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Expression of PDGFR- α in Patients with Valvular Atrial Fibrillation

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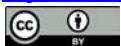
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Abstract

Objective: To investigate the expression of Platelet-derived growth factor receptor alpha (PDGFR- α) in patients who have valvular atrial fibrillation.

Methods: In this research, eighty-four patients with rheumatic heart disease who were going to undertake cardiac surgery were included. The subjects were divided into two groups: the AF group and the sinus rhythm group, the quantities are 39 and 45 respectively. Before the surgery, baseline demographics, physical examination, routine laboratory testing, echocardiography, echocardiographic data and additional clinical data were available for all patients. The right atrial tissue of the subjects was separated during surgery, with an area of approximately 0.3 - 0.5 mm³. Immunofluorescence staining was used to analyze the distribution of PDGFR- α of atrial tissue. mRNA of PDGFR- α in atrial tissue were determined by real-time quantitative PCR (Polymerase Chain Reaction); Western-Blot technique was used to measure the protein of PDGFR- α in atrial tissue. **Results:** There were no significant differences ($P > 0.05$) in sex ratio, age, blood pressure, blood biochemistry, and other aspects of medical history between the two groups. However, the right and left atrium diameters in the AF group were markedly larger than those in the SR group ($P < 0.05$). Moreover, the mRNA and protein of PDGFR- α from right atrial tissue were obviously higher in the AF group than that in the SR group ($P < 0.05$). **Conclusion:** The expression of PDGFR- α in the right atrial tissue of patients with atrial fibrillation was found to be significantly higher.

Keywords

Rheumatic Heart Disease, Atrial Fibrillation, Atrial Remodeling, Platelet-Derived Growth Factor Receptor- α , Cardiology

1. Introduction

Nowadays, in clinic, AF (atrial fibrillation) is the most common supraventricular

arrhythmia, and also one of the key factors of life-threatening diseases such as stroke and congestive heart failure [1] [2]. PDGFR- α is a member of Platelet-derived growth factors (PDGFs), PDGFR- α is mainly distributed in cells of mesenchymal or glial origin: fibroblasts, chondrocytes, osteoblasts, glial cells and smooth muscle cells. PDGFR- α It plays an important role in the development of myocardial fibrosis by binding to its specific ligand PDGF-A. This study collected the right atrial appendage tissue, blood and basic clinical data of patients with clinical valvular atrial fibrillation, and observed PDGFR- α distribution and expression of PDGFR- α in the atrium of patients with valvular atrial fibrillation. It lays a theoretical foundation for the relationship between atrial fibrosis and atrial fibrillation.

2. Materials and Methods

2.1. Patient Enrollment and Data Collecting

Among 84 patients admitted to the hospital for cardiac valve disease and underwent cardiac valve replacement in 2012, 39 were AF patients and 45 were SR patients. Inclusion criteria: patients with rheumatic valvular disease, senile degenerative valvular disease and valvular malformation who underwent cardiac valve replacement with or without AF. Exclusion criteria: people over 65 years old, liver and kidney failure, hyperthyroidism, cardiomyopathy, chronic pulmonary heart disease, left ventricular EF value less than 40%, accompanied by obvious acute infection, tumor and other diseases. The selected patients were divided into the sinus rhythm group and the AF group. All selected subjects signed the informed consent form and passed the review by the ethics committee.

2.2. Treatment of Clinical Data and Specimens

Collect and sort out the clinical baseline data, biochemical examination, ECG and imaging examination data of patients. All the selected patients who are on an empty stomach collected 5 mL of elbow vein blood in the morning and put it in a refrigerator at four degrees Celsius for three hours. The serum was separated by a centrifuge (room temperature, twenty minutes, 1000 r/min), and stored at -80°C . During cardiac surgery, the right atrial tissue (0.3 - 0.5 cm^3) of selected patients was taken, and after removing blood, adipose tissue and other impurities, each atrial tissue was cut into three pieces for storage.

2.3. Analysis of PDGFR- α in Atrial Tissue of Patients in AF Group and SR Group by Immunofluorescence Technique Protein Expression

The fresh right atrium tissue obtained from surgery was frozen and then embedded with OTC embedding gel. The frozen section machine 7 μm thick slices, attached to the slide, placed at room temperature for 30 minutes, fixed in four degrees Celsius acetone for 10 min, dried at room temperature and stored in the refrigerator for standby. Slices were rinsed with 0.01 mol/L PBS for 10 min \times 3

times; Normal bovine serum was blocked for 30 min; 0.01 mol/L PBS rinsing for 10 min \times 1 time; PDGFR- α The first antibody was diluted at 1:150, incubated at 37°C for 1 hour, and stayed at 4°C overnight; 0.01 mol/L PBS rinsing for 10 min \times 3 times; Add fluorescent secondary antibody working solution PDGFR- α , Sheep anti-mouse/FITC, diluted 1:100, incubated at 37°C for 1 hour; 0.01 mol/L PBS rinsing for 10 min \times 3 times; Add DAPI dye working solution (1:100 dilution) and incubate at room temperature for 15 minutes; 0.01 mol/L PBS rinsing for 10 min \times 3 times; Buffer glycerin seal; Observe and take photos under laser confocal microscope.

2.4. Determination of PDGFR- α in Atrial Tissue by Real-Time Fluorescent Quantitative PCR mRNA Expression of

To detect the relative amount of mRNA expression, extract the total RNA from the atrial tissue of selected patients, and then reverse transcribe it into cDNA using real-time fluorescence quantitative PCR. The target primer sequence is upstream primer 5'-GCTACATCATTCCTGCC-3'; Downstream primer 5'-AGGTGGAAGCTGCTGGAACC-3'. Real-time fluorescence quantitative PCR program: 95°C for 3 min for 40 cycles, 95°C for 10 seconds, 57.3°C for 10 s and 72°C for 30 s. The experiment was repeated three times. At the end of the reaction, read the CT values and dissolution and amplification curves of each reaction well for data analysis.

2.5. Determination of PDGFR- α in Atrial Tissue by Western Blot Protein Expression

Extract total protein from atrial tissue, use BAC kit to measure the total protein concentration, draw a standard curve, and calculate the actual mass concentration of the sample ($\mu\text{g}/\mu\text{L}$). Electrophoresis on 100 g/LSDS-PAGE gel for 2 h at 80 mV. PVDF film rotates for 70 min at 250 mA. Seal at room temperature for 2 hours in the skimmed milk powder sealing solution, and wash the film with TBST three times. Join PDGFR- α (1:500 dilution) β -Actin (1:500 dilution) first antibody, incubated at 4°C for a night; After washing the film three times, add the second antibody (1:500 dilution) and incubate it at room temperature for two hours, TBST wash the film three times for 10 min, and put it in the strong chemiluminescence reagent ECL substrate for the chemical reaction for 5 min, and the darkroom exposure shows the specific protein signal. Use the GelDoc100 imaging system for image processing and record the results. Use the target strip and internal reference (The expression of target protein- β was semi-quantitatively analyzed by the ratio of actin) band absorbance area integral.

