Chapter one

Introduction and literature review

1.1 Introduction:

Blastocystis Hominis is a protozoan intestinal parasite belonging to the Blastocystis genus organisms including brown algae, water molds, and diatoms. It has a widespread geographic distribution and is found in countries of all income levels across the world. It's status as a true pathogen is controversial while it has been found in patients with gastrointestinal symptoms it is not proven to be the cause, and many carriers are asymptomatic. Research on *Blastocystis Hominis* is limited, with large gaps remaining in our understanding of its life cycle, transmission mechanisms, incubation period, epidemiology, and treatment options (Silberman et al., 1996). Although they present relatively in large numbers, the pathogenic species are few and of limited affect as far as they are traces immediate symptoms remain invisible and the direct effect is restricted (Rook, 2007). Parasites are much smaller than their host, and show high degree of specialization for their mode of life and produce more quickly and in greater number than their host, and harm and benefit in parasitic interaction concern the biological fitness of the organisms involved. They reduced host fitness in many ways, ranging from general or specialized pathology such as

(castration), impairment of secondary sex characteristic to modification of host behavior hence parasites increased their fitness by exploiting hosts for food, inhabitant and dispersal (Ghimire and Mishra, 2005).

1.1.1 Intestinal parasites:

Intestinal parasites are those parasites which populate the gastrointestinal tract. They are often spread by poor hygiene related to faeces contact with animal or poorly cooked food containing parasites and have primary been attributed to poor socio-economic concern (Cheesbrough, 1987). The involvement of protozoan agents and intestinal nematodes constitutes the highest group of parasites known to infect the human, this have been attributed to the absence of potable drinking water, proper sanitary habitat, good faeces disposable systems, and proper health care and over dispersion of parasites within the human communities. Hence intestinal parasitosis is highly prevalent in rural communities and constitutes an important cause of mortality and morbidity, low birth weight, low reproductivity in adulthood, stunted growth, low haemoglobin concentration, chronic loss of blood and iron related to parasitic infection. Also, interfere in mental activity leading to apathy, cause irritability and increased intestinal permeability which increased during course of protozoan infection that cause damage to intestinal wall. On the other hand pathogenic protozoan infection such as *Entamoeba coli* have no effect on intestinal permeability (Ghimire and Mishra, 2005). Intestinal permeability (IP) increased in patients infected with pathogenic parasites such as *Blastocystis hominis* (parasites of our

interest) brings forth the idea that *B.hominis* and other protozoan parasites can be pathogenic (Kucik *et al.*, 2004).

1.1.2 Intestinal symptoms:

Usually signs and symptoms of parasitic infection are not recognized because symptoms can occur weeks or even years after initial infection. The most common symptoms include fever, nausea, vomiting, abdominal pain, diarrhoea, constipation, gas, bloating, and irritable bowel syndrome related symptoms in which intestinal parasites dig in and attach themselves to the intestinal wall. This often causes irritation and inflammation that can in term lead to muscle spasm, intestinal blockage and malabsorption of nutrients, fatty substance in particular can be difficult to digest (Rapeeporn *et al.*, 2007)

1.2 Biology classification

1.2.1 *Blastocystis hominis*:

Blastocystis hominis is ubiquitous enteric protistan intestinal parasites that has extensive genetic diversity and infect wide range of animals including human (Stensvold *et al*,2006). Distinct molecular methodologies developed to detect variation and obtain information about transmission pattern and clinical importance (Stenzel *et al.*, 1996). Over the last decade, public health interest in human for intestinal protozoan parasites *B.hominis* increased as further evidence of it is pathogenecity is identified (Al-Tawil *et al.*, 1994). First reports of *Blastocystis hominis* infection were by Perroncito in 1899 (19) and Lynch in 1917 (Nassir *et al.*, 2004).Recently case reports concerning *B.hominis* have increased in numbers, even though some investigator considered *B.hominis* a potential pathogen, it is still an open and

