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The Effect of Anti-inflammatory Diet and Vitamin D Supplementation on the Amelioration of the Clinical Status and Cognitive functions of Familial Mediterranean Fever Patients

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Familial Mediterranean Fever (FMF) is an autosomal recessive disorder, characterized by recurrent attacks of fever, serositis and articular pain. Mutations in the MEFV gene causes inflammation that may trigger cognitive impairment in FMF patients. The objectives were to identify the effect of anti-inflammatory diet containing curcumin, flaxseed and vitamin D supplementation on the clinical presentation and cognitive functions of FMF patients. The study included 73 FMF patients, that followed in addition to their regular colchicine doses an anti-inflammatory diet (rich in fresh vegetables and fruits, low in saturated and unsaturated fats and carbohydrates, low in food additives, sugar, fast foods and processed foods). In addition, to dietary supplementation with vitamin D, curcumin and flax seeds. Results: Statistically significant improvement was observed regarding clinical presentation, cognitive functions, CRP and subjective wellbeing. Conclusion: Our study highlights the importance of anti-inflammatory diet in the amelioration of the clinical presentation, cognitive functions and general wellbeing of FMF patients. We recommend that our findings would be confirmed by a randomized controlled trial.

BACKGROUND

Familial Mediterranean Fever (FMF), is an autosomal recessive disorder, that represent by recurrent attacks of serositis, fever, and articular pain, lasting 24-72 hours. FMF mainly occurs in citizens of Mediterranean ancestry [1]. *MEFV* gene is the gene mutated in patients with FMF, that encodes pyrin, involved in inflammation by distorting caspase-1 activation, apoptosis, activation of the NF-κB pathway and secretion of interleukin (IL)-1β in innate immune system [2,3,4,5]. The main impediment of familial Mediterranean fever is the development of renal amyloidosis [6]. Cognitive impairment and depression are other problems of FMF associated with the chronic inflammatory process and may be to dysbiosis (the imbalance of microbiota) [7,8]. Clinically, resistance to colchicine detected in about 10% of the patients, is defined as suffering of monthly attacks or persistently elevated acute phase markers although the adherence of maximally tolerated dose of colchicine [9,10]. FMF attacks may be precipitated by external non genetic factors, including diet, seasonal variation, and unpredictability [11,12]. Phenotypic variation and severity of FMF is mainly determined by MEFV genotypes however environmental factors contribute with a lesser extent [13]. Intestinal microbiota modulates the clinical expression of the FMF and colchicine effectiveness. Since microbiota is directly affected by diet, the essential role of diet in controlling FMF is attaining increasing support [14,15]. Macronutrients and micronutrients in the diet are essential for maintaining the function of immune cells [16]. Nutrient deficiencies adversely affect the immune system. Moreover, a dietary pattern rich in nutrients with favorable anti-inflammatory properties and poor in pro-inflammatory nutrients may play an important role in controlling the inflammatory status in FMF [17]. Many studies reported the correlation of vitamin D level and FMF clinical presentation, as vitamin D deficiency was reported to aggravate clinical symptoms of FMF and increase resistance to colchicine [18,19]. The therapeutic effects of turmeric are believed to come from its phytochemical curcumin, that contains anti-inflammatory, antioxidant, and antibacterial properties. It is used as a major ingredient of many dietary supplements with anti-inflammatory effects [20,21]. Moreover, long-chain omega-3

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polyunsaturated fatty acids (PUFAs) decrease the production of inflammatory eicosanoids, reactive oxygen species, cytokines, and the expression of adhesion molecules [22]. Consequently, omega-3 PUFAs potentially are considered as anti-inflammatory agents. Accordingly, Flax seeds with their high content of long-chain omega-3 PUFAs, play a role as an anti-inflammatory diet [23, 24,25]. The study aimed to spotlight the effect of anti-inflammatory diet containing curcumin, flaxseed and vitamin D supplementation on the clinical presentation and cognitive functions of a group of FMF patients.

