

Effect of Age and Sex On Immunological Activation In Relation To Infection With *Echinococcus granulosus*

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Abstract

Background: Human cystic echinococcosis (CE) was caused by the larval stage of *Echinococcus granulosus* (*E. granulosus*) and characterized by its chronicity. It had been believed that chronicity of CE might be favored by a skewing of the host's immune response to a Th2 response which was affected by age and sex.

Aim of study: to assess some cytokines (IL-2, IL-6, IL-12 and TNF- α) in relation to age and sex as a part of host-parasite relationship.

Materials and Methods: A total of 50 patients suffering from CE and 38 apparently healthy control that consulted Bagdad Teaching Hospital/Medical City from February to July, 2005 were enrolled in this study. Ultrasonography was done to all patients and control while Computed Tomography was done to confirm the Ultrasonography diagnosis. Blood sera were stored in (-20°C) to be used for evaluation of the serum cytokines level of IL-2, TNF- α , IL-6 and IL-12 by enzyme immunoassay (ELISA).

Results: This study showed that in male patients with CE more than 60 years, the IL-2, TNF- α , IL-6 and IL-12 were (1348 $\pm$ 97.33; 11.51 $\pm$ 1.10; 29996 $\pm$ 385.10 and 27.91 $\pm$ 3.8) in comparison to (1804 $\pm$ 111.49; 5.46 $\pm$ 0.84; 20987 $\pm$ 223.54 and 42.6 $\pm$ 3.56) in patients aged (40-60) and (1820 $\pm$ 103.50; 4.89 $\pm$ 1.03; 21228 $\pm$ 209.71 and 40.04 $\pm$ 4.47) in patients aged < 40 years with statistical significance (P value < 0.05). The IL-2, TNF- α and IL-6 were significantly changed (2425 $\pm$ 104.52; 14.2 $\pm$ 2.85; 19973 $\pm$ 266.75) in menopausal patient group in comparison to childbearing age (2103 $\pm$ 354.19; 10.3 $\pm$ 1.20; 23412 $\pm$ 539.4) while they were insignificantly changed (2425 $\pm$ 104.52; 14.2 $\pm$ 2.85; 19973 $\pm$ 266.75) (P value > 0.05) in menopausal female patients in comparison to aged matched male patients (2211 $\pm$ 132.7; 9.92 $\pm$ 1.32; 21039 $\pm$ 315.29) and significantly changed in childbearing female patients (2103 $\pm$ 354.19; 10.3 $\pm$ 1.20; 23412 $\pm$ 539.4) in comparison to aged matched male patients (1832 $\pm$ 355.6; 7.9 $\pm$ 1.06; 20873 $\pm$ 638.21) respectively (P value < 0.05).

Conclusions: young and middle age groups male patients with CE had a more predominant protective Th1 immune response than elderly male patients while the female menopausal women had a more protective Th1 immune response than childbearing women. Female in the childbearing age had a more protective Th1 immune response against CE in comparison to aged matched male group while there was insignificant immune response in menopausal women in relation to aged matched male group. Predominant Th2 immune response in elderly male and childbearing female might favor more parasite growth. Higher estrogen level might augment more growth of CE, and it is advisable to decrease the frequency of pregnancy rate unless surgical treatment of CE performed. Hormonal replacement therapy containing estrogen in menopause should be given with caution for patients with CE as they may favor more parasite growth.

Key words: cystic echinococcosis, hydatid cyst, immunology of hydatid cyst

الخلاصة

الخلفية: داء الحويصلات المائية البشري هو عدوى مزمنة تسببها المرحلة البرقية للحبيبية الشريطية المشوكة. ويعتقد أن الحالة المزمنة قد تفضل انحراف في استجابة المضيف المناعي نحو استجابة Th2 والتي تتأثر بحسب العمر والجنس.

الهدف من الدراسة: تقييم بعض الحركات المناعية (IL-2, IL-6, IL-12 و TNF- α) تبعاً للعمر والجنس كجزء من علاقة المضيف مع الطفيلي.