times, controversial issue. Although asymptomatic infection are an established fact (Nassir *et al.*, 2004). Hence taxonomy of *Blastocystis* species remains controversial and history of the organism reflects the difficulty in defining it is taxonomic position The ultra structural studied by Zierdt et al (1988). Provide the first indisputable evidence that the organism was not yeast or fungus as previously suggested or cyst of anther organisms such as *Trichomona*s spp (Stenzel and Boreham1996). It is classified to the subphylum Sarcodina, order Amoebida in separate suborder Blastocystina however, data to support this reclassification have not been provided (Suresh *et al.*, 2000). *Blastocystis* species isolates from human named *Blastocystis hominis*, commonly found in human intestine, and it is believed to be food or water borne protozoan (Zierdt, 1988). Symptoms commonly attributed to infection with *Blastocystis hominis* are non specific and include diarrhoea (particularity in preschool and school age children in poor hygienic groups);abdominal pain, cramps and nausea. In the other hand fatigue, anorexia, flatulence, and other non specific gastrointestinal effects may also be associated with *B.hominis* infection. Large number of *B.hominis* cells may be present in faecal material from patients who do not show symptoms, so presence of *B.hominis* in stool samples from patients showing gastrointestinal symptoms dose not imply that symptoms are due to this organisms, and other infective and non infective causes should be investigated (Stark *et al.*, 2007).

1.2.1.1Classification

Kingdom: Protista

Subkingdom: Protozoa

Phylum: Sarcomastigophora

Subphylum: Sarcodina

Superclass: Rhizopoda

Class: Lobosea

Subclass: Gymnamoeba

Order: Amoebida

Suborder: Blastocystina

Genus: Blastocystis

Species: Hominis (Zierdt, 1988)

1.2.1.2 Morphology:

Blastocystis horninis is a polymorphic protozoan, from culture sample commonly have noted three major forms of organisms (vacular, granular and amoeboid) (Stenzel and Boreham,1996).Vacular form is the most common form found in feacal sample also referred to as" central body" form of *B.hominis* and considered to be the typical *Blastocystis* cell form and generally used for diagnosis of *B.hominis* and most predominant form of organisms in culture (Hussain,1997). Granular form of *B.hominis* has ultra

structure similar to that of vacular form. Amoeboid form has been reported only rarely and there are number of conflicting reports on it‘s morphology (Dunn *et al.,* 1989Tan and Zierdt,1973).There are different sizes and morphologies frequently are noted within *B.hominis* cells from feacal material and morphological heterogeneity was seen in *B.hominis* isolates from fresh feacal samples, This may indicate the presence of different deems or even different species of *Blastocystis* in the host (Stenzel and Boreham,1991).

1.2.1.3 Pathothogencity:

It is unknown whether *B.hominis* is a truly pathogenic organism or a commensal or perhaps is capable of being a pathogenic in specific circumstances. There have been many reports suggesting that *B.hominis* causes disease (Telalbasic *et al.,* 1991).*B.hominis* has been suggested to cause toxic-allergic reactions, leading to non-specific inflammation of the colonic mucosa, but this has not been verified. The review by Zierdt (1988) give previous information from studies involving isolated segments of rabbit ileum, which suggested the presence of toxins in fractions taken from *B.hominis* culture medium (Babb,1989,Blova,1992). The possibility of contamination of these fractions with bacterial or other toxins was not considered. Detailed studies of *B.hominis* to determine the presence of toxins or other substances likely to be harmful to the gastrointestinal tract have not been published and do not appear to have been performed (Stenzel and Boreham,1996).

1.2.1.4 Life cycle:

Blastocystis hominis lives in colon under anearobic conditions. A small avacular form (approx, 5 mm, with 1 or 2 nuclei) lives in colon, it may transform to an ameboid form. which small in size because are degenerate after passage. The avacular form change to a multi-vacular then to centeral vacular form and then to a granular form (all 5-20mm with 1-4 nuclei) when cultured or passed from colon. These form are thought to be the typical and only form of *B.hominis* but they are not the forms present in the colon. The avacular form give rise to the cyst stage. This may be the transmissible stage. Cyst have one or more nuclei, a thick cyst wall and very small vacules. Infectivity to a new host, and it is ability to withstand environmental conditions, have not been determined.

