

Evaluation of IL-4 and IL-12 concentration in patient of Hydatid Cyst

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Abstract

Infection with the larval stage of *Echinococcus granulosus*, a 2-7-millimeter-long tapeworm found in puppies (definitive host), sheep, cattle, goats, and pigs, reasons cystic echinococcosis (CE), generally referred to as hydatid ailment (intermediate hosts). Despite the truth that maximum human infections are asymptomatic, CE generates dangerous, slowly growing cysts in the liver, lungs, and different organs. The aim of this observe turned into to peer how useful serum concentrations of two cytokines, Interleukin-four (IL4) and Interleukin-12 (IL12), are for detecting CE. This case/manage studies covered 27 sufferers with hepatic, mind, belly, lung, and between-vertebral for this situation/manage research, different reputedly wholesome volunteers among the a while of 10 and fifty five had been recruited. Anti-echinococcal IgG antibodies and blood concentrations of IL-4 and IL-12 had been determined through Enzyme Linked Immunosorbent Assay for every affected person (ELISA). The diagnostic application of those cytokines become assessed using a Receiver Operating Characteristic (ROC) curve. Anti-echinococcal IgG antibodies had been found in all of the sufferers. There were tremendous adjustments in IL-4 serum concentrations between sufferers and controls, however no corresponding variations in IL-12 serum concentrations. IL-4 has a moderate diagnostic price, in keeping with a discrete ROC analysis. However, the mixed ROC analysis for that cytokine led to a very good discriminative price wherein the area underneath the curve (AUC) changed into 0.976, ninety five% CI= 0.942-1.00, $P < 0.001$. The sensitivity and specificity of the take a look at at 242.5 pg/ml reduce-off fee for IL-4 were ninety two.6% and eighty% respectively, serum concentration of IL-four however not IL-12 may be applied as an extra diagnostic device for definitive prognosis of suspected CE. To similarly refine this diagnostic assay, more studies on other Th2-associated cytokines or chemokines in CE patients is needed.

Keywords: Cystic Echinococcus, Th2 interleukins, Antigen B , receiver operator characteristic.

INTRODUCTION

Cystic echinococcosis (CE), a zoonotic disease, is found all over the world. The larval degree of the cestode parasite *Echinococcus granulosus* is to blame. In temperate locations, such as Iraq, the illness is very common (1). Around two to three million people worldwide are affected with CE illnesses (2). CE was found in a variety of organs, according to a recent study (three). A human is used as an intermediary host by the

parasite that shelters the larval degree. As a result, no developmental stages of the parasite are excreted outside the human body, making prediction more difficult. As a result, clinical prediction is focused on indirect procedures rather than direct parasite identification. Imaging modalities (especially ultrasonography (US)) are considered the most important of them. Serological assays, especially enzyme-connected immunoassay (ELISA), based on *E. Granulosus* hydatid cyst fluid antigen, are also

commonly employed (4). All of the diagnostic techniques have significant limitations, making it difficult to name one as the gold standard for CE diagnosis (5). The antigen B found in hydatid cyst fluid has an effect on parasite survival. This is a highly immunogenic (12 kDa) protein that can cause the immune response to be directed toward the non-defensive Th2 arm (6). As a result, extremely high levels of Th2-related cytokines like IL-4 are reasonable to expect (7). These cytokines may be used to test for disease severity and are a valuable resource in CE analysis. This study used ROC analysis to determine the importance of IL-4, a Th2 cytokine, and IL-12, a Th1 cytokine, in the analysis of CE. (8).

Subjects and Methods

The Study Population

This turned into a case-manipulate research concerning 27 patients with CE in many organs who visited Baghdad Medical City for the duration of the period from February 2020 to October 2021. All patients underwent physical examinations, ultrasonography and MRI. To constitute the manage institution, any other 35 age- and intercourse-matched health donors were recruited. Each situation signed a consent shape that blanketed demographic records along with age, sex, residence, and educational level.

