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Association among both IL-10 and IL-12p70 with LILRB4 between fertile and sterile Hydatid cyst Patients.

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ABSTRACT

Hydatid cyst disease is a chronic and parasitic disease caused by genus of tapeworm called *Echinococcus*. This review will discuss different parameter in the study to differentiate between the immune response of patients based on both fertile and sterile hydatid cyst by measured interleukins IL-10, IL-12p70 and the immune receptor Leukocyte immunoglobulin like receptor(LILRB4) .All of them increase in the sera of post surgery patients compared to pre surgery patients in fertile cyst, while in patients with sterile cyst sera concentration non significantly increased in presurgery patients. IL-12p70 significantly increase in pre- surgery patients compared to the post surgery and significant increase in patients with fertile hydatid cyst more than in sterile cyst. The immune receptor (LILRB4), shows a significant differences of it receptor concentration in serum of patients after surgery compared with the level of concentration before surgery and higher than in the control group with non significant differences in the immune response between two types of fertile and sterile cyst.

Keywords: fertilesterile cyst, Hydatidosis, IL-10 , IL-12p70, LILRb4 receptor.

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INTRODUCTION

Hydatid disease, also known as echinococcosis or hydatidosis, has been known from the time of Hippocrates, who refers to the disease as "liver full of water" for cases of echinococcosis (Ezer et al., 2006). Hydatidosis, a problem of worldwide importance (approximately 2-3 million human cases are thought to occur worldwide) is caused by adult or larval (metacestode) stages of cestodes belonging to the genus *Echinococcus* of the family Taeniidae (Brunetti et al., 2010).

Interleukin 10 (IL-10), known as human cytokine synthesis inhibitory factor (CSIF), is an anti-inflammatory cytokine. In humans, interleukin 10 is encoded by the IL10 gene. It signals through a receptor complex consisting of two IL-10 receptors-1 and two proteins, through cytokine with multiple, pleiotropic, effects in immune regulation and inflammation. It down regulates the expression of Th1 cytokines, MHC class II antigens, and co-stimulatory molecules on macrophages. It also enhances B cell survival, proliferation, and antibody production (Moore et al., 2001). Interleukin 12 (IL-12 is composed of a bundle of four alpha helices, it is a heterodimeric cytokine encoded by two separate genes, IL-12A (p35) and IL-12B (p40). The active heterodimer (referred to as 'p70'), and a homodimer of p40 are formed following protein synthesis (Temblay et al., 2007). Leukocyte immunoglobulin like receptor (LILR), LILRB4 was first identified as an inhibitory receptor expressed on myeloid antigen presenting cells which associates with the protein tyrosine phosphatase SHP-1, LILRB4 and LILRB2 may differ in their mechanisms of immune regulation. LILRB4 ligation was therefore performed to determine the phenotypic effects of receptor engagement on antigen presenting cell phenotype (Vlad et al., 2008). In order to understand the relationship among all previous parameters present study was designed.

MATERIALS AND METHODS

The current study included thirty patients suffering from hydatid cyst disease in different organs, by consultant specialist of the surgery department in Baghdad Teaching Hospital, Al Yarmouk Teaching Hospital, Gastroenterology and liver Hospital, Al Karama Hospital, Al Kindi Teaching Hospital, Al Kadhimiya Teaching Hospital, Al Sadr Hospital and Al Qamah National during the period from the 1st March 2017 to February 2018. These patients were followed before and after surgery to comparison of immune responses between them. Blood samples were collected from healthy group included twenty-eight individuals is a control group. Diagnosis of hydatid cysts is based currently on identification of the parasite's structures by imaging techniques, including serological tests, ultrasound and surgery of each patient. Blood collection, for each participant, (5 ml) venous blood samples withdrawn, placing in a plain test tube and left to stand for 30 min at room temperature for clot formation for serum collection, the tubes were centrifuged at (3000 rpm) for (10 minutes), for serum test by Eliza technique and the ELISA Kit provided is typical, the serum was aspirated using a Pasteur pipette and dispensed into sterile eppendorf tubes and stored at (- 20°C) until used.

Assay Procedure of Interleukin IL-10 and Interleukin IL-12p70

According to the manufacturers instruction (Besancon Cedex, France), No. 950.060.096, of IL-10 and according to the manufacturers instruction (Besancon Cedex, France), No. 950.070.096o of IL-12p70, based on sandwich enzyme-linked immune-sorbent assay technology.

Assay Procedure of LILRB4 receptor

According to the manufacturers instruction (MyBioSource, USA), No. MBS764047, by a solid phase sandwich ELISA.

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to effect of different factors in study parameters. Least significant difference –LSD test (ANOVA) t-Test was used to significant compare between means.

RESULTS AND DISCUSSION

Presented study included Fifty-eight personnel, which divided into two main groups: twenty eight healthy individuals as a control with thirty hydatiosis patients.

