British Journal of Medicine & Medical Research
2(4): 527-535, 2012

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Refractory Coeliac Disease; Role of *Nigella sativa* as Immunomodulator

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Authors' contributions

This work was carried out in collaboration between all authors. We declare that work was done by all the authors named in this article and all the liabilities pertaining to claims relating to the content of this article will be borne by the authors. MTO coordinated the study design and participated in the all laboratory work and data collection and analysis and drafted the manuscript. GAD and BIT participated in the serological work. LAM participated in clinical work. All the authors read the final manuscript.

Research Article

Received 3rd June 2012
Accepted 25th July 2012
Published 23rd August 2012

ABSTRACT

Aim: The aim of this study was to assess the immunological and histological profiles of adult coeliac patients after commencing *Nigella sativa (NS)* oil with gluten free diet (GFD) for a period of 1 year ± 1month to prove its validity in treatment of refractory coeliac disease (CD).
Methodology: Thirty two adult coeliac patients who all accepted to do endoscopy and duodenal biopsy in addition to serological assessment before and after treatment of GFD alone or with NS oil capsules for a period of 1 year ± 1 month. Duodenal biopsies were interpreted histologically according to modified Marsh criteria and the sera were tested for antigliadin antibody (AGA), anti tissue transglutaminase antibody (tTG) and endomysium antibody (EMA).
Results: The response to gluten withdrawal with NS oil for a period of 1 year ± 1 month in CD patients was better than GFD alone with significant response to serological markers.

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Conclusion: The administration of *NS* oil with GFD to CD patients leads to a significant decreases more than GFD alone in the levels of all immunological parameters with histological improvement and stop the disease process (P=0.001). Ultimately, the results emerging from this study may substantially improve the immunotherapeutic application of *NS* in clinical management of refractory CD cases.

Keywords: Coeliac disease; gluten free diet; antigliadin antibodies; endomysial antibody; anti tissue-transglutaminase antibodies; Nigella sativa oil.

1. INTRODUCTION

The *Nigella sativa* (NS) seed (Black seed) is an annual *Ranunculaceae* herbaceous plant, has been used for centuries in the Middle East, northern Africa, the Far East and Asia as a traditional treatment for many diseases. *NS* contains 30 w/w of a fixed oil and 0.40–0.45 w/w of a volatile oil. The volatile oil has been shown to contain 18.4–24% Thymoquinone and 46% monoterpenes, such as *p-cymene* and *a-pinene* (El-Tahir Keh et al., 1993). Many clinical and experimental studies have demonstrated the therapeutic benefits of *NS* extracts, including immunomodulative (Al-Ali, et al., 2008), anti-inflammatory (Ali and Blunden, 2003) , antitumour (L. Ait Mbarek et al., 2007; Mohammad and Mastour, 2011) antidiabetic (Nabiela et al., 2010) and anti cardiovascular effects (Yar et al., 2008).

Coeliac disease (CD) is a permanent state of intolerance to gluten, i.e, alcohol-insoluble proteins of wheat, rye and barley (Anderson, 2008). The immunologic response to gluten in gluten-sensitive people causes appearance of coeliac serological antibodies beside histological abnormalities of the small intestinal mucosa, that are comprising influx of lymphocytes into the epithelium, crypt hyperplasia and ultimately, villous atrophy (Cummins et al., 2011). This results in a diversity of symptoms and signs of malabsorption (Anderson, 2008; Cummins et al., 2011). Therefore, removal of gluten from the diet is essential for patients with CD (Cummins et al., 2011). Serological testing has a well-established place in the diagnosis and screening of CD and in this setting; anti-gliadin antibodies (AGA), anti-endomysial antibodies (EMA) and anti- tissue transglutaminase antibodies (tTG) are useful and have been compared and reviewed in many studies (Pietzak, 2005; Bardella, 2007; Anderson, 2008; Hopper et al., 2008; Cummins et al., 2011).

After commencing a gluten free diet (GFD), symptoms improve within weeks and coeliac antibodies may normalize within 6–12 months (Lee et al., 2003). Guidelines have traditionally recommended that, follow-up endoscopy and biopsies should be performed after commencing a GFD to document the histological improvement and to confirm the clinical remission and dietary compliance (Pietzak, 2005; Bardella, 2007; Hopper, et al. 2008). Follow-up endoscopy after 4-6 months on a GFD is considered as the gold standard in evaluating dietary compliance, (Lee et al., 2003; Martin et al., 2006; British Society of Gastroenterology, 2012), meanwhile frequently repeated biopsies are neither practical nor cost-effective so that the follow-up data on serologic markers negativity and small intestinal recovery in CD are scarce and contradictory.

