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Gender-associated difference following COVID-19 virus infection: Implications for thymosin alpha-1 therapy

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ABSTRACT

Gender influences clinical presentations, duration and severity of symptoms, and therapy outcome in coronavirus disease 2019 (COVID-19) infection. Whether the immune response to Tα1 treatment for SARS-CoV-2 differs between the sexes, and whether this difference explains the male susceptibility to COVID-19, is unclear. This study aimed to investigate the efficiency and safety of Tα1 treatment and provide a basis for practically identifying gender differences characteristics and features of COVID-19. One hundred twenty-seven patients with COVID-19 symptoms and tested COVID19-positive (female 42.52%) in Wuhan union hospital were enrolled for medication. They were randomly divided into groups Control and Tα1 intervention. Seventy-eight patients received a subcutaneous injection of 1.6 mg Tα1, based on supportive treatment for 15 days. The control group included untreated 49 COVID19 patients closely matched for gender and age and received regular supportive treatment. In this retrospective analysis, we found that COVID-19-infected males reported more symptoms than COVID-19-infected females. A high degree of gender differences-related variability was observed in CRP and PCT levels and the cell counts of many lymphocyte subpopulations in the COVID-19 patients after Tα1 intervention. Levels of CRP and IL-6 were higher in Tα1-treated male group than Tα1-treated female group, while the level of PCT was significantly lower in Tα1-treated male group. Gender differences may be a factor in sustaining COVID-19 immunity responded to Tα1, male and female show statistically significant differences in relevance to cytokine production associated with the development of a more significant number of symptoms. This leaves the question of identifying gender-specific risk factors to explain these differences.

1. Introduction

In December 2019, an alarmingly contagious and newly discovered atypical viral pneumonia broke out in Wuhan, China. It has been identified as a zoonotic coronavirus, similar to SARS and MERS coronavirus and named SARS-CoV-2 [1,2]. As of May 11, 2020, coronavirus disease 2019 (COVID-19) has been confirmed in 4,196,972 people worldwide, carrying mortality of approximately 6.77%, compared with a mortality rate of < 1% from influenza [3]. Currently, no medications or vaccines have been verified for the treatment or prevention of COVID-19.

National Health Commission of the People’s Republic of China and State Administration of Traditional Chinese Medicine issued a Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7) in March 2020, which including suggestions of clinical characteristics, case definitions, clinical classification, differential diagnosis, and treatment, to promote the establishment of a therapeutic regimen [4]. Based on nutritional support, antiviral treatments should
be useful in fighting COVID-19. Interferons, intravenous gamma globulin, thymosin-α1, thymopentin, levamisole, cyclosporine A and traditional Chinese medicine are also used for clinical intervention [5].

Tα1 is a thymic peptide that demonstrates a peculiar ability to restore immune system homeostasis in different physiological and pathological conditions acting as multitasking protein depending on the host state of inflammation or immune dysfunction [6]. It is a heat-stable highly acidic molecule composed of 28 amino acid residues, that regulates the immune system by enhancing the function of T cell [7]. Tα1 affects thymocytes by stimulating thymocytes differentiation or converting them into active T cells [8]. It has been documented that Tα1 can enhance thymocytes by stimulating thymocytes differentiation or converting them into active T cells [8]. It has been documented that Tα1 intervention significantly reduces the mortality of severe COVID-19 patients [10].

Currently, there are no specific therapies or human vaccines available to treat and prevent COVID-19 infection and hardly understand about the host factors affecting the immune response to COVID-19. The therapeutic strategies to deal with the infection are only supportive, and respiratory failure from acute respiratory distress syndrome (ARDS) is the leading cause of mortality [11]. Cytokine storm (CS) is one of ARDS's main mechanisms, its deadly uncontrolled systemic inflammatory response resulting from the release of large amounts of pro-inflammatory cytokines and chemokines by immune effector cells [12–14]. Accumulating evidence suggests that patients with severe COVID-19 might have a cytokine storm syndrome [15,16], SARS-CoV-2 invades through the respiratory mucosa and infects other cells, systemically inducing cytokine storm [17]. In severe cases of SARS-CoV-2 infection, CS will trigger an intense attack by the immune system to the body, cause ARDS and multiple organ failure, and finally lead to death [18]. In the severe patients, the inflammatory factors (IL-6, IL-10, TNF-α), neutrophil count, D-dimer, blood urea and creatinine levels were higher significantly, and the lymphocyte counts continued to decrease [19].

