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Immunomodulatory role of Nanocurcumin in COVID-19 patients with dropped natural killer cells frequency and function

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ABSTRACT

The ongoing COVID-19 pandemic is still a challenging problem in the case of infection treatment. The immunomodulatory effect of Nanocurcumin was investigated in the present study in an attempt to counterbalance the immune response and improve the patients’ clinical symptoms. 60 confirmed COVID-19 patients and 60 healthy controls enrolled in the study. COVID-19 patients were divided into Nanocurcumin and placebo received groups. Due to the importance of the role of NK cells in this disease, the frequency, cytotoxicity, receptor gene expression of NK cells, and serum secretion levels of inflammatory cytokines IL-1β, IL-6, TNF-α, as well as circulating C5a as a chemotactic factor an inflammatory mediator was evaluated by flow cytometry, real-time PCR and enzyme-linked immunosorbent assay in both experimental groups before and after the intervention. The role of measured factors in the progression and pathogenesis of COVID-19 disease, the results can help find appropriate treatments. The results of this study indicated that the Nanocurcumin could significantly increase the frequency and function of NK cells compared to the placebo-treated group. As an immunomodulatory agent, Nanocurcumin may be a helpful choice to improve NK cell function in COVID-19 patients and improve the clinical outcome of patients.

1. Introduction

An investigation was initiated to uncover the etiology of atypical pneumonia cases reported in Wuhan city, China, in late December 2019 (Guo et al., 2020). As a result, viral particles were extracted from patients’ broncho-alveolar lavages, later proving to belong to an emerging type of beta-coronavirus (Wang et al., 2020). Previously, six members of the coronavirus family were known to cause respiratory tract infections. Severe acute respiratory coronavirus (SARS-CoV) and middle eastern respiratory syndrome coronavirus (MERS-CoV) cause severe respiratory diseases, while other members are regarded as low-pathogenic coronaviruses. Further gene sequencing analysis of newly identified...
coronavirus determined a considerable genetic identity of 79.0% and 51.7% with SARS-CoV and MERS-CoV, respectively (Guo et al., 2020). In terms of statistics, novel coronavirus appeared to be substantially less lethal, with a 4% mortality risk compared to SARS-CoV and MERS-CoV with 9.6% and 36%, respectively (Wu et al., 2020). However, the mysterious viral pneumonia, initially called 2019 novel coronavirus (2019-nCoV) infected pneumonia, soon raised the alarms for health institutions due to its high transmission rate (Guo et al., 2020). Subsequently, local health officials of Wuhan city suspended all measures of public transformation in an attempt to stop the 2019-nCoV from spreading (Yan et al., 2020). On Feb. 11, 2020, the International Committee on Taxonomy of viruses named the novel coronavirus SARS-CoV-2. Considering the imminent threat, World Health Organization announced that the disease caused by SARS-CoV-2 would be called COVID-19 from Feb. 12, 2020 (Wang et al., 2020). Despite the implemented preventive measures, SARS-CoV-2 had spread at such a staggering rate that 89,294 people from 47 countries were reported to be infected within weeks of the Wuhan lockdown (Marofi et al., 2021; Zheng, 2020).

Although not all aspects of COVID-19 pathogenesis are fully explained, the key role of the immune system is among the well-established ones (Ghaebi et al., 2021). Laboratory findings such as a reduction in lymphocyte count, an increase in inflammatory cytokines and D-dimer levels, and hepatic dysfunction are all consistent with immune dysregulation (Sadeghi et al., 2021; Tahmasebi et al., 2020). In an attempt to counterbalance lymphocyte malfunction, pro-inflammatory cytokines are disproportionately produced by macrophages, neutrophils, and monocytes, which contribute to further deterioration in patients' clinical status (Fathi and Rezaei, 2020; Giamarellos-Bourboulis et al., 2020). While there was no completely effective treatment against COVID-19 antivirals, including oseltamivir, ganciclovir, and Kaletra (Lopinavir and ritonavir combination), ribavirin, Tocilizumab, cortico-steroids, chloroquine, etc. That were proposed as candidates for mono-threries and combination use in COVID-19 patients (Marofi et al., 2021).

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Clinical efficacy on NK cells, mentioned pro-inflammatory cytokines (IL-1β, IL-6, TNF-α), and C5a in COVID-19 patients compared to healthy controls (Koutsakos et al., 2021). NK cells are accounted for early responses in acute SARS-CoV-2 infection by recruiting the CD56\textsuperscript{bright} and CD56\textsuperscript{dim} NK cell population from the peripheral blood into the lungs, resulting in NK cell depletion in blood circulation (Maucourant and Filipovic, 2020; Wilk and Rustagi, 2020). The blood circulating NK cells are characterized by increased proliferation with higher expression levels of perforin and granzyme B cycling, and the activated phenotype of NK cells was found in acute SARS-CoV-2 infection with increased expression levels of HLA-DR, CD38, CD69, and K67 activating markers as well as TIM3, LAG3 and PD1 inhibitory markers. Moreover, less differentiated NKG2ACD62L\textsuperscript{dim} KIR- cells in the CD56\textsuperscript{dim} NK cells were the main population during acute SARS-CoV-2 infection (Maucourant and Filipovic, 2020; Wilk and Rustagi, 2020). As a result of lymphopenia and leukocyte dysfunction during SARS-CoV-2 infection, declined count of NK cells and their impaired cytotoxic activity have been reported repeatedly, indicating the importance of these kinds of cells in defense against the virus.

On the other hand, NK cell absence leads to ARDS derived from severe hyperinflammation in the severe stage of the COVID-19 disease. Additionally, it is essential to highlight the importance of the complement system’s role in SARS-CoV-2 infection. It is worth noting that C5a, an inflammatory factor, stimulates the release of cytokines and chemokines from various immune cells by linking to its receptor, C5aR (Yan and Gao, 2012). Elevated expression of C5aR receptor on NK cells and NKT cells and higher production of inflammatory cytokines were found after exposure of these kinds of cells to a sepsis-like immune environment such as SARS-CoV2 infection (Carvelli et al., 2020; Fusakio et al., 2021). Altogether, the production of proinflammatory cytokines and C5a mediators’ engagement with NK and NKT cells would contribute to hyperinflammation and subsequent occurrence of cytokine storm in COVID-19.

We hypothesize that Nanocurcumin could adjust immune dysregulation using NK cell activity as the biomarker and mitigate the inflammation by targeting inflammatory mediators. Therefore, we conducted this randomized double-blinded clinical trial to evaluate curcumin’s clinical efficacy on NK cells, mentioned pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α), and C5a in COVID-19 patients compared to placebo.