2.6. Statistical Analysis

In the experiment, all data in the experiment were analyzed using the SPSS19.0 statistical software package. The mean \pm standard deviation is used to represent

the measured data, and it is proved that the data conforms to the normal distribution through the normality test. In the two sets of data in the experiment, the mean values were compared using a t-test, and the ratios were compared using the χ^2 test. $P < 0.05$ has statistical significance.

3. Results

3.1. Analysis of Basic Clinical Data of SR Group and AF Group

Patients participating in the experiment were divided into SR (including 19 males and 26 females) group and AF group, in AF group, 39 cases (male/female, 24/15). The characteristics of baseline data of patients are shown as follows. From the analysis of the results in the table, between the two groups there is no marked difference in the statistical treatment of various medical history data such as sex constituent ratio, age, blood pressure, blood routine, biochemical indicators, etc. ($P > 0.05$), which is comparable, see **Table 1**.

Table 1. Clinical characteristics.

	AF (n = 39)	SR (n = 45)	t/χ^2 values	<i>P</i>
Basic data				
Age (years)	51.1 ± 9.0	48.1 ± 10.9	-1.405	0.164
Sex	24/15	19/26	3.083	0.086
Male/Female (n)				
SBP* (mmHg)	112.4 ± 10.4	115.8 ± 14.3	1.242	0.218
DBP* (mmHg)	71.7 ± 7.4	70.5 ± 11.5	-0.567	0.572
Laboratory				
Examinations				
WBC* ($10^9/l$)	6.29 ± 1.55	6.0 ± 1.91	-0.763	0.448
RBC* ($10^{12}/l$)	4.55 ± 0.67	4.44 ± 0.51	-0.811	0.420
HB* (g/l)	137.1 ± 16.6	135.3 ± 16.8	-0.482	0.631
CR* ($\mu\text{mol}/l$)	92.47 ± 12.46	91.58 ± 12.43	-0.225	0.823
Echocardiogram				
Hic Parameters				
LVEF* (%)	53.10 ± 5.90	54.47 ± 5.90	1.041	0.301
LAD* (mm)	58.03 ± 13.93*	49.55 ± 17.17*	-2.420	0.018
RAD* (mm)	26.88 ± 4.81*	24.32 ± 4.26*	1.721	0.038

SBP, systolic blood pressure; DBP, diastolic blood pressure; WBC, white blood cell; RBC, red blood cell; HB, hemoglobin; CR, serum creatinine; LVEF, left ventricular ejection fraction; LAD, left atrium diameter; RAD, right atrium diameter.

3.2. Detection of PDGFR- α in Atrial Tissue of Patients in SR Group and AF Group by Laser Confocal Imaging after Double Labeling of Blue-Green Fluorescent Protein Immunofluorescence Staining Results

Using immunofluorescence technology to detect the protein expression of PDGFR- α . Laser confocal microscopy showed: PDGFR- α is expressed in green fluorescence, and the nucleus is blue fluorescence. PDGFR- α in the SR group and AF group. The protein was positive and localized on the cell membrane near the nucleus of the myocardium or fibroblasts. As shown in **Figure 1**. PDGF-A protein expression was weak in the SR group, and for PDGFR- α in the AF group the fluorescence intensity and density of protein expression were apparently greater than those of the SR group.

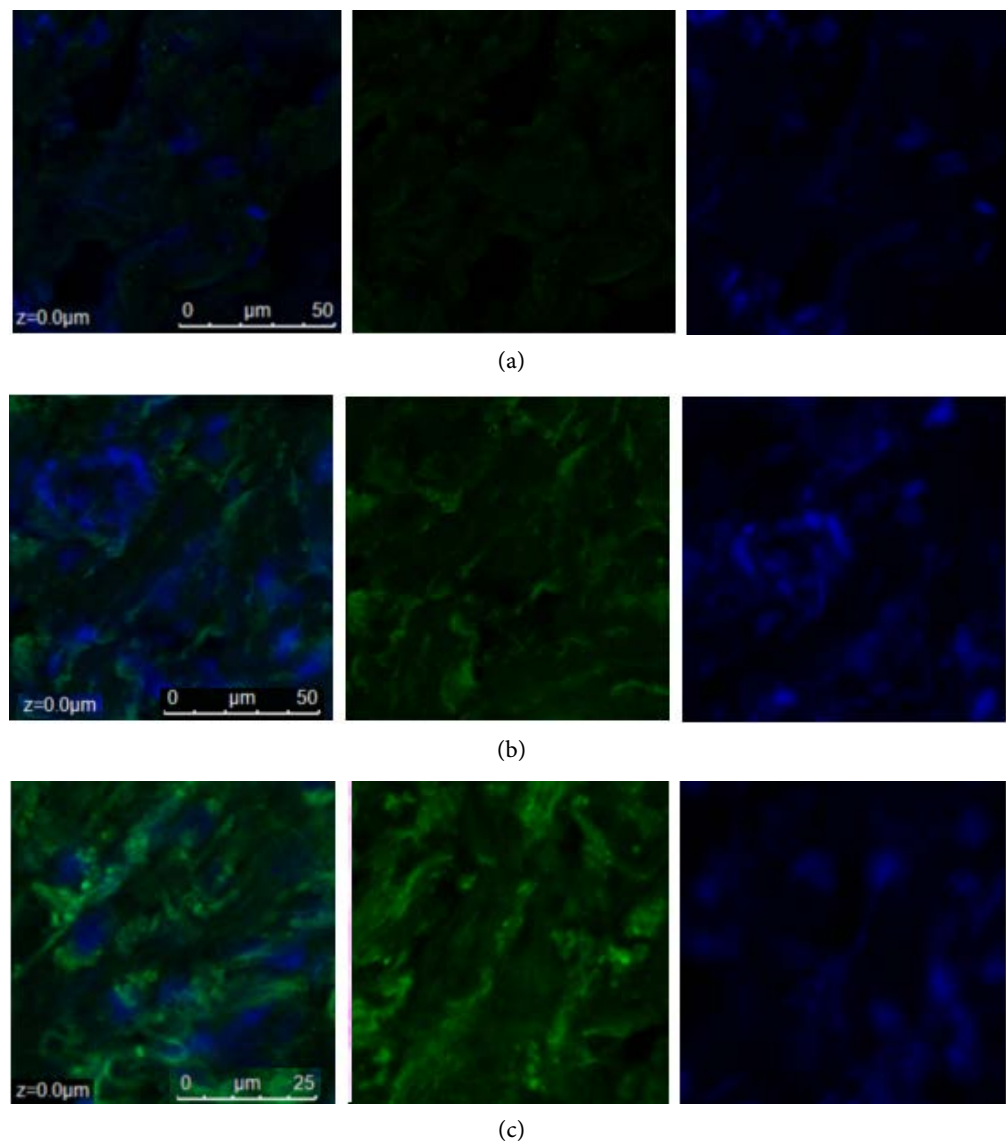


Figure 1. PDGFR- α of atrial tissue in SR group and AF group Confocal immunofluorescence laser \times 500 times ((a) negative control; (b) SR group; (c) AF group).

