SUST Journal of Natural and Medical Sciences (JNMS)vol. **16** (2) 2015
ISSN (Print): 1858-6805 e-ISSN (Online): 1858-6813

SUST
Journal of Natural and Medical Sciences

Journal homepage: http://journals.sustech.edu/

Epidemiological and Clinical Association of Malaria-Intestinal Helminthes Co-Infections in Abu-Naama, Sinnar State, Sudan

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Article history: Recieved: May 2015
Accepted: October 2015

ABSTRACT

This study aimed to determine the epidemiological and clinical association of malaria-intestinal helminthes co-infections in Abu-Naama area in Sinnar State, Sudan. A cross- sectional study was carried out during the period from November 2013 to April 2014. A random total number of 100 subjects, 44 (44%) were males and 56 (56%) were females, with an age ranging between 2-70 years old and average age of 20 ± 18 years were included in this study. Blood and stool samples were taken from all subjects. Clinical and parasitological data were obtained and recorded. Out of 100 stool samples, 7 (7%), 15 (15%) were found to be positive for intestinal helminthes eggs when examined by direct wet mount and formal ether concentration technique (FECT) respectively. When using FECT to determine an intensity of infections, out of 15 positive samples, 3 (20%) samples were presented as few infections, 2 (13%) as moderate, while 10 (67%) stool samples were presented as severe infections. Seventy three out of 100 (73%) blood samples were found to be positive for *P. falciparum* when stained by Giemsa stain. When results were analyzed statistically, the study indicates that there is no correlation between malaria and intestinal helminthes infections in the area under investigation.

KEYWORDS:FECT, Epidemiological correlation, Co-infections.

المستخلص:

هدفت الدراسة لتحديد العلاقة الوبائية و الإكلينيكية للإصابة المشتركة بالملاريا والديدان المعوية في منطقة أبو نعامة، ولاية سنار. الدراسة المستعرضة نفذت خلال الفترة مابين نوفمبر 2013 الي ابريل 2014م. وكان مجموع عدد الاشخاص الذين تم إشراكهم في الدراسة 100 شخص (44 (44%) ذكور و 56 (56%) إناث) وكانت أعمارهم مابين 2 إلى 70 عاماً وكان متوسط العمر20 عاماً.أخذت عينات من الدم و الفسحة من كل شخص كما تم الحصول علي البيانات السريرية والطفيلية لكل شخص. تم تحديد إصابة الديدان المعوية في البراز بواسطة التحضير الرطب في 7 (7%) عينة من أصل 100 وعند إستخدام تقنية الترسيب بواسطة FECT إرتفعت الإصابة الي 15 (15%) من اصل 100 شخص. تم أيضاً استخدام طريقة الترسيب لتحديد شدة الإصابة، من بين 15 عينة إيجابية، 3(20 %) أظهرت إصابات خفيفة، 2 (13%) أظهرت إصابات متوسطة، بينما 10 (67%) من العينات الإيجابية أظهرت إصابات شديدة. بلغت إصابات

الملاريا 73 (73%) إصابة من أصل 100 شخص، تم الحصول علي عينات من الدم إيجابية للملاريا للمصورة المنجلية (*P.falciparum*) بإستخدام صباغة جيمسا. تلخص هذه الدراسة أنه لا توجد علاقة وبائية و إكلينيكية للإصابة المشتركة بالملاريا والديدان المعوية فى المنطقة موضوع الدراسة كما وضح بتحليل النتائج إحصائياً.

INTRODUCTION

Co-infection with multiple parasites is common in malaria endemic area. Although much is known about the epidemiology and immunology of specific parasitic illness, little is known about the interaction of concurrent infections. Mounting evidence suggests an interaction occurring between helminthic and malaria infections, although it is unclear as to whether this effect harms or protects the host [1]. Malaria is the most important tropical disease known to man. It remains a significant problem in many tropical areas, especially in sub-Saharan Africa. Malaria is spreading as a result of environmental changes, including global warming, civil disturbances, increasing travel and drug resistance[2]. Throughout evolutionary history humans have been infected with parasites. Today, it is estimated that over a third of the world's population, mainly those individuals living in the tropics and sub-tropics, are infected by parasitic helminthes (worms) or one or more of the species of *Plasmodium* [3,1]. The ubiquity of these parasites results in high rates of co-infection [4]. It has increasingly been speculated that helminthes infections may alter susceptibility to clinical malaria [5]and there is now increasing interest in investigating the consequences of co-infection [6]. This is, however, not a new research topic. Nearly, thirty years ago, it was suggested that infection with the intestinal nematode *Ascaris lumbricoides* was associated with the suppression of malaria symptoms and that anti-helminthic treatment led to a recrudescence of malaria [7,8]. The mechanisms underlying this finding, and those of more recent studies, are based on the assumption that helminth infections induce a potent and highly polarized immune response [9] which has been proposed to modify the acquisition of immunity to malaria[10].

