The Role of Probiotics in Celiac Disease and their Potential Effect on Immunological and Clinical Markers of The Disease

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Abstract:

Development an adjuvant therapies to gluten free diet in celiac disease (CD) is essential. Intestinal microbiota may have a task in celiac disease pathogenesis, a lower count of *Lactobacillus* has been noticed in the feces of CD patients on a gluten-free diet when was compared with healthy patients. On the other hand, enterobacteria have been shown to be increased, the imbalance that characterizes the gut microbiota of celiac patients may be restored by administration and usage of probiotics. There are very limited clinical studies assessing the hypotheses of probiotics effect on celiac disease patients. This study was designed to investigate the effect of probiotics on CD patients.

From November 2015 to December 2016 a group of forty of newly diagnosed and poorly controlled celiac disease patients with mean age 28.38 ±12.89 yrs(mean ±S. D) were enrolled in a placebo-controlled intervention trial. Twenty two (22) out of forty were administered capsules containingvitalactic B Capsules (Lactobacilli culture 50mg 2Billion *Lactobacillus plantarum* and 2 Billion *L-acidophilus*) and eighteen (18) were administered placebo capsules (rice flour) celiac patients on a daily basis for 3 months besides a gluten-free diet. Serum tissue transglutaminase (tTG) antibody concentrations and serum antiendomysial IgA (EMA) antibody concentration at base line and post intervention (3 months) were assayed using ELISA kits. Gastrointestinal symptoms including presence or absence of (abdominal pain, abdominal distention, heart burn, diarrhea) at base line and post intervention occurrence were assessed using the Gastrointestinal Symptoms Rating Scale (GSRS) questionnaire.

There was significant decline in serum tTG IgA after 3 months administration of Vitalactic B capsule; while the decline was non-significant in serum EMA IgA concentration after the Vitalactic B administration. In placebo group the decline was non-significant in both serum antibodies. There is a significant reduction in abdominal pain and heart burn after 3 months intervention with Vitalactic B, but the decline was non-significant regarding diarrhea and abdominal distention after administration of Vitalactic B. Non-significant reaction in clinical symptoms was reported in the placebo group.

These findings indicate that,Vitalactic B capsules (probiotics) have a health advantage to status of celiac disease patients on gluten free diet. Vitalactic B probiotic supplement improve celiac disease specific antibodies tissue transglutaminase result and CD clinical symptoms including abdominal pain, heart burn after three months of probiotic administration.

Introduction:

Celiac disease (CD) is an autoimmune disease that was diagnosis commonly among Iraqi children and adults (1). Until now, adherence to strict gluten free diet is the most effective treatment for this disease. However, complete adherence with a strict gluten free diet is very difficult due to the appearance of gluten in most processed foods; besides of the social limitation implied. An incomplete adherence to gluten free diet lead to loss of improvement in patient's nutritional status and elevate health risks (2). The proximate goal of the CD therapy is the mucosal healing and the complete healing of the mucosa is achieved in a minority of patients on gluten free diet (3). Therefore, there is a real request and desire to develop adjuvant therapies.

Intestinal microbiota may have a task in celiac disease pathogenesis (4) a lower count of *Lactobacillus* has been noticed in the feces of CD patients on a gluten-free diet when was compared with healthy patients. On the other hand, enterobacteria have been showed to be increased in CD patient's feces (5).

The imbalance that characterizes the gut microbiota of celiac patients may be restored by administration and usage of probiotics (6). There are several preclinical studies concerning the effect of probiotics on causal mechanisms of CD damage.

To our knowledge, there are very limited clinical studies assessing the hypotheses of probiotics effect on celiac disease patients; in addition, there are no clinical studies were performed in Middle East. Therefore, we have designed an exploratory trial to determine the potential effect of probiotic on the CD specific antibodies and CD clinical symptom in Iraqi CD patients in Basrah city.