3.3. Amplification Curve and Dissolution Curve

The dissolution curves of real-time fluorescence quantitative PCR were all single peaks, indicating that the amplification reaction was specific. As shown in **Figure 2**, PDGFR- α , the inflection point of the mRNA amplification curve is clear, the exponential phase and the platform phase are obvious, the slope of the exponential phase is large, and the curve parallelism is good, indicating that the amplification efficiency of each reaction is similar.

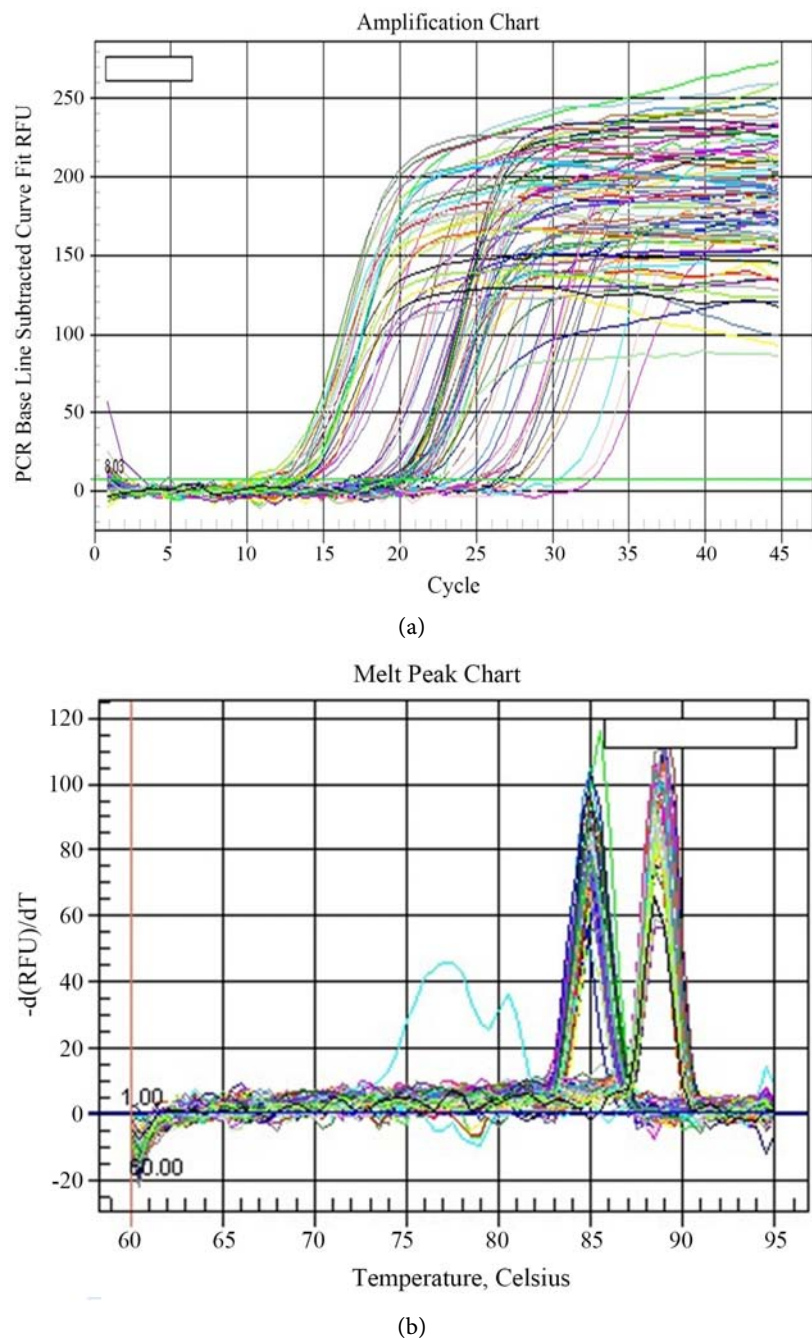


Figure 2. PDGFR- α mRNA amplification curve and fusion curve (a) amplification curve, (b) fusion curve.

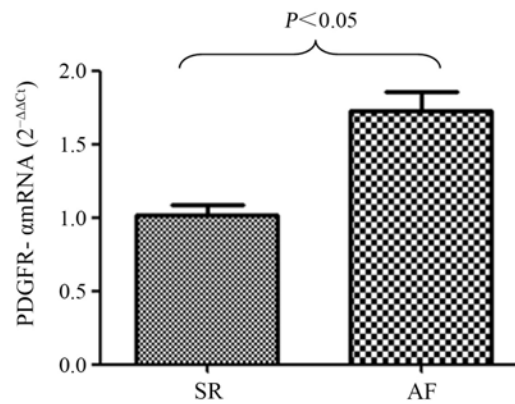


Figure 3. PDGFR- α of atrial tissue in the SR group and AF group. Comparison of mRNA expression.

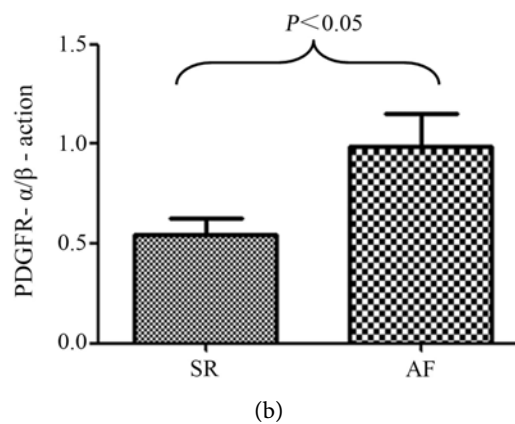
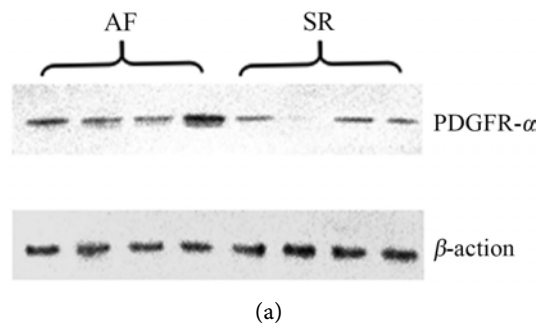


Figure 4. PDGFR- α of atrial tissue in the SR group and AF group. Comparison of protein expression.

3.4. Determination of PDGFR- α in Atrial Tissues of Patients in SR Group and AF Group by Real-Time Fluorescent Quantitative PCR mRNA Relative Expression of (2^{-ΔΔCt})

PDGFR- α in atrial tissue was detected in the relative mRNA expression (2^{-ΔΔCt}) in the AF group and SR group was 1.725 ± 0.135 and 1.017 ± 0.068, respectively. After statistical processing, the result shows PDGFR- α , the relative expression of mRNA (2^{-ΔΔCt}) in the AF group was also markedly greater than SR group, with remarkable statistics significance between groups ($P < 0.05$, $t = 3.922$), as shown in **Figure 3**.

3.5. PDGFR- α of Atrial Tissue between SR Group and AF Group Results of Protein Level Determination

Application of Western Blot Technique to measure PDGFR- α in atrial tissue of patients' relative expression of proteins. The relative expression of the protein was scanned and imaged with the GelDoc100 imaging system, and the results were recorded. Relative expression of PDGFR- α/β -Action) in the AF group and SR group were 0.989 ± 0.162 and 0.546 ± 0.08 , respectively. After statistical treatment, the results showed that the PDGFR- α of AF patients. Relative expression of PDGFR- α/β -Action) was also significantly higher than that of the SR group, with a significant statistical difference between groups ($P < 0.05$, $t = 2.482$), as shown in **Figure 4**.

