

Sudan University of Science and Technology
College of Graduate Studies



**Relationship between Gastrointestinal Parasites and
Irritable Bowel Syndrome in Khartoum state**

**العلاقة بين الطفيليات المعوية ومتلازمة المصران العصبي (المتهيج) في
ولاية الخرطوم**

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الآية

بسم الله الرحمن الرحيم

قال تعالى: ﴿يَا أَيُّهَا الَّذِينَ آمَنُوا أَنْ تَخْشَعَ قُلُوبُهُمْ
لِذِكْرِ اللَّهِ وَمَا نَزَلَ مِنَ الْحَقِّ وَلَا يَكُونُوا كَالَّذِينَ أُوتُوا
الْكِتَابَ مِنْ قَبْلُ فَطَالَ عَلَيْهِمُ الْأَمْتُ فَفَسَسَتْ قُلُوبُهُمْ
وَكَثِيرٌ مِنْهُمْ فَاسِقُونَ﴾.

سورة الحديد الآية 16

Dedication

I dedicate this work to

The one who covered me with love and support

My mother.

The one who gave my life meaning, my guide.....

My father.

*Those who habitually support me and lead me to achieve my
dreams...*

My brother.

*Those who always hope to see me big star in the laboratory
sky.....*

My sisters.

*Those who made my way beacons, example of fidelity, morality
and altruism....*

My teachers.

*Those who enlighten my way and who stand with me step by
step...*

My friends and my colleagues.

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Abstract

This study was conducted in different hospitals in Khartoum state. The study involved two hundred stool samples collected from 100 patients with irritable bowel syndrome (IBS) and another 100 as controls, during the period between February and June (2016). The results showed that 51 (51%) of irritable bowel syndrome patients as well as 33 (33%) of the control were harboring gastrointestinal parasites.

The study revealed that *Entamoeba histolytica* was seen in 22% of the IBS cases followed by *Entamoeba coli* in 18%, *Giardia lamblia* in 16%, *Chilomastix mesnili* in 4%, *Hymenolepis nana*, *Taenia spp* and *Enterobius vermicularis* in 3% each and *Ascaris lumbricoides* in 1%. Lower rates were reported among the control group where *Entamoeba histolytica* was seen in 19% of the control followed by *Entamoeba coli* in 15%, *Giardia lamblia* in 11%, *Hymenolepis nana* in 2%, *Chilomastix mesnili*, hook worm and *Ascaris lumbricoides* in 1% each.

The results demonstrated that the prevalence rate of gastrointestinal parasites among males and females was almost close (23% and 28% respectively).

On the other hand, the prevalence of gastrointestinal parasites among males and females in the control group was found to be 18% and 15% respectively.

The highest prevalence rate (63.6% and 57.6%) were found among the 31-40 and 21-30 years age groups respectively, while the lowest rate (38.5%) was found among the 11-20 years age group for the IBS patients. For the control group, the highest prevalence rate (66.6%) was found among the 0-10 years age group, while the lowest prevalence rate (18.5%) was found among the 21-30 years age group.

The highest prevalence rates (63.6%) was found among the illiterate group, while the lowest rate (20%) was found among the primary school group for the IBS patients. For the control group, the highest prevalence rate (63.6%) was found among the university graduate group, while the lowest prevalence rate (17.6%) was found among the illiterate group.

When using different techniques for all samples (IBS patients and controls), formal ether concentration technique proved to detect higher rates of different parasites encountered, the highest rate was for *Entamoeba histolytica* (20.5%), followed by *Entamoeba coli* (16.5%). The result also showed that using floatation technique was useless in recovering any single case of *Hook worm*, *Ascaris lumbricoides*, *Taenia spp* and *Enterobius vermicularis*.

مستخلص الدراسة

تم إجراء هذا البحث في المستشفيات المختلفة من ولاية الخرطوم، حيث شملت الدراسة 200 عينة براز جمعت من 100 مريض مصاب بالمصران المتهيج (العصبي) ومن 100 آخرين لا تظهر عليهم أعراض المصران العصبي في خلال الفترة من فبراير إلى يونيو 2016. أظهرت النتيجة أن 51 (51%) من مرضى المصران العصبي مصابين بالطفيليات المعوية وكذلك في 33 (33%) من الأشخاص السليمين.

أظهرت الدراسة أن *Entamoeba histolytica* ظهرت في 22% من حالات المصران العصبي يليها *Entamoeba coli* في 18% من الحالات، *Giardia lamblia* في 16% ، *Chilomastix mesnili* في 4% ، *H. nana* ، *Taenia spp* و *E.vermicularis* في 3% من الحالات لكل، و *Ascaris lumbricoides* في 1% .

سجلت معدلات أقل في الأشخاص السليمين ، *Entamoeba histolytica* ظهرت في 19% من الحالات، تليها *Entamoeba coli* في 15% ، *Giardia lamblia* في 11% ، *H. nana* في 2% ، *Hook worm* و *Ascaris lumbricoides* في 1% من الحالات لكل. بينت الدراسة أن معدل الانتشار للطفيليات المعوية كان متقارباً في الإناث والذكور (28% و 23% على التوالي).

من جهة أخرى، كان معدل الانتشار في الأشخاص السليمين في الإناث والذكور 15% و 18% على التوالي.

أعلى معدل انتشار (63.6% و 57.6%) في حالة المصابين بالمصران العصبي سجل في الفئات العمرية 31-40 و 21-30 على التوالي، بينما أقل معدل 38.5% فقد تم تسجيله فالفئة العمرية 11-20 سنة.

في الأشخاص السليمين، تم تسجيل أعلى معدل انتشار 66.6% في الفئة العمرية 0-10 سنوات، بينما أقل معدل انتشار 18.5% تم تسجيله في الفئة العمرية 21-30 سنة.

أعلى معدل انتشار (63.6%) في حالة المصابين بالمصران العصبي تم تسجيله في فئة الأميين، بينما أقل معدل انتشار (20%) تم تسجيله في فئة المدارس الابتدائية. في الأشخاص السليمين تم تسجيل أعلى معدل انتشار (63.6%) في فئة الجامعيين، بينما أقل معدل انتشار (17.6%) تم تسجيله في فئة الأميين.

عند استخدام تقنيات مختلفة لكل العينات (مرضى المصمران العصبي المصابين وغير المصابين)، تقنية التركيز بالفورمالين والكحول أثبت قدرته على التحقق من نسبة عالية من الطفيليات المعوية المختلفة التي وجدت.

أعلى معدل سجل كان *Entamoeba histolytica* (20.5%) وتليها *Entamoeba coli* (16.5%). وأظهرت الدراسة أيضا أن تقنية الطفؤ باستعمال الصوديوم كلورايد المشبع لم تظهر أي نتيجة موجبة لكل من *Hook worm*، *Ascaris lumbricoides*، *Taenia spp* و *Enterobius vermicularis*.