المواد وطرق العمل: تمت دراستهم مجموع ٥٠ مريضاً يعانون من داء الحويصلات المائية و ٣٨ شخص كمجموعة سيطرة على ما يبدو اصحاء الذين راجعوا مستشفى بغداد التعليمي/المدينة الطبية للفترة من شباط إلى تموز ٢٠٠٥. وقد تم التصوير بالموجات فوق الصوتية لجميع المرضى ومجموعة السيطرة في حين تم الفحص بجهاز المفراس الحزوني لتأكيد التشخيص بالموجات فوق الصوتية. تم تخزين

امصال الدم في (-٢٠ °C) ليتم استخدامها لتقييم مستوى الحركيات المناعية IL-2، α -TNF، IL-6 و IL-12 بواسطة الفحص بانزيم المناعية (ELISA).

النتائج: أظهرت هذه الدراسة أن في المرضى الذكور المصابين بداء الحويصلات المائية الذين تكون أعمارهم أكبر من ٦٠ عاما كانت نتائج IL-2، α -TNF، IL-6 و IL-12 (1348 ± 97.33 ، 11.01 ± 1.10 ، 29996 ± 385.10 و 27.91 ± 3.8) مقارنة مع (180.4 ± 111.49 ، 0.84 ± 0.46 ، 20987 ± 223.54 و 42.6 ± 3.06) في المرضى الذين تتراوح أعمارهم بين (٤٠-٦٠) و (1820 ± 103.50 ، 4.89 ± 1.03 ، 21228 ± 209.71 و 4.47 ± 4.00) في المرضى الذين تتراوح أعمارهم > ٤٠ عام وكانت ذات قيمة احصائية معنوية ($P < 0.05$). و تغيير كل من IL-2، α -TNF و IL-6 بشكل ملحوظ (2425 ± 104.52 ، 14.2 ± 2.85 ، 19973 ± 266.75) في مجموعة مرضى سن اليأس بالمقارنة مع سن الإنجاب (2103 ± 354.19 ، 1.20 ± 1.03 ، 23412 ± 539.4) في حين أنها تغيرت بفارق احصائي غير معنوي (2425 ± 104.52 ، 14.2 ± 2.85 ، 19973 ± 266.75) في المرضى من النساء بعد سن اليأس بالمقارنة مع الذكور من نفس العمر (2211 ± 132.7 ، 9.92 ± 1.32 ، 21039 ± 315.29) وتغيرت بشكل كبير في المرضى بسن الإنجاب (2103 ± 354.19 ، 1.20 ± 1.03 ، 23412 ± 539.4) بالمقارنة مع المرضى الذكور من نفس العمر (20873 ± 638.21 ، 1.06 ± 0.79 ، 355.6 ± 1832) على التوالي مع فرق احصائي معنوي ($P < 0.05$ value).

الاستنتاجات: الفئات العمرية الشابة والمتوسطة من المرضى الذكور المصابين بداء الحويصلات المائية كانت لديهم استجابة مناعية وقائية (Th1) أكثر من المرضى المسنين بينما النساء اللاتي في سن اليأس تكون لديهن الاستجابة المناعية الوقائية (Th1) أكثر من النساء في عمر الإنجاب. وتكون النساء في سن الإنجاب لديها مناعة وقائية أكثر من الذكور من نفس الأعمار ولا يوجد اختلاف بين الاستجابة المناعية بين النساء في سن اليأس بالمقارنة مع الذكور بنفس الأعمار. الاستجابة المناعية (Th2) السائدة في الذكور كبار السن والنساء بعمر الإنجاب ممكن ان تحبذ نمو طفيلي داء الحويصلات المائية. مستوى هرمون الاستروجين العالي قد يزيد نمو داء الحويصلات المائية أكثر ، ويستحسن أن يتم تخفيض وتيرة معدل الحمل ما لم يتم العلاج الجراحي لداء الحويصلات المائية. وينبغي تعاطي العلاج الهرموني البديل الحاوي على الاستروجين في سن اليأس بحذر للمرضى الذين يعانون من داء الحويصلات المائية لأنه قد يفضل مزيد من نمو الطفيلي.

