Jinhua Qinggan granule, a Chinese herbal medicine against COVID-19, induces rapid changes in the neutrophil/lymphocyte ratio and plasma levels of IL-6 and IFN-γ: An open-label, single-arm pilot study

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Received May 5, 2021; Accepted November 15, 2021

DOI: 10.3892/wasj.2021.137

Abstract. Traditional Chinese herbal medicine has provided clinical benefits to patients infected with coronavirus 2019 (COVID-19) in China. Jinhua Qinggan granule (JHQGG) is a Chinese multi-herbal formula previously developed for the treatment of H1N1 influenza and has been encouraged for use in patients with clinically suspected COVID-19 infection. However, the immunopharmacological mechanism for the efficacy of JHQGG has not yet been confirmed. To obtain insight into this issue, the present study examined the acute effects of JHQGG ingestion on hematological and immunological parameters using uninfected individuals as subjects. For this purpose, 18 healthy volunteers were enrolled, all of whom tested negative for prior and current severe acute respiratory syndrome coronavirus 2 infection. Peripheral blood samples were collected 1 h after a single oral JHQGG administration and subjected to hematological, biochemical and cytokine tests. JHQGG rapidly induced a significant decrease in the plasma level of interleukin (IL)-6 (P=0.00309) and an increase in the plasma level of interferon (IFN)-γ (P=0.0268). A decrease in IL-6 and an increase in IFN-γ levels were observed in 14 (77.8%) and 13 (72.2%) subjects, respectively. Notably, JHQGG significantly decreased the proportion of neutrophils (P=0.00561) and increased that of lymphocytes (P=0.00485); accordingly, the neutrophil/lymphocyte ratio (NLR) was significantly reduced by JHQGG (P=0.00649). These findings suggest that the clinical benefits of the use of JHQGG against COVID-19 are, at least in part, associated with its rapid modulatory effects on IL-6, IFN-γ and NLR. Considering that IL-6 and NLR are critical biomarkers for severe COVID-19 infection, JHQGG may thus be suitable not only for suppressing disease onset in suspected and asymptomatic cases, but also for preventing disease progression in patients with mild to severe infection. The present open-label, single-arm study has been prospectively registered on the University Hospital Medical Information Network-Clinical Trials Registry (UMIN-CTR) under the trial no. UMIN000040407 on May 15, 2020.

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Abbreviations: JHQGG, Jinhua Qinggan granule; Th1, type 1 helper T; NLR, neutrophil/lymphocyte ratio

Key words: Chinese herbal medicine, coronavirus 2019, cytokines, inflammation, interferon-γ, interleukin-6, Jinhua Qinggan granule, lymphocyte, neutrophil, severe acute respiratory syndrome coronavirus 2

Introduction

Traditional herbal medicine has provided clinical benefits to patients with coronavirus 2019 (COVID-19) in China (1-3). Several herbal formulas are encouraged for use in the treatment of COVID-19 in the latest version of the diagnosis and treatment protocol for novel coronavirus pneumonia (Trial Version 7) released by the National Health Commission of China (4). One of these is Jinhua Qinggan granule (JHQGG), which was formulated specifically for the treatment of H1N1 influenza in the 2009-2010 pandemic (5-7). JHQGG contains 12 herbal components, namely Jinyin hua (*Lonicerae Japonicae* Flos, honeysuckle), Shigao (Gypsum fibrosum moles, Gypsum fibrosum), Ma huang (*Ephedra herba*, Ephedra), Kuxing ren (Amygdalus Communis Vas, Armeniacae Semen Amarum), Huangqin (*Scutellariae* Radix, *Scutellaria baicalensis*), Lianqiao (Forsythiae Fructus, *Fructus forsythiae*), Zhebeimu (*Fritillariae thunbergii* Bulbus, Thunberg Fritillary Bulb), Zhimu (Anemarrhenae Rhizoma, Rhizoma Anemarrhenae), Niubangzi (Fructus Arctii, Arctii Fructus), Qinghao (*Artemisia annua* L., *Artemisiae annua herba*), Bohe (*Menthae Herba*, *Menthae haplocalycis* Herba) and Gancao (Licorice, Liquorice), that exert medicinal effects on symptoms of

respiratory viral infection. Previous preclinical studies have demonstrated that JHQGG reduces pulmonary lesions and mortality in mice infected with the H1N1 influenza virus (5). Clinical studies have also demonstrated that JHQGG reduces the duration of fever and alleviates respiratory symptoms in patients with H1N1 influenza (5,6).

