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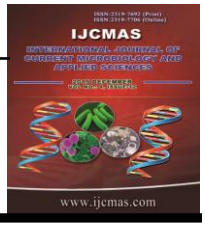

Original Research Article

Role of Il-17 in Toxoplasma Lymphadenitis

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Keywords

Toxoplasma lymphadenitis, Immunohisto-chemistry (IHC), Haematoxylin and Eosin (H&E), Toxoplasma antigen (P30)

A B S T R A C T

Toxoplasmosis is the general term for infection and disease in man and animal caused by a parasite called *Toxoplasma gondii*. There are six members in the IL-17 cytokine family, including IL-17A (commonly referred to as IL-17).In Toxoplasmosis the role of IL-17 was involved in the development and early recruitment of neutrophils, which are essential to clear the parasites during initial stages of infection. This study was carried out to diagnosis the Toxoplasma lymphadenitis and to determine the role of IL-17 in this disease. A total of 45 lymph node tissue samples (paraffin block) were enrolled in this study. Three sections were made from the paraffin-embedded tissue blocks, one section for H&E staining. Two sections were processed by immunohistochemistry (IHC) procedure. The first for diagnosis Toxoplasma antigen (P30) while the second section for diagnosis IL-17byusing specific immunohistochemistry (IHC) detection kits. From 45 patients who examined by H&E, only 25 patients had evidence of Toxoplasma. The lymph nodes with histological evidence of Toxoplasmosis showed positive Immunohistochemical Toxoplasma antigen expression, but, neither reactive lymph nodes nor normal ones showed positive immunohistochemical Toxoplasma antigen expression. Immunohistochemical expression of IL-17 was assessed by two methods. The first one is represented by the percentage of inflammatory cells that express the marker IL-17, and the second method is named "IL-17 score" In this study we concluded there are highly significant expressions in levels of IL-17 by immunohistochemistry assay in both the percent and score methodswhich can give an idea about the association between IL-17 and toxoplasma lymphadenitis.

Introduction

Toxoplasmosis is the general term for infection and disease in man and animal caused by a parasite called *Toxoplasma gondii*, it is responsible for congenital birth defects and toxoplasmic encephalitis (Zhou *et al*., 2011). In humans, the result of infection may range from asymptomatic to severe disease. Infection in congenital and immunosuppressed individual usually more severe (Dubey and Jones, 2008) Lymphadenitis (Infectious of lymph nodes)

is the most common clinical form of the disease (Hill *et al*., 2005).Lymph nodes produce specialized immune system cells called lymphocytes that detect and combat pathogens in the body. When an infection is present, the nodes swell as they produce larger than normal quantities of lymphocytes (Kerawala *et al*., 2010).The diagnosis of Toxoplasma lymphadenitis is clinically significant because it may rule out lymphoma, Hodgkin lymphoma and non-Hodgkin lymphoma, (Iaochim and Medeiros, 2008).

Interleukin 17-A or IL-17 identified by (Rouvier *et al*., 1993).There are six members in the IL-17 cytokine family, including IL-17A (commonly referred to as IL-17), IL-17B, IL-17C, IL-17D, IL-17E (also known as IL-25) and IL-17F. Produce of IL-17 derived from innate and adaptive sources of immune response. Specialized T cells, called Th17 cells, are the major source of IL-17 in many types of adaptive immunity. There are several types of innate immune cells that also produce IL-17. These cells are localized to mucosal tissues, such as the intestine, skin and lung, which constantly interact with the outside environment and can respond via their expression of Toll-like receptors(Chien and Bonneville, 2006). In Toxoplasmosis the role of IL-17 was involved in the development and early recruitment of neutrophils, which are essential to clear the parasites during initial stages of infection. The initial of innate immune response by neutrophils has also been reported to be critical for successful (Yisong and Richard, 2009).

Materials and Methods

A total of 45 lymph node tissue samples (paraffin block) were enrolled in this study. The patients were divided as follow (25) positive Toxoplasma lymphadenitis, ten reactive lymphadenitis and 10 normal lymph node.

The blocks were collected from Al-Khadhmiya Teaching Hospital and private laboratories from January-2013 to June-2013, and the samples of normal lymph node tissue were taken from the Institute of Forensic Medicine (autopsy) as a control group. These specimens were processed and paraffin embedded in the unit of histopathology of Al-Khadhmiya Teaching Hospital and the department of pathology at Collage of Medicine, Al-Nahrain University. The questionnaire and clinic pathological parameters were obtained from patients who include admission case sheets. Three sections were made from the paraffin-embedded tissue blocks of 5µm thickness to study the following parameters, one section for H&E staining. The following Two sections were processed by immunohistochemistry (IHC) procedure. The first for diagnosis Toxoplasma antigen (P30) while the second section for diagnosis IL-17 by using specific immunohistochemistry (IHC) detection kits. Slides were stained by Haematoxylin and Eosin(H&E)according to (Bancroft and Steven, 1975) and examined by histopathologist.