2. Methods and materials
2.1. Study design and patient selection

This is a placebo-controlled study that received the approval of the Research Ethics Committee of Tabriz University of Medical Sciences. Patients were selected among those admitted to Imam Reza hospital, affiliated with Tabriz University of Medical Sciences (IR.TBZMED.REC.1398.1314). Moreover, the trial was registered in the Iranian Registry of Clinical Trials (IRCTID: IRCT20200324046851N1). After applying inclusion criteria, a total number of 60 confirmed COVID-19 patients and 60 healthy control subjects were considered eligible. The COVID-19 diagnosis was made by a pulmonologist based on laboratory
data and clinical symptoms with respect to protocols. Then, a pre-designed informed consent form was obtained from all participants either in the patient or control group. COVID-19 patients group was later separated into placebo and Nanocurcumin groups. The inclusion criteria were: age between 18 and 70, eagerness to participate, and COVID-19 confirmed by RT-PCR. 10% (3 out of 30) of patients in the Nano-curcumin and 6% (2 out of 30) of patients in the placebo group were on ventilators, with no significant difference between both groups.

In the case of a compromising immune condition (like immunosuppressive agent usage or human immunodeficiency virus (HIV) infection), pregnancy, and breastfeeding, subjects were excluded. The subject’s information, including clinical symptoms and laboratory findings, is summarized in Table 1.

### 2.2. Therapeutic intervention

The Nanocurcumin group received 160 mg of nanocurcumin as two 80 mg dosages for 21 consecutive days, and on the other hand, the placebo group received placebo capsules. The oral curcumin capsules used in this study were “SinaCurcumin®”, a registered product from curcuminoids in Iran (IRC:1228225765), which were developed in the nanotechnology research center of Mashhad University of Medical Sciences and marketed by Exir nano Sina company (Hatamipour et al., 2019). Nanocurcumin oral capsules were administered to patients, known as improved oral bioavailability formulation of curcumin. Sina-Curcumin is a soft gelatin capsule containing 40 mg of curcuminoids, the dietary polyphenols extracted from the dried rhizomes of *Curcuma longa* L. (turmeric), which is introduced as C3 complex [curcumin, bisdemethoxycurcumin (BDMC), and desmethoxycurcumin (DMC)] (Hatami L. (2018)). Differences that make Nanocurcumin more applicable and beneficial than curcumin for therapeutic applications include Particle size, hydrophobicity, as well as charge, and area of the surface that increases effectiveness, solubility, oral bioavailability, active targeting, and pharmacokinetic profile (Karthikeyan et al., 2020). Small particle size (mostly 10–100 nm size nanoparticles) improve the permeability, accessibility, and effectiveness of Nanocurcumin in different medicinal applications compared to curcumin. Nanocurcumin possesses a higher absorption capability into cells and can enter body organs more feasible than normal curcumin, especially in infections, by targeting intracellular pathogens (Flora et al., 2013). Thereby, Nanocurcumin could be considered as a useful drug choice compared to normal curcumin. Increased systemic bioavailability and higher distribution of Nanocurcumin in tissues than normal curcumin with 60 folds of enhancement in the biological half-life could be mentioned as another advantage (Ma et al., 2007). Curcumin nanof ormulation has also been reported to augment its mean residence-retention- and circulation time inside the body (Mythri et al., 2007).

On the other hand, a larger surface area of Nanocurcumin elevates its solubility, improves pharmacological activity, fast drug-releasing, and specific responsiveness to target molecules (Mohanty et al., 2012). Interestingly, surface charges defined as zeta potential, established in Nanocurcumin formulation, avoid the aggregation of nanomaterials and increases the solubility and stability in suspension. At the same time, free curcumin is susceptible to aggregations and opsonization due to its lower solution in water (Muller and Keck, 2004).

In addition to Nanocurcumin, atorvastatin, bromhexine, azithromycin, protease inhibitors, broad-spectrum antibiotics, and corticosteroids were also the routine therapeutics used in both Nanocurcumin- and placebo-treated COVID-19 patients; therefore, did not affect the study findings, and the differences between groups were only related to the placebo and Nanocurcumin.

### 2.3. Blood sampling and sample preparation

Peripheral blood samples of healthy control and COVID-19 groups were obtained before treatment, and NK cell frequency, cytotoxicity, inflammatory cytokines, C5a serum level, and NK cell receptor gene expression were evaluated and compared between the two groups. Sampling was also done for Nanocurcumin and placebo group after treatment to assess the mentioned parameters. The PBMCs isolation was done using Ficoll (lymphosep) 1.077 g/ml (Biosera, UK) density gradient centrifugation method. The isolated PBMCs were used for flow cytometry analysis and mRNA expression assessment.

### 2.4. NK cells frequency assessment

To evaluate NK cell proportion, immunostaining and triple-color immunofluorescence analyses were done. Immunostaining was carried out by fluorescein isothiocyanate (FITC; BD Biosciences) labeled anti-CD3, phycoerythrin (PE; BD Biosciences) labeled Anti-CD16, and allophycocyanin (APC; BD Biosciences) labeled anti-CD56 antibodies to distinguish NK cells. Finally, the NK cell population was determined as CD3+CD56dimCD16+ cells. FITC, PE, and APC mouse IgG2a were used as the isotype controls. The gating strategy was based on the cells’ forward and side scatter profiles. The FACSCalibur flow cytometer (BD Biosciences) and the Cell Quest Pro software (BD Biosciences, San Jose, CA) were used to measure the stained cells and data analysis, respectively.