Materials and Methods:

1. **Patients and the Study Design:**

From November 2015 to December 2016 a group of forty two of newly diagnosed and poorly controlled celiac disease patients aged 14-58 years with mean 28.38 ±12.89 yrs (mean ±S.D) from Faiha Specialized Diabetes, Endocrine and Metabolism centerin Basrahwere enrolled in a placebo-controlled intervention trial to estimate the effects of the involvements with probiotic *Lactobacilli* culture of *Lactobacillus plantarum* and *L. acidophilus* (vitalactic B capsules)(Vitanepharma company, United States of America) together with the gluten free diet

on the CD specific antibodies and patients clinical symptoms after exclusion of patients on antibiotic and immunosuppressive therapy, malignant disease,food allergy.

An experimental trial was conducted on 42 celiac disease patients(Ethic agreement number 76 in 28-1-2016,Iraqi Ministry of Health)from those celiac disease patients 23 were administered capsules containing Vitalactic B Capsules (*Lactobacilli* culture 50mg 2Billion *L-plantarum* and 2 Billion *L-acidophilus*) and 19 were administered placebo capsules (rice flour) celiac patients daily for 3 months besides a gluten-free diet (table 1).

Table 1: Characteristic of patients included in the study

Characteristics		Vitalactic B groups	Placebo groups
Total number		23	19
Gender	Male	7	4
	Female	16	15

Histology (marsh categorization)	Marsh I	1	2
	Marsh II	2	4
	Marsh IIIa	2	2
	Marsh IIIb	10	6
	Marsh IIIc	8	5
Associated endocrine diseases	Type 1 diabetes	7	7
	Primary hypothyroidism	2	1
	Adison disease	1	2

2. Outcome measures (at baseline and post intervention) included:

- **Celiac disease specific antibodies:**

- Tissue transglutaminaseIgA (tTG)

- Anti-EndomysialIgA (EMA IgA)

Blood samples were taken from patients that were entered the experimental trial. Blood was accumulated in clot activator tubes then centrifuged for 3-5 minute on 1500 g. The serum was collected and divided into 2 plain tubes then stored in -20 c. Serum tissue transglutaminase antibody concentrations were assayed using ELISA kit (AESKULISA tTGIgA,Germany) and serum antiendomysial IgA antibody concentration was assayed using ELISA kit (my BioSource company MBS7235065,USA).

- **Clinical Symptoms:**

Gastrointestinal symptoms including presence or absence of (abdominal pain, abdominal distention, heart burn, diarrhea at base line and post intervention occurrence were assessed using the Gastrointestinal Symptoms Rating Scale (GSRS) questionnaire, which assess common symptoms of gastrointestinal disorders including celiac disease.

At each visit, patients were asked about their abdominal pain, abdominal distention, heart burn, diarrhea (symptoms were reported as improved, worsen or similar).

Statistical Analysis:

Statistical analysis was performed by using spss version 19. Results was depicted as independent sample. T-test to compare between patients baseline and post intervention outcomes measures (CD specific antibodies,weight changes), values of < 0.05 was considered to indicate statistical significance, chi square (fisher tests) was used to compare between patients baseline and post intervention outcomes measures (clinical symptoms) values of < 0.05 was considered to indicate statistical significance. The general characteristics of study groups were explored using mean and standard deviation (S.D).

Results:

From the forty two patients, there were two withdrawals registrations, due to the poor adherence to gluten free diet, of those withdrawals, one belonged to the Vitalactic B group and one to the placebo group.In this study, there was a try to compare patient's outcome

measures (baseline and post intervention) that involve patients' serum specific celiac disease antibodies concentrations and evaluation of clinical symptom outcomes.

- ***specific celiac disease antibodies:***

a-Tissuetransglutaminase IgA:

In vitalactic B group the mean baseline level of serum tTG IgA for celiac patients was 147.363 ± 136.057U/ml (mean ±S.D) while estimated levels after three months administration of vitalatic B capsules (post intervention)was 63.852±102.0482U/ml. There was statistically significant difference ($P < 0.05$) (Table 2).

inplacebo group the baseline level of serum tTG IgA for celiac patients was 221.794± 126.35001U/ml (mean ±S.D) while estimated levels after three months administration of placebo capsules (post intervention) was152.666±133.212U/mlthe difference was statistically non-significant ($P > 0.05$) (Table 2).

b-Antiendomysial (EMA) IgA:

In vitalactic B group the baseline level of serum EMA IgA for celiac patients was 223.729± 94.4786 ng/ml(mean ±S.D) while estimated levels after three months administration of vitalatic B capsules (post intervention) was 143.188±63.439ng/ml the difference was statistically non-significant ($P >0.05$).

