates a comparison of amylase, phosphorus P, magnesium Mg and zinc Zn levels between (NC) and (NP) groups. Also, the mean±SD of (PC) and (PP) groups were compared.

Table 2: Mean± SD of Amylase, Zn,Mg, and Phosphorus concentration of the four studied groups.

Group	NC	NP	PC	PP
Amylase ((IU/L)) mean± SD	77.923±33.487	58.869±20.363	63.000±14.470	58.650± 31.771
p-Value	0.015	0.200	0.200	0.200
Zn (µg/dL) mean± SD	94.7677±25.786	103.410±16.740	79.625±32.998	72.622±33.337
p-Value	0.192	0.005	0.200*	0.002
Magnisum (Mg) mean± SD (mg/dl)	2.5046±0 .402	1.7596±0.642	1.3186 ±0 .743	2.774 ±0.228
p-Value	0.065	0.007	0.023*	0.014*
Phosphorus (P) mean± SD *(mg/dl)*	5.5077 ± 1.931	5.8913 ± 2.32005	4.5743±0.78189	21.258±14.897
p-Value	0.003**	0.000**	0.200	0.000**

NC: Negative *T.gondii* Control, **PC**: Positive *T. gondii* Control, **NP:** Negative *T. gondii* Patients **PP:** Positive *T. gondii* Patients.

**The difference is highly significant at $p < 0.001$. *The difference is significant at $p < 0.05$

By observing the results in Table (2), it can be stated that the decrease in the level of amylase in the positive patients and the negative patients compared to the positive and negative healthy patients was a non- significant. The level of zinc in the sterile patients was significantly higher ($p<0.05$) compared to the negative controls, while the zinc level was decreased in the positive patients compared to the positive healthy controls, the magnesium level increased significantly ($p<0.05$) in the positive patients compared to all of the positive and negative healthy controls and negative patients, there was a non-significant decrease of ($p>0.05$) in the magnesium level in the negative patients compared to the negative healthy controls. In addition, phosphorous level was significantly increased ($p<0.05$) in the positive patients compared to the positive healthy controls. Inflammation and OS are deleterious conditions for cells; inflammatory reactions fundamentally involve the generation of free radicals (FR), and so trigger OS, which influences fertilization, ovulation, implantation, embryo development, and idiopathic recurrent pregnancy loss, leads to female infertility [17-19]. Meanwhile, highly ROS have been specified in the pathogenesis of diverse parasitic infections, including *T. gondii* [20]. The results of the current study may indicate that Toxoplasmosis generate particular effects on the antioxidant defense mechanisms due to the disease inflammatory character [21]. Several factors associated with inflammation are implicated in imbalance in the ROS/antioxidant system, when the body faced OS, alterations in antioxidant activity of the enzymes were formed, it can be either induction or suppression of these enzymes. At times, these enzymes can be inactivated by FRs and/or by several other factors, such as hormonal imbalance, environmental pollution under chronic conditions. Cells are able to overcome stress by increasing the antioxidant response after a mild increment in ROS generation [22-31]. This study's data show that seropositive groups (PC and PP groups) had a decrease in amylase activity, which was lower than the seronegative groups (NC and NP groups). Surprisingly, although the level of Mg was significantly decreased in patients with chronic toxoplasmosis, the Zn level did not show similar results. Even though the mechanism is unknown, the decrease in Mg level in patients with chronic toxoplasmosis can be the result of the decrease of enzyme systems (e.g. ATPase and alkaline phosphatase) or vice versa. However, our results show that although chronic toxoplasmosis somehow decreased Mg levels, it did not affect the Zn level. In other words, it did not affect the Zn-linked enzyme systems (such as carbonic dehydrogenase, alcohol dehydrogenase) and, consequently, did not decrease the Zn level. Zn is known to be vital for immunity,

especially when it comes to parasites (19). Figure (1) shows the level of p(mg/dl) between groups, Figure (2) shows the level of Mg (mg/dl) between groups, the level of Zn (mg/l) between groups is shown in Figure (3), finally the increase level of amylase in Np when compered with other groups in figure 4.