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CHAPTER ONE
INTRODUCTION AND
LITERATURE REVIEW

Chapter one

Introduction and literature review

1.1 Introduction:

About one third of the world (more than two billion people) is infected with intestinal parasites (Chan, 1997). Poverty, illiteracy, poor hygiene, lack of access to potable water, and hot and humid tropical climate are some of the common factors attributed to intestinal parasitic infections (IPI). About 39 million disability adjusted life years (DALYs) are attributed to IPI and thus, represents a substantial economic burden due to these infections (Stephenson *et al*, 2000). Intestinal parasites are parasites that populate the gastrointestinal tract. The term is not merely a collective term but it can include a group of diverse parasites that vary greatly in many aspects e.g: biology, pathology and epidemiology (WHO, 1985). Taxonomically, the intestinal parasites are composed of two major subgroups: Protozoa: it includes *Giardia lamblia*, *Entamoeba histolytica*, *Balantidium coli* and *Cryptosporidium parvum*. Helminthes: the intestinal helminthes are represented by both flat worms and nematodes (WHO, 1985). Intestinal nematodes constitute by far the most common parasitic infection in human. It includes *Ascaris lumbricoides*, *Enterobius vermicularis*, *Strongyloides stercoralis* and *Trichuris trichiura*. In the other branch, it includes flat worms (trematodes and cestodes). They include *Hymenolepis nana* and *Taenia species* (WHO, 1985). Many of the infections of the gastrointestinal tract (GI) are caused by parasites that are cosmopolitan in distribution. Protozoa can be directly infectious for man when they are passed in the feces into the environment, but helminths require a period of maturation while in the soil, where they become infectious. Others such as *Taenia saginata* require the involvement of an intermediate host during their life cycle. Infections of the GI tract account for a high proportion of deaths in infants where the

standards of hygiene and nutrition are low. Fecal-oral transmission of the pathogens is the most common mode of GI infections, whereby water, food and hands become contaminated with fecal material which then come in contact with the mouth (Garcia and Bruckner, 1997).

1.2 Intestinal parasites:

Parasitic infections, caused by intestinal helminthes and protozoan parasites, are among the most prevalent infections in humans in developing countries (Savioli and Albonico, 2004).

1.2.1 Intestinal protozoa:

In developed countries, protozoan parasites more commonly cause gastrointestinal infections compared to helminthes (Savioli and Albonico, 2004). The most common intestinal protozoan parasites are: *Giardia lamblia*, *Entamoeba histolytica*, *Cyclospora cayetanensis*, and *Cryptosporidium spp.* The diseases caused by these intestinal protozoan parasites are known as giardiasis, amoebiasis, cyclosporiasis, and cryptosporidiosis respectively, and they are associated with diarrhea (Davis *et al*, 2002). *G. lamblia* is the most prevalent parasitic cause of diarrhea in the developed world, and this infection is also very common in developing countries. Amoebiasis is the third leading cause of death from parasitic diseases worldwide, with its greatest impact on the people of developing countries. The World Health Organization (WHO) estimates that approximately 50 million people worldwide suffer from invasive amoebic infection each year, resulting in 40-100 thousand deaths annually (WHO, 1997). Cryptosporidiosis is becoming most prevalent in both developed and developing countries among patients with AIDS and among children aged less than five years. Several outbreaks of diarrhoeal disease caused by *C. cayetanensis* have been reported during the last decade (Herwaldt, 2000).

1.2.1.1 *Entamoeba histolytica*:

E. histolytica is an aerobic parasitic protozoan belonging to the genus *Entamoeba* and an etiology agent of amoebiasis. *E. histolytica* is pathogenic in the caecum and colon of human being. The term '*histolytica*' literally means "tissue dissolving" referring to the carnivorous habit of the organism. *E. histolytica* is the most unique among the Amoebas because of its ability to hydrolyse host tissue. It can become a highly virulent and invasive organism causing diarrhea. Acute infection of amoebiasis may be presented with other infection apart from bloody diarrhea such as ulceration of the colonic mucosa, abdominal pain and a palpable mass in corresponding areas of the abdomen. Amoebiasis may give rise to amoebic liver abscess and intestinal pathologies (Aribodor *et al*, 2012).

Entamoeba histolytica infection is one of the most common parasitic infections worldwide, infecting about 50 million people, often in developing countries, resulting in 40,000 to 100,000 deaths per year. It has long been known that although about 500 million people each year have amoebiasis, only about 10% experience symptomatic disease (WHO, 1997). There are only two stages to the life cycle of *E. histolytica*, the infectious cyst stage and the multiplying, disease-causing trophozoite stage. In the majority of cases, infection results from the ingestion of fecally-contaminated water or food that contains *E. histolytica* cysts. Much less often the cyst or the trophozoite forms can be transmitted as a result of oral or oral/anal sexual practices (Abhay *et al*, 2009). Trophozoites vary remarkably in size from 10 to 50 μm in diameter and when they are alive they may be actively motile (Mahon and Manuselis, 2000). The amoebae are anaerobic organisms which do not have mitochondria. The finely granular endoplasm contains the nucleus and food vacuoles, which in turn may contain bacteria or red blood cells. The

parasite is sheathed by a clear outer ectoplasm. The nucleus has a distinctive central karyosome and a rim of finely beaded chromatin lining the nuclear membrane (Sodeman and William, 1990). The cyst is a spherical structure, 10 to 20 µm in diameter, with a thin transparent wall (Sodeman and William, 1990). Fully mature cysts contain four nuclei with the characteristic amoebic morphology. Rod like structures (chromatoidal bars) are present variably, but are more common in immature cysts. Inclusions in the form of glycogen masses also may be present (Beaver *et al*, 1984).

Approximately, 90% of infected individuals are asymptotically colonized, amoebic dysentery usually occurs gradually, with symptoms (such as abdominal pain and tenderness, painful sudden bowel evacuation (tenesmus) and diarrhoea followed by weight loss. Liver abscess is overwhelmingly the most common extra-intestinal manifestation of amoebiasis. The typical patient with amoebic liver abscess is a 20 to 40 years old male with a 1 to 2 week history of fever and diffuse or right, upper quadrant abdominal pain (Manson and Bell, 1987). The less common extra intestinal form of invasive amoebiasis was CNS, lung, pericardium and genitourinary system (Manson and Bell, 1987).

1.2.1.2 *Giardia lamblia*:

Giardia lamblia, also called *Giardia intestinalis* and *Giardia duodenalis*, is one of the most common intestinal parasites in the world, occurring in both industrialized and developing countries with an estimated 2.8 million new cases annually. First observed by Anton Van Leeuwenhoek in 1681 in a sample of his own diarrheal stool, and later described in greater detail by Vilem Lamble, *Giardia* was initially thought to be commensal and has only been recognized as a pathogen since the mid 1900s (Abhay *et al*, 2009).

G. lamblia is the most commonly isolated intestinal parasite throughout the world. Prevalence rates of 20-40% are reported in developing countries, especially in children (Fraser, 1994).

Giardia transmission occurs by the fecal-oral route, either directly, via person to person contact or indirectly, via contamination of surface water or food. The salient features of *Giardia* cysts that influence disease transmission include their stability in the environment, their immediate infectivity upon leaving the host and the small number of cysts required to cause infection (Abhay *et al*, 2009). *Giardia lamblia* has a simple life cycle consisting of two stages: trophozoite and cyst, cysts are the transmission stage and are excreted in the feces of infected individuals into the environment where they can survive for weeks (Abhay *et al*, 2009).

Trophozoite of *Giardia lamblia* has two nuclei and each nucleus contains a prominent karyosome, giving the parasite its characteristic face like appearance. In addition, it has four pairs of flagella, an axostyle (a microtubule containing organelle to which the flagella attach), a ventral disk and two median bodies, organelles whose function is not known. Cysts, which are slightly smaller than trophozoites, have a carbohydrate rich cell wall which likely protects them from the environment and two to four nuclei (Abhay *et al*, 2009).

Giardia infection may be asymptomatic or it may cause disease ranging from self limiting diarrhoea to a severe chronic syndrome (Meyer, 1990). The length of the incubation period, usually 1 to 3 weeks, depends at least partly on the number of cysts ingested. Normal human hosts with giardiasis may have any or all of the following signs and symptoms: loose, foul smelling stools, steatorrhea (fatty diarrhea), malaise, abdominal cramps, excessive flatulence, fatigue and weight loss or a coeliac disease like syndrome (Washington *et al*, 2006). Although most

cases are seen in hosts with some concurrent condition, such as an immune deficiency, protein calorie malnutrition, or bacterial overgrowth of the small intestine, some cases of severe giardiasis occur in apparently normal hosts. Different strains of *G. lamblia* possibly vary in virulence (Smith and Wolfe, 1980).