الكلمات المفتاحية: مناعة الاكياس المائية، الاكياس المائية ، مرض الاكياس المائية.

Introduction

Human CE was caused by the larval stage of *E. granulosus* and was a chronic infection and led to significant public health problem in many world regions (Brunetti, *et al.*, 2010). In the face of its wide-ranging distribution and the substantial economical and sanitary affliction that carried out on the healthcare systems, financial support allotted to this disease was restricted (Budke, *et al.*, 2006; Budke, *et al.*, 2009). It had been proposed that chronicity of CE might be preferred by a skewing of immune response of the host towards a Th2 response and as it was affected by age which become of increasing importance, because the cohort of older adults constitutes the fastest growing population in the world for the first time in history. It had been predicted that by the year 2025, the population older than 65 years will be increasing 3.5 times as rapidly as the total population (Oeppen and Vaupel, 2002). The proportion of the population above 60 years has increased from 8% in 1950 to 10% in 2000 and is expected to reach 21% by 2050 (McCusker, *et al.*, 1998). Decline in immune function was a hallmark of aging. Indeed, older people presented with increasing rates and severity of bacterial and viral infections, cancer and reduced vaccine responses (Ongradi, and Kovesdi, 2010) with increasing risk of adverse outcomes after hospitalization, such as a loss of function and independence, increased length of hospital stay, institutionalization and even death (Anpalahan, and Gibson, 2008; Ongradi, and Kovesdi, 2010). Aging was associated with a decline in the function of the immune system (immunesenescence) is a well-described phenomena (Gruver, *et al.*, 2007; Weiskopf, *et al.*, 2009) characterized by dramatic changes on the

cellular and systemic level that affect the innate as well as the adaptive immune system. The innate immune system, represented e.g. by natural killer (NK) cells, macrophages and dendritic cells (DCs), shows an age-associated impaired function to trigger T-cell responses (Villanueva, et al., 1990). The output of naive T cells, part of the adaptive immunity, declines with age as a result of the thymic involution (Kovaiou, *et al.*, 2007). However, elderly people have significantly higher number of T cells that are specific for persistent viruses (Akbar, *et al.*, 2004). This so called immunosenescence was further characterized by an inverted CD4+/CD8+ T cell ratio, a shift from Th1 to Th2 cell induction following T cell activation that influences the balance between humoral and cell mediated immune responses. This altered cytokine profile is probably the result of an increased ratio of memory to naive T cells (Stacy, 2002; Hsu, and Mountz, 2003). In addition to that sex hormones critically affects immune functions and activity which were affected by age and sex (Whitacre, 2001; Salem, 2004; Ackerman, 2006) and led to modulation of host responses by which parasites would ensure successful establishment and persistence. Host counteraction against this modulation may be required for the host to develop resistance to infection (Riganò *et al.*, 2007). Therefore, studying of the age-dependent molecular and cellular mechanisms are of rising interest. For this purpose, this study focus on the immunological assessment of some cytokines (IL-2, IL-6, IL-12 and TNF- α) in relation to age and sex as a part of host-parasite biology.

Materials and Methods

Patients and control: A total of 50 patients suffering from CE and 38 apparently healthy control that had consults Bagdad Teaching Hospital/Medical City from February to July 2005 were enrolled in this study. From each patient, a full history, general information, clinical assessment and the findings of the Ultrasonography (US) and Computer Tomography (CT) scan reports were taken. Ultrasonography was done to all patients and control while Computed Tomography was done to confirm the ultrasonography diagnosis. The US and CT scan reports provide a diagnosis of CE disease and the surgery provide the clinical confirmation. Out of 50 patients with CE, 19 out of 50 (38 %) patients were males (6 patients were less than 40 years, 8 patients were 40-60 years and 5 patients were more than 60 years) and 31 out of 50 (62%) were females (18 patients were childbearing age and 13 patients were in menopausal age). Twenty cases out of fifty (40%) were inpatients from the surgical wards that were surgically confirmed as CE disease cases later on. Fifteen volunteers were males and twenty three were females.

