Title page

Title: Epidemiological evidence that helminthes infestation is associated with less risk of idiopathic inflammatory bowel disease.

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Abstract

The UC and Crohn's disease are chronic, idiopathic, inflammatory diseases of the GIT that share common symptoms such as diarrhea, abdominal pain, fever, and weight loss. Ulcerative colitis involves all or part of the colon, whereas, Crohn’s disease commonly involves the terminal ileum and proximal colon.The two major forms of IBD share many clinical and epidemiological characteristics, suggesting that underlying causation may be similar. Yet, UC & Crohn's disease are distinct syndromes with divergent treatment and prognosis.

Aims of the study

1- Study a relationship between some parasitic infections such as helminthes infestation, and the development of IBD.
2- Understanding the correlation between parasitic infections and autoimmune disorders may be helpful in prediction, early identification and conceivably the prevention of these diseases
3- To help confirm a clinical diagnosis of IBD disease.
4- To determine whether a patient may respond well to IBD disease medications.
5- To help differentiate Crohn's disease from ulcerative colitis patients.

Conclusions

1. The present study provided significant epidemiological evidence that infestation with round worm (helminthes) provided protection against acquisition of inflammatory bowel disease.
2. The combined infestation with multiple intestinal helminthes has a better protective role than single parasite in protection against inflammatory bowel disease.

3. The best method for diagnosis of helminthes infestation in terms of sensitivity and specificity was conventional PCR.

Key words: Crohn's disease, ulcerative colitis, IL1B and IL10**,**IBD.

Introduction

Inflammatory Bowel Disease (IBD) comprises those conditions which tend to be chronic or relapsing immune activation and inflammation within gastrointestinal tract (GIT). Ulcerative Colitis (UC) and Crohn's disease are the two major forms of this disease with unidentified etiopathology (Sands, 2002). The incidence of IBD has been rising not only in Western countries, but also in Asia, including Korea (Yang *et al*., 2008). Thanks to the inventions of vaccines and anti-microbial agents that dramatically reduced the rate of infectious disorders. The big picture in the developed world can be summarized by two main trends. The first tread is that infectious disorders such as mumps, rubella, T.B, pneumonia, meningitis, etc., have reached very low incidence rates in these developed countries; the second trend however; on the other hand is that a number of disorders such type 1 diabetes mellitus, hay fever, celiac disease, asthma, Crohn’s disease and ulcerative colitis have witnessed marked increase in incidence rate particularly when compared with their incidence rates in developing countries (Scudellari, 2017).

Epidemiologic studies have shown that the prevalence rates of idiopathic inflammatory bowel disease, Crohn’s disease and ulcerative colitis, are higher in developed countries such as USA and Western Europe than in underdeveloped and developing countries. On the other hand, the prevalence rate of parasitic infestation with round worms, helminthes, is significantly lower in developed countries in comparison with developing countries. Based on these epidemiologic data, a number of authors has suggested a link between high incidence rate of Crohn’s

disease and ulcerative colitis and low incidence rate of helminthes infestation in developed countries and has hypothesized that under exposure to children in their early lives to helminthes infestation resulted in maldevelopment of their immune system with subsequent predisposition to autoimmunity that may manifest itself in the form of either Crohn's disease of ulcerative colitis (Sýkora *et al*., 2018; M'Koma, 2013). However; because of the lack of clear consensus about this suggestion and because of the high prevalence of helminthes infestation in our community in Iraq (Saheb *et al*., 2017), the present study was planned and conducted to investigate the possible association among the immune system function, the prevalence of helminth infestation and the prevalence and pathogenesis of idiopathic inflammatory bowel diseases.

Patients and methods

After sterilizing the area with alcohol (70%), aspiration blood sample (5ml) was collected from cubital fossa vein from GIT patients and control groups.

