Indirect Hemagglutination as an Immunodiagnostic Technique for Cystic Echinococcosis in Iraqi Patients

Iraklı Hastalarda Kistik Ekinokokkozisin İmmunodiag nostik Teknikle Tanısı için İndirekt Hemaglutinasyonun Kullanılması

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ABSTRACT

Objective: The aim is to determine the effectiveness of indirect Haemagglutination (IHA) as diagnostic techniques for Cystic Echinococcosis in Iraqi and the possible correlation of age and gender in disease occurrence.

Material and Methods: IHA technique using sensitized sheep red blood cells coated with an Echinococcus granulosus antigen. A chi square, Pearson correlation used for analysis of categorical variables.

Results: Patients presented with clinical manifestation of hydatidosis (202) selectively enrolled in this study. The mean age was 31.64±8.98 years. The minimum age was 18 years and the maximum age was 70 years. Males represent 50%, with the mean age of 33.50±7.8 years. The rest of patients were females with a mean age of 29.78±9.6 years. Positive cases represent 19.3%, and the 80.7% were negative. The number of positive cases was higher among females (22.8%) compared with males (15.8%) with no significant difference (p= 0.212) or correlation between gender and IHA results (p = 0.214). The majority of patients had a the rage of 24-30 years, 31-36 years and 18-23 years. Among males, a higher number of positive cases at 31-36 years followed by 24-30 years and 49-54 years. Among females, a higher number of positive cases with 24-30 years of age was followed by those with 37-42 years of age and 18-23 years of age. There was a significant difference (p= 0.001) between age groups according to gender without a significant correlation between the age group and IHA results according to gender (p= 0.268).

Conclusion: The IHA is considered as rapid, reliable and adequate technique that makes it possible to process a large number of samples simultaneously. It does not require highly trained technical personnel. Females appear to be more susceptible to CE than males especially in younger age groups.

Key Words: Indirect haemagglutination, hydatidosis, age, gender

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ÖZET

Amaç: Irak’ta kistik ekinokokkus tanısı için indirekt hemaglutinasyonun (IHA) etkinliğinin değerlendirilmesi ve hastalığın ortaya çıkmasında yaş ve cinsiyetin ilişkisini araştırılmıştır.

Gereç ve Yöntem: IHA tekniği kistik ekinokokkus antijeni ile kaplanmış sensitize koyun eritositleri ile uygulanmıştır. Ki kare ve Pearson korelasyon testi analiz-lerde kullanılmıştır