In the study of immunological, gender is a biological variable that should be considered. A growing body of evidence indicates gender difference in the clinical outcomes of COVID-19 [20–23]. Numerous studies have shown that females have higher innate and adaptive immune responses than males, leading to faster clearance of viruses and contributes to increased development of immunopathology [24–26]. After virus infection, females have been observed to mount more robust humoral and adaptive immune responses than males [27]. As a result of heightened immunity to viruses, both the intensity and prevalence of viral infections are often lower for females than males [28]. However, reports on the gender difference regulation of the human cytokine response to COVID-19 in significant shortage. Tα1 has been recommended for some patients to enhance cellular immunity for the resistance of viral infection [29]. Although Tα1 intervention has been recommended for adjuvant immunoregulation therapy in COVID-19 patients, the efficiency and security of Tα1 cannot be determined due to the influence of many factors on curative effect. To establish a scientific and rigorous Tα1 intervention therapeutic regimen, the retrospective study of clinical outcomes is incredibly essential. The effect of Tα1 intervention on patients with COVID-19, patients were divided into Tα1 intervention group and control group (without Tα1 intervention) and compared T lymphocyte subsets, cytokine and other laboratory examinations levels.

2. Methods

2.1. Subject enrollment

After careful medical chart review, we compiled the clinical data of laboratory-confirmed hospitalized cases, from the Union Hospital of Huazhong University of Science and Technology in Wuhan between January 30th, 2020, and April 2nd, 2020. Patients diagnosed with

| Table 1 | Patient groups and basic clinical information. |
| --- | --- |
| Characteristics | All patients (n = 127) | Tα1 intervention group (n = 78) | Control group (n = 49) |
| Demographic | | | |
| Female (%) | 42.52 | 44.87 | 38.78 |
| Age [Mean value (S.D.)] | 62.08(12.11) | 62.71(11.13) | 61.08(13.57) |
| Symptoms and Conditions (%) | | | |
| Disease Condition | | | |
| Severe cases (84.25) | Severe cases (83.53) | Severe cases (87.76) |
| Critical illness (14.96) | Critical illness (16.67) | Critical illness (12.24) |
| Respiratory support | 85.04 | 91.03 | 75.51 |
| Fever | 81.10 | 80.77 | 81.63 |
| Cough | 77.95 | 78.20 | 77.55 |
| Expectoration | 29.92 | 26.92 | 34.69 |
| Fatigue | 40.94 | 44.87 | 34.69 |
| Headache | 8.61 | 7.69 | 10.20 |
| Chest distress | 43.31 | 38.46 | 51.02 |
| Muscle soreness | 22.83 | 19.23 | 28.57 |
| Nausea | 6.30 | 5.13 | 8.16 |
| Emesis | 8.61 | 6.41 | 12.24 |
| Diarrhea | 16.54 | 20.51 | 10.20 |
| Prognosis (%) | | | |
| Cure Rate | 94.49 | 94.87 | 93.88 |

