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Molecular Study of Toxoplasmosis and Its Relationship With Some Parameters (TSP, Globulin and Albumin) Among pregnant and aborted women in the Babylon Province Iraq

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Abstract : Molecular and biochemical study were conducted for pregnant and aborted women to investigate the infected person with Toxoplasmosis in the Babylon province during the period from September 2015 till March2016, ELISA technique for 300 serum samples for pregnant and 100 serum samples for aborted women by using polymerase chain reaction technique for 96 blood samples (where positive with ELISA technique), according to epidemiological criterions (Residence area , Age group ,Existence of cats in houses , Eating of the fresh the vegetable and it's modes of sterilization , times number of abortion ,trimester of pregnancy). The results showed that PCR technique was for IgG and IgM and both (IgG & IgM) in pregnant and aborted women its (22.8% ,35.1%and 19.3%), (20.5%, 59.0% and 20.5%) respectively.

The results of relationship of infection with residence area (rural and urban area)the positive cases of IgG and IgM and both (IgG & IgM) as the following (32.3%,35.5% and 19.4%) ,(16.9%,49.2% and 20.0%)respectively. Whereas forage groups the result for IgG and IgM and both (IgG & IgM) for age group (22-26),(27-31)and(22-26),(32-36) and (32-36) years which were (16.2%),(28.6%) and (42.9%),(45.0%) and (30%), respectively. The infected persons those keeping the cats or not in their houses for IgG and IgM both (IgG & IgM)shows (30.4%,45.7% and 23.9%) and (14%,44% and 16%), respectively. The IgG and IgM and both (IgG&IgM) for these eating fresh vegetables and using sterilization or not or completely not eating fresh vegetables for the pregnant and aborted women reveals which were (28.8%,27.3% and 25.8%),(6.9%, 86.2% and 6.9%) and (0%,0% and 0%) respectively. According to pregnant periods number (1-3 month), (3-6 month) and (6-9 month) which were(16.6%,33.3% and 50%), (22.2%,55.6% and 22.2%) and (23.8%.31% and 14.3%) respectively. The abortion times numbers (First, second and more than second) and relationship with positive cases of IgG and IgM and both (IgG & IgM) which were (41.7%,63.6% and 0%), (11.1%,55.6% and 33.3%) and (11.1%.61.1% and 27.8%) respectively.

Toxoplasmosis infection rates were among pregnant and aborted women that infected and it's control, the concentrations mean for total serum protein , among pregnant women and aborted for all positive antibodies (5.60±0.77), (6.12±0.71) g/dl, respectively, compared with control group(pregnant not infected) (5.99±0.75), (non pregnant and non infected) (6.85±0.67) g/dl, while the concentration of albumin protein was a slightly increase in pregnant women and aborted which was (3.57±0.73), (3.99±0.63) g/dl, respectively, compared to the control group (pregnant not infected) (2.75±0.74), (non pregnant and non infected) (4.20±0.68) g/dl, either globulin protein concentration, in pregnant women and aborted (2.0±1.0), (2.18±0.91) g/dl, respectively, with a significant decrease compared to the control group (pregnant not infected) (3.31± 0.62) and (non pregnant and non infected) (2.67± 0.56) g/dl, respectively.

Introduction

Toxoplasma gondii is protozoan parasite an obligate intracellular, that causes toxoplasmosis. Its distributed worldwide, and capable infecting virtually all warm-blooded animals [1]. Its capable of causing severe and life threatening conditions in pregnant women and immunecompromised individuals [2]

Congenital infection can leads to severe disease when the infection is acquired in the first trimester [3]. *T. gondii* increase with the stage of pregnancy, from 5 to 15% in the first half to 60 to 80% in the second half of pregnancy. Conversely the chances of serious lesions and death decrease from 70 to 80% in the first half to less than 10% in the second half of pregnancy[4].

Direct detection of parasite-specific DNA in biological samples using polymerase chain reaction that based on molecular methods, The molecular diagnosis is more accurate and cost effective than the conventional methods[5]. PCR should be considered the gold standard for diagnosis of in all infection. Blood serum consists of two main protein consisting albumin and globulin[6] . Many factors, consisting parasite and contagious diseases, may affect the vacillation of those serum proteins, and its relationship with the serum protein profile and the infection with toxoplasmosis has been demonstrated in humans[7] .The measurement of serum protein was done via spectronic method reaction[8]. The aims of the present study its using PCR technique special gene primer Tg1,Tg2 (469bp) to detection the positive cases with ELISA (IgG, IgM) for pregnant and aborted women and the relationship between the rate of infection of toxoplasmosis with some aspects like (Residence area, Age group, Existence of the cats in the houses, Eating of the fresh vegetable and it's modes of sterilization, times number of abortion and trimester periods of pregnancy).