Bulgular: Bu çalışmaya kistik ekinokokkus kliniği olan toplam 202 hasta dahil edilmiştir. Ortalama yaş 31.64±8.98 yıldır. En düşük yaş 18 ve en ileri yaş ise 70 yaştır. Erkekler, hastaların %50’sini oluşturmaktakı birlikte ortalama yaş 33.50±7.8 yıldır. Kadınlarda ise ortalama yaş 29.78±9.6 yıl olarak saaptanmıştır. Pozitif olgular %19.3 iken negatif olanlar %80.7 idi. Pozitif olgular kadınlar arasında daha yüksek orandayken (%22.8) erkeklerde ise %15.8 olarak bulunmuş ve aralarında anlamlı bir ilişki bulunmamıştır. Ayrıca, IHA sonuçları ve cinsiyet arasında da korelasyon yoktur (p = 0.214). Hastaların %50’sini oluşturan birlikte ortalama yaş 33.50±7.8 yıldır. Kadınlarda ise ortalama yaş 29.78±9.6 yıl olarak saaptanmıştır. Pozitif olgular kadınlar arasında daha yüksek orandayken (%22.8) erkeklerde ise %15.8 olarak bulunmuş ve aralarında anlamlı bir ilişki bulunmamıştır. Ayrıca, IHA sonuçları ve cinsiyet arasında da korelasyon yoktur (p = 0.214). Hastaların %50’sini oluşturan birlikte ortalama yaş 33.50±7.8 yıldır. Kadınlarda ise ortalama yaş 29.78±9.6 yıl olarak saaptanmıştır. Pozitif olgular kadınlar arasında daha yüksek orandayken (%22.8) erkeklerde ise %15.8 olarak bulunmuş ve aralarında anlamlı bir ilişki bulunmamıştır. Ayrıca, IHA sonuçları ve cinsiyet arasında da korelasyon yoktur (p = 0.214). Hastaların %50’sini oluşturan birlikte ortalama yaş 33.50±7.8 yıldır. Kadınlarda ise ortalama yaş 29.78±9.6 yıl olarak saaptanmıştır. Pozitif olgular kadınlar arasında daha yüksek orandayken (%22.8) erkeklerde ise %15.8 olarak bulunmış ve aralarında anlamlı bir ilişki bulunmamıştır. Ayrıca, IHA sonuçları ve cinsiyet arasında da korelasyon yoktur (p = 0.214). Hastaların %50’sini oluşturan birlikte ortalama yaş 33.50±7.8 yıldır. Kadınlarda ise ortalama yaş 29.78±9.6 yıl olarak saaptanmıştır. Pozitif olgular kadınlar arasında daha yüksek orandayken (%22.8) erkeklerde ise %15.8 olarak bulunmuş ve aralarında anlamlı bir ilişki bulunmamıştır. Ayrıca, IHA sonuçları ve cinsiyet arasında da korelasyon yoktur (p = 0.214). Hastaların %50’sini oluşturan birlikte ortalama yaş 33.50±7.8 yıldır. Kadınlarda ise ortalama yaş 29.78±9.6 yıl olarak saaptanmıştır. Pozitif olgular kadınlar arasında daha yüksek orandayken (%22.8) erkeklerde ise %15.8 olarak bulunmuş ve aralarında anlamlı bir ilişki bulunmamıştır. Ayrıca, IHA sonuçları ve cinsiyet arasında da korelasyon yoktur (p = 0.214). Hastaların %50’sini oluşturan birlikte ortalama yaş 33.50±7.8 yıldır. Kadınlarda ise ortalama yaş 29.78±9.6 yıl olarak saaptanGITM. Pozitif olgular kadınlar arasında daha yüksek orandayken (%22.8) erkeklerde ise %15.8 olarak bulunmuş ve aralarında anlamlı bir ilişki bulunmamıştır. Ayrıca, IHA sonuçları ve cinsiyet arasında da korelasyon yoktur (p = 0.214). Hastaların %50’sini oluşturan birlikte ortalama yaş 33.50±7.8 yıldır. Kadınlarda ise ortalama yaş 29.78±9.6 yıl olarak saaptanmıştır. Pozitif olgular kadınlar arasında daha yüksek orandayken (%22.8) erkeklerde ise %15.8 olarak bulunmuş ve aralarında anlamlı bir ilişki bulunmamıştır. Ayrıca, IHA sonuçları ve cinsiyet arasında da korelasyon yoktur (p = 0.214). Hastaların %50’sini oluşturan birlikte ortalama yaş 33.50±7.8 yıldır. Kadınlarda ise ortalama yaş 29.78±9.6 yıl olarak saaptanיתGITM. Pozitif olgular kadınlar arasında daha yüksek orandayken (%22.8) erkeklerde ise %15.8 olarak bulunmuş ve aralarında anlamlı bir ilişki bulunmamıştır. Ayrıca, IHA sonuçları ve cinsiyet arasında da korelasyon yokt

Anahtar Sözcükler: indirect hemagglutination, hidatidozis, yaş, cinsiyet

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INTRODUCTION

Cystic Echinococcosis, a zoonotic disease occurs throughout the world and causes economical losses and public health problems in many countries. Domestic intermediate hosts (sheep, goats, and cattle) are major reservoirs for the disease in humans. Infection of humans occurs during the natural transmission of the parasite between the definitive hosts and domestic livestock intermediate host [1].

Diagnosis of Cystic Echinococcosis is based currently on the identification of the parasite's structures by imaging techniques, including ultrasound, computed tomography and magnetic resonance imaging (2). However, imaging techniques are relatively complex, do not always offer a good prospect for early diagnosis, and produce data that are sometimes difficult to interpret, being often confused with those from abscesses and neoplasms. In addition, imaging technology is not always available in developing countries (1). Clinical symptoms do not appear until the larva of the parasite has reached a certain size, which normally requires many years after the primary infection. Therefore, the clinical diagnosis is based only on assumption, and needs specific tests. For these reasons, serological methods are important not only for the confirmation of CE case, but also to differentiate hydatidosis from other cystic lesions and tumors, as well as for epidemiological studies in endemic areas. Serological techniques are very useful for the follow-up of patients after surgical or pharmacological treatment(1). Serological tests for diagnosing hydatid infections in people living in areas where the disease is endemic are useful because of the low cost and ease of performance. Meanwhile, radiological techniques are often too expensive or are not available in many areas where hydatidosis is highly endemic (3). The presence of raised specific antibody titers in patients with cystic hydatid disease has been assayed by various techniques, such as indirect haemagglutination(IHA) or latex agglutination, immunoelectrophoresis, complement fixation, immunoenzymatic, and indirect fluorescent antibody tests (4). In many countries including Iraq hydatidosis is important both to human and animal health, because of the morbidity and the occasional mortality cases from the infection of humans with this disease.

