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EFFECT OF PREGNANCY ON SOME BIOCHEMICAL AND IMMUNOLOGICAL MEASURES FOR WOMEN WITH *TOXOPLASMA GONDII*

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ABSTRACT : The current study included measuring the level of calcium and vitamin D and some biochemical variables of 58 samples of women, who were referred to outpatient clinics for the period from the beginning of November 2017 to the end of March 2018. They ranged in age from 15-40 years of pregnant and non- Toxoplasmosis. The results of the statistical analysis showed a significant decrease in the concentration of vitamin D in pregnant women (10.93 ± 3.17pg / dl) compared to non-pregnant women (15.44 ± 5.94 pg / dl) at a significant level of 0.05 P. P.

In the study of the concentration of electrolytes in the serum of pregnant and non-pregnant women, the results of the statistical analysis showed no significant differences in calcium at a significant level of 0.05 P P, while in magnesium, the results of the statistical analysis showed a significant increase in the infected pregnant women (3.26 ± 0.55 compared with non-pregnant women (2.55 ± 0.85mlu / ml) at a significant level of 0.05 P. P. In terms of antibodies, the results of the statistical analysis showed that the percentage of pregnant women infected with IgG antibody was 57% Total. While the percentage of non-infected women was 68%. As for the antibody result, IgM did not appear in infected women.

Key words : Parasite Toxoplasma gondii, vitamin D, magnesium, calcium, IgG, IgM.

INTRODUCTION

The vitamin D is a vitamin that is considered as one important biochemical scales to humans, a vitamin soluble in fat, which is found naturally in some foods and is available as a dietary supplement and the vast majority of vitamin D is manufactured by the human body, while the skin is exposed to sunlight, and plays an important role in bones and maintained by regulating calcium and phosphorus levels of growth, and is also necessary for the proper functioning of the immune system, hormones and cardiovascular health (Deschasaux *et al*, 2016). Some studies have shown that vitamin D is affected by toxoplasmosis, an anthropozoonic disease caused by the toxoplasmosis gondii, a parasitic parasite that lives within cells (obligate living) and can infect all blood-bearing animals. Most of the birds and mammals are considered as intermediate intermediates and are therefore the most widely spread parasites on earth (Joanne, 2012; Jimenas-Coello *et al*, 2012). Domestic and terrestrial cats are endemic specimens that play an active role in parasite transmission, where egg bags (oocyst) are deposited in their faeces (Cenci-Goga *et al*, 2011). These bags are a threat to public health because they are resistant to harsh environmental conditions. The infection is caused by direct contact with programmed cat feces or by ingesting egg bags in food, drink, contaminated soil, blood transfusions or transplantation or trans-placenta is a container on tissue bags. Studies of Cenci- Goga *et al* (2011), Rogerio *et al* (2011), Jimenez-Coello *et al* (2012) indicate that up to one-third of the world's population is infected with T. gondii and symptoms are mild or invisible in immunologically competent people (Zhou, 2011 Kay, 2011). Infection is transmitted in pregnant women from mother to fetus, leading to birth defects or miscarriage (Villena *et al*, 2010).

The calcium ion is also affected by the occurrence of parasites, which is a necessary element in the parasite and host body (Alhasry and Zheiri, 2010), as it is necessary for the movement of parasitic parasite also owns the parasite several stores of calcium, the most important is the acid calcium gap Acidocalcisome and is the largest store of calcium in the parasite which is characterized by its dense and acidic nature (Dubey, 2010). Vitamin D is an essential vitamin for the body. Its most important role is to maintain the level of calcium in the blood and to follow it with the help of thyroid hormone and calcitonin (Deschasaux *et al*, 2016). Therefore, the present study aimed at:

1. Studying the relationship between *T. gondii* and vitamin D in pregnant and non-pregnant women.
2. Studying the changes in IgG and IgM level in pregnant and non-pregnant women with anticoagulants.
3. Effect of T. gondii on electrolytes (calcium, magnesium) in pregnant and non-pregnant women.

