

Intestinal permeability in migraine male patients upon omega-3 and tocopherol administration

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25

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ABSTRACT

Background. Gut microbiome may affect CNS, while an important neurotransmitter in migraine patients, can be expressed in enteric neurons. Omega-3 PUFA (polyunsaturated fatty acid) and vitamin E are reported to decrease neuroinflammation, have beneficial effect on intestinal wall and microbiota of the small intestine. This study aims to find the effect of omega 3 PUFA and vitamin E on intestinal permeability in migrainous, and their possible modulation effect on certain vitamins that have relations for both migraine and intestinal integrity.

Methods. A total 21 migrainous men are included in this study. Blood samples withdrawn before and after intake of omega-3 PUFA and vitamin E supplementation softgel once daily for 30 days to compare with 13 apparently healthy men (control group). The blood samples are used to measure serum lipopolysaccharide, zonulin, amylase, vitamin B12, vitamin D, and albumin. serum lipopolysaccharide, and zonulin.

Results. Body mass index, serum lipopolysaccharide and serum zonulin of migrainous men are higher, but vitamin B12 and vitamin D are lower than control group. Upon intake of supplement, serum zonulin and lipopolysaccharide are decreased in migrainous indicating an improvement of intestinal integrity, but vitamin B12 and vitamin D showed no significant difference. Serum albumin and amylase showed no significant differences.

Conclusion. Omega 3 PUFA and vitamin E supplements decreased intestinal permeability, and can be a useful as an adjunct therapy for migrainous men because of its positive effect on intestinal integrity and gut brain axis, but it has no significant effect on vitamin B12 and vitamin D levels.

Keywords: Intestinal Permeability, Migraine, Omega 3, Vitamin E

INTRODUCTION

Migraine headache is due to release of vasoactive peptides from trigeminal fibers such as substance P, neurokinin and calcitonin gene related peptide (CGRP) resulting in activation of nociceptive sensory nerve terminals in the meningeal vessels [1]. It has been reported that gastrointestinal disorder is more frequent in migraine patients than other people such as dyspepsia and gastro esophageal reflux [3,4]. The same factors; neuroendocrine, immunity, and intestinal microbiota can share in the pathogenesis of migraine and irritable bowel syndrome [5]. Moreover, gastric emptying is delayed in migraine patients during the attack and outside of migraine attack [6,7]. While, nausea and vomiting are usually associated with migraine symptoms profile [2,8].

There are strong connection between brain and gut; brain and pituitary regulate gastrointestinal tract, while the gut may affect central nervous system [9]. Dysfunction of gut-brain axis has been reported in migraine, anxiety and parkinsonism [10,11]. Gut microbiome may affect CNS by stimulation of vagus nerve end terminals or by production of inflammatory molecules [12]. Moreover, intestinal bacteria can produce tryptophan metabolite and affect the level of serotonin [13,14], the latter is decreased in migraine patient but increased during migraine attack [15]. CGRP an important neurotransmitter in migraine patients expressed in enteric neurons can inhibit gastric acid secretion [16,17] affect pancreatic enzymes [18], and has antimicrobial activity for some intestinal bacteria [19]. On the other hand, dysbiosis of intestinal microbiota may influence CGRP signaling and increase its secretion [12]. So, there are strict connection between GIT and migraine. Different factors may affect in intestinal permeability such as (gut microbiota, mucous layer, epithelial layer, in addition to dietary factors) [20,21].

This study involves administration of omega-3 polyunsaturated fatty acid (PUFA) and vitamin E supplement because of their reported usefulness for gut and brain. Omega-3 PUFA has been reported to decrease reactive oxygen species and nitric oxide production in the microglia of the brain and decrease neuroinflammation [22]. DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) of omega-3 when metabolized produce resolvins and protectins which have anti-inflammatory activity and inhibit neutrophil migration [23]. Concerning intestine, omega-3 PUFA and vitamin E have beneficial effect on intestinal wall [24,25] and affect microbiota composition in the small intestine [26–28]. Tocopherol- α is the

biologically active form of vitamin E, used as an adjuvant therapy to treat neuroinflammation of epilepsy [29], showed protective activity for intestinal barrier in mouse [30] by decreasing reactive oxygen species metabolite and by decreasing formation of Arachidonic metabolites [31], which tend to increase status of inflammation [32]. Vitamin E influence intestinal microbiota composition [33], and has antimicrobial activity and can change gut redox potential [34] but its bioavailability decreases upon bacterial endotoxemia [35].

