The role T-helper-17 in toxoplasmosis among women with abortion

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الخلاصة:

اجريت الدراسه الحاليه لتقييم دور(T-helper-17) من خلال قياس مستوى الانترلوكين السابع عشر (Interleukin-17) في مصل الدم والغايه المتوخاة من ذلك هو لمعرفة الاستجابه المناعيه ضد مرض داء المقوسات لدى النساء اللاتى عانين اسقاطا ، تضمنت تلك الدراسه جمع عينات من دم (٣٥) من اولئك المرضى و (٢٠) من النساء الاصحاء كمجموعه ظابطه ، تراوحت اعمار من شاركت في تلك الدراسه مابين ١٥-٥٠ سنه من النساء الوافدات الى مستشفى الولاده والاطفال التعليمي في النجف ،مستشفى الحكيم العام وبعض العيادات الخاصه وللفتره مابين مايس ٢٠١٠ ومايس ٢٠١١ وقد تم قياس مستوى السايتوكاينات الاتيه -Interleukin (Gamma Interferon 17,Interleukin-10) في مصول دم اولئك المرضى والمجموعه الضابطه اضافة الى اعاده قياسها لمجموعه المرضى يعد اخذ العلاج اللازم.

اظهرت تلك الدراسه زياده وبصوره معنويه في مستوى الانترلوكين السابع عشر(٢٦٦.٥٨٩ pg/ml) في دم اولئك المرضى في المراحل المبكره من الاصابه عند مقارنتها للمستوى في المجموعه الضابطه ومجموعه المرضى بعد اخذهم العلاج اللازم (54.182pg/ml, 54.875pg/ml) وعلى التوالي.

Abstract:

The present study was carried out to evaluate the role of T-helper-17 through measuring serum level of IL-17 in infection with *Toxoplasma gondii* among women with abortion. The present study was carried out on(35) women patients ,all of them having abortion and proved by confirmatoy tests that they have an active form of toxoplasma infection. It also included twenty apparently healthy women as a control group . The patients were between 15 to 50 years of age, they were attending AL-Zahraa Maternity and Child Teaching Hospital, AL-Hakeem Hospital and some private clinics, in Najaf governorate during the period between May 2010 to May 2011. The Cytokines that were measured by ELISA technique were Interleukin-17(IL17),Interleukin-10(IL10) and Gamma Interferon(IFNγ).

The study showed a high significant increase in serum level of IL-17 in patients with IgM seropositivity (266.589pg/ml) when compared with serum levels in control and post treated groups (54.875pg/ml,54.182pg/ml) respectively, whereas there was no significant differences in serum levels between those with seropositivity with both IgM and IgG and levels in post treated and control groups.

The role T-helper-17 in the immune response against toxoplasmosis was studied in the current infection, it was found that there was a concomitant increase of IL-17 with the increase of T-helper-1 cytokine.

Introduction:

T-Helper-17 (Th17) cells are a newly identified class of effector T cells attracting much attention recently. Th17 cells produce IL-17A, E and F among the six members of IL-17 family, IL-17A, B, C, D, E (or IL-25) and F (1,2,3). It is now becoming clear that Th17 cells also produce IL-21 and IL-22 (4,5).

The Th17 response seems to share commonality with both Th1 and Th-2 responses.

Th17 cells have been suggested to contribute to the resistance to, Toxoplasma (6).

Differentiation of CD4+ T-helper cell into polarized Th1 and Th2 cells revealed that these subsets were capable of considerable cross-regulation. Thus, Th1 and Th2 cells inhibit the development of one another through the action of their lineage-specific cytokines IFNγ and IL-4 respectively (7, 8) This principle also held true for Th17 cells with evidence that IFNγ and IL-4 could antagonize initial Th17 development (9, 10).

It was found that fully matured Th17 cells are resistant to the suppressive effects of IFNγ and IL-4 in vitro providing evidence that this subset of Th cells represents a committed phenotype (11). However, to date there has been no evidence that Th17 cells can antagonize the development of Th1 or Th2 responses in the same fashion (9, 10).

The role of IL-17 in toxoplasmosois : the initial innate immune response led by neutrophils has also been reported to be critical for successful resolution of the infection (12, 13, 14). Several studies have shown that their loss leads to exacerbation of infection (15 , 16).

The early induction of neutrophil induction during *T. gondii* infection is dependent on IL-17-mediated signaling. The neutrophils clear the parasites during initial stages of infection so that adaptive immunity, which is induced later, is not overburdened. In the presence of suboptimal levels of neutrophils, parasite load is not efficiently reduced and adaptive immunity is unable to handle this elevated burden, which ultimately leads to increased mortality (17).