MATERIALS AND METHODS

Blood samples were collected from women and all study groups and 5 mm in venous blood using syringe syringes. They were placed in test tubes with a gel free of anticoagulant (EDTA) and left for 10 min at room temperature (25°C). Centrifuge was centrifuged at 3000 cycles per minute for 5 minutes to separate the serum. The serum was then withdrawn using a micropipette pipette and distributed to Eppendroff tubes. The diagnostic tests for congenital toxoplasmosis were then performed for all samples of the study, then checked by ELISA to investigate the specific antibodies of the convex curve (IgG and IgM). The other freezer tubes were then frozen at 20°C until further biochemical testing

Determination of calcium ion concentration in serum

Determination of calcium ion concentration in blood serum (Ca^{2+}) Serum (Ca^{2+}) was assessed using a pre-test kit from German company Human. The color method followed by Pollard and Marun (1956) was followed.

Determination of serum magnesium concentration in serum

Determination of magnesium ion concentration in serum (Mg^{2+}) Mg^{2+} was evaluated in the blood serum using the Heth and KhayamBashi method using the pre-test kit from the French company Biolabo.

Estimation of Vitamin D in blood serum

The ELISA test kit is used and it is prepared by the Chinese company Sunlong Biotech using the Sandwich-ELISA method.

Method of action procedure

1. Mitigation Standards: Ten measuring vessels are used in the stripe plate MicroelisaStripplate. Where, lì 100 of the standard solution and 50 ìL of the buffer are added to the standard dilution in pot 1 and pot 2 and mixed together well.

In container 3 and pot 4, 100 ìl of the first container solution (1) and the second (2) are added respectively, and then add 50 ìL of the buffer for standard dilution and mixed well. 50 ìl of the third and fourth vessel solution.

The seventh container (7) and the eighth (8) lì 50 from the fifth container (5) and the sixth (6) are added respectively, after which add 50 lì of the buffer buffer for standard dilution and mix well.

In the ninth and tenth containers, add 50 ìl of the seventh container (7) and the eighth (8) respectively and then add 50 ìL of the buffer for standard dilution and mix thoroughly, then 50 ìL of the solution from the ninth and tenth containers.

The total volume in all vessels is 50 ìL and the concentrations are 48 pg/ml, 32 pg/ml, 16 pg/ml, 8 pg/ml 4 pg/ml, respectively.

2. In the Stripllate Microelisa tape plate, leave one empty container as the control sample. While, 40lμ is added from buffer to dilution and 10μl from sample (dilution factor 5) in sample vessels. Samples should be placed in the bottom of the vessel without touching the wall of the vessel and mixing and mixing well.

3. Custody : Close the container with duct tape and incubate for 30 minutes at 37°C.

4. Relief : The concentrated washing solution is diluted with distilled water.

5. Washing : Adhesive tapes are closed and are perfusion and re-filled with washing solution after stabilizing for 30 seconds, the washing process is repeated 5 times.

6. Add the 50μl of the HRP-Conjugate reagent to each pot except the empty control container.

7. Incubation : The brood is as described in step 3.

8. Washing: Re-wash the same way as mentioned in step (5).

9. Coloring: Add 50lμ of chromogen solution (A) and lì50 of Chromogen (B) solution to each pot, mix and stir vigorously and incubate at 37°C for 15 minutes, taking into account the avoidance of light while coloring.

10. Finish : Add 50μl of the solution of the stop to each pot to finish the reaction. It should change the colors in the container from blue to yellow.

11. Read optical density (OD): Read optical density at wavelength 450 nm using a reader readerMicrotiter The optical density value (OD) is reset to the empty control vessel and the test should be performed within 15 minutes after adding the soulution.

Quick test of IgG / IgMOnsitToxoIgG/IgM combo Rapid Test

The combined analysis from CTK Biotech, Inc. of America used the IgG and IgM side-by-side immunoglobulin analysis to detect and discriminate simultaneously on: anti-toxoplasmqgondi in serum, plasma or human blood.