Cases of refractory CD have been reported (Ryan and Kelleher, 2000; Georgia Malamut and Christophe Cellier, 2011). Refractory CD is defined as a condition with persistent or recurrent malabsorptive symptoms and villous atrophy despite strict adherence to a gluten-free diet (GFD) for at least 6-12 months in the absence of other causes of non-responsive

treated coeliac disease and overt malignancy (Georgia Malamut and Christophe Cellier, 2011). However the condition often resulting from poor absorption of nutrients from the intestines leads to a poor prognosis including small bowel cancers (Daum et al., 2005; Georgia Malamut and Christophe Cellier, 2011). Congdon et al., in 1981; found persisting villous atrophy in 2 of 10 children with CD. Grefte et al., in 1988; reported slow and incomplete histological and functional recovery in 22 adults with celiac disease after 24 to 48 months of a gluten-free diet. Refractory CD is treated by GFD, nutritional supplement, corticosteroid therapy and immunosuppressive drugs (Ryan and Kelleher, 2000). This fact motivated us to do this research, since to our knowledge; till now there are no available data regarding the effect of using *Nigella sativa* in addition to GFD for the management of CD. Therefore, the aim of this study was to assess the immunological and histological profiles of adult coeliac patients after treatment with *NS* in addition to GFD for at least 1 year ± 1month.

2. MATERIALS AND METHODS

2.1 Subjects and Methods

This study included 32 untreated adult coeliac patients (14 females, 18 males, median age, 28 years; range, 18-66 years) referred to Medical City Hospitals in Baghdad, during a period of five years. Patients were diagnosed as CD patients after performing duodenal biopsy on the bases of clinical history and serological assessment, including AGA, EMA, tTG antibodies testing.

Biopsies were interpreted by two pathologists who were not informed about the clinical status of the patients and interpreted small intestinal histological features, according to the Marsh classification and the modified Marsh criteria (Marsh, 1992; Oberhuber et al., 1999). Marsh I consists of raised intraepithelial lymphocytes (IELs) with >40 lymphocytes per 100 enterocytes, Marsh II consists of raised intraepithelial lymphocytes and crypt hyperplasia, Marsh IIIa partial villous atrophy, Marsh IIIb subtotal villous atrophy and Marsh IIIc total villous atrophy. Diagnosis of CD was dependant on the presence of Marsh III only. Any report, which did not include the features of Marsh III was considered as non-coeliac patient.

Patients were randomized into two groups, 16 patients each; Group 1: CD patients were treated by GFD for a period of 1 year ± 1 month. Group 2: CD patients were treated by GFD plus *Nigella sativa* oil, these patients were given respectively *NS* oil capsules orally (one capsule with a dose of 450mg, twice a day), as dietary supplement for a period of 1 year ± 1 month. The *NS* oil capsules were purchased from local market (from Pharco Pharmaceuticals, Egypt).

Follow-up endoscopies with duodenal biopsies and serological monitoring were repeated at 1 year ± 1 month after treatment. Serum from each patient was separated within 4 hours of collection and stored until analysis of serological markers of CD. Serum IgA EMA was detected qualitatively by indirect imunofluorescent (IIF) method using commercial slides of monkey esophagus (from Medic Company. Italy), with reticular staining of the muscularis mucosa at serum dilution of 1:3 reported as positive. However, AGA and tTG were performed by enzyme-linked immunosorbent assay (ELISA) in duplicate and according to the manufacturers' instructions.

All patients were matched for age, gender, chronic disease status other than CD.

2.2 Statistical Analysis

Analysis comprised of summary statistics for gender and age, comparative analysis between the findings before and after GFD alone or with NS oil capsules. Data were analyzed using SPSS v10 for Windows and paired *t*-tests were used to compare the change in histopathology findings (Marsh grade) after the follow-up period. Data values were adjusted for age and initial values. Analyses where the *P*-value was =0.05 were considered to be statistically significant.

3. RESULTS

At the time of study enrollment, all subjects of 2 groups had to have villous atrophy (Marsh stage III), to be eligible to participate in the follow up study, moreover all patients shouldn't have complained from any other chronic diseases under treatment other than CD (e.g diabetes mellitus). Table 1 shows a comparison between histopathological results in both groups of coeliac patients before and after commencing treatment.