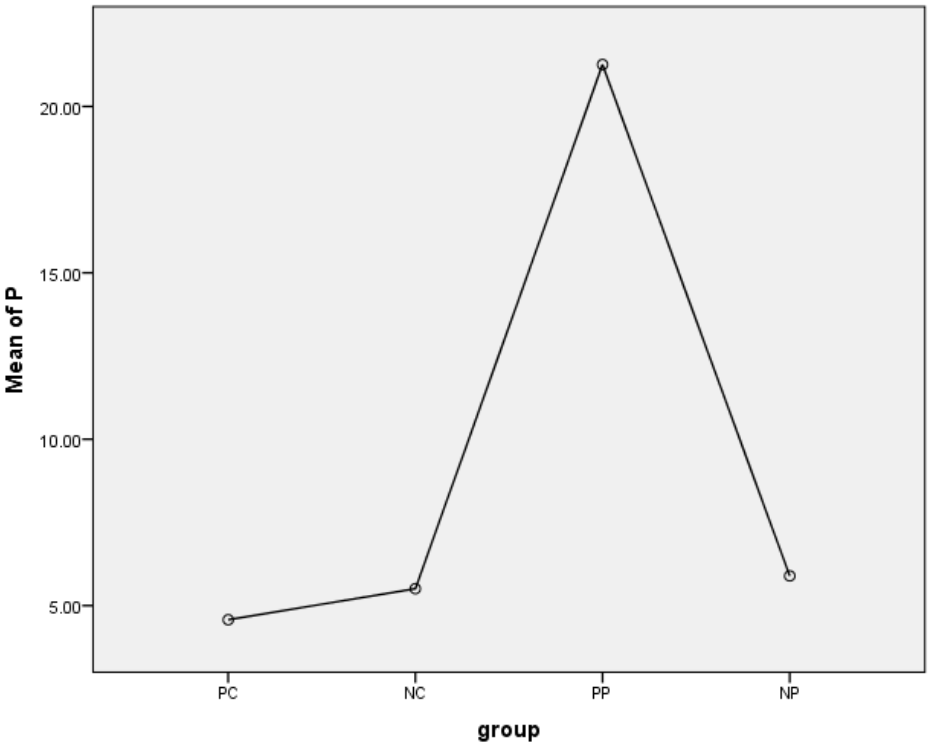

Figure 1: The level of p(mg/dl) between groups

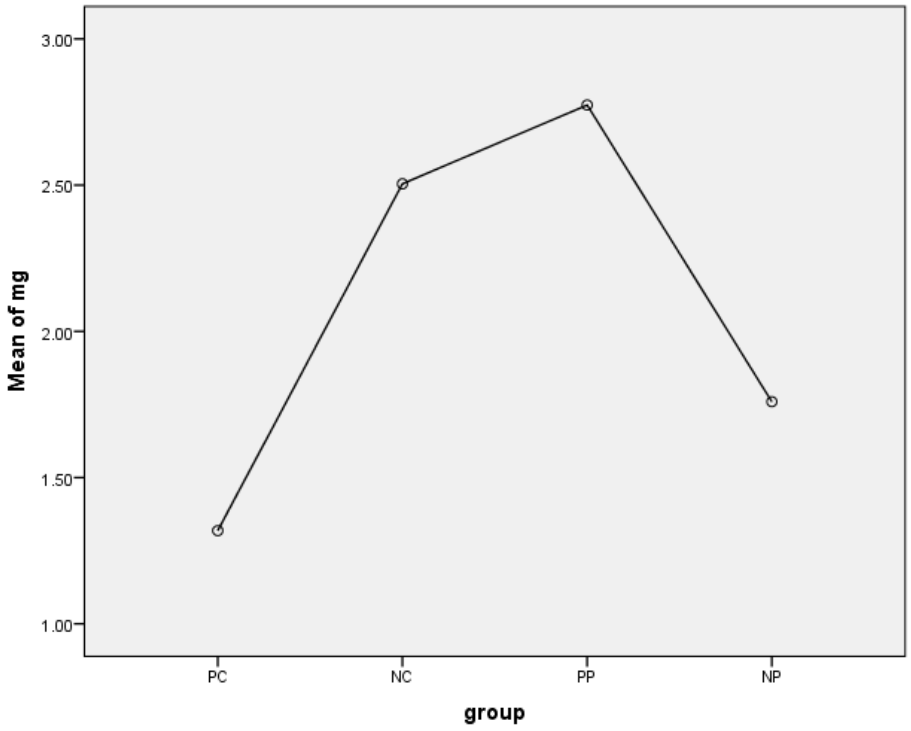

Figure2: The level of Mg(mg/dl) between groups

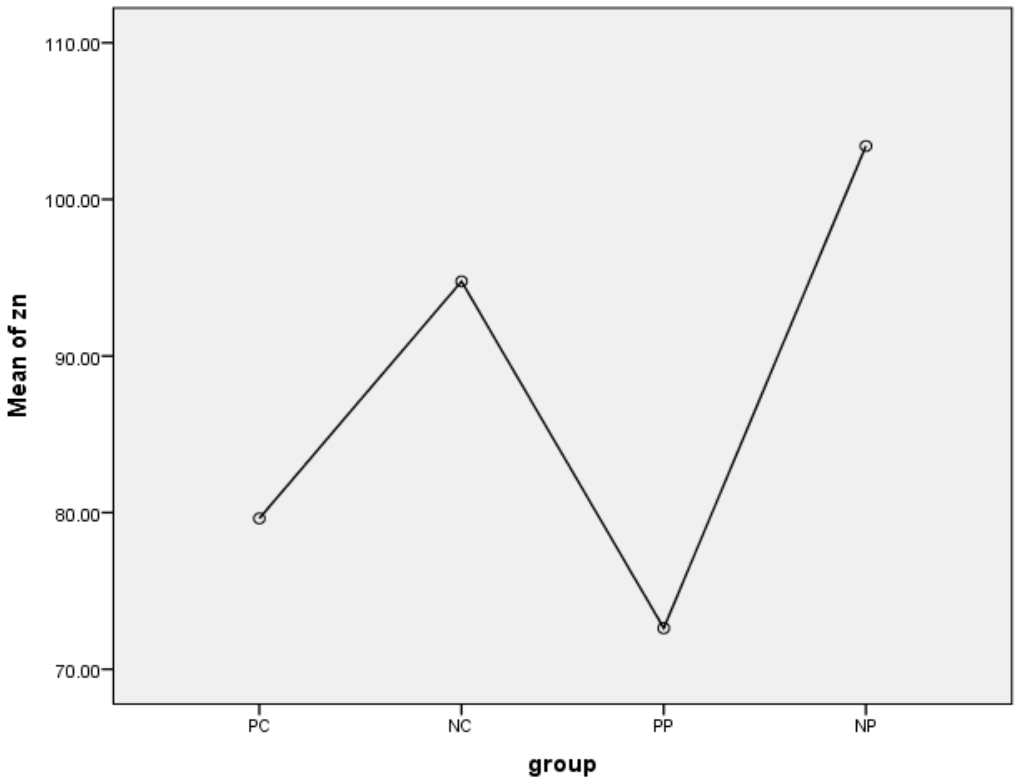

Figure 3: The level of Zn (mg/l) between groups

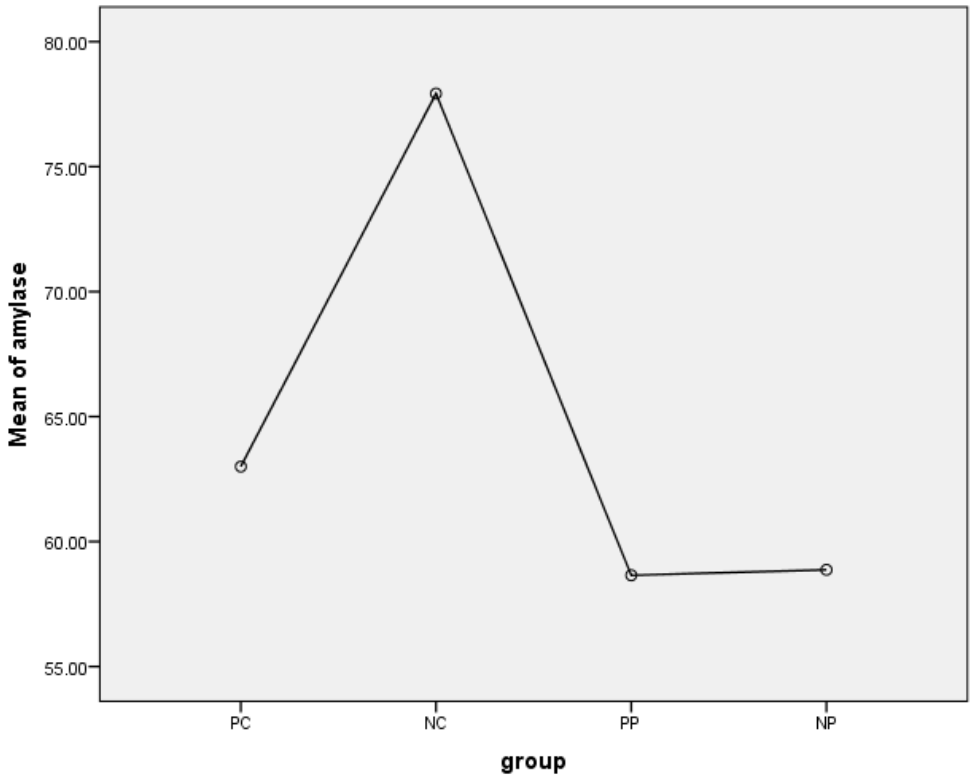

Figure 4: The level of amylase between groups

To investigate the Pearson correlation of the relationship between amylase activity and the other trace element studied parameters.

Table 3: Pearson correlation of studied groups. Correlation of Np negative patient

	Zn	Mg	p
Amylase r, p-value	-0.192, 0.379	0.093, 0.674	-0.199, 0.364
Zn r, p-value		-0.500*, 0.015	0.772**, 0.000
Mg r, p-value			-0.461*, 0.027

Table 4: Pearson correlation of studied groups. Correlation of PC positive control

	Zn	Mg	p
Amylase r, p-value	-0.436, 0.180	-0.045, 0.896	-0.461, 0.154
Zn r, p-value		0118, 0.730	0.519, 0.102
Mg r, p-value			-0.181, 0.595

Table 5: Pearson correlation of studied groups. Correlation of NC negative control

	Zn	Mg	p
Amylase r, p-value	0.404, 0.193	0.465, 0.128	0.083, 0.799
Zn r, p-value		0.329, 0.272	0.802**, 0.001