Material and Methods

Primers That Used in the Present Study

Usedone a primers and manufactured from a company (Bioneer –Korea) of the purpose of investigating of *Tgondii*and the genes its Tg1,Tg2 (469bp)[9]. That may be prevalence in the Babylon province.

Table(1):Primer Tg1,Tg2(469bp)used in present study with sequence nucleotides

Primers (Gene name)	Direction	Primer sequences
Tg1,Tg2(469bp)	Forward	AAAAATGTGGGAATGAAAGAG
	Reverse	ACGAATCAACGGAACTGTAAT

PCR Master Mix That Used With First Primers Tg1,Tg2

Attended mixed chain reaction polymerization by use kit (Accupower PCR Premix) by company follows instructions :

1. Attended mixed chain reaction polymerization in the tube PCR equipped with a kit containing components chain reaction. polymerization and added other components of the reaction mixture .

Table (2):Mixture chain reaction polymerization volume 20 micro liter

Ingredients	Volume (micro liter)
Master mix	5
DNA template	3
Forward primer (F)	1.5
Reverse primer(R)	1.5
Nuclease free water	9
Total	20

2. After completing preparation , Mixture chain reaction polymerization the closure of the tube and mix carefully by vortex for five second.

3. Tubes transferred to PCR thermo cycler for cases of thermal cycles.

Table (3):Thermo Cycler for to Amplify of DNA

Interactions conditions used in thermo cycler:

Steps	Temperature c°	Time	Number of Cycles
Initial Denaturation	94	10(min)	30
Denaturation	94	45(sec)	
Annealing	48	30(sec)	
Extension	72	1(min)	
Final Extension	72	5(min)	

Agarose electrophoresis

Make electrophoresis to gel Agarose 1% the Polymerase chain reaction product analysis as, [10] following:

1. Melted one gram from gel Agarose in 100 ml from (TBE Buffer) a concentration of 1X.
2. Leave the gel cooled at room temperature and after the dye was added Ethidiumbromide nuclear and radioactive and mix well with gel.
3. Agaros gel was poured in deportation tray containing the comb template to locate samples PCR and then left the gel solidifies at room temperature for 15 minutes and then removed from the comb gel removed carefully.
4. Used DNA ladder sequence from 100 bpto measure the result of the interaction in the first pit.
5. Immersion agarose gel by used (TBE buffer) a concentration of 1X and operation of the device electrophoresis current 100 volt and 70 AMP for one hours.
6. After complete the electrophoresis is checked gels containing the product PCR by use ultraviolet rays at a wave length 260 nm to determine the results are positive and matched with measurement by with DNA ladder.and then photography of bands that revealed by digital camera.

Biochemical tests

Estemation of Albumin in Blood Serum.

In buffered solution at pH 4.2 bromocresol green binds albumin to form a coloured compound which absorbance, measure at 630 nm (620-640) is proportional to the albumin concentration in the specimen[11].

Reagent composition

Bromocresol green - Succinic acid 83 mmol/L ,Bromocresol green (BCG) 167 µmol/L ,Sodium Hydroxide 50 mmol/L ,Polyoxythylenemonolauryl ether 1, 00 g/L .

Standard :- Bovine Albumin 5.0 g/dL (725µmol/L).

Manual procedure: Let stand reagents and specimens at room temperature

Table (4): Illustrated the reagent and other materials that used to detect the albumin

Pipette into well identified test tubes	Blank	Standard	Assay
Reagent	2ml	2ml	2ml
Demineralized water	10µL		
Specimen			10µL
Standard		10µL	
Mix well . Record absorbances at 630 nm (620-640) within 3 minutes against reagent blank or better after exactly one minute.			

Calculation of results

$$\text{Result} = (\text{Abs (Assay)}))/(\text{Abs (standard)}) \times \text{Standard concentration}$$

Estimationof Total Serum Protein in blood serum Principle .

Colorimetric method described by gornall and their collegeous the peptide bonds of proteins react with cu^{+2}in alkaline solution to form a colored complex which absorbance proportional to the concentration of total protein in the specimen, is measured at 550 nm . The biuret reagent contains sodium potassium tartrate to complex cupric ions and maintaintheir solubilitysolution[12] .

