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Immunomodulatory Effect of *Nigella Sativa* Oil Treatment in Iron Deficiency Anemia Caused by Refractory Coeliac Disease

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ABSTRACT

The purpose of this study was to assess the hematological, immunological and histological profiles of iron deficiency anemia (IDA) caused by refractory coeliac disease (CD) after commencing *Nigella sativa (NS)* oil with gluten free diet (GFD) for a period of 1 year to prove its immunomodulatory effect in these conditions. Eighteen adult unexplained IDA patients diagnosed as CD patients were recruited in the study after performing endoscopy and duodenal biopsy in addition to hematological and serological assessment before and after treatment of GFD alone or with *NS* oil capsules for a period of 1 year. Duodenal biopsies were interpreted histologically according to modified Marsh criteria and the sera were tested for antigliadin antibodies (AGA), endomysium antibodies (EMA), and anti-tissue transglutaminase antibodies (tTG). The response to gluten withdrawal plus *NS* oil for a period of 1 year in unexplained IDA patients caused by refractory CD was better than GFD alone with significant response of hematological parameters, serological markers, and histological changes. Using *NS* oil in addition to GFD as treatment of IDA associated with refractory CD effectively elevates the levels of all hematological parameters due to complete duodenal histology remission and complete absence of serological CD antibodies.

Keywords: Iron deficiency anemia, Coeliac disease, *Nigella sativa* oil, Gluten free diet, Coeliac antibodies.

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INTRODUCTION

Celiac disease (CD) is an immune-mediated enteropathy caused by permanent intolerance to gluten, the major storage protein of wheat and similar grains, which causes damage to the small intestinal mucosa by an autoimmune mechanism in genetically susceptible individuals [1].The immunologic response to gluten in CD patients 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 [2]. The clinical presentation of CD is extremely heterogeneous [3]. Classical symptoms include chronic diarrhea, steatorrhea and abdominal complaints (4-5). However, only a few patients with CD show clinical malabsorption, while most patients have subtle symptoms, therefore, the disease is clearly underdiagnosed (3-5).

Because of the improvement in diagnostic methods for identifying CD, there has been a marked increase in the proportion of subjects identified as coeliac patients, who do not have the classical manifestations of disease [1]. Anemia without other clinical clues of intestinal malabsorption is one of the most common extraintestinal manifestations of coeliac disease [6-7]. Although folate and cobalamin deficiency are known complications of CD, the most common nutritional anemia associated with CD is iron deficiency [8]. Iron deficiency anemia (IDA) was reported in up to 46% of patients with subclinical coeliac disease in one study [6, 9], and its prevalence was higher in adults than in children. Similarly, among patients identified by population screening, consisting mostly of young or middle-aged adults, 50% were anemic. However, most patients have subclinical or silent forms of CD in which IDA can be the sole presentation (10), whereas, a characteristic feature of IDA associated with CD is its refractoriness to oral iron treatment [10].

However, regarding management of CD, the guidelines have recommended that follow-up endoscopy and biopsies should be performed after commencing a GFD to document the serological antibodies disappearance and histological improvement and to confirm the clinical remission and dietary compliance [11]. Follow-up endoscopy after 6-12 months on a GFD is considered as the gold standard in evaluating dietary compliance. [11]

The seeds of *Nigella sativa* commonly known as black seed or black cumin, are used in herbal medicine all over the world for the treatment and prevention of a number of diseases and conditions [12]. The pharmacological actions of the crude extracts of the seeds (and some of its active constituents, e.g. volatile oil and thymoquinone) that have been reported include protection against nephrotoxicity and hepatotoxicity induced by either disease or chemicals [12]. The NS oil has anti-inflammatory, analgesic, antipyretic, antimicrobial and antitumor activities [13-14]. It has also anti oral ulcer effects [15], antidiabetic effects (16), anti-cardiovascular effects (17), gastroprotective effects [18] and immunomodulatory effects [19]. The seeds and oil are characterized by a very low degree of toxicity since it would appear that the beneficial effects of the use of NS might be related to their cytoprotective and antioxidant actions, and to their effect on some mediators of inflammation [19].