On the basis of its therapeutic efficacy for influenza, JHQGG has been recommended for use in patients clinically suspected of COVID-19 infection to prevent disease onset (8-11). In randomized controlled trials on patients with COVID-19, the combined administration of JHQGG with Western medicine was shown to significantly promote viral clearance, ameliorate respiratory symptoms, shorten the recovery time from pneumonia and relieve psychological anxiety compared with the administration of Western medicine alone (8-11). Recent network pharmacological analyses have identified a large number of active natural compounds contained in the herbal ingredients of JHQGG, including rutin, luteolin, wogonin, myricetin, quercetin, ursolic acid, chrysoeriol and glabridin (12,13). These compounds interact with a wide variety of target proteins and modulate the complex webs of signaling and metabolic pathways associated with immune regulation, anti-inflammation, and protection from oxidative stress and tissue injury (12,13). Several active compounds also have the potential to directly inhibit severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection by down-regulating angiotensin I converting enzyme 2, the viral entry receptor (13).

Although network pharmacological studies have provided *in silico* predictions on the possible compound-target and target-function networks modulated by JHQGG (12,13), there is limited information available on the *in vivo* immunological mechanisms underlying the prophylactic and therapeutic benefits of JHQGG against COVID-19. To obtain insight into this issue, the present study examined the acute effects of JHQGG on hematological and immunological parameters.

Subjects and methods

Subjects and inclusion/exclusion criteria. Since JHQGG has been recommended for use in individuals clinically suspected of COVID-19 infection under medical observation to prevent disease onset, the present study employed uninfected individuals as subjects in a single-arm trial. Participants were recruited through the University Hospital Medical Information Network-Clinical Trials Registry (UMIN-CTR) website, the authors' clinical website (https://takanawa-clinic.com/), announcements in an e-mail newsletter and personal contacts. Individuals who met all the following inclusion criteria were enrolled in the trial: Adults between the ages of 20 and 70, and having negative RT-PCR and IgM/IgG antibody tests for SARS-CoV-2 at study entry. Individuals were excluded from the trial if they met any of the following exclusion criteria: Were pregnant or breastfeeding; had duplicate enrollment in other clinical trials; a history of infectious diseases within the prior 6 months; a current or past history of chronic inflammatory or immune-related diseases, or malignancies; a history of drug use within the past 6 months; had any underlying conditions associated with a higher risk of COVID-19 infection, including hypertension, cardiovascular disease, cerebrovascular disease, diabetes, obesity (body mass index ≥30), chronic obstructive pulmonary disease and chronic kidney disease.

Administration of JHQGG. JHQGG was kindly provided by Dr Hugh Wang, Juxiechang (Beijing) Pharmaceutical Co., Ltd. The subjects were instructed to take a packet (5 g) orally 40 min their lunchtime meal in accordance with the administration protocol of the Chinese official guideline (4). This dose is known to be effective for the treatment of influenza (6).

Hematological, biochemical and cytokine analyses. To examine the acute hematological and immunological effects of JHQGG, peripheral blood samples were obtained from each subject immediately prior to and at 1 h following the administration of JHQGG. Hematological and blood biochemical tests were outsourced to SRL, Inc. Plasma cytokine levels were quantified using the V-PLEX Proinflammatory Panel 1 Human kit (K15049D-1; Meso Scale Diagnostics) and the Human IL-18 ELISA kit (ab215539; Abcam). The primary outcome measure was changes in the plasma levels of inflammatory-related cytokines [interleukin (IL)-6, IL-1β, IL-18, IL-12, IL-2, IL-8, IL-10, interferon (IFN)-γ and tumor necrosis factor (TNF)-α] at 1 h after the JHQGG ingestion compared with the baseline levels. The secondary outcome measure was changes in hematological parameters (as listed in Table I) at 1 h after the JHQGG ingestion compared with baseline levels.

Statistical analysis. No outliers were taken into account, and all collected data from all patients (n=18) were subjected to statistical analysis. The normality of the data was tested using the Shapiro-Wilk test. On the basis of the results from the normality test, the two-tailed Wilcoxon signed-rank test was employed at the significance level (α) of 0.05 for subsequent statistical analysis of the data. All statistical analyses were performed using EZR version 1.53 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) (14). Post hoc power analysis was performed using G*Power version 3.1.9.2 (15). A P-value <0.05 was considered to indicate a statistically significant difference.