Reagents : Vial R1:Biuret Reagent , Sodium hydroxide 370 mmol/L , Na-K Tartrate 10 mmol/L , Potassium iodide 3 mmol/L , Copper ll sulfate 3 mmol/L , Vial R2:Standard , Bovine albumin 6 g/dL .

Manual procedure : Let stand reagents and specimens at room temperature

Table(5): Illustrated the reagent and other materials that used todetect the TSP.

Pipette into well identified test tubes	Reagent Blank	Specimen Blank	Standard	Assay
Saline solution		1ml		
Reagent R1	1ml		1ml	1ml
Standard			20µL	
Specimen		20µL		20µL
Demineralized water	20 ml			
Mix well. Let stand for 10 minutes at room temperature. Record absorbance at 550 nm (530-570)against reagent blank. Red specimen blank against saline solution				

Calculation of results

$$\text{Result} = \frac{\text{Abs (Assay)} - Abs\ \text{(specimen blank)}}{\text{Abs (standard)}} \times \text{Standard concentration}$$

Estimation of Globulin concentration in blood serum.

Calculation globulin concentration throughout minus albumin concentration from protein concentration[13] .

Results

A total of 400 blood samples (300 pregnant and 100 aborted) women that attending to Maternity and Children's Hospital and External laboratory in the Al-Hilla city, Babylon province, during the period from

September 2015 till December 2015, their age groups ranged between, less than(17years) to 41years and more than years .

Table(6):Percentage of infection toxoplasmosis among aborted and pregnant women, diagnosed by PCR technique.

(%)PCR for (IgG&IgM)	Positive cases PCR for (IgG&IgM)	(%) PCR for IgM	Positive cases PCR for IgM	(%) PCR for IgG	Positive cases PCR for IgG	Examined No.	Women status
19.3	11	35.1	20	22.8*	13	57	Pregnancy
20.5*	8	59.0*	23	20.5	8	39	Abortion
19.79	19	44.79	43	21.87	21	96	Total
(X^2) - Calculated = 1.4 sign (X^2) - Tablated = 0.1							

***Significant differences, P ≤ 0.05.**

Table(7):Percentage of infection with toxoplasmosis among aborted and pregnant women according to the residence area, diagnosed by PCR technique.

(%)PCR FOR (IgG & IgM)	Positive cases PCR for (IgG & IgM)	(%) PCR for IgM	Positive cases PCR for IgM	(%) PCR for IgG	Positive cases PCR for IgG	Examined No.	Residence Area
19.4	6	35.5	11	32.3*	10	31	Rural
20.0*	13	49.2*	32	16.9	11	65	Urban
19.79	19	44.79	43	21.87	21	96	Total
(X^2) - Calculated =1.4 sign (X^2) - Tablated = 0.1							

***Significant differences, P ≤ 0.05.**

Table(8):Percentage of infection of toxoplasmosis among aborted and pregnant women according to age groups diagnosed by PCR technique.

(%)PCR for (IgG & IgM)	Positive cases PCR for (IgG & IgM)	(%) PCR for IgM	Positive cases PCR for IgM	(%) PCR for IgG	Positive cases PCR for IgG	Examined No.	Age group (years)
0	0	50	1	50	1	2	17≤
10	1	40	4	40	4	10	17 – 21
18.9*	7	45.9*	17	16.2*	6	37	22 – 26
23.8	5	42.9*	9	28.6*	6	21	27 – 31
30	6	45.0*	9	15	3	20	32 – 36
0	0	50	3	16.66	1	6	37 – 41
0	0	0	0	0	0	0	≥41
19.79	19	44.79	43	21.87	21	96	Total
3.3 sign (22 – 26)		4.3 sign (27 – 31) , (32-36)		2.2sign(22 – 26) , (27-31)			LSD
(X^2) - Calculated =6.5 sign (X^2) - Tablated = 5.2							

***Significant differences , P ≤ 0.05.**

Table(9):Percentage of infection toxoplasmosis among pregnant and aborted women according to the presence of the cats in the houses, diagnosed by PCR technique.