In this study slides were stained by IHC according to (Albert *et al*., 1941) by using immunoperoxidase secondary detection kit (Dako Cytomation IHC kit LSAB2 System-HRP, code K0679) and Brown cytoplasm staining is considered positive reaction in both toxoplasma Ag ((P30) and IL-17.

Results and Discussion

The mean age of the (25) patients, were diagnosed as toxoplasma lymphadenitis histological, was (16.76+4.61years) with a

range of 12-34 years. The (10) patients, were diagnosed as having reactive lymphadenitis histological, was (18.50+2.84) years with a range of (14-23)years. And for 10 subjects with no histological evidence of any pathology (normal lymph nodes) was (19.90+2.60) years with a range of 17-24 years. The majority of cases were found to be infected with toxoplasma lymphadenitis 15(60%) in age group between (15-19 years), 5(50%) and 6 (60%) with reactive and control respectively. Although there was some difference in mean ages among the three groups, nevertheless, this difference was not statistically significant P-value = 0.178 (Table 1).

The total number of all cases (patients and control) include in this study was (45) the number of female was 32(71.11%) and for male were 13(28.89%). With Toxoplasma lymphadenitis patients there were only 3 (12%) male and 22(88%) female out of 25 patients. (M: F ratio =1:7.33). In reactive group 5 male: 5 female (M: F= 1:1), in control group there were 8 male and 2 female (M: F= 4:1) (Table 2).

Histology

From 45 patients who examined by H&E, only 25 patients had evidence of Toxoplasma, showed the architecture microscopically which include reactive hyperplasia, small epitheloid cell within at the periphery of the follicle margin encroaching and blurred their margin, medullary sinusoid filled with Monocytoid B cells (Figure 1).

Immunohistochemical Expression of Toxoplasma antigene (P30)

The lymph nodes with histological evidence of Toxoplasmosis showed positive Immunohistochemical Toxoplasma antigen expression, but, neither reactive lymph nodes nor normal ones showed positive immunohistochemical Toxoplasma antigen expression. P-value was highly significant <0.001 (Table 3)

The expression of Toxoplasma antigen was granular cytoplasmic with brown color, involving both lymphocytes and histeocytes (Figure 2).

Immunohistochemical Expression of IL-17

Immunohistochemical expression of IL-17 was assessed by two methods. The first one is represented by the percentage of inflammatory cells that express the marker IL-17, and the second method is named "IL-17 score" it refers to the intensity of staining (weak =1, moderate =2, strong =3) with disregard to number of cells expressing the marker.In Toxoplasma groups the mean of IL-17 percent was significantly higher when compared to reactive group (47.2+18.82%) and (21.5+7.47%) respectively, while in control group was undetectable; p<0.001 (Figure 3).

The strong IL-17 score was more frequent in Toxoplasma cases than in reactive cases (52% versus 0%) respectively and IL-17 in control group was undetectable. Unfortunately p-value was not valid due to small cell size (Table 4and figure 4).

Toxoplasma gondii is a protozoan parasite that causes the disease toxoplasmosis. It is one of the most common parasitic infections in humans**.** Toxoplasmosis can be asymptomatic or can have more severe consequences such as congenital birth defects, toxoplasmic encephalitis (Feustel *et al*., 2012). Lymphadenitis is the most common clinical form of the disease (Abu-

Madi *et al*., 2008).In this study, result showed that The mean age of participant patients with toxoplasmosis was (16.76±4.61) years with age ranged 10- 34 years which is approximately similar to the study carried out in the United Arabic Emirates (Abu-Zeid, 2002). Who stated that mean age of patients was 19.7 years with age ranged (12-52) years. This may be explained by the fact that exposure to the parasite is concentrated around this age in which contact with main carrier for the parasite, cat, is maximum.

In current study result showed highest number of patients from female than male was 88% and 12% respectively, this result agreement with (Yasodhara *et al*., 2004;Tabbara and Saleh, 2005) and with study in China which found 10.5%, male versus 14.3%, female (Xiao *et al*., 2010). This may be interpreted by continuous exposure of women to the risk factors of *T. gondii* infection through their routine house works like minced contaminated meat products, gardening and contact with soil especially in rural area, eating of raw or unwashed vegetables and fruits and drinking of municipal water from contaminated reservoirs.

