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Non-Aborted women toxoplasmosis: Immunological status in Duhok governorate

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Abstract:

Toxoplasmosis antibodies were demonstrated in 200 serum of women that are randomly chosen as a non-aborted women in a cross sectional study. Anti-Toxoplasma IgM antibodies were seen in 65 (32.5%), but IgG antibodies were observed in 9 cases (4.5%) only and for total the ratio is 74 (37%). These results were obtained by ELISA double sandwich method.

The average of examined non-aborted women age was reach 28. 9 years where mostly in ≤25 to ≤40 years (85.5%). Most cases of Toxoplasmosis here were seen at 9^{th}, 11^{th} and 12^{th} months (62%). Urban area contributed with 51.5% of cases in comparison with rural area (48.5%).

Spearman's rho correlation test as a statistical test was used to explain the results in current research. This method helps to explaining why Toxoplasmosis infection could be found in any age, or understanding how Toxoplasmosis is related to any level of IgM or IgG. Also it indicate that Toxoplasmosis disease is not specific to any living or resident area.

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Lately the study revealed that Toxoplasmosis may be seen at any of months of the year but in more frequent months of both late of autumn and the beginning of winter.

- ## Introduction:

Toxoplasmosis is considered among the most important protozoan parasitic diseases related directly to abortion, preterm labor, stillbirth, and fetal abnormalities of women all over the world (1, 2). The parasite nature is correlated with the presence of cat as a final host and human as an intermediate host as well as other mammalian animals and birds (2, 3). Infection takes place via fecal-oral methods through eating contaminated food and water (4, 5).

Toxoplasmosis clinical symptoms are observed in both of men and women. These symptoms begin with fever, muscle ache, eye injuries, CNS disorders, etc. (6, 7). In women Toxoplasmosis is important because of occurrence of repeated abortion (8). This disease is also very important for people who have been suspected of infection with this disease (8, 9). The immunological state of Toxoplasmosis patients is quite significant and for that we can use immunological tests as a clinical marker in health-profile management (10, 11). There are many diagnostic tests have been performed to track the infection of Toxoplasmosis from the basic to the more complex type, including the agglutination of ELISA and PCR (8,15).

Current research is focused on IgM and IgG detections by ELISA for screening of the distribution of these two immunoglobulins among women of Duhok governorate in Kurdistan region / IRAQ.

- ## Material and methods:

1-Blood samples of 200 randomly chosen non-aborted women suspected to have Toxoplasmosis according to clinical history rather than abortion, with respect to area of living as in Duhok city/IRAQ

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and around area. Samples are collected in sterile method according to usual hematological laboratory protocol (4, 13, 14, and 15).

2- According to previous studies (4, 16 and 17) the ELISA protocol (Double or dual sandwich type) has been used according to kit manufacturing manual (Jackson immunology Research, West Grove, PA-USA), briefly, the buffered (100 µl) solution containing standard antigen has been well mixed with 100 µl of collected serum and 100 µl of IgM samples and human IgG. The batch was incubated separately with antigen free buffer for approximately 20 minutes at 37 °C, followed by three washes with washing buffer. Then the plate put in the reader (detector module) to detect the cutoffs reading for control positive and control negative. In the following, 50 µl of anti-human IgM or anti-human IgG were applied for 10 minutes at 37 ° C. For IgM antibodies concentration of < 0.9 is account as negative, but 0.91-0.99 is equivocal and > 1 is positive. Then for IgG concentration, if value is < 32, it considered negative but more it will be positive.

4. Statistical analyses includes: Frequency tables, histograms, and Spearman correlation tests. Cross section study design will be used and the analysis takes place based on the version of SPSS 21 statistical software.