Serum Lipopolysaccharide and zonulin protein are measured as indicators of intestinal permeability [36,37]. Serum amylase is reported to increase in mucosal disease [38]. The loss of bowel integrity due to infarction or perforation result in hyperamylasemia due to absorption of amylase from intestinal lumen [39]. The serum amylase increases in pancreatic and kidney disease, but this cause is excluded as all of the patients and control group in this study have no pancreatic or kidney problem. Concerning serum albumin, the intact endothelial barrier restrict albumin and other molecules [40], while increase capillary permeability cause albumin release. In conditions of disturbances of intestinal integrity, both endothelial and epithelial permeability are increased [41,42], that may result in albumin leakage. The patients in this study have normal liver and kidney function, therefore the change of serum albumin is not related to its synthesis or renal excretion

Vitamins can affect gut microbiome and hence intestinal permeability [34], therefore. Vitamins B12 and vitamin D are measured. Vitamin D is highly associated with intestinal integrity [43,44], microbiome composition [45,46], while its deficiency is found in irritable bowel syndrome [47]. Both vitamin B12 and vitamin D are measured before and after omega3 PUFA and vitamin E supplement intake to show their effect on intestinal permeability through modulating these vitamins levels, possibly by microbiome modulation [48,49].

This study aims to compare intestinal permeability between migrainous and healthy individuals by measuring serum lipopolysaccharide, zonulin protein, amylase and albumin, and to find the effect of omega-3 PUFA combined and vitamin E supplement on intestinal permeability in migrainous. In addition to the effect of this supplement on serum vitamins D and B12 due to their relation with migraine as well as intestinal permeability

SUBJECTS, MATERIALS, AND METHODS

A total of 21 men previously diagnosed of migraine by specialist physician, are included in this study. They do not take dietary supplements three months before this study. All of them

are from AL-Qyara province, and they have no liver, kidney or pancreatic functions disorders. They agree to provide random blood samples before and after intake of omega-3 PUFA and vitamin E supplementation (Mera Omega-3 plus vitamin E)¹® softgel once daily for 30 days. Each softgel contain fish oil including Eicosapentaenoic acid(EPA) 180 mg,⁸ Docosahexaenoic acid(DHA) 120 mg and DL-alpha-tocopherol(vitamin E) 3 mg, manufactured by Starpharma Ltd/Poland for Mera Pharma GmbH/Switzerland. All of patients complete the supplement course and provide the second blood samples. Only two patients couldn't tolerate the supplement, one of them feel of dizziness, while the other one do not give any reason. All the patients provide their samples during interictal migraine phase(in period free of migraine attack)

Excluded criteria : Patients with pancreatic, kidney and liver problems. Those with diabetes mellitus and on dietary supplements.

Control group: Thirteen healthy men (have no migraine before) who agreed to provide blood samples to compare with migrainous group.

The blood samples are used to measure serum lipopolysaccharide, zonulin, amylase, albumin ,vitamin B12, and vitamin D.

Zonulin are measured by using kit (human Zonulin) ELISA, catalog NO: YLA1319HU supplied from Shanghai YL Biotech CO; Ltd. Website: www.YLbiont.com. Lipopolysaccharide of translocated bacteria in human serum are measured by kit(Human Lipopolysaccharides(LPS) ELISA kit) catalog NO: YLA1854HU supplied by Shanghai YL Biotech CO; Ltd. Website:www.YLbiont.com. Amylase, serum albumin are measured by Automatic Clinical Chemistry Analyzer "Accent-200" manufactured by P Z Cormay S.A.¹³ Headquarters: Wiosenna 22 str;05-092 Lomianki, Poland. The kits used are: ACCENT-200- Amylase, and Accent-200 Albumin respectively, all of them supplied from PZ CORMAY. S.A.

