The Effects of Nano-Curcumin as A Nutritional Strategy on Clinical and Inflammatory Factors in Children with Cystic Fibrosis: A Protocol Study and Review Article

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Study protocol

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Abstract

Background: Cystic fibrosis(CF) is a genetic disorder, which is caused by the CFTR protein defects. Along with CFTR dysfunction, inflammation plays a key role in the disease outcomes. Inflammation may develop due to the internal dysfunction of the CFTR protein or external factors. Curcumin affects the CFTR protein function primarily as a corrector and potentiator and secondary as an anti-inflammatory and antimicrobial agent. The present study aims to assess the impact of Nano-curcumin on clinical and inflammatory markers in children with CF.

Methods: This prospective, double blind control trial will be conducted at Akbar Children's Hospital in Mashhad, Iran. children with CF will be enrolled based on the eligibility criteria. Placebo and curcumin with the maximum dose of 80 milligrams considering the body surface of the patients will be administrated for three months. The primary outcome is to evaluate inflammation based on serum interleukin-6, interleukin-10, and hs-CRP, stool calprotectin, and neutrophil count of nasopharyngeal swab. The secondary outcome involved clinical assessment via spirometry, anthropometrics, and quality of life. They will be assessed before and after three mounts.

Discussion: Due to the multifarious effects of curcumin on CF disease, it can be used as a nutritional strategy in the treatment of cystic fibrosis.

Trial registration: Iranian Registry of Clinical Trials, IRCT20200705048018N1, Registered on 2020-07-10, <https://en.irct.ir/search/result?query=IRCT20200705048018N1>

Introduction

Background and rationale {6a}

Cystic fibrosis (CF) is a common, life-shortening, autosomal recessive, genetic disorder in Caucasian populations. The disease prognosis has improved in recent decades with the advances in the treatment procedures, and the mortality rate has decreased by 2% annually. The median age of survival in the male and female patients with CF has been reported to be 65 and 56 years, respectively (1). Inflammation plays a critical role in CF lung pathology and disease progression. The abnormal function of cystic fibrosis transmembrane conductance regulator (CFTR) alters the chloride ion transport in the epithelial cells. Hence, leading to the dehydration of the airway surface liquid. This condition causes the secreted mucin to thicken and contribute to airway obstruction, thereby predisposing the patients to infection. This is considered to be the leading cause to trigger inflammation (2). The dysregulation of the pro-inflammatory mediators secondary to the innate and adaptive immune dysfunction in CF patients is another cause of excessive inflammation. It remains unclear whether the intrinsic effects of mutant CFTR on the immune system are the major cause of inflammation or exposure to the CF airway infection and inflammatory environment could exert extrinsic effects toward this phenomenal (3, 4). With the advent of new modified CFTR protein drugs such as ivacaftor, lumacaftor, tezacaftor, a significant impact has been observed on the treatment of patients. Ivacaftor and tezacaftor act as the potentiator through enabling

the opening chloride channel, while lumacaftor acts as the corrector through increasing the trafficking of the CFTR proteins to the outer cell membrane (5, 6). Although these drugs could reduce airway inflammation, they cannot eliminate it completely (7). On the other hand, these drugs affect specific mutations and do not cover all patients with various mutations (7). Therefore, anti-inflammatory treatments are an inevitable therapy for CF patients. Curcumin is the main ingredient in turmeric with a substantial impact on the reduction of inflammatory factors (8) (9). Previous findings have indicated that curcumin could correct the CFTR protein function (corrector) in the F508del mutation, which is associated with the CFTR protein misfolding (10). Moreover, curcumin has been reported to be acts as a potentiator and release the gating defect of CFTR protein and promoting the channel activity (11, 12). According to the literature, curcumin can reduce inflammation through antimicrobial effects, which contribute to its extrinsic impact. On the other hand, curcumin is soluble in water and has a short half-life and low bioavailability. Various products have been obtained for the improvement of bioavailability through increasing the absorption rate from the gastrointestinal tract and reducing the liver metabolism (13-15).

The present study aimed to evaluate the effects of Nano-curcumin on CF patients in order to achieve the intrinsic and extrinsic effects of curcumin on the inflammatory process in these patients.

This is a prospective, single center, double-blind, parallel, randomized placebo-controlled clinical trial. The study protocol was written following the standard Protocol Items Recommendation for Interventional Trials (SPIRIT) Checklist. (Additional file 1)

Objectives {7}

The present study aimed to evaluate the effects of Nano-curcumin on CF patients in order to achieve the intrinsic and extrinsic effects of curcumin on the inflammatory process in these patients.