- **Results:**

The Average ages of patients with antibodies against Toxoplasmosis is shown in table (1). The group or classes which are most common for investigated non-aborted women shows to be that of ages ≤25 to ≤ 40 years. The cumulative percentage of these women reach 85.5 % in compare to other groups. The average age of patients with was 28.9 ±7.044 years .The effect of months of the year (Fig.1), shows that the highest number of patients who have Toxoplasmosis are seen

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in September, November and December with ratio of 62% (n= 124 of 200 cases) **.**In relationship to living area or residency approximately more than 51.5% of patients come from Duhok center city (Tab. 2) with 53.8% of positive for IgM, then followed by other regions around Duhok center city. IgG data are ignored here because of their small number which make it not reliable for statistical analysis, but they are included in the final results.

For total positive cases (Tab. 2) it has been shows that the ratio reach 37% for both of IgM and IgG.

Spearman's rho correlation test (Tab. 3)

Tab. (1): Descriptive frequency of age of patients screened for Toxoplasmosis Immunoglobulins (IgM and IgG).

Age Groups (Years)	Frequency	Percent (%)	Valid Percent (%)	Cumulative Percent (%)
1 (≤ 20)	20	10.0	10.0	
2 (≤ 25)*	50	24.9*	25.0	25.0
3 (≤ 30)*	59	29.4*	29.5	54.5
4 (≤ 35)*	35	17.4*	17.5	72.0
5 (≤ 40)*	27	13.4*	13.5	85.5
6 (≤45)	8	4.0	4.0	
7 (≤ 50)	1	.50	.50	
Total	200	100.0	100.0	
* = The cumulative percentage of these are 85.5% of total.				

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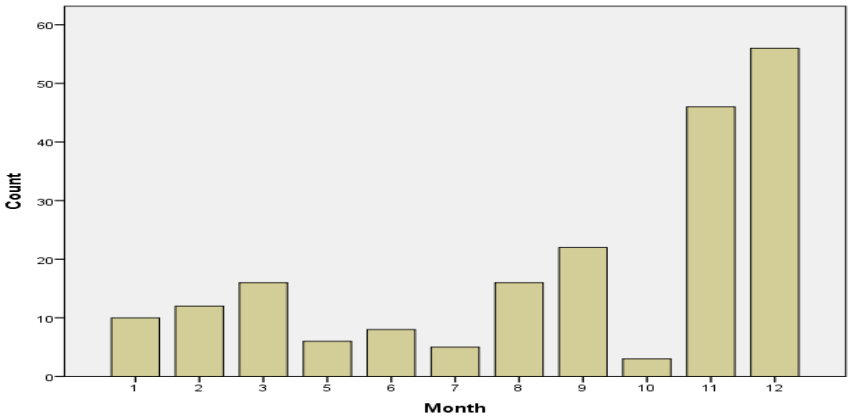

Fig (1): Cases distribution according to months of screened Toxoplasmosis Immunoglobulins (IgM and IgG)

Table (2): Cases distribution according to living area of Duhok center and around area for Toxoplasmosis Immunoglobulin (IgM and IgG)

Duhok area		Number of cases (%)	IgM positive cases (%)	IgG positive cases	Total Positive cases
1	Duhok center	103 (51.5%)	35 (53.8%)	8	43
2	Semel	27 (13.5%)	18 (27.7%)	1	19
3	Zaweeta	20 (10%)	2 (3.07%)	-	2
4	Sharia	15 (7.5%)	2 (3.07%)	-	2
5	Mesereek	12 (6%)	2 (3.07%)	-	2
6	Xankee	12 (6%)	2 (3.07%)	-	2
7	Sendoree	6 ((3%)	2 (	-	2

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			3.07%)		
8	Beseree	5 (2.5%)	2 (3.07%)	-	2
Total		200	65 (32.5%)	9 (4.5%)	74 (37%)

Tab. (3): Spearman's rho correlation test values of various research criteria of patients screened for Toxoplasmosis Immunoglobulins (IgM and IgG).