MATERIAL AND METHODS

This retrospective cross-sectional study was conducted in the outpatient clinic of the Clinical Genetics Department. Patients were seen during the period from December 2017 –December 2018. This study was reviewed and approved by the Research Ethics Committee according to the "World Medical Association Declaration of Helsinki "in 1995 (as revised in Seoul 2008) and written consent was obtained from each patient or guardians.

Seventy-three patients were comprised in this study, their age ranged 7-24 years, 39 were males and 34 females in a ratio of 1.15: 1. The FMF patients (diagnosed according to clinical and molecular analysis), in addition to their regular colchicine doses received training sessions to modify their dietary habits to include anti-inflammatory diet (rich in fresh vegetables and fruits, low in saturated and unsaturated fats and carbohydrates, low in food additives, sugar, fast foods and processed foods). In addition, to dietary supplementation with vitamin D, curcumin and flax seeds. They were followed up for 6 months as this period is sufficient for the effect of nutritional intervention to appear on the symptoms, cognitive functions and the biochemical analysis.

MEFV genotyping

Genomic DNA isolation, PCR amplification and sequencing

Peripheral blood was collected from each participant and the genomic DNA samples were extracted from blood lymphocytes using DNA Isolation Kit for Mammalian Blood (Roche Diagnostics, Mannheim, Germany). For each patient, both *MEFV* exon 2 and 10, which are considered as mutation hot spots, were individually amplified by PCR using 2 pairs of corresponding primers:

Exon 2: F: 5′- GCCTGAAGACTCCAGACCACCCCG-3′, R: 5′- AGGCCCTCCGAGGCCTTCTCTCTG-3′

Exon 10: F: 5′- GAGGTGGAGGTTGGAGACAA-3′, R: 5′- TGACCACCCACTGGACAGAT-3′.

PCR will be performed in a 25 ml reaction volume containing 60 ng of genomic DNA, 5 U of Taq160 (Invitrogen), 20 pmol of each primer, 50 mM $MgCl_2$, 10 mM d NTP, and 10× PCR buffer (Invitrogen) in the Veriti 96-well Thermal Cycler 9902 (Applied Biosystems, Foster City, CA, USA). The PCR conditions will be as follows: initial denaturation at 94 °C for 5 min, 35 cycles at 94 °C for 30 s and 58 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min. Bidirectional direct sequencing of purified PCR products will be performed using the BigDye Terminator V1.1 Cycle Sequencing kit (ABI prism, Foster City, CA, USA) and an Applied Biosystems 3500DX Genetic analyzer. The resulting chromatogram will be analyzed using the Sequencing Analysis SeqA V5.4 (Applied Biosystems) program. The sequencing results will be compared with the MEFV reference coding sequence available at NCBI with GenBank http://blast.ncbi.nlm.nih.gov/Blast.cgi

Dietary intervention

Dietary pattern of the participating FMF patients was modified to include an anti-inflammatory diet high in fresh vegetables and fruits, low in saturated, trans-fats and carbohydrates, low unsaturated fat and low in food additives, sugar, fast foods and processed foods.

Supplement with anti-inflammatory natural sources: flax seeds (2 small spoons daily), curcumin (turmeric) 10 mg daily and vitamin D supplementation (4000 IU daily) was given for 6 months [18].

The evaluation was done at baseline and repeated after 6 months of administrating an anti-inflammatory diet. Each patient was educated concerning the importance of diet in improving or aggravating the clinical presentation and prognoses of the case. In most cases, mothers were the target of our nutritional education session since most of the cases were children. They were given a sheet that included clear instructions about what to eat and what not to eat. The diet composed of high fresh fruits and vegetables, high proteins, low fat, and simple carbohydrates. All food additives were forbidden. Besides, vitamin D supplements, curcumin and flaxseeds were given as an anti-inflammatory agent.

The following procedures were performed at baseline and repeated six months later:

1- Classification and clinical grading according to symptoms including abdominal and chest pains, temperature, arthritis, myalgia, erysipelas-like erythema (duration, frequency, and severity of attacks) in the FMF patients (score of attack) into: mild, moderate, or severe [26,27].