3.1 Histological Results

Complete histological remission (Table1) was seen in 10 patients (62.5%) of 16 patients in group of treated CD patients with only GFD (group1) within 1year ± 1 month, meanwhile, 4 (25%) of Group 1 showed Marsh I, 1 (6.2%) showed MarshII changes and 1 patient (6.2%) showed MarshIIIa, however these patients known as histological 'non-responders'. Regarding histological results in group 2 (patients treated with NS oil in addition to GFD). Complete histological remission was observed in 13 patients (81.2%) (*P*=001), while the other 3 (18.7%) patients showed histological changes of Marsh I, and none of the group 2 patients showed Marsh III, (Table1).

3.2 Serological Results

At baseline, 22 (68.75%) of 32 CD patients in both groups were positive to AGA while 10 patients (31.25%) were negative. After 1year ± I month-follow-up, the AGA level was normalized in all patients (100%) of the 32 subjects. Overall, there was a significant reduction in AGA levels (*P*=0.0001) following a GFD alone or with NS oil treatment. This was strongly significant reduction in AGA (P=0.00001), Table 2.

As shown in table 2, at baseline; 30(93.75%) of 32 CD patients were positive to IgA EMA while 2 patients (6.25%) were negative. After, a 1year ± 1month-follow-up, the EMA was normalized in 15 (93.75%) of the 16 patients in group 1, while all 16 patients in group 2 showed negative EMA (0%) after the same period of treatment. The reduction in EMA was strongly significant (*P*=0.0001) in both groups.

31 (96.87%) of 32 CD patients (with same results of EMA at baseline) were positive to IgA tTG antibodies while only one patient (3.13%) was negative. After the 1year ± 1 month-follow-up, the tTG antibodies level had normalized in 15 (93.75%) of the 16 patients. Meanwhile all 16 patients in group 2 showed negative tTG (0%) after the same period of treatment. Overall, there was strongly significant reduction in tTG levels (*P*=0.0001) following the period of treatment in both groups (Table2).

Table 1. Comparison between histopathological results in coeliac patients before and after GFD alone or with *Nigella sativa*

Histopathology	Before GFD		After GFD alone		After GFD with NS		P-value
	No.	%	No.	%	No.	%	
Marsh I	0	0	4	25	3	18.7	
Marsh II	0	0	1	6.2	0	0	
Marsh IIIa	8	25	1	6.2	0	0	
Marsh IIIb	15	46.9	0	0	0	0	
Marsh IIIc	9	28.1	0	0	0	0	
Normal histology	0	0	10	62.5	13	81.2	
Total	32	100	16	100	16	100	0.001

Table 2. Comparison between AGA, EMA and tTG Ab tests in coeliac patients before and after GFD alone or with NS

		Before GFD		After GFD Alone		After GFD With NS		P-value
		No.	%	No.	%	No.	%	
AGA	Positive	22	68.75	0	0	0	0	
	Negative	10	31.25	16	100	16	100	0.00001
EMA	Positive	30	93.75	1	6.25	0	0	
	Negative	2	6.25	15	93.75	16	100	0.00001
tTG Ab	Positive	31	96.87	1	6.25	0	0	
	Negative	1	3.13	15	93.75	16	100	0.00001
	Total	32	100	16	100	16	100	

4. DISCUSSION

Many medicinal plants and their products have been documented to have immunomodulatory and therapeutic properties. *Nigella sativa* is one of such plants (Randhawa and Al-Ghamdi, 2002; Salem, 2005). To the best of our knowledge there is no documentation on effects of *NS* oil in coeliac disease. Therefore, this trial study was done to assess the potential benefits of *NS* oil administration when used with GFD as an adjunct treatment modality in the treatment of coeliac disease and its related complications.

NS oil is believed to share similar properties to the benzoquinones already in use as therapeutic drugs and its effect has been demonstrated in many human and animals studies (Swamy and Tan, 2000). In a mouse model of allergic airway inflammation, the administration of *NS* oil reduced the number of inflammatory cells in lung tissue (Haq et al., 1999). In another study, the intraperitoneal administration of NS oil inhibited the synthesis of both prostaglandin D2 and T-helper2 cytokines (Szejda et al., 1984). However, in splenic mononuclear cells isolated from allergen sensitized mice given NS oil, cytokine production was unchanged (Hanafy and Hatem, 1991). The exposure of mouse-bone marrow derived dendritic cells to NS compromised their maturation, cytokine release and survival (Ragheb et al., 2009). Possibly, dendritic cells regulate the anti-inflammatory action of black seeds. From the overall results of these experiments, it may be concluded that black seed produce a maximum effect on immune response and resistance in humans and animals (Abbas et al., 2005; Buyukozturk et al., 2005; El Mezayen et al., 2006; Nabiela et al., 2010; Xuan et al., 2010).