### Table 1

| Subject | Nanocurcumin group (n = 30) | Placebo group (n = 30) | Healthy Control (n = 60) |
|---------|----------------------------|------------------------|--------------------------|
| Age, Years | 18-67 (52.7 ± 4.6) | 20-68 (52.4 ± 7.6) | 21-69 (50.3 ± 8.4) |
| Sex | Men 21 (67.0) | Women 9 (30) | 10 (66.7) |
| Current smoking | 5 (16.6) | 6 (20) | 10 (66.7) |
| Diabetes | 1 (3.3) | 2 (6.6) | 0 |
| Hypertension | 2 (6.6) | 2 (6.6) | 0 |
| Cardiovascular disease | 0 | 2 (6.6) | 0 |
| Chronic kidney disease | 0 | 2 (6.6) | 0 |
| Fever | <37.3 °C | 3 (10) | 1 (3.3) |
| 37.3–38.0 °C | 11 (36.6) | 14 (46.6) | 0 |
| 38.0–39.0 °C | 9 (30) | 9 (30) | 0 |
| 39.0–39.9 °C | 7 (23.3) | 6 (20) | 0 |
| Gough | 18 (66) | 20 (66) | 0 |
| Headache | 2 (6.6) | 3 (10) | 0 |
| Dympnea | 8 (26.6) | 7 (23.3) | 0 |
| White blood cell count, × 10^9/L | <4 | 8 (26.6) | 7 (23.3) |
| 4–10 | 12 (40) | 14 (46.6) | 0 |
| Lymphocyte count, × 10^6/L | >10 | 10 (33.3) | 9 (30) |
| 1–10 | 20 (66.6) | 18 (60) | 2 (3.3) |
| Platelet count, × 10^9/L | >100 | 19 (66.3) | 21 (70) |
| ≥100 | 11 (36.6) | 9 (30) | 0 (100) |
| Creatinine, μmol/L | ≤133 | 25 (83.3) | 27 (90) |
| >133 | 5 (16.6) | 3 (10) | 0 |
| Lactate dehydrogenase, U/L | ≤245 | 20 (66.6) | 19 (60) |
| >245 | 10 (33.3) | 6 (30) | 0 |
| Bilateral involvement of chest radiographs | 28 (90.3) | 28 (90) | 0 |
| Mechanical ventilation | 3 (10) | 2 (6) | 0 |
2.5. NK cells cytotoxicity assessment

The relative proportion of killed K562 target cells by NK cells accounted for NK cells cytotoxicity and was evaluated by flow cytometry. After co-incubation of K562 target cells with patient mononuclear cells, including NK cells, propidium-iodide uptake was quantified. After 2 h of co-incubation at 37 °C with 5% CO2, the permeability of Killed K562 cells by NK cells allowed DNA staining by propidium iodide. Subsequently, the percentage of killed target cells was measured using flow cytometry. Beyond 15% of target cell killing is determined as increased cytotoxicity in an effector to target ratio of 50:1.

2.6. mRNA expression assessment of NK cells inhibitory and activating receptors

The expression of NK cells receptors, including KIR and C-type lectin receptors (KIR2DL1, KIR2DL2, KIR2DL3, and NKG2A) and KIR2DS1, KIR2DS4, and NKG2C, activating receptors were evaluated by qRT-PCR based on SYBR-Green; therefore, total RNA of isolated PBMCs were extracted by RNX-PLUS Solution (SinaClon, Tehran, Iran). Subsequently, cDNA was synthesized by reverse transcription of extracted mRNA using the RevertAid Reverse Transcriptase kit (Thermo Fisher, Waltham, Massachusetts). An SYBR GREEN qRT-PCR Master Mix was used for amplification, and data analysis was carried out by a Light Cycler 2.0 Real-Time PCR System machine (Roche Applied Science, Germany). Beta-2 microglobulin (β2M) was selected as an endogenous control gene. The sequence of primers has been presented in Table 2.

2.7. Measurements of IL-1β, IL-6, TNF-α, and C5a secretion levels by ELISA

Serum secretion levels of IL-1β, IL-6, TNF-α, and C5a cytokines, as well as circulating C5a were assessed in the serum samples of COVID-19 patients (before and after treatment) and controlled by enzyme-linked immunosorbent assay (ELISA) technique using ELISA Kit (MyBioSource) for cytokines and HK349 ELISA Kit (Hycult Biotech) for C5a, according to the Manufacturer’s protocols. HRP-conjugated secondary antibodies and tetramethylbenzidine, a peroxidase substrate, were applied to detect the abovementioned mediators. After stopping the reaction with acidification (H2SO4), the absorbance values were read by the Medgenix ELISA reader (BP-800; Biohit) at 450 nm. Standard curves were utilized to calculate the concentrations.

2.8. Statistical analysis

Statistical analysis was done by SPSS PC Statistics (version 19.0; SPSS Inc.). An unpaired T-test was utilized to compare the differences in immunologic parameters between COVID-19 and the control group. To compare the parameters before and after treatment, paired T-test was used. The descriptive data were reported as the mean ± SD, and p values < 0.05 were considered to be statistically significant. The GraphPad Prism (version 7.00 for Windows) (GraphPad Software, La Jolla, California, www.graphpad.com) was used for graph drawing.

3. Results

3.1. Population and function of NK cells

NK cell population was evaluated by flow cytometry to compare the frequency of NK cells in COVID-19 patients and the control group. The results showed that NK cell frequency was 16.45 ± 4.02 percent in each ml of whole blood in the control group, while it was decreased in COVID-19 patients (11.77 ± 4.87), and the difference was statistically significant (P < 0.001) (Fig. 1A). The results of cytotoxicity evaluation also showed that NK cell cytotoxicity was significantly reduced in COVID-19 patients (12.42 ± 6.15) compared with the control group (18.92 ± 4.6) (P < 0.001) (Fig. 1B).

NK cell population was also compared in Nanocurcumin and placebo groups before and after the treatment. Before the treatment, the frequency of NK cells in the Nanocurcumin group was 11.94 ± 3.5%, while after the treatment, the frequency was dramatically elevated to 17.05 ± 4.3% (P < 0.001) (Fig. 2A).

The comparison of NK cells’ cytotoxicity pre-treatment (13.94 ± 3.7%) and post-treatment (19.21 ± 5.0%) demonstrated that Nanocurcumin was able to increase the cytotoxicity in the Nanocurcumin group notably (p < 0.001) (Fig. 2B).

3.2. Expression of NK cells receptors

QRT-PCR assessment was done to evaluate the mRNA expression level of NK cells activating and inhibitory receptors. The expression levels of KIR2DS1, KIR2DS4, and NKG2C activating receptors of NK cells in the COVID-19 group were significantly lower than in the control group (p = 0.009, p = 0.026, and p = 0.015, respectively) (Fig. 3A). In the case of inhibitory receptors, the expression levels of KIR2DL3, KIR2DL2, KIR2DL1, and NKG2A were increased in COVID-19 patients compared with the control group (p = 0.01, p = 0.008, p = 0.006, and p = 0.004, respectively) (Fig. 3B).