Materials and methods:

Collection of Blood Samples:

Thirty five blood samples were collected from women with active form of toxoplasmosis after confirmation with IgM test ,the age was ranged between 16 to 45 years old from different health centers in Najaf governorate),five ml of venous blood was drawn using a 5 ml size disposable syringe then transferred to 10 ml disposable sterile serum tube. The blood samples were left to clot then centrifuged at 3000 rpm for 5 minutes to separate the serum. Serum samples transferred to eppendorf tubes and stored at 4 - 8 °C for 24 hrs. If long period of storage is required, the sera will be kept frozen at – 20 °C until use.

ELISA tests:

The following tests were done according to manufacturer's instructions:

1-IgG and IgM ELISA tests. (Biocheck/USA)
2-Interleukin-17 (IL-17). (Causobio/China)
3-Gamma Interferon (IFN-γ).(Immunotech/France)
4- Interleukin-10 (IL-10). (USBiologica/USA)

Results:

Interleukin-17:

It was observed that there are an increased of of serum level of IL-17 (266.589±78.225) among women with acute stage of toxoplasmosis who have only positive IgM .Statistically this level was significantly higher (P ▪ 0.000) than level among post treatment and control groups (63.631±26.212 , 54.87575±16.4017) respectively. In case of patients with positive both IgM &IgG there was no statistical difference among studied groups (P=0.067) (table: 1).

Table (1) : Serume IL-17 levels in study groups.

Clinical group \ Cytokine		Mean concentration ±SD of IL-17 No.(Conc. Pg/ml)
Acute cases	With positive IgM only	266.589±78.225*
	With positive IgM/IgG	54.182±17.952**
	Total	114.871±106.421***
Post treatment		63.631±26.212* ,**
Control group		54.87575±16.4017***
P value		**Paired t-test=8.967 p value=0.000* Paired t-test=1.919 p value=0.067** Independent t-test=2.49 pvalue=0.01*** ** **

*****paired t-test between acute (IgM only) group & post treatment group.
** paired t-test between acute (IgM/IgG) group & post treatment group.
***Independent t-test between acute group & control group.

Gamma Interferon:

A high significant increase (p▪ 0.000) of serum IFNγ was recorded in acute cases with only positive IgM (19.023±2.881pg/) when compared with post treatment and control serum level (3.052±1.416 , 2.211±1.193) **,**whereas there was no significant difference between serum level of this cytokine in acute cases with both positive IgG and IgM and serum level in post treatment and control groups (P=0.689) figure (1).

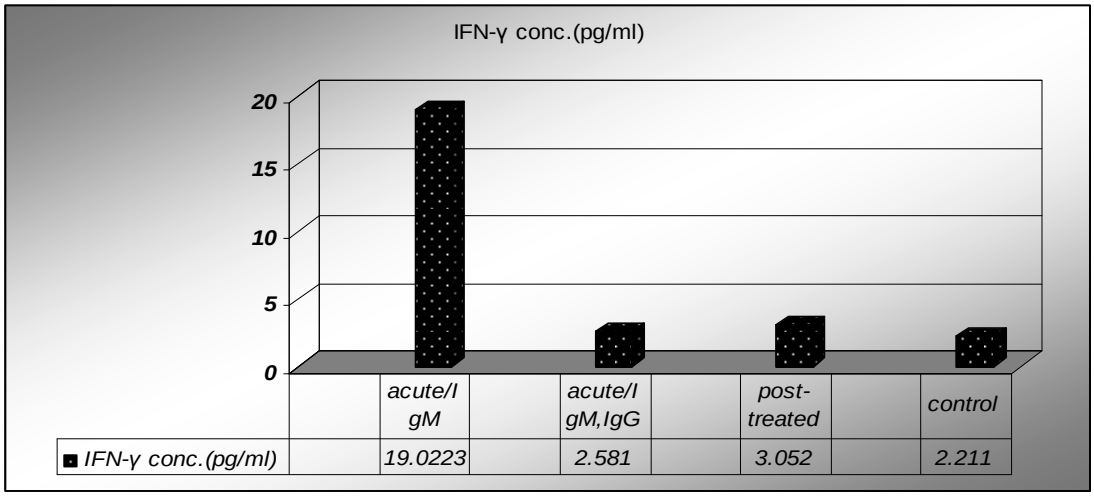

Figure (1): mean of serum IFN Gamma level in different study groups.

Note: *paired t-test between acute (IgM only) group & post treatment group=**14.256; P=0.000**.
paired t-test between acute (IgM/IgG) group & post treatment group=0.392;P=0.698**.
*****Independent t-test between acute group & control group= **P=0.006**.

Interleukin-10:

It was obvious that there was a high significant increase in IL-10 level in the sera of patients with acute toxoplasmosis having both positive IgG and IgM (132.258±97.488pg/ml) in comparison with control and post treated groups (5.331±1.312pg/ml, 6.394±1.591pg/ml). The same was applied in acute cases with only positive IgM (14.942±3.539pg/ml) where serum level was significantly higher than other studied groups (P=0.000), (Figure 2).