4. Discussion

PDGFs are 24 ku cationic glycoproteins, mainly present in platelets α Granules and also existing in damaged endothelial cells, macrophages migrating under the endothelium, smooth muscle cells, fibroblasts, mesangial cells and other cells. These cells release a large amount of PDGFs in the form of autocrine and paracrine [3] [4] chain amplification reactions, and mediate the interaction between endothelium and stroma in various tissues [5] [6]. PDGFs can promote the mitosis and chemotaxis of fibroblasts and vascular smooth muscle cells, and also regulate the content of collagen, fibronectin, proteoglycan, hyaluronidase, and collagenase in the extracellular matrix, in connective tissue remodeling playing an important role. Due to its important role in tissue repair, fibrosis, tumor cell proliferation, and immune response, it has become a research hotspot for many scholars. PDGF-A is a member of the PDGF family. Its peptide chain consists of 196 and 211 amino acid residues. It is highly expressed in the heart, skeletal muscle and pancreas [7] [8] [9] and passes through the specific receptor PDGFR- α on the plasma membrane. It binds and crosses the plasma membrane to transmit information to the cell interior, and generates response through a series of actions. PDGFR- α is mainly distributed in cells of mesenchymal or glial origin: osteoblasts, chondrocytes, fibroblasts, glial cells and smooth muscle cells.

Studies confirm PDGF-A and its receptor PDGFR- α play an important role in the development of myocardial fibrosis. PDGFR- α was in acute rejection of heart transplantation. The expression of PDGF-A and PDGF cmRNAs was significantly up-regulated in chronic heart transplantation rejection [10]. Studies confirm PDGF-A and its receptor PDGFR- α play a significant role in the development of myocardial fibrosis. The expression of PDGFR- α was significantly up-regulated in acute cardiac allograft rejection, while the expression of PDGF-A and PDGF-cmRNAs was significantly up-regulated in chronic cardiac allograft rejection [10]. Relevant studies confirm that: in the process of myocardial fibrosis, PDGFR- α is more important than its ligand PDGF-A. Intravenous PDGFR- α Specific antibodies can alleviate atrial fibrosis [10] [11]. In previous studies, the characteristics of cardiac structural remodeling were compared between the AF group and SR group. The studies indicated that the atrial diameters in the atrial

fibrillation group were apparently greater than those in the SR group; Atrial tissue in the atrial fibrillation group has visible fibrosis changes; the relative expression of type I collagen mRNA ($2^{-\Delta\Delta Ct}$) in the atrial muscle samples from patients with atrial fibrillation was markedly higher than that in patients with sinus rhythm. It is suggested that the occurrence and development of atrial fibrosis in patients with rheumatic valvular disease and atrial fibrillation may be related to the up-regulation of the transcription level of type I collagen gene, the increase of synthesis and secretion of type I collagen, which leads to the abnormal accumulation of collagen in atrial tissue and the imbalance of I/III collagen deposition ratio, which provides a pathological matrix for the occurrence and maintenance of atrial fibrillation [12] [13] [14]. Whether PDGF/PDGFR signal is involved in the occurrence, development and maintenance of atrial fibrosis due to valvular atrial fibrillation by up-regulating the transcription level of type I collagen gene, it leads to the increase of fibroblast synthesis and secretion of type I collagen, and abnormal accumulation of collagen in atrial tissue remains to be further studied.

In this part, real-time fluorescence quantitative PCR was used to determine PDGFR- α in atrial muscle tissue samples of patients with sinus rhythm and atrial fibrillation mRNA relative expression. PDGFR- α was found in atrial muscle tissue samples of patients with AF, and the relative expression of mRNA in the AF group was markedly higher than that in the SR group ($P < 0.05$). Furthermore, the relative expression of PDGFR- α protein in atrial muscle tissue samples of patients with AF was also significantly greater than that in the SR group ($P < 0.05$), as determined by the Western Blot technique. This provides a theoretical basis for further studies on the relationship between the PDGF/PDGFR signaling pathway [15]-[20] and atrial fibrosis in patients with AF.

5. Funding Information

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Clinical Evaluation of the Safety and Efficacy of the Phytomedicine APIVIRINE Based on Aqueous Extracts of *Dichrostachys glomerata* (Forssk.). Chiov. (*D. cinerea*) in COVID-19 Patients without Signs of Severity

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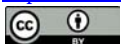
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Abstract

Background: Since the appearance of the COVID-19 pandemic, several drugs have also been proposed for the treatment of the COVID-19, but the therapeutic effectiveness of those drugs is not satisfactory. This situation has led to the search for therapeutic solutions based on recipes from traditional medicine. **Aims:** This study aimed to evaluate the clinical safety, efficacy and tolerability of the phytomedicine APIVIRINE in patients with non-severe COVID-19. **Methods:** Patients were included following defined criteria and followed on an outpatient basis until recovery in accordance with national guidelines for the management of single cases of COVID-19 in Burkina Faso. Vital signs, anthropometric parameters as well as electrocardiographic, hematological and biochemical examinations were measured on D4, D7, D14 and D21. Adverse events were recorded during maintenance. **Results:** The present study included 45 patients. The clinical signs present at inclusion were mostly cough (44.44%), asthenia (42.22%), headache (40%), and anosmia (35.55%). Dyspnoea and chest pain were less represented in 05 (11.11%) and 06 (13.33%) patients. Cough, dyspnoea, chest pain, fever, sore throat, headache, and nasal obstruction present at inclusion disappeared before Day 4 of treatment. Anosmia and asthenia disappeared before Day 7. At the inclusion visit (Day 1), CRP, WBC, and blood glucose were abnormal in 15

(33.33%), 13 (28.89%), and 11 (24.44%) patients respectively. In addition, 3 (6.66%) patients had elevated creatinine levels. Transaminases Alanine aminotransferase (ALAT) were elevated in 05 (11.11%) patients while Aspartate aminotransferase (ASAT) was elevated in 04 (8.89%) patients. After 4 days of treatment, the cure rate was 33.33% of patients and 48.89% after 7 days. The cumulative cure rate was 86.67% after 14 days of treatment. **Conclusion:** No serious side effects or allergic reactions were observed during treatment. No clinical complications were observed and all symptoms present resolved on the 7th day of treatment.

Keywords

APIVIRINE, COVID-19, Clinical Study, Safety

1. Introduction

The COVID-19 outbreak occurred in Wuhan, China at the end of 2019, and gradually spread worldwide. Sub-Saharan Africa, which had long been spared, declared its first case of COVID-19 on 27 February 2020 in Nigeria. The spread of the pandemic then rapidly spread to all African countries [1]. Burkina Faso reported its first case of COVID-19 on 9 March 2020. As of October 2022, a total of 676,609,955 cases had been reported worldwide, including 6,881,955 deaths [2]. Thus, COVID-19 is a public health emergency of international concern more than ever.