Collected sample was transferred immediately in to two tubes as follows:

A. Two milliliter of blood in 5 ml tube (EDTA tube) used for PCR technique to detect NOD/CARD15 gene polymorphism

Two groups were included in this study;

A- Patients Group : A total of fifty patients from Al-Diwaniyah province (males and females) with inflammatory bowel disease; 31 patients with Ulcerative Colitis and 19with Crohn's Diseases patients, who have been diagnosed by specialist physicians in Al-Diwaniyah Teaching Hospital for Gastrointestinal Tract and Hepatic diseases unit, depending on clinical features, biopsy for

histopathology, and endoscopy. All were regularly attending the consultant clinic for treatment and follow-up during the period from January 2018 to august 2018.

B- Control Group; A total number of thirty individuals, who were apparently healthy, were involved as a control group. They matched the patients group regarding sex, and age and had no history of / or clinical features of IBD, no obvious abnormalities, none of them had an acute or chronic diseases.

Study Protocol and Sampling Members of the two groups were subjected all were subjected to the following assays;

1- GSE.

Stool examination for parasites

1. Saline wet mount: It is used to detect worms, bile stained eggs, larvae, protozoan trophozoites and cysts. In addition, it can reveal the presence of RBCs and WBCs.

2. Iodine wet mount: It is used to stain the glycogen and nuclei of the cysts. A cyst is appreciated better in an iodine preparation, but the motility of the trophozoite is inhibited in the iodine preparation.

Procedure:

• Place a drop of saline on the left half of the slide and one drop of iodine on the right half.

• With an applicator stick, pick up a small portion of the specimen (equivalent to the size of a match head) and mix it with a saline drop.

• Similarly, pick up a similar amount and mix with a drop of iodine.

• Put the cover slip separately on both and examine under the microscope.

• The ova, cysts, trophozoites and adult worms can be identified as per their characteristic features.

2- Screening the bellow:

. EDTA tube for blood extraction and then RFLP PCR.

..Tube for stool extraction and PCR.

Results

Demographic characteristics

The mean age of patients with IBD was 36.68 years and the range was 12 – 63 years, whereas the mean age of control subject was 37.22 years and he range was 12 – 63 years. Indeed, there was no significant difference in mean age between patients and control subjects (P = 0.828), table 1. According to gender, patients included 38 males (76 %) and 12 females (24 %) while control subjects included 33 males (66 %) and 17 females (34 %); the difference in the distribution of patients and control subjects was statistically insignificant (P = 0.978), table 4.2. Moreover, there was no statistical significance difference in the distribution of patients with IBD and control subjects with respect to residency (P = 0.829), as shown in table 3. Alcoholism was seen neither in control group nor in patients group, table 4; however, smoking was observed in 14 % of patients (7 out of 50) and in 12 % of control subjects (6 out of 50); the difference in distribution of patients and control subjects according to smoking was statistically insignificant (P = 0.766), as shown in table 4. Positive family history was seen in 8 patients out of 50 (16 %) and in 6 out of 50 control subjects (12 %); there was no statistical significant difference in the distribution of patients with IBD and control subjects

with respect to family history of idiopathic inflammatory bowel disease, as shown in table 5.

Table 1: Distribution of patients with IBD and control subjects according to age

Age (years)	**IBD** ***n* = 50**	**Control** ***n* = 50**	***P***
Mean ± SD	36.68 ±12.24	37.22 ±12.56	0.828 † NS
Range	12 – 63	12 - 63	
<20 years, *n* (%)	4 (8 %)	4 (8 %)	0.978 ¥ NS
20-40 years, *n* (%)	28 (56 %)	27 (54 %)	
> 40 years, *n* (%)	18 (36 %)	19 (38 %)	

IBD: inflammatory bowel diseases; *n*: number of cases; SD: standard deviation; †: independent samples t-test; ¥: Chi-square test; NS: not significant at $P \leq 0.05$