The aim of this study is to determine the effectiveness of indirect haemagglutination as immunodiagnostic technique for hydatid cyst in Iraqi patients and the possible correlation of age and gender in disease occurrence.

MATERIALS AND METHODS

Selection of patients

Two hundred and two patients who presented with clinical manifestation of hydatidosis and attended to private and public outpatient clinics in Baghdad during a period from June 2013 to May 2015 were selectively enrolled in this study. All cases proved to have a cystic growth in abdominal cavity via ultrasounds. This study was conducted according to the principles of Helsinki declaration. Dully-filled consent form obtained from all patients participating in the study. Approval of ethical review Committee (Issue No. 1/232-15, Januaryr2012) of College of medicine –Diwali University-Iraq taken prior to initiation of the work.

Methods

Five milliliters venous blood taken aseptically from (202) patients presented with clinical manifestation of hydatidosis. The samples centrifuged at 2000 x g at room temperature for five min to separate sera. Separated sera were stored at -20°C until used for indirect haemagglutination test (IHA) techniques. Indirect haemagglutination test (IHA) techniques from Fumouze Diagnostics-France (5). Principle of the test based on sensitized sheep red blood cells coated with an Echinococcus granulosus antigen. Serum antibodies against Echinococcus granulosus are revealed by an agglutination of sensitized sheep red blood cells. A reddish brown film can be observed in the well. In the absence of specific antibodies, these red blood cells deposit forming a ring in the well bottom. The unsensitized red blood cells ensuring the reaction specificity and allow the elimination of interferences due to the reaction is performed in U-microplate. The test procedure is easy and rapid the results are obtained in two hours.

RESULTS

Table 1 shows the mean age of 202 patients with a clinical manifestation of hydatidosis to be 31.64±8.98 years. One hundred and one patients 50% were males, with the mean age of 33.50±7.8 years. The mean age of females was 29.78±9.6 years. As shown in table 2, 39 (19.3%) of cases were positive. Number of positive cases was higher among females (22.8%) as compared with males (15.8%) without a significant difference (p= 0.212) or correlation between gender and IHA results (p= 0.214) as shown in table 3.

As shown in table 4, the majority of patients were in the age group of 24-30 years, and then in that of 31-36 years and finally in that of 18-23 years. The higher number of positive cases among males in the 31-36 year group was followed by the 24-30 year group and the 49-54 year group. Among females, a higher number of positive cases in the 24-30 year group was followed by 37-42 year group and the 18-23 year group. There was a significant difference (p= 0.002) between males by age without a significant correlation between males' age group and their IHA results (p= 0.302). There was a significant difference (p= 0.001) between females according to their age group without a significant correlation between the female age groups and their IHA results (p= 0.340). There was a significant difference (p= 0.001) between both genders by age without a significant correlation between the gender and IHA results (p= 0.268) as shown in table 4.

Table 1. Description of patients with clinical manifestations of hydatidosis.

| Parameter | Minimum | Maximum |
|-----------|---------|---------|
| Age (years) | 18 | 70 |
| Gender | Male | Female |
| Age according to gender | Mean ± SD | Male | Female |
| Minimum | 20 | 18 |
| Maximum | 56 | 70 |
| Mean ± SD | 33.50±7.86 | 29.78±9.6 |

Table 2. Results of IHA test in patients presented with cystic hydatidosis.

| IHA test | No.[%] |
|----------|--------|
| Positive cases | 39(19.3%) |
| Negative cases | 163(80.7%) |
| Total | 202(100%) |

Table 3. IHA test according to gender of patients presented with cystic hydatidosis.

| Gender | IHA test | X2 | Pearson's correlation value | p |
|--------|----------|----|-----------------------------|---|
|         | Positive No.[%] | Negative No.[%] | value | p | value | p |
| Male    | 16(15.8%) | 85(84.2%) | 1.557 | 0.212 | -0.088 | 0.214 |
| Female  | 23(22.8%) | 78(77.2%) |       |     |       |     |
| Total   | 39(19.3%) | 163(80.7%) |       |     |       |     |
In this study, the overall prevalence of CE in humans was (19.3%) which is higher than that of other studies, which reported the prevalence rate to range from 2.3% to 8.5%. It's lower than findings of others [13-16]. The results of IHA indicate a higher incidence in females (22.8%) as compared with males (15.8%).