Measurement of IgG and IgM concentrations of ELISA

The ELISA assay prepared by the American Bio Tech Company using the Sandwich-ELISA method was used.

Method of action procedure

1. Bring all reagents and samples to room temperature (25-18^{0}C) for assay, gently tug before use.

2. Prepare the required number of receptacles, including one opening bowl, two negative control bays, two positive control bins, one pot per sample and then write the serial numbers of the controls and samples in the datasheet.

3. Add 20 ml of diluted sample to each pot.

4. Add 100 mol of the sample and negative control and positive control to each suitable receptacle according to the data sheet. (Reserving a pot for whitening).

5. Press the mixing plate taking into account not to spray liquid on the clipboard.

6. The plate is incubated at 37°C in a water bath or incubator for 60 minutes.

7. Add 50 ml of enzyme conjugated to each pot while avoiding touching the container folder to avoid error.

8. Incubate the dish at 37°C in a water bath or incubator for 30 minutes.

9. Wash each pot five times with the washing machine by washing process:

A) Taking into consideration washing thoroughly according to the instructions as incomplete washing will adversely affect the test.

B) Put the contents of the entire container in a waste flask and then fill the vessels with a wash (350 m or more).

Make sure there are no fluids on the tape carrier (eg, through blotting with absorbent tissue)

10. Add 50 m of color A and 50 m of color B to each pot In order, press the mixing plate.

11. Incubate the plate in C37 into a water bath or incubator for 30 minutes.

12. Stop the reaction by adding 50 ml of solution to each pot

13. We read the absorption of the solution in each vessel at 450 nm (single wavelength) or 450 and 630 nm as reference (double wavelength).

RESULTS AND DISCUSSION

The results showed that there were significant differences in the level of P 0.05, where vitamin D was reduced in the infected pregnant women. The concentration of vitamin D in pregnant women (10.93 ± 3.17 Pg/dl) compared with non-pregnant women (5.94 ± 15.44 Pg / dl) as shown in Table 1.

The result was close to that of researchers, Ambrish and Sanjay (2018), Nada *et al* (2018). This may be because during pregnancy the fetus derives its nutrition from the mother, as the fetus receives vitamin D from the mother's body and when there is a decrease in the amount of vitamin in the mother's diet, the fetus takes the mother's vitamin D, which causes her to develop multiple diseases. Vitamin D is essential in the pregnant woman because any harm it causes causes serious complications on her body and on the fetus's body (Bruce W Hollis *et al*, 2011).

The results showed no significant differences in the concentration of calcium at a significant level P 0.05. The concentration of calcium in pregnant women was 9.08 ± 1.20 and non-pregnant women (9.15 + 1.15 Mg / dl). This result was similar to that of Hatswell *et al* (2015), which showed no change in calcium during pregnancy, which begins to decline after pregnancy during lactation. That calcium has not decreased in pregnant women although the parasite is highly correlated with calcium (Coppenis and Joine, 2001).

Magnesium showed significant differences at P 0.05, with an increase in Mg concentration in infected women (0.55±3.26mlu/ml) compared to non-infected women (0.85 ± 2.55mlu/ml). Most pregnant women are more likely to eat foods that provide them with important nutrients to the mother and fetus, which can be easily obtained from nuts, grains, yogurt, tea, kaku, and leafy vegetables such as sapang, a nutrient rich in magnesium (Larsson et al, 2008).

Table 3 shows the antibody concentration of IgG in pregnant women who are not pregnant with toxoplasmosis. Statistical results showed that the percentage of pregnant women infected with IgG was 58% of total pregnant women. While the percentage of non-infected women was 68%. IgM did not appear in infected women. The percentage of non-pregnant women is higher than the percentage of pregnant women, and this may be due to the inhibitory effects of progesterone and estrogen on the functions of infected cells, especially the production of Nitric Oxide (No), which is one of the mechanisms of the elimination of Tachyzoite increase susceptibility of pregnant women to infection with *T. gondii* (Bodgan, 2001).

