

Circulating Immune Complexes In Relation To Polymorphonuclear Leucocytes in Patients Infected With Toxoplasmosis

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ABSTRACT

Circulating immune complexes (CICs) play an important role in many diseases in human. They can cause damage to kidney, joints, skin, and CNS through complement activation with or without local deposition. They can also modulate humoral and cellular immune response through binding to surface receptors of lymphocytes & phagocytes. CICs like activity has been reported in sera from patients with variety of infectious diseases caused by viral, bacterial, protozoal & helminthes. Total 25 patients with different problems of kidney and abortion history (14 male & 11 female) and 25 control groups (13 male & 12 female). The CICs was detected by platelets aggregation test (PI.A.test) and polymorphonuclear (PMN) were counted automatically. Polymorphonuclear cells depleted in number in patients with Toxoplasmosis 63.9 percentage and 77.4% percentage in control group, there is no relationship between age and % of PMNs ($P > 0.001$) in patients and control group, 13% of patients had CICs detected by PL.A. test and 4% of control group had CICs, There were no of patients had IgM of *T.gondii* and 8(32%) of them had IgG of *T.gondii* and 3(12%) of control group with IgM of *T.gondii* and 8% of them with IgG of *T.gondii*. PMN play an important role in the defense against toxoplasmosis and the clearance of CICs from circulation. Most of CICs formed in the circulation contained IgG of *T.gondii*, since most patients group of the present study did not have IgM of *T.gondii* but those with IgG of *T.gondii* containing IC and the number of PMNs was decreased in large number after *T.gondii* invasion.

Keywords: Immune Complexes, Polymorphonuclear, Leucocytes.

INTRODUCTION

Toxoplasma gondii, an intracellular coccidian, infects a wide range of eukaryotic cells and is an important opportunistic pathogen in humans and animals. The infection is frequently encountered without any symptom but there are two groups of high-risk individuals, the human fetuses and the immunosuppressed persons, particularly those with acquired immunodeficiency syndrome (AIDS), that frequently develop fatal toxoplasmic encephalitis (TE)^{1,2,3}.

Toxoplasmosis is a disease affecting 500 million people worldwide. The sero prevalence varies from 5% to 90% depending on geographical location, age, habit of eating raw meat or unwashed fruit and vegetables, and general level of hygiene. The incidence of infections is higher in warmer and humid climate and increases with age. The disease can be congenital or acquired⁴.

Circulating immune complexes (CICs) play an important role in many diseases in human. They can cause damage to kidney, joints, skin, CNS

through complement activation with or without local deposition⁵.

Circulating immune complexes (CICs) like activity has been reported in sera of patients with variety of infectious diseases caused by viral, bacterial, protozoal and helminthes agents⁶. They can also modulate humoral & cellular immune responses through binding to surface receptors of lymphocytes⁵.

Antigen-antibody complexes can damage tissues by triggering inflammation. Recent studies have enabled the description of sequence of steps, which depend on intra- or perivascular location of complex formation. The lesions associated with perivascular complexes are characterized by plasma leakage and the recruitment of polymorphonuclear leukocytes⁷.

Presence of immune complex, associated with the absence of detectable levels of antibodies or free P30 can be an indicative of the stage of immune response to the infection. Predominance of immune complex in HIV positive samples may be associated with compromising of the mononuclear phagocytic system responsible for IC clearance from CSF. This could promote a longer persistence of the IC in this and other organics^{8,9}.

A neutropenia is a risk factor associated with *Aspergillus fumigatus*, *Candida albicans*, *Mycobacterium tuberculosis* and *T.gondii* infection¹⁰⁻¹⁸. In some cases neutropenia has been correlated with impaired protective acquired immunity, suggesting that neutrophil function as immunomodulators of acquired immunity^{16,17}.

Moreover, neutrophil-depleted mice harbored an increased parasite burden. It was found that neutrophil depletion at the time of infection lead to development of lesions in multiple organs, including spleen, lung, liver, and brain and was associated with an impaired ability to produce early gamma interferon (INF- γ), tumor necrosis factor (TNF) and interleukin-12. This is leading to fact that neutrophils are important immunomodulators early in the early course of *T.gondii* infection and play critical role in protecting the host from uncontrolled tachyzoite replication⁽¹⁹⁾. In group of seropositive

pregnant women with toxoplasmosis, CIC detection rates were noticeably higher in the samples showing both IgG and IgM antibodies²⁰.

Both immunoglobulin G (IgG) and IgM were found in the CICs; however IgG was seen in the majority of sera were selected from patients with clinical symptoms generally associated with toxoplasmosis, more CICs were also again demonstrated²¹.