Activating and inhibitory receptor expression was also assessed in Nanocurcumin and placebo group after treatment. Initially, a comparison was made between the expression of receptors in the control group with Nanocurcumin and the placebo group. The expression levels of KIR2DL3, KIR2DL2, KIR2DL1, and NKG2A inhibitory receptors were higher in the placebo group in comparison with before treatment (P < 0.001, P < 0.001, P = 0.011, and P = 0.001, respectively). However, the expression level of mentioned receptors was decreased after Nanocurcumin therapy when compared to the placebo group (P = 0.028, P < 0.001, p = 0.004, and P < 0.001, respectively) (Fig. 4A). The same analysis was done about activating receptors. The mRNA expression levels of KIR2DS1, KIR2DS4, and NKG2C activating receptors were significantly lower in the placebo group pre-treatment (P = 0.011, p = 0.001, and P = 0.003, respectively). The post-treatment results showed that Nanocurcumin therapy was able to upregulate the expression levels of activating receptors when compared to the placebo group (p = 0.004, p = 0.002, and p = 0.016, respectively) (Fig. 4B). All the results are also summarized in Table 3.

3.3. Secretion levels IL-1β, IL-6, TNF-α, and C5a

Serum levels of IL-1β, IL-6, TNF-α, and C5a were measured by ELISA in COVID-19 patients before and after treatment with Nanocurcumin and healthy control individuals. Based on obtained results, serum

Table 2

| Gene                | Forward (5’-3’) | Reverse (3’-5’) |
|---------------------|----------------|----------------|
| **Activating receptors** |                |                 |
| KIR2DS1 F           | AGATGCGACCTGTAGAAGATCA | TCTTCACACGACGGACAG |
| KIR2DS4 F           | AGAGAATAACAAAGACTGAGC | AGATGATGTGGTTTCTCAGGCC |
| NKG2C F             | GCAAAAGACATCTCACAAC | AACTCTCCGACACACCCTCAG |
| **Inhibitory receptors** |                |                 |
| KIR2DL1 F           | GTGGGTACAGTGCTGTGGTGA | CCTGCAGCTGTCGGC |
| KIR2DL2 F           | AACACTTCTCTCTCAGGCCA | GCCGTGAGAAGAACCACCA |
| KIR2DL3 F           | AGACCTCCAGAGGAGTGA | AGAGAAGACACTTGGTACA |
| NKG2A F             | GTAGATGATGAAGAGATGGATAC | TAAATTTCCTTGAAACTGCCG |
Fig. 1. NK cells frequency and function in COVID-19 patients and control group. A1) The results pertaining to the flow cytometry analysis of the control group, and COVID-19 patients. A2) Flowcytometry analysis of NK cells frequency showed that NK cells population was significantly lower in patients in comparison with control group (p < 0.001). B) NK cells cytotoxicity was also reduced in COVID-19 patients when compared to control group (p < 0.001). COVID-19 patient group, n = 60. Control group, n = 60. Results were presented as mean ± SD. P < 0.05 was described as statistically significant. COVID-19, coronavirus disease 2019; NK cells, Natural killer cells.

Fig. 2. NK cells frequency and function in Nanocurcumin and placebo group. A1 & A2) Flowcytometry analysis of NK cells frequency showed that NK cells population was significantly increased in Nanocurcumin group after treatment (p = 0.001). B) NK cells cytotoxicity was also elevated after Nanocurcumin therapy (p < 0.001). COVID-19 patient group, n = 60. Results were presented as mean ± SD. P < 0.05 was described as statistically significant. COVID-19, coronavirus disease 2019; NK cells, Natural killer cells.
secretion levels of all IL-1β (p = 0.001), IL-6 (p = 0.001), TNF-α (p < 0.001), and CSa (p = 0.002) were significantly higher in COVID-19 patients compared to control group (Fig. 5). In the Nanocurcumin group, findings represented that the Nanocurcumin could meaningfully decrease the serum secretion levels of all IL-1β (p = 0.003), IL-6 (p = 0.008), TNF-α (p = 0.002), and CSa (p = 0.002) after treatment when compared to pre-treatment condition (Fig. 6). All the results are also summarized in Table 4.

3.4. Clinical outcomes

A subject evaluation was done prior to the treatment in the case of clinical manifestation and laboratory tests, which are summarized in Table 1. Clinical symptoms such as fever, cough, headache, or chronic diseases like diabetes and hypertension were evaluated besides the laboratory tests, including white blood cells (WBCs) and platelet count. There were also chest radiographs to monitor the lung situation. No laboratory tests, including white blood cells (WBCs) and platelet count. As a result, the mortality rate was found to be higher in the placebo-treated group than in the Nanocurcumin-treated group (p < 0.001).

4. Discussion

The COVID-19 pandemic has so far claimed over Four million lives globally. Moreover, the ongoing pandemic threatens to become a global humanitarian crisis since no aspect of human life has remained untouched by it (Curković et al., 2020). Various studies have concentrated on lightning up the shadowy transition of SARS-CoV-2 through a possible intermediate host(s) to humans (Yan et al., 2020). These efforts may provide invaluable data on effectively controlling or preventing future pandemics. Nonetheless, we believe that a decent understanding of COVID-19 immunopathology is pivotal for better managing the ongoing pandemic (Giamarellos-Bourboulis et al., 2020).

Acute lung injury (ALI) that could lead to acute respiratory distress syndrome (ARDS) is COVID-19 patients’ significant morbidity and mortality cause (Li et al., 2020). It is hypothesized that lung inflammation triggers a series of events underlying acute lung injury (ALI). The unconstrained inflammatory response to SARS-CoV-2 infection may be partly due to the increased production of inflammatory cytokines (including IL-6 and TNF-α) (Market et al., 2020). These cytokines were even suggested to be used as predicting parameters for disease prognosis since numerous studies reported their increased concentration in severe COVID-19 cases (Fathi and Rezaei, 2020). The results of our previous study also confirmed that the expression and secretion level of inflammatory cytokines, including IL-1β, IL-6, TNF-α, and IL-18, were significantly increased in COVID-19 patients when compared to healthy controls (Valizadeh et al., 2020a).

As more evidence regarding the immunopathology of COVID-19 was accumulating, administration of immune-modulatory agents as a potential treatment came under consideration (Esmailizadeh et al., 2021a, 2021b). Anti-cytokines (i.e. Tocilizumab), corticosteroids (i.e. dexamethasone), and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) were among the evaluated medications for alleviating immunopathology COVID-19 trials. However, various studies concentrated on natural health substances with well-established immunomodulatory effects (Market et al., 2020). Curcumin (diferuloylmethane) is a natural pharmacologically active compound with a safe adverse reaction profile. As a consequence of extensive investigations, curcumin’s capability to modulate diverse molecular signaling pathways has been directly linked to its different biological effects, such as anti-inflammatory, antioxidant, and antimicrobial (Aggarwal et al., 2003).