⁹ Vitamin D are measured by the kit "MAGlumi® 25-OH Vitamin D(CLIA)" Snibe Diagnostic manufactured by Shenzhen new Industries Biomedical Engineering CO; Ltd. China; using the Analyzer Maglumi® fully- auto-chemiluminescence immunoassay analyzer Model: Maglumi X3⁷ Supplied from Shenzhen New industries Biomedical Engineering Co; Ltd. Vitamin B12 are measured by the fully automated immunoassay analyzer-LIASON® XL-DiaSorin which depend on chemiluminescence technology supplied by DiaSorin S.A (11 rue G.Besse-

Bât.Galilée, 92160 Antony) using LIAISON® vitamin B12 kit supplied by DiaSorin S.P.A Italy.

5 Statistical analysis: The statistical analysis were conducted using GraphPad prism software. Data expressed as mean±SD. One way ANOVA with post hoc Duncan test used to compare between parametric variables. P values of less than 0.05 considered as significance.

RESULTS

The study involves administration of 1 omega-3 PUFA and vitamin E supplement in the form of pharmaceutical softgel "Mera pharma GmbH/Switzerland" as a dietary supplement for migraine male patients. There are three groups in this study : control (healthy individuals with no migraine), 20 migrainous before supplement intake, and migrainous after intake of supplement for 1 month (Table 1).

Table 1. Demographic parameters of the studied groups.

Parameters	Control	Migraine patients
Age (years)	33.15±6.02	36.875±7.79
BMI	25.55±2.18	29.47±5.12
Duration of Migraine(years)	-----	10.22±3.8

Data expressed as Mean±SD

Serum zonulin and lipopolysaccharide are higher in migrainous before supplement intake, indicating a higher intestinal permeability than control. Upon intake of omega3 and vitamin E, serum zonulin and lipopolysaccharide have been decreased indicating improvement of intestinal integrity (Figure 1).

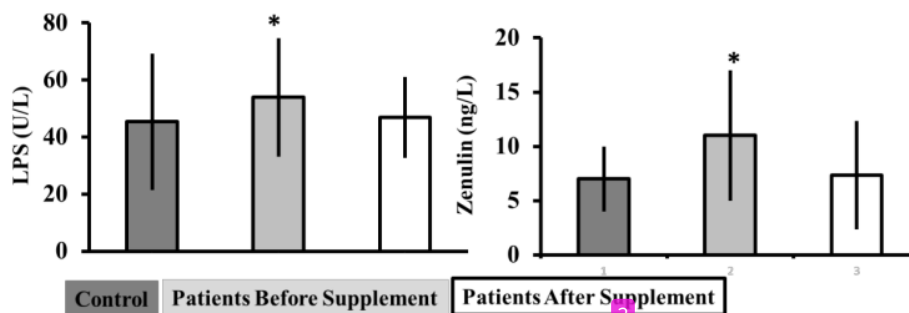


Figure 1. Supplement modulated serum zonulin and lipopolysaccharide in migraine patients compared to control group or patients group after therapy. *p<0.05, * as compared to other group. Data expressed as mean±SD, one-way ANOVA test with series of t-test between groups to determine the difference.

Amylase has no significant differences between the three groups, while serum albumin has no differences between control group and migrainous before supplement intake, indicating no

mucosal disease resulted in increased amylase absorption, or intestinal protein loosing. Serum albumin exhibit no difference between migrainous before and after supplement intake, but migrainous group after supplement intake showed lower albumin level than control healthy group which may be attributed to down regulation effect of omega 3 PUFA (Figure 2).

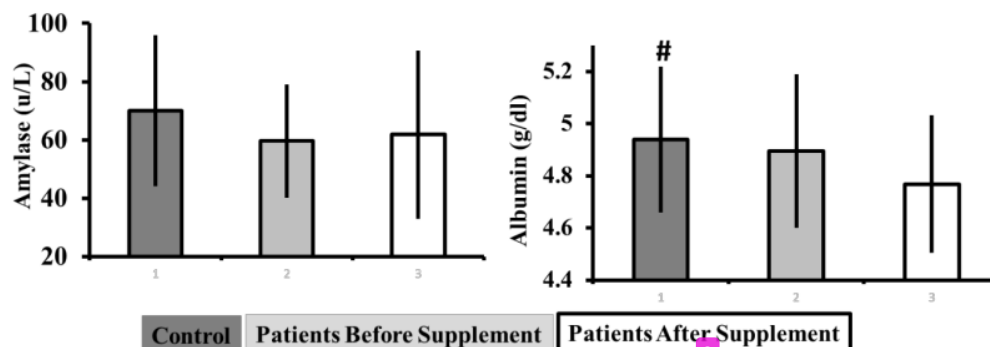


Figure 2. Supplement effect on serum amylase ¹⁴ albumin parameters in migraine patients compared to control group or patients group after therapy. *#p<0.05, ³ as compared to control group. #as compared to patients group after therapy. Data expressed as mean±SD, one-way ANOVA test with series of t-test between groups to determine the difference.