Samples

A easy tube become used to collect 5 milliliters of peripheral blood from each participant. Sera have been separated and maintained at -20 °C. Following the manufacturer's commands, a business ready package (DIESSE, Italy) become used to estimate serum stages of anti-echinococcal IgG antibodies in sufferers and controls using the ELISA technique. When the IgG concentration became greater than 1.2 U/ml, the ELISA became dominated fine.

Cytokine Assays

The concentrations of IL-four and IL-12 in the blood were decided using ELISA commercial kits (Komabiotech, Korea) consistent with the manufacturer's instructions. 31.25-3000 pg/ml and 31.25-3000 pg/ml had been the detection levels for these kits, respectively.

Statistical Analysis

SPSS software program for Windows was used to research all the records (model 19). The median and range of continuous statistics were used, while frequency and percentage had been used for dichotomous variables. The area under the curve (AUC), sensitivity, specificity, and cut-off value for the cytokines had been decided using the ROC curve. The significance threshold changed into mounted at $P < 0.05$.

Results

Study Population Demographics

In any of the demographic factors examined, the hydatid cyst-infected group did not vary significantly from controls (Table 1).

Titer of IgG Antibodies

All CE patients (100%) tested positive for anti-echinococcal IgG, but all controls tested negative, according to the kit manufacturer's technique (0 percent)

Cytokine Concentrations in the Blood

Table 2 shows the blood levels of numerous cytokines in both patients and controls. The median levels of IL-4 in cases (1800 pg/mL) were much greater than in controls (148 pg/mL), with significant differences ($P=0.001$). The median level of IL-12, on the other hand, did not differ significantly across the individuals and controls (124 pg/ml vs 187 pg/ml).

IL-4 levels in patients and control groups

To evaluate the IL-4 cut-off value as well as to predict the H. Cyst as diagnostic tests or adjuvant diagnostic tests, receiver operator characteristic (ROC) curve analysis was carried out and the results are shown in (Table3), and figure (1). The IL-4 cut-off value was > 242 . Five with sensitivity, specificity, superb predictive cost (PPV), bad predictive value (NPV), and Area beneath curve of 92.6%, eighty.0%, 78.1%, ninety three.3% and zero.976 (0.942- 1.00).

IL-12 stages in sufferers and manage organizations

To compare the IL-12 cut-off fee as well as to expect the H.Cyst as diagnostic tests or adjuvant diagnostic tests, receiver operator

function (ROC) curve evaluation become completed and the results are proven in (Table4), and determine (2). The IL-12 reduce-off cost turned into > 207.Zero with sensitivity, specificity, superb predictive fee (PPV), negative predictive fee (NPV), and Area below curve of 70.4%,sixty eight.6%, 63.Three%, 75.Zero% and 0.743 (0.614- 0.842).

Discussion

Diagnosing CE, specifically in its early degrees, is hard because of bad sensitivity or a loss of appropriate facilities. As a end result, new ways to CE prognosis will need to be researched. Despite the reality that serum cytokine concentrations are sensitive to a ramification of inner and outside stimuli, they may be utilized by myself or in association with other scientific and lab parameters to become aware of a huge variety of infectious and non-infectious illnesses. Interferon-gamma (IFN- γ) degrees within the blood, for instance, have lately been used to make extensive progress in the detection of latent tuberculosis (nine,10). CE sufferers had notably better serum levels of IL-4, but not IL-12, than healthful controls in the contemporary take a look at. As a result, this cytokine (IL-four) has the functionality to detect CE. Similar findings have already been obtained in some of investigations. A Th2 profile and high levels of IL-four are not unusual in patients who have lively CE, in line with Rigano et al (eleven). An environment that minimizes proto-scolecis killing will help parasites survive with the aid of decreasing proto-scolecis killing (12). It has been shown that IL-four reciprocity reduces the protective Th1 response and promotes CE survival, that's consistent with present day findings (13). A high attention of IL-4 became determined in non-responders, whereas Th1-related cytokines have been discovered in those who replied to remedy (specifically IFN- γ). IL-12 ranges in CE sufferers, alternatively, have been stated to be non-tremendous or maybe low in multiple investigations. Retrospective evaluation with the aid of Tamarozzi et al (14) located no variations in IL-12 levels between CE-sufferers and controls, however appreciably higher IL-four ranges among CE-patients. In an Algerian look at concerning 51 sufferers and 12 healthy people, Amri et al (12) observed that each IL-12 and IL-8 concentrations had been extensively better in patients than in controls.