The preliminary results of our CD research group have proved that *NS* oil has immunomodulatory effects on refractory CD in general [20]. These cases are defined as conditions 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 CD and overt malignancy (21-22). Refractory CD is treated by GFD, nutritional supplement, corticosteroid therapy and immunosuppressive drugs (21). To the best of our knowledge till now there are no available data regarding the effect of using *Nigella sativa* as immunomodulator in addition to GFD for the management of associated diseases with refractory CD.

The aim of this study was to assess the hematological, immunological and histological profiles of adult IDA caused by refractory CD after treatment with *NS* oil in addition to GFD for at least 1 year.

MATERIALS AND METHODS

Subjects and methods

Eighteen (18) patients [12 females, 6 males, median age, 34 years; range, 18-66 years] were recruited in this study. These patients were diagnosed to have unexplained IDA with refractoriness to oral iron treatment, referred to GIT Teaching Hospital in Baghdad, Iraq from different medical centers. Initially, the primary evaluation of anemia was done by the hematologists who referred the patients for exclusion of gastrointestinal causes of IDA. The IDA was defined as: hemoglobin level less than lower limits (13.5 g/dl for male adult, 12.0 g/dl for female adult), serum ferritin level <15 ng/ml (normal 20- 300 ng/ml), transferrin saturation less than 15%, and mean corpuscular volume (MCV) less than 80 fL. Patients with the following conditions were excluded from the study: age <16 years or >80 years, known or previous investigation for CD, acute or chronic obvious blood loss (e.g. melena, hematochezia, hemoptysis, recurrent epistaxis, hematuria, trauma), present treatment for IDA, known malignancies or chronic diseases (e.g. chronic renal or liver disease, severe respiratory or cardiac disease, connective tissue disorders, diabetes mellitus), pregnancy, hypermenorrhea (cycles ≥7 days), menometrorrhagia, alcoholism, gastric surgery.

Before patient's recruitment an extensive evaluation of IDA etiology was performed to each patient, including detailed bleeding questionnaire, physical examination, blood tests, urine analysis for hematuria, occult blood loss analysis in feces, upper GI endoscopy, and colonoscopy.

After this evaluation, these 18 IDA patients were diagnosed as CD patients and recruited into study after performing duodenal biopsy on the bases of clinical history and serological assessment, including AGA, EMA, tTG antibodies testing.

Biopsies were interpreted by one expert pathologist who was 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 [23-24]. 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 while Marsh I and Marsh II were considered as gluten sensitive subjects.

Patients were randomized into two groups, 9 patients each; (1) Group 1: CD patients were treated by GFD for a period of 1 year; (2) 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. The *NS* oil capsules were purchased from local market (from Pharco Pharmaceuticals, Egypt).

Follow-up endoscopies with duodenal biopsies, hematological, and serological monitoring were repeated at 1 year after treatment. Blood from each patient was collected to examine the hematological indices [Hb (hemoglobin), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), and serum Ferritin], then serum 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. Meanwhile, AGA and tTG were performed by enzyme-linked immunosorbent assay (ELISA) in duplicate and according to the manufacturers' instructions.

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

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. Analyses where the *P*-value was =0.05 were considered to be statistically significant.

RESULTS

Figure 1 shows the hematological profile of all patients before and after treatment. At baseline, all patients were anemic with hematological indices ranges below normal (Hb= 10.7g/dl, MCV= 71fl, MHC= 25pg, MHCH= 28g/dl, and s.Ferritin= 8ng/ml. Meanwhile, after 1year treatment there was an increase in all parameters in both doses of treatment with significant effect with GFD plus NS oil (group 2). This was strongly significant and mean ranges

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were elevated to normal (Hb= 15.4g/dl, MCV= 90fl, MHC= 30pg, MHCH= 34g/dl, in addition to restoration of Ferritin to normal (33ng/ml) (Fig.1).