Results

A total of 18 healthy volunteers were screened for eligibility, found to be eligible and enrolled in the trial in the present study (Fig. 1). These subjects were the same as those that used in a previous study by the authors on Qingfei Paidu decoction (QFPD) (16). JHQGG was administered to all the enrolled participants from May 28-29, 2020 following a 2-week washout period from the completion of the QFPD trial. Consequently, 18 subjects (5 males and 13 females; age range, 22-58 years; mean ± SD, 33.8±10.7 years) completed the intervention, and the data were subjected to statistical analysis (Fig. 1).

It was found that hematocrit levels (Z=2.06, P=0.0394, r=0.486) and mean corpuscular volume (Z=2.30, P=0.0216, r=0.542) were slightly altered within the reference ranges, although there were no significant differences in the other measurements of complete blood count and blood biochemistry

Table I. Alterations in hematological parameters and cytokine levels in subjects administered JHQGG.

Measurements	Pre-JHQGG administration		Post-JHQGG administration		Z value	P-value	r value
	Median	(IQR)	Median	(IQR)			
Complete blood count							
Red blood cell count (x$10^4/\mu$l)	454	(419-468)	457	(416-469)	1.26	0.206	0.298
Hemoglobin (g/dl)	13.5	(12.3-14.4)	13.6	(12.2-14.0)	1.60	0.111	0.376
Hematocrit (%)	41.0	(36.5-43.7)	40.6	(36.2-42.8)	**2.06**	**0.0394**[a]	**0.486**
MCV (fl)	90.7	(88.2-94.7)	90.3	(87.8-95.4)	**2.30**	**0.0216**[a]	**0.542**
MCH (pg)	30.1	(29.4-31.5)	30.3	(29.5-31.7)	0.166	0.868	0.0392
MCHC (%)	33.2	(32.6-33.7)	33.4	(32.9-33.7)	1.58	0.115	0.372
White blood cell count (/μl)	6200	(5730-6680)	6450	(6030-6780)	1.11	0.265	0.263
Platelet count (x$10^4/\mu$l)	24.4	(22.1-28.7)	24.7	(22.7-28.3)	0.214	0.831	0.0504
White blood cell differential							
Neutrophils (%)	62.3	(59.6-68.5)	61.7	(58.9-65.6)	**2.77**	**0.00561**[b]	**0.653**
Eosinophils (%)	1.10	(0.850-2.08)	1.05	(0.725-2.38)	0.0713	0.943	0.0168
Basophils (%)	0.450	(0.300-0.575)	0.500	(0.325-0.600)	0.431	0.666	0.102
Monocytes (%)	5.25	(4.40-6.38)	5.65	(4.73-6.48)	1.06	0.288	0.250
Lymphocytes (%)	29.1	(23.6-32.9)	29.9	(25.3-33.0)	**2.82**	**0.00485**[b]	**0.664**
Neutrophil/lymphocyte ratio	2.24	(1.82-2.97)	2.08	(1.79-2.52)	**2.72**	**0.00649**[b]	**0.642**
Blood biochemistry							
AST (U/l)	16.5	(15.0-19.5)	16.0	(14.3-18.8)	0.00	1.00	0.00
ALT (U/l)	15.0	(10.3-18.5)	15.0	(10.3-18.5)	0.632	0.527	0.149
γ-GT (U/l)	14.0	(12.3-21.0)	14.0	(13.0-21.0)	1.51	0.132	0.355
LDH (U/l)	143	(134-170)	145	(131-166)	1.07	0.285	0.252
Albumin (g/dl)	4.70	(4.50-4.88)	4.65	(4.53-4.90)	0.206	0.837	0.0486
Urea nitrogen (mg/dl)	12.2	(11.8-14.2)	12.4	(11.4-14.4)	0.0237	0.981	0.00559
HDL cholesterol (mg/dl)	69.0	(61.8-77.8)	68.5	(61.3-77.0)	1.02	0.306	0.241
LDL cholesterol (mg/dl)	109	(96.5-124)	109	(91.8-123)	1.30	0.193	0.307
Triglycerides (mg/dl)	62.0	(41.3-108)	66.0	(42.0-111)	0.687	0.492	0.162
CRP (mg/dl)	0.0500	(0.0225-0.0875)	0.0550	(0.0225-0.0875)	1.61	0.107	0.380
Cytokines							
IFN-γ (pg/ml)	3.33	(1.80-4.72)	3.85	(2.99-5.00)	**2.20**	**0.0268**[a]	**0.518**
IL-6 (pg/ml)	1.40	(0.731-3.31)	0.988	(0.690-1.59)	**2.96**	**0.00309**[b]	**0.697**
TNF-α (pg/ml)	2.49	(2.01-3.55)	2.63	(2.29-3.39)	0.893	0.393	0.210
IL-1β (pg/ml)	0.504	(0.336-1.45)	0.377	(0.224-2.36)	0.806	0.442	0.190
IL-18 (pg/ml)	145	(99.6-232)	109	(90.1-304)	**1.98**	**0.0483**[a]	**0.467**
IL-12 (pg/ml)	0.262	(0.106-0.452)	0.330	(0.185-0.472)	1.02	0.325	0.241
IL-2 (pg/ml)	0.280	(0.0955-0.655)	0.389	(0.157-0.891)	1.94	0.0539	0.457
IL-8 (pg/ml)	448	(105-1220)	415	(115-766)	0.719	0.495	0.169
IL-10 (pg/ml)	0.259	(0.184-0.500)	0.268	(0.213-0.556)	0.327	0.766	0.0770