In placebo group the baseline level of serum EMA IgA for celiac patients was192.9307± 90.5972 ng/ml (mean ±S.D) while estimated levels after three months administration of

placebo capsules (post intervention) was 185.5138±104.576 ng/ml, the difference was statistically non-significant (P >0.05) (Table 2, Fig. 1 & 2).

Table 2: shows difference between mean serum level of celiac disease specific IgA before and after administration of Vitalactic B and placebo capsules

CD specific IgA	Baseline	Post intervention	p.value	sig
tTG IgA* (Vitalactic B groups)	147.363 ± 136.05U/ml	63.852±102.0482U/ml	0.019	significant
tTG IgA*(placebo groups)	221.794± 126.351U/ml	152.666±133.212U/ml	0.402	Non significant
EMA IgA**(Vitalactic B groups)	223.729± 94.47 ng/ml	143.188±63.439ng/ml	0.183	Non significant
EMA IgA** (placebo groups)	192.9307± 90.59 ng/ml	185.5138±104.576 ng/ml	0.405	Non significant

*tTG IgA = Tissue transglutaminase IgA

**EMA IgA= Anti-Endomysial IgA

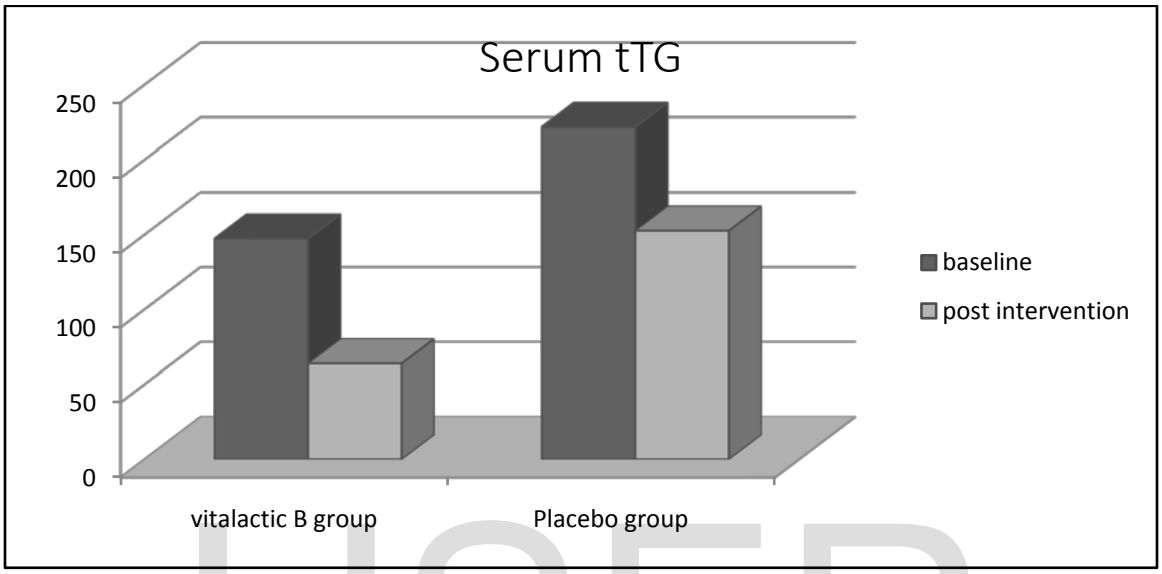

Figure 1: Histogram image shows difference between mean serum level of tTG IgA before and after administration of Vitalactic B capsules