All slides examined by H&E, for 25 patients had evidence of Toxoplasma, the architecture microscopically showed, reactive hyperplasia, small epitheloid cell within and at the periphery of the follicle margin encroaching and blurred their margin, medullary sinusoid filled with monocytoid B cells(Juan, 2011).There are many granulomatous diseases, that toxoplasmosis confused with them such as leishmaniasis, tuberculosis (common), sarcoidosis, cat-scratch disease and brucellosis (uncommon), (Juna, 2011). So must also be differentiated from it by using other diagnostic methods such as Toxoplasma antigen expression by (IHC). Immunohistochemistry expression of Toxoplasma antigen showed that, the total population infected with *T. gondii* was 55, 56% in this study, This study was agreement with study in Ethiopia 60% (Negash *et al*., 2008), Indonesia 50.0% but disagreement with study in Iran 20.9%, Slovakia 20.5%, Somalia 37.5%, Nigeria 23.8%, (Uneke, 2005).The discrepancy in seroprevalence in different countries may be due to different ethnicity, traditional culture, and food habits (Cenci Goga, 2011). One explanation may be about personal hygiene, people whose not have enough knowledge, thus increasing the possibility of infection. Also this variation is presumably due to the presence or absence of cats or dogs, climatic factors, playing in the soil, and consumption of raw or improperly cooked meat or vegetables, or unboiled water (Alvarado, 2011). Occupations also were reported that a farmer or livestock worker had a higher probability of acquiring *T. gondii* infection than a businessman or civil servant (Kamani, 2009).The mean of IL-17 percentage was significantly higher in Toxoplasma group compared to reactive group (47.2±18.82 and 21.5±7.47) respectively. The early increase in level of IL-17 in the present study matches the results of several researchers (Kelly *et al*., 2005, Yisong and Richard, 2009, Ye *et al*., 2001) who found that an increase in IL-17 had been reported in early stage of infection found that IL-17was involved in the development and early recruitment of neutrophils, which are essential to clear the parasites during initial stages of infection (Yisong and Richard, 2009). Another possibility is that during toxoplasmosis, the splenic accessory cells produce additional cytokines/ cofactors that cooperate with IL-6, IL-23, and TGF-β to promote IL-17 secretion. This result is in agreement with study by (Al-Dahmoshi *et al*., 2012) in Iraq.

Table.1 Frequency of *Toxoplasma* According to Age Among Study Groups

Age interval (years)	Toxoplasma		Reactive		Normal		Total	
	No.	%	No.	%	No.	%	No.	%
10-14 years	7	28	1	10	0	0	8	17.78
15-19 years	**15**	60	**5**	50	4	40	24	**57.78**
20-24 years	1	4	4	40	**6**	60	11	20.00
25-29 years	1	4	0	0	0	0	1	2.22
30-34 years	1	4	0	0	0	0	1	2.22
Total	**25**	**100**	**10**	**100**	**10**	**100**	**45**	**100**
Mean ages	16.76±4.61		18.50±2.84		19.90±2.60		17.84±4.04	
Age range	12-32		14-23		17-24		12-32	
P-value*	0.096 (not significant)							

Table.2 Distributions According to the Gender (sex) for all Groups

Group	Male		Female		Total	
	No.	%	No.	%	No.	%
Toxoplasma	3	12	22	88	25	100
Reactive	5	50	5	50	10	100
Normal	8	80	2	20	10	100
Total	16	35.55	29	64.44	45	100

P= 0.020 (not valid because more than 20% of cells have expected values less than5)

Table.3 Immunohistochemical Toxoplasma Antigen Expression in all Groups

	Toxoplasma		Reactive		Normal		Total	
	No.	%	No.	%	No.	%	No.	%
Positive *Toxoplasma* antigen	25	100	0	0	0	0	25	55.56
Negative *Toxoplasma*antigene	0	0	10	100	10	100	20	44.44
Total	25	100	10	100	10	100	45	100
P1	**<0.001** " is not valid because 2 cells (33.33%) have expected count less than 5							

Table.4 Comparison of IL-17 Score Between Toxoplasma Lymphadenopathy Group and Reactive Lymphadenitis Group

		Toxoplasma		Reactive		control		Total	
		No.	%	No.	%	No.	%	No.	%
IL-17 score	Weak	7	28	7	70	0	0	14	40
	Moderate	5	20	3	30	0	0	8	22.86
	Strong	13	52	0	0	0	0	13	37.14
	Total	25	100	10	100	0	0	35	100
P=0.013 (not valid)									

Figure.1 Haematoxylin and Eosin Staining of Lymph Node (Toxoplasma Positive)