2- Dietary recalls:

A 24 hours of dietary intake and food frequency questionnaires were performed to assess the dietary intakes and patterns.

3- Cognitive functions evaluation:

Mini-Mental State Examination (MMSE) was implemented for the assessment of mental and cognitive status. The Mini-Mental State Examination (MMSE) is the most common test for screening memory and attention in different ages. Performing the test takes between 5–10 minutes, the examined functions include: registration, attention, recall, language, calculations, commands, and orientation [28]. The modified mini-mental status test was used for children between 5 and 14 scorings out of 38. The cutoff point was as follows 6-8 years was 28, 9-11 years was 30 and 12-14 years was 35.

4- Subjective well-being and school achievement Questionnaire:

The questionnaire included ten items for the wellbeing and five for school achievement, each one was presented on 1-5 scale, where 1 was the least score and 5 was its maximum. These items were: general well-being, sleep duration, and quality, sense of gratitude, general activity, happiness, memory, mood, anger, anxiety, and appetite. As for school achievement: school satisfaction, days off from school, success and scoring in school, understanding most of the lessons and sharing in school activities.

5- Blood sampling and Biochemical analysis: Complete blood count and C-reactive protein (CRP):

Fasting blood samples were drawn from patients in the attack free period. The blood was collected on plain tubes and was allowed to clot, and the serum samples were aliquoted and stored at -70 °c until used for further analysis. Fasting C-reactive (CRP) protein was estimated by the enzyme linked immunosorbent assay (ELISA) kit supplied by DRG® International Inc. (EIA-3954), USA. Serum 25 hydroxyvitamin D (25 (OH) D) was assessed by vitamin D direct ELISA kit (EIA-4696) DRG ® International, Inc. USA [29,30].

Statistics

The collected data were statistically analyzed using SPSS version.10 (SPSS Inc. Chicago, IL). Results were expressed as Mean ± SD. A comparison of different variables in various groups were done using dependent and independent samples t-test. P-values less than 0.05 was considered statistically significant.

RESULTS

The 73 studied patients at the baseline had borderline cognitive functions (cutoff point) regarding their MMSE. The children had different school problems, mainly impaired attention and concentration, and frequent absence at school due to severe abdominal colic, arthritis, and fever. The clinical presentation, cognitive tests, and wellbeing showed a significant ($p < 0.05$) improvement after 6 months of dietary intervention, with anti-inflammatory diet and nutritional supplements (Curcumin, vitamin D and flaxseed) Table I, Fig 1. The mean CRP was elevated (normal range 0-10 mg/dl) and serum vitamin D was below the normal range (<50 ng ml) before intervention. After the intervention, they revealed significant improvement ($p < 0.01$), were the level of CRP was reduced and the level of vitamin D ($p < 0.01$) was markedly elevated (Table II). Mutation analysis performed for all patients revealed M694I, M694V, M680I, E148Q and V726A mutations (Table III).

Table I. Statistical significance of different cognitive function tests before and after the dietary intervention (Mean± SD)

Variables	Before intervention	After intervention	P-value
Scores of FMF attacks	2.7±0.51	1.3±0.56	0.05
MMSE	25.32±2.2	27.10±2.4	0.05
Subjective well being	3	4	0.05
Schooling	2	3.5	0.01

MMSE: Mini-Mental State Examination

The parameters are represented as Mean± SD

p value <0.05 is significant, p value is highly significant at< 0.01

Table II. Statistical significance of vitamin D and CRP before and after the dietary intervention (Mean± SD).

parameters	Before Intervention	After Intervention	P-value
Vitamin D3 (ng ml)	12.41 ± 1.60	16.18 ± 1.03	0.01
CRP (mg dl)	17.8 2±3.12	13.14 ±2.63	0.01

The parameters are represented as Mean± SD
p value <0.05 is significant, p value is highly significant at< 0.01

Table III. Mutational analysis in the studied patients.

