



Serodiagnosis of schistosomiasis: IHA test as field, routine, easy and quick approach

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Abstract:

Schistosomiasis is a global infectious disease that endangers human health as well as it impairs the socioeconomic development. The most prevalent species of *Schistosoma* in Egypt are *S.mansoni* and *S.haematobium*. Nevertheless, the traditional parasitological methods still the gold standard methods for detection of schistosomiasis; it was encountered by several obstacles. Therefore, the emerging of the need for more sensitive technique became a must. So, this study tried to put light on IHA test to be used as field, easy, quick, quantitative and cheap test, in addition to give a simple insight about the epidemiological state of the parasites in the area of study. IHA test was found to be very useful in clinical and epidemiological setting. As well as IHA test is potentially helpful in diagnosis of disease in endemic area in condition of knowing the history of patient. During the current survey the statistical analysis (Chi-square test) revealed very high significance of infection in older persons than younger one where the $P < 0.0001$. As well as the gender was having role, since the males received infestation rate more than those of female and showed only high significance value $P < 0.05$. Ultimately, the study recommends that IHA test should be modified to be used as routine test for all parasites.

Key words: Schistosomiasis, Serological tests, IHA test, Egypt.

Introduction

The human population on the earth is always in accelerated growth and the question arise is do the agricultural production meet this human growth rate? Moreover, about more than half of human population suffer from pain and problems and economic losses due to parasitic diseases (Ambrosio and De waal, 1990). The parasites are constituting a major origin of disease in human and livestock. Therefore, several solutions were adopted to increase food production, some of them

are related to expansion of agricultural technology development and the other is increasing of animal production by reducing animal diseases including parasites (Ristic and Montenegro-james, 1987). This case is representing a major problem in developing regions like our country. Consequently, diagnosis of parasitic diseases is a critical step to reach to efficient treatment, effective control of parasite. So, the diagnoses of parasites require using reliable and quick, easy,

sensitive and specific techniques. In our study we are aiming to evaluate and assess the diagnostic efficacy of the IHAT in diagnosing of schistosomiasis, in addition to evaluate the epidemic state of *Schistosoma* in selected region of the country. Schistosomiasis is considered one from worldwide neglected endemic infectious disease especially in tropical and subtropical region and has public health consequences. According to WHO 2014, it is estimated that over 240 million people in 78 countries are infected and around 8 million people are at the risk. Nevertheless, the harmful consequences of schistosomiasis can be controlled if only there are accurate diagnosis and prompt treatment especially in the early stage of the disease. Therefore, the use of sensitive and specific diagnostic tools is very critical and play key role in controlling of schistosomiasis. The direct detection of *Schistosoma* egg in stool and urine (using direct smear method, kato-katz, natural precipitation method and urine precipitation) whether in case of intestinal or urogenital schistosomiasis is most early and sensitive procedure for diagnosis and it is still golden methods for centuries (Yu et al., 2007 and De Vlas et al., 1997). Furthermore, it is relatively simple and inexpensive even under field condition. However, several drawbacks have arisen such as low sensitivity in low intensity of infection, as well as the intermittent shedding of eggs and daily variation of egg deposition particularly in light infection (Engels et al., 1996 and Yu et al., 1998) or after chemotherapy (Degarege et al., 2014). So, to overcome the lack of sensitivity of parasitological methods in situation of light intensity, repeated urine and stool samples should be collected or make several slides from single sample or (sequential), but this increase the cost and might hinder the survey for the need of repeated samples. Furthermore, confuse control strategies that based on screening and treatment (Rabello and Enk, 2006). Another one of pitfalls of microscopic

