

Effect of Some Biochemical Parameters on the Level of Enzymes in the Serum and Placenta of the Infected Pregnant Woman with Toxoplasmosis

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Abstract: The present study was conducted aiming at investigating the medical, biological and biochemical importance of toxoplasmosis and the effect of the causative agent, *Toxoplasma gondii*, on the level of the enzymes in the serum and placenta of infected pregnant women compared to non-pregnant, non-infected women (control group). The study included 450 specimens of women, 225 out of them were non-infected and 225 were infected with toxoplasmosis. A significant increase ($p \leq 0.01$) in activity of Px, GST, LDH and AchE whereas non-significant decrease in activity of SOD and AE was noticed, compared to control group. Interference between infection and different pregnancy periods showed a significant increase ($p \leq 0.01$) in activity of SOD, Px, LDH and AE in serum of pregnant, infected women during 1-3 months pregnancy period, whereas GST increased significantly ($p \leq 0.01$) in activity during 3-6 months pregnancy period and AchE during 6-9 months pregnancy period. The result related to the placenta of the pregnancy women who are infected with Toxoplasmosis showed a non-significant increasing in activity of: SOD, GST, LDH, and AchE through period of pregnancy ranged (6-9) months. Also; efficiency of AE increased through period of pregnancy ranged (1-3) months; but activity of Px through period of pregnancy in placenta of the pregnant infected women decreased.

Keywords: Toxoplasmosis, Arylesterase, Acetylcholineesterase, Peroxidase

Introduction

Toxoplasmosis is a disease caused by a kind of the parasitic prophylaxis called *Toxoplasma gondii* parasite, which considered one of the intracellular parasites, that can cause many kinds of the intermediate hosts like: the rodents, birds, wild animals and the human beings. (Remington *et. al.*, 2000).

Cats and the other kinds related to the family felidae are considered specialist final enforcing and intermediate hosts in the life cycle of the parasite. (Dubey, 2003). The name of *Toxoplasma* was derived from its Crescent Shape; whereas toxin means an arch and plasma means a form. (Buxton; 1983).

The patients are classified into two groups according to the way of causing with the disease: the first one represents the placenta Toxoplasmosis which occurs as a result of transposing the parasite from the mother to the embryo across the placenta; and the second kind is produced by many ways including the mouth through taking some materials like: water, fruits and vegetables polluted in Sporulated Oocytes; dealing with polluted soil with the excrement of the caused cats; or taking the tissue hosts existing in the raw or uncooked well intermediate tissues, less common ways like through the nose by inhaling. (Prescott and *et. al.*, 2002). Besides; through blood transfusion whereas the parasite can stay in the blood stored in 4°C for more than 50 days, or by members transplantation. (Al-Nuaimy, 2010). Also; pregnancy is considered a kind of the oxidative stress: that causes to increase oxidation levels and decrease anti-oxidation because the oxidative stress represents the unbalance state among oxidants (free radicals and their products resulting from the metabolic processes) and anti-oxidants, more than anti-oxidants causing to destroy the biochemical particles in the body like: proteins, fats, and nucleic acids. (Robinson and *et. al.*, 2008).

Then, it is necessary to point out that the free *radicals* are chemical compounds consist of one electron or more undoubled in the cell; whereas; they are severe in the interaction process; that the particle tries to reach at state of stability as quick as possible then attacks the neighboring particles and obtains the electron required from the stable particle. (Gleeson and *et. al.*, 2006).

Free radicals play an important role in many diseases that cause the human being. (Liu; 2003). So; the reactive oxygen species (ROS) generated inside the body attack the cells and destroy their components of protein, fats, and nucleic acids like: Ribonucleic acid RNA and Deoxy Ribonucleic acid DNA, carbohydrate and other components in the cell then cause to damage the cellular wall. (Stahl & Sies; 1997, Bartosikova *et. al.*, 2003). Also; reactive oxygen species affect on the proteins through their effect on the double bond and its oxidation, besides; oxidation of SH group that causes to change in composition of the proteins with modifications on them that affect on their work and inhabit enzymes activities (Tritto & Ambrosio; 1999, Michael, 2001).

Therefore; the study is an attempt to limit the change that can be taken place in efficiency of the enzymes like: Superoxide Dismutase (SOD), Peroxidase, Glutathione- S- transferase, Lactet dehydrogenase LDH, Arylesterase, and Acetyl Choline esterase as anti-oxidants in serum of the women caused with the disease whether the aborted or non-aborted ones.