Circulating immune complexes (CICs) remained detectable for several weeks with non virulent strain of *Toxoplasma*, this period characterized by clinically healthy animals, indicating a subacute stage of the *Toxoplasma* infection. The positive CICs test requires great care but may provide useful information about the activity of a toxoplasma infection²².

The data suggest that phagocytosis of circulating immune complexes by neutrophils may interfere with the function of these cells in combating infection and also render them susceptible to removal from the circulation thus leading to the development of neutropenia²³.

MATERIALS AND METHODS

Total number of 50 Iraqi patients were included 25 patients with different problems of kidney and abortion history (14 male & 11 female) and 25 control groups (13 male & 12 female). 5 ml of blood sample collected from each patients and control group under sterile and the serum was divided into 2ml. for PMN counting and 3ml. of blood put in screw capped sterile plastic tube after centrifugation then the serum was divided in sterile appendrof tube each with 0.5ml. for platelet aggregations test (PI.A.test)²⁴ for detection CICs. The CICs was detected by platelets aggregation test (PI.A.test) and polymorphonuclear (PMNs) were counted automatically. The study was carried out during the period from November / 2015 to February / 2016. All patients were obtained from those who had been admitted to / or attended the following health institution:

1. Renal Transplant Center / out patients clinic of Ghazi Al-Harriri hospital.
2. Baghdad Teaching Hospital/Gynecology and

- Delivery Department.
3. Laboratory of Blood Bank Center /Baghdad.
*All laboratories tests were done in laboratories of Blood Bank Center.

The PL.A.test test was done to supposed circulating immune complexes(CICs) in the sera of patients with toxoplasmosis. The platelets were used on the day of preparation , with made a pool of three lots of platelets .It was always recommended to decrease the effect of varying sensitivity of different lots of platelets from different donors(24,25).Platelets was counted using(26) viable methods for platelets count are still used extensively which are valuable when the count is low. The diluents consist of 1% aqueous ammonium oxalate in which the red blood cells (RBCs) are lysed. There is a possibility that (RBCs) debris may be mistaken for platelets. The method is preferred to that using formal-citrate as diluents, which leaves the red cells intact and more likely to give incorrect results, when the platelets count is low. The method was done as following:

1. Two hundred (200µl) of platelets suspension was added to 0.38ml of diluting fluid (1%ammonium oxalate)in a small plastic tube.
2. The suspension was mixed with mechanical mixer or manually with Pasture pipette for 10-15 min .
3. The Neubauer counting chamber was filled with the suspension using a stout glass capillary or Pasteur pipette.
4. The counting chamber was placed in a moist Petridish and was left untouched for at least 20min .
5. The platelets when examined in the preparation under ordinary illumination appeared as a small highly refractile particles.

Calculation: $\text{Count of platelets}/\mu\text{l} = N. \times \text{dilution} (\text{diluent's volume} / \text{volume counted } \mu\text{l}) \times 10(\text{depth}).$ N= number of platelets counted in an area of 1 mm² .

If the number of platelets counted was high ,it is referable to use 2ml of 1% ammonium oxalate to 20µl of platelets suspension with consideration to dilution factor in the law above

The PL.A. test was performed using disposable microplates U shaped (cooke engineering

Co. Alexandria, Virginia).

1. Two fold serial dilution of patients sera in PBS (pH =7.8)without glucose (1/2–1/4096) were made , using 25µl automatic micropipette. The sera were heated and inactivated at 56° C for 30 min. before use (24).
2. Then 25µl of platelets suspension (200,000 platelets/mm³)was added to each well-containing diluted patients' serum or control sera.
3. The microtiter plate was agitated gently by side away movements for several minutes, then was covered with paraffin foil and incubated overnight at a temperature (5-8° C).
4. The sedimentation patterns were read in the following morning, using ordinary light microscope.

The results were recorded as follows:

- Fully aggregated platelets were considered as positive result.
- Not aggregated platelets were considered as negative result.
- While not fully aggregate of platelets were considered as intermediate result.

The Results

The study included of 25 patients group (14male and 11 female) infected with toxoplasmosis and with different diseases(*e.g.* R.T.,N.S.and SLE) and 25 control healthy group(13, the mean of ages of patients group was (35.4±13.72) and (34.6±4.34)of control group (Table-1-).The mean of percentage of PMN was(63.91±11.39)of patients group and (77.48±8.89)of control group. There was no significant differences between the means of ages of patients and control groups(P>0.001).

Table-2-showed the prevalence of CICs in patients and control groups ,there was 13(68.4%) patients with CICs in their sera and 6(31.6%)patients without CICs in their sera, while there was 4(16%) of control group with CICs in their sera and 21(84%) without CICs in their sera. There was a significant differences between their prevalence of CICs in the sera of the groups (P<0.0001).