Trial design {8}

This is a prospective, single center, double-blind, parallel, randomized placebo-controlled clinical trial. The study protocol was written following the standard Protocol Items Recommendation for Interventional Trials (SPIRIT) Checklist. (Additional file 1)

Methods: Participants, Interventions And Outcomes

Study setting {9}

The study population will consist of the patients with known CF referring to the Cystic Fibrosis Clinic at Akbar Children's Hospital, Mashhad, Iran.

Eligibility criteria {10}

Inclusion Criteria

The inclusion criteria of the study are as follows: 1) one or more typical phenotypic features of CF and a minimum of an elevated sweat chloride concentration on two/more occasions or two mutations known to cause CF on separate alleles; 2) age of 5-18 years; 3) pulmonary and gastrointestinal involvement; 4) ability to perform spirometry maneuvers and the minimum FEV1 of $\geq 30\%$ compared to the same age, gender, and height in the normal population; 5) the percentage of oxygen saturation based on pulse oximetry of $\geq 90\%$ at room temperature; 6) no cardiovascular, hepatic, and renal failure; 7) absence of celiac disease and rheumatoid arthritis; 8) no acute pulmonary exacerbation requiring hospitalization within the past four weeks; 9) absence of acute respiratory tract infection and 10) informed consent for participation.

Exclusion Criteria

The exclusion criteria of the study are the lack of adherence to the drug regimen and presence of drug intolerance like nausea or vomiting or any allergic reaction.

Who will take informed consent? {26a}

Initially, the objectives of the trial will be clarified by the main administrator to the children's parents and legal guardians, and written informed consent will be obtained from them at the time of enrolment.

Additional consent provisions for collection and use of participant data and biological specimens {26b}

We do not need any additional consent provisions

Interventions

Explanation for the choice of comparators {6b}

The Patients with eligible criteria will be enrolled to the study.

Intervention description{11a}

The Patients with eligible criteria will be enrolled to the study. Thereafter, With the use of the stratified randomization procedure, participants will be divided into two groups according to disease severity (severe/mild-to-moderate) using spirometry with 40% FEV1 cutoff range. Following that, simple sampling method will be used to select the patients from each group to receive either Nano-curcumin drop or Nano-curcumin-like placebo drop. (Figure 1)

Nano-curcumin (Exir Nano Sina Drug Company, Iran) is prepared as Nano micelle in the form of 70 milligrams of drops in 1 cc, and the placebo with the same color, taste and odor.

To adjust the drug dose for different ages and considering that the maximum acceptable dose based with the most significant impact and minimum side-effects was 80 milligrams, the required amount will be obtained for each subject based on the ratio of the body surface area of the patients. The curcumin

and placebo glasses will be labeled A and B by Exir Nano Sina company, respectively and made available to the patients through the double-blind design. The duration of the treatment period is three months.

Criteria for discontinuing or modifying allocated interventions {11b}

Although no special side effect has been reported until now, with any intervention related side effects we will stop the intervention and report it to the Ethics committee of Mashhad university of medical sciences (MUMS) for decision making.

Strategies to improve adherence to interventions {11c}

In order to control patients for taking curcumin and placebo, they will be followed-up by phone every month. curcumin and placebo will be given to the patients for one month and patients will be asked to bring the bottle of drug in their next visit for assessing their compliance and if anyone used less than 70% of drop, he or she will be excluded.

Relevant concomitant care permitted or prohibited during the trial {11d}

Concomitant intervention will be:

1-Pancreatic Enzyme Replacement Therapy(PERT): All CF patients with pancreatic deficiency need PERT for enzyme replacement as a regard we enrolled CF patients with gastrointestinal involvement, all of our patients use Creon as a usual treatment that doses are adjusted to the level of dietary fat. 2-Antibiotic: Tobramycin inhalation is an antibiotic which is used for pseudomonas infection. 3-Anti-inflammatory: Low dose azithromycin treatment frequently prescribed chronically as an anti-inflammatory effect in CF patients six years and older. We use it in our patients. 4-Anti Acid: suggested for patient who fail to respond to maximal dose of PERT and in patient with reflux disease.

5-Inhaled hypertonic saline: nebulized hypertonic (7%)saline is recommended twice daily to all patients six years and older. We use it in our patients. 6-Inhaled Beta2 agonist: is administrated in CF patient with moderate to severe lung disease twice a week.