In this study, younger male age groups appear to be more frequently infected. IHA for CE was positive in 2.97% among males at the age group of 31-36 years. It was less frequent in the 24-30 year group (1.98%) and the 49-54 year age groups. These results are in line with others in Yemen [13, 17].

A significant difference (p = 0.002) was reported between males according to age without a significant correlation between male age groups and their IHA results (p = 0.302). In this study, younger female age groups appear to be more frequently infected. IHA for CE was detected in 3.96% of the 24-30 year group. A significant difference (p = 0.001) was observed between female age groups and their IHA results (p = 0.302). In this study, younger female age groups appear to be more frequently infected. IHA for CE was detected in 3.96% of the 24-30 year group. In 29.4% of the 31-36 year group, 3.47% of the 37-42 year group, and 2.97% in the 49-54 year age groups. These results are in line with others in Yemen [13, 17]. A significant difference (p = 0.001) between female age groups was observed without a correlation between female age groups and their IHA results (p = 0.340). There was a significant difference (p = 0.001) between both genders according to age without a correlation between age group and IHA results (p = 0.268).

The highest risk groups in Iraq are women and children. Traditionally, rural women especially of young age groups still bear the biggest burden of infection, according by age without a correlation between female age groups and their IHA results (p = 0.302). In this study, younger female age groups appear to be more frequently infected. IHA for CE was detected in 3.96% of the 24-30 year group. A significant difference (p = 0.001) was observed between female age groups and their IHA results (p = 0.302). In this study, younger female age groups appear to be more frequently infected. IHA for CE was detected in 3.96% of the 24-30 year group. In 29.4% of the 31-36 year group, 3.47% of the 37-42 year group, and 2.97% in the 49-54 year age groups. These results are in line with others in Yemen [13, 17].

A significant difference (p = 0.001) between female age groups was observed without a correlation between female age groups and their IHA results (p = 0.340). There was a significant difference (p = 0.001) between both genders according to age without a correlation between age group and IHA results (p = 0.268).

The highest risk groups in Iraq are women and children. Traditionally, rural women especially of young age groups still bear the biggest burden of tending animals whether breeding, milking, or wool-shearing- and are found usually in such environments where domestic or stray dogs are never far away. The chore of women in preparing and cooking contaminated food and vegetables using little clean water increases considerably the risk of infection. In many parts of middle east during springtime, it is a common practice to gather berries and various wild plants which are eaten unwashed and increase the possibility of infection [6].

In this study, the overall prevalence of CE in humans was (19.3%) which is higher than that of other studies, which reported the prevalence rate to range from 2.3% to 8.5%. It's lower than findings of others [13-16]. The results of IHA indicate a higher incidence in females (22.8%) as compared with males (15.8%).

E. granulosus is known to be endemic in several areas of the world. Cystic echinoccosis is often considered to be a disease of developing countries and of populations with low socio-economic status. CE is an extensive epidemiological problem in developing countries like Iraq, particularly in the cattle and sheep raising areas. In the present study 202 patients with clinical manifestations of hydatidosis were enrolled. The minimum age was 18 years while the maximum age of patients was 70 years with a mean age of 30.82 ± 0.52 years. This result is in accordance with a recent local study [6]. In an Ethiopian study, the age was quite similar to that of Iraq (39.33 ± 2.28) [7] and disagree with others reporting the incidence for the age of 61.3 ± 17.1 years [8]. The outstanding result in the current study was the registration of CE among younger age groups such as 24-29, 30-36 and 37-42 years of age compared with reports from northern Iraq [1], Latin America [8] and China [9] in which CE were more frequent among older ages (60-69) and (70-79) years.

The disease occurs in males and females, the sex ratio being different in the different endemic areas, depending on the characteristics of the parasite cycle in this area, and of particular behaviors in the communities. In the present study male patients represent 50%, females 50% without a significant difference (p = 0.212) between IHA positive and negative cases. This result disagree with that reported in Qatar [10] and Egypt [11]. However in China and Argentina, a higher prevalence was reported in females [8, 12].

Housewives, especially in rural areas, where the most infected cases can be found, have the highest chance of contact with the sources of infection. Contact with contaminated vegetables, cleaning the house containing dog faeces, desire to eat soil (Geophagy) as longing in the pregnant concern the etiological issues. High infection rate in females come in agreement with other studies [6, 13, 14].

