Intestinal parasites, including *Cryptosporidium* species, in Iraqi patients with sickle-cell anaemia

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الطفيليات المعوية بما فيها خفيّات الأبواغ لدى العراقيين المرضى بفقر الدم المنجلي

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الخلاصة: تم الحصول على عينات برازية مفردة من الأشخاص الذين أدخلوا في ثلاث مستشفيات في البصرة في الفترة بين تشرين الثاني/نوفمبر 1997 وأيار/مايو 1998، ومن بين المرضى الأربعين المصابين بفقر الدم المنجلي كان لدى 25 منهم (62.5٪) طفيليات معوية بما فيها الحيوانات الأوالي الصامدة للحمض. أما في المجموعات التي تتمتع بالصحة والتي أخذت كشواهد للمقارنة فإن الطفيليات المعوية وجدت لدى 26 من أصل 175 منهم (14.8٪) مما يشكل فرقاً يُعتدُّ به إحصائياً. وأكثر الطفيليات التي استفردت من مرض فقر الدم المنجلي شيوعاً كانت المتكيّسات البشرية التي استفردت لدى 36٪ منهم والجيارديا اللامبلية التي استفردت لدى 28٪ منهم، أما معدلات استفراد أنواع خفيّات الأبواغ لدى المصابين بفقر الدم المنجلي فقد كانت 5٪، وهي لا تختلف اختلافاً يُعتدُّ به إحصائياً عما هي عليه لدى الأصحاء (1.14 ٪). ونحن هنا نسجّل وللمرة الأولى استفراد متساويات الأبواغ من مرض فقر الدم المنجلي في العراق في إقليم شرق المتوسط.

ABSTRACT Stool samples were obtained from individuals admitted to three hospitals in Basra during November 1997–May 1998. Of 40 patients with sickle-cell anaemia, 25 (62.5%) had parasitic infections. In the apparently healthy comparison group, 26 of 175 individuals (14.8%) had intestinal parasitic infections, a statistically significant difference. The most common intestinal parasites isolated in the sickle-cell patients were *Blastocystis hominis* (36%) and *Giardia lamblia* (28%). The isolation rate of *Cryptosporidium* species in sickle-cell patients (5%) was not significantly different from that in apparently healthy individuals (1.14%). We report for the first time the isolation of *Isospora belli* from a sickle-cell patient in Iraq and the Mediterranean region.

Les parasites intestinaux, y compris *Cryptosporidium* spp., chez des patients iraquiens atteints de drépanocytose

RESUME Des échantillons de selles ont été recueillis chez des personnes admises dans trois hôpitaux de Bassora de novembre 1997 à mai 1998. Sur 40 patients atteints de drépanocytose, 25 (62,5 %) avaient une parasitose intestinale. Dans le groupe témoin de patients apparemment en bonne santé, 26 des 175 sujets (14,8 %) avaient une parasitose intestinale, une différence statistiquement significative. Les parasites les plus couramment isolés chez les patients atteints de drépanocytose étaient *Blastocystis hominis* (36 %) et *Giardia lamblia* (28 %). Le taux d'isolement d'espèces de *Cryptosporidium* chez les sujets atteints de drépanocytose (5 %) n'était pas significativement différent de celui chez les sujets apparemment en bonne santé (1,14 %). Pour la première fois, nous signalons l'isolement de *Isospora belli* chez un patient atteint de drépanocytose en Iraq et dans la région de la Méditerranée.

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Introduction

Sickling disorders are among the most common abnormalities affecting the stability and integrity of red blood cells. They comprise a group of genetic diseases resulting from homozygosity of haemoglobin S (HbS), double heterozygosity of HbS and other abnormal haemoglobins, and the presence of HbS in association with various types of thalassaemias [*1*]. HbS is the most common variant of normal adult haemoglobin (HbA). The phenotypic frequency for the S gene is 10%–45% in black Africans, 8%–12% in African–Americans and African–Caribbean populations, and 5%–30% in Greece, Turkey, and parts of India and South-East Asia [*2*]. Individuals, particularly children, with such disorders have an increased susceptibility to bacterial infections, and this is one of the main causes of morbidity and mortality [*3–8*].

Cryptosporidium species have been implicated as an etiological agent in persistent diarrhoea, with significant morbidity and mortality in those with chronic diseases [*9,10*]. Intestinal parasitic infections are relatively common in the developing countries, including Iraq [*11*].

In the absence of previous reports, we aimed to examine the prevalence of such parasitic species in patients with sickle-cell anaemia compared with individuals with no sickle-cell disorder.

Methods

The study involved 40 patients (20 males and 20 females) with sickle-cell anaemia who had been admitted to Abu Al-Khasib General Hospital, Basra General Hospital or Basra Teaching Hospital for blood transfusion or recovery from crisis. Their ages ranged from 2 years to 60 years with a mean of 21.6 ± 17.4 years. As a comparison group, 175 apparently healthy individuals (105 males and 70 females) with no episodes of diarrhoea in the previous 2 months were studied. Their mean age was 23.7 ± 20.1 year.

Single stool samples were collected from these 215 individuals from November 1997 to May 1998. All samples were screened for the presence of non-acid fast parasites by direct smear method and the Ritchie formalin–ether sedimentation concentration method [*12*]. Faecal smears were prepared from the sediment and stained by the modified Ziehl–Neelsen method [*12*] for the detection of red-pink oocysts of acid-fast parasites (*Cryptosporidium*, *Cyclospora* and *Isospora* species).

The SND (standard normal deviate) test was used as a test of significance. Differences were recorded as significant when $P > 0.05$. The odds ratio and 95% confidence intervals of the odds ratio were estimated for a single 2 × 2 table (to determine whether there was a crude disease exposure association).