7-Fat soluble vitamins: for all CF patients with pancreatic deficiency.

Provisions for post-trial care {30}

Outcomes {12}

The primary outcomes of the current trial are changes from the baseline to three months of the intervention in the inflammation at three levels: systemic inflammation by assessing IL-6 as an inflammatory agent, IL-10 as an anti-inflammatory agent, and hs-CRP level in the blood samples, pulmonary inflammation with the neutrophil count, and bacterial/viral culture on the nasopharyngeal swab, and gastrointestinal inflammation with the calprotectin level in the fecal samples.

The secondary outcomes are changes in clinical assessment of the pulmonary symptoms via spirometry, anthropometric assessment stand on the BMI Z score, and evaluation of the quality of life using the CFQ from the baseline to three months of the intervention.

Participant timeline {13}

Nutritional recommendations will be provided to all the patients and/or their parents and legal guardians stand on the CF requirements and adjustable CREON dosing to the level of dietary fat. In addition, the patients and their parents completed the CFQ depend on age.

Anthropometric measurements (weight and height) will be performed using a digital scale (model: SECA). Body weight will be measured without shoes, and height will be measured in the standing position without shoes with the heels stuck to the wall and the head looking frontwards with the accuracy of 0.5 centimeter.

The fecal examination will be performed before and after the intervention. Before intervention, fecal sample will be evaluated for bacterial over growth and parasite specially giardia by checking stool PH and trophozoites or cysts of giardia. Any positive result will be treated with antibiotics before stating the trials.

Blood sampling will be performed before and after the intervention. Approximately five milliliters of blood will be collected, immediately centrifuged, the serum will be separated from the sediment, and preserved at the minimum of temperature of -20°C.

The primary and secondary consequences of the treatment will be investigated before and three months after the treatment. The clinical evaluation and follow-up of medication use and side-effects will also be carried out via phone call and paying monthly visit to the clinic, and the findings will privately be presented to the patients. (Table 2)

Sample size {14}

According to the lack of complete similar clinical trials which evaluate inflammatory effect of Nano-curcumin in children with cystic fibrosis, we used HSCRP indices as an effect size. Based on related article, the mean $\pm$ SD of hs-CRP indices in the curcumin and placebo groups, were 5.9(2.57) and 3.6(1.58). (16). G- power analyzer was applied for calculating sample size with a confidence interval of 95% and power of 80%. By considering 10% dropout and using Deff (deign effect) for stratified sample, a total of 30 samples for each group was calculated. In total, 60 eligible patients with CF are needed to include of randomization procedures.

Recruitment {15}

During regular monthly visit of patients at the cystic fibrosis clinic, treatment team specially nutritionist will explain the trial and discuss the advantages and disadvantages of therapy and answer any questions that participants may have.

Assignment of interventions: allocation

Sequence generation {16a}

The Patients with eligible criteria will be enrolled to the study. Thereafter, the first time with the use of the stratified randomization procedure, participants will be divided into two groups according to disease severity (severe/mild-to-moderate) using spirometry with 40% FEV1 cutoff range. The second time, simple sampling method will be used to select the patients from each group to receive either Nano-curcumin drop or Nano-curcumin-like placebo drop by using table of random numbers. (Figure 1)

Concealment mechanism {16b}

Table of random number is used to select patients from each group after stratified randomization based on disease severity. Also curcumin and placebo labelling is done by Exir Nano Sina company.

Implementation {16c}

Generate the allocation sequence, enrol participants, and assign participants to interventions will be done by the main administrative.

Assignment of interventions: Blinding

Who will be blinded {17a}

The curcumin and placebo glasses will be labeled A and B by Exir Nano Sina company, respectively and made available to the patients through the double-blind design in which neither the participant nor the experimenter are aware of which group.

Procedure for unblinding if needed {17b}

Data collection and management

Plans for assessment and collection of outcomes {18a}

Electronic registry form of cystic fibrosis in Khorasan province is an instrument for collecting the outcomes result.

Plans to promote participant retention and complete follow-up {18b}

In order to control patients for taking curcumin and placebo they will be followed-up by phone every month. curcumin and placebo will be given to the patients for one month and patients will be asked to bring the bottle of drug in their next visit for assessing their compliance and if anyone used less than 70% of drop, he or she will be excluded.

