ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Immunotherapeutic Application of *Nigella sativa* Oil in Management of Dermatitis Herpetiformis Associated with Refractory Coeliac Disease

Muhamed T Osman[1*], and Methil Kannan Kutty[1]

[1]*Department of Pathology, Cluster of Laboratory Medical Sciences, Faculty of Medicine, Universiti Teknologi MARA (UiTM), Sg. Buloh Campus, 47000 Sg Buloh, Selangor, Malaysia.*

ABSTRACT

Dermatitis herpetiformis (DH) is an autoimmune skin disorder associated with a gluten-sensitive enteropathy, characterized by extremely itchy, burning and stinging skin rash. DH is currently considered to be coeliac disease (CD) of the skin which is treated by lifelong strict gluten-free diet (GFD), however after follow-up period of at least 12 months, diagnosis of refractory CD is established where presentations are still present despite strict GFD. This study was carried out to assess the immunological and histological profiles of DH caused by refractory CD after commencing *Nigella sativa* (*NS*) oil with GFD for a period of 6 months to prove its immunomodulatory effect in these conditions. Fourteen DH patients diagnosed as CD cases were recruited in the study. After follow up period of 12 months with strict GFD, three DH cases were considered as refractory CD. Commencing of *NS* oil capsules in addition to GFD was started for additional period of 6 months. Diagnosis of DH with CD before and after treatment was performed by endoscopy, duodenal and skin biopsies in addition to serological assessment. Duodenal biopsies were interpreted histologically according to modified Marsh criteria; meanwhile, skin biopsies were assessed by direct immunofluorescent technique. Moreover, the sera were tested for antigliadin antibodies, anti-endomysium antibodies, and anti-tissue transglutaminase antibodies. The response to gluten withdrawal plus *NS* oil for a period of 6 months in DH patients caused by refractory CD was better than GFD alone. Using *NS* oil in addition to GFD as treatment of DH associated with refractory CD effectively lead to complete clinical remission due to complete duodenal and skin histology remission in addition to absence of serological CD antibodies. Ultimately, the results emerging from this study may substantially improve the immunotherapeutic application of *NS* in clinical management of diseases.

Keywords: dermatitis herpetiformis, coeliac disease, *Nigella sativa*, duodenal, biopsy, Marsh criteria, coeliac antibodies

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Coeliac disease (CD) is a chronic immune mediated gluten-dependent enteropathy, resulting from an inappropriate T-cell-mediated immune response, against ingested gluten in genetically predisposed people [1-2]. It classically presents with gastrointestinal symptoms including chronic diarrhoea, abdominal pain, weight loss, abdominal bloating and anorexia. However, there is a large variety of clinical presentations characterized by the presence of extra-intestinal manifestations, including autoimmune diseases and cutaneous manifestations including dermatitis herpetiformis (DH) [3-4].

Dermatitis Herpetiformis (DH) is an autoimmune skin disorder associated with CD. DH is presenting as an itchy, chronic, papulovesicular eruption. Classically, skin lesions are characterized by a symmetrical eruption on the extensor surfaces of the body such as the knees, elbows, buttocks, and back [4-6]. Treatment of DH with CD is based on strict, lifelong adherence to gluten-free diet (GFD). Majority of DH and CD patients respond well to GFD [7-8]. However a significant minority of patients will continue to be symptomatic. These patients can present a difficult diagnostic and therapeutic challenge and called Refractory CD which 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 [9] Refractory CD with associated DH treatment strategies have focused on immunosuppression as glucocorticoids in addition to GFD [10].

Natural immunomodulators are going to be a central part of 21st medicine helping the body help itself by optimizing the immune system is of central importance in a society so stressed, unhealthily nourished and exposed to toxins that most of peoples are likely to have compromised immune systems [11]. Among these natural immunomodulators is the black seeds " *Nigella sativa*" [12].