Table 2: Distribution of patients and control subjects according to gender

Gender	**IBD** ***n* = 50**		**Control** ***n* = 50**		χ^2	***P***
	n	**%**	***n***	**%**		
Male	38	76	33	66	1.214	0.271¥ NS
Female	12	24	17	34		

IBD: inflammatory bowel diseases; *n*: number of cases; ¥: Chi-square test; NS: not significant at $P \leq 0.05$

Table 3: Distribution of patients and control subjects according to residency

Region	**IBD** ***n* = 50**		**Control** ***n* = 50**		χ^2	***P***
	n	**%**	***N***	**%**		
Urban	34	68	35	70	0.047	0.829 ¥ NS
Rural	16	32	15	30		

IBD: inflammatory bowel diseases; *n*: number of cases; ¥: Chi-square test; NS: not significant at $P \leq 0.05$

Table 4: Distribution of patients and control subjects according to smoking and alcoholism

Bad habits	IBD *n* = 50		Control *n* = 50		χ^2	*P*
	n	%	*N*	%		
Smoking	7	14	6	12	0.088	0.766 ¥ NS
Alcoholism	0	0	0	0	---	---

IBD: inflammatory bowel diseases; *n*: number of cases; ¥: Chi-square test; NS: not significant at $P \leq 0.05$

Table 5: Distribution of patients and control subjects according to family history

Family history	IBD *n* = 50		Control *n* = 50		χ^2	*P*
	n	%	*n*	%		
Positive	8	16	6	12	0.332	0.564 ¥ NS
Negative	42	84	44	88		

IBD: inflammatory bowel diseases; *n*: number of cases; ¥: Chi-square test; NS: not significant at $P \leq 0.05$

Association between parasitic infestation and inflammatory bowel disease

The distribution of patients with inflammatory bowel disease and control subjects according to presence and type of parasitic infestation is demonstrated in table 9. First of all, the helminthes (round worms) that were detected in the current study included *Ascaris lumbricoides*, *Enterobius vermicularis,* and *Strongyloides stercoralis.*

Ascaris lumbricoides was seen in 12 % and 26 % of patients and control subjects, respectively, that is it is more common in control subjects than in patients with IBD; however, the difference did not reach statistical significance (*P* = 0.074); in terms of risk the odds ratio was 0.43 which means that patient with

Ascaris lumbricoides are less liable to get IBD by a fraction of 0.57 and the preventive fraction was 0.30, table 9.

Enterobius vermicularis was seen in 34 % and 50 % of patients and control subjects, respectively, that is it is more common in control subjects than in patients with IBD; however, the difference did not reach statistical significance (P = 0.105); in terms of risk the odds ratio was 0.52 which means that patient with *Enterobius vermicularis* are less liable to get IBD by a fraction of 0.48 and the preventive fraction was 0.28, table 9.

Strongyloides stercoralis was seen in 28 % and 38 % of patients and control subjects, respectively, that is it is more common in control subjects than in patients with IBD; however, the difference did not reach statistical significance (P = 0.288); in terms of risk the odds ratio was 0.63 which means that patient with *Strongyloides stercoralis* are less liable to get IBD by a fraction of 0.37 and the preventive fraction was 0.20, table 9.

Total parasite burden was seen in 52 % and 62 % of patients and control subjects, respectively, that is it is more common in control subjects than in patients with IBD; moreover, the difference was statistically significance (P = 0.039); in terms of risk the odds ratio was 0.42 which means that patient with round worm infestation are less liable to get IBD by a fraction of 0.58 and the preventive fraction was 0.37, table 9.