These results are consistent with the findings of Fox *et al* (1991) and may be due to immune changes in pregnancy that help in the successful implantation of the egg and placenta and ensure the survival of the developing

Table 1 :

Data	Samples	Number	Mean ± S.D	P Value
Vit. D (Pg/dl)	Infected pregnant	11	10.93 ± 3.17	P ≤ 0.05
	Non-infected pregnant	47	15.44±5.49**	P ≤ 0.05

**P ≤ 0.05 refers that there is a significant differences with significant levels.

Table 2 : Concentration of electrolytes in the blood serum of pregnant women and non-pregnant women with cones.

Data	Samples	Number	Mean ± S.D	P Value
Calcium (Mg/dl)	Infected pregnant	11	9.08±1.20	P ≤ 0.05
	Non-infected pregnant	47	9.15±1.15n.s	
Manganese (mlu/ml)	Infected pregnant	11	3.26 ± 0.55	P ≤ 0.05
	Non-infected pregnant	47	2.55 ± 0.85**	

ó P ≤ 0.05 refers that there is not significant differences, **P ≤ 0.05 indicates that there is significant differences.

Table 3 : Concentration of antibodies (IgG) in pregnant and non-pregnant women with toxoplasmosis.

The antibody		Total number	Number of infections	Percentage
IgG	Pregnant	19	11	58%
	Non-pregnant	69	47	68%
	Total		88	58

fetus. The number of hormones in pregnancy increases such as estrogen, estosterone and progesterone. The cause of IgG may be attributed (Roberts *et al*, 2001) (Progesterone). This may be due to the factor that most pregnant women's attention to their health status during pregnancy, review of medical clinics, and analysis throughout pregnancy, and studies have shown that the presence of IgG antibodies in the absence of IgM antibody Pregnant women at a fixed level do not offer intrauterine fetus for posterior infection during pregnancy because they have immunity to infection (Foulon *et al*, 2000; Devi *et al*, 2008).

CONCLUSION

From our study of this research, we conclude:

1. Low vitamin D in pregnant women with alopecia.

2. There were no significant differences in calcium ion in pregnant and non-pregnant women with alopecia.

3. High magnesium ion in pregnant women with cones.

4. IgG reduction in pregnant women with no significant differences in IgM.

Recommendations

1. Expanding studies on hormones in women with alopecia.

2. Study the relationship of other vitamins (vitamin B and vitamin E) to the incidence of toxoplasmosis.

3. Study the effect of other elements such as zinc on the incidence of toxoplasmosis.

4. Measure current study variables on acute cases of IgM and compare them with chronic conditions (IgG).

REFERENCES

Alhasry Nabil Ahmed and Zuhairi Ahmed Saleh (2010) Effects of Crocheting and Brucellosis on Some Biochemical Standards in Local Iraqi Production. *Medical Sciences* **24**(2), 73-80:

Mohammed Medhat Hussein Khalil (2005) *Endocrinology*. Second Edition. University Book House. Eye. U.A.E.

Ambrish M and Sanjay K (2014) Vitamin D Supplementation in Pregnancy. *Indian J Endocrinol Metab*. **18**(5), 593–596. doi: 10.4103/2230-8210.139204

Hatswell B L, Allan C A, Teng J, Wong P, Ebeling P R, Wallace E M and Milat F (2015) Management of hypoparathyroidism in pregnancy and lactation–A report of 10 cases. *Bone Reports* **3**, 15-19.

Bogdan C (2001) Nitric Oxide and the immune response. *Nature Publishing Group* **2**(10), 907-916.

Bruce W Hollis, Donna Johnson, Thomas C Hulsey, Myla Ebeling, Carol L and Wagner (2011) Vitamin D supplementation during pregnancy: Double blind, randomized clinical trial of safety and effectiveness. *Journal of Bone and Mineral Research* 26(10).