The black seeds of the *Nigella sativa* plant that belongs to the *Ranunculaceae* plant family have an extensive history of medicinal use that dates back thousands of years as a spice and food preservative, and was used in traditional medicine by physicians to treat many kinds of illnesses. Many clinical and experimental studies have demonstrated the therapeutic effects of *NS* extracts, including immunomodulative, anti-inflammatory, anti-tumour, anti-diabetic, and anti- cardiovascular diseases [12-13].

Our previous findings demonstrated the immunomodulatory effect of *Nigella sativa 'NS'* oil with gluten free diet (GFD) in treatment of intestinal tissue injuries of refractory CD [14], its immunomodulatory effect in management of extra-intestinal features of refractory CD [15], and its immunomodulatory effect in treatment of refractory iron deficiency anemia caused by CD [16].

This clinical-trial was carried out to assess the potential immunomodulatory effect of black seeds on DH caused by refractory CD.

ISSN: 0975-8585

SUBJECTS, MATERIALS AND METHODS

The study was carried out in Medical city hospitals, Baghdad, Iraq. Fourteen DH patients diagnosed as CD cases were recruited in the study (8 females, 6 males; range 22-52 years). Patients were initially diagnosed as CD patients after performing duodenal biopsy on the bases of clinical history and serological assessment, including testing of antigliadin antibodies (AGA), endomysial antibodies (EMA), and anti tissue transglutaminase antibodies (tTG). All cases then diagnosed as cases of DH secondary to CD after performing skin biopsies.

After follow up period of 12 months with strict GFD, three from these 14 DH cases were considered as DH caused by refractory CD. Commencing of *NS* oil capsules (with a dose of 450mg, twice a day), as dietary supplement in addition to strict GFD was started for additional period of 6 months. The *NS* oil capsules were purchased from local market (Product of Pharco Pharmaceuticals, Egypt). Follow-up endoscopies with duodenal biopsies and serological monitoring were repeated after these 6 months.

Duodenal biopsies were interpreted before and after treatment 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 [17-18]. 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; however, skin biopsies of DH were assessed by direct immunofluorescent technique. They were considered as DH when showed deposits of IgA and complement at the junction of the dermal and epidermal layers of the skin [19].

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.
Data analysis was analyzed using SPSS v16 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

There were clear histological remissions in 11 patients (78.6%) treated with GFD after 12months. However, 3 patients (21.4%) showed MarshIIIa criteria (table1).
The skin biopsies of these 3 patients showed deposits of IgA and complement at the junction of the dermal and epidermal layers of the skin; in addition they have at least one positive antibody test .

Treatment with *NS* oil in addition to GFD resulted in complete duodenal and skin histological remission with significant absence of all immunological antibodies (table2).

ISSN: 0975-8585

Table 1: Histological results in DH patients before and after GFD alone or with *Nigella sativa* oil.

Histopathology	Before GFD		After GFD alone		After GFD with NS		P-value
	No.	%	No.	%	No.	%	
Marsh I	0	0	1	7.1	0	0	
Marsh II	0	0	0	0	0	0	
Marsh IIIa	2	14.3	2	14.4	0	0	
Marsh IIIb	8	57.2	1	7.1	0	0	
Marsh IIIc	4	28.5	0	0	0	0	
Normal histology	0	0	10	71.4	14	100	
Total	14	100	14	100	14	100	0.001

GFD: Gluten-free diet
NS: *Nigella sativa*
No.: number

Table 2: Serological results of AGA, EMA, and tTG Ab tests in DH 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	13	92.8	0	0	0	0	
	Negative	1	7.2	14	100	14	100	0.00001
EMA	Positive	12	85.6	1	6.25	0	0	
	Negative	2	14.4	13	93.75	14	100	0.00001
tTG Ab	Positive	14	100	2	6.25	0	0	
	Negative	0	0	12	93.75	14	100	0.00001
	Total	14	100	14	100	14	100	

GFD: Gluten-free diet
NS: *Nigella sativa*
No.: number

DISCUSSION

The principle finding of this study is using of black seed oil with GFD in treatment of DH associated with refractory CD effectively leads to complete clinical remission due to complete duodenal and skin histology remission in addition to absence of serological CD antibodies. To the best of our knowledge; the results emerging from this study may substantially provide for the first time a new immunotherapeutic application of black seed to be recommended in clinical management of DH associated with CD.

