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Immunological comparison for sHLA-G and some receptors in Iraq patients with sterile and fertile *Echinococcus granulosus*

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Abstract. Hydatidosis, a problem of worldwide importance is caused by larval (metacestode) stages of cestodes belonging to the genus *Echinococcus*. Non-classical human leukocyte antigen (HLA-G) plays an remarkable role in liver disease. This review will discuss sHLA-G and some immunological parameters in the study of Iraq patients with sterile and fertile hydated cyst. A study the immunological parameters include effect of Human Leukocyte Antigen-G which are investigated by ELISA, showed significant differentiation ($P < 0.05$) at the serum level concentration in 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. So, results of KIR2DL4 and ILT4 immunological receptor, which were investigated in patients' blood using the flow cytometry, showed significant increase in concentration from these receptors for patients who suffer fertile and sterile hydatid cysts compared to the control group. Therefore changed immune responses in two type cysts with hydatidosis patients in presence sHLA-G expression.

Keywords. Hydatid cyst disease, sHLA-G molecule, KIR2DL4 receptor, ILT4 receptor.

1. Introduction

Cystic echinococcosis (CE) it is widely endemic helminthic illness caused by infection metacetodes (larval stage) from tapeworm *Echinococcus granulosus*, one of the most important types of parasitic disease, especially in underdeveloped and developing countries, the parasite affects humans and a wide range of livestock species (Taherkhani and Rogan, 2000). *E. granulosus* possess a significant economic and a public health problem in many portions of world, particularly in pastoral areas where dogs and livestock are put forward together, the disease is usually asymptomatic, however, it can clinically manifest as a complicated cyst, the most frequent complication is compression or rupture of pericystic structures (Daali *et al.*, 2001). Human leukocyte antigen (HLA-G), a non-classical HLA class I molecule (Brenol *et al.*, 2012). HLA-G molecule differs from classic HLA class I molecule by its expression, structure, genetic diversity and functions, several pathological cases, HLA-G gene could be expression induced by non-rejected allograft, damage infiltrating antigen presenting cells (APCs) through inflammatory sickness and tumor tissues and their tumor infiltrating antigen presenting cells, anywise, its tolerogenic function can be suitable or prejudicial for the patient (Morandi *et al.*, 2007).



Killer cell immunoglobulin-like receptors (KIRs), KIR2DL4 belong to family of killer cell Ig-like receptor (KIR), believed to contribute in innate immunity to infection and tumors, yet, KIR2DL4 is only MHC receptor whose gene is reduplicate in all natural killer (NK) cells, in disparity with clonal apportionment seen in all other KIR (Young *et al.*, 2001). Immunoglobulin-like transcripts (ILTs), ILT family receptors are made up of active and inhibitory organs, LILRs inhibitory which transmit signals through their long cytoplasmic tails. Best-characterized inhibitory receptors are ILT2 (LILRB1), ILT3 (LILRB4) and ILT4 (LIRB2), ILT4 is expressed fundamentally by macrophages, monocytes and dendritic cells, ILT4 ligands are class I HLA molecules. (Anderson and Allen, 2009).

2. Materials and Methods

The current study included thirty patients suffering from hydatidiosis disease in several organs, by consultants specialist of the surgery department in Baghdad Teaching Hospital, Al Yarmouk Teaching Hospital, Gastroenterology and liver Hospital during the period from March 2017 to February 2018. Data were recorded for each patients by using special form designing for the purpose of the study. The diagnosis of cystic echinococcosis is currently based on the determination of parasite structures by imaging techniques, including serological, ultrasound and surgical procedures for each patient.

3. Blood collection

For each patient, (5 ml) venous blood samples withdrawn, (3 ml) were placed in plain tube with Ethylene Diamine Tetra Acetic Acid (EDTA) for blood tests by flow cytometry technique, which measures simultaneously and decomposes the multiple physical properties of single particles, usually cells, flow as they flow into the liquid stream through the beam of light. While (2 ml) placing in a plain test tube and left to stand for 30 minute at room temperature for clot formation for serum collection, the tubes were centrifuge at (3000 rpm) for (10 minutes), for serum test by ELISA 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 - 20C° until used.

4. Assay Procedure of Soluble Human Leukocyte antigen- G

According to the manufacturers instruction (MyBioSource. USA), No. MBS2600014 of s(HLA-G) based on sandwich enzyme-linked immune-sorbent assay technology.

5. Assay Procedure of immune receptors (KIR2DL4) and (ILT4)

According to the manufacturers instruction (R&D systems bio-technique, USA), No. MBS9316732, of Human Killer Cell Immunoglobulin-Like Receptor 2DL4 (KIR2DL4) and according to the manufacturers instruction (BD Bioscience, British), No. MBS765388, of Human LILRB2 (Leukocyte immunoglobulin-like receptor subfamily B member 2) (ILT4) based on the Flow Cytometry.