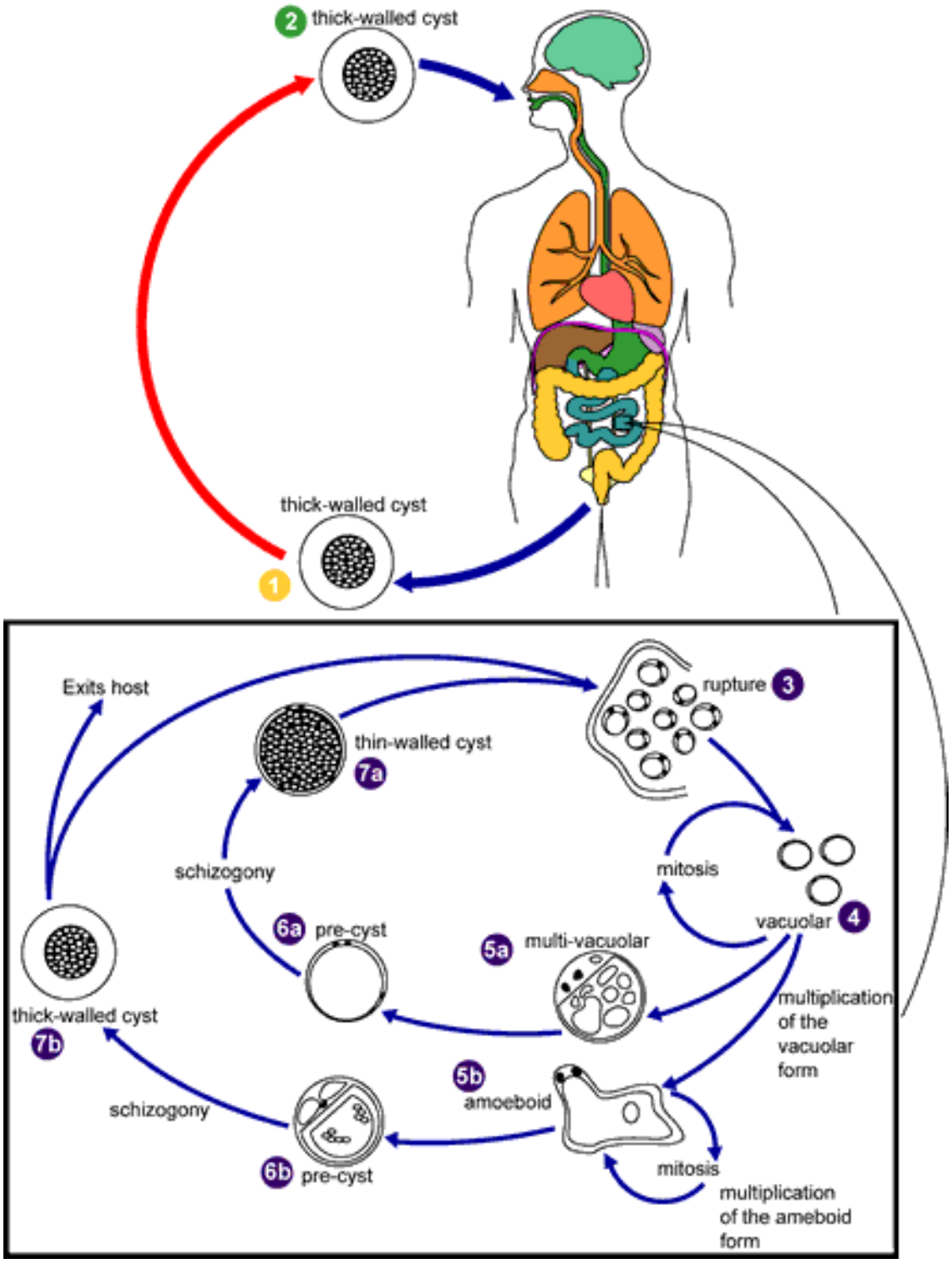

Figure 1:1CDC diagram of *B. hominis*‘ life cycle.