Blood sample

Blood was drawn from the patients with CE using a 5 ml disposable syringe from all cases then these samples were putted in a disposable centrifuged tube and lifted for half hour to clot. After that the serum was separated by centrifugation and putted in a small screw capped tubes and each was labeled with serial number. Sera were stored in (-20 °C) to be used for evaluation of the serum cytokines level of IL-2 (Biomaghreb), TNF- α (DRG company, USA), IL-6 (Biomegrab) and IL-12 (Biosource, Europe) by enzyme immunoassay (ELISA).

Statistical analysis

All data are presented as means and the deviation are presented as standard deviation, and to test the significance in means of different quantitative data, Independent-Sample t-

test of significance was applied. Statistical analysis was done by SPSS 14.0 for Windows 98. P values below 0.05 were accepted as statistically significant.

Results

IL-2	Male patients with CE (< 60 years)		Male patients with CE (> 60 years)	Control
	< 40 years	40-60 years		
mean	1820	1804	1348	1786
SD	103.50	111.49	97.33	122.26
P value	< 0.05			

Table (1): mean IL-2 levels in male patients with CE in relation to age group

TNF- α	Male patients with CE (< 60 years)		Male patients with CE (> 60 years)	Control < 40 years
	< 40 years	40-60 years		
mean	4.89	5.46	11.51	5.12
SD	1.03	0.84	1.10	0.93
P value	< 0.05			

Table (2): mean TNF- α levels in male patients with CE in relation to age group

IL-6	Male patients with CE (< 60 years)		Male patients with CE (> 60 years)	Control < 40 years
	< 40 years	40-60 years		
mean	21228	20987	29996	21487
SD	209.71	223.54	385.10	234.90
P value	< 0.05			

Table (3): The level of IL-6 (pg/ml) in male patients in different age group

IL-12	Male patients with CE (< 60 years)	Male patients with CE (> 60 years)	Control
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Table (4): mean IL-12 levels in male patients with CE in relation to age group

	< 40 year	40-60 years		
mean	40.04	42.6	27.91	38.2
SD	4.47	3.56	3.8	3.8
P value	< 0.05			

Table (5): Mean IL-2, TNF- α and IL-6 between female patients with CE

	Childbearing age (< 45 year)with CE	Menopausal age (>45year) with CE	P value
IL-2	2103 $\pm$ 354.19	2425 $\pm$ 104.52	< 0.05
TNF- α	10.3 $\pm$ 1.20	14.2 $\pm$ 2.85	< 0.05
IL-6	23412 $\pm$ 539.4	19973 $\pm$ 266.75	< 0.05

Table (6): mean IL-2, TNF- α and IL-6 among male and female patients(< 45 years old) with CE and control

	Female		Male*		P value
	Childbearing age (< 45 year) with HC	Control	< 45 years with HC	Control	
IL-2	2103 $\pm$ 354.19	1812 $\pm$ 231.4	1832 $\pm$ 355.6	1754 $\pm$ 125.3	< 0.05
TNF- α	10.3 $\pm$ 1.20	5.11 $\pm$ 0.92	7.9 $\pm$ 1.06	4.99 $\pm$ 0.87	< 0.05
IL-6	23412 $\pm$ 539.4	18298 $\pm$ 467.3	20873 $\pm$ 638.21	18795 $\pm$ 467.93	< 0.05

Table (7): mean IL-2, TNF- α and IL-6 between male and female patients(> 45 years old) with CE

	Female (menopausal age)(>45 year)	Male* > 45 years	P value
IL-2	2425 $\pm$ 104.52	2211 $\pm$ 132.7	> 0.05
TNF- α	14.2 $\pm$ 2.85	9.92 $\pm$ 1.32	> 0.05
IL-6	19973 $\pm$ 266.75	21039 $\pm$ 315.29	> 0.05

* male age groups were divided (< 45 years and > 45 years) for statistic purpose in regard to age matching with female