Allele	No in patients (%)
M694I	26 (35.6%)
M694V	19 (26.1%)
M680I	16 (21.9%)
E148Q	7 (9.6%)
V726A	5 (6.8%)

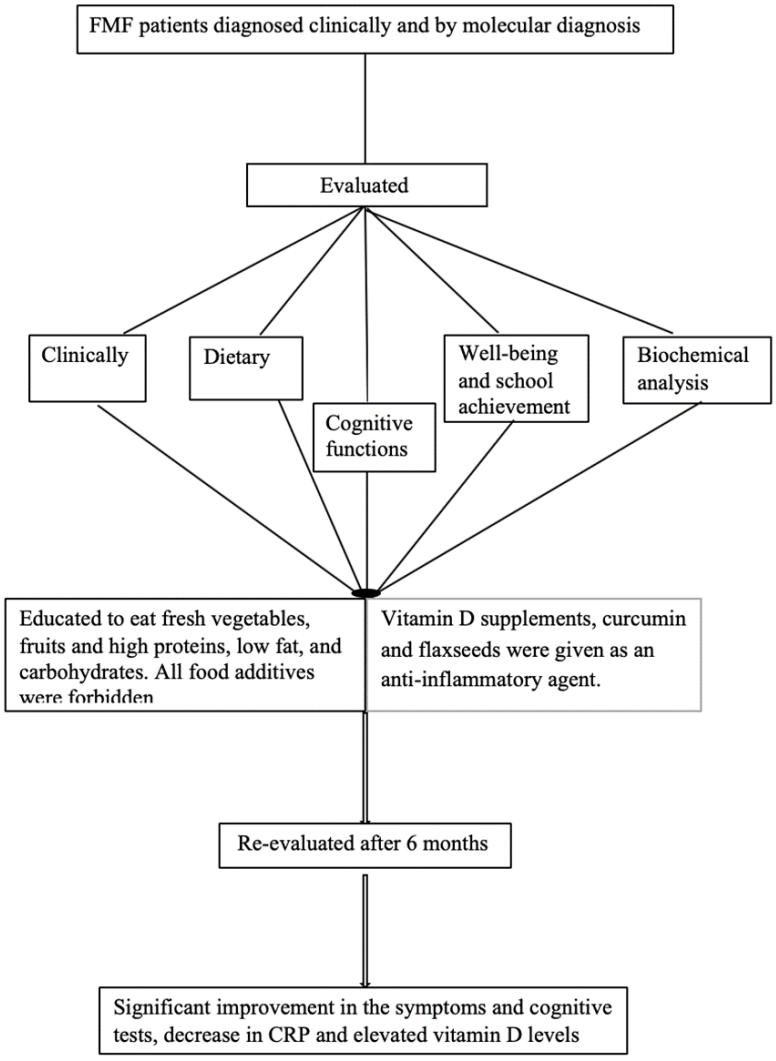

Figure 1. algorithm illustrating our findings

DISCUSSION

In this work, significant improvement of the clinical presentation was detected regarding duration, frequency and severity of attacks with the anti-inflammatory diet including vitamin D, flax seeds and curcumin intake. Previous investigators delineated that omega-3 PUFA alters the expression of inflammatory genes through the activation of transcription factor [17,22]. Accordingly, flaxseeds rich in omega-3 PUFAs are effective anti-inflammatory and therapeutic agents [23]. Our findings were supported by previous studies who reported that turmeric is a strong anti-inflammatory agent and an active neurogenic compound that reduces the inflammatory status resulting in better general condition, decrease in the frequency of attacks and improved cognitive functions. Similarly, the low fat, low carbohydrates, high fresh fruits, and vegetables diet, proved to improve the general condition and reduce the inflammatory status of FMF resulting in better prognosis and less frequency and intensity of the attaches [21, 25].