Serum Level of cytokines

Interleukin IL-10

Current results show non significant increasing level of IL-10 (Th1) in the serum of patient groups with fertile and sterile hydatid cysts, in pre- surgery (20.49 pg/ml) and (23.51 pg/ml) respectively, in contrast post-surgery patient groups showed non significant high level of IL-10 in both fertile and sterile cysts groups (28.30 pg/ml) and (22.73 pg/ml) respectively, while all patients groups show non significant increasing concentration compared to the control group was (16.40 pg/ml) (table 1).

Table (1): Comparison among study groups in thelevel of IL-10 cytokines

Hydatid Cyst	Concentration of IL-10 (pg/ml) (Mean ± SE)			LSD
	Pre-surgery	Post surgery	Control	
Fertile Cyst	20.49 ± 2.72	28.30 ± 5.41	16.40 ± 1.57	9.421 NS
Sterile Cyst	23.51 ± 3.23	22.73 ± 4.18		7.372 NS
t-test	8.596 NS	14.32 NS	---	---

NS: Non-Significant

Present result goes with previous results done by Bayraktar (2005), Mezioug and Touil-Boukoffa (2009), which showed that increasing level of IL-10 refer into different immune regulatory events and the cytokine response during hydatiosis may be partially associated with a slight inflammation observed in hydatid cysts disease patients. As well like other helminthes infections, echinococcosis induces two very distinct Th1 and Th2 cytokine secretion patterns, also produced IL-10 in large quantities in hydatid disease patients (Zhang et al., 2008). In addition IL-10 considered liver factors , which produced by the liver during liver transplantation and liver disease(Ingelsten et al., 2014).

Human echinococcosis demonstrated that cytokine profile varies with the age of cyst, as it has been shown that Th1 response in early infection and Th2 response in chronic and late infections (Bayraktar et al., 2005).Therefore, the reason for the increase of IL-10 in post surgery may be due to the type of infection may be second or third and not the primary infection of the hydatiosis.

Interleukin IL-12 p70

Increasing level of interleukin (IL-12p70) in pre- surgery patients group (41.98 pg/ml) recorded compared with control (34.68 g/ml), while post surgery patients group (30.89 pg/ml) showed decreasing level of (IL12p70) compared to this control for patients in fertile cysts,therefore , present results showed significant increasing between patients groups and control and between the two types of fertile and sterile cysts . In contrast, pre and post surgery patients groups (31.48 pg/ml and 33.49 pg/ml) respectively, of sterile cysts showed non significant elevation level of (IL-12p70) compared to control group (table 2).

Increasing level of IL-12p70 due to, in hydatid infections, both cell population profiles (Th1 and Th2), remain highly expressing, at least in cysts that survive the immune response, but, yet is not understood why hydatid infection can induce high levels of both Th1 and Th2 cytokines since they usually down-regulate each other (Vuitton , 2015).

Table (2): Comparison among study groups in the level of IL-12p70 cytokines

Hydatid Cysts	Concentration of IL-12p70 (pg/ml) (Mean ± SE)			LSD
	Pre-surgery	Post surgery	Control	
Fertile Cyst	41.98 ± 3.34 a	30.89 ± 1.79b	34.68 ± 3.15ab	9.325 *
Sterile Cyst	31.48 ± 1.29	33.49 ± 1.82		5.266 NS
t-test	7.731 **	5.26 NS	---	---

** (P<0.01) , NS: Non-Significant.
The different letters in the same row means there are significant differences

Since it is cystic echinococcosis induces two very distinct Th1 such as (IL12p70) and Th2 such as (IL-10) cytokine secretion patterns, they were implicated in the sterile and fertile stages of hydatid disease, therefore, the roles of cytokines in host immunity seem to be quite complex and may differ by genera and species of helminth, its size and location within the host (Trinchieri, 2014). This correspond with the study of Amri et al. (2008), Mezioug and Touil-Boukoffa (2009) and Tamarozzi et al. (2010) by increasing the level IL-12p70 in the serum of patients with hydatidiosis. So, IL-12 has an important role in innate defense against the parasite as the increased resistance to hydatid infection (Al-Qaoudet al., 2008).

Serum level of LIRB4

Leukocyte immunoglobulin-like receptor subfamily B member 4 (LIRB4), showed significant increasing level in the serum of hydatid patients between pre- surgery patients group (9.31 ng/ml and 9.12 ng/ml) and post surgery group (11.31ng/ml and 7.91 ng/ml) of fertile and sterile cysts respectively, but compared with control group (9.87 ng/ml) was non significant differences (table 3).

This may be due to LILRB4, although the biological function and clinical significance of these receptors (LIRB4) are not well understood, (Kang et al., 2016). LILRBs considered immune checkpoint factors as well as the receptor is expressed on immune cells where it binds to MHC class I molecules on antigen-presenting cells and transduce a negative signal that inhibits stimulation of an immune response. (Park et al., 2017). So increasing level of it make causes inhibit immune resistance.