A small percentage of symptomatic individuals will have chronic infection, lasting months or longer, Chronic giardiasis is frequently accompanied by weight loss, which can be significant, and malabsorption of fats, vitamins A and B12, disaccharides, especially lactose, and protein are observed, with malabsorption of fats and lactose being most common (Abhay *et al*, 2009).

1.2.1.3 The coccidia:

The coccidia are small protozoa within the subphylum sporozoa. The important intestinal coccidia include *Cryptosporidium parvum* and *Isospora belli*. They are obligate tissue parasites with sexual and asexual stages in their life cycle (Washington *et al*, 2006).

1.2.1.3.1 *Cryptosporidium parvum*:

Members of the genus *Cryptosporidium* (Apicomplexa, Cryptosporidiidae) are small coccidian protozoan parasites that infect the microvillus region of epithelial cells in the digestive and respiratory tracts of vertebrates (Palmer, 1990). Although the first human cases of *Cryptosporidium* were described in 1976, the contribution of this protozoan parasite to gastrointestinal disease was not fully appreciated until the 1980s when scores of cases were described among patients with acquired immunodeficiency syndrome (AIDS) (Abhay *et al*, 2009). *Cryptosporidium* was initially thought to be an opportunistic pathogen of immune compromised persons, but a number of water borne out breaks, plus frequent cases in immunocompetent individuals, have disproved this. Indeed, *C. parvum* is now one of the most commonly identified intestinal

pathogens throughout the world. Its occurrence is dependent on factors that include season, and the age and other demographic characteristics of a population: among children aged 1 to 5 years with diarrhoea, *C. parvum* may be the most frequently found pathogen (Palmer, 1990). The organism is transmitted by the fecal oral route. Out breaks have most commonly been associated with person to person (day care center) and water borne (drinking and recreational water) modes of spread. Food borne and animal (especially calves) to person spread has also been documented, infected hosts shed oocysts, and these oocysts are spherical with a diameter of 4 to 6 microns. Thick and thin walled oocysts are formed. The thin walled oocysts may excyst within the same host and start a new life cycle (autoinfection) (Fayer and Ungar, 1986).

The illness is usually symptomatic, but self limited. Major symptoms such as acute or chronic profuse watery diarrhea and abdominal pain commonly occur in children under the age of five years and immunocompromized patients, especially those with HIV/AIDS. Frequent exposure is associated with development of immunity, and this reduces symptoms in infected individuals (Tadesse *et al*, 2008).

1.2.1.3.2 *Isospora belli*:

Isospora belongs to the coccidia subclass in the family Eimeridae and in the phylum Apicomplexa, *Isospora belli* is only known to infect humans (Soave and Johnson, 1988). *I. belli* is distributed world wide particularly endemic in

tropical and subtropical regions but has been infrequently detected in stool specimens. Although the infection frequently occurs in the immunocompromized patient, it can also cause disease in adults and children (Garcia and Bruckner, 1997). The life cycle of *Isospora belli* is similar to that of *Cryptosporidium parvum*, except that oocyst maturation occurs in the external environment after sporulation before it becomes

infective (Tadesse *et al*, 2008). Infection is acquired by consumption of infective oocysts through fecal-oral route (Tadesse *et al*, 2008).

Oocysts are passed in feces unsporulated or partially sporulated (sporoblast stage). They can sporulate in less than 24 h. Oocysts are elongate and ellipsoidal with slightly tapered ends, or one end may be tapered and the other end blunts (Mojon *et al*, 1981).

Acute infection begins abruptly with fever, abdominal pain and diarrhea. It is usually self limited, but its manifestations resemble that of cryptosporidiosis (with chronic, profuse diarrhea) in patients with immunodeficiency such as HIV/AIDS (Tadesse *et al*, 2008).

Diarrhea produced by *I. belli* in AIDS patients is often very fluid and secretory like and leads to dehydration requiring hospitalization. Fever and weight loss are also common findings (Mojon *et al*, 1981).

1.2.1.4 Diagnosis of intestinal protozoa:

Intestinal protozoa are diagnosed by identifying cysts or trophozoites in fecal specimens or histologically by visualizing cysts in biopsy specimens or secretions of intestinal mucosa (Sheehan *et al*, 1979). The conventional direct wet mount preparation for microscopy to identify motile trophozoites and a formalin ethyl acetate concentration step to identify cysts (Qvarnstrom *et al*, 2005), and when appropriately conducted with or without iodine stain, the conventional wet mount establishes the diagnosis of *Giardia lamblia* in up to 70-85% of cases after two stool examinations (Sodeman and William, 1990). The sensitivity of the acid fast stain for oocysts of *Cryptosporidium* in the direct examination of stools is approximately 30% after one sample examination (Sodeman and William, 1990).

Serological diagnostic methods have been developed such as the Immunofluorescence (IF), Enzyme Linked Immunosorbent Assay

(ELISA), culture and subsequent differentiation by isoenzyme analysis and the Polymerase Chain Reaction (PCR) (Nunez *et al*, 2001).

1.2.2 Intestinal helminthes:

Intestinal worm infections are widely prevalent in tropical and subtropical countries and occur where there is poverty and poor sanitation. Soil transmitted helminthes (STH) infections form the most important group of intestinal worms affecting two billion people world wide and the main species are *Ascaris lumbricoides* (roundworms), *Trichuris trichiura* (whip worms) and *Necator americanus*/*Ancylostoma duodenale* (hookworms) (Sodeman and William, 1990).

Recent estimates suggest that *A. lumbricoides* can infect over a billion, *T. trichiura* 795 million, and hookworms 740 million people (de Silva *et al*, 2003). Other species of intestinal helminthes are not widely prevalent. Intestinal helminthes rarely cause death. Instead, the burden of disease is related to less mortality than to the chronic and insidious effects on health and nutritional status of the host (Stephenson *et al*, 2000). In addition to their health effects, intestinal helminthes infections also impair physical and mental growth of children, thwart educational achievement, and hinder economic development (Drake *et al*, 2000).

1.2.2.1 *Taenia* species:

Taenia spp are long, segmented, parasitic tapeworms (family: Taeniidae, subclass: Cestoda). These parasites have an indirect life cycle, cycling between a definitive and an intermediate host (Beaver *et al*, 1984).

Taenia infections are estimated to affect 100 million people world wide, with major endemic areas located primarily in the developing countries of South America, Africa, India, China and Southeast Asia. *Taenia* infections are less common in North America; however, neurocysticercosis has been recognized as an important health problem in California. Although this disease is mainly seen in migrant workers from

Latin American, it has also been reported in US residents who have not traveled to endemic countries (Abhay *et al*, 2009).

Taenia species are hermaphrodite and are very long enough to measure in metres (*T. solium* measure 3-5 meters while that of *T. saginata* measures 5- 10 meters). The body is divided into head, neck and a long segmented body (strobilla). The head size is about 1 mm in diameter. Head of both species bears four suckers. The head of *T. solium* has a rostellum armed with hooklets. In contrast, the head of *T. saginata* does not have the armed rostellum instead a depression. Each segment contains independent male and female sex organs. The terminal mature segments measuring 15-25 x 5-7 mm keep on detaching from the body and are passed in the feces. There are more than 15 lateral uterine branches in each segment of *T. saginata* whereas the *T. solium* segment contains less than 15 lateral uterine branches. The eggs are golden brown in color, measure 30-40 μ m in diameter and are indistinguishable morphologically. The onchosphere bears three pairs of hooklets (Rai *et al*, 1996). Humans become infected after eating uncooked or under cooked beef or pork containing cysticerci. After ingestion, the cysticerci attach to the intestinal mucosa and develop into adult worms (Tadesse *et al*, 2008).