Our study detected low serum vitamin D level among all patients at baseline, which was increased after supplementation, accompanied by a significant improvement in clinical presentation and decrease in CRP level together with improved cognitive functions. These findings were reported in previous studies, which stated that the FMF cases have low vitamin D serum level then reported significant improvement with supplementation. Our data corroborate with the results previously mentioned in literature [31, 32, 33]. An important finding of our study was the mild cognitive impairment seen among most of our patients at baseline directly related to the chronic inflammatory status of FMF patients. In our study, significant improvement in cognitive functions and scholastic achievement was revealed after following the anti-inflammatory diet and subsequently correcting the CRP and vitamin D levels. Similarly, several studies reported that low-grade chronic inflammation, affect several brain functions [27, 34, 35]. Diet affects the pattern of microbiota in the gut, which in turn plays an important role in regulating the inflammatory status of the body. We suggest that through diet, the degree of inflammation in FMF patients can be controled, not only through direct antioxidant and its anti-inflammtory properties but also mediated by the microbiotic flora in the gut. A previous study emphasized the relation between the innate immune system and microbiota that may affect the inflammatory status [36]. Some reports concluded that bacterial decontamination improved the clinical course and response to colchicine in the FMF patients [8, 36].

Furthermore, several studies strongly indicated that high consumption of vegetables and fruits or diet rich in antioxidants, serum carotenoids, vitamins, fiber, and magnesium are beneficial in reducing inflammation and the CRP levels [37, 38,39,40].

The study limitation includes the small sample size.

CONCLUSION

The anti-inflammatory diet containing curcumin, flaxseed and vitamin D supplementation ameliorated the clinical presentation and the cognitive functions. We recommend that our findings would be confirmed by larger studies.

List of abbreviations:
FMF; CRP; PUFAs; MMSE; ELISA
FMF: Familial Mediterranean Fever
CRP: C-reactive protein
PUFAs: polyunsaturated fatty acids
MMSE: Mini-Mental State Examination
ELISA: enzyme linked immunosorbent assay

DECLARATIONS

Ethics approval and consent to participate: This study was reviewed and approved by the Research Ethics Committee according to the "World Medical Association Declaration of Helsinki "in 1995 (as revised in Seoul 2008).
Consent for publication: written consent was obtained from each patient or guardians.
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REFERENCES