Data management {19} Research nurses and assistants collect the data at baseline and follow up and record it on electronic registry forms of cystic fibrosis in Khorasan province. Other additional data that is not available in the electronic registry form, will be recorded in the Excel file and in the paper forms at the same time. Also CFQ will be answered via electronic validated questionnaire by patients older than twelve years old. For patients younger than this age, clinical psychologist will ask them their questionnaire in the clinic.

Confidentiality {27}

Participants' study information will be stored at the security site. All laboratory specimens, data collected, and reports will be identified by a coded ID number.

Plans for collection, laboratory evaluation and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}

We will try to collect a minimum two millilitre of blood sample for pharmacogenetic and pharmacokinetic study in future use.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}

Data analysis will be performed in SPSS version 20. The differences in the quantitative dependent variables will be evaluated using paired t-test or Wilcoxon test. The difference in the quantitative independent variables between two groups, Mann-Whitney test or independent t test will be considered. The analysis of variance (ANOVA) or Kruskal-Wallis test will be applied for quantitative variable more than two groups. In all the statistical analyses, the P-value of ≤ 0.05 will be considered significant.

Interim analyses {21b}

Interim analysis is not considered. In case of frequent side effects (more than previous reports), we will stop the intervention and present the results to the Ethics Committee of Mashhad University of Medical Sciences (MUMS) for further decision.

Methods for additional analyses (e.g. subgroup analyses) {20b}

Any covariates will be controlled by ANCOVA or binary logistic regression.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

For incomplete block of data's, we will use intention to treat analysis.

Plans to give access to the full protocol, participant level-data and statistical code {31c}

We will attempt to release the full study protocol and results as soon as possible, regardless of the magnitude or direction of effect. The anonymized data set and statistical code may be available from the corresponding author (Email: kianifar HR@mums.ac.ir) on reasonable request.

Oversight and monitoring

Composition of the coordinating center and trial steering committee {5d}

The ethical committee and vice chancellery of Mashhad University of Medical Sciences, supervises all the study stages. It is an academic committee and has no competing interest

Composition of the data monitoring committee, its role and reporting structure {21a}

We do not have a DMC

Adverse event reporting and harms {22}

Frequency and plans for auditing trial conduct {23}

Auditing trial conduct is not considered

Plans for communicating important protocol amendments to relevant parties (e.g. trial participants, ethical committees) {25}

Any modification to protocol which may impact on the conduct of the study, will be approved by ethical committee of MUMS prior to implementation.

Dissemination plans {31a}

We will attempt to release the full study protocol and results as soon as possible, regardless of the magnitude or direction of effect. The anonymized data set and statistical code may be available from the corresponding author (Email: kianifar HR@mums.ac.ir) on reasonable request.

Discussion

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], is a polyphenolic compound isolated from the rhizomes of *Curcuma longa* (turmeric).(17)

Secondary metabolites of this component are phenolic acids, flavonoids, alkaloids, terpenoids, tannins and saponins all of which are known to have biological effects. The antibacterial effect of curcumin has been exhibited since 1949 (18) and several studies have revealed its anti-inflammatory, anticancer, antioxidant, wound-healing, antiviral effects which are believed to have therapeutic effects in many human related diseases. (19) Curcumin is suggested as a line of treatment for cystic fibrosis disease. Since CF is the most common life shortening disease, Early initiation of treatments, including anti-

inflammatory agent, are very important in the final prognosis of the disease. It appears cystic fibrosis children are the best candidate for using this treatment.

According to previous research it is shown that Cystic fibrosis transmembrane conductance regulator (CFTR), is a cAMP-activated Cl ion channel in the apical membrane of epithelial cells(20), which is a well-studied ion channel target for curcumin. (Fig. 2)

Due to the limited trial that evaluated the effect of curcumin in cystic fibrosis patient, here we reviewed animal and cellular based studies to recognize the main mechanism of curcumin on CFTR protein. It acts as a CFTR modulatory, anti-inflammatory and anti-microbial agent.