examination is the *Schistosoma* eggs still shedding after the worm have died in addition to young worm (schistosomula) and worm that are stopped shedding cannot be easily detected (Corstjens et al., 2017). Moreover, two months of pre-patent period of *Schistosoma* result in camouflage of early detection (Gray et al., 2011). Therefore, the attentions have been drawn for antibody based detection techniques such as indirect immunofluorescence (IFAT), enzyme-linked immunosorbent assay (ELISA), indirect hemagglutination (IHA), all of them are designed to detect antibodies in the serum of the infected people. However, serological techniques still have limitation of indiscrimination between past and current exposure but is very useful in less endemic area and symptomatic travellers (Tsang and Wilkins, 1997). The two widely used immunodiagnostic techniques for schistosomiasis are IHA and ELISA. IHA showed high accuracy result in diagnosis of schistosomiasis (Wang et al., 2017). As well as (Weifeng et al., 2018) categorized all diagnostic methods for schistosomiasis and referred to IHA as highly sensitive, highly specific and the easiest operated methods. The diagnostic performance of IHA was higher than that of ELISA (Wang et al., 2012). Furthermore, (Chen et al., 2011) proved the relative high diagnostic efficiency of IHA Kits in addition of using them as mass screen indicator of schistosomiasis in field sites. Other confirmation for using IHA as community screening test was provided by (Yu et al., 2007 and Zhou et al., 2008) but it is of poor specificity in individual screening. However, (Van Gool et al., 2002) showed that combination of both ELISA and IHA is sensitive and specific for *Schistosoma* detection in travellers from tropics. In a comparative study between IFT, ELISA and IHA for diagnosis of schistosomiasis, Sorgho et al., 2005 suggested that IHA is an accurate diagnostic test in endemic area.

Material and methods

Collection of samples:

A total of 170 sera samples were randomly collected from people live at the area of Mutubis city and its villages, Kafr EL Sheikh province during period between March and June 2018. In this study, human sera were preferred to make sure that absence or at least reduce other parasitic infection to get ride cross reaction with *Schistosoma spp.* as it is much difficult to ensure that in animal sera. Each case during this survey provided at least one sample each of serum, stool and urine. During this survey some data was taken such as age, gender, previous treatment, and history of previous exposure to infection.

IHA assay:

IHA test was carried out according to manufacturer's instructions using U-microtitre plates (SCHISTO-IHA-FAST, ABC diagnostics®). In brief, U-microtitre plate wells receive 50 µl of buffer (sample diluent), then 50 µl of 1:40 initial diluted serum are added to first well containing buffer after that serial dilution was done for at least titre of 1:2560. Following that, 20 µl of sensitized RBCs are dropped over the wells having serum and buffer. In addition, we assign another two wells as a control negative, one of them contains (buffer, serum of titre <1:80> and non-sensitized RBCs) called serum control, the other well contains only buffer called reagent control. As well as we have the option to make control positive and control negative as the kits have positive serum of titre $\geq 1:320$ and negative serum as well. Gently mix the plate and let it in horizontal position for at least one hour. The positive result appear as cloudy red/brown deposit coating the well while the negative result interpreting as ring like deposit at the bottom of the well. According to manufacturer's instruction cases of titre less than 1/160 considers past infection or already treated cases while as titre equal or

over than 1/160 it considers a significant reaction and presumptive current infection. Alongside, antibodies detection of *Schistosoma*, stool samples were taken and microscopically examined using sedimentation technique and direct smear methods for detection of *Schistosoma mansoni* egg. As well as urine samples are collected and analysed by precipitating of about 10 ml for detection of *Schistosoma haematobium* egg in the precipitate.

Statistical analysis:

Statistical analysis was performed by Chi-square test to compare diversity of infestation rate between age groups and gender as well. A significance level of $p < 0.05$.

Result

A total of 170 human cases were serologically and parasitologically examined for detection of schistosomiasis in area of Mutubis and its villages, Kafr EL Sheikh Province. All of cases provided each blood, stool and urine sample to investigate the presence of *Schistosoma* in each sample. The blood samples (sera) were taken and analysed for detection of antibodies of schistosomiasis as well as determine their titre. For each serum sample, stool sample and urine sample are parasitologically examined as well for detection of *Schistosoma* eggs. Out of 170 sera samples, only 2 (1.18%) stool samples were positive for presence of *Schistosoma* egg. While as 71 (41.76%) sera samples were found to be serologically positive for presence of *Schistosoma*'s antibodies. This result ensure that relying on stool and urine samples for detection of schistosomiasis is controversial and less sensitive and it needs intensive lab work and sequential sampling and this is very difficult in case of large scale epidemiological screening. As well as intermittent shedding of egg is another defect in parasitological examination. As well as, some schistosomiasis cases do not enter the parasitological state therefore, only antibodies are to be detected in sera while diagnostic stage are yet not