Statistically significant results are presented in bold font ([a]P<0.05 and [b]P<0.01). The undetectable IL-6 data in one subject and the undetectable IFN-γ data in two subjects were corrected with the values of 0.5 x lower limit of detection (IL-6, 0.03 pg/ml; IFN-γ, 0.185 pg/ml) (Fig. 2). MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, γ-glutamyltransferase; LDH, lactate dehydrogenase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; IQR, interquartile range; Z value, test statistic for the Wilcoxon signed-rank test; r value, effect size.

between pre- and post-JHQGG ingestion (Table I). Notably, JHQGG intake induced a rapid decrease in the proportion of neutrophils (Z=2.77, P=0.00561, r=0.653) and a concomitant increase in the proportion of lymphocytes (Z=2.82, P=0.00485, r=0.664), resulting in a significant decrease in the neutrophil/lymphocyte ratio (NLR; Z=2.72, P=0.00649, r=0.642 (Table I). A decreased NLR was observed in 13 (72.2%) out of the 18 subjects (Fig. 2).

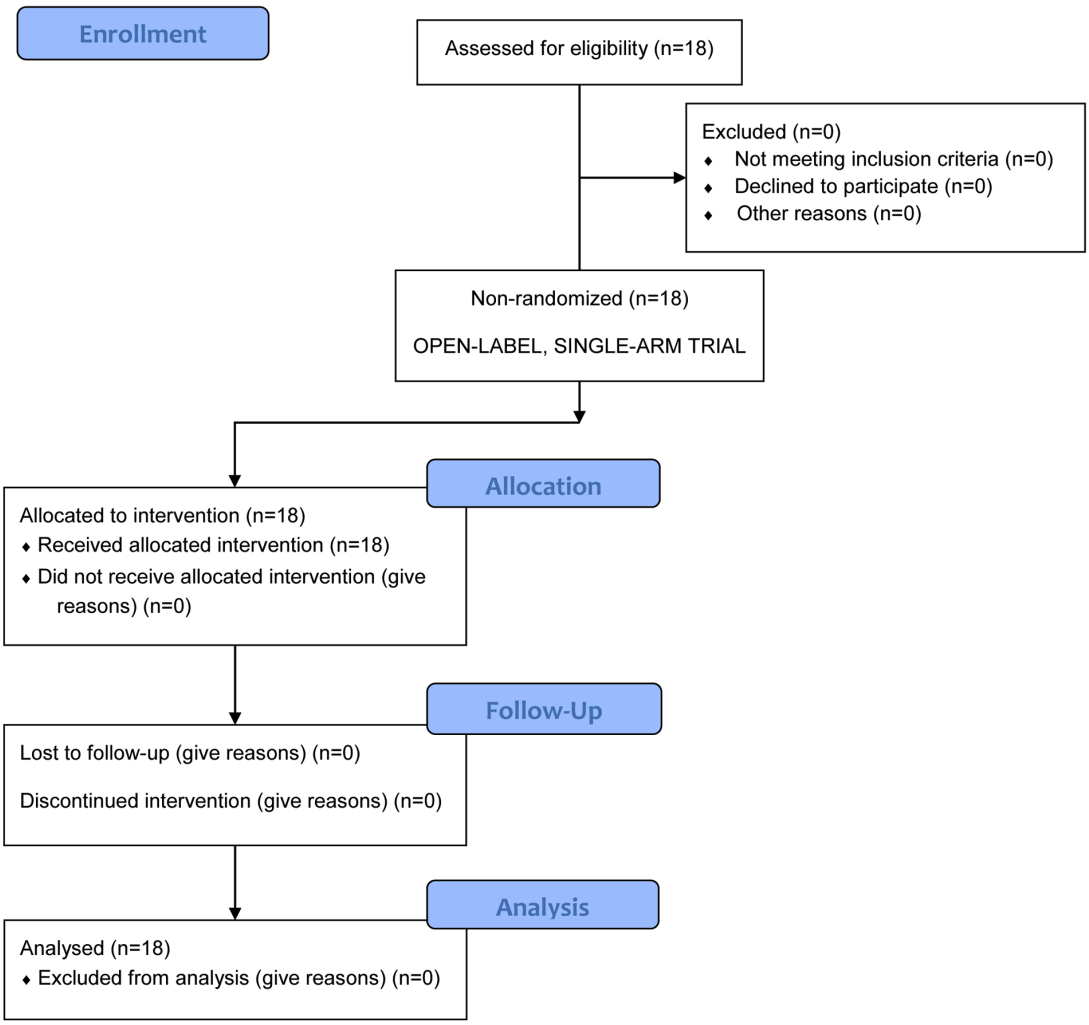

Figure 1. CONSORT flow diagram of the study trial.

In the blood cytokine analysis, the plasma levels of IL-6 and IFN-γ were significantly decreased and increased, respectively, compared with those in the pre-JHQGG ingestion (IL-6: Z=2.96, P=0.00309, r=0.697; IFN-γ: Z=2.20, P=0.0268, r=0.518) (Table I). The plasma IL-6 levels were decreased in 14 (77.8%) subjects, and the plasma IFN-γ levels were increased in 13 (72.2%) subjects (Fig. 2). A marginally significant decrease was also found in the plasma IL-18 levels (Z=1.98, P=0.0483, r=0.467) (Table I).

Post hoc two-tailed power analysis was performed (significance level, α=0.05; sample size, n=18) and statistical powers were obtained (1 - β) of 0.720 (neutrophils), 0.735 (lymphocytes), 0.706 (NLR), 0.775 (IL-6), 0.524 (IFN-γ) and 0.445 (IL-18) following the completion of the trial.

Discussion

Patients with severe COVID-19 infection present with neutrophilia, lymphocytopenia and a resulting elevated NLR, which are closely associated with poor clinical outcomes (17,18). Extensive neutrophil infiltration into pulmonary capillaries and the alveolar space has been observed in autopsy specimens from patients with COVID-19 (19). Neutrophils stimulate alveolar macrophages to release pro-inflammatory cytokines, such as IL-1β during respiratory viral infection (20). Neutrophilia can also cause the formation of excessive neutrophil extracellular traps (NETs), which induce deregulated cytokine release, respiratory failure, microthrombosis and oxidative stress-induced tissue damage (19,21,22). In the present study,