1.2.1.5 Diagnosis of Blastocystis hominis:

Blastocystis hominis is diagnosed using microscopy on a stool sample. The CDC recommends that samples be concentrated and at least three separate samples should be taken before a negative result is confirmed. The two most common methods for preparing slides are a wet mount and a trichrome stain. In the wet mount *Blastocystis hominis* is stained with iodine and appears as a large vacuole in the middle of many small nuclei, though it may be difficult to see. In the trichrome stain, the parasite is stained with trichrome giving the large central body a gray or green appearance and the cytoplasm elements a dark red color.

1.2.2 Irritable bowel syndrome:

In gastroenterology, irritable bowel syndrome (IBS) or spastic colon is a functional bowel disorder characterized by abdominal pain and changes in bowel habits which are not associated with any abnormalities seen on routine clinical testing. It is fairly common and makes up 20-50% of visits to gastroenterologists. Lower abdominal pain and bloating associated with alteration of bowel habits and abdominal discomfort relieved with defecation are the most frequent symptoms. The abdominal pain type is usually described in a patient as either diarrhoea-predominant (IBS-D), constipation-predominant (IBS-C) or IBS with alternating stool pattern (IBS-A). In some individuals, IBS may have an acute onset and develop after an infectious illness characterised by two or more of the following: fever, vomiting, acute diarrhoea, or positive stool culture (Benetton *et al* 1999). It is one of the most commonly diagnosed gastrointestinal illnesses with prevalence rate of(10-15%) in north America and Europe, similar rates have been documented

in developing countries; although it is difficult to know how aggressively alternative diagnosis with excluded in these population (Curioso *et al.*, 2002).IBS affects all ages and all sexes with 2:1 female predominance. The pathophisiology of IBS remain elusive and no mechanism is unique to or characteristics, IBS. There is probably several inter connected factors which occur to varying degrees in patients account for the clinical symptoms of IBS. These include altered gut reactivity (colonic and small bowel motility). In response to luminal or physiological stimuli, visceral afferent hypersensitivity in the gut enhanced visceral perception and pain (Levy *et al* 2001). It is un clear whether this is hypersensitivity is mediated via the local or central nervous system or dysregulation of the brain-gut axis. In addition, hereditary, environmental and dietary factors probably play a role(Fass *et al.*, 2001**).**

1.2.2.1 Diagnosis of Irritable bowel syndrome:

Diagnosis of IBS involves excluding conditions which produce with IBS-like symptoms, and Irritable bowel syndrome then following a procedure to categorize the patient's symptoms. Because there are many causes of diarrhoea and IBS-like symptoms, the American Gastroenterological Association has published a set of guidelines for tests to be performed to diagnose other conditions which may have symptoms similar to IBS. These include gastrointestinal infections, lactose intolerance and Coeliac disease. Research has suggested that these guidelines are not always followed, Once other causes have been excluded, the diagnosis of IBS is performed using a diagnostic algorithm (Yakoob *et al.*,2004). Well-known algorithms include the Manning Criteria, the Rome I Criteria, the Rome II Process, the Kruis

Criteria, and studies have compared their reliability. The more recent (Rome III) process was establishied in (Houghton *et al.,* 2006).

1.2.2.2Manning criteria:

Is a diagnostic algorithm used in the diagnosis of irritable bowel syndrome. The criteria consists of a list of questions the physician can ask the patient (Manning *et al.*, 1987). The answers are used in a process to produce a diagnostic decision regarding whether the patient can be considered to have Irritable Bowel Syndrome. The Manning Criteria include onset of pain linked to more frequent bowel movements, Looser stools associated with onset of pain, pain relieved by passage of stool, noticeable abdominal bloating, sensation of incomplete evacuation more than 25% of the time, and diarrhoea with mucous more than 25% of the time .

1.3 Protozoan parasite and irritable bowel syndrome:

1.3.1 *Blastocystis hominis* and IBS:

There is research to support IBS being caused by an as-yet undiscovered active infection. Most recently, a study has found that the antibiotic Rifaximin provides sustained relief for IBS patients. While some researchers see this as evidence that IBS is related to an undiscovered agent, others believe IBS patients suffer from overgrowth of intestinal flora and the antibiotics are effective in reducing the overgrowth (known as small intestinal bacterial overgrowth). Other researchers have focused on an unrecognized protozoal infection as a cause of IBS as certain protozoal infections occur more frequently in IBS patients (Stendvold *et al.*, 2006).