**Table 4. IHA test according to age group of patients presented with cystic hydatidosis.**

| Age group (years) | Total No. (%) | IHA test | χ² | R |
|-------------------|---------------|----------|----|---|
|                   | Total male    | Male positive IHA | Female positive IHA | P value | χ² value | R Value | Male Female | P value |
| 18-23             | 42 (21.56%)   | 7 (3.47%)     | 2 (0.99%)        | 2 (12.73%) | 35 (17.33%) | 6 (2.97%) | 29 (14.36%) | 60.55 |
| 24-30             | 55 (27.22%)   | 15 (6.18%)   | 4 (1.96%)        | 28 (14.86%) | 24 (12.81%) | 9 (4.96%) | 15 (7.43%) | 0.001 |
| 31-36             | 48 (23.76%)   | 22 (6.53%)   | 6 (2.97%)        | 28 (14.86%) | 15 (7.43%) | 0 (0%)   | 15 (7.43%) | -0.078 |
| 37-42             | 34 (16.83%)   | 14 (6.39%)   | 0 (0%)           | 14 (6.39%)  | 19 (9.14%)  | 7 (3.47%) | 12 (5.94%) | 0.268 |
| 43-48             | 13 (6.43%)    | 8 (3.69%)    | 0 (0%)           | 8 (3.69%)   | 5 (2.48%)   | 0 (0%)   | 5 (2.48%)  | 60.55 |
| 49-54             | 7 (3.43%)     | 4 (1.89%)    | 0 (0%)           | 2 (0.99%)   | 0 (0%)      | 0 (0%)   | 0 (0%)    | 0.001 |
| 55-61             | 2 (0.98%)     | 0 (0%)       | 0 (0%)           | 0 (0%)      | 0 (0%)      | 0 (0%)   | 0 (0%)    | 0.001 |
| 62-67             | 0 (0%)        | 0 (0%)       | 0 (0%)           | 0 (0%)      | 0 (0%)      | 0 (0%)   | 0 (0%)    | 0.001 |
| 68-73             | 1 (0.49%)     | 0 (0%)       | 0 (0%)           | 0 (0%)      | 1 (0.54%)   | 1 (0.54%) | 0 (0%)    | 0.001 |
| Total             | 202 (100%)    | 101 (50%)    | 16 (7.92%)       | 85 (42.08%) | 101 (51.39%) | 23 (11.39%) | 78 (38.61%) | 47.870 |

**DISCUSSION**

E. granulosus is known to be endemic in several areas of the world. Cystic echinoccosis is often considered to be a disease of developing countries and of populations with low socio-economic status. CE is an extensive epidemiological problem in developing countries like Iraq, particularly in the cattle and sheep raising areas. In the present study 202 patients with clinical manifestations of hydatidosis were enrolled. The minimum age was 18 years while the maximum age of patients was 70 years with a mean age of 30.82 ± 0.52 years. This result is in accordance with a recent local study [6]. In an Ethiopian study, the age was quite similar to that of Iraq (39.33 ± 2.28) [7] and disagree with others reporting the incidence for the age of 61.3 ± 17.1 years [8]. The outstanding result in the current study was the registration of CE among younger age groups such as 24-29, 30-36 and 37-42 years of age compared with reports from northern Iraq [1], Latin America [8] and China [9] in which CE was more frequent among older ages (60-69) and (70-79) years.

The disease occurs in males and females, the sex ratio being different in the different endemic areas, depending on the characteristics of the parasite cycle in this area, and of particular behaviors in the communities. In the present study male patients represent 50%, females 50% without a significant difference (p = 0.212) between IHA positive and negative cases. This result disagree with that reported in Qatar [10] and Egypt [11]. However in China and Argentina, a higher prevalence was reported in females [8, 12].

Housewives, especially in rural areas, where the most infected cases can be found, have the highest chance of contact with the sources of infection. Contact with contaminated vegetables, cleaning the house containing dog faeces, desire to eat soil (Geophagy) as longing in the pregnant concern the etiological issues. High infection rate in females come in agreement with other studies [6, 13, 14].
CONCLUSION

The IHA is considered to be a rapid, reliable and adequate technique that makes it possible to process a large number of samples simultaneously without requiring highly trained technical personnel to be present. Females appear to be more susceptible to CE than males especially in younger age groups.

Conflict of interest
No conflict of interest was declared by the authors.

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