The main objectives of this study were to determine the epidemiological and clinical association of malaria-intestinal helminthes co-infections in Abu-Naama area in Sinnar State, Sudan and to study the prevalence of intestinal helminthes and malaria infections in the study area, to detect intensity of intestinal helminthes infection by FECT, to determine relationship between intensity of intestinal helminthes infection and age groups, to determine relationship between malaria severity and age groups, to determine relationship between intestinal infections, gender and age groups, to determine relation between previous helminthes infection and current malaria co-infection and to compare between direct wet mount and FECT in detection of intestinal helminthes.

MATERIALS and METHODS

Study area

This study was carried out in Abu-Naama area, Sinnar State, in West coast of Blue Nile, which is considered as farm land, because ¾ of Sinnar State farms are present in Abu-Namma. Therefore, the majority of the population are farmers, depending on agriculture and raising animals. Agriculture in Abu-Naama depends mainly on irrigation canals, which many of them are blocked forming swage disposable places; houses and animal wastes are seen in streets, next to houses, empty irrigation canals and small water collections. The area is

considered to be endemic for malaria collection of water that provide breeding sites for mosquitoes. Most animals (cows, goats, poultry, and dogs) are raised inside houses, where their wastes are accumulated. As the area is lacking. The peaks of the malaria transmission are reported in October and continue to December. *Plasmodium falciparum* is considered to be the major malaria species in the area. *Plasmodium vivax* is also endemic in the area. Mixed infection may also be present. The area is endemic with many intestinal helminthes mainly *Hymenolepis nana*.

General characteristics of the studied population and ethical clearance.

The study was conducted on 100 subjects from Abu-Naama area, Sinnar State, Sudan with an age ranging between 2-70 years old and the mean age of 20 ± 18 years, 44 of them were males (44%) and 56 were females (56%). The subjects were categorized into six age groups: less than 6, 6-12, 13-18, 19-40, 41-64 and more than 65 years. The frequency of each age group was 27 (27%), 12 (12%), 18 (18%), 27 (27%), 12 (12%) and 4 (4%) of the total subjects. Ethical clearance for this study was obtained from College of Medical Laboratory Science- Sudan University of Science and Technology. An informed consent was obtained from all subjects included in this study.

Design of questionnaire

The design of questionnaire contains simple investigative questions or indicators, which include gender, age, signs and symptoms of diseases such as fever, abdominal pain, diarrhea, previous malaria infection, previous intestinal helminthes infection and previous co-infections.

Blood examination

For detection of *P. falciparum*, stained blood films were screened using the method described by WHO (1993) [11]. The number of parasites was counted in thick film against white blood cells using formula: Parasitemia per µl= Parasite count x 8000/200 as described by WHO (1993) [11].

Stool examination

Intestinal parasites were detected in stool samples using direct wet mount and FECT method as described by WHO (1993) [11] and Young*et al*. (1979) [12] respectively. The intensity of infection was determined by the method of Young*et al*. (1979) [12]. Results were expressed as more than 3 cysts per high-power field, or more than 20 eggs or larvae per mount presented as many infection, 2 cysts per high-power field, or 10 to 19 eggs or larvae per mount as moderate infection, 1 cyst per high-power field, or 3 to 9 eggs or larvae per mount as few infection and less than 1 cyst per high-power field, or less than 2 eggs or larvae per mount as rare infection.

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) under windows, version 15.0. Chi square test statistical analysis was performed and the *p* values of less than 0.05 were considered statistically significant.Data were presented in tables using Excel after analysis using SPSS.

RESULTS

Overall prevalence rate of *P. falciparum* using blood films (B.F) according to gender

Out of 100 study subjects, 73 (73%) were found to be harboring *P.falciparum* parasite when using blood films. From the 73 positive cases, 34 (46.6%) were males and 39 (53.4%) were females (table 1). The differences in prevalence according to gender was found to be statistically significant (p=0.000).