Two of the protozoa investigated have a high prevalence in industrialized countries and infect the bowel, but little is known about them as they are recently emerged pathogens. Studies from research hospitals in various countries have identified high *Blastocystis* infection rates in IBS patients, with 38% being reported from London School of Hygiene and Tropical Medicine, 47% reported from the Department of Gastroenterology at Aga Khan University in Pakistan and 18.1% reported from the Institute of Diseases and Public Health at University of Ancona in Italy. Reports from all three groups indicate a Blastocystis prevalence of approximately 7% in non-IBS patients. Researchers have noted that clinical diagnostics fail to identify infection, and *Blastocystis* may not respond to treatment with common antiprotozoals(Yoshimasa *et al.*, 2000). Hussein et al, in 1997 found the symptoms *of Blastocystis hominis* (diarrhea anorexia, and flatulence) resembled the symptoms of IBS theyfound levels of *B.hominis* lg G antibodies were significant elevated in patients with IBS compared with asymptomatic controls (Giacmett i *et al.*, 1999).In 1999, in the same context Eur and coworkers found that a significant number of IBS patients were infected with the parasite *Blastocystis hominis* the another concluded there was asset of patients with irritable bowel syndrome in whom the presence of *Blastocystis hominis* not be accidental (Benetton *et al.*,1999) .In 2000 researchers were interested enough in the possible connection between irritable bowel syndrome and parasitic infection to test over 1000 people diagnosed with IBS. Their result confirmed that parasites were significant in this group. An extremely and much overlooked aspect of the research was that parasite and ova testing result were not reliant on a single stool sample instead three samples were tested and specialized lab testing was used

In the year 2000 in the United Kingdom tested stool samples of IBS patients using special collection and testing methods as opposed to standard single stool tests and found that 40% of IBS patients were infected with either *Blastocystis hominis* or *Dientamoeba fragilis*. Mixed infections are also common. Other studies shown that, both *Dientameoba fragilis* are found as commonly as *Giardia* when these specific stool collections and testing are employed. In March 2000, stool samples were collected from 17 symptomatic patients in a study in Canada to test parasites. All patients had suffered at least one of the following: nausea, vomiting, diarrhea, abdominal cramps, bloating. Two stool samples were requested for testing at the provincial reference laboratory. Further 10 symptomatic individuals were evaluated by their physicians and 2 stool samples from each were sent to seven different laboratories. The governmentlaboratory reported all parasites found in the samples but 5 private labs did not report all parasites, in particular *Blastocystis hominis* as they beloved this parasites was non pathogenic. If it was found they did not report it to the treating physician (Stenzel and Boreham, 1991). The American society of tropical medicine and hygiene in the year 2004, designed a study to examine stool specimen of irritable bowel syndrome patients for *Blastocystis hominis*, a common intestinal parasite. One hundred fifty patients were enrolled, 95 IBS cases and 55 controls. These patients provided a medical history, and underwent physical and laboratory evaluations that included stool microscopy and culture for *B. hominis* and colonoscopy. Out of 95 cases (51 males and 44 females), 30 stool microscopy were positive for *B. hominis* besides 4 of the control. Stool culture was positive in 46% (44 of 95) of the cases and 7 (4 of 55) of the control. *B. hominis* was frequently demonstrated in the stool

samples of IBS patients, however its significance in IBS still needs to be investigated (Giacmetti *et al.*, 1999).

Rationale

There are no biological markers for IBS, diagnosis based on a cluster of clinical symptoms (Rome 11 criteria) and also no firm recommendations about the extent and type of testing required to exclude other organic pathology (Houghton *et al.*, 2006).

On the other hand, the growing interest of candidate parasites necessitate researches for the relevance to variety of disorders and the intestinal manifestation is now represent the peak of the iceberg.

Objectives

General objective:

To determine the prevalence of *Blastocystis hominis* among the patients with IBS in Kkartoum State.