1. **Alghamdi, M.** 2017. Familial Mediterranean fever, review of the literature. Clin Rheumatol **36**: 1707–1713.
2. **Ozen, S., Kone-Paut, I., and Gül, A.** 2017. Colchicine resistance and intolerance in familial Mediterranean fever: definition, causes, and alternative treatments. Semin Arthritis and Rheum **47**: 115–120.
3. **Zarouk, W.A., El-Bassyouni, H.T., Ramadan, A., Fayez, A.G., Esmaiel, N.N., Foda, B.M., et al.** 2018. Screening of the most common MEFV mutations in a large cohort of Egyptian patients with Familial Mediterranean fever. Gene Rep **11**: 23–28.
4. **Salehzadeh, F., Mohammadikebar, Y., Haghi, R.N., Asl, S.H., and Enteshary, A.** 2019. Familial Mediterranean Fever Gene Mutations and Gout as an Auto Inflammatory Arthropathy. Med Arch. **73**: 55-57.
5. **Bashardoust, B.** (2015) Familial Mediterranean fever; diagnosis, treatment, and complications. J Nephropharmacol; **4**: 5–8.
6. **Mimouni, A., Magal, N., Stoffman, N., Shohat, T., Minasian, A., Krasnov, M., et al.** 2000. Familial Mediterranean fever: effects of genotype and ethnicity on inflammatory attacks and amyloidosis. Pediatrics **105**: E70.
7. **Singh-Manoux, A., Dugravot, A., Brunner, E., and Kumari, M.** 2014. Interleukin-6 and C-reactive protein as predictors of cognitive decline in late midlife. Neurology **83**:486-93.
8. **Özer, S., Bozkurt, H., Yılmaz, R., Sönmezgöz, E., and Bütün, I.** 2017. Evaluation of executive functions in children and adolescents with familial Mediterranean fever. Child Neuropsychology **23**:332-342.
9. **Grattagliano, I., Bonfrate, L., Ruggiero, V., Scaccianoce, G., Palasciano, G., and Portincasa, P.** 2014. Novel therapeutics for the treatment of familial Mediterranean fever: from colchicine to biologics. Clin Pharmacol Ther **95**:89–97.
10. **Cerquaglia, C., Diaco, M., Nucera, G., La Regina, M., Montalto, M., and Manna, R.** 2005. Pharmacological and clinical basis of treatment of familial Mediterranean fever (FMF) with colchicine or analogues: an update. Curr Drug Targets Inflamm Allergy **4**: 117–124.
11. **Álvarez-Errico, D., Vento-Tormo, R., and Ballestar, E.** 2017. Genetic and Epigenetic Determinants in Autoinflammatory Diseases. Front Immunol **8**:318.
12. **Yenokyan, G., and Armenian, H.K.** 2012. Triggers for attacks in familial Mediterranean fever: application of the case-crossover design. Am J Epidemiol **175**:1054-61.
13. **Ben-Zvi, I., Brandt, B., Berkun, Y., Lidar, M., and Livneh, A.** 2012. The Relative Contribution of Environmental and Genetic Factors to Phenotypic Variation in Familial Mediterranean Fever (FMF). Gene **491**:260-3.
14. **Verrecchia, E., Sicignano, L.L., La Regina, M., Nucera, G., Patisso, I., and Cerrito, L.** 2017. Small Intestinal Bacterial Overgrowth Affects the Responsiveness to Colchicine in Familial Mediterranean Fever. Mediat Inflamm **2017**:7461426.
15. **Hentgen, V., Grateau, G., Kone-Paut, I., Livneh, A., Padeh, S., Rozenbaum, M., et al.** 2013. Evidence-based recommendations for the practical management of Familial Mediterranean fever. Semin Arthritis Rheum **43**:387–391.
16. **Gabrielli, M., D'Angelo, G., Di Rienzo, T., Scarpellini, E., and Ojetti, V.** 2013. Diagnosis of small intestinal bacterial overgrowth in the clinical practice. Eur Rev Med Pharmacol Sci **17**: 30–35.
17. **Lopez-Garcia, E., Schulze, M.B., Fung, T.T., Meigs, J.B., Rifai, N., Manson, J.E., et al.** 2004. Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. Am J Clin Nutr **80**:1029-35.
18. **Warnberg, J., Gomez-Martinez, S., Romeo, J., Diaz, L.E., and Marcos, A.** 2009. Nutrition, inflammation, and cognitive function. Ann N Y Acad Sci **1153**:164–75.
19. **Onur, H., Aral, H., Arica, V., Bercem, G.A., and Kasapcopur, O.** 2016. Vitamin D levels in children with familial Mediterranean fever. Pediatr Rheumatol Online J **14**:28.
20. **Ozer, I., Mete, T., Sezer, O.T., Ozgen, G.K., and Kucuk, G.O.** 2015. Association between colchicine resistance and vitamin D in familial Mediterranean fever. Ren Fail **37**:1122-5.
21. **Strimpakos, A.S., and Sharma, R.A.** 2008. Curcumin: Preventive and therapeutic properties in laboratory studies and clinical trials. Antioxid Redox signal **10**:511-45.
22. **Aggarwal, B.B., and Harikumar, K.B.** 2009. Potential Therapeutic Effects of Curcumin, the Anti-inflammatory Agent, Against Neurodegenerative, Cardiovascular, Pulmonary, Metabolic, Autoimmune and Neoplastic Diseases. Int J Biochem Cell Biol **41**: 40–59.
23. **Lemos, J.R., Alencastro, M.G., Konrath, A.V., Cargnin, M., and Manfro, R.C.** 2012. Flaxseed oil supplementation decreases C-reactive protein levels in chronic hemodialysis patients. Nutr. Res

32:921–927.