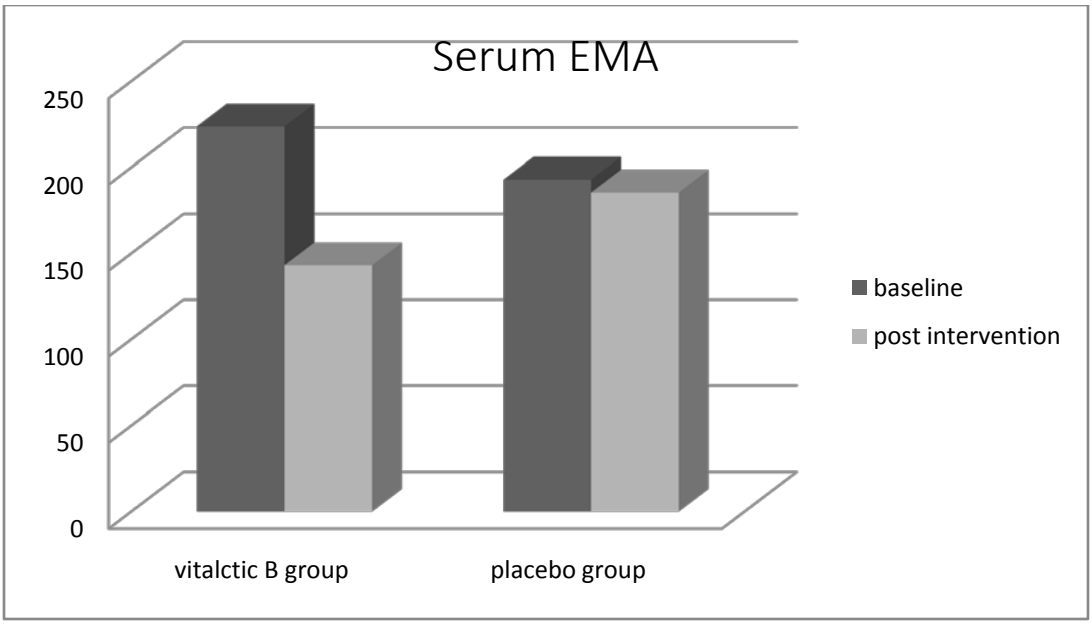

Figure 2: Histogram imageshows difference between mean serum level of EMA IgA before and after administration of placebo capsules

- **Clinical Symptoms:**

In Vitalactic B group fifteen patients suffer from abdominal pain at the onset of study, after administration of Vitalactic B capsules only four patients have abdominal pain, the difference was boarder line significant(P=0.05) while in placebo group ten patients suffer from abdominal pain at the onsetof study, after administration of placebo capsules only seven patients had abdominal pain, the difference was statistically non-significant (p >0.05).

Regarding heart burn, in vitalactic B group fourteen patients suffer from heart burn at the onset of study, after administration of vitalactic B capsules only three patients have heart burn, the difference was statistically significant (p <0.05), in placebo group ten patients

suffer from heart burn at start of study, after administration of placebo capsules only six patients have heart burn, the difference was statistically non-significant (p >0.05).

In regard to, abdominal distention, thirteen patients suffer from abdominal distention in vitalactic B group at the onset of study, after administration of vitalactic B capsules only seven patients have abdominal distention, the difference was statistically non-significant (p >0.05) while in placebo group eleven patients suffer from abdominal distention at the start of the study, after administration of placebo capsules only six patients had abdominal distention,the difference was statistically non-significant (p >0.05).

Performance of diarrhea was also assessed in this study; in vitalactic B group ten patients suffer from diarrhea at beginning of study, after administration of vitalactic B capsules only three patients were remain having diarrhea, the difference was statistically non-significant (p >0.05) but in placebo group only two patients suffer from diarrhea at beginning of study and after administration of placebo capsules only one patients were having a diarrhea, the difference was statistically non-significant (p >0.05) (Table 3).