Taeniasis is an infection with the adult tapeworm which usually remains confined to the small intestine. Most often, such infection results in minor gastrointestinal irritation and is frequently accompanied by nausea, diarrhea, constipation, hunger pains, chronic indigestion and passage of proglottids in the feces. Although these symptoms are usually milder when the infection is caused by *T. solium*, the risk of developing cysticercosis remains high (Abhay *et al*, 2009).

1.2.2.2 *Hymenolepis nana*:

Hymenolepis nana (dwarf tapeworm) is a common human parasite and the smallest tapeworm known to infect humans. The lifecycle of *H. nana*

does not require an intermediate host, complete development occurring within a single host (Tadesse *et al*, 2008).

Hymenolepis nana is widely distributed in countries with warm climates including those of Africa, South America, Mediterranean region, and South

East Asia, the infection is more frequently seen in children although adults are also infected (Tadesse *et al*, 2008).

Adult worm measures 1-3 cm in length. It is made up of head (scolex), neck and segmented body. The head carries four suckers and a rostellum armed with one row of hooks. The segments of the body are divided into mature and gravid segments. In the mature segment, there are three testes in the middle. The egg, which is immediately infective when passed by the patient, is rounded, about 40 microns in diameter. It contains a six hooked oncosphere within a rigid membrane (the embryophore). This embryophore has two polar thickening or knobs from which project 4 to 8 long, thin filaments called polar filaments (Assafa *et al*, 2004).

Infection in man takes place by ingestion of eggs through contaminated foods or drinks. Autoinfection (the onchosphere hatched while the eggs being inside the intestine penetrate the villi and develop into cysticercoid larva that later develop into adult worm) also occurs (Rai *et al*, 1996).

Light infections produce no symptoms. In fairly heavy infections, children may show lack of appetite, abdominal pain and diarrhea (Assafa *et al*, 2004).

1.2.2.3 *Ascaris lumbricoides*:

Round worm (*Ascaris lumbricoides*) is the largest of the human intestinal parasites. It lives and matures in the ileum and sometimes jejunum of the small intestine. Roundworm is often regarded as a parasite of children, but people of all ages may be infected (Obeng, 1997).

Ascariasis, a soil transmitted infection, is the most common human helminthes infection. Current estimates indicate that more than 1.4 billion people are infected world wide. In the United States, there are an estimated 4 million people infected, primarily in the southeastern states and among immigrants (Abhay *et al*, 2009).

Important factors associated with an increased prevalence of disease include socio-economic status, defecation practices and cultural differences relating to personal and food hygiene as well as housing and sewage systems. Most infections are sub clinical; more severe complications occur in children who tend to suffer from the highest worm burdens (Abhay *et al*, 2009).

Round worm is long, cylindrical and tapers toward both ends. Female worms are longer than the males. Females measure from 200 to 400 mm long and the males are 150 to 300 mm (Obeng, 1997). At the anterior end, there are three prominent lips with dentigerous ridge. Posterior end of male is curved ventral. The tail is bluntly pointed. The spicules in male genital organ are simple and measure 2-3 mm in length. In female, vulva is present at about one third of the body length from the anterior end (Assafa *et al*, 2004). Since the sexes are separate, it requires infection with both male and female worms to produce fertile eggs in the host. It has been reported that, generally, infected persons harbor more females than male worms with an estimated ratio of 10 female worms to 3 male worms. There is always the possibility that a host may be infected only by female or by male worms. In such cases, the female worms produce the unfertilized eggs, which are incapable of developing further. Each female worm lays about 200,000 eggs per day, for as long as she is fertilized and in the intestine. Adult worms in the human host live for less than 10 months, with maximum life spans of up to 1.5 years. The fertile eggs are ovoid and measure 45 to 70 micrometers by 35 to 50 micrometers in size.

Each has a protective durable shell. The eggs are discharged into the lumen of the intestine and leave the host with the feces into the environment (Obeng, 1997). Infection in man takes place by ingestion of embryonated eggs through contaminated food or drinks (unfertilized eggs are non infectious) (Assafa *et al*, 2004).

The adult worm normally feeds on partly digested food from the intestine in humans. It has been reported that the host (having about 26 worms) may lose 10 per cent of his/her total daily intake of protein (Obeng, 1997).

Although most individuals infected with *Ascaris lumbricoides* are essentially asymptomatic, the burden of symptomatic infection is relatively high as a result of the high prevalence of infection on a worldwide basis. Symptomatic disease is usually related to either the larval migration stage or manifests as pulmonary disease, or to the intestinal stage of the adult worm (Abhay *et al*, 2009). The early symptoms of round worm infection are a pneumonitis with cough and sometimes blood stained sputum (which may contain larvae), dyspnea, wheezing, persistent non productive cough, substernal pain, fever and diarrhea; These symptoms begin 5 to 6 days after infection, usually last 10 to 12 days and are caused by the round worm larvae migrating and developing inside the human body. A heavy presence of adult worms in the small intestine may cause digestive disorders, nausea, abdominal pain, vomiting, restlessness and disturbed sleep (Obeng, 1997).

The large size of the adult worms also presents problems, especially if the worms physically block the gastrointestinal tract. *Ascaris* seems to be especially sensitive to anesthetics, and numerous cases have been documented where patients in surgical recovery rooms have had worms migrate from the small intestine, through the stomach, and out the patient's nose or mouth (Assafa *et al*, 2004).

1.2.2.4 *Enterobius vermicularis*:

Enterobius vermicularis commonly referred to as pin worm, has the largest geographical distribution of any helminth. Discovered by Linnaeus in 1758, it was originally named *Oxyuris vermicularis* and the disease was referred to as oxyuriasis for many years. It is believed to be the oldest parasite described and was recently discovered in ancient Egyptian mummified human remains as well as in DNA samples from ancient human coprolite remains from North and South America (Abhay *et al*, 2009). *Enterobius* is one of the most prevalent nematodes in the United States and in Western Europe. At one time, in the United States there are an estimated 42 million infected individuals. It is found worldwide in both temperate and tropical areas. Prevalence is highest among the 5-10 year-old age group and infection is uncommon in children less than two years old. Enterobiasis has been reported in every socioeconomic level; however, spread is much more likely within families of infected individuals, or in institutions such as child care centers, orphanages, hospitals and mental institutions. Humans are the only natural host for the parasite (Abhay *et al*, 2009).

Enterobius vermicularis is a spindle shaped parasite of man and attaches to the mucosa of the lower ileum, ceacum and ascending colon. Pinworm eggs are infective shortly after being excreted. After ingestion, the eggs hatch in the upper intestine and liberate larvae which migrate to the region of the ileum. Copulation (mating) of the worms takes place in the lower small intestine, and then the females migrate to the ceacum or lower bowel and pass through the anus where upon contact with the air they shower their sticky eggs on the perianal skin (Tadesse *et al*, 2008). The eggs of *E. vermicularis* naturally transparent and colorless, measure 50-60 x 20-30 mm in size, and are ovoid and asymmetrical, one side being more convex than the other. They embryonate in 6 h and can

remain viable for 20 days in the environment (Burkhart and Burkhart, 2005).

Infection is facilitated by factors including over crowding, wearing soiled clothing, lack of adequate bathing and poor hand hygiene, especially among young school aged children. Infestation follows ingestion of eggs which usually reach the mouth on soiled hands or contaminated food. Transmission occurs via direct anus to mouth spread from an infected person or via air borne eggs that are in the environment such as contaminated clothing or bed linen (Abhay *et al*, 2009).

The majority of enterobiasis cases are asymptomatic; however the most common symptom is perianal or perineal pruritus. This varies from mild itching to acute pain. Symptoms tend to be most troublesome at night and as a result, infected individuals often report sleep disturbances, restlessness and insomnia. The most common complication of infection is secondary bacterial infection of excoriated skin. Folliculitis has been seen in adults with enterobiasis. Gravid female worms can migrate from the anus into the female genital tract. Vaginal infections can lead to vulvitis, serous discharge and pelvic pain (Abhay *et al*, 2009).