6. Statistical analysis

The statistical analysis system- SAS (2016) programme was applied to impact of various operators in study parameters. Least Significant Difference –LSD test (ANOVA) and T-test was used to a great comparison between the means.

7. Results and Discussion

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

8. Serum levels of sHLA-G

Serum levels of sHLA-G showed significant increasing between pre- surgery patient group (0.310 u/ml and 0.304 u/ml) and post surgery group (0.317 u/ml and 0.303 u/ml) of fertile and sterile cysts respectively. So, decreasing level of sHLA-G in control group (0.301 u/ml) with non significant variation. (Table 1). The highest level of sHLA-G in post surgery patients group, may be due to this

increasement occur when taking drugs after the operation of (sHLA-G) to escape from the immune system before surgery. Upregulation of sHLA-G in fertile more than sterile cysts due to be associated for cyst activity (Vuitton et al., 2014). HLA-G expression could be constitute an immune evasion strategy in a host parasite reaction (Amiot et al., 2015).

Yet, HLA-G protein is discovered in liver tissue and in plasma of patients distress from hepatocellular cancer, hepatitis C or B, visceral leishmaniasis, viral infections and at liver disease, i.e. it does not expression through health conditions, thus, in normal liver, HLA-G transcripts could be identified, but there isn't from HLA-G protein type (Nystrom et al., 2014).

Table 1. Comparison between fertile and sterile groups pre and post surgery in level of sHLA-G

Hydatid Cyst	(Mean \pm SE) sHLA-G (u/ml) Level		Control	LSD
	Pre- surgery	post-surgery		
Fertile Cyst	0.310 \pm 0.006	0.317 \pm 0.005		0.041NS
Sterile Cyst	0.304 \pm 0.002	0.303 \pm 0.002	0.301 \pm 0.001	0.036 NS
t-test	0.0158 NS	0.012 *	---	---

* (P<0.05); NS: Non-Significant.

9. Expression of immune receptors

9.1. Killer cell immunoglobulin-like receptor, KIR2DL4 (CD58d)

The relationship between KIR2DL4 receptors and hydatidosis patients represented in a (Table 2). KIR2DL4 showed significant increasing levels in the blood of patients group with fertile and sterile hydatid cysts pre-surgery compared to others groups (23.51 % and 24.47 %) respectively, while post-surgery patients groups of both fertile and sterile cysts showed significant moderate level, compared to control group (11.21 % and 12.80 %) respectively. While the healthy individual (2.63%) with fertile and sterile cyst groups recorded the significant lowest level of present receptor. But, there were no significant differences in blood level of KIR2DL4 between the two types groups of cysts pre and post-surgery.

Table 2. Comparison between fertile and sterile groups pre and post-surgery in KIR2DL4 blood level

Hydatid Cyst	(Mean \pm SE) KIR2DL4 level (%)		Control	LSD
	Pre- surgery	Post-surgery		
Fertile cyst	23.51 \pm 1.93 a	11.21 \pm 1.14 b	2.63 \pm 0.19 c	7.526 **
Sterile cyst	24.47 \pm 1.97 a	12.80 \pm 1.30 b		7.698 **
t-test	5.684 NS	3.52 NS	---	---

** (P<0.01); NS: non-significant.

Different letters in the same row mean there are significant differences.

KIR2DL4, is one of the most important immunological receptors of sHLA-G, which express on the natural killer cells and some CD8+ T cell. When sHLA-G protein is discovered in patients suffering from liver diseases, such as hydatid cysts in our study, this receptors result in weakness of lymphocyte responder function, such as cytotoxic properties or cytokine secretion ability (Ristich et al., 2005).

9.2. Immunoglobulin- Like Transcript 4 Receptor; ILT-4 (CD85d)

ILT4 investigated in the blood of hydatid patients, so that there was significant increasing in the expression of this receptor infertile and sterile pre-surgery patients (35.24 % and 31.62 %) respectively compared to control (3.06 %) and decreased in the patients post-surgery for fertile (17.98 %) and sterile cysts (15.35 %), but remain more than the control. (Table 3).

Table 3. Comparison between fertile and sterile groups pre and post-surgery in ILT4 level

Hydatid cyst	(Mean \pm SE) ILT4 level (%)			LSD
	Pre- surgery	Post-surgery	Control	
Fertile Cyst	35.24 \pm 2.46 a	17.98 \pm 3.10 b		14.055 **
Sterile Cyst	31.62 \pm 1.45 a	15.35 \pm 0.92 b	3.06 \pm 0.32 c	10.241 **
t-test	6.076 NS	7.049 NS	---	---

** (P<0.01); NS: non-significant.

Different letters in the same row means there are significant differences.