Vitamin B12 and vitamin D are higher in control group than migrainous before and after supplement intake (Figure 3).

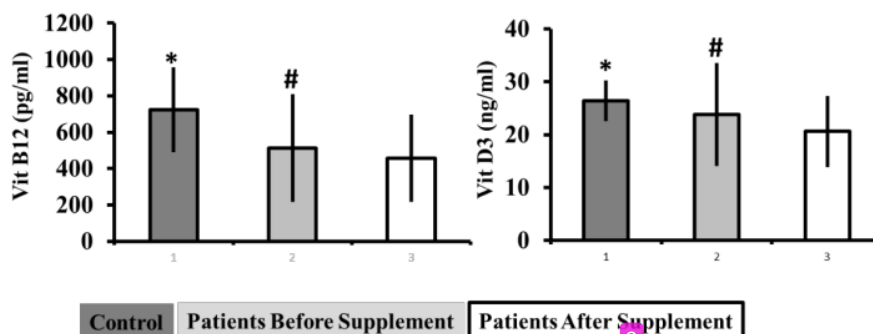


Figure 3. Supplement modulated vitamin D ³⁰ d B12 serum concentration in migraine patients comp ¹⁰ to control group or patients before therapy. *#p<0.05, * as compared to other groups. #as compared to control group. Data expressed as mean±SD, one-way ANOVA test with series of t-test between groups to determine the difference.

DISCUSSION

Intestine prevent the entrance of pathogenic bacteria and toxic substances to the circulation [50], while increased intestinal permeability lead to entrance of endotoxin, pathogen and inflammatory substances to circulation leading to systemic inflammation and disease [51]. The translocation of lipopolysaccharide derived from intestinal microbiome trigger a state of inflammation and oxidative stress which further augment the problem of permeability [52].

Moreover, the inflammation enhance expression of zonulin, the protein that modulate tight junction in intestine [53]. Serum lipopolysaccharide and serum zonulin are used for measurement of intestinal permeability, as a higher zonulin indicates a more permeable tight junction that lead to increase microbial translocation to systemic circulation [54]. Therefore, serum zonulin and lipopolysaccharide are reported as non-invasive sensitive markers to determine intestinal permeability [55].

Nutrition and gut microbiota are implicated in inflammatory status which may alter intestinal permeability [20,21,56]. Therefore, the addition of dietary supplement may help intestinal permeability. Omega-3 PUFA possess ant-inflammatory activity by binding to COX-enzyme binding site and inhibit conversion of Arachidonic acid to prostaglandin and leukotriene [57]. Moreover, omega-3 polyunsaturated fatty acids enter in the synthesis of mediators that resolve inflammation [58–60]. The effect of omega 3 PUFA on microbiota composition is evident in mice but less evident in human [24], While, vitamin E has antimicrobial activity, modulate intestinal microbiota, decrease inflammation and has antioxidant activity [34].

This study involves administration of omega 3 PUFA and vitamin E supplementation to migrainous men in the form of pharmaceutical formulation softgel "mera pharms GmbH/Switzerland". The study involves three groups: control healthy individual with no migraine, migrainous before supplement intake, and migrainous after one month of supplement intake. The study revealed that migrainous patients have higher body mass index than control healthy individual (Table 1). The higher body mass index in migrainous may be attributed to their abnormal eating behavior [61].

After comparison of the three groups, significant differences in serum lipopolysaccharide and zonulin levels are reported in migraine patients before supplement intake when compared to control (Figure 1). Which means a higher intestinal permeability in migrainous when compared to control, but serum amylase and serum albumin showed no differences (Figure2). In migraine patients, both serum zonulin and lipopolysaccharide showed significant decreases after Omega-3 PUFA and vitamin E supplement (Figure 1), which suggest a supplement positive effect on intestinal integrity, as the decrease of zonulin and lipopolysaccharide mean a decrease in intestinal permeability [55,62]. The positive effect of omega 3 PUFA and vitamin E on intestinal permeability may be related to the effective dose of supplement and its resolving effect on stimulus that increase migrainous intestinal permeability [24].