The cytokine storm is associated with severe COVID-19 cases mainly characterized by acute respiratory distress syndrome (ARDS), multi-organ failure, and death (Coperchini et al., 2020; Li et al., 2020). In this regard, we found dramatically higher levels of IL-1β, IL-6, and TNF-α in blood samples of COVID-19 patients than healthy controls. Interestingly, as we expected, Nanocurcumin could strikingly decline the secretion levels of the cytokines mentioned above after treatment.

In line with our study, the therapeutic effect of Nanocurcumin in 2019-nCoV patients was recently proven in similar studies. The findings of recent studies documented the immunomodulatory and anti-inflammatory role of Nanocurcumin in reducing the proinflammatory cytokines (IL-1β and IL-6) in mRNA and protein levels (Valizadeh et al., 2020b), mitigating inflammatory Th17 cell responses (Tahmasebi et al., 2020) and increasing T regulatory (Treg) cell responses (Tahmasebi et al., 2021b) in COVID-19-treated patients, which was suggested as a potent natural-based immunomodulatory agent for improving the patients’ condition. Moreover, in this line, the immunomodulatory role of Nanocurcumin has been proven in various inflammatory and autoimmune diseases such as ankylosing spondylitis (AS), multiple sclerosis (MS), inflammatory bowel disease (IBD), diabetes, and neurologic disorders, which increasingly emphasizes the therapeutic role of...
Nanocurcumin in inflammatory conditions and confirms our obtained results (Dolati et al., 2018a, 2018b; Ghosh et al., 2015; Hajialilo et al., 2019). It seems that nanocurcumin exerts a part of its anti-inflammatory function by inhibiting the expression and activation of different transcription factor, including NF-κβ, inflammatory cytokines including TNF-α, IL-1β, and IL-6 and various chemokines including MIP-1α (Trivedi et al., 2017). However, all aspects of nanocurcumin’s immunomodulation have not been discovered, and more studies are needed for this purpose (Fig. 7).

Therefore, we decided to investigate the theory that curcumin application in COVID-19 patients could regulate the defect in the immune system. Imbalance of the immune system responses, which is characterized by an increase in inflammatory cells (Th1 and Th17), increased production of inflammatory mediators, as well as a decrease and dysfunction of regulatory cells (Tregs), is the leading cause of inflammation in SARS-CoV2 infected patients (Sadeghi et al., 2021). Following this fact, the disturbance of the immune system responses has been studied and proved in many autoimmune and inflammatory diseases, including Behçet’s disease (Ahmadi and Yousefi, 2019), preeclampsia (Eghbal-Fard and Yousefi, 2019), MS (Izadi et al., 2020; Tahmasebi et al., 2021a), AS (Ahmadi et al., 2020), and recurrent implantation failure (RIF) (Ahmadi et al., 2019), which emphasize the immune system role in the pathogenesis of these type of disorders.

The profound lymphopenia seen in COVID-19 patients is due to depletion of T-cells, B-cells, and natural killer (NK) cells, probably caused by rapid virus replication. This lymphocyte exhaustion phenomenon was also reported in patients infected with SARS-CoV (Market et al., 2020). NK-cells, as innate responders, hold quite an important role in fighting viral infections and immune modulation (van Eeden et al., 2020). There is a lack of data on how NK-cells may directly contribute to failed control of SARS-CoV2. However, evidence supports the hypothesis that NK-cell diminished function is linked to COVID-19 severity. Interestingly, obesity, old age, and malignancy are described by CDC (centers for disease control and prevention) as predisposing conditions against COVID-19 and are also associated with decreased NK-cell activity (Market et al., 2020).

Although the mechanism of NK cell depletion during SARS-COV2 infection is not clearly understood, it is probably due to the absence or
decreased expression of activating receptors (KIR2DS and NKG2C) along with increased expression of inhibitory receptors (KIR2DL and NKG2A) on NK cells (Maucourant and Filipovic, 2020; Osman et al., 2020).

Our study results indicated the significantly lower counts of circulating NK cells in COVID-19 patients, which is consistent with previously published results reporting the notably declined number of NK cells in peripheral blood mononuclear cells (PBMCs) of COVID-19 patients (Taghiloo et al., 2021).

Further, our findings represented the meaningfully higher expression of inhibitory receptors on NK cells that may lead to NK cell dysfunction (Fig. 7). In this context, the upregulated expression level of inhibitory receptors on NK cells has been documented as one of the main reasons for NK cell depletion and dysregulated function in SARS-COV2 infection. Cytotoxicity of NK cells is prevented by NKG2A binding to the HLA-E molecule (Zheng et al., 2020). It was found that the upregulation of NKG2A on NK cell surface results in limited production of cytokines and Granzyme B during SAS-COV2 infection. Furthermore, COVID-19 infection elicits the exhausted phenotype of NK cells by

| mRNA expression level of activating and inhibitory receptors | Prior to treatment | Post treatment |
|---------------------------------------------------------------|-------------------|---------------|
| KIR2DL3                                                       | 1.437 ± 0.50      | 0.975 ± 0.06  | 0.01 | 0.998 ± 0.09 | 1.717 ± 0.63 | <0.001 | 0.998 ± 0.09 | 1.205 ± 0.59 | 0.081 | 0.028 |
| KIR2DL2                                                       | 1.587 ± 0.65      |               | 0.008 | 1.495 ± 0.66 | <0.001 | 0.870 ± 0.38 | 0.082 | <0.001 |
| KIR2DL1                                                       | 1.778 ± 0.83      |               | 0.006 | 1.478 ± 0.85 | 0.011 | 1.030 ± 0.54 | 0.769 | 0.004 |
| NKG2A                                                        | 1.769 ± 0.77      |               | 0.004 | 2.273 ± 0.94 | <0.001 | 1.060 ± 0.63 | 0.623 | <0.001 |
| KIR2DS1                                                      | 0.805 ± 0.16      | 1.011 ± 0.11   | 0.009 | 0.994 ± 0.06 | 0.688 ± 0.41 | 0.011 | 0.994 ± 0.06 | 1.100 ± 0.29 | 0.145 | 0.004 |
| KIR2DS4                                                      | 0.722 ± 0.30      |               | 0.026 | 0.649 ± 0.34 | 0.001 | 0.996 ± 0.19 | 0.959 | 0.002 |
| NKG2C                                                        | 0.814 ± 0.17      |               | 0.015 | 0.685 ± 0.35 | 0.003 | 0.936 ± 0.15 | 0.179 | 0.016 |