Discussion

Table (1) showed that the IL-2 was significantly reduced in those male patients aged more than 60 years in comparison to the age groups less than 60 years. This might be due to normally age associated strong decrease in IL-2 due to the effect of Dehydroepiandrosterone (DHEA) with its precursor, dehydroepiandrosterone sulfate (DHEA-S) (Ferrari, *et al.*, 2008) and to decreased expression of CD28 which was a costimulatory molecule critical for T cell activation (Posnett, *et al.*, 1994). In younger age group, DHEA modulates immune function by enhancing more IL-2 release following

immune stimulation (Goncharova, and Lapin, 2000; Hazeldine, *et al.*, 2010; Schmitz, *et al.*, 2010). Elderly individuals have decreased cell proliferative responses to mitogens in vitro and less IL-2 secretion from antigen stimulated T cells compared with young people (Gillis, *et al.*, 1981; Nagel, *et al.*, 1989; Miller, 1999), so as there was progression in the age to older age, so IL2 decreased and as it was major Th1 cytokine, so this reflected decreased Th1 activity that might favor parasite growth.

Table (2) showed that TNF- α was significantly increased in those male patients more than > 60 years old in comparison to those < 60 years. As the transforming growth factor-beta 1(TGF- β) was shown to be decreases in those more than 60 years old, and it was is another immune modulator that acts in a time, dose, , and cell type-dependent manner to inhibit TNF- α (Turner, *et al.*, 1990; van der Kraan, *et al.*, 2010), so the increase in TNF- α might be due to lack of inhibitory effect of TGF- β . Also DHEA modulates immune function by decreasing TNF- α following immune stimulation and as DHEA decrease in elderly age (Ferrari, *et al.*, 2009), so TNF- α would increase in elderly age due to less concentration of inhibitory DHEA (Goncharova, and Lapin, 2000; Hazeldine, *et al.*, 2010; Schmitz, *et al.*, 2010). As the TNF- α is acute phase reactant and was thought to be produced primarily by macrophages, in addition to a broad variety of cell types including lymphoid cells, mast cells, endothelial cells, cardiac myocytes, adipose tissue, fibroblasts, and neurons(Olszewski, *et al.* , 2007), so its isolated increase in elderly would not reflect Th1 activity and might be increased due to other causes .

Table (3): showed that IL-6 was significantly increased in those male patients more than 60 years in comparison to other age groups and control (P value < 0.05). This might be due to increase of TNF- α in dependent and accumulative manner(van der Kraan, *et al.*, 2010) due to HC effect (Rigano, *et al.*, 1996) in part and to aging process in the other part (Pedersen, 2003; Deeks, 2010). The major role of this cytokine was to induce differentiation of B cells into plasma cells, thus contributing to the development of antigen-specific humoral responses (Van Snick, 1990). It had been reported that hydatid cyst fluid (HF) evade immune mechanism by its ability for IL-6 mimicry (MacIntyre, 2000). In addition to that the increase in IL-6 and TNF- α might had other systemic effect on human as increased levels of IL-6 and TNF- α and their receptors are significant independent risk factors for mortality and morbidity in the aged population (Pedersen, *et al.*, 2003) which were further augmented in the existence of CE.

Table(4): showed that the IL-12 significantly reduced in those male patients aged more than 60 years than those less than 60 years. This might be partly due to the ability of HF to impair secretion of IL-12 which was a fundamental cytokine requisite for the encouragement of Th1 responses. It had been shown previously, if the cells cultured in the existence of HF, there would be a prominently impaired capability to secrete IL-12 in response to lipopolysaccharide stimulation (Emery, *et al.*, 1998; Amiot, *et al.*, 1999). In addition to that of immunesenescence would lead to change in phenotypic composition of T cells. Studies demonstrate a decline in naïve T cells along with an accumulation of late differentiated T cell types in aging, particularly in CD8+ T cells (Koch, *et al.*, 2008). However, studies with a closely related parasite, *Echinococcus multilocularis*, have shown that IL-12 and TNF- α and/or lymphotoxin- α were vital to inhibit larval growth representing that a Th1 response was advantageous to the host. In addition to induction of fast discharge of these inflammatory mediators, HF had a long-term effects on DC differentiation which was markedly affected in elderly(Koch, *et al.*, 2008). So it can be concluded that there was a more predominate Th1 immune response with a more protective action in young and

middle age groups in comparison to elderly age group. In addition to that, increase in TNF- α alone without increase in IL-12 and IL-2 was of no benefit in limitation of parasite growth, and TNF- α increase might be due to other causes that increase it in elderly patients.