Method

Collection of Placenta Specimens

Placenta specimens were collected from aborted and non-aborted women. Then, 24 specimens were collected from Al-Khansa' Gynecology and Obstetrics Hospital. Hence, 18 placentae specimens were collected from women suffered from repeated abortion cases through various pregnancy periods as shown (1-3), (3-6) and (6-9) months. Thereafter it became certain to be caused with Toxoplasmosis by using the direct microscopic examination of the placenta extract and isolation the parasite in its pure form from the infected placentae. (Sharma & Dubey; 1981, Dubey & Livingston, 1986; Al-Khaffaf ; 2001).

Collection of Blood Specimens

Blood specimens were taken from various groups of the pregnant women aiming at estimation of some enzymes as following: healthy pregnant and infected women with Toxoplasmosis for each period of pregnancy through (1-3), (3-6) and (6-9) months.

Group of infected women and non-infected with *Toxoplasma Condi*

Scientifically speaking, 450 blood specimens were collected from pregnant women who were non-infected and infected with Toxoplasma Condi whose cases were diagnosed by female diagnosticians Obstetrics Hospital / Consultative Section; whose ages ranged (20-45) years; whereas 10 milliliters of the venious blood were taken and put in a plain tubes then left for clotting for 10 minutes in a water bath at 37°C; thereafter a centrifugal was made at speed 5000 rpm to get the blood serum to be maintained at -20°C for estimation the biochemical variables. (Tietz; 1999).

Estimation of SuperOxide Dismutase (SOD)

SuperOxid Dismutase (SOD) was estimated by using the modification method; Tetrazolum Blue Nitro/Riboflavin Modified. (Beyer *et. al.*, 1991).

Estimation of Peroxidase

A colouring method was used for estimation the total Peroxidase efficiency by using the material called amino antipyrine-4 which interacts with hydrogen peroxide with existence of peroxidase enzymes to give pink complex; whereas the absorbance of which was estimated at 510 nm resulting from decomposition of hydrogen peroxide (Tinder; 1996).

Estimation of Glutathione-S-Transferase

The enzyme was estimated according to the method used by the researchers (Habig *et. al.*, 1974).

Estimation of Lactate Dehydrogenase and Arylesterase

Lactate Dehydrogenase was estimated by using ready equipment from SRYBio company; and an Aryle esterase according to the method of (Thomas *et. al.*, 2000).

Estimation of Acetyl Choline Esterase

Electrometric Techniques was used to estimate Acetyl Choline Esterase. (Mohammed & Omar, 1982).

Results and Discussion

The results showed on table (5) highly significant effect for each of: Peroxidase, Glutathione-S-Transferase, Lactet Dehydrogenase LDH, and Acetyl Choline Esterase in the serum of the pregnant women who are infected and non-infected through the periods of pregnancy; besides the interaction among the cases and periods of pregnancy at the probability level estimated $P \geq 0.01$. But; Superoxid Dismutase (SOD) and Arylesterase did not show a significant effect in the serum of the pregnant women. According to the results appeared; it was noted a significant increasing in each of Peroxidase and Glutathione-S-Transferase. Lactate Dehydrogenase LDH, and Acetyl choline Esterase in the serum of the pregnant women who are infected with *Toxoplasmosis* reaching 22.55 ± 16.66 , 24.35 ± 13.42 , 279.87 ± 129.38 , IU/L, 0.69 ± 0.20 $\mu\text{mol/L}$ in comparison with their counterparts the pregnants who are non-infected; whereas Superoxide Dismutase and Arylesterase decreased ; that the values did not reach at the statistical significant.

Therefore; results of the study conducted on the interaction among the cases and periods of pregnancy showed an significant increasing in: Superoxide dismutase, Peroxidase, Lactate dehydrogenase LDH, and Arylesterase reaching $0.09 \pm 0.001 \pm 0.09$ $\mu\text{mol/L}$, 33.75 ± 19.71 , 339.06 ± 15.09 , 158.001 ± 12 IU/L in the serum of the pregnant women caused in the period of pregnancy (1-3) months; then the activity of Glutathione-S-transferase through period of pregnancy increased (3-6) months reaching 26.21 ± 13.2 IU/L. but; activity Acetyl choline esterase decreased in the serum of the pregnant women infected with disease in the period of pregnancy (6-9) months in comparison of their non-infected counterparts reaching 0.772 ± 0.17 $\mu\text{mol/L}$.