Table 9: Association between parasitic infestation and inflammatory bowel disease

Parasite	IBD n = 50		Control n = 50		*P* ¥	OR	95 % CI		PF
	n	%	*n*	%			Lower	Upper	
Total parasite	26	52	36	62	0.039 S	0.42	0.18	0.97	0.37
Ascaris lumbricoides	6	12	13	26	0.074 NS	0.43	0.15	1.26	0.30
Enterobius vermicularis	17	34	25	50	0.105 NS	0.52	0.23	1.15	0.28
Strongyloides stercoralis	14	28	19	38	0.288 NS	0.63	0.27	1.47	0.20

IBS: inflammatory bowel diseases; *n*: number of cases; ¥: Chi-square test; NS: not significant at $P \leq 0.05$; OR: odds ratio; CI: confidence intervals; PF: preventive fraction

Distribution of patients and control group according to parasite infestation by residency (urban versus rural)

Evidence of round worm infestation was seen in a total of 62 out of 100 individuals enrolled in the current study; regarding control group, in urban area the prevalence rate of round worm infestation was 77.1 % whereas in rural areas the prevalence rate of round worm infestation was 60.0 %; the difference was statistically insignificant ($P = 0.372$), as shown in table 4.10. Regarding UC group, in urban area the prevalence rate of round worm infestation was 63.6 % whereas in rural areas the prevalence rate of round worm infestation was 55.6 %; the difference was statistically insignificant ($P = 0.990$), as shown in table 4.10. regarding CD group, in urban area the prevalence rate of round worm infestation was 75.0 % whereas in rural areas the prevalence rate of round worm infestation was 42.9 %; the difference was statistically insignificant ($P = 0.364$), as shown in table 4.10. Regarding all enrolled subjects, in urban area the prevalence rate of round worm infestation was 63.8 % whereas in rural areas the prevalence rate of

round worm infestation was 58.1 %; the difference was statistically insignificant (*P* = 0.587), as shown in table 10.

Table 10: Distribution of patients and control group according to parasite infestation by residency (urban versus rural)

Group	Parasite	Total		Urban		Rural		χ^2	*P*
		n	%	*n*	%	*n*	%		
Control	Positive	36	72.0	27	77.1	9	60.0	0.798	0.372 † NS
	Negative	14	28.0	8	22.9	6	40.0		
UC	Positive	19	61.3	14	63.6	5	55.6	0.001	0.990 † NS
	Negative	12	38.7	8	36.4	4	44.4		
CD	Positive	7	36.8	3	25.0	4	57.1	0.825	0.364 † NS
	Negative	12	63.2	9	75.0	3	42.9		
Total	Positive	62	62.0	44	63.8	18	58.1	0.295	0.587 ¥ NS
	Negative	38	38.0	25	36.2	13	41.9		

n: number of cases; †: Yates correction for continuity; ¥: Chi-square test; NS: not significant at $P \leq 0.05$

Discussion

The present study showed that the mean age of patients with IBD (ulcerative colitis and Crohn's disease) was 36.68 years and the range was from 12 to 63 years. This finding agrees to the findings of several other studies which described a mean age ranging from 35 to 37 (Prelipcean *et al*., 2014); this study also agree with Velonias *et al.* (2017), who found that the mean age of majority of patients with ulcerative colitis and Crohn's disease was 36 years. In the current study, majority of cases were male patients and female patients constituted a minority, 75 % versus 24 %. This finding agrees with Baars *et al*., (2012), who found the majority of patients with inflammatory bowel disease were males (64 %); also agrees with the finding of Yang *et al*., (2014) who found that male patients constituted 66.7 % out of all patients with Crohn's disease; however, the result of the current study disagree with Gasparini *et al*., (2018) who found that inflammatory bowel disease was more common in female patients (59.7 %). On the other hand, some authors

described no sex predilection for inflammatory disease (Shah *et al*., 2018). Collectively, the results of the present study, in addition to previous studies indicated the lack of clear consensus on sex predilection of inflammatory disease; this may explain the absence of factors related to either gender in the predisposition to inflammatory bowel disease, namely hormonal influences.