(%) PCR for (IgG & IgM)	Positive cases PCR for (IgG & IgM)	(%) PCR for IgM	Positive cases PCR for IgM	(%) PCR for IgG	Positive cases PCR for IgG	Examined No.	Present of cats in the houses
23.9*	11	45.7*	21	30.4*	14	46	Exist the cats
16	8	44	22	14	7	50	Not exist the cats
91.79	19	44.79	43	21.87	21	96	Total
(X^2) - Calculated = 2.3sign (X^2) - Tablated = 0.1							

***Significant differences, P ≤ 0.05.**

Table(10):Percentage of infection with toxoplasmosis among pregnant and aborted women, according to the eating fresh vegetables and using sterilization diagnosed by PCR technique.

(%)PCR for (IgG & IgM)	Positive cases PCR for (IgG & IgM)	(%) PCR for IgM	Positive cases PCR for IgM	(%) PCR for IgG	Positive cases PCR for IgG	Examined No.	Eating fresh vegetables and modes of sterilization for it
25.8*	17	27.3	18	28. 8*	19	66	Eat and sterilization
6.9	2	86.2*	25	6. 9	2	29	Eat and without sterilization
0	0	0	0	0	0	1	Do not eat vegetables
19.79	19	44.79	43	21.87	21	96	Total
(X^2) - Calculated = 21.04sign (X^2) - Tablated = 0.71							

***Significant differences, P ≤ 0.05 .**

Table(11):Percentage of infection with toxoplasmosis among pregnant women, according to the periods of pregnancy, diagnosed by PCR technique.

(%)PCR for (IgG & IgM)	Positive cases PCR for (IgG & IgM)	(%) PCR for IgM	Positive cases PCR for IgM	(%) PCR for IgG	Positive cases PCR for IgG	Examined No.	Pregnancy Period (Month)
50*	3	33.3	2	16.6	1	6	First period (1 – 3) months
22.2	2	55.6*	5	22.2	2	9	Second period (3-6) months
14.3	6	31	13	23.8*	10	42	Third period (6-9) months
19.29	11	35.08	20	22.80	13	57	Total
(X^2) - Calculated =2.9 sign (X^2) - Tablated = 0.71							

***Significant differences, P ≤ 0.05.**

Table(12):Percentage of infection with toxoplasmosis among aborted women according to the times number of abortion status, diagnosed by PCR technique.

(%) PCR for (IgG & IgM)	Positive cases PCR for (IgG & IgM)	(%) PCR for IgM	Positive cases PCR for IgM	(%) PCR for IgG	Positive cases PCR for IgG	Examined No.	Abortion times No.
0	0	63.6	7	41.7*	5	12	First
33.3*	3	55.6	5	11.1	1	9	Second
27.8	5	61.1*	11	11.1	2	18	More than Second
20.51	8	58.97	23	20.51	8	39	Total
(X^2) - Calculated =7.4 sign (X^2) - Tablated = 0.71							

***Significant differences, P ≤ 0.05.**

Table(13):The concentration of biochemical parameters for pregnant and aborted women and control.

Control		Abortion			Pregnancy			Biochemical test
Non infected and non-pregnant M±S.D. n=20	Non infected pregnant M±S.D. n=20	Positive cases for antibody IgG-IgM M±S.D. n=19	Positive cases for antibody IgM M±S.D. n=31	Positive cases for antibody IgG M±S.D. n=25	Positive cases for antibody IgG-IgM M±S.D. n=19	Positive cases for antibody IgM M±S.D. n=59	Positive cases for antibody IgG M±S.D. n=30	
6.85±0.67	5.99±0.75	5.69±0.68*	6.06±0.63*	6.20±0.80*	5.41±0.63*	5.61±0.75*	5.59±0.81*	Total serum protein (gm/dl)
4.20±0.68	2.75±0.74	3.88±0.15	3.77±0.92	4.15±0.51	3.55±0.59	3.50±0.78	3.69±0.62	Albumen (gm/dl)
2.67±0.56	3.31±0.62	1.81±0.69	2.30±0.99	2.02±0.78	1.76±0.79	2.01±0.92	1.92±1.12	Globulin (gm/dl)
		3.3sign total serum	2.8 sign total serum	3.1 sign total serum	2.7 sign total serum	1.8sign total serum	2.4 sign total serum	LSD

***Significant differences, P ≤ 0.05 .**

M= Mean. S.D. = Standard deviation. n = Samples number .

Table (14):The concentration of biochemical parameters among the positive cases of pregnant and aborted women comparison with control.