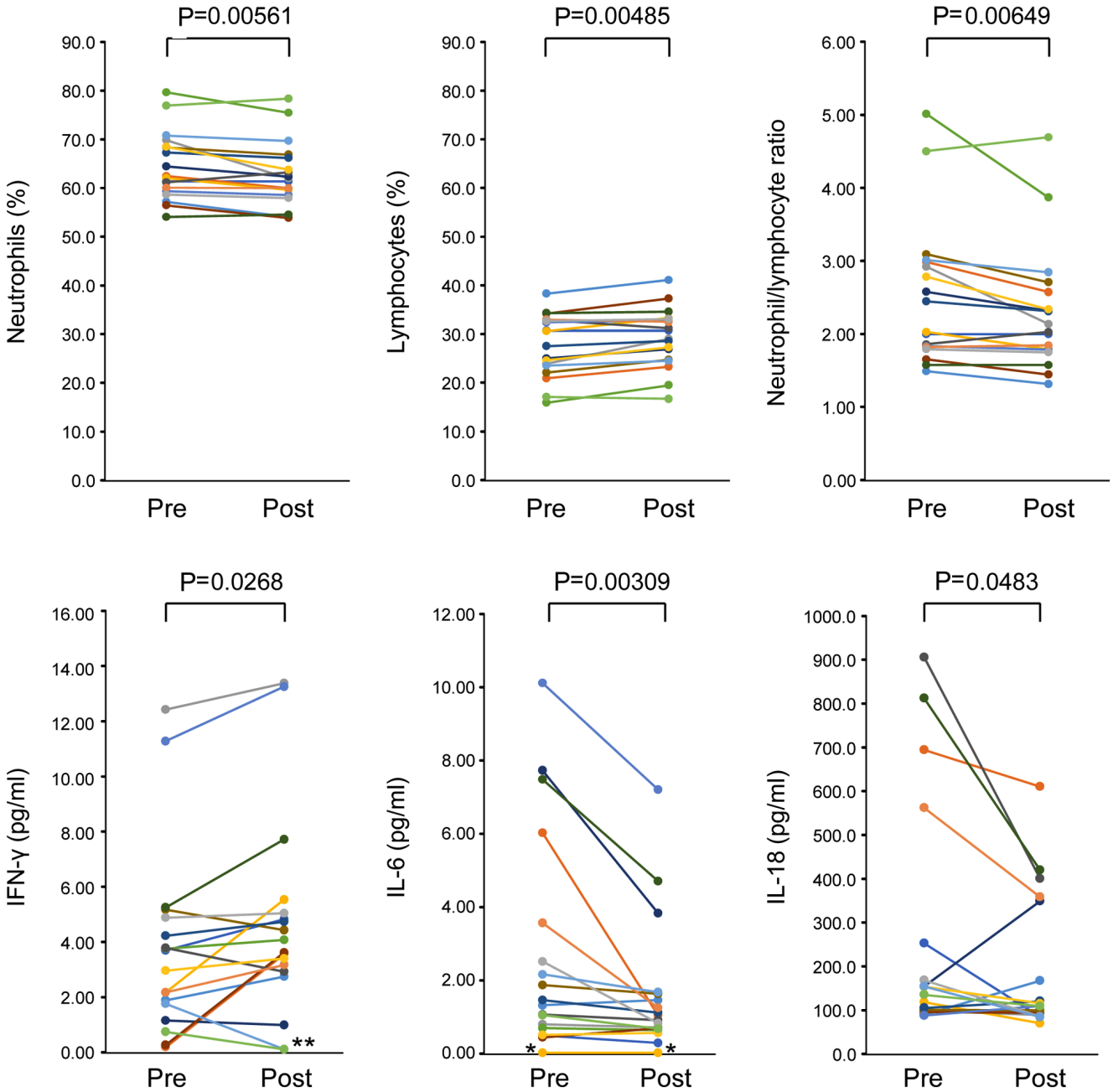

Figure 2. JHQGG modulates the proportions of neutrophils and lymphocytes, neutrophil-to-lymphocyte ratio, and the plasma levels of IL-6 and IFN-γ. Data (n=18 subjects) were analyzed using the two-tailed Wilcoxon signed-rank test at the significance level (α) of 0.05. The single asterisk (*) indicates one subject with undetectable IL-6 before and following oral JHQGG administration and double asterisks (**) indicate two subjects with undetectable IFN-γ following oral JHQGG administration. The values of 0.5 x lower limit of detection (IL-6, 0.03 pg/ml; IFN-γ, 0.185 pg/ml) were used to correct the undetectable IL-6 and IFN-γ data. Pre, pre-ingestion (baseline); Post, post-ingestion; JHQGG, Jinhua Qinggan granule.

JHQGG decreased the proportion of neutrophils and increased that of lymphocytes within 1 h following ingestion, yielding a significantly lower NLR. The rapid effects on the proportions of neutrophils and lymphocytes may be suitable for rebalancing immune cell dysregulation in patients with COVID-19.

JHQGG contains various active pharmaceutical ingredients, mainly rutin, luteolin, wogonin, myricetin, quercetin, ursolic acid, chrysoeriol and glabridin (12,13). There are only a limited number of studies available assessing the pharmacokinetic properties of these representative active ingredients in humans. The oral bioavailability values of the active compounds are known to range from 3.20-46.43% (rutin, 3.20%; myricetin, 13.75%; ursolic acid, 16.77%; chrysoeriol, 28.41%; wogonin, 30.68%; luteolin, 36.16%; glabridin, 46.42%; and quercetin, 46.43%) (12,13). By contrast, the time to peak blood concentration (Tmax) of each active ingredient remains poorly understood, apart from quercetin. As previously discussed (23), Erlund *et al* (24) demonstrated that the Tmax of oral quercetin aglycone in healthy humans ranged from 1.9±1.2 to 4.9±2.1 h in a dose-dependent manner, and Graefe *et al* (25) demonstrated that the Tmax of oral quercetin glycosides in humans was 0.7±0.2 to 0.3 h. Lee and Mitchell (26) reported that the Tmax of dietary quercetin from onion powder and apple peel powder was 2.0±1.7 and 2.9±2.0 h