Mg r, p-value			0.487, 0.092

**correlation is significant at the 0.01level (2-tailed)

Table 6: Pearson correlation of studied groups. Correlation PP positive patient

	Zn	Mg	p
Amylase r, p-value	0.290, 0.215	0.265, 0.259	-0.024, 0.921
Zn r, p-value		-0.206, 0.383	0.854**, 0.000
Mg r, p-value			0.399, 0.082

By observing the data in the tables (1-6) above, it can be argued that there is no correlation among the mean values of Mg and Zn and age in sero-positive males and females, as well as controls. Moreover, there is no significant correlation between Mg levels and blood Zn in sero-positive female and male patients and controls. It has been known that the deficiency of zinc and magnesium affects many different enzyme systems and vice versa [30]. This is the first report indicating that the levels of some elements significantly decrease in patients with chronic toxoplasmosis; this can have possible effects on some specific enzyme systems, which can, consequently, cause some serious pathology, such as severe neurological disorders, in addition to pneumonia, hepatitis, and blindness.

3.1. Study limitations: The main limitation of this study was the small number of cases, which hindered our ability to obtain stronger statistical support.

3.2. Future research directions: A Larger sample size with more comprehensive data collection. Longitudinal studies to investigate the temporal relationship between infection and biomarker changes. Mechanistic studies to elucidate the underlying biological pathways

4. Conclusions

According to the findings, it can be concluded that a significant increase in the levels of magnesium and zinc has affected the Toxoplasmosis patients, while there was no significant effect on the rest of the elements and amylase among the four groups of Toxoplasmosis patients.

Acknowledgments: Thanks to all the fertile and infertile women (volunteers) who participated in this study.

Conflicts of Interest: The authors declare no conflict of interest.

Funding statement: This work was financially supported by the author. No funds, research grants or other support were received for the purpose of

conducting this work. The authors have no relevant financial or non-financial interests to disclose.