Control (Non pregnant and non infected) M±S.D. n=20	Control (pregnant Non infected) M±S.D. n=20	Abortion M±S.D. n=56	Pregnancy M±S.D. n=94	Biochemical Test
6.85±0.67	5.99 ±0. 75	6.12 ±0. 71*	5.60±0. 77*	Total serum protein (gm/dl)
4.20±0.68	2.75 ±0. 74	3.99 ±0. 63	3.57 ±0.73	Albumin (gm/dl)
2.67±0.56	3.31 ± 0.62	2.18 ±0. 91	2.0 ± 1.0	Globulin (gm/dl)
		3.7 sign Total serum	3.2 sign Total serum	LSD

***Significant differences, P ≤ 0.05**

M=Mean. S.D.=Standard deviation. n=Samples number .

Discussion

Prevalence of toxoplasmosis among pregnant and aborted women .

PCR technique in pregnant women the percentage of IgG were 22.8% higher than in aborted women were 20.5% , but for the IgM ,(Igg & IgM), found in aborted women high percentage than pregnant women were 59.0%,20.5%,35.1%,19.3%, respectively. In study [14] it was examined 180 cases from pregnant and aborted women who found 30 case by using PCR technique that found 16.6% and this agreement with the present study.

The reason for this fluctuations for the rates of infection may be explain as the antibodies IgM the concentration in the first weeks of infection and about a week to three weeks is very high and then begin to decrease concentration and high concentration antibody IgG significantly and that too those seen in aborted women where they have been subjected to infection predecessor and thus stimulate the immune system in advance which increases of high concentrations of these antibodies no these fall into chronic infections or may be consider the most patients in this study have acute infection (IgM) or subacute infection (IgG & IgM) and chronic in factor (IgG) [15].

Prevalence of toxoplasmosis among pregnant and aborted women according to the residence area.

PCR technique the total percentage for IgG, IgM in rural area was 67.8%,in urban was 66.1% , agree with study [16] in Romania who revealed that higher seropositivity of toxoplasmosis was among rural environment 63.68% compared to the urban one 55.12% and with study of [17] in Poland also showed that human living in farms had significantly greater percentage of anti-*Toxoplasma* antibodies 59% compared to urban dwellers 41.0%. In present study in the urban area the IgM,was 49.2% higher than rural area was 35.5%, also agreed with study of[18] who found IgM 8.3% in urban area and 7.1% rural area , the prevalence of *toxoplasmosis* schizophrenia among patient is higher in urban areas more than in rural areas[19] . Also disagreed with study of [20] who found 80.92% in the urban area and 66.67% in the rural area. in the present study Also IgG was 32.3% in rural area higher than in the urban area was 16.9% disagreement with study of [18] who found 37.5% in urban area and 14.3% rural area.

Differences in the readings *toxoplasmosis* according to a residence area may be because lack of health education in some rural areas, insufficient appropriate of treatment and difficulty of early diagnosis of symptoms are similar to symptoms of the disease influenza including sore throat and enlarged lymph glands and the lack of obvious symptoms, the infection ratio in the rural area higher than in the urban area because the women in the villages are more in contact with the soil and more exposure to cat and direct contact with cats and other domestic animals, and food and water contamination by oocyst and also using raw or fresh vegetables and not washing vegetables properly , as they generate animals in the houses garden[21]. Perhaps the presence of poultry in urban area and possible explanation increased exposure to infection during pregnancy and children due to the more crowded living conditions or prenatal period of living conditions in urban areas [22].

The low level of IgM antibody may be due to transform the parasite from tachyzoite in the blood to bradyzoite that stay in the tissues, and they can be limited the disease since its start and elimination, and also the low level of antibody IgM be faster than the lower level of antibody IgG and that are found in people inefficient immune after infection for a long duration may be for many years, as the half-life of the antibody IgG be larger than the half-life of antibody IgM and also for that for the ability antibody IgG to replacing the large molecules in the absence of antibody generators[23]. As well as the disparity inof the qualities that distinguish the antibodies IgM, IgG in terms of the number of units and the weights of the molecular and also to the types of chains peptide and splits, and those qualities are IgG antibody has been able to survive for a long time in the body after infection[24].

Prevalence of toxoplasmosis amongpregnant and aborted women according to the age group.

PCR technique the pregnant and aborted women according to age group for IgG among age group (22-26) , (27-31) years which 16.2% ,28.6%,respectively, disagreement with study [18] found the percentage rates of infection with age group (37- 41) year was 50% ,in IgM who found in age group (27-31),(32-36) years was 45.9%,45%, respectively, also the current study disagreement with study of [18]who found in age group (22-26) years was 23.1%.