completed to be in stool or in urine. This study also included simple epidemiological study, out of 170 cases, 71 (41.76%) case were serologically positive and 99 case were negative (58.23%). Alongside general prevalence, the cases are categorized into 5 groups to detect the effect of age on the infestation, Group from 10-20 years old, 21-30 years old, 31-40 years old, 41-50 years old and 51-60 years old. The result showed that very high significance in older age than younger ages. Where group from 10-20 years old received infestation rate of (13.51%), group from 21-30 revealed infestation rate of (29.16%), then group of 31-40 years old showed (60%) infestation rate then group of 41-50 years old found to be infested by (66.66%) and finally group of 51-60 years old showed (57.14%) infestation rate. In addition the chi-square test was used to analysed these result it was very prominent that age played role in

the infestation rate since the test showed very high significance among older and younger ages ($P < 0.0001$), table (1) and Figure (1). Furthermore, when narrowing the difference between ages and combined them in only two groups, one from 10-30 years old and the other one from 31-60 years old we obtained the same infestation rate while the younger age (10-30) recorded smallest (22.35%) infestation rate and older ages (31-60) recorded the greatest infestation rate (61.17%), table (2) & Figure (1) also chi-square test showed very high significance $P < 0.0001$. The study also tried to evaluate the infestation rate among genders while males were found to be exposed to schistosomiasis more than females since the infestation rate was 53.73% in males and 33.98 % in females, table (3) & Figure (2) in addition the value of chi-square test depicted only high significance $P < 0.05$.

Table 1: Depicting the infestation rate among different categories of age and their value and significance level.

Age / year	Total No.	Positive cases %	Negative cases %	Chi-square value and significance level
10-20	37	5 (13.51%)	32 (86.48%)	28.90 *** $P < 0.0001$
21-30	48	14 (29.16%)	34 (70.83%)	
31-40	40	24 (60%)	16 (40%)	
41-50	24	16 (66.66%)	8 (33.33%)	
51-60	21	12 (57.14%)	9 (42.85%)	
Total	170	71 (41.76%)	99 (58.23%)	

Table 2: Showing the infestation rate among only two categories of age and their value and significance level.

Age / year	Total No.	Positive cases %	Negative cases %	Chi-square value and significance level
10-30	85	19 (22.35%)	66 (77.64%)	26.34*** $P < 0.0001$
31-60	85	52 (61.17%)	33 (38.82%)	
Total	170	71 (41.76%)	99 (58.23%)	

Table 3: Showing the infestation rate among genders and their value and significance level.

Gender	Total No. and %	Positive cases %	Negative cases %	Chi-square value and significance level
Male	67/170 (39.41)	36 (53.73)	31(46.26)	6.51* $P < 0.05$
Female	103/170 (60.58)	35 (33.98)	68(66.20)	
Total	170	71 (41.76%)	99 (58.24%)	