Specific objectives:

-To correlate the prevalence of *B.hominis* in patients with IBS.

- To study the prevalence of *B.hominis* among different age group.

- To study the prevalence of *B.hominis* among gender.

Chapter two

Materials and methods

2.1 Study design:

This is a descriptive facility based and cross sectional study with a qualitative approach, in which patient with IBS and control group were enrolled

2.2 Study area and study population:

Samples were collected from different gastroenterology clinics in Khartoum State .Three groups of study subjects were nrolled in the study as follows; group of people with symptoms suggesting IBS according to Rome II criteria, the second group includes patients with IBS but without symptoms, the third group includes the control group which contain the healthy individuals.

2.3 Study variables:

The main variables include the status of individuals with IBS, and patients symptoms. Result will be obtained by examination of feacal specimen as well as constructed questionnaire.

2.4 Sampling and sample size:

Non probability samples namely convenience sampling method is going to be followed.

2.5 Methods:

2.5.1 Direct wet preparation:

Sample was collected into dry air tighted leak - proof plastic stool container. Approximately, 2 mg of fecal specimen was thoroughly mixed and emulsified on glass slide in one drop of physiological saline and then covered gently with cover glass. Similar preparation was made on another slide using lougl"s iodine.These preparations were examined under both low power (10x) and high dry power (40x) objectives.

2.5.2 Trichrome stain:

Thin air dried smear of faeces was prepared on clean slide. The formed faeces was first emulsified in physiological saline. Smear was fixed in absolute methanol for 3 min, then the trichrome stain was placed for 90 min, decolorized in acidified ethanol. The smear was dehydrated in two containers of 95% ethanol and then absolute methanol 2-5 min. The smear was placed in xylene for 10 min.The smear was drained and mounted with cover slip using DPX mounting media. The smear was examined microscopically at oil immersion 100X.

2.5.3 Formal ether concentration technique:

About 1 ml of emulsified faecal suspension in 10% formal water .3-4 ml of 10% formal saline was added and mixed by shaking. The emulsified faeces were sieved and the suspension was transferred to conical centrifuge tube and equal volume of ether was added.The tube was stoppered and mixed for l min then centrifuged at medium speed. Cotton swab was used to remove ether and faecal debris layer. The sediment was mixed by pasteur pipette and

entire sediment was transferred to slide and covered with cover glass. Entire preparation was examined using the (l0X and 40X) objectives.

2.6 Quality control:

Quality control was performed at each step and procedure during this study (from construction of questionnaires to data analysis) to ensure the reliable performance and correct reporting of results. Stains were checked regularly for contamination. On the other hand, results were checked and confirmed by supervisor and senior staff in parasitology department, as well as negative and positive control is run in staining procedure.

2.7 Tool for data collection:

Questionnaire was used for collection of demographic and clinical data and observation check list for stool specimen.

2.8 Data analysis:

Data was computerized using standard sheet and Statistical Package of Social Sciences (SPSS) version 11.0 software.

2.9 Ethical consideration:

Permission from the College of Medical Laboratory science at Sudan University of Science and Technology and hospital management was taken. And verbal consent from study units on explanation of study objectives was taken.

Chapter three

Results

3.1 Detectoin of *Blastocystis hominis* in healthy people and IBS patient:

In one hundred thirty populations, 50 were healthy people and 80 were diagnosed as IBS patients from sign and symptoms. A total of 130 samples were examined by using wet preparation, 17samples were positive for *B. hominis* (table 3.1).

Table 3.1 Over all prevalance of B.h among the patient with IBS

	Healthy	**IBS**	**Total**
positive	0	17	17
negative	50	63	113
Total	50	80	130

3.2 Relation between *B.hominis and IBS patient::*

Out of the 80 IBS patients, 17(21.3%) were found positive for *B.hominis* and 63 (78.8%) were negative for *B.hominis* (table 3.3).

Table 3.3: frequency and Percent age of *B.hominis* in IBS patients

B.hominis	**Frequency**	**Percentage**	**p.value**
positive	17	21.3	0.39
negative	63	78.8	
Total	80	100	

3.3 Relation between *Blastocystis hominis and gender:*

Out of the 17 infected with *B.hominis*, 6 were males (35.2%) and 11 were females (64.8%) (table 3.3).