1.2.2.5 *Trichuris trichiura*:

Trichuris trichiura is an intestinal nematode in the family Trichuridae, also known as whipworm due to its characteristic shape, *Trichuris* can be classified as a soil transmitted helminth because its life cycle mandates embryonic development of its eggs in the soil. It is the second most common nematode found in humans, behind *Ascaris* (Abhay *et al*, 2009). Both larval and adult whipworms are normally found only in the intestines. They do not undergo tissue migration (Beaver *et al*, 1984).

Trichuris trichiura infection is endemic in tropical and subtropical countries, but few sporadic cases have occurred in nonendemic areas, mainly as a result of immigration (Hong *et al*, 2006).

Adult worm measures 30-50 mm in length, males are relatively smaller with coiled posterior end. The anterior part of the body is thin and long (two third of the body length) whereas the posterior part is thick and stout, thus appears as a whip. Mouth is simply an opening and does not contain any lips. Anus is located near the tip of tail. Male has single spicule surrounded by a spiny sheath. One fertilized female worm lays 1,000- 7,000 eggs per day. Eggs are barrel shaped, golden brown in color and measure 50 x 25 mm in size. Embryonation takes place in environment. Approximately 10% of world populations are infected by this parasite. Infection takes place by ingestion of embryonated eggs through contaminated food or drinks (Rai *et al*, 1996).

Trichuriasis is often a symptomatic in humans but heavy infections can cause chronic diarrhea, which may be bloody. Other symptoms may include abdominal pain and distention, nausea, vomiting, flatulence, headache, weight loss, malnutrition and anemia. Nonspecific signs such as nervousness, anorexia and urticaria have been reported in some individuals. Untreated severe infections can lead to clubbing of the fingers in children, through an unknown mechanism. Complications may include rectal prolapse (particularly in children), appendicitis, colitis and proctitis (Beaver *et al*, 1984).

1.2.2.6 Hookworms:

Hookworms are nematodes in the superfamily Ancylostomatoidea, Human hookworm infection is a soil transmitted helminthes infection caused primarily by the nematode parasites *Necator americanus* and *Ancylostoma duodenale*. It is one of the most important parasitic infections worldwide, ranking second only to malaria in terms of its impact on child and maternal health. An estimated 576 million people are chronically infected with hookworm and another 3.2 billion are at risk, with the largest number of afflicted individuals living in impoverished

rural areas of sub-Saharan Africa, Southeast Asia and tropical regions of the Americas. *N. americanus* is the most widespread hookworm globally, whereas *A. duodenale* is more geographically restricted in distribution (Abhay *et al*, 2009). The adult worms are brown at the time of passing. The buccal capsule is big and is armed with cutting plate or teeth. Lips are not present. Males have a conspicuous copulatory bursa, consisting of two broad lateral lobes and a smaller dorsal lobe supported by a fleshy ray. Spicules are simple and needle-like in appearance (Rai *et al*, 1996). Females' worms are bigger than males, Females have simple conical tail. The vulva is located at about three fifth of the body length from the anterior end (Rai *et al*, 1996). The eggs are colorless, oval in shape, measure 70 x 40 mm in size and contain four blastomeres in it. A rhabditiform larva (250-300 mm) is hatched from the egg which further grows into a non feeding filariform larva (500-700 mm) (Rai *et al*, 1996). The route through which both *A. duodenale* and *N. americanus* enter the human host occurs by the infective larvae penetrating the skin of bare feet or hands that have contacted with contaminated soil (Beaver *et al*, 1984). Adult worms bite into the tissues and suck blood. When a hookworm feeds on blood, it releases the secretion of anticoagulant, causing the lesions it created to continue bleeding even when the worm has stopped sucking blood. It has been estimated that a single *N. americanus* is responsible for a mean blood loss of 0.031ml (plus or minus 0.015 ml) per day. Similarly, an *A. duodenale* will cause a mean blood loss of about 0.08-ml (plus or minus 0.02 ml) per day. In case of heavy infections, there may be 500 to 1,000 worms in the host. At that level, the infected person would lose about 50 ml of blood per day (Beaver *et al*, 1984). The clinical features of hookworm infection can be separated into the acute manifestations associated with larval migration through the skin and other tissues and the acute and chronic manifestations resulting from

parasitism of the gastrointestinal tract by adult worms (Abhay *et al*, 2009).

Repeated exposure to *N. americanus* and *A. duodenale* filariform larvae can result in a hypersensitivity reaction known as “ground itch”, a pruritic local erythematous and papular rash that appears most commonly on the hands and feet (Abhay *et al*, 2009).

Symptoms or signs of hookworm infection are abdominal pain, nausea, headache, rash, itching, weakness, fever, vomiting, diarrhea, dysentery and gastrointestinal bleeding. Hookworm infection causes iron deficiency anemia. Anemia is believed to be associated with high risk of maternal mortality and morbidity. In areas of very intense transmission, heavy worm burdens can be built up in early childhood. In such cases, there may be retardation of mental and physical development (Beaver *et al*, 1984).

1.2.2.7 *Strongyloides stercoralis*:

Strongyloides stercoralis also called threadworms is a nematode helminth parasite that causes strongyloidiasis. There are an estimated 100 million to 200 million people infected with *S. stercoralis* residing in 70 different countries. The true prevalence of an *S. stercoralis* infection is under estimated because a majority of the cases are sub-clinical (Vadlamudi *et al*, 2006).

The mortality rate for patients requiring hospitalization with *Strongyloides* infection is about 16.7%. It is endemic in warm moist tropical and sub-tropical climates and is widespread in Eastern Europe, South and Southeast Asia, Central America, South America and sub-Saharan Africa. It has also been reported in a non endemic area (Rivero *et al*, 2006). Strongyloidiasis is more commonly found in institutional settings, rural areas and lower socioeconomic groups (Genta, 1989). The *Strongyloides* life cycle is complicated when compared with other

nematodes alternating between a free living cycle and a parasitic cycle. *Strongyloides* is the only helminthic parasite that secretes larvae (not eggs) in feces (Carvalho *et al*, 2004).

The parasitic female produce thin shelled and partially embryonated eggs (50 x 30 mm) in the intestinal mucosa. The larva measuring 250-300 mm long hatched from the egg comes to the intestinal lumen and then passed in the faeces. In the environment, the larva either develops into a free living adult worm or into an infective filariform larva of 500-700 mm in length. Infection in man takes place by skin penetration by filariform larvae. However, autoinfection (sometimes the larvae hatched inside the intestine develop into the filariform larvae by the time they reach to anus and then enter into the body by penetrating the skin and mucosa around anus) also does occur (Rai *et al*, 1996).

Clinically, strongyloidiasis is significant because infection persists for many years in the human host, usually as an undetected, asymptomatic condition. In areas of endemicity and non endemicity (because of travel, etc.), this prolonged course of infection results in a significant but unsuspected worm load in the infected population. Under specific circumstances (immunosuppression), this undetected worm load produces extensive tissue invasion by parasite larvae, the so called hyper infection syndrome. This syndrome is a serious medical condition that necessitates a high index of suspicion and aggressive, early therapeutic intervention (King, 1993).