The SARS-Cov-2 infection causes a range of cases ranging from mild with mild clinical symptoms without pneumonia to severe and often fatal (respiratory distress, hypoxia ($SpO_2 \leq 93\%$), abnormal blood gases) [3] [4].

The availability of current vaccines is a severe problem for developing countries despite the COVAX initiative set up by the ACT Accelerator led by the Coalition for Epidemic Preparedness Innovation (CEPI), the GAVI Alliance (formerly the Global Alliance for Vaccines and Immunization) and the WHO. In addition, their populations are reluctant to accept these vaccines. Anti-malarial drugs (chloroquine, hydroxychloroquine), anti-inflammatory drugs (dexamethasone) and antiviral drugs (remdesivir, lopinavir, etc.) have also been proposed for the treatment of COVID-19, but their therapeutic effectiveness is not satisfactory. [5]. The populations of 93% of countries in the Western Pacific region compared to 80% to 90% in developing countries such as Burkina Faso use traditional medicine for their primary health needs [6] [7]. This situation has led to the search for therapeutic solutions based on recipes from traditional medicine to address the pandemic in Africa and around the world [8]. This is the case in countries such as Madagascar (COVID Organics), India (Ayurvedic Kadha) and China (Guduchi Ghan Vati), which have embarked on research into plant-based medicines to provide a solution for the treatment of patients suffering from COVID-19 [9]. Also, clinical trials have already been conducted

on herbal preparations such as Qingfei Paidu Decoction (QFPDD) and Jinhua Qinggan (JHQG) [10] [11] [12]. In the same vein, in Burkina Faso, a phytomedicine called APIVIRINE, which is made from aqueous extracts of *Dichrostachys glomerata* (Forssk.). Chiov. (*D. cinerea*) (Mimosaceae) has been proposed in a clinical evaluation of patients with COVID-19. This phytomedicine was already used as an antiretroviral against HIV/AIDS and as an antibiotic. The chemical compounds mainly present in *Dichrostachys glomerata* are gallic tannins and catechin tannins; three are abundant: Alkaloids, Flavonoids and Saponosides [13] [14] [15]. Among the phenolic compounds, molecules such as quercetin-3-O-*apiosyl*-3''O-gallate, myricetin-3-O-rhamnopyranoside, myricetin-3-O-glucopyranoside, quercetin-3-O-Lrhamnopyranosyl (1''-6'')-O-D-glucopyranoside, quercetin-3-O-rhamnopyranoside, quercetin-3-O-glucopyranoside, quercetin-3-O-galactopyranoside, apigenin, kaempferol, have been isolated from the aerial parts of the plant [13]. Molecules such as (-)-festidinol and (-)-epicatechin (from the flavan-3-ols) have been isolated from the extracts [16]. Studies have shown that molecules such as myricetin, kaempferol, quercetin, apigenin and epicatechin have antiviral activity on SARS-CoV-2. These molecules affect SARS-CoV-2 by inhibiting ACE2 receptors either by protease inhibition or by immunomodulation of inflammation by regulating the infection-induced cytokine storm [17] [18] [19] [20]. This study aimed to evaluate the clinical safety, efficacy and tolerability of the phytomedicine APIVIRINE in patients with non-severe COVID-19. Specifically, the aim was to determine the safety and tolerability of APIVIRINE in terms of adverse events during treatment; to determine the effect of APIVIRINE on the improvement of COVID-19 symptoms.

2. Patients and Methods

2.1. Study Population

The study was conducted in male and female patients 20 to 65 years old, who tested positive for SARS-CoV-2 by RT-PCR. All patients had no severe symptoms.

2.2. Inclusion and Exclusion Criteria

Only patients who met the following inclusion criteria were included in this study: 1) diagnosed positive for COVID-19 without signs of severity and by RT-PCR; 2) at least 20 years of age and no more than 65 years of age; 3) signed the informed consent; 4) willingness and ability to comply with the study protocol during the study period.

However, patients were excluded from the study if they: 1) have renal failure; 2) have heart failure; 3) have a QTc Interval > 500 ms; 4) be diabetic with complications; 5) are immunocompromised; 6) have chronic liver disease with transaminases greater than 5 times normal; 7) have known chronic diseases (cancers, tuberculosis...); 8) are a pregnant or breastfeeding woman; 9) participate in any other ongoing clinical study; 10) are of legal age and unable to

comply with the study protocol; 11) have other conditions that, in the opinion of the investigator, would jeopardise the safety or rights of the study participant or render the subject unable to comply with the protocol.

2.3. Study Design

A clinical evaluation of APIVIRINE was conducted in patients with COVID-19. Each patient was followed until the cure. Inclusion was from 30 October 2020 to 4 January 2021. The total duration of the study was 10 weeks.

Patients were recruited at COVID-19 screening sites in Ouagadougou. They were then followed up on an outpatient basis (home confinement), following the national guidelines for managing single cases of COVID-19 in Burkina Faso (Ministry of Health, 2020).

The National Influenza Reference Laboratory (LNR-G) of the “Institut de Recherche en Sciences de la Santé (IRSS)” carried out the diagnostic tests for COVID-19 by RT-PCR in Ouagadougou. Nasopharyngeal samples collected for RT-PCR were stored at 2°C - 8°C and sent to the National Influenza Reference Laboratory (LNR-G). According to the manufacturer’s instructions, the virus RNA was extracted from oropharyngeal and/or nasopharyngeal swabs using the QIAamp RNA Viral Kit (Qiagen, Heiden, Germany). Real-time RT-PCR was performed using primers and probes from the ThermoFisher TaqPath COVID-19 CE-IVD RT-PCR kit. The ORF1ab, N and S genes of the novel 2019 coronavirus (COVID-19) are selected as target regions for amplification.

The Applied Biosystems™ 7500 Fast Real-Time instrument was used for amplification. A specimen is positive if it shows an obvious amplification curve with a CT value, cycle threshold less than or equal to 37 ($CT \leq 37$) for the three ORF1ab, N, S genes or for two of the genes. The identification of each gene was made by the number of TC (cycle threshold).

2.4. Sample Size

The open-label, controlled clinical trial considers a cohort of patients who are administered APIVIRINE with a repeated dose during the time of management.

Patients will be treated for a fortnight, which is sufficient time to observe negatiation of SARS-CoV-2 viral carriage. A medical visit to assess possible late side effects will be carried out one week after the end of treatment.

Under the assumption of an approximation between the binomial distribution and the standard reduced normal distribution and the hypothesis that APIVIRINE treatment produces a cure rate of more than 80% after 14 days of treatment, the minimum sample size necessary to test this hypothesis was calculated to be 41 patients. Assuming a 10% non-respondent rate, a sample size of 45 patients was included in the study. To test this hypothesis the sample size is obtained by applying the Cohen following formula [21].

$$n = 2 \left(\frac{Z_{1-\alpha} - Z_{1-\beta}}{\phi(\pi) - \phi(\pi_0)} \right)^2$$

where

- ✓ $\alpha = 5\%$ the significance level;
- ✓ $Z_{1-\alpha/2}$ is the fractile of order $1 - \alpha/2$;
- ✓ $1 - \beta$ is the power to detect a difference between the healing proportions is set at 80%;
- ✓ $Z_{1-\beta}$ is the fractile of order $1 - \beta$ of the normal distribution;
- ✓ ϕ is the distribution function of a normal distribution;
- ✓ $\phi(\pi) - \phi(\pi_0)$ is the effect size considered, *i.e.* the difference between the proportion π_0 of cured patients treated according to the national protocol and the proportion π of cured patients treated with APIVIRINE after one week of treatment.