The increase in the expression of this receptor may be due to immunoglobulin-like transcript 4 receptors, ILT-4 (CD85d) is known to be distinguished by dendritic cells (DCs), monocyte and macrophage, all of which are existing in the liver. ILT-4/ HLA-G interaction may prevent antigen-presenting mission of these cells, weakening adaptive immunity and permit liver infection to evasion host immunity (Amiot *et al.*, 2015). Thus, the parasite may escape and survival in the body for long periods. Since it is neutrophils as well express ILT4 inhibitory receptor and interaction between that receptor and HLA-G prevent phagocytic function of neutrophils ,therefore this modification of neutrophil activity may be helpful in patients with hydatid disease, preventing neutrophil impairment observed during inflammatory disorder (Baudhuin *et al.*, 2013).

10. Correlation between sHLA-G and immunological receptors

Present study showed (table 4) negative correlation between sHLA-G and KIR2DL4. Since, KIR2DL4 receptors (expressed by natural killer cells) with HLA-G leading to the suppression of the cytolytic NK cell action, this may be HLA-G expression in hydatid cyst cell line plays essential role in conserved these cells against the attack of NK cell, these important connection between HLA-G expression (with assist of its receptors) and NK cell lysis denote that uncommon HLA-G expression may participate to the mechanism of salvation from host immune monitoring to hydatid cysts (Amiot *et al.*, 2015). But non significant correlation of sHLA-G with ILT4, although sHLA-G play a role in immune tolerance and in presence suppression receptors (ILT4) on dendritic cell (DC) is a vigorous way to alter their functions to obtain hypo responsiveness or tolerance recruitment, therefore ILT4 receptor attachment by HLA-G resulted in down-regulation of MHC class II expression and co-stimulatory molecules, then modification of cytokine output on DC which express unrivaled set of co stimulatory molecules which declaration the activation of naive T cells and consequently permit of primary immune response during cytokines they secrete, includ IL-10 and IL-12. (Liang *et al.*, 2003).

Table 4. Correlation coefficient between parameters

Parameter	sHLA-G	KIR2DL4	ILT4
sHLA-G	-	-0.06 NS	0.16 NS
KIR2DL4		-	0.48 **
ILT4			-

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

References

- [1] Amiot L., Nicolas V. And Samson M.(2015). Biology of the immunomodulatory molecule HLA-G in human liver diseases., J. of Hepatology., 62 :1430–1437.
- [2] Anderson K., and Allen R.(2009). Regulation of T-cell immunity by leukocyte immunoglobulin-like receptors: innate immune receptors for self on antigen-presenting cells. Immunol.,127(1): 8–17.
- [3] Baudhuin J., Migraine J., Faivre V., Loumagne L., Lukaszewicz A. and Payen D.(2013).

- Exocytosis acts as a modulator of the ILT4-mediated inhibition of neutrophil functions. *Proc. Natl. Acad. Sci. U S A.*, 110:17957–17962.
- [4] Brenol C., Veit D., Chies A. and Xavier M.(2012). The role of the HLA-G gene and molecule on the clinical expression of rheumatologic diseases ., *Rev. Bras. Reumatol.*, 52(1):82-91.
 - [5] Daali M., Fakir A., Hssaida R., and Had A. (2001). Hydatid cysts of the liver opening in the biliary tract., *Ann Chir.*, 126: 242-245.
 - [6] Liang A. and Horuzsko S. (2003). Mobilizing dendritic cells for tolerance by engagement of immune inhibitory receptors for HLA-G. *Hum. Immunol.*, 64:1025.
 - [7] Morandi F., Levreri I., Bocca P., Galleni B., Raffaghello L. and Ferrone S. (2007). Human neuroblastoma cells trigger an immunosuppressive program in monocytes by stimulating soluble HLA-G release, *Cancer Res.*, 67 : 6433.
 - [8] Nystrom J.(2014). Rapid increase of interleukin-10 plasma levels after combined auxiliary liver-kidney transplantation in presensitized patients. *Transplantation.*, 98:208–215.
 - [9] Ristich V., Liang S., Zhang W., Wu J. and Horuzsko A.(2005). Tolerization of dendritic cells by HLA-G. *Eur. J. Immunol.*, 35 : 1133 – 1142 .
 - [10] SAS. (2016). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
 - [11] Taherkhani H. and Rogan M.(2000). General characterization of laminated layer of *Echinococcus granulosus*., *Iran J. Med. Sci.*, 25:95-104.
 - [12] Vuitton D., Millon L., Gottstein B. and Giraudoux P.(2014). Proceedings of the International Symposium., *Parasite.*, 21:28.
 - [13] Young N., Uhrberg M., Phillips J., Lanier H., and Parham P.(2001). Differential expression of leukocyte receptor complex encoded Ig-like receptors correlates with the transition from effector to memory., *CTL. J. Immunol.*, 166: 3933–3941.