The clinical manifestations of uncomplicated strongyloidiasis are cutaneous, Pulmonary and gastrointestinal. Following penetration of the skin, there may be a localized, erythematous, papular, pruritic eruption. Migrating larvae may produce a serpiginous urticarial rash, larva currens that can progress as fast as 10 cm/hr. This is frequently seen on the buttocks, perineum and thighs and may represent autoinfection. Within a

few days of initial infection, pulmonary symptoms such as cough, wheezing or shortness of breath may develop as well as fleeting pulmonary infiltrates and eosinophilia. With the development of gastrointestinal involvement there may be epigastric discomfort suggestive of peptic ulcer disease, bloating, nausea and diarrhea (Abhay *et al*, 2009). In this case, the larva may invade not only the intestinal and the lung tissues, but also the central nervous system, liver and kidneys. Patients may have severe and often bloody diarrhea, bowel inflammation with multiple micro perforations, bacterial peritonitis and paralytic ileus, gram negative sepsis, hemoptysis, pleural effusion and hypoxia, and encephalitis and bacterial meningitis (Tadesse *et al*, 2008).

1.2.2.8 Diagnosis of intestinal helminthes:

Although clinical signs may evoke the suspicion of helminthiasis, diagnosis is still dependent upon the isolation and identification of helminthes from the feces. Adult worms or their segments can also be demonstrated macroscopically when the adult worm is spontaneously passed in stool or vomitus; administration of an antihelminthic drug may result in expulsion of the worm. The definitive methods usually involve microscopic detection of helminth eggs from fecal preparations via smears or after concentration. Microscopy, however, requires trained experts, has low sensitivity for detection of light and moderate infections, and may result in misdiagnosis leading to delayed or inadequate treatment (Verweij *et al*, 2007). Numerous flotation and concentration methods are available, such as the Kato-Katz, formol ethyl acetate sedimentation and zinc sulphate flotation technique techniques (Martin and Beaver, 1968). Harada-Mori filter paper strip technique or charcoal culture method is the method of choice to distinguish the larvae of *A. duodenale* and *N. americanus* on epidemiological ground (Cooper, 1999).

Commercial antibody detection tests are available for some STH infections (Verweij *et al*, 2007).

Colonoscopy is useful for the detection of whipworms in the rectum (Cooper, 1999).

The most successful diagnostic method for *Enterobius* is the “Scotch tape” or “cellophane tape” method; this is best done immediately after arising in the morning before the individual defecates or bathes (Abhay *et al*, 2009).

1.3 Irritable bowel syndrome:

Irritable bowel syndrome (IBS) is a chronic, functional gastrointestinal (GI) disorder which is common among adults in the West with a prevalence of 5-10% in Asia (Gwee *et al*, 2003). IBS is recognized as one of the commonest causes of consultation in gastroenterology clinics worldwide, resulting in a major economic burden for healthcare. The actual cause of IBS is still questionable, but major mechanisms are thought to include psychosocial factors, altered motility and hypersensitivity of the GI tract. Irritable bowel syndrome was defined based on the Rome III criteria (Longstreth and Thompson, 2006). Whereby gastrointestinal symptoms varied from diarrhoea, excessive intestinal gas, abdominal pain and bloating. Recent discovery of persistent, subtle inflammatory changes in adults with post-infectious IBS (i.e. IBS symptoms developing after an acute enteric infection) have alluded to an additional mechanism of intestinal infection mediated-inflammation (Gwee, 2005). Pathogens such as bacteria and parasites have also been a factor in contributing to the symptoms of IBS (Stojanović *et al*, 2011).

To diagnose IBS, a health care provider will conduct a physical exams and take a complete medical history. The medical history will include questions about symptoms, family history of GI disorders, recent

infections, medications, and stressful events related to the onset of symptoms (Grundmann and Yoon, 2010). An IBS diagnosis requires that symptoms started at least 6 months prior and occurred at least three times a month for the previous 3 months. Further testing is not usually needed, though the health care provider may perform a blood test to screen for other problems (Grundmann and Yoon, 2010).

1.4 Irritable bowel syndrome and intestinal parasites:

Tungtrongchitr *et al*, (2004) performed a research to evaluate the prevalence of parasitic infections and the possible association of IBS and parasitic infections. Fifty nine IBS patients were recruited using symptoms based criteria (Rome Criteria II) with an absence of intestinal parasitic infection by direct smear method. Stool samples of individual patients were examined using 7 methods, *i.e.* examination for stool occult blood, simple saline smear method, formalin ether technique, culture for *Blastocystis hominis*, modified trichrome stain, modified Ziehl-Neelsen method and trichrome stain for parasitic and bacterial infections. Of the 59 patients, stool samples of 13 patients (22.1%) were positive for parasites. These were *B. hominis* (13.6%), *Strongyloides stercoralis* larvae (1.7%), *Giardia lamblia* cysts (1.7%), and non pathogenic protozoa, *i.e.* *Endolimax nana* cysts (5.1%). The prevalence rate of parasitic infections in the control group (20%) was not statistically different from the patients. There was no statistical difference between *B. hominis* infection in IBS patients and control was found in this study ($p = 0.87$). In the IBS group, *B. hominis* infection predominated (13.6%), while other parasitic infections were found in 8.5%. The culture method for *B. hominis* is more sensitive than the direct (simple) stool smear method, which is the routine diagnostic method in most laboratories. These results were also found in control group.

In Sudan, a study involved three hundred participants (200 IBS patients and 100 control group) in Khartoum state showed that *Giardia lamblia* was seen in 28.5% of the IBS cases followed by *Entamoeba histolytica* in 9.2% of cases, *Hymenolepis nana* (7%), *Taenia spp* (4%), *Ascaris lumbricoides* and *Entamoeba coli* (3.5% each), the difference in rates was found to be statistically significant ($p=0.004$). Lower rates were reported among the control group where *G. lamblia* was seen in 10% of the cases, *E. coli* in 5% *E. histolytica* in 1%, *H. nana* in 2% and *Taenia spp*. In 2% of the cases the difference was found to be statistically insignificant ($p=0.115$) (Abdalla *et al*, 2014).

Stark *et al*, (2007) have described a possible role for protozoan parasites, such as *Blastocystis hominis* and *Dientamoeba fragilis*, in the etiology of the IBS. They stated that *Dientamoeba fragilis* is known to cause IBS-like symptoms and has propensity to cause chronic infection but its diagnosis relies on microscopy of stained smears, which many laboratories do not perform thereby, leading to the misdiagnosis of *Dientamoeba* in IBS. The role of *B. hominis* as an etiological agent is inconclusive because of the contradictory reports and the controversial nature of *B. hominis* as a human pathogen. Although *Entamoeba histolytica* infections occur predominately in developing regions of the world, clinical diagnosis of amoebiasis is often difficult because symptoms of patients with IBS may mimic those patients with non dysenteric amoebic colitis. They observed that clinical manifestations of *Giardia lamblia* infection are also varying from asymptomatic diarrhea to acute and chronic diarrhea with abdominal pain. They noticed that these IBS-like symptoms can be continuous, intermittent, sporadic or recurrent, sometimes lasting years without correct diagnosis and suggested that it is essential that all patients with IBS undergo routine parasitological

investigations in order to rule out the presence of protozoan parasites as the causative agents of the clinical signs.

Mohemmi *et al* in 2013 indicated that since the infection rate in the case group was higher than that in the control group, a relationship between *Blastocystis hominis* infection and IBS is possible. Speaking about infection diagnosis, culture technique was more sensitive than other techniques used to diagnose *Blastocystis hominis* infection.

Studies from non-Asian countries showed that *Giardia lamblia* infection could lead to development of functional bowel disease, including IBS. In a study from Norway, structured interview and questionnaires given 12-30 months after the onset of *Giardia* infection revealed that 66 of 82 (81%) of patients had symptoms of IBS according to Rome II criteria (Ekdahl and Andersson, 2005).