Considering an objective of detecting a mean effect according to Cohen's criterion, the minimum sample size determined using the values below is 41 patients. Considering a no response proportion of 10%, this gives a sample size of 45 patients to be included in the study.

2.5. Treatment and Follow-Up

The phytomedicine APIVIRINE 350 mg capsule was administered at home as follows: 3 capsules 4 times daily, *i.e.* 3 capsules every 6 hours for 14 days.

After inclusion, participants were followed for 21 days.

Vital signs such as temperature, pulse rate, blood pressure, respiratory rate, pulse oxygen saturation (POS) and heart rate were taken during the follow up at home. Anthropometric parameters such as weight and height were also measured.

The clinical doctors interviewed the participants' about their medical and surgical history, looking for potentially adverse comorbidities. Functional respiratory signs such as cough and dyspnoea were explored during the interview. Other symptoms of COVID-19 were also sought through a thorough physical examination of each patient.

The paraclinical examination included: electrocardiogram (ECG); computed tomography (CT); a biological assessment carried out from a blood sample taken on day 1 before starting the treatment: and including a haematological examination (blood count, ESR) and a biochemical assessment [transaminases (ALT, AST), creatinemia, glycaemia, C-Reactive Protein (CRP)]; a nasopharyngeal swab for RT-PCR of the coronavirus.

The follow-up visits V2 to V5 at D4, Day7 (D7), Day14 (D14) and D21 [22] [23]. At each visit at home, a complete interview, physical examinations were performed and adverse events were recorded.

2.6. Judgment Criteria

Primary endpoint: Systemic reactions

The rate of immediate reactions (IRs) and reactions within days of the first dose as well as any serious adverse events (SAEs) that occurred between enrol-

ment and discharge from the study. The relationship of the SAEs to the trial drug should be established by the investigator using the following definitions: unrelated, possible relationship, probable relationship, established relationship.

Secondary endpoints

The time to the fever clearance and clinical signs present at patient admission were assessed. The efficacy of APIVIRINE phytodrug on COVID-19 viral load were evaluated through the viral load clearance time based on the Cycle Thresholds (CTs) of RT-PCR. Moreover, the proportion of RT-PCR negative was calculated at D4, D7, D14, D21.

Ethical considerations

The protocol received a favorable opinion after examination by the “Comité d’Ethique pour la Recherche en Santé (CERS)” (Ethics Committee for Health Research) of the Burkina Faso Ministry of Health under Deliberation number N°2020-7-121 of 1 July 2020.

2.7. Statistical Analyses

Statistical processing was done on the 45 patients of the study, considering an intention-to-treat analysis. An exploratory analysis including completeness assessment, examination of the tails of the distribution of quantitative data, and identification and correction of erroneous data was done first. This was followed by an analysis of the sample descriptors to define the socio-demographic, clinical and biological profiles of patients at inclusion. Central tendency and dispersion statistics were calculated.

3. Results

3.1. Patients Characteristics

Sociodemographic characteristics

The present study included 45 patients from the 09 districts of Ouagadougou. The female sex was represented by 53.3% (24 patients). The median age was 31 years (27 years; 46 years) with extremes of 20 years and 64 years. The distribution of patients by sex and age is shown in **Table 1**.

Table 1. Distribution of patients by sex and age (n = 45).

Variables	n	Percentage
Sex		
Male	21	46.7
Female	24	53.3
Age (year)		
<30	16	35.6
[30 - 49]	23	51.1
[50 - 65]	06	13.3

Biological and CT profile of patients at inclusion

In this study, one (01) patient (2.22%) was a carrier of AS haemoglobinopathy, and the rest were AA homozygotes.

Chest CT was performed in all patients at inclusion. Abnormal ground glass images were found in 10 (22.22%) patients.

3.2. Evolution of Biological Parameters

Assessment of clinical tolerance

During this study, no serious adverse events were reported. However, one (01) case of non-persistent diarrhoea was recorded in one (01) patient during treatment which stopped after taking a loperamide tablet.

The course of symptoms during treatment is recorded in **Table 2**. Analysis of this table shows the existence of respiratory distress, nausea, pruritus, rash, constipation and dizziness in 01 (2.22%), 02 (4.44%), 02 (4.44%), 01 (2.22), 01 (2.22%) and 02 (4.44%) patients respectively at inclusion. Only nausea persisted in 01 patient [01 (2.22%)] until D7 before disappearing.

Electrocardiograms were performed in all patients at baseline and at follow-up visits (data not shown). No heart rhythm abnormalities were observed in all patients during the entire treatment period.

Three (6.66%) patients in the study missed doses of the drug according to the treatment schedule, including one dose in 01 patient (2.22%) and three doses in 02 patients (4.44%). Forgetfulness was the only reason cited for this non-compliance. Thus, the taste of the drug was not cited as a reason for non-adherence. The acceptability of the smell and the number of drugs taken per dose were not assessed during the treatment. Nevertheless, some patients reported that the regimen was restrictive due to the high number of doses per day.

Table 2. Evolution of symptoms throughout the treatment.

Adverse effects	Day1 = Inclusion (n = 45) n (%)	Day4 (n = 45) n (%)	Day7 (n = 30) n (%)	Day14 (n = 20) n (%)	Day21 (n = 3) n (%)
Paresthesia	0 (0)	1 (2.22)	0 (0)	0 (0)	0 (0)
Respiratory distress	1 (2.22)	0 (0)	0 (0)	0 (0)	0 (0)
Dyspnea	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Nausea	2 (4.44)	2 (4.44)	1 (3.33)	0 (0)	0 (0)
Diarrhea	0 (0)	0 (0)	1 (3.33)	0 (0)	0 (0)
Vomiting	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Abdominal pain	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pruritus	2 (4.44)	0 (0)	0 (0)	0 (0)	0 (0)
Skin rash	1 (2.22)	1 (2.22)	0 (0)	0 (0)	0 (0)
Bone pain	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Limb inflammations (oedemas)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Other symptoms	- Constipation 01 (2.22); - Dizziness 02 (4.44)	Anosmia/agueusia 01 (2.22);	Nothing	Nothing	Nothing

3.3. Evolution of Biological Parameters

Biological data of the patients were systematically collected during the different medical visits, including the inclusion visit (**Table 3**).

Table 3. Evolution of biological data during medical visits.