The incompatibility with previous studies may be due to the kinds of samples in the present study it were pregnancy and abortion women only, that possibly due reason for the high of positive cases in the age groups that the infection without symptoms has to be attention to conduct tests for the detection of the disease and its treatment[25].

The positive cases of high proportion in the age groups(22-36) in most women under study they are of childbearing age and pregnant revisions to care centers to ensure the safety of the fetus and the pregnancy either age group(≥41) any non- child-bearing age because the infection is under clinical and asymptomatic so it does not have interest in conducting tests for the detection of the disease and its treatment in addition to low immunity with age ,concentrated infection within the age groups (22-36) year because this age represent the optimum period of fertility and reproduction and because the pregnancy reduce the immunity of the body thus the critical period of the life of the woman has a greater chance to activate latent infection *T. gondii* that can be vertically transmitted to the fetus [26].

Prevalence of toxoplasmosis among aborted and pregnant women according to the exist or not exist of the cat in the houses .

By used PCR technique for those have exist cats in their houses for IgG, IgM (IgG&IgM) were 30.4%,45.7%,23.9%, respectively, higher than from those have not exist cats in their houses were 14%, 44%,16%, respectively and present study agreed [27],who found high prevalence about 45.7% for those keeping animals higher than for those not keeping animals were 43.9% and the present study disagreement with study of [18],who found close percentage rates for keeping animals 29.7% and not keeping animals 31%.

The infection may occur by exposure to the millions of oocysts from the cat feces that contaminated the environment, food and water reach of these oocyst to other domesticated animals and become infected meat from these animals to humans if it is not cooked well or be tasted or when dealing with human [28].

The distribution of disease may be attributable to the stray cats which get infection from birds and rodents, eating, and were aborted fetus in slaughterhouse and shedding their oocysts which lead to contamination of gardens, animal food stuff, and thus resulting to infection and abortions of human and animals[29].

Prevalence of toxoplasmosis among pregnant and aborted women according to the eating fresh vegetables and modes of sterilization it.

By used PCR technique IgG ,(IGg&IgM) for those eating fresh vegetable and sterilization was 28.8% , 25.8%, respectively ,higher than compared with eat fresh vegetable and without sterilization and do not eating fresh vegetables 6.9% ,6.9% , 0%,0%, respectively ,while in IgM the high percentage in eat fresh vegetable and without sterilization was 86.2%, when compared with those eating fresh vegetable and sterilization and do not eating vegetables were 27.3%,0%. respectively, and the reasons it's the same that illustrated above for ELISA technique.

Prevalence of toxoplasmosis among pregnant women according to trimester period of pregnancy.

By used PCR technique the results indicate that the highest percentage rates of positive cases for IgG were 23.8% in third period (6-9) months higher than when compared with seconed period (3-6) months and first period (1-3) months were 22.2%,16.6% , respectively, while in IgMthe highest percentage rates for the seconed period (3-6) months were 55.6%,compared with first period (1-3) and third period (6-9) months were 33.3%,31%, respectively, and in together (IgG&IgM) high percentage rates for the first period (1-3) when compared with seconed period (3-6) months and third period (6-9) months were 22.2%,14.3%, respectively ,and the reasons illustrated in the above when explain the ELISA technique.

Prevalence of toxoplasmosis among aborted women according to the times number of abortion.

By used PCR technique in the times number of abortionthe highest rates of IgG ,IgM, were 41.7%,63.6%,respectively,in the one times number of abortion the reason for that because the abortion for the first time when pregnant women requires a review of hospitals and health centers for the purpose of diagnosis of the reason for taking the treatment and this reduces the recurrence of abortion either women who are not

attending antenatal health institutions recur abortion to have and because of the parasite reactivity developed by the underlying during the second pregnancy and the fact that It has become a chronic infection or maybe therapy is not effective to eliminate the parasite this result consists with[30], while (IgG&IgM) was 33.3% in second aborted. [31] who found that the incidence of abortion due to uterine anomalies were vary from 0.13% to 4.0% , also [32]support this study where he found that uterine anomalies causes 15% of abortion.

The incidence of the female genital tract anomalies may be cause of abortion in the present study is double the incidence in other regions. Also found that cervical incompetence was responsible for 6.8% of abortion's incidence, single and recurrent, but the higher percentage 4.5%, was related to recurrent abortion group of the patients, there is significant differences ($P \leq 0.05$) between single and recurrent abortion groups. This may be due to that cervical incompetence is one of the causes that required treatment, so if it not treated probably so recurrent abortion will result[25]. All of those patients occure the abortion in the second trimester of their pregnancy, the explanation is that with the progression of the pregnancy, the pressure of the baby on the cervix will increase, and because of the weakness of the cervix, so abortion will take place. this result consists with many studies [32,33].