Modified CFTR effect

Cystic fibrosis is caused by mutations, or errors, in the CFTR gene. This can result in either no CFTR protein being made or a malformed CFTR protein being created which cannot perform its key function in the cell.(21)

Curcumin has several effects on CFTR protein such as increase trafficking of channel on cell membrane (corrector) and or potentiation (activity). The first study published by Egan et al demonstrated that curcumin induced accumulation of complex-glycosylated F508 CFTR and increased cell surface density. (22) Curcumin as a sarcoplasmic/endoplasmic reticulum calcium (SERCA) pump inhibitor, could increase the appearance of functional DF508 CFTR on the plasma membrane cells. (22) On the other hand, several chaperone proteins are associated with the quality control of CFTR. Chaperons could bind to the mutant CFTR protein and carry this complex to the ER. One important chaperone is called ER chaperone calreticulin (CRT) which is one of the negative regulators of CFTR expression and function. Some studies have also demonstrated that curcumin suppressed the endogenous calreticulin mRNA transcription that regulate level of this protein in wild type and DF508 CFTR protein so that CFTR protein can be stabilized on the cell membrane.(23) In addition, curcumin enhanced trafficking of DF508 CFTR protein by phosphorylation of ser52 on keratin 18 intermediate filaments on CF pancreatic epithelial cells(24)

Potentiation function of curcumin has been reviewed and confirmed in a number of studies. Curcumin can react as a potentiator that repairs sub activation of CFTR protein activity, and also accelerates CFTR protein activity which has been increased by other CFTR modulation in mutations such as S1251N, G551D and F508del.(25) Considering G551D-CFTR mutation, where normal trafficking on the plasma membrane is characterized with a very short time on opening channel. Therefore, Curcumin can increase G551D-CFTR opening channel and its activity on its own, or when it is added to the channel that is further affected by genistein. Thus, these neutral components are suggested to have different mechanisms as potentiations of channels. (12) Similarly, curcumin stimulates channels which lack NBD2 due to deletion of G551D CFTR mutants, where effect is persistent and irreversible.(26)

W1282X is another common CF mutations which reduces CFTR function by two mechanisms: firstly through reduction of steady state levels of CFTR mRNA, and secondly by lowering the channel activity

due to the defect of the second cytosolic nucleotide binding domain (NBD2), that is a surface bind to ATP molecules (27, 28). According to several studies, curcumin stimulated W1282X-CFTR after adding a saturating dose of VX-770, thus it increased the rates of channel opening. With regards to channel opening, ATP is required for binding of both nucleotide-binding domains (NBDs), which probably results in the dimerization of the two NBDs, and phosphorylation of the R domain. Therefore, both curcumin and VX-770 are acting as allosteric modulators since they stimulate activity of the CFTR channel without ATP binding, but with different mechanisms.(11)

Finally, CFTR-S1251N gating mutation has been evaluated and clinical effects of the three CFTR potentiator treatments curcumin, genistein and ivacaftor have been studied. Based on previous research no clear response was seen on clinical parameters of cystic fibrosis patients, and the main reason was the plasma concentration of curcumin which was lower than the in vitro effective experimental range. (3.25–200µMcurcumin). (29)

Anti-inflammatory effect

Inflammation has a significant role in the outcome of cystic fibrosis patients. Studies have shown an extensive inflammation on pulmonary, gastrointestinal and systemic levels in CF patients.

Mucus plugging is suitable for microorganism growth and neutrophilic inflammation as it has many nutrients. It is believed that, delayed neutrophil apoptosis and long lifespan of neutrophils could increase the levels of neutrophil extracellular traps (NETs), thus inflammation is induced. (30) Moreover, differentiation of monocyte into macrophage and division into M1 or M2 phenotype are unsuccessful in CF patients, and hence it does not produce IL-13Ra1 on the surface. This impaired process contributes to excessive inflammation in CF lung disease.(33). Last but not least, the highest number of T-cell subsets, which are Th17 cells, are present in the CF lung. Th17 cells produce both IL-8 and IL-17 and promote neutrophil accumulation. These T-cells are activated by IL-6, IL-23, and TGF- β , and suppressed by active T regulatory-lymphocytes (Tregs). Otherwise, a CFTR abnormality or an unknown imbalance between Th17 cells and Tregs can induce inflammation by neutrophil recruitment.(2)

Antimicrobial effect

Toll-like receptor-2(TLR2) is a receptor of pathogenic bacteria like *Staphylococcus aureus* peptidoglycan (PGN), is found in epithelial cell and has a key role in recognizing the infected pathogen, and increases inflammatory cytokines as a result, and is up regulated in CF patients.