In the present study, we noted that *NS* oil, if added to GFD has significant effect on histological recovery profiles of 16 subjects with coeliac disease more than the effect of GFD alone. CD patients who were administrated NS oil with GFD (Group 2) reached complete histological remission within 1 year ±1month except 3 cases that showed just increase in intraepithelial lymphocytes (Marsh I), these cases were non coeliac disease (Table 1). Meanwhile 6 of 16 CD subjects (Group 1) who commenced GFD alone without NS oil still had at least partial villous atrophy (Marsh IIIa). These are considered to have clinically refractory coeliac disease (Table 1).

Histological non-response to GFD manifests as persistent features of enteropathy on mucosal biopsy (Peter J. Wahab et al., 2002). This feature provided the clue to search for other causes with CD to find treatment other than GFD. Other causes include pancreatic insufficiency, secondary lactose deficiency, bacterial and parasitic overgrowth, coexisting inflammatory bowel disease, collagenous colitis and lymphocytic colitis (Barbara et al., 2000). Hence, it is important to determine the presence of persistent villous atrophy and then refractory coeliac disease is diagnosed (Barbara et al., 2000; Peter et al., 2002; Bai et al., 2005).

The response to gluten withdrawal in CD patients is variable and notably, the clinical, histological and serological responses often do not occur in parallel. Clinically, a marked symptomatic improvement may occur within several days, whereas mucosal improvement may take up to 2 years (Pardeep Brar et al., 2006). Histological response is characterized by a significant increase in villous size (reduction in villous atrophy), reduction in crypt hyperplasia and finally a reduction in the IELs count. Many responding patients may have continued mild elevation of the IELs count (Marsh I) (6 patients in group1 of this study), despite normalization of villous architecture and normal crypt size. This indicated that the expression of gluten hypersensitivity as enteropathy, measurable only if a count of IELs was performed. This was consistent with other studies (Pardeep Brar et al., 2006; lmars Lidums et al., 2011).

There were high numbers of negative coeliac antibodies in this work among CD subjects of group 1 after commencing GFD only. There were 2 patients even after a period of 1 year ±1month showed positive serology either in one test or more than one test. The positive results indicate either that patient was not on strict GFD, or non-responsive to GFD as in cases of refractory CD. Meanwhile, none of the 16 CD patients after commencing *NS* oil plus GFD for a period of 1 year ±1month showed positive serology results in all markers.

Effect of NS oil on CD autoantibodies observed in this study demonstrated that an immunological effect occurred in parallel to the histological effect of this oil. Indeed, the effect of *NS* on immune system was reported in many studies over a two decades ago suggested that ongoing usage of *NS* enhances immune response in human (Salem, 2005). The majority of patients treated with *NS* oil for 4 weeks showed a 55% increase in CD4 to CD8 T cells ration and a 30% increase in natural killer (NK) cell function (Salem, 2005). It was also reported that *NS enhanced* the production of interleukin-3 by human lymphocytes and increased interleukin-1β *in vitro* (Haq et al., 1995), suggesting that it has an effect on macrophages, thus *NS* can enhance immune response. Previous studies showed that the constituents of *NS* seed possess potent potentiating effects on the cellular (T cell-mediated) immunity, while they have a tendency to downregulate (suppress) B cell-mediated (humoral) immunity (Haq et al., 1995; Salem, 2005). Moreover, a study reported that treatment with *NS* oil induced a 2-fold decrease in the antibody production in response to typhoid vaccination in

albino rats (Islam et al., 2004). In contrast to the above mentioned reports, our findings demonstrated that *NS* oil exhibits its immunological activity through the downregulation of B cell-mediated immunity, as evidenced from decreased antibodies production, hence histological recovery was happened.

Our preliminary findings also supported the traditional usage of *NS* for the prevention of diseases especially by immune protection, but the actual mechanism by which *NS* oil exerts its anti-coeliac disease effects needs to be further investigated.

5. CONCLUSIONS

Preliminary findings demonstrated that administration of *NS* oil with GFD in treatment of CD can lead to complete histological recovery and complete absence of CD antibodies. Ultimately, results emerging from this study may help to provide a scientific basis for the immunotherapeutic application of NS in clinical management of refractory CD patients.

ACKNOWLEDGEMENTS

The authors would like to thank staff of Pathology and Immunology units at Teaching Laboratories of Medical City in Baghdad for technical support for this work.

CONSENT AND ETHICAL APPROVAL

The study was approved by the Ethics Committee of College of Medicine/ University of Baghdad prior to commencement of study and informed written consent was obtained from all participants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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