Fig. 5. Serum levels of IL-1β, IL-6, TNF-α and C5a in COVID-19 patient and healthy control groups. The results of ELISA measurement revealed the significantly higher serum levels of IL-1β (p < 0.001), IL-6 (p = 0.001), TNF-α (p < 0.001) and C5a (p = 0.002) in COVID-19 patients compared to the healthy control individuals. COVID-19 patient group, n = 60. Control group, n = 60. Results were presented as mean ± SD. P < 0.05 was described as statistically significant. COVID-19, coronavirus disease 2019; IL, interleukin; TNF-α, tumor necrosis factor alpha.
Fig. 6. Serum levels of IL-1β, IL-6, TNF-α and C5a in Nanocurcumin- and placebo-treated groups. After treatment, findings indicated that the Nano-curcumin could considerably decreased the serum secretion levels of IL-1β ($p < 0.001$), IL-6 ($p = 0.008$), and C5a ($p = 0.002$) in Nanocurcumin-treated group than in placebo-treated group. COVID-19 patient group, $n = 60$. Results were presented as mean ± SD. $P < 0.05$ was described as statistically significant. COVID-19, coronavirus disease 2019; IL, interleukin; TNF-α, tumor necrosis factor alpha.

Table 4
ELISA results.

|                      | Prior to treatment (n = 60) | Post treatment                          |
|----------------------|-----------------------------|-----------------------------------------|
|                      | COVID-19 patients (Mean ± SD) | Healthy control (Mean ± SD) | p-value | Nano-curcumin group (Mean ± SD) (n = 28) | Placebo group (Mean ± SD) (n = 23) | p-value |
|                      | Before After p-value        | Before After p-value                  | Before After p-value |
| IL-1β                | 16.93 ± 9.76 8.34 ± 6.07 0.001 | 15.82 ± 7.43 11.48 ± 6.81 0.003 | 15.99 ± 8.63 15.38 ± 8.63 0.738 |
| IL-6                 | 4.97 ± 3.2 3.04 ± 1.77 0.001 | 4.95 ± 3.06 3.82 ± 2.13 0.008 | 5.42 ± 4 4.95 ± 3.44 0.564 |
| TNF-α                | 289.5 ± 122.3 197.3 ± 88.27 <0.001 | 293.7 ± 234.8 ± 0.002 | 285.4 ± 279.5 ± 0.762 |
| C5a                  | 24.25 ± 28.07 12.76 ± 6.94 0.002 | 28.57 ± 29.07 17.96 ± 16.18 0.002 | 24.67 ± 20.24 ± 0.533 |

Table 5
Subjects clinical symptoms and laboratory finding before and after the treatment.

|                      | Nano-curcumin group (n = 28) | Placebo group (n = 23) | p value |
|----------------------|-----------------------------|------------------------|---------|
|                      | Before (n = 30) | After (n = 28) | P value | Before (n = 30) | After (n = 23) | P value |
| Fever                | <37.3 °C | 3 (10%) | 21 (75%) | <0.001 | 1 (3%) | 2 (7%) | 0.546 |
|                      | 37.3–38.0 °C | 11 (36.6%) | 7 (25%) | 9 (30%) | 4 (46.6%) | 10 (43%) |
|                      | 38.1–39.0 °C | 9 (30%) | 0 (0%) | 6 (20%) | 4 (8%) | 0.124 |
|                      | >39.0 °C | 7 (23.3%) | 0 (0%) | <0.001 | 6 (24) | 6 (26%) | 0.038 |
| Cough                | 18 (60) | 3 (10%) | 6 (26%) | 0.038 |
| Headache             | 2 (6.6) | 0 (0%) | 3 (10%) | 0.011 |
| Dyspnea              | 8 (26.6) | 3 (10%) | 7 (21%) | 0.124 |
| White blood cell count, × 10^9/L | <4 | 8 (26.6) | 3 (10%) | 0.001 |
|                      | 4–10 | 12 (40) | 20 (60) | 0.124 |
|                      | >10 | 10 (33.3) | 5 (17) | 0.001 |
| Lymphocyte count, × 10^9/L | <1 | 20 (66.6) | 7 (25%) | 0.001 |
|                      | ≥1 | 10 (33.3) | 21 (75%) | 0.038 |
| Platelet count, × 10^9/L | <100 | 19 (63.3) | 6 (21%) | 0.367 |
|                      | ≥100 | 11 (36.6) | 22 (78%) | 0.367 |
| Creatinine, μmol/L | ≤133 | 25 (83.3) | 27 (96%) | 0.367 |
|                      | >133 | 5 (16.6) | 1 (3%) | 0.001 |
| Lactate dehydrogenase, U/L | ≤245 | 20 (66.6) | 26 (92%) | 0.367 |
|                      | >245 | 10 (33.3) | 2 (6.6) | 0.367 |
| Bilateral involvement of chest radiographs mechanical ventilation | 28 (96.3) | 9 (32%) | 28 (96.3) | 9 (32%) | 0.011 |
inducing the expression of PDCD1, HAVCR, and LAG3 on their surface (Wilk and Rustagi, 2020). Some other evidence documented that downregulated expression of NKG2C, an activating receptor of NK cells, leads to a severe condition in COVID-19 (Vietzen et al., 2021). As a result, due to the reduction in the number of cells and their activity in patients, maybe increasing the reserves, activity, and restoration of these cells can help improve the condition of COVID-19 patients (Giarmarellos-Bourboulis et al., 2020). The results of this study showed that Nanocurcumin, as a natural treatment with its immunomodulatory function, could increase the frequency of NK cells and their function in SARS-COV2 infected patients by upregulating the expression of activating receptors and downregulating inhibitory receptors on these cells. In our study, NK-cell number was used as a biomarker to determine Nanocurcumin efficacy against COVID-19 immune pathology. As stated in the results, Nanocurcumin could increase the lethal activity of cells in patients receiving Nanocurcumin compared with placebo. Consistent with these findings, previously, it was demonstrated that curcumin could intensify the NK cell cytotoxicity and killing activity (Trivedi et al., 2017). In a study, it was reported that Nanocurcumin could boost the function of NK cells by increasing the expression of CD16^+ and CD56^dim on NK cells and activation of STAT4 and STAT5 in NK cells (Lee and Cho, 2018). Besides, another study has shown that curcuminoids could elevate the NK cells' cytotoxicity and killing function by stimulating the NK cells to secrete IFN-γ (Fiala et al., 2015). Considering the importance of the C5a complement fragment in the COVID-19 immunopathogenesis, serum levels of this mediator were evaluated in the current study. We found significantly increased serum levels of C5a in COVID-19 patients than in controls, which were significantly decreased after treatment with Nanocurcumin compared to placebo. It was demonstrated that C5a-C5aR involved in inflammatory responses and development of coagulopathy in SARS-COV2 infected patients (Risitano and Mastellos, 2020). Monocytes, macrophages, neutrophils, NK and NKT cells expressing C5aR were recruited to the lungs by binding to C5, which leads to the production of IL-1β, IL-6, and TNF-α pro-inflammatory cytokines and subsequently cytokine storm (Bosmann and Ward, 2012; Mastellos et al., 2020). Using anti-C5a antibody as a treatment choice in patients supports the abovementioned facts by elevating the lung oxygenation, mitigating the systemic inflammation, and mediating clinical improvement (Gao et al., 2020). In line with our obtained results, the increased serum levels of C5a have been reported recently in COVID-19 patients which was correlated with disease severity and were significantly higher in patients suffering from lung damage and ARDS. Also, it was suggested that the elevated level of C5a in the patients with the most-severe conditions has an important role in developing the inflammation mostly in ARDS patients (Carvelli et al., 2020; Gao et al., 2020).