Thus, activity increasing of some enzymes may belong to increasing of the free radicals production as a result of the case that to loss of balance state among levels of anti-oxidations and the reactive oxygen species; that generated a state of oxidative stress; then emphasized the necessity of giving anti-oxidation like the vitamins such as C and E (Vasdev *et. al.*, 2006), (Senti *et. al.*, 2003), and (Mullah Alo, 2011).

Whereas reason for decreasing in other enzymes activity belongs to effect of oxidative stress especially through the advanced stages of the pregnancy on the enzymatic anti-oxidant and changing of these enzymes like Glutathione peroxidase GPX and Glutathione reductase GR; accordingly this go with previous study results conducted on the blood- pressure patients; whereas a decreasing activity of some enzymes was noted in comparison with the control group. (Rodrigo *et. al.*, 2007, Al Sabawi,2009)

Table1.Show the average and standard deviation for enzymes in serum of infected and non infected women

| Parameters | Mean ±SD µmol/L | Mean ±SD U/L | | | | Mean ±SD µmol/ L | NO |
|---|-------------------------------|------------------------|-----------------------------|---------------------------------------|----------------------|-------------------------|-----|
| | Acetyl choline esterase | Arylester ase | Lactet dehydroge nase | Glutathion e-S- Transfera se | Peroxida se | Superoxide Dismutase | |
| non- infected pregnant women | 0.67±0.13 B | 147.91±11 .14 A | 109.03±6. 22B | 13.97±7.31 B | 7.84±3.4 3B | 0.06±0.03A | 225 |
| infected pregnant women | 0.69±0.20 A | 147.22±22 .10 A | 279.87±1 29.3 8A | 24.35±13.4 7A | 22.55±16 .66 A | 0.05±0.8A | 225 |
| Interference between infection and different pregnancy periods | | | | | | | |
| non- infected(1- 3)months | 0.68±0.05 B | 151.81±17 .37B C | 107.71±4. 35C | 7.11±2.77 D | 7.58±3.0 7D | 0.005±0.001 C | 75 |
| non- infected(3- 6)months | 0.54±0.12 D | 142.62±4. 52D | 109.12±7. 14C | 19.88±6.01 B | 6.86±4.1 0D | 0.006±0.001 BC | 75 |
| non- infected(6- 9)months | 0.77±0.09 A | 149.30±2. 77C | 110.25±6. 61C | 14.92±5.84 C | 9.07±2.5 9D | 0.007±0.001 B | 75 |
| infected(1- 3)months | 0.61±0.18 C | 158.01±12 .46 A | 339.06±1 5.09 A | 22.78±13.0 8 AB | 33.75±19 .71 A | 0.09±0.001 A | 75 |
| infected(3- 6)months | 0.70±0.21 B | 127.44±18 .70E | 258.28±1 2.48 B | 26.21±13.0 2 A | 19.99±14 .17 B | 0.003±0.001 D | 75 |
| infected(6- 9)months | 0.77±0.17 A | 156.36±19 .28 AB | 243.06±8 3.82 B | 24.04±14.2 4A | 14.07±6. 90 C | 0.003±0.001 D | 75 |

Within columns, means having the same letters don't differ significantly at $p \leq 0.05$ probability level

Activity of Enzymes in the Placentae of Infected and Non-Infected Pregnant Women

Clarifying results showed on table (2) indicate to existence of high significant effect in activity of Glutathione - S- transferase and Arylesterase in the placentae of the pregnant infected and non-infected women at the $P < 0.01$. Also; according to what the clarified studies showed on table (2); it was noted that there was a significant increasing in activity of: Superoxide dismutase, Glutathione-S-transferase, Lactate dehydrogenase, and Acetyl choline esterase in placentae of the pregnant infected and non-infected women through period of pregnancy (6-9) months. Also; it was noted that each of Lactate dehydrogenase, Acetyl choline esterase did not reach at that statistical significant; whereas the values estimated 0.010 ± 0.001 µmol/L, 10.13 ± 3.90 , 88.37 ± 6.13 IUL 0.140 ± 0.05 µmol/L respectively.