Table (5): showed that menopausal women encounter significant increase in IL-2 and TNF- α more than childbearing age while the IL-6 increase significantly in those of childbearing age more than those in the menopausal period (P value < 0.05) and there was significant change of these cytokine between female in the childbearing age in comparison with aged matched male group (table 6) (P value < 0.05). This might be due to the effect of sex hormones which critically affect immune functions. Estrogen was suggested to foster immunological processes driven by CD4⁺ Th2 cells and B cells and androgens to foster Th1 CD4⁺ and CD8⁺ cell activity (Whitacre, 2001; Salem, 2004; Ackerman, 2006). Despite that there was significant reduction in IL-2 and TNF- α level and significant increase in IL-6 of childbearing age female in comparison to menopause but the mean level of IL-2 and TNF- α were more in female of childbearing age than aged matched male group (table 6). This reflect that despite the effect of estrogen in the development of Th2 immune response, which generally down regulate Th1 immune response, but, astonishingly, the Th1 immune response is also stronger in women than men, hence affording them a better protection against HC than men (Wilkinson, and Shaw, 1999; Zandman-Goddard, *et al.*, 2007). This might be due to the effect of DHEA which is higher in male than female (Webb, *et al.*, 2006). Powell *et al.*, (Powell, and Sonnenfeld, 2006) had shown that DHEA increases Th2 and decreases Th1 cytokines production. DHEA induces the production of mature dendritic cells with a possible Th1-skewing potential (Canning, 2000), therefore, in humans, DHEA, represents a pivotal up-regulator of interleukin-2 production and hence Th1 immune response.

It was concluded that due to the decrease of physiologic reproductive period levels of estrogen after menopause, Th1 cytokines are augmented in postmenopausal women giving more protective effect against HC than the childbearing period. It was likely that these hormones adjust immune responses by altering patterns of gene expression which was mediated by the binding of the receptor/hormone complex to a specific DNA sequence (Kanda, and Tamaki, 1999; Goldsby, *et al.*, 2003). Childbearing female had increase the physiologic levels of estrogen produced during menstrual cycle with a shift of female immune system towards a Th2 response. Furthermore, high levels of estrogen in pregnancy induce a Th2 dominant immune response which allows tolerance to fetal antigens and successful continuation of pregnancy (Faas, *et al.*, 2000), so higher estrogen level might augment more hydatid cyst growth in this age group, and it is advisable to decrease the frequency of pregnancy rate unless surgical treatment of CE performed.

Table (7): showed that there was insignificant change in IL2, IL6 and TNF- α between menopausal patients and aged matched male patients. As in menopausal women, hormonal replacement therapy might be used, so this might prevent the immune enhancement and improves the aberration of Th1/Th2 balance that was implicated in menopausal period and may partly reverse the pathologic condition (Kamada, *et al.*, 2001), so hormonal replacement therapy containing estrogen in menopausal female should be given with caution if the CE was untreated as they may favor more parasite growth.

Conclusions

It can be concluded that young and middle age groups male patients with CE had a more predominant protective Th1 immune response than elderly male patients while the female menopausal women had a more protective Th1 immune response than childbearing women. Female in the childbearing age had a more protective Th1 immune response against CE in comparison to aged matched male group while there was insignificant immune response in menopausal women in relation to aged matched male group. Predominant Th2 immune response in elderly male and childbearing female might favor more parasite growth. Higher estrogen level might augment more growth of CE, and it is advisable to decrease the frequency of pregnancy rate unless surgical treatment of CE performed. Hormonal replacement therapy containing estrogen in menopause should be given with caution for patients with CE as they may favor more parasite growth.

Recommendations

1. Further studies with larger sample size were recommended
2. Further studies that include the whole cytokines of both Th1 and Th2 in the serum or their protein and /or gene expression are recommended to confirm Th1 and Th2 cytokine profile and anticipate their activity.

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