There may be variations in cystic stage, survivability, chemical therapy, and ethnicity among the 2 studies that designate the discrepancy in findings. The quantity of 1 cytokine IL-four that become drastically more in CE sufferers than controls had been subjected to ROC evaluation, either independently or in mixture. Based on findings from a single observe, cytokine has identical diagnostic cost (mild discrimination). In the mixed ROC version, the AUC has improved, indicating that the cytokine has a very good diagnostic cost, with a sensitivity and specificity of ninety two.6 and eighty%, at cut-off values of 242.Five pg/mL. There isn't any evidence to signify that this approach have to be utilized for medical CE analysis, but the blood levels of those cytokines and their reduce-off values can be used to beautify different diagnostic assessments for CE.

In order to make the cytokine-dependent assay a reliable approach, extra studies on different Th2-associated cytokines or chemokines in CE sufferers is needed

Table (1): *Distribution of patient groups and control subject according to age*

Characteristic	Control n = 35	H. cyst n=27	P
Age (years)			
Mean \pmSD	30.54 \pm 12.72	36.48 \pm 12.14	0.008† S
Range	06 - 55	10 - 55	

Table (2): *Cytokines level in control and patient groups.*

Cytokines	Comparison		
	Control n = 35	H. cyst n=27	P value
IL-4	148.0 (113.0)	1800.0 (966.9)	< 0.001 HS
IL-12	187.0 (148.0)	124.0 (93.0)	0.025 S

IQR: inter-quartile range; †: Mann Whitney U test; HS: Highly significant at $P \leq 0.001$

Table (3): Sensitivity and specificity of IL-4 level (> 242.5 -fold) in *H. cyst*

IL-4 level (fold)	<i>H. cyst</i> n = 27	Control n = 35
> 242.5	25 (%)	7 (%)
≤ 242.5	2 (%)	28 (%)
Sensitivity %	92.6%	
Specificity %	80.0 %	
PPV %	78.1%	
NPV %	93.3%	
AUC (95% CI)	0.976 (0.942-1.00)	

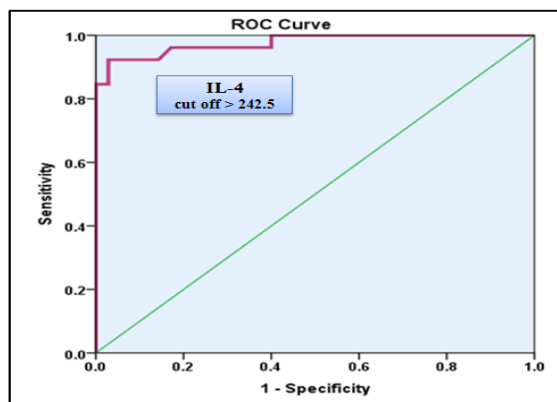


Figure (1): Receiver operator characteristic curve analysis for the calculation of IL-4 possible diagnostic cutoff value.

Table (4): Sensitivity and specificity of IL-12 level (> 207.0 -fold) in *H. cyst*

IL-12 level (fold)	<i>H. cyst</i> n = 27	Control n = 35
> 207.0	19 (%)	11 (%)
≤ 207.0	8 (%)	24 (%)

Sensitivity %	70.4%
Specificity %	68.6 %
PPV %	63.3%
NPV %	75.0%
AUC (95% CI)	0.743 (0.614- 0.842)