5. Conclusion

The present study confirmed the presence of a dysregulated immune response, including diminished frequency and function of NK cells in addition to higher expression of inhibitory receptors and lower
expression of stimulatory receptors in COVID-19 patients. The results of Nanocurcumin therapy as an immunomodulatory agent demonstrated that controlling the exaggerated immune response may improve NK cells function and infected patients’ situation. Also, as a hopeful outcome, Nanocurcumin could considerably decrease the mortality rate in treated patients compared to the placebo-treated group. Therefore, Nanocurcumin may be a helpful choice in modulating immune system dysregulated responses like increased NK cells in COVID-19 patients.

CRediT authorship contribution statement

Sanaz Abbaspour-Aghdam: Investigation, Writing – original draft, Writing – review & editing. Ali Hazerati: Investigation, Writing – original draft, Writing – review & editing. Samaneh Abdolmohammadi-Vahid: Validation, Visualization. Saba Tahmasebi: Data curation, Formal analysis. Jafar Mohseni: Validation, Visualization. Hamed Valizadeh: Conceptualization, Supervision, Writing – review & editing. Mehdi Nadjiri: Data curation, Formal analysis. Haleigh Mikaeili: Methodology, Software. Armin Sadeghi: Validation, Visualization. Mehdi Yousefi: Conceptualization, Resources. Leila Roshangar: Conceptualization, Resources. Behzad Nikzad: Data curation, Formal analysis. Fariborz Jadidi-Niaragh: Methodology, Project administration. Hossein Samadi Kafi: Methodology, Project administration. Kosar Malekpour: Validation, Visualization. Majid Ahmadi: Conceptualization, Supervision, Writing – review & editing.

Declarations of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be considered as a potential conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejphar.2022.175267.

References

Aggarwal, B.B., Kumar, A., Bharti, A.C., 2003. Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Res. 23, 963-998.
Ahmadi, M., Abdolmohammadi-Vahid, S., Ghaebi, M., Dolati, S., Abbaspour-Aghdam, S., Danaii, S., Berjis, K., Madadi-Javid, R., Nouri, Z., Siahmansouri, H., 2019. Sirolimus therapy for COVID-19: an overview of the involvement of the chemokine/chemokine-receptor axis. Cytokine Growth Factor Rev. 53, 25–32.
Bjørkstrøm, N.K., Ljunggren, H.G., Michaelsson, J., 2016. Emerging insights into natural killer cells in human peripheral tissues. Nat. Rev. Immunol. 16, 310-320.
Bjørkstrøm, N.K., Ponzetta, A., 2021. Natural killer cells and unconventional T cells in COVID-19. Curr. Opin. Virol. 49, 176-182.
Boomsma, M., Ward, P.A., 2012. Role of CD3, CD28 and CD134/49 receptor in acute lung injury and in sepsis. Adv. Exp. Med. Biol. 946, 147–159.
Carvell, J., Demaria, O., Vely, F., Batista, L., Chouaki Benmansour, N., Fares, J., Carpenter, S., Thibult, M.L., Morel, A., Remark, R., Andrè, P., Represa, J., Pipereggl, C., Cordier, F.Y., Le Daut, E., Guervilly, C., Simeone, P., 2020. Association of COVID-19 inflammation with activation of the Csa-Csra1 axis. Regular and young investigator award abstracts 588, 146–150.
Coppolino, F., Chiavato, L., Rocchi, M., Flora, G., Gupta, D., Tiwari, A., 2021a. Nanocurcumin restores aberrant miRNA expression profile in multiple sclerosis, randomized, double-blind, placebo-controlled trial. J. Cell. Physiol. 236, 1082-1090.
Coppolino, F., Chiavato, L., Rocchi, M., Flora, G., Gupta, D., Tiwari, A., 2021b. Nanocurcumin restores aberrant miRNA expression profile in multiple sclerosis, randomized, double-blind, placebo-controlled trial. J. Cell. Physiol. 236, 1082-1090.
Dolati, S., Aghebati-Maleki, L., Ahmadi, M., Marofi, F., Babaloo, Z., Ayramloo, H., Jafari, D., Tahmasebi, S., Elahi, R., Khosh, E., 2020a. Immune-based therapy for COVID-19. Adv. Exp. Med. Biol. 1318, 449-468.
Esmaeilzadeh, A., Rostami, S., Yeganeh, P.M., Tahmasebi, S., 2021b. Recent advances in antibody-based immunotherapy strategies for COVID-19. J. Cell. Biochem. 122, 1543-1552.
Fathi, N., Rezaei, N., 2020. Lymphopenia in COVID-19: therapeutic opportunities. Cell Biol. Int. 44, 1792-1797.
Faria, N., Rezaei, N., 2020. Lymphopenia in COVID-19: therapeutic opportunities. Cell Biol. Int. 44, 1792-1797.
Faria, N., Rezaei, N., 2020. Lymphopenia in COVID-19: therapeutic opportunities. Cell Biol. Int. 44, 1792-1797.
Faria, N., Rezaei, N., 2020. Lymphopenia in COVID-19: therapeutic opportunities. Cell Biol. Int. 44, 1792-1797.
Faria, N., Rezaei, N., 2020. Lymphopenia in COVID-19: therapeutic opportunities. Cell Biol. Int. 44, 1792-1797.
Faria, N., Rezaei, N., 2020. Lymphopenia in COVID-19: therapeutic opportunities. Cell Biol. Int. 44, 1792-1797.
Faria, N., Rezaei, N., 2020. Lymphopenia in COVID-19: therapeutic opportunities. Cell Biol. Int. 44, 1792-1797.
Faria, N., Rezaei, N., 2020. Lymphopenia in COVID-19: therapeutic opportunities. Cell Biol. Int. 44, 1792-1797.
Faria, N., Rezaei, N., 2020. Lymphopenia in COVID-19: therapeutic opportunities. Cell Biol. Int. 44, 1792-1797.
Osman, M., Faridi, R.M., Sligl, W., Shabani-Rad, M.T., Dharmani-Khan, P., Parker, A., Mythri, R.B., Jagatha, B., Pradhan, N., Andersen, J., Bharath, M.M., 2007. Mitochondrial complex I inhibition in Parkinson disease: how can curcumin protect? J. Biotechnol. 113, 151–157.