Association between *Giardia* infection and IBS would be of importance even in non-Asian countries due to the high frequency of giardiasis (5.3 of 100000) in travelers returning from endemic areas. Highest frequencies have been noted among travelers returning from the Indian subcontinent (628 of 100000), East Africa (358 of 100000), and West Africa (169 of 100000) (Chaudhary and Truelove, 1962)

Since as high as 80% of patient contracting *Giardia* infection may develop chronicity and symptoms of IBS, the role of travel-acquired infection with *Giardia* may be of major importance. Initial studies suggested that *E. histolytica* may also play a role in IBS (Taylor *et al*, 1988; Awole *et al*, 2002).

Rationale

Irritable bowel syndrome is a condition that affects the function and behavior of the intestines. Many people experience only mild symptoms of IBS, but for some, symptoms can be severe. Symptoms might include cramping, abdominal pain, bloating, gas, mucus in the stool, diarrhea and/or constipation. However, there is no reliable diagnosis for IBS: symptoms remain the only method for identifying of the disease. Some gastrointestinal parasites are said to be involved in causing IBS. More reseach is needed to prove if there is any associaction. The aim of this study is to investigate the relationship between the occurrence of the IBS and parasitic infections in Khartoum state.

Objectives

General objectives:

- To establish an association (if any) between gastrointestinal parasites and irritable bowel syndrome (IBS).

Specific objectives:

- To determine the prevalence of gastrointestinal parasites in IBS patients according to gender, education level and age group.
- To determine the prevalence of gastrointestinal parasites in the control group according to gender, education level and age group.
- To identify the frequency of each parasite.
- To compare between different parasitological techniques used in the diagnosis of gastrointestinal parasites.

CHAPTER TWO
MATERIALS AND
METHODS

Chapter two

Materials and methods

2.1 Study design:

The study was a case control study.

2.2 Study area:

The study was conducted in different health centers, hospitals and other health facilities in Khartoum state.

2.3 Study population:

The study was carried out on 100 patients presented with irritable bowel syndrome and 100 healthy as the control group selected by physician in hospitals and health centers. The participants were categorized according to gender, education level and age groups. Age groups were as follows: 1-10, 11-20, 21-30, 31-40 and over 40.

2.4 Ethical consideration:

Approval was taken from the faculty and hospital administration. A verbal consent was taken from each patient.

2.5 Study duration:

The study started in 1st february and ended in 18th june of 2016.

2.6 Sample size:

200 stool samples were collected from individuals under study. 100 samples as the study cases and other 100 samples as the study controls.

2.7 Sampling collection:

Each selected patient with irritable bowel syndrome and healthy individuals with out irritable bowel syndrome was provided with a labeled container which is transparent, clean and with wide mouth for faecal sample collection.

2.8 Data collection:

A questionnaire was designed to collect data on gender, education level and age (appendix).

2.9 Methodology:

The direct smear examination, formal ether concentration technique and the

saturated sodium chloride floatation technique were used for the detection of different gastrointestinal parasites.

2.9.1 Direct smear examination:

Wet preparation was made by mixing small portion of stool taken with an applicator wooden stick with a drop of normal saline on slide and covered with cover slip and examined systematically under microscope using 10X and the high magnification 40X for observation of more details.

2.9.2 Formal ether concentration technique:

Approximately, one gram of feces was collected from different parts of the specimen and emulsified in 5 ml of formal saline in glass beaker. Further 5 ml from same solution was added and mixed. The resulting suspension was strained through the sieve. The filtered sample was poured back into a centrifuge tube and then equal volume of ether was added. The tube was mixed for one minute and then centrifuged for 5 minutes at 2000 rpm.

All upper 3 layers were discarded and the sediment was transferred into slide which was covered with cover slip and examined under microscope using 10X and 40X magnifications.

2.9.3 Saturated sodium chloride floatation technique:

Approximately, half gram of feces was collected from different parts of the specimen and emulsified in long glass tube half filled with saturated sodium chloride solution and then the tube was filled with the same solution until convex shape was formed. Carefully, a cover glass was put and air bubbles were avoided. 30 to 45 minutes after, a cover glass was taken and put on clean and dry slide and examined under microscope using 10X and 40X magnifications.

2.9 Data analysis:

The data was analyzed using statistical package for social science (SPSS) computer program version 21.

CHAPTER THREE
RESULTS

Chapter three

Results

The results showed that out of the 100 patients with irritable syndrome, 51 were found infected with gastrointestinal parasites. This constituted an overall infection rate of 51%, while out of the 100 control group (those who were not suffering from IBS), 33 were found infected with gastrointestinal parasites. This constituted an overall infection rate of 33% (table 1). This difference in rates was found to be statistically insignificant at $p=0.076$. The overall prevalence rate among both IBS and control patients was found to be 42% (table 1). In IBS patients, the prevalence among males and females was almost close (23% and 28% respectively), however, this difference was found to be statistically insignificant at $p=0.317$ (table2). On the other hand, the prevalence of gastrointestinal parasites among males and females in the control group was found to be 18% and 15% respectively, however, this difference was found to be statistically insignificant at $p=0.523$ (table 3). The highest prevalence rates (63.6% and 57.6%) were reported among the 31-40 and 21-30 years age groups respectively, while the lowest rate (38.5%) was reported among the 11-20 years age group for the IBS patients (table 4). These differences in rates were found to be statistically insignificant at $p=0.290$. For the control group, the highest prevalence rate (66.6%) was reported among the 0-10 years age group, while the lowest prevalence rate (18.5%) was reported among the 21-30 years age group (table 5). This difference in rates was found to be statistically insignificant at $p=0.071$. The highest prevalence rate (63.6%) was reported among the illiterate group, while the lowest rate (20%) was reported among the primary school group for the IBS patients (table 6). These differences in rates were found to be statistically insignificant at $p=0.467$. For the control group, the highest prevalence rate (63.6%) was reported among

the university graduate group, while the lowest prevalence rate (17.6%) was reported among the illiterate group (table 7). This difference in rates was found to be statistically insignificant at $p=0.128$.

The results revealed that *Entamoeba histolytica* was seen in 22% of the IBS cases followed by *Entamoeba coli* in 18% of cases, *Giardia lamblia* in 16%, *Chilomastix mesnili* in 4%, *Hymenolepis nana*, *Taenia spp* and *Enterobius vermicularis* in 3% each and *Ascaris lumbricoides* in 1% (table 8). Lower rates were reported among the control group where *Entamoeba histolytica* was seen in 19%, followed by *Entamoeba coli* in 15% of controls, *Giardia lamblia* in 11%, *Hymenolepis nana* in 2%, *Chilomastix mesnili*, *Hook worm* and *Ascaris lumbricoides* in 1% each (table 9).

When using different techniques for all samples (IBS patients and controls), formal ether concentration technique proved to detect higher rates of different parasites encountered (table 10). The highest rate reported was for *Entamoeba histolytica* (20.5%), followed by *Entamoeba coli* (16.5%). The table also showed that using floatation technique could not recover a single case of *Hook worm*, *Ascaris lumbricoides*, *Taenia spp* and *Enterobius vermicularis*.