Cenic Goga B T, Rossitto P V, Sechi P, Mecrindle C M and Cullor J S (2011) Toxoplasma in animals, food and humans : an old parasite of new Concern. *Food born Pathog. Dis*. **8**, 751-762.

Coppens I and Joiner K A (2001) Parasite–host cell interactions in toxoplasmosis: new avenues for intervention?. *Expert Reviews in Molecular Medicine* **3**(2), 1-20.

Dabrit H A and Conrad P A (2011) Cats and toxoplasma: Implications for public health. *J. Vet. Med*. **57**, 34-52.

Deschasaux M, Souberbielle J C, Partula V, Lécuyer L, Gonzalez R, Srour B, Guinot C, Malvy D, Latino-Martel P, Druesne-Pecollo N and Galan P (2016) What do people know and believe about vitamin D?. *Nutrients* **8**(11), 718.

Devi K S, Devi Y G, Singh N S, Singh A M and Singh I D (2008) Seroprevalence of TORCH in women with Still Brith in RIMS hospital. *J. Med*. **22**, 2-4.

Dubey J (2010) *Toxoplasmosis of Auimals and Humans*. **2**nd ed. pp6-8, 60-68

Fox H S, Bond B L and ParSlow T G (1991) Estrogen regulates thIFN_gamma promoter. *J. Immunol*. **146**, 4362-4367.

Foulon W, Naessens A and Ho-Yen D (2000) Prevention of Congential toxoplasmosis *J. Perinat. Med.* **28**, 337-345.

Holick M F (2006) High Prevalence of Vitamin D inadequacy and

implications for health. *Mayo Clin. Proc*. **81**(3), 353-373.

Jimenez_Coello M, Acosta-Viand K Y, Guzman_Marin E, Puerto_Solis M and Ortega_pacheco A (2012) Toxoplasmosis: A relevant zoonotic Food borne disease in tropical conditions. *Afr. J. Microbiol. Res.* **6912**, 2956-2964.

Joanne P M (2012) Review of Toxoplasmosis of animal and human (Second edition) by J.P. Duey. *Parasite and Vector* **3**, 112.

Kaye A (2011) Toxoplasmosis : diagnosis, treatment and prevehtion in Congenitally exposed infants. *Journal of Pediatric Health Care* **25**, 355-364.

Larsson S C, Virtanen M J, Mars M, Mannisto S, Pietinen P, Albanes D and Virtamo J (2008) Magresium, Calcium, Potassium and Sodium intakes and risk of stroke in male Smokers. *Arch. Intern Med*. **168**(5), 459-465.

Nada A, Qays A M and Maha S H (2018) Serum Vitamin D and TNF_ α in Iraqi intertilewemen with positive 196 toxoplasma gondii : ls there a correlation between in fertility and Vitamin D. *Journal of Eudocrinobgy and Metabolism* **9**(1), 628-639.

Pollard F H and Marun J V (1956) The spectrophotometric determination of the alkaline-earth metals with murexide, eriochrome black T and with o-cresolphthalein complexone. *Analyst*. **81**, 348-353.

Roberts C W, Walker W and Alexander J (2001) Sex_Associted hormone and immunity to protozoan parasites. *Clin. Microbial. Rev*. **14**(3), 2476-2488.

Rogerio S V, Patricia R, Rosiane G M and Marco A (2011) Congenital Toxoplasmosis : A neglected Diseaes Current Brazilian public health policy. *Field Actions Sa. Rep*. **3**.

Smith C, Marks A D and Lieberman M (2005) *Baismedica Biochemisty*. 2nd edition. Lippincott Willisams-Willinks. P 580-584.

Villena I, Ancelle T, Delmas C, Garcia P, Brezin A P, Thulliez P and Goulet V (2010) Congenital toxoplasmosis in France in 2007: first results from a national surveillance system. *Eurosurveillance* **15**(25), 19600.

Zhou P, Chen Li H, Zheng H H, He S, Lin R and Zhu X (2011) *Toxoplasma gondii* infection in human in China. *Parasites and Vectors* **4**(156), 1-9.