In the current study, majority of cases with inflammatory bowel disease were urban areas in comparison with rural areas, 68 % versus 32 %. This result agrees with the majority of studies as Soon *et al*. in 2012 described 40 studies in their meta-analysis that support the finding that both ulcerative colitis and Crohn's disease are more common in urban than in rural areas. Increased urbanization is one hypothesis for the rising incidence of IBD. Urban residence is associated with higher incidence of both Crohn's disease (CD) and ulcerative colitis (UC) (Benchimol *et al*., 2017). The mechanism by which rurality protects against IBD is uncertain, and may include dietary and lifestyle factors, environmental exposures, or segregation of individuals with different genetic risk profiles (Benchimol *et al*., 2017). However, the higher prevalence rate of helminthes infestation in early childhood in individuals with rural inhabitant in comparison with those lining in urban areas may partly explain this protective effect through the immune modulation effect of these parasites .

The current study showed no significant association between smoking and inflammatory bowel disease since rates of smoking were 14 % versus 12 % in IBD patients and control group respectively. The finding of this study agrees with Ng *et al*. (2015) who found no significant association with smoking whereas it disagrees with Wang et al., (2018) who found that smoking increases the risk of Crohn's disease. Several other studies have assessed the association between smoking and

inflammatory bowel disease; however, the results were controversial (Niu *et al*., 2016; Moon *et al*., 2014; Reif *et al*., 2000).

In the present study the prevalence rate of positive family history was 16 %; however, there was no significant association between the IBD and family history of IBD. In agreement with the present study, it was found the rate of positive family history in patients with IBD approaches 13 % (Childers *et al*., 2014). Some authors found no significant association between family history of IBD and occurrence of inflammatory bowel disease (Gupta *et al*., 2017; Chung *et al*., 2014), in agreement with the findings of the current study. However, some studies found significant association between inflammatory disease and positive family history (Torres *et al*., 2016).

The current study has shown that apparently healthy control individuals have higher rates of round helminthes infestation than patients with inflammatory bowel disease since *Ascaris lumbricoides* was seen in 12 % and 26 % of patients and control subjects, respectively, that is it is more common in control subjects than in patients with IBD; *Enterobius vermicularis* was seen in 34 % and 50 % of patients and control subjects, respectively, that is it is more common in control subjects than in patients with IBD; *Strongyloides stercoralis* was seen in 28 % and 38 % of patients and control subjects, respectively, that is it is more common in control subjects than in patients with IBD. In addition, we found that the total burden of parasite was significantly higher in control subjects than in patients with IBD.

These findings suggested a protective role for helminthes against development of inflammatory bowel disease. The authors of the current study were able to find that multiple parasitic infestations were significantly protective against

both ulcerative colitis and Crohn's disease with an estimated odds ratio of 0.42 (95 % confidence interval of 0.18-097). Similar observations have been recorded by a number of cross sectional studies. In a case control study from South Africa, childhood exposure to helminthes was protective against both CD and UC development (adjusted OR of 0.2 [95% CI 0.1–0.4] for CD and adjusted OR of 0.2 [95% CI 0.1–0.6] for UC) (Chu *et al*., 2013).

It is known that environmental factors, including helminthes exposure and smoking, show close association with the risk of developing IBD, (Cosnes *et al*., 2011; Weinstock and Elliott, 2009) despite 18.8% and 50% of contribution from genes to UC and CD, respectively (Halfvarson *et al*., 2003). For instance, in sub-Saharan Africa where helminthes of intestinal infestation were frequent, the prevalence of IBD was surprisingly low in these local black populations; however, the incidence of these diseases is approaching to white populations when African people immigrated in USA and UK, which cannot be explained only by genetic factors (Fiasse and Latinne, 2006). In other words, helminthes are seemed to be inversely associated with the development of IBD. So far and in view of these data obtained from the present study, supported by the cross sectional study of Chu *et al*. (2013), the hypothesis that helminthes infestation is protective against inflammatory bowel disease, namely ulcerative colitis and Crohn's disease becomes epidemiologically supported.

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