Biological analyses	Day1 = Inclusion (n = 45) n (%)	Day7 (n = 26) n (%)	Day14 (n = 16) n (%)	Day21 (n = 1) n (%)
High sensitivity C-reactive protein (mg/L)				
<6	27 (60.00)	19 (73.08)	15 (93.75)	1 (100.00)
≤6	18 (40.00)	7 (26.92)	1 (6.25)	
Erythrocyte sedimentation rate (mm/h)				
≤10	12 (26.67)	12 (46.15)	13 (81.25)	1 (100.00)
>10	33 (73.33)	14 (53.85)	3 (18.75)	
White blood cells (/mm³)				
<4000	3 (6.67)			
[4000 - 10,000]	32 (71.11)	22 (84.62)	16 (100.00)	1 (100.00)
>10,000	10 (22.22)	4 (15.38)		
Neutrophil count/(mm³)				
<1500	7 (15.55)	3 (11.54)	3 (18.75)	
[1500 - 7500]	35 (77.78)	22 (84.61)	13 (81.25)	1 (100.00)
>7500	3 (6.67)	1 (3.85)		
Lymphocyte count/mm³)				
<1000				
[1000 - 4000]	31 (68.89)	17 (65.38)	11 (68.75)	1 (100.00)
>4000	14 (31.11)	9 (34.62)	5 (31.25)	
Blood glucose level (mmol/L)				
<4.1	7 (15.56)			
[4.1 - 6.1]	26 (57.78)			
>6.1	12 (26.67)			
Hemoglobin level (g/dL)				
<13	30 (66.67)	16 (61.54)	10 (62.50)	1 (100.00)
[13 - 17]	15 (33.33)	10 (38.46)	6 (37.50)	
>17				
Platelets (/mm³)				
<100,000	1 (2.22)			
[100,000 - 300,000]	37 (82.22)	15 (57.69)	8 (50.00)	1 (100.00)
>300,000	7 (15.56)	11 (42.31)	8 (50.00)	

Continued

Alanine aminotransferase (U/L)				
Males				
≤40	19 (90.48)	11 (91.67)	8 (100.00)	
>40	2 (9.52)	1 (8.33)		
Females				
≤31	22 (91.67)	13 (92.86)	7 (87.50)	1 (100.00)
>31	2 (8.33)	1 (7.14)	1 (12.50)	
Aspartate aminotransferase (U/L)				
Males				
≤38	20 (95.24)	12 (100.00)	8 (100.00)	
>38	1 (4.76)			
Females				
≤31	21 (87.50)	12 (85.71)	7 (87.50)	1 (100.00)
>31	3 (12.50)	2 (14.29)	1 (12.50)	
Creatinemia (μmol/l)				
Males				
<61.8				
[61.8; 123.7]	18 (85.71)	12 (100.00)	8 (100.00)	---
>123.7	3 (14.29)			
Females				
<53	1 (4.17)			
[53; 97.2]	21 (87.50)	12 (85.71)	8 (100.00)	1 (100.00)
>97.2	2 (8.33)	2 (14.29)		

The results showed that at the inclusion visit (D1), CRP, White Blood Cell (WBC) and blood glucose were abnormal in 15 (33.33%), 13 (28.89%) and 11 (24.44%) patients respectively. In addition, three (03) patients had elevated creatinine levels. Transaminases levels in patients were variable. Alanine aminotransferase (ALAT) were elevated in 05 (11.11%) patients, while Aspartate aminotransferase (ASAT) was elevated in 04 (8.89%) patients. At the end of the treatment (D21), all patients normalized these parameters.

RT-PCR was performed in all patients at the second visit (D4) and at the third (D7), fourth (D14) and fifth (D21) scheduled visits when the previous visit result was still positive.

After 4 days of treatment, the cure rate was 33.33% (15/45) of patients and 48.89% (22/45) after 7 days. The cumulative cure rate was 86.67% after 14 days of treatment. However, the cumulative cure rate was 93.33% on day 15 of treatment.

The statistical test comparing the cure rate of this study to 80% concludes that

there is no evidence to support the superiority hypothesis. The proportion of cures would not be statistically different from 80% ($p = 0.1318$).

3.4. Evolution of the Patients' Clinical Parameters

Median temperature values were normal at all medical visits. However, high maximum temperature values were recorded during the inclusion visit, ranging from 38.7°C to 39.2°C. All maximum temperatures were normalized by the second medical visit (D4) until the end of treatment (Table 4).

3.5. Evolution of the Patients' Clinical Signs

Positive contacts were the most represented with 22 patients (48.8%). The clinical signs present at inclusion were mostly cough (44.44%), asthenia (42.22%), headache (40%) and anosmia (35.55%). In the study, dyspnoea and chest pain were poorly represented in 05 (11.11%) and 06 (13.33%) patients. In particular, cough, dyspnoea, chest pain, fever, sore throat, headache and nasal obstruction, which were present at inclusion, disappeared before D4 of treatment. Only anosmia and asthenia disappeared before D7. It should be noted that the same patient could present several clinical signs at inclusion. The evolution of the functional signs during the treatment is recorded in Table 5.

4. Discussion

This study aimed to evaluate the safety, efficacy and tolerability of APIVIRINE, a phytomedicine based on the leaves of *Dichrostachys glomerata* (Forssk.) Chiov. (Mimosaceae) in adult patients with COVID-19.

Table 4. Evolution of clinical constants during the treatment.

Constants	D1 = Inclusion (n = 45)	D4 (n = 45)	D7 (n = 30)	D14 (n = 20)	D21 (n = 3)
	Median (Min; Max)	Median (Min; Max)	Median (Min; Max)	Median (Min; Max)	Median (Min; Max)
Body temperature. Median (IQR) (°C) 1	37.2 (35.9; 38.7)	37 (36; 37.8)	36.9 (36.1; 37.9)	37 (36.5; 37.7)	36.7 (36.7; 37.6)
Body temperature. Median (IQR) (°C) 2	37.1 (35.9; 38.9)	37 (36.1; 37.8)	36.8 (36.1; 37.8)	37 (36.5; 37.6)	36.7 (36.7; 37.7)
Body temperature. Median (IQR) (°C) 3	37.1 (35.8; 39.2)	37 (36.1; 37.8)	36.9 (36.1; 37.8)	36.9 (36.5; 37.7)	36.8 (36.6; 37.6)
Breathing rate. Median (IQR)	19 (16; 30)	18 (17; 23)	18 (16; 22)	18.5 (18; 19)	18 (18; 20)
Saturation (SpO ₂)	98 (94; 100)	--	---	---	---
Heart Rate. Median (IQR)	81 (52; 118)	84 (52; 117)	84.5 (52; 101)	84.5 (53; 89)	86 (67; 88)
Systolic blood pressure (mmHg)	127 (100; 100)	128 (100; 75)	128 (113; 167)	128.5 (120; 57)	125 (125; 137)
Diastolic blood pressure (mmHg)	77 (63; 109)	76.5 (67; 97)	77 (67; 97)	79 (69; 92)	76 (69; 82)

Table 5. Evolution of symptoms during treatment.