Results

Table 1 shows the distribution of parasitic infections in the sickle-cell patients and the comparison group. Of the 40 sickle-cell patients examined, 25 (62.5%) were found to be infected with intestinal parasites (including *Cryptosporidium* species). In the comparison group only 26 (14.8%) individuals were infected (Table 1). The difference was statistically significant (SND = 6.44, $P < 0.01$, odds ratio = 9.55). Of the 40 sickle-cell patients, only 2 (5.0%) were found to be only excreting *Cryptosporidium* oocysts. The *Cryptosporidium* infection rate among the comparison group was 1.14%

Table 1 **Distribution of parasitic and *Cryptosporidium* infections among the sickle-cell patients and an apparently healthy comparison group according to age**

Age group (years)	Sickle-cell patients					Healthy comparison group				
	No. examined	*Cryptosporidium* infection		Parasitic infection		No. examined	*Cryptosporidium* infection		Parasitic infection	
		No.	%	No.	%		No.	%	No.	%
<6	10	1	1.0	7	70.0	60	1	1.7	9	15.0
6–15	8	0	0	7	87.5	20	0	0	5	25.0
16–25	8	0	0	5	62.5	20	0	0	4	20.0
26–35[a]	5	0	0	2	40.0	17	0	0	4	23.5
36–45	4	0	0	2	50.0	25	0	0	2	8.0
46–55	3	1	33.3	1	33.3	18	0	0	1	5.6
56–65	2	0	0	1	50.0	15	1	6.7	1	6.7
Total	40	2	5.0[a]	25	62.5[b]	175	2	1.14[a]	26	14.8[b]

[a] *SND = 1.77, P > 0.05.*
[b] *SND = 6.44, P < 0.01, odds ratio = 9.55.*

but the difference was not statistically significant (SND = 1.77, $P > 0.05$).

Table 2 shows the distribution of the different parasitic infections in the sickle-cell and comparison groups by sex. The most common parasites identified in the sickle-cell group were *Blastocystis hominis* (36%) and *Giardia lamblia* (28%). A single case of *I. belli* was diagnosed in a male sickle-cell patient. The infection rate was

Table 2 **Distribution of parasitic infections including acid-fast protozoa among the sickle-cell patients and an apparently healthy comparison group according to sex**

Parasites	Sickle-cell patients ($n = 40$)			Healthy comparison group ($n = 175$)		
	Male	Female	Total	Male	Female	Total
Single infection						
Cryptosporidium species	1	1	2	–	–	–
Isospora belli	1	–	1	–	–	–
Blastocystis hominis	5	4	9	9	2	11
Giardia lamblia	5	2	7	3	0	3
Entamoeba histolytica	1	2	3	1	1	2
Enterobius vermicularis	–	1	1	2	4	6
Trichiuris trichiura	–	1	1	–	–	–
Taenia saginata	–	1	1	–	–	–
Mixed infection						
Cryptosporidium + *B. hominis*	–	–	–	1	1	2
B. hominis + *G. lamblia*	–	–	–	1	–	1
B. hominis + *E. vermicularis*	–	–	–	–	1	1
Total no. (%)	13 (32.5)	12 (30.0)	25 (62.5)	17 (9.7)	9 (5.1)	26 (14.8)

similar in both males and females within each group (Table 2).

Discussion

Our results show that patients with sickle-cell anaemia are much more susceptible to both acid- and non-acid-fast parasitic infections in comparison with the non-sicklers (odds ratio = 9.55). Patients under 25 years were particularly affected by intestinal parasites, possibly due to the severity of the sickle-cell anaemia in this age group.

Thus parasitic infections, including cryptosporidiosis, can be added to the long list of bacterial infections, such as pneumonia, osteomyelitis, meningitis, septicaemia, typhoid fever and urinary tract infections [*1–8,13–15*] to which sickle-cell patients are vulnerable. Such infections can be considered a main cause of morbidity and mortality and may be life-threatening to those with chronic disease such as sickle-cell anaemia or in individuals with certain immunological conditions [*1,2,16*].

To our knowledge, we report the first cases of cryptosporidiosis and isosporiasis among sickle-cell patients in Iraq and the Mediterranean region. The combination of formalin–ether sedimentation and modified acid-fast stain methods clearly provides high sensitivity and specificity [*10*].

The rate of parasitic infection found here is of significance from the public health point of view, and the actual rate is likely to be higher due to the intermittent shedding of acid-fast oocysts [*17*] and *G. lamblia* [*18*].

Bacterial and parasitic infections are a major presenting manifestation in sickle-cell patients [*19*]. The mechanisms contributing in the increased vulnerability to infection are splenic dysfunction, decreased IgM levels and a defective alternative complement pathway [*20*]. The spleen has a dual role in infection: biological filtrating and phagocytosis of microorganisms, and production of specific IgM antibodies and tuftsin [*2*]. Some sickle cells cause blockage of the vessels supplying blood to an organ, leading to hypoxia of the organ and resultant cell death and progressive scarring. This forms an ideal site for the growth of certain bacteria and leads to an increased susceptibility to infections [*1*]. These patients have also been shown to have decreased levels of functionally active factor B, due to an increased rate of factor B catabolism [*21,22*]. Inadequate levels of C_3b lead to deficient opsonic activity and inefficient phagocytosis [*2*].

Our observations in sickle-cell patients suggest that both acid- and non-acid-fast intestinal parasites should be considered in the differential diagnosis of undiagnosed chronic diarrhoea in order to arrive at the proper treatment. Management of complications, including infections, will improve the quality of life of such patients.

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