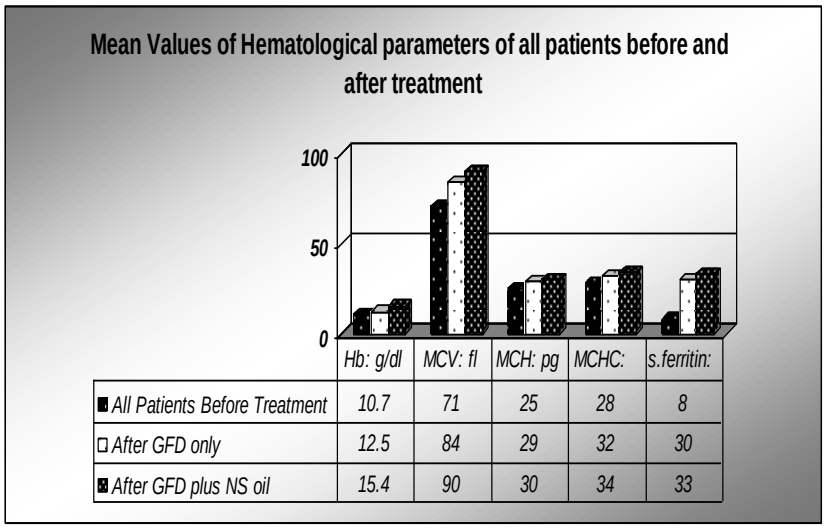

Fig.1. Histogram of hematological findings of all patients with IDA and CD before and after GFD alone or with *NS* oil.

Figure 2 shows the histological results of all 18 patients. After 1 year treatment with GFD only (group 1) there were still one patient had CD (Marsh IIIa changes) in addition to 2 patients without complete recovery from gluten sensitivity (Marsh I & Marsh II changes respectively). However, after 1 year treatment with GFD plus NS oil (group 2) there was complete histological remission in 8 of 9 CD patients while 1 CD patient still had gluten sensitivity only but not CD (Marsh I changes). This was strongly significant.

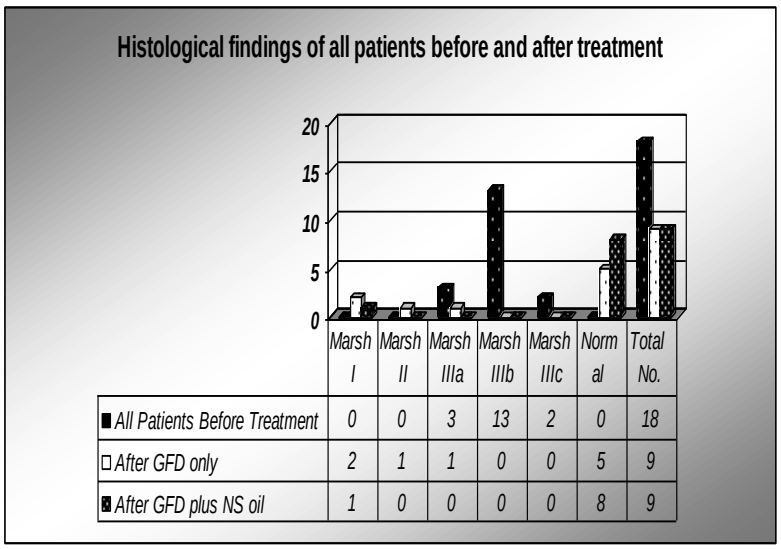

Fig 2. Histogram shows histopathological results in patients before and after GFD alone or with *NS* oil.

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As shown in figure 3; at base line from 18 CD patients there were 13, 16 and 17 patients had AGA positive, EMA positive, tTG antibodies positive respectively. After 1 year treatment the AGA levels were normalized in all patients of 2 study groups, however there was still one patient had positive EMA and one patient had positive tTG after GFD only while all immunological parameters levels were negative when the treatment was GFD plus NS oil. This was strongly significant (fig.3).