Table -3-Showed the there was no patient with IgM of *T.gondii* ,while there was 25(100%) of

patients with IgM of *T.gondii*. comparison control group there was 3(12%) with IgM of *T.gondii*, while there was 22(88%) control group without IgM of *T.gondii*, there was no significant differences between patients and control group $P>0.01$.

Table-4-There was 8(32%) of patients with IgG of *T.gondii*, while there was 17(68%) of patients without IgG of *T.gondii*. In control group there was 12(48%) healthy persons had IgG-Abs. of *T.gondii*, while there was 13(52%) healthy persons without IgG of *T.gondii*. There was no significant differences ($P<0.05$) between patients and control groups $P>0.248$.

Table (5) showed the prevalence of circulating immune complexes (CICs) detected by P.L.A. test and polymorphonuclear cells (PMNs) in patients group. There was 16 patients with CICs in their sera the mean of their ages was (35.1 ± 16.402) , and there was 9 patients without CICs in their sera the mean of their ages was (35.55 ± 7.77) . There was 16 patients with PMNs% (64.268 ± 10.39) , while there was 9 patients without CICs their sera and PMN% was (63.27 ± 13.65) . There was no significant $P>0.840$. differences between the two groups

There was 3(18.8%) patients with *T.gondii* IgG Abs (Table-6) and CICs detected P.L.A. test in

Table 1: Age of patients with toxoplasmosis and control group in relation with PMNs percentages

	Groups	N	Mean	Std. Deviation	Std. Error Mean
Age	Patient	25	35.4000	13.72346	2.74469
	Control	25	34.6000	4.34933	.86987
PMN%	Patient	25	63.9120	11.39694	2.27939
	Control	25	77.4840	8.89933	1.77987

Table 2: Prevalence of Circulating Immune Complexes in Patients with Toxoplasmosis and control groups

Groups		CiCs		Total
		+ve	-ve	
Patient	Count	13	6	19
	% within Group	68.4%	31.6%	100.0%
Control	Count	4	21	25
	% within Group	16.0%	84.0%	100.0%
Total	Count	17	27	44
	% within Group	38.6%	61.4%	100.0%

Table 3: Prevalence of IgM in patients with Toxoplasmosis in comparison to healthy control

Groups		<i>T.gondii</i> IgM		Total
		+ve	-ve	
Patient	Count	0	25	25
	% within Group	.0%	100.0%	100.0%
Control	Count	3	22	25
	% within Group	12.0%	88.0%	100.0%
Total	Count	3	47	50

their sera, while there was 5(55.6%) patients without *T.gondii* IgG and CiCs detected PL.A. test in their sera. There was no significant difference $P > 0.058$ between the two groups of patients and control.

DISCUSSION

Toxoplasmosis is caused by a coccidian parasite *Toxoplasma gondii*. It is world-wide in distribution and infects most of the vertebrates.

The Felids are its definitive host. The humans are infected either through contaminated food, water, transfusion of infected blood, organ transplantation or from mother-to-foetus through placenta⁽²⁷⁾. *Toxoplasma gondii* is an obligate intracellular protozoan parasite that infects at least a third of the world's population. Infection with the parasite is divided into a limited acute stage. Followed by a persistent chronic stage. In the chronic stage of *T. gondii* forms cyst, found mainly in brain and muscle

Table 4: Prevalence of IgG of *T.gondii* in patients with Toxoplasmosis in comparison with healthy control

Groups		<i>T.gondii</i> IgG		Total
		+ve	-ve	
Patient	Count	8	17	25
	% within Group	32.0%	68.0%	100.0%
Control	Count	12	13	25
	% within Group	48.0%	52.0%	100.0%
Total	Count	20	30	50
	% within Group	40.0%	60.0%	100.0%

Table 5: The prevalence of circulating immune complexes detected by Platelets Aggregation Test and polymorphonucleus cells (PMNs) in patients with toxoplasmosis and control

Circulating immune complexes detected by Platelet aggregation test	N	Mean	Std. Deviation	Std. Error Mean
Age	+ve	16	35.3125	16.40211
	-ve	9	35.5556	7.77996
PMN%	+ve	16	64.2688	10.39447
	-ve	9	63.2778	13.65209

Table 6: Correlation between the presence of *T.gondii* IgG Abs. and CiCs detected by PL.A.test

CiCs detected by PL.A.test		<i>T.gondii</i> IgG		Total
		+ve	-ve	
+ve	Count	3	13	16
	% within CiCs PL.A.	18.8%	81.3%	100.0%
-ve	Count	5	4	9
	% within CiCs PL.A.	55.6%	44.4%	100.0%
Total	Count	8	17	25
	% within CiCs PL.A.	32.0%	68.0%	100.0%

tissues, which can persist for the lifetime of the host⁽²⁸⁾.