Table 1: Overall prevalence rate of P. falciparum using blood films according to gender

P. falciparum	Gender		Total
	Male	Female	
B.F positive	34	39	73

p=0.000 p‹0.001

Intensity of malaria parasite

Malaria parasite intensity was available for 73 (73%) individuals (individuals with positive results).Parasitemia expressed as number of parasite per µl of blood, ranged between 1000- 71520 parasite/µl, with mean count 8437 parasite/µl of blood (table 2).

Table 2: Mean of parasitemia among age groups and sex

		Mean of parasitemia per µl of blood
Age groups	Less than 6	9674
	6-12	8837
	13-18	7869
	19-40	7638
	41-64	6650
	More than 65›b5	11892
Sex	Males	9613
	Females	7513

p=0.727

Relation between presence of malaria and presence of clinical features

Chi-square test was used to determine the relation between presence of malaria and appearance of clinical features; individuals where examined clinically by physician for presence of fever (p=0.329), diarrhea (p=0.061) and abdominal pain (p=0.141) (table 3). And the mean count was determined in presence and absence of each feature as shown in (table 4).

Table 3: Relation between presence of malaria and presence of clinical features

Presence of symptoms		Malaria		Total
		Positive	Negative	
Fever	Presence	67 (67%)	23 (23%)	90 (90%)
	Absence	6 (6%)	4 (4%)	10 (10%)
Diarrhea	Presence	23 (23%)	14 (14%)	37 (37%)
	Absence	50 (50%)	13 (13%)	63 (63%)
Abdominal pain	Presence	26 (26%)	14 (14%)	40 (40%)
	Absence	47 (47%)	13 (13%)	60 (60%)

Fever (p=0.329), diarrhea (p=0.061), abdominal pain (p=0.141)

Table 4: Mean of malaria parasitemia among different clinical status

Symptoms		Mean of count per µl of blood
Fever	Presence	8292
	Absence	9744
Diarrhea	Presence	8123
	Absence	8621
Abdominal pain	Presence	8384
	Absence	8472

Fever (p=0.633), diarrhea (p=0.452), abdominal pain (p=0.563)

Overall prevalence rate of intestinal helminthes infection using direct wet mount and FECT according to gender

A total of 100 stool samples were examined for intestinal helminthes eggs. Out of these, 7 (7%) were found to be positive for *H. nana* when detected by using direct wet mount method and 15 (15%) when using FECT. From the 7 positive cases, 3 (42.9%) were males and 4 (57.1%) were females and from the 15 positive cases, 5 (33.3%) were males and 10 (66.7%) were females (table 5). The differences in prevalence rate according to gender was found to be statistically significant (p=0.000). p‹001

Table 5: Overall prevalence of H. nana infection using direct wet mount and FECT according to gender

H. nana	Gender		Total
	Male	Female	
Direct wet mount	3	4	7
FECT	5	10	15

p=0.000

Overall prevalence rate of intestinal helminthes infection using direct wet mount and FECT according to age groups

The positive cases within each age group were 2 (2%), 1 (1%), 1 (1%), 1 (1%) 1 (1%), and 1 (1%) respectively when using direct wet mount and were 4 (4%), 4 (4%), 2 (2%), 2 (2%), 2 (2%) and 1 (1%) respectively when using FECT (table 6). The differences in prevalence rate according to age groups was highly significant (p=0.000).

Table 6: Overall prevalence of intestinal helminthes infection using direct wet mount and FECT according to age groups

Age groups (years)	*H. nana*	
	Direct wet mount	FECT
Less than 6	2	4
6-12	1	4
13-18	1	2
19-40	1	2
41-64	1	2
More than 65	1	1
Total	7	15

p=0.000 p‹001

Intensity of intestinal helminthes among age groups

The intensity of *H.nana* infection was obtained by counting the number of eggs per 1 gram of stool using FECT. Eggs per 1 gram of stool presented as rare, few, moderate and many infection (Young *et al.*, 1979)[12] (table 7).