References

[1] Cañedo-Solares, I.; Gómez-Chávez, F.; Luna-Pastén, H.; Ortiz-Alegría, L. B.; Flores-García, Y.; Figueroa-Damián, R.; Macedo-Romero, C. A.; Correa, D.; "What do anti-Toxoplasma gondii IgA and IgG subclasses in human saliva indicate?". Parasite Immunol. 40(5): p.e12526, 2018.

[2] Cañedo-Solares, I.; de la Luz Galván-Ramírez, M.; Luna-Pastén, H.; Pérez, L. R. R.; Ortiz-Alegría, L. B.; Rico-Torres, C. P.; Vela-Amieva, M.; Pérez-Andrade, M.; Figueroa-Damián, R.; Correa, D.; "Congenital toxoplasmosis: specific IgG subclasses in mother/newborn pairs." J. Pediatr. Infect. Dis. 27(5):469-474, 2008.

[3] Dubey, J. P.; "The history of Toxoplasma gondii—the first 100 years." J. Eukaryot. Microbiol. 55(6): 467-475, 2008.

[4] Fischer, S.; Agmon-Levin, N.; Shapira, Y.; Porat Katz, B. S.; Graell, E.; Cervera, R.; Stojanovich, L.; Gómez Puerta, J. A.; Sanmartí, R.; Shoenfeld, Y.; "Toxoplasma gondii: bystander or cofactor in rheumatoid arthritis." Immunol. Res. 56: 287-292, 2013.

[5] Mousa, M. A.; Soliman, H. E.; El-Shafie, M. S.; Abdel-Baky, M. S.; Aly, M. M.; "Toxoplasma seropositivity in patients with rheumatoid arthritis". J Egypt Soc Parasitol. 18(1): 345-351, 1988.

[6] Ketcheson, M. R.; Barron, G. P.; Cox, D. H.; "Relationship of maternal dietary zinc during gestation and lactation to development and zinc, iron and copper content of the postnatal rat." J Nutr. 98(3): 303-311, 1969.

[7] Kinnamon, K. E.; "Some independent and combined effects of copper, molybdenum, and zinc on the placental transfer of zinc-65 in the rat". J. Nutr. 81(4): 312-320, 1963.

[8] Kumar, S.; "Effect of zinc supplementation on rats during pregnancy". Clin. Chem. 23: 1834–1837, 1976.

[9] Campbell, J. K.; Mills, C. F.; "The toxicity of zinc to pregnant sheep". Environ. Res. 20(1): 1-13, 1979.

[10] Rajput, R.; Denniston, A. K.; Murray, P. I.; "False negative Toxoplasma serology in an immunocompromised patient with PCR positive ocular toxoplasmosis". Ocul. Immunol. Inflamm. 26(8): 1200-1202, 2018.

[11] Murdaca, G.; Tonacci, A.; Negrini, S.; Greco, M.; Borro, M.; Puppo, F.; Gangemi, S.; "Emerging role of vitamin D in autoimmune diseases: An update on evidence and therapeutic implications". Autoimmun. Rev. 18(9): 102350, 2019.

[12] Chistiakov, D. A.; Melnichenko, A. A.; Orekhov, A. N.; Bobryshev, Y. V.; "Paraoxonase and atherosclerosis-related cardiovascular diseases." Biochimie. 132: 19-27, 2017.

[13] Abbas, I. E.; Villena, I.; Dubey, J. P.; "A review on toxoplasmosis in humans and animals from Egypt". Parasitol. 147(2): 135-159, 2020.

[14] Yang, J.; Yang, C.; Qian, J.; Li, F.; Zhao, J.; Fang, R.; "Toxoplasma gondii α-amylase deletion mutant is a promising vaccine against acute and chronic toxoplasmosis". Microb. Biotechnol. 13(6): 2057-2069, 2020.

[15] Sindhu, R.; Binod, P.; Madhavan, A.; Beevi, U. S.; Mathew, A. K.; Abraham, A.; Pandey, A.; Kumar, V.; "Molecular improvements in microbial α-amylases for enhanced stability and catalytic efficiency." Bioresour. Technol. 245: 1740-1748, 2017.