However, it has been reported that omega 3 PUFA supplement has no effect on tight junction protein expression [63].

Serum amylase has been shown to increase after inflammatory disease of small intestine, and gastroenteritis [64]. However, there were no differences in serum amylase between control migrainous before and after supplement intake (Figure2). Although of that fecal albumin is used to assess intestinal permeability but this test is difficult to perform and fit obese not healthy individuals [62], while, serum albumin is reported to decrease in protein losing enteropathies and intestinal leakage [65]. However, there was no difference in serum albumin between control and migrainous before intake of supplement (Figure 2), and no difference between migrainous before and after supplement (Figure 2). But, serum albumin is lower in migrainous men after intake of omega 3 PUFA and vitamin E supplement when compared to control healthy group (Figure 2). The result may be related to albumin downregulation effect of omega 3 PUFA [66].

Other biochemical parameters may relate directly or indirectly to intestinal permeability and to migraine pathophysiology are measured such as vitamin D, and Vitamin B12. Both of the two vitamins were higher in the control healthy group and lower in migrainous (Figure 3), and upon supplement intake their levels do not differed significantly (Figure 3). One study showed that vitamin D has a negative correlation with the severity of migraine headache [67], while vitamin D receptor are highly expressed in epithelial cells of intestine playing a major role in regulating intestinal permeability [68]. The decreased vitamin D level results in dysregulation of intestinal microbiota [69–72], and disruption of tight junction integrity [73] by up regulation of zonulin expression [74]. This study revealed a significant higher vitamin D levels in control group when compared to migraine patients before and after intake of supplements (Figure3). This result is in agree with the review of Zeinab Ghorbani et al, who revealed that most of migraine patients have vitamin D deficiency [75]. However, upon omega 3 PUFA and vitamin E supplement intake, vitamin D showed no significant difference (Figure 3), this result may be attributed to the low dose of supplement ,as omega 3 PUFA is reported to affect vitamin D level when its dose is more than 1000 mg/day and for more than 8 week [76].

This study showed significant higher level of Vitamin12 in control group when compared to migraine male groups before and after supplement intake. While, another study showed no difference of vitamin B12 Level between migrainous and non migrainous men [77].

Concerning vitamin B12, it is highly associated with migraine and tension type headache [78], and reshape intestinal microbiota [79]. There is a strong connection between vitamin B12 and butyrate producing microbiota [80], while Butyrate and keto-diet can benefit migrainous by decreasing pain and frequency of attack [81,82]. Moreover, vitamin B12 deficiency decrease intestinal barrier by reduction of cell differentiation [47], shortening of intestinal villi, accumulation of homocysteine and induction of inflammatory reaction [83]. However, supplement of omega 3 PUFA and vitamin E has no effect on the level of vitamin B12 and vitamin D directly, or through possible effect on intestinal microbiota (Figure 3)

The effect of omega 3 PUFA on intestinal permeability may be due to the attenuation of pro-inflammatory cytokines by EPA and DHA [84], or by their ability to decrease transepithelial electrical resistance [85,86], or by the ability of omega 3 PUFA [22] and or vitamin E [87] to reduce oxidative stress and reciprocally minimizing migraine-attack associated gut dysfunction, but may not by omega 3 PUFA effect on microbiota that produce vitamin B12 or by influencing vitamin D level.

CONCLUSION

Omega 3 PUFA and vitamin E supplements in the form of "mera" softgel once daily for one month for migraine patients decrease intestinal permeability. Migraine patients exhibited lower level of vitamin B12 and vitamin D than non migrainous group, and upon supplement intake their level do not differed significantly. The modulation effect of omega 3 PUFA and vitamin E on intestinal permeability may not related to microbiota producing vitamin B12 modulation or vitamin D absorption, but may due to their anti-inflammatory effect. Omega3 PUFA and vitamin E supplement can be a useful as an adjunct therapy to migrainous men because of its positive effect on intestinal permeability and gut brain axis involved in migraine pathophysiology.

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