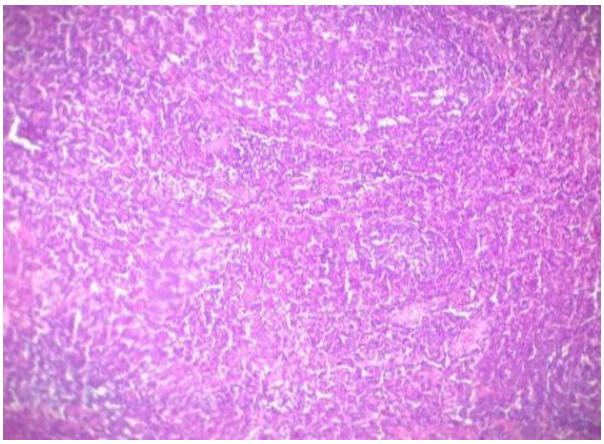


Figure.2 Immunohistochemical Stain of Positive Toxoplasma Antigen Show Brown Cytoplasm of Lymphocytes (40 X)

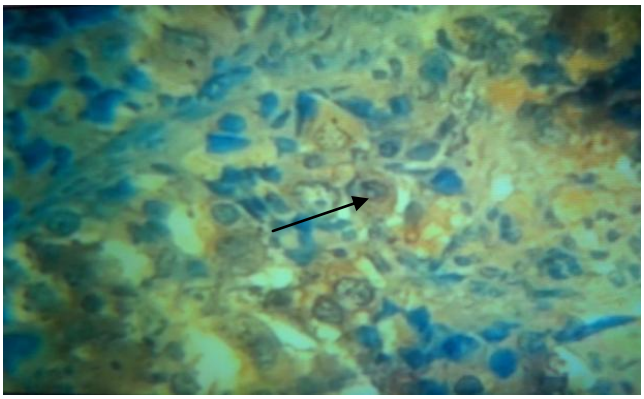


Figure.3 Comparisonof IL-17 Expression Between Toxoplasma Lymphadenopathy Groups and Reactive Lymphadenitis Groups

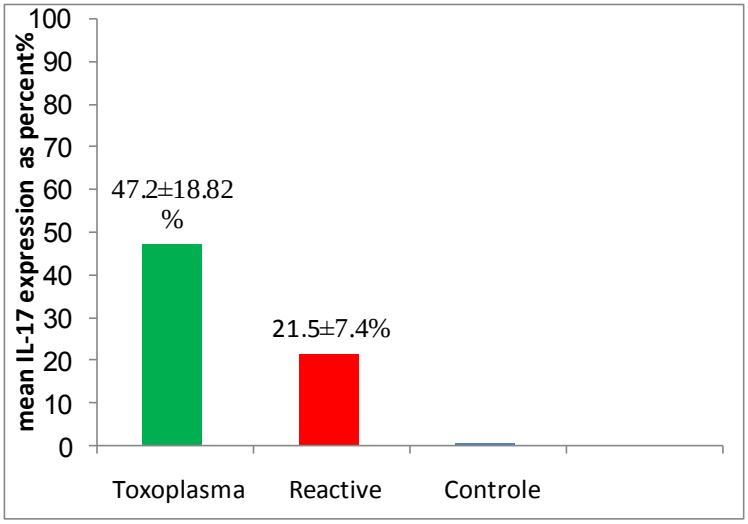
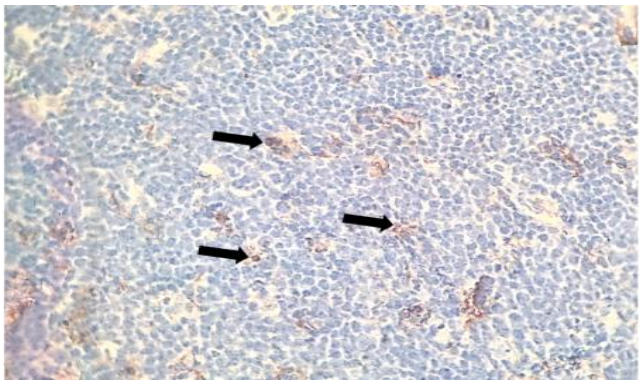


Figure.4 Immunohistochemical Staining Positive IL-17 Marker Show Brown Cytoplasm 40 X



In conclusion, in this study we concluded there are highly significant expression in levels of IL-17 by immunohistochemistry assay in both the percent and score methods when compared with the reactive groups in the percent and score methods which was not so significantly increase, while in control group IL-17 was undetectable, which can give an idea about the association between IL-17 and toxoplasma lymphadenitis.

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