Table 1: The overall prevalence rate of gastrointestinal parasites among patients with irritable bowel syndrome compared to the control groups:

Patients	Number examined	Number positive	Prevalence
Cases	100	51	51%
Controls	100	33	33%
Total	200	84	42%

$p.value=0.076$

Table 2: Prevalence of gastrointestinal parasites among irritable bowel syndrome patients according to gender:

Gender	Number examined	Number positive	Prevalence
Males	50	23	46%
Females	50	28	56%
Total	100	51	51%

p.value= 0.317

Table 3: Prevalence of gastrointestinal parasites among control groups according to gender:

Gender	Number examined	Number positive	Prevalence
Males	50	18	36%
Females	50	15	30%
Total	100	33	33%

p.value= 0.523

Table 4: The prevalence of gastrointestinal parasites among irritable bowel syndrome patients according to age group:

Age groups	Number examined	Number positive	Prevalence
0-10	0	0	0%
11-20	26	10	38.5%
21-30	26	15	57.6%
31-40	22	14	63.6
> 40	26	12	46.2%
Total	100	51	51%

p.vlue=0.290

Table 5: The prevalence of gastrointestinal parasites among control groups according to age group:

Age groups	Number examined	Number positive	Prevalence
0-10	3	2	66.6%
11-20	18	10	55.5%
21-30	27	5	18.5%
31-40	19	5	26.3%
> 40	33	11	33.3%
Total	100	33	33%

p.value=0.071

Table 6: The prevalence of gastrointestinal parasites among irritable bowel syndrome patients according to education level:

Education level	Number examined	Number positive	Prevalence
Illiterate	22	14	63.6%
Primary school	5	1	20%
Secondary school	13	6	46.2%
University graduate	47	23	48.9%
Post university graduate	13	7	53.8%
Total	100	51	51%

p.value= 0.467

Table 7: The prevalence of gastrointestinal parasites among control groups according to level of education:

Education level	Number examined	Number positive	Prevalence
Illiterate	17	3	17.6%
Primary school	12	5	41.7%
Secondary school	15	5	33.3%
University graduate	11	7	63.6%
Post University graduate	45	13	28.9%
Total	100	33	33%

p.value= 0.128

Table 8: Prevalence of different gastrointestinal parasites encountered among irritable bowel syndrome patients:

GIT parasites	Number examined	Number positive	Prevalence
<i>Entamoeba histolytica</i>	100	22	22%
<i>Giardia lamblia</i>	100	16	16%
<i>Entamoeba coli</i>	100	18	18%
<i>Chilomastix mesnili</i>	100	4	4%
<i>Hymenolepis nana</i>	100	3	3%
<i>Taenia spp.</i>	100	3	3%
<i>Ascaris lumbricoides</i>	100	1	1%
<i>Enterobius vermicularis</i>	100	3	3%

Table 9: Prevalence of different gastrointestinal parasites encountered among control groups:

GIT parasites	Number examined	Number positive	Prevalence
<i>Entamoeba histolytica</i>	100	19	19%
<i>Giardia lamblia</i>	100	11	11%
<i>Entamoeba coli</i>	100	15	15%
<i>Chilomastix mesnili</i>	100	1	1%
<i>Hymenolepis nana</i>	100	2	2%
<i>Hook worm</i>	100	1	1%
<i>Ascaris lumbricoides</i>	100	1	1%

Table 10: Comparison between different techniques among all study population:

GIT parasites	Number examined	Number positive (prevalence) by wet preparation	Number positive (prevalence) by FECT	Number positive (prevalence) by floatation
<i>Entamoeba histolytica</i>	200	31(15.5%)	41(20.5%)	25(12.5%)
<i>Giardia lamblia</i>	200	11(5.5%)	27(13.7%)	22(12%)
<i>Entamoeba coli</i>	200	17(8.5%)	33(16.5%)	10(5%)
<i>Chilomastix mesnili</i>	200	3(1.5%)	5(2.5%)	0(0%)
<i>Hymenolepis nana</i>	200	4(2%)	5(2.5%)	5(2.5%)
<i>Hook worm</i>	200	1(0.5%)	1(0.5%)	0(0%)
<i>Ascaris lumbricoides</i>	200	1(0.5%)	2(1%)	0(0%)
<i>Taenia spp</i>	200	0(0%)	3(1.5%)	0(0%)
<i>E.vermicularis</i>	200	2(1%)	3(105%)	0(0%)

p.value = 0.005

CHAPTER FOUR
DISCUSSION,
CONCLUSIONS AND
RECOMMENDATION

Chapter four

Discussion

From the results, it is obvious that the overall prevalence rate of gastrointestinal parasites among patients with IBS was extremely high (51%). This rate was found to be higher than the rate reported by Awole *et, al* (2002) in Ethiopia (34.4%). However, our rate was almost near to rate reported by Abdalla *et, al* (2014) in Khartoum state (56%), Eisa (2005) (56.6%) in Keryab Village and Develoux *et, al* (1986) in Niger (57.5%). On the other hand, our rate was lower than the rate reported by Homeida (1991) in Juba (Southern Sudan) (66%). As far as the control group is concerned, the overall prevalence rate reported was 33%. This rate is lower than all the rates mentioned above. The difference in rates between the control group and patients with IBS was insignificant. This in our opinion might probably reveal that there is no association between the establishment of gastrointestinal parasites and IBS. The prevalence rates in males and females with IBS and control showed insignificant statistical difference with p.value 0.317 and 0.523 respectively. The highest prevalence rates (63.6% and 57.6%) were reported among the age groups 31-40 and 21-30 respectively in the IBS patients and for the control group, the highest prevalence rate (66.6%) was reported among the 0-10 years age group. The findings were in disagreement with Abdalla *et, al* (2014), who reported higher rates among the 11 -20 and 21-40 years age groups.

From table 6, the highest prevalence rate (63.6%) was reported among the illiterate group, while the lowest rate (20%) was reported among the primary school group for the IBS patients. These differences in rates were found to be statistically insignificant ($p=0.467$). For the control group, the highest prevalence rate (63.6%) was reported among the university graduate group, while the lowest prevalence rate (17.6%) was reported

among the illiterate group. This difference in rates was found to be statistically insignificant ($p= 0.128$).

The result also showed that *Entamoeba histolytica* was seen in 22% of the IBS cases followed by *Entamoeba coli* in 18% of cases, *Giardia lamblia* in 16%, *Chilomastix mesnili* in 4%, *Hymenolepis nana*, *Taenia spp* and *Enterobius vermicularis* in 3% each and *Ascaris lumbricoides* in 1%. Lower rates were reported among the control group where *Entamoeba histolytica* was seen in 19% of the control followed by *Entamoeba coli* in 15%, *Giardia lamblia* in 11%, *Hymenolepis nana* in 2%, *Chilomastix mesnili*, hook worm and *Ascaris lumbricoides* in 1% each.

From table 10, different prevalence rates among all study population were reported by different techniques and these differences in rates were found to be statistically significant ($p= 0.005$).

Surprisingly, our result proved no significant association between IBS and intestinal parasites, which fortunately was in total agreement with Morgan *et, al* (2012) who also found no significant association. However, our conclusion was in total disagreement with the findings of Abdalla *et, al* (2014), who reported strong association between IBS and intestinal parasites in Khartoum state.

Conclusions

- Gastrointestinal parasites are highly prevalent among IBS patients compared to the control group.
- Infection rate was slightly higher among females.
- The highest infection rate was reported in the 31-40 and 21-30 age groups among the IBS patients and 0-10 and 11-20 years age groups among the control patients.
- Infection with *G. lamblia*, *E. histolytica* and *E. coli* could probably be associated with bowel irritation and should be considered as potential irritable factor.
- Despite what has been mentioned above, our results revealed no clear significant association between IBS and GIT parasites infection.
- Formal ether concentration technique proved to have high sensitivity rate of detection of different gastrointestinal parasites.

Recommendations

Based on this work, it is recommended that:

- Further investigations are required for irritable bowel syndrome patients with respect to gastrointestinal parasites to elucidate more on the association.
- Treat gastrointestinal parasites in irritable bowel syndrome patients.
- Emphasis should be put on increasing the sample size to avoid errors in interpretation of the results.
- The use of formal ether concentration technique as most reliable technique for detection of different parasitic infections instead of wet preparation in routine diagnosis.

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APPENDICES

Sudan University of Science and Technology
Questionnaire

**Relationship between Gastrointestinal Parasites and
Irritable Bowel Syndrome in Khartoum state**

Name:

Gender: ☐ Male ☐ Female.

Age:..... years.

Education level: ☐ Illiterate.

☐ Primary school.

☐ High secondary school.

☐ University graduate.

☐ Post graduate studies.