Table 7: Intensity of intestinal helminthes infection among age groups

Intensity Age groups (years)	Negative	Rare	Few	Moderate	Many
Less than 6	23	0	1	0	3
6-12	10	0	0	0	2
13-18	16	0	0	2	0
19-40	25	0	0	0	2
41-64	10	0	0	0	2

More than 65	3	0	0	0	1
Total	87 %	0 %	1 %	2 %	10 %

p=0.381

Relation between presence of *H.nana* infection and presence of clinical features

Chi-square test was used to determine the relation between presence of *H.nana*and appearance of clinical features; individuals where examined clinically by physician for presence of fever (p=0.161), diarrhea (p=0.139) and abdominal pain (p=0.568) (table 8).

Table 8: Relation between presence of H.nana and presence of clinical features

Presence of symptoms		*H.nana*		Total
		Positive	Negative	
Fever	Presence	15 (15%)	75 (75%)	90 (90%)
	Absence	0 (0%)	10 (10%)	10 (10%)
Diarrhea	Presence	3 (3%)	34 (34%)	37 (37%)
	Absence	12 (12%)	51 (51%)	63 (63%)
Abdominal pain	Presence	7 (7%)	33 (33%)	40 (40%)
	Absence	8 (8%)	52 (52%)	60 (60%)

Fever (p=0.161), diarrhea (p=0.139), abdominal pain (p=0.568)

Co-infections

Intestinal helminthes- *Plasmoduim* co-infection

The present study indicated that 13 individuals (13%) were co-infected with intestinal helminthes and malaria (table 9).

Table 9: Prevalence of intestinal helminthes- Plasmoduim co-infection in the study area

		Malaria		Total
		Positive	Negative	
Helminthes	Positive	13 %	2 %	15 %
	Negative	60 %	25 %	85 %
Total		73 %	27 %	100 %

p=0.196

Mean of *P. falciparum* parasitemia (per µl of blood) in relation to presence of helminthes infection

Mean of *P. falciparum* count (parasite per µl of blood) was determined in relation to presence of intestinal helminthes infection among malaria positive individuals (table 10).

Table 10: Mean of P.falciparum parasitemia in relation to intestinal helminthes infection

	Helminthes infection (*H.nana*)	
	Positive	Negative
Mean of parasitemia (parasite per µl of blood)	13785	7638

The mean of *P. falciparum* parasitemia (parasite per µl of blood) in relation to intestinal helminthes infection intensity

The mean count of *P. falciparum* was determined for each sex and among the different status of intestinal helminthes infection (*H.nana* density) (table 11).

Table 11: Mean of P. falciparum parasitemia in relation to helminthes infection intensity and sex

		Mean of parasitemia per µl of blood
Sex	Male	9613
	Female	7513
H.nana	Rare	0
intensity	Few	5880
	Moderate	6880
	Many	15117

p=0.080

Table 12: Prevalence of co-infections among different age groups

Age Groups (years)	Co-infection Positive	Negative
Less than 6	4	22
6 – 12	2	10
13 – 18	2	16
19 – 40	2	25
41 – 64	2	10
More than 65	1	3
Total	13	87

p=0.892

Relationship between co-infection and fever, abdominal pain and diarrhea

Chi-square test was used to determine the relation between co-infection and fever (p=0.179), abdominal pain (p=0.627) and diarrhea (p=0.058); individuals were examined clinically by physician for presence of these features (table 13).

Table 13: Relationship between co-infection and fever, abdominal pain and diarrhea

		Co-infection		Total
		Positive	Negative	
Fever	Presence	14 (14%)	76 (76%)	90 (90%)
	Absence	0 (0 %)	10 (10%)	10 (10%)
Diarrhea	Presence	2 (2%)	35 (35%)	37 (37%)
	Absence	12 (12%)	51 (51%)	63 (63%)
Abdominal pain	Presence	6 (6%)	34 (34%)	40 (40%)
	Absence	8 (8%)	52 (52%)	60 (60%)
Total		13 (13%)	87 (87%)	100 (100%)

Prevalence of previous intestinal helminthes and current malaria co-infection

The present study indicated that 26 individuals (26%) with malaria had previous intestinal helminthes infection (table 14), (p=0.202).