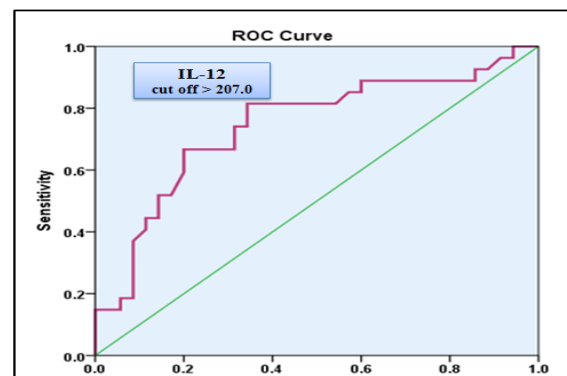


Figure (2): Receiver operator characteristic curve analysis for the calculation of IL-12 possible diagnostic cutoff value

Reference

- [1] Grosso G, Gruttadauria S, Biondi A, Marvenano S, Mistretta A. Worldwide epidemiology of liver hydatidosis including the Mediterranean area. *World J Gastroenterol* 2012; 18(13):1425-1437.
- [2] KHAN, Aisha, et al. Knowledge, attitudes and practices related to cystic echinococcosis endemicity in Pakistan. *Infectious diseases of poverty*, 2018, 7.1: 1-15.
- [3] Eckert J, Deplazes P. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clin Microbiol Rev.* 2004;17(1):107-135. doi:10.1128/CMR.17.1.107-135.2004
- [4] Gessese AT. Review on Epidemiology and Public Health Significance of Hydatidosis. *Vet Med Int.* 2020; 2020:8859116. Published 2020 Dec 3. doi:10.1155/2020/8859116

- [5] Gavidia CM, Gonzalez AE, Zhang W, et al. Diagnosis of cystic echinococcosis, central Peruvian Highlands. *Emerg Infect Dis*. 2008;14(2):260-266. doi:10.3201/eid1402.061101
- [6] Grubor NM, Jovanova-Nesic KD, Shoenfeld Y. Liver cystic echinococcosis and human host immune and autoimmune follow-up: A review. *World J Hepatol*. 2017;9(30):1176-1189. doi:10.4254/wjh.v9.i30.1176
- [7] Seoane, P., Rückerl, D., Casaravilla, C. et al. Particles from the *Echinococcus granulosus* laminated layer inhibit IL-4 and growth factor-driven Akt phosphorylation and proliferative responses in macrophages. *Sci Rep* 6, 39204 (2016).
- [8] Bayraktar MR, Mehmet N, Durmaz R. Th1 and Th2 inducing cytokines in Cystic echinococcosis. *Turkiye Parazitolo Derg*. 2005;29(3):167-70. PMID: 17160815.
- [9] Teklu T, Kwon K, Wondale B, et al. Potential immunological biomarkers for detection of *Mycobacterium tuberculosis* infection in a setting where *M. tuberculosis* is endemic, Ethiopia. *Infect Immun* 2018; 86(4): pii: e00759-17.
- [10] Ilievska-Poposka B, Metodieva M, Zakoska M, Vragoterova C, Trajkov D. Latent tuberculosis infection: diagnosis and treatment. *Macedonian J Med Sci* 2018; 6(4):651-655.
- [11] Rigano R, Buttari B, Profumo E, Ortona E, Delunardo F, Margutti P, et al. *Echinococcus granulosus* antigen B impairs human dendritic cell differentiation and polarizes immature dendritic cell maturation towards a Th2 cell response. *Infect Immun* 2007; 75:1667e78.
- [12] Amri M, Mezioug D, Touil-Boukoffa C. Involvement of IL-10 and IL-4 in evasion strategies of *Echinococcus granulosus* to host immune response. *Eur Cytokine Netw* 2009; 20:63e8.
- [13] Jankovic D, Liu Z, Gause WC. Th1- and Th2- cell commitment during infectious disease: asymmetry in divergent pathways. *Trends Immunol*. 2001; 22:450–457.
- [14] Tamarozzi F, Meroni V, Genco F, et al. Ex vivo assessment of serum cytokines in patients with cystic echinococcosis of the liver. *Parasite Immunol* 2010; 32:696-700