Cystic fibrosis patients are prone to infection since absence of CFTR protein causes demethylation of DNA at the specific CpG sites which overlaps a minimal region to maintain activity of TLR2 promotor.(34) Curcumin can degrade specificity protein 1 (SP1) via oxidative and proteasome degradation pathways. This factor is essential for upregulation of TLR2 expression. Consequently, curcumin decreases basal expression of TLR2.(35) Finally, curcumin has been proved to have a significant effect on suppressing the growth of *P. aeruginosa* on biofilm optical densities. Although the MIC (Minimum Bacterial

concentration) which is needed for growth suppression is higher than other usual *P. aeruginosa* treatments such as imipenem or tobramycin. (36)

Since the reduction of inflammation plays a key role in the conditions of patients with CF and considering the primary effects of curcumin on the CFTR protein, extensive investigations are required regarding the applications of this natural compound in these patients. Recently, the Nano-curcumin formulation has been extensively studied considering its impact on the increased bioavailability of curcumin. This formulation has been reported to have a great effect on the reduction of inflammatory processes.

The main limitation of the current study is the lack of assessment of CFTR protein function by nasal epithelial biopsy and cellular evaluation. However, this method is invasive and cannot be performed easily on children. To the best of our knowledge, this is the first study which will evaluate the use of curcumin as a specific and supportive nutritional agent in children with CF.

Trial Status

Recruitment was started on 11 July 2020 and is estimated to be completed by 2020-11-21. Recruitment was ongoing at the time of submission.

Abbreviations

IL Interleukin, hs-CRP high sensitive C Reactive Protein, BMI Body Mass Index, CFQ Cystic Fibrosis Questioners, CFTR cystic fibrosis transmembrane conductance regulator, RCT Randomize Control Trial, FEV1 Forced Expiratory Volume 1, PERT Pancreatic Enzyme Replacement Therapy, Camp Cyclic adenosine monophosphate, SERCA Sarco/endoplasmic Reticulum Ca ATPase, ER Endoplasmic Reticulum, CRT, ROS Reactive Oxygen Species, TGF Transforming Growth Factor, M Mitosis, TLR Toll Like Receptor, MAPK Mitogen Activated Protein Kinase, RNS Reactive Nitrogen Species, NBDs nucleotide-binding domains, MIC Minimum Inhibitory Concentration, NETs neutrophil extracellular traps.

Declarations

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Authors' contributions {31b}

ST and HK and MS and SS initially conceptualized and designed the study. ST and HK and SS and MS upgraded the protocol design .HK contributed to obtaining initial funding. The manuscript was written by ST and reviewed by all members. ZS was responsible for design optimizing and statistical analysis. GR performed English editing. All authors read and approved the final manuscript.

Funding {4}

This research will be funded by the vice chancellery for research of Mashhad University of Medical Sciences (MUMS), and all study stages such as design of the study and collection, analysis, interpretation of data and in writing the manuscript will be undertaken under its supervision

Availability of data and materials {29}

The datasets generated and/or analyzed during the current study are not publicly available due to ethical considerations, but may be available from the corresponding author on reasonable request.

Ethics approval and consent to participate {24}

Ethical approval was obtained from ethics committee of MUMS. The ethical approval code is IR.MUMS.MEDICAL.REC.1399.144. The informed consent will be obtained from all study participants or their legal guardian.

Consent for publication {32}

No personal identifying information will be published

Competing interests {28}

The authors declare that they have no competing interests

Authors' information (optional)

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Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Table 2: Laboratory measurements, equipment and normal range

Normal range	Company	Test equipment	Test
Children: up to 2.8 mg/L Adult: up to 5 mg/L	Pars azmoon	Biochemical auto analyzer	Hs-CRP
0-16.5 pg/ml (variable by age)	Demeditec	ELISA kit	IL-6
Up to 10 pg/ml (variable by age)	pars gene	ELISA kit	IL-10
50-120 µg/g: borderline ≥120 µg/g : positive	EUROIMMUN	ELISA kit	Calprotectin
Culture: negative WBC Count: up to 2-3	Variable	URT-S&C(gram positive, gram negative, pseudomonas)	Nasopharyngeal Swab

Figures

Figure 1

schematic of study design. First and second evaluation consist of: serum interleukin-6 (IL-6), IL-10, and high-sensitivity C-reactive protein (hs-CRP), fecal calprotectin, weight and body mass index (BMI), and score of quality of life of the cystic fibrosis questionnaire (CFQ).

Figure 2

Diagram effects of curcumin in cystic fibrosis disease as a CFTR modulator, Anti-inflammatory , antioxidant and anti microbial.

Supplementary Files

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- [Table1.JPG](#)