Table 14: Prevalence of previous intestinal helminthes and current malaria co-infection

		Current malaria		Total
		Positive	Negative	
Previous helminthes	Positive	26 %	6 %	32 (32 %)
	Negative	47 %	21 %	68 (68 %)
Total		73 (73 %)	27 (27 %)	100 (100 %)

p=0.202

DISCUSSION

In Sudan, malaria has been subject to a large amount of epidemiological, entomological, and biomedical research. Malaria evidence in Sudan was estimated to be about 9 million episodes in 2002 and the number of deaths was 44000 [13]. Intestinal helminthes infections are major causes of morbidity in all age groups in the developing world. More than a quarter of the world population is infected with soil-transmitted helminthes like hook worms, *H.nana* and *Ascaris*, and 200 million with schistosomiasis [14]. Spatial congruence of both *P. falciparum* and different helminthes remains poorly defined. Preliminary analyses, however, suggest that as many as one quarter of African school children may be coincidentally at risk of *P.falciparum* and hook worms[15]. This spatial coincidence of risk between these two parasite populations would suggest that co-infection is extremely common; although the public health significance of polyparasitic infection remains a topic for which there are many unknowns [9]. So this study was conducted to establish association between helminthes infection and acquiring malaria infection. For this purpose, 100 blood and 100 stool samples were involved and examined to detect co-infection. Helminthes infections diagnosed in Abu-Naama area, 15 (15%) were found to be positive for *H.nana* infection, due to low hygiene and bad environmental conditions. Most individuals (77 % of positive individuals); intensity of infection presented as many, with mean count of 62 eggs/ 1g of stool. The prevalence of *P.falciparum* infection in Abu-Naama area was 73 %, due to presence of its preferred breeding sites, provided feeding and post-feeding places and favorable hosts. The parasitemia of infection showed mean count of 8437 parasite/µl of blood. High parasitemia was detected among males with mean count of 9613 parasite/µl of blood, while mean count for females was 7513 parasite/ µl of blood. The prevalence of malaria-intestinal helminthes co-infection (*P.falciparum*-*H.nana* co-infection) was 13%. The most co-infected individuals were found to be in the less than 6 years age group. Although observations indicate that intestinal helminthes infection can be considered as a risk factor for malaria infection (prevalence ratio=1.22), but the statistical results showed that there is no association between malaria and intestinal helminthes infection (p=0.196). This result disagreed with Andargachew *et al.*[16] and Hartgers and Yazdanbakhsh [17]who proved presence of association. The results showed no association between previous helminthes and current malaria co-infection (p=0.202). The mean of malaria parasitemia in helminthes positive individuals (13785 parasite/ µl of blood) was found to be higher than the mean of parasitemia of non-helminthic individuals (7638 parasite/ µl of blood). The highest mean count associated with highest worm burden as expressed by egg/ 1 g of stool (mean of 15117 parasites/ µl of blood associated with mean of 62 eggs/ 1g of stool in co-infected patients). The results showed no association between co-infection and fever (p=0.179), diarrhea (p=0.058) and abdominal pain (p=0.627). Also, the results showed no association between *H.nana* and fever (p=0.161) with mean count of 8 egg /1 g of stool for individuals suffering from fever, diarrhea (p=0.139) with mean count of 3 egg /1 g of stool and abdominal pain (p=0.568) with mean count of 10 egg /1

g of stool. Results showed no associa-association between *P.falciparum* and fever (p=0.329) with mean count of 8292 parasite/ µl of blood, diarrhea (p=0.061) with mean count of 8123 parasites/ µl of blood and abdominal pain (p=0.141) with mean count of 8384 parasites/ µl of blood. The results obtained by FECT were compared with those obtained using wet preparation, (p=0.000) indicating that FECT is better than the wet preparation in the detection of intestinal parasites.

CONCULUSIONS

The study showed no epidemiological and clinical correlation between malaria and intestinal helminthes in Abu-Naama area in Sinnar State.

RECOMMENDATIONS

From the results obtained, the following recommendations can be drawn:

1. Further studies should be done on immunological effects of intestinal helminthes infections on malaria immune response.
2. Further studies should be done with other species of *Plasmoduim*.
3. Further studies should be done to find epidemiological intestinal helminthes-malaria co-infection.
4. Further studies should be done in prevalence of intestinal helminthes- malaria co-infection in other endemic areas.
5. Formal ether concentration technique should be used as best method for detection of intestinal helminthes eggs than wet preparation.
6. Control activities should be conducted in the study area to reduce infection with malaria and other intestinal parasite infections.
7. Similar study should be performed in the area with relation to protozoal parasites.
8. Increase sample size.

ACKNOWLEDGEMENT

I am grateful for the staff ofAbu-Naama Health Center in Sinnar State. Also, for all those who participated in the study.

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