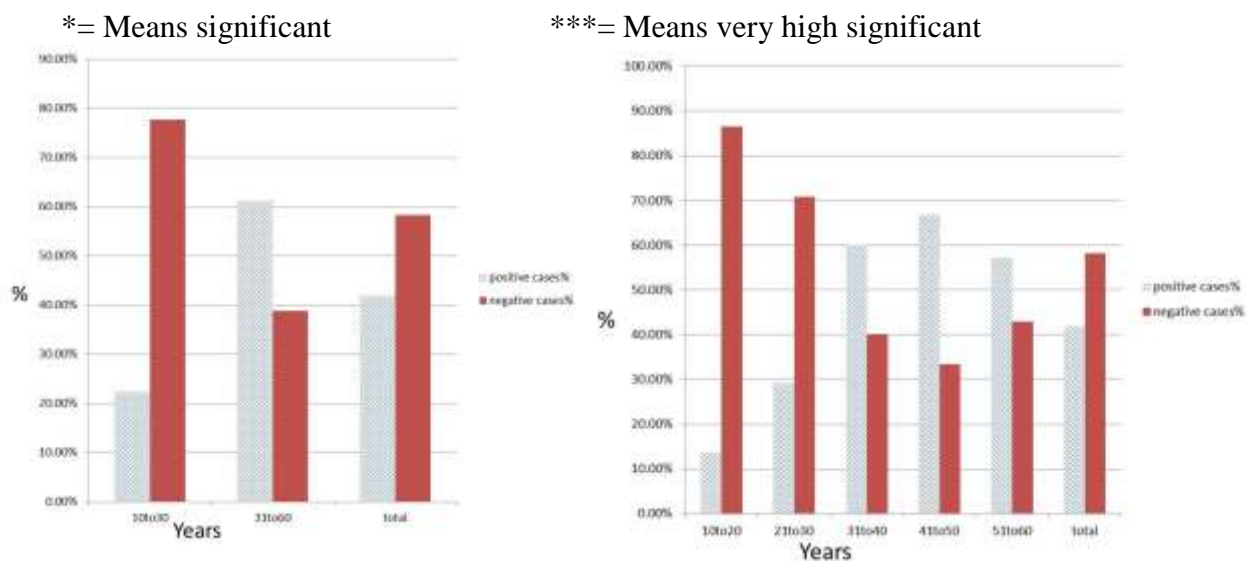


Figure 1: Illustrate the infestation rate among different categories of ages.

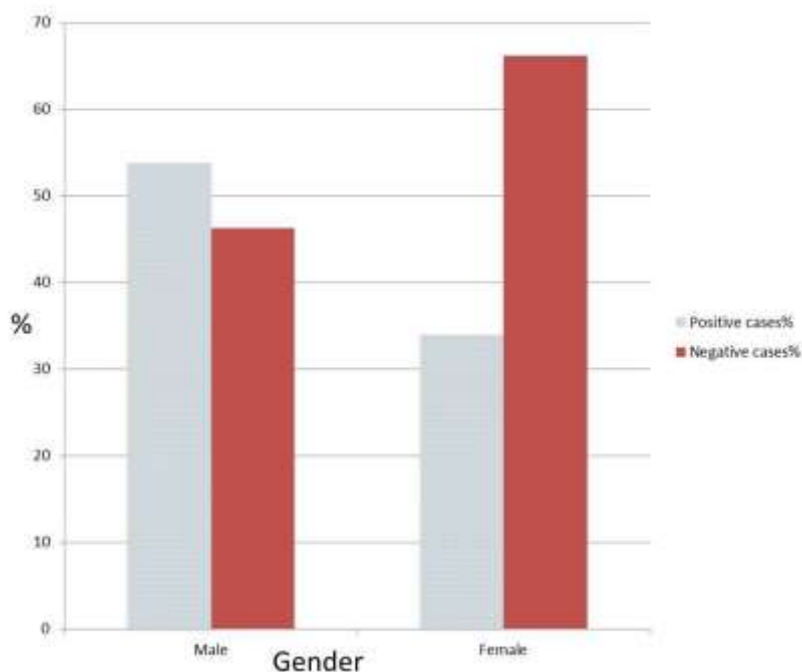


Figure 2: Showing the infestation rate among different gender.

Discussion

The data were based on comparative of 170 sera samples in parallel with parasitological examination of stool and urine samples to give the accurate picture of serological reactivity of schistosomiasis in region of Mutubis, Kafr-ELSheikh Province, Egypt. The result revealed 71 sera samples serologically positive for schistosomiasis in contrast to the results were obtained by the parasitological

examination revealed only two positive stool samples. Therefore, only one stool or urine sample does not give true picture of *Schistosoma* diagnosis and prevalence so, examination of multiple stool and urine samples are required to provide accurate prevalence and diagnosis (Sorgho et al., 2005). However, examination of multiple samples are potential to be helpful in individual surveillance, it seems to be