Table(1) showed that patients with low percentage of PMNs 63.9% ,in comparison with control group 77.4 % of PMNs .The results agreed with that reported by Salyes,1999⁽²⁹⁾ that the previous studies showed neutrophil was play an important role in resistance to acute primary *T.gondii* infection and that depletion of neutrophils reduces the numbers of CD4+ and CD8+ lymphocytes recoverable from peripheral blood of infected but not uninfected mice. Also, it was found that examined the development of immunity during *T. gondii* infection in mice depleted of neutrophils by monoclonal antibody (MAb) administration. *T. gondii*⁽¹⁹⁾

Table- 2- showed that 13% of patients with CICs in their sera in comparison with 4% of healthy group had CICs in their sera ,the results compatible with reported by Sonia,2005⁽⁷⁾ management the deposition of immune complexes in tissues consider the pathogenic mechanism underlying a variety of human diseases. Also, Gladkova *et.al.*,2000⁽²⁰⁾ described that circulating immune complexes(CICs) in the host just in early *T.gondii* invasion can be present in the blood. In addition, there has been no report of CIC in patients with toxoplasmosis. Some aspects of toxoplasmosis, notably the occasional occurrence of glomerulonephritis or congenital nephrotic syndrome⁽³⁰⁾ suggest a pathogenic role for CIC.⁽⁵⁾

The results included (Table-3- and Table-4-) no of patients with IgM of *T.gondii* but 8(32%) of them with IgG of *T.gondii*, while 3(12%) of control group with IgM of *T.gondii* and 12(48%) of them with IgG of *T.gondii* and the results suggested that patients might be chronic phase of toxoplasmosis and might IgG Abs of *T.gondii* form CICs in there circulation ,while control group might had active phase of toxoplasmosis since they with both IgM and IgG of *T.gondii* in there sera and there antibodies did not form immune complexes in their circulation. These results in line with Ghasemian *et al.*,2007⁽³¹⁾ who reported that the disease in immunocompromised individuals such AIDS, transplant recipients,persons receiving immunosuppressive drugs usually is due to reactivation of latent infection but can result from acute infection. Toxoplasmosis in these persons

leads to lethal meningoencephalitis,focal lesions of the CNS, and less commonly,myocarditis or pneumonitis.

Also, IgG antibodies to *Toxoplasma* are usually present 1-2 weeks after acquisition of the infection and usually persist for life. For immunocompetent persons, seroconversion with high concentrations of *Toxoplasma* specific IgM and a 4-fold increase in specific IgG titer is indicative of recent infection. It has been known that 15-58% of humans are infected with *T.gondii*, but the rate of infection varies widely by location, age and other factors⁽³¹⁾ While, Wongkamchai *et al.*,1995⁽³²⁾ mentioned that *Toxoplasma*-specific IgG and IgM antibodies were determined in healthy persons and patients with different symptoms who were suspected of toxoplasmosis. Specific IgG were detected in 3.2% of healthy persons, 12.5% of patients with ocular disease and 42.5% in HIV positive patients. Only 3.1% of patients with ocular disease were positive for specific IgM Ab. No specific IgM were found in the other samples studied .

Table-5- showed that there were 16 patients with circulating immune complexes and with the percentage of PMNs was (64±10.26),while there were only 9 of patients without CICs in their circulation .

Also, these results compatible with that reported by Steffelaar *et al.*,1976⁽³³⁾ who reported that antigen-antibody complexes are eliminated from the circulation by phagocytosis effected in the reticuloendothelial system (RES) and by deposition in renal glomeruli and other tissues⁽³⁴⁾. In addition, neutrophils have long been regarded as one of the most important of the induced host innate defenders primarily because they are the earliest cells to arrive at sites of infection or inflammation in response to chemotactic signals to eliminating invading pathogens⁽³⁵⁾.

Table (6) showed that there 3(18.8%) patients with immune complexes(CICs) and with IgG of *T. gondii* and the result agree with Gladkova *et al.*,2000⁽²⁰⁾ who reported that CICs in serum of pregnant women exhibit only IgG contained mainly *T.gondii* Ag having MW of 67and 30 KD.

Finally deposited ICs cannot be detected by PL.A. test, if only when they are found in soluble form in cases of antigen excess, since PL.A. test detect IgG containing ICs, whether or not they fix complement but does not detect complexes formed with IgM. Also PL.A. test reaction caused by ICs are competitively inhibited by some rheumatoid

factor, C1q and to lesser degree by monomeric IgG.⁽³⁶⁾ The final diagnosis of toxoplasmosis depends on the results of ELISA method that was used for the detection of IgM *Toxoplasma* antibodies chosen in the decision of the final diagnosis of toxoplasmosis and not IgG, because acute toxoplasmosis is usually diagnosed on the basis of IgM antibody detection⁽³⁷⁾.

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