[16] Yazar, S.; Kilic, E.; Saraymen, R.; "Changes of total content of magnesium and zinc status in patients with chronic toxoplasmosis". Biol. Trace Elem. Res. 92: 11-15, 2003.

[17] Jones, T. C.; Hunt, R. D.; King, N. W.; "Veterinary Pathology", 6th ed.; Williams & Wilkins, A Waverly Company: Baltimore, MD, USA, 1996.

[18] Wellinghausen, N.; Jochle, W.; Reuter, S.; Flegel, W. A.; Grunert, A.; Kern, P.; "Zinc status in patients with alveolar echinococcosis is related to disease progression". Parasite Immunol. 21(5): 237-241, 1999.

[19] Asady, B.; Dick, C. F.; Ehrenman, K.; Sahu, T.; Romano, J. D.; Coppens, I.; "A single Na+-Pi cotransporter in Toxoplasma plays key roles in phosphate import and control of parasite osmoregulation". PLoS Pathog. 16(12): e1009067, 2020.

[20] Rodrigues, C. O.; Ruiz, F. A.; Rohloff, P.; Scott, D. A.; Moreno, S. N.; "Characterization of isolated acidocalcisomes from Toxoplasma gondii tachyzoites reveals a novel pool of hydrolyzable polyphosphate". J. Biol. Chem. 277(50): 48650-48656, 2002.

[21] Chaudhry, S. A.; Gad, N.; Koren, G.; "Toxoplasmosis and pregnancy." Can Fam Physician. 60(4): 334-336, 2014.

[22] Afifi, M. A.; Jiman-Fatani, A. A.; Al-Rabia, M. W.; Al-Hussainy, N. H.; El Saadany, S.; Mayah, W.; "More than an association: latent toxoplasmosis might provoke a local oxidative stress that triggers the development of bipolar

disorder". J. Microsc. Ultrastruct. 6(3): 139-144, 2018.
[23] Hussain, T.; Tan, B.; Yin, Y.; Blachier, F.; Tossou, M. C.; Rahu, N.; "Oxidative stress and inflammation: what polyphenols can do for us?". Oxid. Med. Cell. Longev. 2016: 1-9, 2016.
[24] Agarwal, A.; Aponte-Mellado, A.; Premkumar, B. J.; Shaman, A.; Gupta, S.; "The effects of oxidative stress on female reproduction: a review". Reprod. Biol. Endocrinol. 10: 49, 2012.
[25] Shi, L.; Zhang, J.; Lai, Z.; Tian, Y.; Fang, L.; Wu, M.; Xiong, J.; Qin, X.; Luo, A.; Wang, S. "Long-term moderate oxidative stress decreased ovarian reproductive function by reducing follicle quality and progesterone production". PLoS One. 11(9): e0162194, 2016.
[26] Khadhim, N. A.; Al-Khafaji, Q. A. M.; Hussein, M. S.; "Iron Status, Malondialdehyde, and Nitric-oxide Levels in Iraqi Infertile Women with IgG Seropositive Toxoplasma Gondii". J. Glob. Pharma. Technol. 11(9):414-421, 2019.
[27] Kashan, Z. F.; Shojaee, S.; Keshavarz, H.; Arbabi, M.; Delavari, M.; Salimi, M.; "Vitamin D deficiency and Toxoplasma infection". Iran J. Public Health. 48(6): 1184-1186, 2019.
[28] Khadhim, N. A.; Al-Khafaji, Q. A. M.; Hussein, M. S.; "Serum vitamin D and TNF-alpha in Iraqi infertile women with positive IgG toxoplasma gondii: Is there a correlation between infertility and vitamin D deficiency". Res. J. Pharm. Biol. Chem. Sci. 9(1): 628-639, 2018.
[29] Waldron, J. L.; Ashby, H. L.; Cornes, M. P.; Bechervaise, J.; Razavi, C.; Thomas, O. L.; Chugh, S.; Deshpande, S.; Ford, C.; Gama, R.; "Vitamin D: a negative acute phase reactant". J. Clin. Pathol. 66(7): 620-622, 2013.
[30] Ruder, E. H.; Hartman, T. J.; Goldman, M. B.; "Impact of oxidative stress on female fertility". Curr. Opin. Obstet. Gynecol. 21(3): 219-22, 2009.