It is well known that 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 [20]. 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) (1 patient in current 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 [20-22]

Effect of *NS* oil on serum 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 [12]. 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 [12]. It was also reported that *NS* enhanced the production of interleukin-3 by human lymphocytes and increased interleukin-1β *in vitro* [23], 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 [12, 23]. 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 [24]. 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 in duodenum was happened besude the histological recovery in the skin. Findings of current study with our previous findings [14-16] support 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 and its extra-intestinal manifestations including DH needs to be further investigated.

CONCLUSION

Treatment by *NS* oil with GFD in treatment of DH associated with refractory CD effectively lead to complete clinical remission due to complete duodenal and skin histology remission in addition to absence of serological CD antibodies. To the best of our knowledge: the results emerging from this study may substantially provide for the first time a new immunotherapeutic application of *NS* to be recommended in clinical management of DH associated with CD. Since DH and CD are autoimmune diseases, our data may open a wide field to study the effect of *NS* on autoimmune diseases in general.

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.

REFERENCES

[1] Green PH, Jabri B. Coeliac disease. Lancet 2003; 362: 383-391
[2] Schuppan D. Gastroenterology 2000; 119: 234-242.

[3] Corazza GR, Gasbarrini G. Baillieres Clin Gastroenterol 1995; 9: 329-350
[4] Haussmann J, Sekar A. Can J Gastroenterol 2006 ; 20(4):291-3.
[5] Farrell RJ, Kelly CP. Celiac sprue. N Engl J Med 2002; 346: 180-188.
[6] L Abenavoli, I Proietti, L Leggio, A Ferrulli, L Vonghia, R Capizzi, M Rotoli, PL Amerio, G Gasbarrini, G Addolorato. World J Gastroenterol 2006; 14: 12(6): 843-852
[7] Oxentenko AS, Murray JA. Int J Dermatol 2003; 42: 585-587.
[8] Lincoln Hernandez, MD, and Peter H. Green. Current Gastroenterology Reports 2006; 8: 383–389.
[9] Malamut G, Cellier C. The American J Gastroenterol 2100; 106; 929-932.
[10] Ryan, B.M., Kelleher, D. Gastroenterology 2010; 119(1): 243-51.
[11] U.S. Patil, A.V. Jaydeokar, D.D. Bandawane. Int J Pharm Pharm Sci 2012; 4 (Suppl 1): 30-36.
[12] Salem ML. Intl Immunopharmacol 2005; 5(13-14): 1749-1770.
[13] Atta MB. Food Chem 2003; 83(1): 63-68.
[14] Muhamed T Osman, et al. British J Med Med Res 2012; 2(4): 527-535.
[15] Muhamed Osman, and Balsam I Taha. Res J Pharm Chem Sci 2013; 4 (1): 522-526.
[16] Muhamed T Osman, et al. Res J Pharm Chem Sci 2012; 3(4): 887- 895.
[17] Marsh MN, Gluten. Gastroenterol 1992;102: 330–354.
[18] Oberhuber, G., Granditsch, G., Vogelsang, H. Eur J Gastroenterol Hepatol. 1999;1: 1185–1194.
[19] Abenavoli L, Proietti I, Leggio L, et al. World Journal of Gastroenterology 2006; 12(6): 843–852.
[20] Brar, P., Lee, A.R., Lewis, S.K., Bhagat, G., Green, P.H.R. Dig Dis Sci. 2006; 10: 1007.
[21] Pietzak, M.M. Gastroenterology 2005; 128: S135–S141.
[22] Lidums L, Teo E, Field J, Stat A. Clin Translat Gastroenterol 2011; 2(4): 1038**.**
[23] Haq A, Abdullatif M, Lobo PI, Khabar KSA, Sheth KV, Al-Sedairy ST. Immunopharmacol 1995; 30: 147-155**.**
[24] Islam SN, Begum P, Ahsan T, Huque S, Ahsan M. Phytotherapy Res 2004; 18: 395-398.