in humans, respectively. To the best of our knowledge, there are no studies available to date on the pharmacokinetics of luteolin in humans; however, oral luteolin in rats is known to be absorbed efficiently with a Tmax of 1.1 h (27,28). Yasuda *et al* (29) also demonstrated that blood luteolin levels rapidly increased at 0.5 h following the oral ingestion with a Tmax of 1.0 h, as also previously discussed (27). As regards these data on oral bioavailability and Tmax, the current setting of blood sampling (1 h following JHQGG ingestion) was considered to be appropriate for the pilot study of the acute immunological effects of JHQGG.

There is a large body of evidence to indicate that patients with COVID-19 have aberrantly increased blood levels of pro-inflammatory cytokines and chemokines (30). In particular, IL-6 is known to be a critical driver of complex immune dysregulation that causes the excessive production of pro-inflammatory cytokines termed as the 'cytokine storm' and systemic hyperinflammation (31). The blood IL-6 level is positively associated with the severity and mortality of patients with COVID-19 (32). IL-6 receptor blockade using tocilizumab has been shown to reduce mortality and the risk of mechanical ventilation in patients with severe COVID-19 infection (33). The network pharmacological study by Niu *et al* predicted that several natural compounds such as rutin, luteolin, quercetin and ursolic acid, all of which are active ingredients of JHQGG, potentially interact with IL-6 directly to downregulate its pro-inflammation function (12). Considering the critical role of IL-6 as an exacerbating factor for COVID-19, the immunological activity of JHQGG to rapidly downregulate the blood level of IL-6 indicates that it may be suitable for preventing severe cases of infection and/or improving the severity of COVID-19 infection.

INF-γ is produced predominantly by type 1 helper T (Th1) and natural killer (NK) cells and stimulates innate immunity and inflammation, particularly through the activation of macrophages and dendritic cells. In the present study, JHQGG ingestion induced a slight, yet significant increase in the blood level of INF-γ. This pro-inflammatory activity appears to be contradictory to its clinical benefits against COVID-19. Notably, IFN-γ is known to serve as a central mediator of a broad spectrum of antiviral immunity by interfering with viral replication directly, potentiating the effects of IFN-α/β, activating Th1-dependent immune responses, and promoting the activation of the MHC class I pathway (34,35). However, compared with type I IFNs, the roles of IFN-γ in COVID-19 pathogenesis are more complex and have been less clearly defined. Notably, several studies have reported the impaired IFN-γ responses in patients with severe COVID-19 infection (36-38). Kim *et al* (36) reported that the plasma levels of IFN-γ were significantly decreased in patients in the intensive care unit (ICU), compared with outpatients and patients with mild disease. In line with the decrease in the levels of IFN-γ, the levels of IFN-γ-stimulated genes, including *HLA-A*, *HLA-B*, *HLA-DPA1*, *HLA-DRA*, *β2M* and *CIITA*, were downregulated, particularly in patients in the ICU, as compared with outpatients and convalescent patients (36). Ruetsch *et al* (37) demonstrated that the reduced production levels of IFN-γ by *in vitro*-stimulated immune cells from patients with COVID-19 were significantly associated with the increased disease severity and the stimulated IFN-γ levels <15 IU/ml upon hospital admission were significantly associated with a greater number of complications during hospitalization. The multivariate-adjusted logistic regression analysis by Hu *et al* (38) revealed that the circulating IFN-γ levels were inversely associated with the risk of developing lung fibrosis, inflammation-induced lung injury, in hospitalized patients. Thus, the functional exhaustion of IFN-γ production and the impairment of the downstream inflammatory responses are pathologically associated with a severe disease course of COVID-19. It was thus hypothesized that physiological, well-controlled, mild pro-inflammatory conditions, such as the JHQGG-induced minimal increase in INF-γ levels, may play protective roles against viral infection and replication, although pathological, dysregulated hyperinflammation leads to severe symptoms, such as the cytokine storm and acute respiratory distress syndrome.