Table 3.3: frequency and Percentage of *B.hominis in* gender

Gender	**Frequency**	**Percent**	**p.value**
Male	6	35.2%	0.08
Female	11	64.8%	
Total	17	100	

3.4 Relation between *B.hominis* and age groups*:*

The highest infection rate (53%) of *B.hominis* was reported among the more than 41years old, while the lowest infection rate (17.6%) was reported among the less than 20 years old (table 3.4).

Table 3.4 Relation between *Blastocystis hominis* and age group

Age groups	Frequency	Positive number	Positive %	p.value
Less than 20	15	3	17.6%	0.64
21-40	30	5	29.4%	
More than 41	35	9	53%	
Total	80	17	100	

Chapter four

Discussion

Irritable bowel syndrome is functional bowel disorder in which abdominal pain is associated with defect or change in bowel habit. Subtle inflammation especially after infectious enteritis which has been some times suspected as one mechanism of pathogenesis. It is fairly most common diagnosed gastrointestinal disorder and make up 20% -25% of visits to gastroenterologist. Lower abdominal pain, bloating with altering in the bowel habit and diarrhea are more frequent symptoms, reported by Schmulson and Orihel (1990). These finding agree with result obtained from this study among Sudanese patients complain from IBS, bloating (87%), diarrhea with altering bowel habit (62%) are more common symptoms, but vomiting and fever not frequently occur. Published research has demonstrated that some poor patients out comes are due to trample cause of diarrhea being misdiagnosed as IBS common. Example include colic disease and parasitic diseases said by Drisko *et al* (2006). This study showed increased prevalence of *Blastocystis hominis* infections among patients with irritate IBS and this tend to be higher in patients with irritate IBS than health individuals. and also there is exist relationship between the type of parasite, parasite load and severity of diarrhea. So, study showed that feacal carriage of *Blastocystis hominis* more frequently in patients with active IBS and found that low percentage in apparently healthy individuals which is similar to study reported by Jacob *et al.*, (2004).

These evidences suggest that *Blastocystis hominis* play major role in irritable bowel syndrome but the presence of *B.hominis* in stool samples from patients with IBS does not imply that symptoms are due to this organism that is to say *B.hominis* may be a predisposing factor for IBS. The

relatively high prevalence of *B.hominis* in patients with IBS history may probably be attributed to diet factor or nutritional habitat which are established in irritate IBS and give rise to active IBS low percent (16%) of *B.hominis* found in healthy persons this due to asymptomatic carrier which is self limited. The parasites increase in irritable bowel syndrome patients is similar to the study reported by Millet *et al* (2002) were interested enough in the possible connection between irritable bowel syndrome and parasitic infection to test over 1000 people diagnosed with IBS. Their results confirmed that parasites were significant in this group.

Also similar study conducted by Stark *et aI* (2007), has described a possible role for *Blastocystis hominis* in the etiology for IBS. Females are more likely to develop IBS than males.

Chapter five

Conclusion and recommendation

5.1 Conclusions:

- *Blastocystis hominis* is probably the most prevalent unicellular parasite in human feacl specimens in irritable bowel syndrome.
- Infection with *B.hominis* is likely associated with bowel irritation and should be considered a potential irritable factor.

5.2 Recommendation:

-*Blastocystis hominis* should be reported in stool examination whenever detected.
- Further-case control study is required not only on the pathophysiological effect of *B. hominis* in IBS patients, but also large scale prospective trials assessing the role of *B. hominis* in IBS.
- Determine the true prevalence in different populations.

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Sudan University of science and technology
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Questionnaire form

ID No: ☐☐☐☐☐☐ Date: / /2013

Pt Name: …….....

Age ☐ year☐ Gender: Male ☐ female ☐
Nationality: ……
Occupation: …….
Residence: ……
Address: ……
Medication:
Laboratory diagnosis:
Macroscopy:
Colour:……

Consistency…….

Mucus:……
Blood :……
Microscopy

RBCS……..
pus cells……...

trophozoite……..

Cyst……

Other……