Symptoms	D1 = Inclusion (n = 45) n (%)	D4 (n = 45) n (%)	D7 (n = 30) n (%)	D14 (n = 20) n (%)	D21 (n = 3) n (%)
Cough	20 (44.44)	00	00	00	00
Dyspnea	05 (11.11)	00	00	00	00
Chest pain	06 (13.33)	00	00	00	00
Anosmia	16 (35.55)	01 (2.22)	00	00	00
Nasal obstruction	10 (22.22)	00	00	00	00
Asthenia	19 (42.22)	02 (4.44)	00	00	00
Fever	12 (26.67)	00	00	00	00
Headaches	18 (40)	00	00	00	00
Sore throat	10 (22.22)	00	00	00	00

Evaluation of tolerance

The results of this study showed that the study population tolerated well APIVIRINE. Indeed, no serious adverse events were reported in any of the study patients during treatment. These results are comparable to those of clinical studies using phytomedicines in patients with COVID-19 [5] [24]. However, one (01) case of non-persistent diarrhoea was recorded and stopped following loperamide administration. Clinically, all symptoms reported at inclusion disappeared by D7 of treatment, and no clinical complications were found. Furthermore, no symptomatic rebound was noted during treatment, in contrast to retrospective follow-up data in patients with COVID-19 [25]. Similarly, no heart rhythm abnormalities were observed in all patients during the entire treatment period.

These results support those of preclinical studies showing the absence of significant toxicological changes in haematological, biochemical and histological parameters of vital organs up to a dose of 2500 mg/kg/day bodyweight of the methanolic extract of leaves of the plant in rats [26] [27].

Transaminases (AST and ALT) and creatinine levels are liver, heart, and kidney function indicators. Studies have shown that liver and kidney damage is associated with COVID-19 infection [3] [28]. Some patients had undisturbed transaminases and creatinine levels at baseline. However, patients with disturbances at inclusion had normalized transaminases and creatinine levels by day 14 in the present study. Laboratory work has also shown that *D. glomerata* leaves have hepatoprotective properties in rats [26].

Clinical and virological efficacy

Clinically, the predominance of dry cough and fever in the study patients could be explained by the pathophysiology of the disease. Indeed, after an incubation period of about five days, 70% of infected patients develop a cough [3] [29]. In addition, symptoms such as fever, dyspnoea, chest pain and asthenia

have also been described in other patients in China [30]. The viral invasion phase is followed, in some patients, by an inadequate immune response marked by worsening respiratory symptomatology and inflammatory syndrome, usually eight to ten days after the first symptoms [3]. Indeed, the study results showed that an elevated CRP at baseline in some patients was normalized after 14 days of treatment.

In this study, cough, dyspnoea, chest pain, fever, sore throat, headache and nasal obstruction, present at inclusion, disappeared before D4 of treatment. Standardized prospective follow-up studies in untreated patients have shown that the duration of symptoms often exceeded 2 weeks [31]. A retrospective study of untreated outpatients with COVID-19 showed that asthenia and anosmia persisted for more than 14 days [25]. A population-based cohort study also showed that only 80% of 2904 COVID-19 patients had symptomatic resolution after 30 days [32]. Thus, there was a gain in time to resolution of signs by APIVIRINE treatment where only anosmia and asthenia disappeared before the seventh day of treatment.

Moreover, all the high maximum temperatures at inclusion were normalized by the fourth day (D4) of treatment. Preclinical studies have indeed highlighted the anti-inflammatory, analgesic, antipyretic properties of the leaves of the plants [16] [26] [33] [34] [35]. This effect could be attributed to the dose-dependent broncho-relaxant effect of the plant leaf extract on guinea pig tracheal preparations [26].

This study also showed virological cure rates of 33.33%, 48.89% and 86.67% after the fourth, seventh and fourteenth day of treatment, respectively. These rates are significantly higher than the results obtained with 7 days of Lopinavir/Ritonavir treatment, where only 3/55 (5.45%) samples tested negative after 3 to 5 days [36]. In a study comparing traditional Chinese treatments with modern antiviral and antibiotic treatments, the median clearance time for SARS-CoV-2 was 12 and 15.5 days, respectively [37]. Treatment with APIVIRINE would improve the cure rate of patients and avoid complications compared to other treatments and the natural course of the disease. Indeed, a follow-up study of antibodies showed that the rate of neutralizing antibodies only reached 80 to 100% between 14 and 21 days after infection with SARS-CoV-2 [30]. Preclinical studies have highlighted the antiviral properties (Poliovirus, Astrovirus, HSV 1, Equine HSV, Canine Bovine and Parvovirus, Influenza A (H5N1) virus) of the plant's leaves [38]. In addition, clovamide, an antiviral compound active against the influenza A (H5N1) virus (74% at 20 µg/mL) has already been isolated as a major compound from *D. glomerata* leaves [16]. Many studies have shown that molecules such as myricetin, kaempferol, quercetin, apigenin and epicatechin have antiviral activity on SARS-CoV-2. Studies have shown that flavonoids exert their immunomodulatory activity through regulation of inflammatory mediators, inhibition of endothelial activation, NLRP3 inflammasome, toll-like receptors (TLRs), or bromodomain containing protein 4 (BRD4), and activation of

erythroid nuclear factor 2-related factor 2 (Nrf2). Thus, this immunomodulatory activity could be beneficial in regulating the cytokine storm during SARS-CoV-2 infection [20].

Indeed, flavonoids such as kaempferol and quercetin have shown inhibitory properties of protein kinase B and protein kinase phosphorylation with a selective blockade of channel 3a expressed in cells infected with SARS-CoV-2 [39].

Also, quercetin (3,3',4',5,7-pentahydroxyflavone) has been reported as one of the potent inhibitors of inflammasome-mediated IL-1 β production [17]. A recent study has shown that flavonoids such as epicatechin have the potential to naturally block or weaken the entry of SARS-CoV-2 as well as its subsequent invasion via a stable ACE2-epicatechin complex [18].

It has also been demonstrated that apigenin is a good candidate for the inhibition of the SARS-CoV-2 protease Mpro through hydrogen bonds, electronics and hydrophobic interactions [40].

These molecules have also been isolated from the extracts of *D. glomerata* constituting APIVIRINE. This could explain the antiviral effect of this phyto-medicine in patients.

However, it should be noted that the effect of the plant or APIVIRINE on SARS-CoV-2 has not yet been evaluated preclinically and is the immediate prospect of this study.

5. Limitation

The study was carried out on an outpatient basis, and the taking of medication could only be directly observed during scheduled visits.

6. Conclusion

APIVIRINE treatment has been well tolerated in patients with COVID-19 without signs of severity. No serious side effects or allergic reactions were observed during treatment. Patients with abnormal blood creatinine, transaminases and blood glucose parameters of interest experienced normalization of values at the end of treatment. A cure rate of 86% was obtained after two weeks of treatment. All the patients progressed favorably towards recovery on the 21st day of treatment. No clinical complications were observed and all symptoms present resolved on the 7th day of treatment. The patients who were positive between the two controls had a declining viral load. Treatment with APIVIRINE is reportedly well tolerated and reduces healing time as well as the risk of complications in patients with COVID-19.

Authors Contributions

DO and AH conducted clinical study data collection, GT conducted monitoring study, JCRPO and ST have done data analysis, SO, GGO, LB and NO have done study design, critical revision of the manuscript for intellectual content, FBK and ZT have done Lab analysis supervision, MO has been the principal investigator

of the study, SO has been the supervisor of the study. All authors have participated in the manuscript writing and have given approval to the final version.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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