Mythri, R.B., Jagatha, B., Pradhan, N., Andersen, J., Bharath, M.M., 2007. Mitochondrial complex I inhibition in Parkinson’s disease: how can curcumin protect mitochondria? Antioxidants Redox Signal. 9, 399–408.

Osman, M., Faridi, R.M., Sligl, W., Shahani-Rad, M.T., Dharmani-Khan, P., Parker, A., Kolra, A., Tripathi, M.B., Storek, J., Cohen Tervaert, J.W., Khan, F.M., 2020. Impaired natural killer cell counts and cytotoxic activity in patients with severe COVID-19. Blood Adv. 4, 5035–5039.

Pantrip, R., Naime, M., 2021. The deregulated immune reaction and cytokines release storm (CRS) in COVID-19 disease. Int. Immunopharmacol. 90, 107225.

Rahimi, H.R., Mohammadpour, A.H., Dastani, M., Jaafari, M.R., Abnous, K., Mobarakian, M.G., Oskuee, R.K., 2016. The effect of nano-curcumin on HBAlc, fasting blood glucose, and lipid profile in diabetic subjects: a randomized clinical trial. Avicenna J. Phytomed. 6, 567.

Ristano, A.M., Calado, R.T., Lambris, J.D., 2020. Complement C3 vs C5 inhibition in severe COVID-19: early clinical findings reveal differential biological efficacy. Clin. Immunol. 220, 108598.

Sadeghi, A., Tahmasebi, S., Khosh, E., Esmaeizadeh, A., 2020. The outlook for diagnostic purposes of the 2019-novel coronavirus disease. J. Cell. Physiol. 235, 9211–9229.

Tahmasebi, S., Qasim, M.T., Krivenson, M.V., Zekiy, A.O., Thangavelu, L., Arvindvahan, S., Izadi, M., Jadiidi-Niairagh, F., Ghaedi, M., Astani, S., 2021a. The effects of Oxygen-Ozone therapy on regulatory T-cell responses in multiple sclerosis patients. Cell Biol. Int.

Tahmasebi, S., Saeed, B.Q., Temirgulayeva, E., Yamashov, A.E., El-Eswai, M.A., Navashenag, J.G., Valizadeh, H., Sadeghi, A., Aslani, S., Yousefi, M., Jadiidi-Niairagh, F., Adigozalou, J., Ahmadi, M., Roshangar, Li., 2021b. Nanocurcumin Improves Treg Cell Responses in Patients with Mild and Severe SARS-CoV-2. Life sciences. 119437.

Trivedi, M.K., Mondal, S.C., Gangwar, M., Jana, S., 2017. Immunomodulatory potential of nanocurcumin-based formulation. Inflammopharmacology 25, 609–619.

Valizadeh, H., Abdolmohammadi-Vahid, S., Daneshnia, S., Gencer, M.Z., Ammari, A., Sadeghi, A., Roshangar, L., Aslani, S., Esmaeizadeh, A., Ghaedi, M., 2020a. Nano-curcumin therapy, a promising method in modulating inflammatory cytokines in COVID-19 patients. Int. Immunopharmac. 89, 107088.

Valizadeh, H., Abdolmohammadi-Vahid, S., Daneshnia, S., Ziya Gencer, M., Ammari, A., Sadeghi, A., Roshangar, L., Aslani, S., Esmaeizadeh, A., Ghaedi, M., 2020b. Nano-curcumin therapy, a promising method in modulating inflammatory cytokines in COVID-19 patients. Int. Immunopharmac. 89, 107088.

van Eeden, C., Khan, L., Osman, M.S., Cohen Tervaert, J.W., 2020. Natural killer cell dysfunction and its role in COVID-19. Int. J. Mol. Sci. 21, 6351.

Vietzen, H., Zoufaly, A., Traugott, M., Arealje, J., Aberle, S.W., Puchhammer-Stickl, E., 2021. Deletion of the NKG2C receptor encoding KLR2 gene and HL-A variants are risk factors for severe COVID-19. Genet. Med. 23, 963–967.

Wang, L., Wang, Y., Ye, D., Liu, Q., 2020. Review of the 2019 novel coronavirus (SARS-CoV-2) based on current evidence. Int. J. Antimicrob. Agents 55, 105948.

Wilk, A.J., Rustagi, A., 2020. A single-cell atlas of the peripheral immune response in patients with severe COVID-19 26, 1070–1076.

Wu, D., Wu, T., Liu, Q., Yang, Z., 2020. The SARS-CoV-2 outbreak: what we know. Int. J. Infect. Dis. 94, 44–48.

Yadav, V., Mishra, K., Singh, D., Mehrotra, S., Singh, V., 2005. Immunomodulatory effects of curcumin. Immunopharmacol. Immunotoxicol. 27, 485–497.

Yan, C., Gao, H., 2012. New insights for CsA and CsA receptors in sepsis. Front. Immunol. 3, 368.

Yan, Y., Shin, W.I., Pang, Y.X., Meng, Y., Lai, J., 2020. The first 75 Days of novel coronavirus (SARS-CoV-2) outbreak: recent advances, prevention, and treatment. Int. J. Environ. Res. Publ. Health 17.

Zheng, M., Gao, Y., Wang, G., Song, G., Liu, S., Sun, D., Xu, Y., Tian, Z., 2020a. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. Cell. Mol. Immunol. 17, 533–535.

Further reading

Tahmasebi, S., El-Eswai, M.A., Mahmoud, Z.H., Timoshin, A., Valizadeh, H., Roshangar, L., Varshoch, M., Vaez, A., Aslani, S., Navashenag, J.G., Aghabati-Maleki, L., 2021 Jul. Immunomodulatory effects of Nanocurcumin on Th17 cell responses in mild and severe COVID-19 patients. J. Cell. Physiol. 236 (7), 5325–5338.