difficult in large scale epidemiological survey (Rabello and Enk, 2006). As well as, in case of low intensity infestation the yielding of eggs is very poor and considers another camouflage diagnostic factor. Moreover, the variation of egg deposition along the day also play role in misdiagnosis (Engels et al., 1996 and Yu et al., 1998) all of these pitfalls of parasitological stool and urine examination encourage the serological tests specifically IHA test to be routine, sensitive and field test for diagnosis of schistosomiasis. Furthermore, in some cases such as governmental control program that depends on administration of drug and in case of stop of worms to give eggs as well, all of them make microscopic examination unhelpful, this come in the same line with (Rabello and Enk, 2006 and (Corstjens et al., 2017). Therefore, to overcome the limitations of microscopic examination many studies recruited serological test such as indirect immunofluorescence (IFAT), enzyme-linked immunosorbent assay (ELISA), indirect hemagglutination (IHA). Several studies gave privileges of IHA test over the other two tests such as (Bray 1976, Wang et al., 2017 and Weifeng et al., 2018). Therefore, this study tried to give some attentions about advantage of IHA test such as easy and ready to use, cost-effective, quantitative, high sensitivity and specificity, fast result within one hour and simple optical reading without needing of experience. All of these advantage make IHA test routine and field test and satisfactory for *Schistosoma* diagnosis like the result obtained by (Chen et al., 2011). On other hand some studies recommended to combined both ELISA and IHA for more sensitivity and specificity (Van Gool et al., 2002). However, this suggestion increases of cost and disagrees with the idea of field test because we try to find test are useful under conditions minimal equipped laboratories such what was suggested with (El-Adawi et al., 1994). Furthermore, IHA test showed reliable results in our study area

thought of it considers endemic area like the results were obtained by (Sorgho et al., 2005) who suggested that IHA is an accurate diagnostic test in endemic area. Ultimately we recommend using of IHA test as reliable field test for *Schistosoma* detection in condition of taking history of patient such as chemotherapy history. Especially, after the results proved that percentage of cross reaction with other parasites are almost ignored or of minimal importance (El-Adawi et al., 1994) where most of anti-parasitic chemotherapy are safe and do not cause serious harms if they were taken in false positive cases. As well as, using of IHA test and increase studies work on improvement of its sensitivity and specificity will overcome the other expensive serological test such as ELISA and IFAT. We recommend also improving IHA test to be able to detect all types of parasites, to be widely used in Veterinary Parasitology branch. From epidemiological view, the current study revealed that schistosomiasis is abundant in the older age than younger ages. This result consider realistic because increasing of campaign of awareness about how to avoid infection by *Schistosoma* that was lack in the past. In addition the percentage of infestation in males was higher than those of females; we found also it is logical result because the males are in more exposure to infection during their work in the agricultural field and in direct contact with polluted water with infective cercaria.

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الملخص العربي

التشخيص السيروولوجي لداء البلهارسيا: إختبار التلازن الدموي غير المباشر (IHA)

كإختبار حقلى وروتينى وسهل وسريع

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داء البلهارسيا واسع الإنتشار ومعدى، يعرض صحة الإنسان للخطر، كما أنه يعوق التنمية الإجتماعية والإقتصادية في المناطق الموبوءة. أكثر أنواع البلهارسيا إنتشارا في مصر هي بلهارسيا معوية (مانسونية) و بلهارسيا بولية (هيماتوبيم). وبالرغم من إن الطرق التقليدية لتشخيص الطفيليات لا تزال هي الطرق المعيارية الأكثر تطبيقاً للكشف عن داء البلهارسيا، إلا إنها تواجهها عدة سلبيات. لذلك، أصبح الحاجة لتطبيق تقنية أكثر حساسية أمراً ضرورياً. وقد حاولت هذه الدراسة وضع ضوء على إختبار التلازن الدموي غير المباشر IHA لإستخدامه كإختبار حقلى سهل وسريع وكمي ورخيص. بالإضافة إلى استخلاص دلالة بسيطة عن الحالة الوبائية للطفيل في المنطقة محل الدراسة. وقد وجد أن إختبار IHA مفيد جداً في الحالات السريرية والوبائية. وكذلك هذا الإختبار مفيداً في تشخيص المرض في المنطقة الموبوءة في حالة معرفة تاريخ الحالة المرضية.

خلال المسح الحالى، أظهر التحليل الإحصائي (إختبار Chi-square) إختلافات عالية جداً للإصابة في كبار السن عن الشباب حيث $P < 0.0001$. كذلك كان للجنس دور أيضاً في تباين نسب الإصابات، فقد وصلت نسبة الإصابة في الذكور معدل أكثر من التى كانت عليها في الإناث، وأظهرت قيمة عالية فقط $P < 0.05$. لهذا، توصي الدراسة بتعديل إختبار IHA ليتم استخدامه كإختبار روتينى للتشخيص المعملى لجميع الطفيليات.