Patients with severe COVID-19 infection have significantly lower numbers of $CD4^+$ T-, $CD8^+$ T- and NK cells, with a decreased capacity to produce IFN-γ, which results in a decrease in IFN-γ production by $CD4^+$ T-cells (39-41). Similarly, in a previous study using IFN-γ-deficient mice, Kyuwa and Sugiura (42) demonstrated that IFN-γ and $CD8^+$ T-cells were essential for the clearance of murine coronavirus, a virus within the same genus (betacoronavirus) as SARS-CoV-2. Previous studies have reported the decreased plasma IFN-γ levels in patients with severe COVID-19 infection (36-38). Furthermore, the reduced cytotoxic potential of $CD4^+$ T-, $CD8^+$ T- and NK cells is IL-6-dependent, and cases with severe COVID-19 infection have a higher IL-6/IFN-γ ratio than cases with moderate infection (40,43). The transfusion of COVID-19 convalescent plasma to patients with severe infection has been shown to significantly decrease the IL-6/IFN-γ ratio (44). Thus, the ability of JHQGG to decrease IL-6 and increase IFN-γ levels may be a suitable property for correcting the aberrantly elevated IL-6/IFN-γ ratio.

The main limitations of the present study are the small number of participants and the selection of uninfected individuals as the study subjects. Since JHQGG has been recommended for use in individuals clinically suspected of COVID-19 infection under medical observation or asymptomatic patients to prevent disease onset, uninfected individuals were employed as the study subjects. The present study demonstrated that JHQGG significantly up- and downregulated the plasma levels of IFN-γ and IL-6, respectively. Further studies with larger cohorts of patients with moderate to severe infection are thus essential to confirm the conclusion in patients and determine generalizability. Randomized controlled trials in patients with moderate to severe infection are also essential to confirm whether cytokine responses to JHQGG are sustained and whether they are associated with significant clinical improvement. Further validation studies are also required to determine whether the blood levels of JHQGG-derived compounds increase at 1 h following oral administration. The present study tried to measure the blood levels of representative active ingredients of JHQGG before and after oral administration; however, since some difficulties were encountered in finding optimal Liquid chromatography-mass spectrometry conditions, such data could not be obtained. In addition, since JHQGG contains 12 herbal components, the quality control or batch-to-batch quality consistency poses a great challenge in clinical practice.

In conclusion, the findings of the present study suggest that the clinical benefits of JHQGG against COVID-19 are, at least in part, associated with its rapid immunomodulatory effects; JHQGG can rapidly decrease the blood levels of neutrophils and IL-6, two critical exacerbating factors of COVID-19, and increase the blood levels of lymphocytes and IFN-γ that are essential for coordinated antiviral immune responses. JHQGG has been recommended for use in suspected and asymptomatic cases of COVID-19 to suppress disease onset, as per the Chinese official clinical guideline (4). Considering the rapid immunomodulatory effects on neutrophils, lymphocytes, IFN-γ and IL-6, JHQGG may also be effective for preventing disease progression in patients with moderate to severe infection as an option of adjunctive pharmacotherapy against COVID-19.

Acknowledgements

The authors would like to thank Dr Hugh Wang [Juxiechang (Beijing) Pharmaceutical Co., Ltd.] for generously providing Jinhua Qinggan granule and for his helpful advice for clinical trial planning.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YK and TE were involved in the conceptualization and methodology of the study, as well as in performing the experiments and data collection, obtaining resources, data curation, reviewing and editing of the manuscript, and project administration. KA, KK and MM were involved in performing the experiments and data collection, obtaining resources, and in the reviewing and editing of the manuscript. TA was involved in the conceptualization of the study, and in the reviewing and editing of the manuscript. TN was involved in the conceptualization and methodology of the study, as well as in formal analysis, and in the writing of the original draft, as well as in the preparation and creation of the published figures and tables. YK, TA and TN were also involved in study supervision. YK and TN confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). All procedures were reviewed and approved by the Ethics Committees of Takanawa Clinic (approval no. 2020-2). A signed informed consent form was obtained from each participant prior to inclusion in this study.

Patient consent for publication

Not applicable.

Competing interests

YK, KA, KK, MM and TE are employees of Takanawa Clinic. TA and TN serve as research advisers to Takanawa Clinic and receive advisory fees. The authors declare that there is no conflict of interest between the study group and Juxiechang (Beijing) Pharmaceutical Co., Ltd., the Chinese pharmaceutical company that provided Jinhua Qinggan granule for the study.

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