Clinical symptoms	Vitalactic B group	P-value Vitalactic B	Placebo group	P-value Placebo
Abdominal Pain				
Baseline	15	0.05	10	0.256
Post intervention	4	Border line	7	Non-significant
Heart burn				
Baseline	14	0.04	10	0.229
Post intervention	3	Significant	6	Non-significant
Abdominal distention				
Baseline	13	0.13	11	0.31
Post intervention	7	Non significant	6	Non-significant
Diarrhea				
Baseline	10	0.055	2	0.6
Post intervention	3	Non significant	1	Non-significant

Table 3: number of cases with celiac disease clinical symptoms before and after administration of Vitalactic B and placebo capsule.

Discussion:

In this study, there was observable changes regarding the CD specific antibody production, which might have resulted from the treatment. As expected, all patients had abnormally increased serum concentrations of the two diagnostic important antibodies tTGIgA and anti-EMA IgAat baseline. After the 3-months trial; patients on the probiotic capsules experienced a more than half declinein serum values of anti-tTG IgA which was statistically significant decrease.

Simultaneously, a non-significant decrease was observed in patients on placebo capsules when compared with baseline values. This result agreed with results obtained in Argentina were twenty-two adult celiac disease patients; twelve of them employed to take

*Bifidobacteriuminfantis*natren life start strain capsule the remaining ten patients received placebo capsules on 3 weeks period. The patients were also taken 12 g of gluten per day at least were patients on probiotic experienced a 10% reduction in serum values of anti-tTG IgA; contrary, a 7% increment was observed in patients on placebo capsules when compared with baseline values (7). The significant decrease in anti-tTG IgA might be due to that; the administration of probiotic in newly diagnosed CD patients caused a decline in mature lymphocytes (CD3þ) and HLA-DRþ T- lymphocytes greater than in patients on gluten free diet alone, which is most probably lead to better recovery from the inflammatory status associated with celiac disease, due to increase pro-inflammatory cytokine production (8).

Also, there is established correlations between the densities of mucosal CD3þ with serum tTG antibody levels in treated and untreated celiac patients, besides that the count of intraepithelial lymphocyte correlated significantly with serum tTGantibody level (9).

In regarding to anti-EMA IgA antibody, an observable change was noticed in anti-EMA IgA antibody production, which might have resulted from the treatment with vitalactic B probiotics. As expected all patients have raised baseline serum level of anti-endomysial IgA antibody, and after 3 months intervention with vitalactic B capsules there was more than half decline in the mean serum level of anti-EMA IgA antibody. Whereas, patients on placebo capsules intervention shows only a minority decline in serum level of anti-EMA IgA antibody after 3 months intervention. Both changes were statistically non-significant, but the decline in

anti-EMA IgA antibody production was more in group on vitalactic B capsules than in group on placebo capsules which indicated better reaction however, the changes manner of both antibodies suggests that the effect could be associated with administration of vitalactic B capsules probiotic, as more decline in serum levels of antibodies observed after administration of probiotic capsules (Vitalactic B).

The serum concentrations of auto antibodies decreased over time after starting gluten free diet so it can assess the degree of compliance with gluten free diet, but the time required to normalize anti-EMA IgA antibody was about 6 months of strict gluten free diet and a one year of gluten free diet was required to normalize serum level of tTG IgA antibody (10).

In another study, a three months of strict gluten-free diet treatment produced significant decrease in CD-related antibodies which may be helpful for assessment dietary compliance (11). However, in this study there is a non-significant decline in CD-related antibodies in placebo groups, and the significance result obtained only in group on probiotic capsules (Vitalactic B capsules) in tTG IgA antibody.

In this study, the effect of consumption of vitalactic B capsules were also studied as an adjuvant to gluten free diet for three months on some clinical symptoms of celiac disease using gastrointestinal rating scale questionnaire as a guide for the result interpretation. Regarding to abdominal pain in vitalactic B group; patients experienced about three quarter

reduction in abdominal pain; this reduction was borderline in significance (p. value = 0.05). However, in placebo group the obtained mean result was about onequarter reduction in abdominal pain which is statistically non-significant reduction. This result was homogenized with previous result reported in Argentina using *Bifidobacteriuminfantis*Natren Life Start probiotic (7) and parallel to result obtained in children with irritable bowel syndrome were *Lactobacillus* significantly reduces the rate and severity of abdominal pain. This effect may be produced as a result to improvement of the gut barrier (12) or due to the probiotic anti-inflammatory effect on the enteric mucosa which may diminish visceral hypersensitivity and consequently abdominal pain and may reduce bloating (13).