Table (3): Comparison among study groups in the level of LIRB4 receptor

Hydatid Cysts	LILRB4 Level (ng/ml) (Mean ± SE)			LSD
	Pre- surgery	Post surgery	Control	
Fertile Cyst	9.31 ± 1.10	11.31± 0.89	9.87 ± 0.84	2.961 NS
Sterile Cyst	9.12 ± 1.28	7.91 ± 1.04		2.749 NS
t-test	3.455 NS	2.802 **	---	---

** (P<0.01), NS: Non-Significant.

Dendritic cells (DCs) that express high levels of LILRB4 of regulatory T cells (Treg), because these cells (DCs) are antigen-presenting cells (also known as accessory cells) of the mammalian immune system, their main function is to process antigen material and present it on the cell surface to the T cells of the immune system as well as they act as messengers between the innate and the adaptive immune systems. (Katrin et al., 2014). But in the liver diseases, including the hydatid cysts , presence of these receptors (LILRB4) on immune cells (DCs) inhibited its main function, which may affects of host immunity in the presence of the parasite.

Correlation between the concentration of immunological parameters and hours number for post surgery patients samples

The immune responses of hydatidiosis patients changed of each immunological parameter according sample time of the post surgery group figure (1).

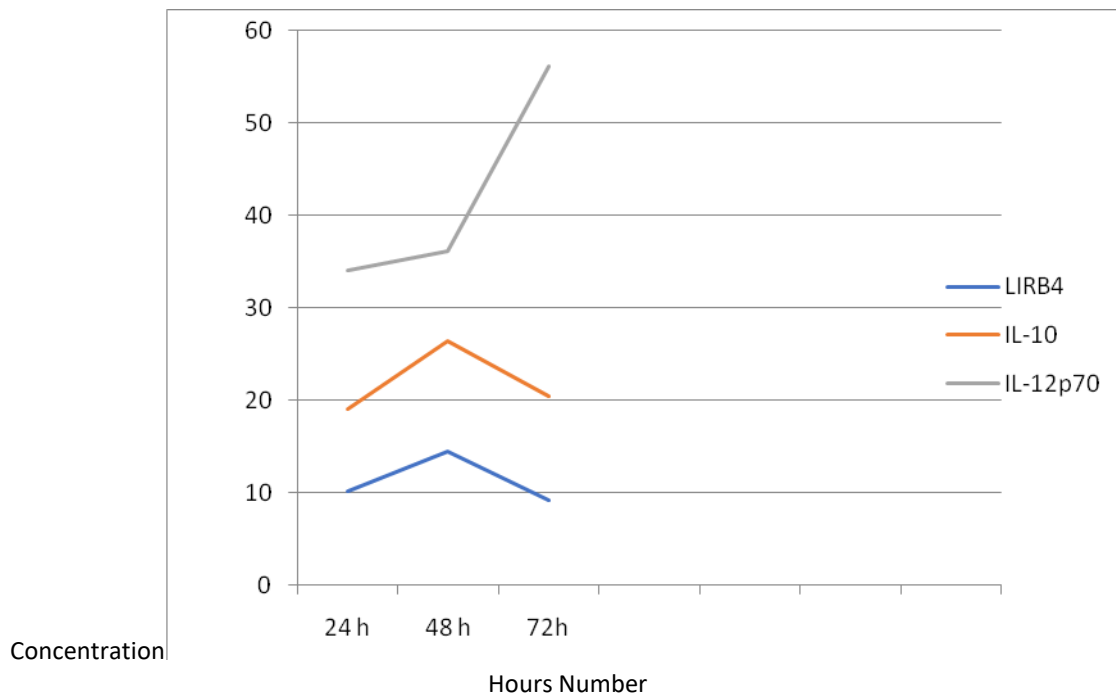


Figure (1): Comparison between immunological parameters of post surgery patient group

Release of IL-10 remained almost balanced despite the fluctuation of its levels, this may be due to the nature of its work, which is the opposite of IL-12, it inhibits IFN- γ and TNF- α in addition to suppress the antigen presentation capacity of antigen presenting cells (Hassuneh et al., 2014). IL-10 decreases initially until 72h as compared with its level of acceptance and then increases after 144h (Nayak et al., 2012) this finding agreed with (Yang et al., 2013), who reported that IL-10 protein was detectable on day 2 and further increased on days 3 and 6 as measured by ELISA. On the other hand, the secretion of cytokines IL-12p70 increased its concentration levels significantly with increase sample time of patients post surgery may be due to its main functions which include production of TNF- α (it does regulation of immune cells) from many immune cells such as activated macrophage and NK cells and regulate activities of NKs and T lymphocyte. In addition IL-12 is useful in treatment of many diseases including hydatidosis. This increasing level may be due to the fact that this interleukin has a very small half-life of 12 hours only, so it is excreted in ascending to return the immune responses to its correct function after removing the hydatid cysts from the host (Cavalcanti et al., 2012).

Levels of inhibitory receptor (LILRB4) level close to concentration of IL-10, which increased slightly after 48 hours and decreased after 72 hours. This may be due to the main function of the receptor which is expressed on immune cells where it binds to MHC class I molecules on antigen-presenting cells and transduces a negative signal that inhibits stimulation of an immune response (Morandi et al., 2016), and reduce these responses after removing the parasite from host, resulting in lower levels of these receptors in the patients' serum post surgery.

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