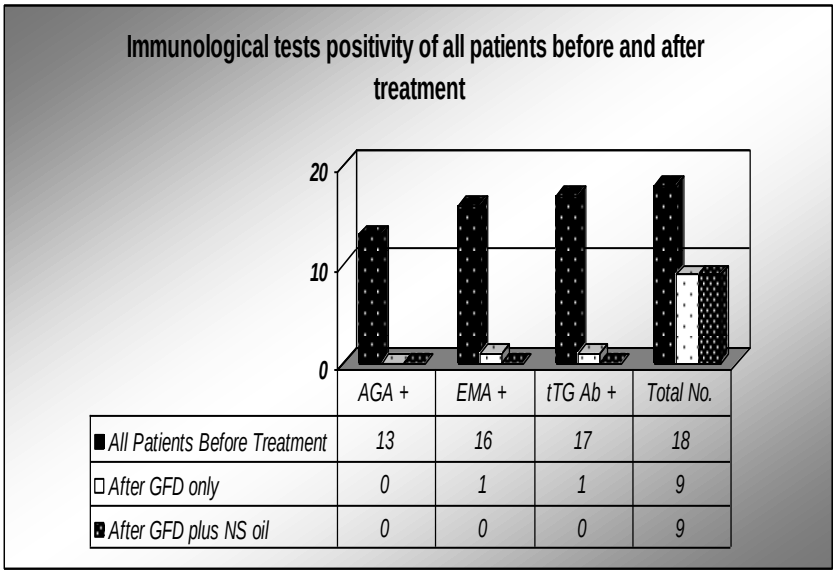

Fig.3: Histogram shows the levels of AGA, EMA, and tTG Ab tests in patients before and after GFD alone or with *NS* oil.

DISCUSSION

This study was our second trial to examine the effect of *NS* oil on CD after 1 year treatment in addition to GFD which is the only treatment been used in CD, but it was not effective against refractory CD cases [20]. The present study demonstrates for the first time, to the best of our knowledge, the effects of *NS* oil on unexplained IDA cases associated with refractory CD. Our principle finding is using NS oil in addition to GFD as treatment of IDA associated with refractory CD effectively elevates the levels of all hematological parameters of blood due to complete duodenal histology remission and complete absence of serological CD antibodies.

It has been suggested that patients with isolated iron or folate deficiency should be referred directly for endoscopic duodenal biopsy. However, at present many of these patients are not referred at all for investigation for coeliac disease [25], however coeliac disease has a wide clinical spectrum including GI and extra-GI findings, which can be diagnosed at any age from childhood to the elderly (4). Classical or typical form of CD is associated with features of malabsorption; however, a substantial number of CD patients have atypical manifestations, including hematologic, endocrinologic, renal, neurologic, psychiatric, dermatologic, and cardiovascular symptoms. Anemia, especially IDA, is a frequent feature in CD and may be the

only presenting symptom (4). An early identification of CD in patients with IDA has great importance, since a strict adherence to a gluten- free diet not only provides management of anemia but also prevents the severe complications such as ulcerative jejunoileitis, intestinal lymphoma and neoplasm [26].

While in most of CD patients, the process of inflammation in duodenum and serological antibodies gets better when gluten is removed from the diet (2 thirds of the patients in present study), there are some cases called refractory CD which occur when we are confident that the gluten has been removed entirely from the diet, and yet the intestine remains inflamed and or serological antibodies are still positive. The explanation of no improvement may be because it could be that some gluten is still in patient's diet or some patients are very sensitive to gluten and may react to even the small amounts of gluten that are present in the GFD products. Refractory CD patients may be helped by the use of corticosteroid therapy and immunosuppressive drugs [21-22]. That's why may be we need to use some medication with minimal side effects like *NS* oil.

Nigella sativa has been known to have immunomodulatory effects, anti-inflammatory, and therapeutic properties [19, 27-29]. *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 [29]. In a mouse model of allergic airway inflammation, the administration of *NS* oil reduced the number of inflammatory cells in lung tissue [28]. In another study, the intraperitoneal administration of *NS* oil inhibited the synthesis of both prostaglandin D2 and T-helper2 cytokines [30]. The exposure of mouse-bone marrow derived dendritic cells to *NS* compromised their maturation, cytokine release and survival [31]. From the overall results of these studies, it may be concluded that NS produces a maximum effect on immune response and resistance in humans and animals [19, 32].

The pharmacological investigations of the effect of *NS* on hemostasis and blood diseases are few. Studies reported an enhancement of coagulation after administration of *NS* in addition they revealed that *NS* is effective on blood hemostatic function but with unclear explanation [33-35]. While in the present study, we noted that *NS* oil, if added to GFD for 1 year treatment has significant effect on hematological parameters in unexplained IDA associated with refractory CD with complete histological recovery profiles and complete absence of CD antibodies (group2) more than the effect of GFD alone (group 1) except 1 case that showed just increase in intraepithelial lymphocytes (Marsh I), however this case was non coeliac disease (fig.2). This effect on hematological parameters was most probably due to immunomodulatory effect of NS on the disease process of autoimmune CD [20].

These findings 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.

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CONCLUSIONS

Our findings demonstrated that administration of *NS* oil with GFD in treatment of IDA associated with refractory CD can lead to increase all hematological indices in addition to complete histological recovery with 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 associated diseases of refractory CD.

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