Whereas Peroxidase decreased in the placenta of the pregnant infected and non-infected women through period of pregnancy (6-9) months that reached 1.85 ± 0.28 IUL ; but Arylesterase in placentae of the pregnant infected and non-infected women through period of pregnancy (1-3) months; whereas reached 91.62 ± 0.01 IUL. Perhaps the reason for that is attributed to anti-toxoplasmosis drugs taken by the pregnant infected women through period of pregnancy that may play a role in increasing the activity of anti-oxidant; whereas sparmycin, one of the drug used in treatment of toxoplasmosis, its concentration in the placenta ranged (3-5) times higher than its concentration in the other tissues (Stray, 1999), (Mulla Allo, 2011), or increasing the oxidation in large quantities inside the body of the pregnant infected women causing certain increasing in production the harmful compounds resulting from oxidation the fats or proteins; then increasing in activity of enzyme in taking off the

poisonous compounds inside the body by way of Glutathione-S-transferase which is called also Detoxification Enzyme.

Besides; results of this study went with the results offered by many researchers like: (Al-Gubori *et. al.*, 2004; Kaynar *et. al.*, 2005, and Mullah Allo, 2011).

Also; we note existence of non-significant decreasing in activity of Lactate dehydrogenase in placenta of the infected women through various stages of pregnancy in comparison with the non-infected placenta; whereas the results of these studies agree with the study of (Dawood, 2007); also with results of many researchers like: (Al-Gubori *et. al.*, 2004; Kaynar *et. al.*, 2005; and Mullah Alo, 2011).

Thus, the results of this study agree with results of previous of other diseases; whereas it was noted an important increasing as to efficiency of other enzymes came synchronous with other diseases such as cancerous tumours. (Al-Abachi, 2006).

Thereafter, enzymes in the serum of healthy non-infected with any disease individuals were estimated; and it was noted also that some of them are affected came and decreased with old-aged according to sex, variety of ecological factors, difference in nutrition, pattern of life, in addition to genetic factors and mutations which affect on the azo enzymes. (Nevin *et. al.*, 1996). Also; that was happened with those patients caused with certain diseases like: arteriosclerosis, blood- pressure, and diabetes that caused decreasing in activity of oxidative stress processes with the patients (Al-Rubaiie, 2006). Also; they agree with results of previous studies conducted on those patients who are caused with blood pressure; whereas it was noted decreasing in activity of enzymes in comparison with the control group. (Rodrigo *et. al.*, 2007; Al-Sabawi; 2009). Besides; they agree with results of studies related to other diseases like those patients who are caused with Rheumatoid arthritis drugs; whereas the activity of enzyme decreased after treatment using one medicine with those who were treated using common medicine in comparison with those who were infected but not treated. (Al-Jammas, 2006), with patients caused with amnesia who were treated with Vitamin E which increases composition of nervous carrier in nervous clashes places like Acetyl choline through staying of nerves in the brain area (Morris; 2002).

Table 2. Show the average and standard deviation for enzymes in placenta of infected and non-infected women

| Placenta in pregnancy periods | Mean ± SDµmol/L | Mean ± SDU/L | | | | | Mean ± SDµmol/L | No. |
|-------------------------------|-------------------------|--------------|----------------------|---------------------------|-------------|----------------------|-----------------|-----|
| | Acetyl choline esterase | Arylesterase | Lactet dehydrogenase | Glutathione-S-Transferase | Peroxidase | Superoxide Dismutase | | |
| non-infected placenta | 0.10±0.05A | 74.62±9.64B | 80.14±20.11A | 5.11±1.80B | 18.63±1.99A | 0.008±0.001A | 6 | |
| infected(1-3)months | 0.14±0.01A | 91.62±0.01A | 76.90±0.01A | 7.18±0.01B | 5.80±0.01AB | 0.004±0.001B | 6 | |
| Infected3-6)months | 0.10±0.01A | 69.78±0.01B | 70.42±2.21A | 4.12±2.22B | 3.08±0.69B | 0.005±0.001B | 6 | |
| Infected(6-9)months | 0.14±0.05A | 73.12±2.49B | 88.37±6.13A | 10.13±3.90A | 1.85±0.28B | 0.01±0.001A | 6 | |

Within columns, means having the same letters don't differ significantly at p≤ 0.05 probability level

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