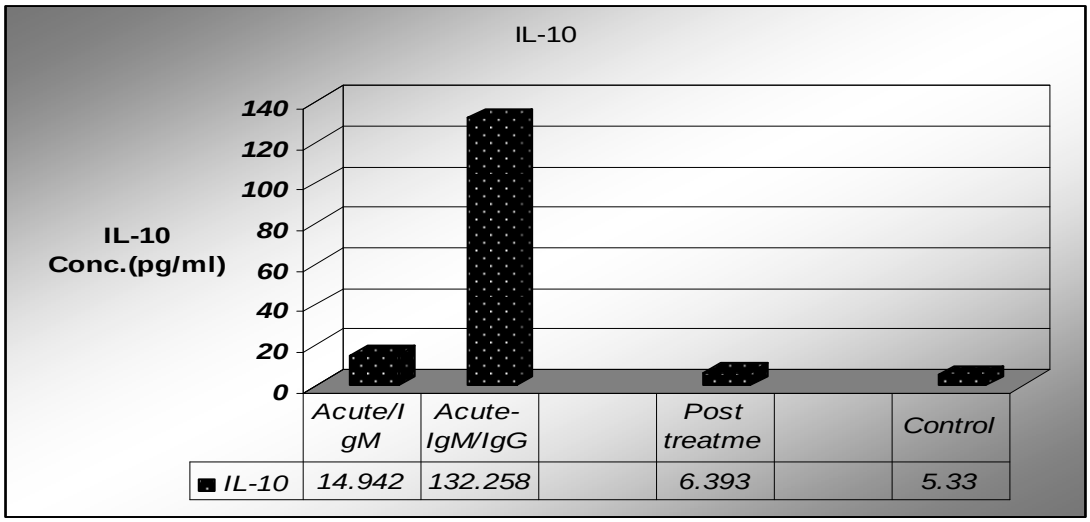

Figure (2): mean of serum IL-10 in different study groups.

Note: *paired t-test between acute (IgM only) group & post treatment group=**7.211; Pvalue=0.000**.
****paired t-test** between acute (IgM/IgG) group & post treatment group=**6.488 P value=0.000**.
*****Independent t-test** between acute group & control group=**4.245 ; Pvalue=0.000**.

DISCUSSION:

The present study showed a highly significant increase in the mean serum level of IL-17 in patients with IgM seropositivity (266.589 pg/ml) when compared with serum levels in control and post treated groups, in case of patients with seropositivity with both IgM and IgG ,the serum levels had no significant difference from levels in control and post treated groups.

The early increase in serum level of IL-17 in the present study match the results of several researchers (12) who found that an early increase in IL-17 had been reported in early stage of infection. Ye *et al*.,(16) found that IL-17 was involved in the development and early recruitment of neutrophils ,which are essential to clear the parasites during initial stages of infection ,in case of low level of this cytokine , Kelly *et al*.,(17) found that the adaptive immunity will not deal correctly with infection ,and animals without IL-17 gene are more susceptible to oral infection.

Interleukin-17 is secreted by T-helper-17cells.Th17cells are a newly identified class of effector T cells that attracting much attention recently ,while it was accepted that Th17 is a T cell lineage distinct from Th1 and Th2 , and its evolution in relation to Th1 and Th2 is under debate (18). In addition to its role in resistance and clearance of many pathogens ,IL-17 expression has been also found to be associated with many autoimmune diseases such as multiple sclerosis and psoriasis (19).

Concerning relation of IL-17 and Il-10, the present finding was compatible with finding of Park et al. 2005(9) who found that IL-10 was able to inhibit accessory cell functions required for optimal T-cell response. This includes the ability to suppress the production of IL-12 p40 required to polarize and expand Th1 cells as well as IL-1β and IL-6 that are now recognized as being important in Th-17 development.

In case of relation of IL-17 and IFNγ, the present study revealed that there was an association between increase serum level of Il-17 and IFNγ whereas there was an inverse relationship between serum level of IL-17 and IL-10,that mean there was a concomitant increase of both IL-17 and IFNγ in the early stage of infection ,while the bulk of increment of serum level of IL-10 was in late stage of acute infection. The present finding was in agreement with finding of Nurieva et al.,and Zheng et al.,(20,21), who found that absence of IL-10 would not only result in enhanced Th1 responses but also lead to increased levels of IL-23 and IL17 activity , that is mean there is an inverse realation between IL-10 and IL17 concentrations, that revealed there is close association between serum concentration of Th1 cytokines profile and serum level IL-17. Absence of IL10 can lead to exacerbation of Th1 cell activity in a number of experimental settings including infection (22). The present study found that there is an increase in IL-17 level especially in the early stage of acute infection , that interpret the synergism between IL-17 and Th1 cytokines in generating an optimal immune response against *T.gondii* infection which has not previously studied or discussed in Iraq ,however the subject of Th17 is new debatable subject and information about its relation to *T.gondii* infection may be liable to change till adequate researches cover the whole aspects of the subject.

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