Adherence to the gluten free diet, with and without vitalactic B supplementation, led to the positive effects on abdominal distention associated with CD, in this study there is about a half reduction in abdominal distention after three months supplementation of both vitalactic B and placebo capsules. but in children *Lactobacillus* probiotic is superior to placebo in relieving apparent abdominal distention (14). In functional bowel disorders patient, *Lactobacillus acidophilus* improve symptoms of bloating (15) and treatment with *L. plantarum* for 4-week period, in irritable bowel disease patients provide efficient symptom relief, mainly abdominal pain and distention (16).

A significant reduction in heart burn obtained in group of patients that were taken Vitalactic B capsules aboutmore than three quarter reduction and non-significant reduction

had been noticed at the placebo group as more than one third reduction. Heartburn is frequent in untreated CD patient's and gluten free diet may be a helpful mean in reducing heartburn and acid regurgitation and prevention recurrence of gastro esophageal reflux disease associated symptoms (17). Current study demonstrated that addition of probiotic as adjuvant therapy to gluten free diet in treatment of heartburn enhance the effect of gluten free diet, since probiotics are safe and lacking side effect (18). Probiotics are an attractive option for treatment of gastro esophageal reflux disease associated symptoms in celiac disease patients but further insight is required to study their mechanism in treatment of heart burn and acid regurgitation symptoms related to celiac disease.

The other clinical symptom that were assessed in this study was diarrhea which is classic modes of presentation in patients with celiac disease (19). The gluten free diet led to increase in pathogenic bacteria like *Bacteroides fragilis* group and Enterobacteriaceae and declines in the healthy microbiota which can counteracted by equivalent administration of probiotic (8). In fact, in animal models *B. fragilis* and metalloproteases producing strains are often involved in opportunistic infections and aggravate colitis (20). Vitalactic B administration result in about three-quarter decline in diarrhea, this result was border line in significance (p.value =0.055). Symptom's remission in vitalactic B group could be explained with the mechanism of action of probiotic, which can stimulate mucus secretion by the epithelial cells of the intestinal tract which improve gut barrier utility (21). This result is in

parallel to meta-analysis done in children that had been taken *Lactobacillus* orally, authors found that *Lactobacillus* is safe and effective as a therapy for acute infectious diarrhea (22).

Therefore, using of vitalactic B capsules might add a health advantage to status of celiac disease patients on gluten free diet by improving celiac disease specific antibodies (tTG) and clinical symptoms including abdominal pain, heart burn, diarrhea. Probiotic can be used as adjuvant therapy to gluten free diet but not to be a replacement for gluten free diet. more studies required to determine the mechanism by which probiotic improve celiac disease health status.

This study might open insight for further studies on the role of probiotic as adjuvant therapy to gluten free diet in celiac disease.However, there were a limitation whichhad been reported in this study and indicated that the total IgA level wasn't measured which may lead to miss 2.6% of CD patients as selective IgA deficiency that may take place with celiac disease (23), in addition that, many patients with positive tTG refused to do OGD. As well, Children with CD couldn't beincluded in the study because of the status of capsule which was used to administer the probiotic as a dosage form which made it difficult to beused in children, in addition to the OGD difficulties in children.Restricted gluten free diet implied social limitation due to the appearance of gluten in most processed foods besides high glycemic value of gluten free diet that requisite to raise insulin doses.

In conclusion, Vitalactic B capsules (probiotics) have a health advantage to status of celiac disease patients on gluten free diet, that probiotic supplement improves celiac disease specific antibodies tTG result and CD clinical symptoms including abdominal pain, heart burn, diarrhea after three months of probiotic administration.

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