Spearman's rho of studied criteria		Age	IgG	IgM	Living area	Month
Age	Correlation Coefficient	1.000	.013	-0.076	-0.043	-0.085
	Sig. (2-tailed)	-	.8680	.3250	.5740	.2700
	N	200	200	200	200	200
IgG	Correlation Coefficient	.0130	1.000	.2070	.1120	-0.259
	Sig. (2-tailed)	.8680	-	.0070	.1440	.0010
	N	200	200	200	200	200
IgM	Correlation Coefficient	.0760-	.2070	1.000	.1960	.0370-
	Sig. (2-tailed)	.3250	.0070	-	.0100	.6360
	N	200	200	200	200	200
Living area	Correlation Coefficient	.0430-	.1120	.1960	1.000	.2800-
	Sig. (2-tailed)	.5740	.1440	.0100	-	.0000

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	N	**200**	**200**	**200**	**200**	**200**
Month	**Correlation Coefficient**	**.0850-**	**-0.259**	**.0370-**	**-0.280**	**1.000**
	Sig. (2-tailed)	**.2700**	**.0010**	**.6360**	**.0000**	**-**
	N	**200**	**200**	**200**	**200**	**200**

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of the 200 cases shows to be related together either positively or negatively.
The coefficient of correlation (rho = r) for different parameters is between 0.196 and–0.280 in both directions.
In the investigation of both of IgG and IgM the value of correlation shown to be 0.207. But in case of IgG vs. months another value is observed (- 0.259). Then the living area against IgM the result is 0.196 and lately against months of the year result shows another direction (- 0.280) of correlation.

- **Discussion:**

Toxoplasmosis studies for any population or culture should be includes the age, time and living area. Other parameters may be includes the presence and the frequency distribution of antibodies that are correlated with immunological status of toxoplasmosis. In such studies, IgM and IgG are the most investigated types (1, 8).

Current research indicates that toxoplasmosis (Tab.1) infection occurs with an average age of twentieth till fortieth groups of studied non-aborted women.

This observation is seen in many previous studies (1, 3, and 12) but not in details as in the current study. This may be explained by the effects of hormonal status and period cycle which both of them could be decreases immune system efficiency against Toxoplasmosis infection which have incubation period of 20 ± 7 days (18) and that may be interfere with menstrual cycle.

Toxoplasmosis seasonally occurred from September to December over the last months of the year could be clarified that ultimate final host (cat) attachment to people in gardens or parks would be in that time. In previous researches (21, 22) the seasonal phenomenon was observed.

On the other hand the immunological status that are estimated by ELISA to follow-up the immunological situation of women is used widely for many

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diseases as in case of Toxoplasmosis with respect to other methods like immune-fluorescent technique or PCR. These studies are mostly deals with an aborted women (1, 12 and 16) but not non-aborted as here.

Usual ELISA testing requires more precision and sensitivity (14) in compare with the double sandwich ELISA (1, 12). In the current study, anti-Toxoplasmosis immunoglobulin is present in high rate (37%) in compared to other studies (1, 16), especially in the case of the acute toxoplasma phase (16). This high rate may be due to the selected sample which concentrate of non-aborted women that have clinical signs could be related to Toxoplasmosis.

Immunoglobulin IgM is more distributed than IgG in the present study may be due to that the local parasitic strain of Toxoplasmosis parasite which not leads to developing of delayed immunity. This finding have been seen in other previous studies (19, 20).

The findings of Spearman's rho statistical analysis demonstrate how epidemiology functions works against factors leading to the distribution and transmission of diseases of Toxoplasmosis for the first time. This test has not been used in the same way as here before. Previous statistical analysis are compare between two variables each time by ANOVA and Chi-square test in most of researches (16, 23).

In the current analysis, r values have been shown results forwarded into to both directions. This means that the infection could occur at any age, time and area. This correlation can be seen immunologically with the presence of IgM or IgG. This may be attributed to the biological nature of intracellular pathogens like Toxoplasmosis parasite and sources infection like eggs in the feces of cat and cysts in the meat (2, 16).

- **Conclusion:**

Toxoplasmosis antibodies are highly distributed among non-aborted women and that should be taken in consideration for further detailed study..

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