24. **Al-Okbi, S.Y.** 2005. Highlights on functional foods, with special reference to flaxseed. J Nat Fibers **2**:63–68.
25. **Ren, G.Y., Chen, C.Y., Chen, G.C., Chen, W.G., Pan, A., Zhang, Y.H., et al.** 2016. Effect of Flaxseed Intervention on Inflammatory Marker C-Reactive Protein: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Nutr **8**: 136.
26. **Demirkaya, E., Acikel, C., Hashkes, P., Gattorno, M., Gul, A., Ozdogan, H, et al.** 2016. Development and Initial Validation of International Severity Scoring System for Familial Mediterranean Fever (ISSF). Ann Rheum Dis **75**:1051-6.
27. **Federici, S., Sormani, M.P., Ozen, S., Lachmann, H.J., and Amaryan, G.** 2015. Paediatric Rheumatology International Trials Organisation (PRINTO) and Eurofever Project. Evidence-based provisional clinical classification criteria for autoinflammatory periodic fevers. Ann Rheum Dis **74**:799-805.
28. **Folstein, M.F., Folstein, S.E., and McHugh, P.R.** 1975. Mini-mental state. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res **12**: 189–98.
29. **Roberts, W.L., Sedrick, R., Moulton, L., Spencer, A., and Rifai, N.** 2000. Evaluation of Four Automated High-Sensitivity C-Reactive Protein Methods: Implications for Clinical and Epidemiological Applications. Clin Chem **46**: 461-468.
30. **Wielders, J.P., and Wijnberg, F.A.** 2009. Preanalytical stability of 25(OH)-vitamin D3 in human blood or serum at room temperature: solid as a rock. Clin Chem **55**:1584-1585.
31. **Ozen, S., Aktay, N., Lainka, E., Duzova, A., Bakkaloglu, A., and Kallinich, T.** 2009. Disease severity in children and adolescents with familial Mediterranean fever: a comparative study to explore environmental effects on a monogenic disease. Ann Rheum Dis **68**:246–248.
32. **Kazem, Y.I., Moaty, M.I., and El–Shebini, S.** 2014. Low Vitamin D serum levels may be a modifiable risk factor for obesity and cognitive impairment in middle-age Egyptian women. Maced J Med Sci **7**: 279-284.
33. **Zaki, M., El-Bassyouni, H.T., Reyad, H., Yousef, W., Youness, E., Mohamed, G., et al.** 2018. Assessment of physical growth, some oxidative stress biomarkers and vitamin D status in children with Familial Mediterranean Fever. Meta Gene **17**: 212-215.
34. **Adibian, M., Hodaei, H., Nikpayam, O., Sohrab, G., Hekmatdoost, A., and Hedayati, M.** 2019. The effects of curcumin supplementation on high-sensitivity C-reactive protein, serum adiponectin, and lipid profile in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. Phytother Res **33**:1374–83.
35. **Kajla, P., Sharma, A., and Sood, D.R.** 2015. Flaxseed—a potential functional food source. J Food Sci Technol **52**: 1857–1871.
36. **Khachatryan, Z.A., Ktsoyan, Z.A., Manukyan, G.P., Kelly, D., Ghazaryan, K.A., and Aminov, R.I.** 2008. Predominant role of host genetics in controlling the composition of gut microbiota. PloS One **3**: e3064.
37. **Kholoussi, S., Kholoussi, N., Zaki, M.E., El-Bassyouni, H.T., Elnady, H., Morcos, B., and Abo–Shanab, A.** 2018. Immunological Evaluation in Patients with Mediterranean fever. Maced J Med Sci **2**:310-313.
38. **Manna, R., and Rigante, D.** 2019. Familial Mediterranean Fever: Assessing the Overall Clinical Impact and Formulating Treatment Plans. Mediterr J Hematol Infect Dis **11**: e2019027.
39. **Abiri, B., and Vafa, M.** 2020. Effect of vitamin D and/or magnesium supplementation on mood, serum levels of BDNF, inflammatory biomarkers, and SIRT1 in obese women: a study protocol for a double blind, randomized, placebo-controlled trial. Trial **21**: 225.
40. **Adibian, M., Hodaei, H., Nikpayam, O., Sohrab, G., Hekmatdoost, A., and Hedayati, M.** 2019. The effects of curcumin supplementation on high-sensitivity C-reactive protein, serum adiponectin, and lipid profile in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. Phytother Res **33**:1374–83.