The Relationship Between Total Serum Protein, Albumin and Globulin With Toxoplasmosis in Pregnant and Aborted Women.

The relationship between biochemical tests (total serum protein, albumin, globulin), found the highest in total serum protein in pregnant and aborted women (5.60±0.77) and (6.12±0.71) gm /dl, respectively, while the lowest in albumin in pregnant and aborted women it's (3.57±0.73) and (3.99±0.63) gm/dl, respectively, also lowest in globulin in pregnant and aborted women it's (2.0±1.0) and (2.18±0.91) gm/dl. This study agreed with study [34]Which studied individual (male and female) with acute myocardial infection muscle, where they found high concentration total serum protein comparison with albumin and globulin.This may be due reason for the high TSP to the fact that people infected with blood parasites act as stimulate types immune A globulin producing acute phase protein (APP), such as c-reactive protein (CRP) due for infection while the cause of low concentration of albumin may be due to overheating of the liver due to acute infection and the consumption of amino acids to manufacture antibodies on the one hand and the manufacture of proteins and acute phase proteins as well as consumption by the parasites themselves.

The concentration of biochemical test in pregnant and aborted women comparison with control (pregnant non infected and non pregnant and non infected).

In pregnant women total serum protein concentration slightly decreasing which was (5.60±0.77) gm/dl, when compared to control (pregnant non infected and non pregnant and non infected) as (5.99±0.75) and (6.85±0.67) gm/dl respectively, also in aborted women the concentration slightly decreasing (6.12±0.71) gm/dl, when compared to control (non pregnant and non infected) (6.85±0.67) gm/dl, but also slightly increasing when compared with (pregnant non infected) it's (5.99±0.75) gm/dl, this study agreed with study [35]who found in both miscarriage and pregnant infected women with toxoplasmosis revealed significant decrease of T.S.P (4.52±0.35) gm/dl, and (5.19±0.76) gm/dl, respectively in comparison to single infected one (7.59±0.61) gm/dl, and also comparison with negative cases of *Toxoplasmosis* (control) were (7.04±0.41) gm/dl, and (7.48±0.57) gm/dl, respectively.

The decrease level of TSP in the current study in pregnant women which infected with toxoplasmosis indicate that those women may be exposure to the risk of threatened abortion especially when TSP scored normal decline [36], and *Toxoplasma* may be considered another factor of this decreased due to causes extensive and the progressive damage to the liver and another tissues The proliferation of remarkable of organisms such damage in these tissues Metabolism caused the changes [37]. Changes of peotien fractions varied according qualitative difference in the severity of inflammation of parasite strains and host[38]. Low TSP might be associated with abnormal placental implantation and uterine bleeding [39]. The TSP concentration is known to be of limited value on its own, and may be transformed by changes in plasma volume without altering the albumin : globulin ratio, changes in TSP concentration may result from dehydration and over loading with fluid, while a short spell of vigorous exercise and stress can result in measurable increase in TSP concentration, as does prolonged venous stasis during phlebotomy.[40].

Another explanation of the decreased level of TSP in pregnant women may be the protein and vitamin deficiencies are common features is pregnant women belonging to the lower socioeconomic groups, thus affecting serum protein concentrations, A direct relationship of quality and quantity of dietary protein with decrease in plasma protein in cases of protein malnutrition has been reported and maternal malnutrition may be aggravated by pregnancy [41].The whey protein pattern, pointed in the pathogenesis of various parasitic infections including lieshmaniosis, babesiosis and echinococosis[42] . However to his knowledge, has not been reported pattern of whey protein in a previous time in human infected naturally *T.gondii*. While in albumin the concentration increase in aborted and pregnant women which were (3.99±0.63) gm/dl and (3.57±0.73) gm/dl, respectively, when comparison with control (pregnant non infected) (2.75±0.74) gm/dl, but decrease albumin concentration when comparison with control(non pregnant and non infected) it's (4.20±0.68) gm/ml. This study agreement with study of [35] the present results some what increase concentration of albumin in aborted and pregnant women which were (3.13±0.28) gm/dl and (5.05±0.52) gm/dl, respectively, compared with negative cases of *Toxoplasma* (controls) it's (3.91±0.14) gm/dl and (5.06±0.10) gm/dl, respectively.

Is completely albumin production in the liver and is a high degree of importance in regulating the flow of water between the plasma and tissue fluid through their effects on the colloid osmotic pressure [23].A drop in albumin in the bloodlevels in current subjects may the results of decreased Protein synthesis in the liver or increase the loss of protein through the digestive tract or kidney [43]. Other possible causes for low albumin may include malabsorption and increased protein needs secondary to infection, and natural half-life of albumin average of 21 days, and thus a decrease in serum albumin is usually not evident early in the course of liver disease [44] .

It may have contributed to the low level of albumin in the infection of pregnant and aborted in edema notable in their legs since albumin is important in maintaining the colloid plasma osmotic pressure [44] .Has been reported for these changes in serum proteins facilities incidence of other parasitic diseases by many researchers[45]. A significant decrease in pregnant and aborted women in the current study have similarity agreement with the studies of [46] conducted on pregnant women in France, who observed that serum albumins levels were significantly decreasing during normal pregnancy. It is also of interest to note that [47] in the study on certion community in United State of America found no effects of pregnancy or advancing gestation on maternal serum protein profiles. In the current study, the pattern of change in serum albumin during normal pregnancy (pregnant without toxoplasmosis) also have similarity agreement in one way or the other with findings of previous investigators [48].The decreasing concentrations of these parameters in this study are much higher than the fall in studies carried out on subjects from economically advanced countries. Nutritional deficiencies may be implicated for this phenomenon [49] .In females minimal albumin gets due to low activity of hepatic protein production [50] .It can be explained by the low plasma concentrations of albumin in this study by the disturbances in liver function [51] or increase protein catabolism [52] showed studies that Toxoplasmosis pays lipid peroxidation, and reduces the ultra albumin concentrations and activity, and increases the activity of hepatic enzymes such as AST and ALT [25].

In globulin the concentration decrease in pregnant and aborted women as (2.0±1.0) gm/dl and (2.18±0.91) gm/dl, respectively, comparison with control (pregnant non infected and non pregnant and non infected) as (3.31±0.62) gm /dl and (2.67±0.56) gm /dl, respectively, This study agreed with study [34]Which studied individual (male and female) with acute myocardial infection muscle found decrease in globulin was(0.97±27.8) gm /dl in male and (1.24±30.0) gm /dl in female in age groups (50-60) years compared with control (1.22 ±27) and (1.86±26) respectively, also in (≥60) year as (1.14±29) gm /dl in male and (1.38±28.5) gm /dl in femalecompared with control (2.24±28.4) and (2.24±28.4) respectively, the present study disagreement with study of [53]It showed globulin levels and found a steady increase, and the values of showing an increase excessively in postmenopausal women through the gamma globulin compared with premenopausal women.

Suppose that globulin levels work on the production protein based, also has beenChanged by IgM levels, Where the values during the human aging and gender equality for globulin cannot evolve through protein metabolism[50]. In other study [54]disagreed with current study for TSP and globulin but agree with albumin concentration for the present study which found high concentration in TSP and globulin which was (11.02±0.22), (8.38±0.06), (7.52 ± 0.19), (4.58 ±0.03) gm/dl, respectively in mammals (mice) infected with *toxoplasmosis* that effect on liver function (infected non treated and infected and treated, respectively) when compared with control (7.42±0.12) and (2.56±0.03) gm/dl, respectively, while in albumin was (3.50±0.26) and

(3.8±0.05) gm/dl (infected non treated and infected and treated, respectively) when compared with control (4.86±0.09) gm/dl. Also agreed with[55] in gerbils infected toxoplasmosis the decrease concentration in all TSP, albumin and globulin as (6.19 ± 0.49, 2.01 ± 0.21 and0.62±4.18) gm/dl, respectively, compared with control as (7.35 ± 0.79, 0.13±3.31 and 4.03 ± 0.82) gm/dl, respectively

The body's reaction system may reflect differences resistance for toxoplasmosis[56]. Also Changes shown in the enzymes in sera and a tendency to increase after the infection, which may reflect the degree of damage to the liver and other tissues, the production of albumin in the tissue reticuloendothelia liver, kidney and gamma globulin in some tissues [57]. Increased globulin associated mechanisms of antibodies against toxoplasmosis in the acute case, and the defense mechanism of the body is extremely complex mechanisms[58-70].

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