| Table 2 | The level of T lymphocyte subsets and cytokines in patients. |
| --- | --- |
| All patients (n = 127) | Tα1 intervention group (n = 78) | Control group (n = 49) |
| [Mean value (S.D.)] | [Mean value (S.D.)] | [Mean value (S.D.)] |
| CD3⁺ T cells (%) | 71.96 (9.29) | 73.99 (8.53) | 68.74 (9.65) |
| CD4⁺ T cells (%) | 43.41 (9.40) | 43.92 (9.92) | 42.60 (8.53) |
| CD8⁺ T cells (%) | 24.80 (8.55) | 26.41 (9.28) | 22.26 (6.57) |
| CD4⁺/CD8⁺ | 2.03 (0.99) | 1.97 (1.05) | 2.14 (0.90) |
| B lymphocytes (%) | 13.27 (8.33) | 12.23 (7.26) | 14.92 (9.64) |
| NK lymphocytes (%) | 9.15 (6.30) | 8.15 (5.56) | 10.73 (7.11) |
| IL-2 (pg/ml) | 2.73 (0.81) | 2.57 (0.62) | 2.98 (1.00) |
| IL-4 (pg/ml) | 2.38 (1.54) | 2.03 (0.83) | 2.93 (2.16) |
| IL-6 (pg/ml) | 9.66 (12.61) | 10.18 (13.62) | 8.84 (10.88) |
| IL-10 (pg/ml) | 3.94 (3.09) | 3.74 (3.01) | 4.27 (3.21) |
| TNF-α/IFN-γ/p (pg/ml) | 2.26 (0.93) | 2.12 (0.73) | 2.47 (1.16) |
| IFN-γ/p (pg/ml) | 3.79 (17.43) | 4.66 (22.23) | 2.40 (1.28) |
| IL-6/IL-10 | 2.73 (3.62) | 3.09 (4.36) | 2.28 (2.35) |
| TNF-α/IL-10 | 0.65 (0.21) | 0.65 (0.20) | 0.65 (0.22) |

| Table 3 | The level of D-dimer, C-reactive protein, procalcitonin and homocysteine in patients. |
| --- | --- |
| Cases | Mean value | S.D. |
| D-dimer(pg/ml) | All patients | 125 | 2.25 | 2.59 |
| Tα1 intervention group | 76 | 2.66 | 2.71 |
| Control group | 49 | 1.62 | 2.29 |
| CRP (mg/L) | All patients | 127 | 34.94 | 36.71 |
| Tα1 intervention group | 78 | 29.47 | 31.15 |
| Control group | 49 | 43.66 | 43.07 |
| HCY(μmol/L) | All patients | 113 | 10.37 | 4.27 |
| Tα1 intervention group | 71 | 10.75 | 4.77 |
| Control group | 42 | 9.72 | 3.21 |
| PCT (%) | All patients | 125 | 0.23 | 0.08 |
| Tα1 intervention group | 76 | 0.22 | 0.08 |
| Control group | 49 | 0.23 | 0.07 |
COVID-19 based on the World Health Organization interim guidance were enrolled. This study was approved by the Ethics Committee of Union Hospital and registered at the Chinese Clinical Trial Registry (ChiCTR2000030803). The diagnosis of COVID-19 was according to ‘Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia’ Released by National Health Commission & State Administration of Traditional Chinese Medicine and confirmed by RNA detection of the SARS-CoV-2 in the clinical laboratory of Union Hospital. Confirmed cases denoted the patients whose real-time reverse-transcription polymerase-chain-reaction (RT-PCR) assay findings for nasal and pharyngeal swab specimens were positive.

2.2. Clinical evaluations at Wuhan Union Hospital

Clinical data, including recent exposure history, clinical symptoms and signs, comorbidities, and laboratory results at admission, were reviewed and abstracted by senior medical practitioners and entered into a computerized database for further verification. According to Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7), patients are classified as mild cases, ordinary cases, severe cases and critical illness COVID-19. Medical history was collected based on the patient’s self-report at the time of admission. Comorbidities were initially treated as a categorical variable (Yes vs. No) and were subsequently classified according to single and multiple numbers. Besides, comorbidities were classified according to the organ system, including the respiratory system, cardiovascular system, and endocrine system. The endpoint of our study was a synthetic measure, including the intensive-care unit (ICU), invasive ventilation, or death.

2.3. Design and patients

A total of 127 COVID-19 patients [54 female (42.52%) with a mean age of 62.08 years (S.D. 12.11)] were identified. The researchers systematically collected the following data from medical records: demographic characteristics (gender, age), disease condition, respiratory support, clinical characteristics (fever, cough, expectoration, hypodynamia, headache, chest distress, muscle soreness, nausea, emesis and diarrhea), therapeutic medication, Lymphocyte subsets (CD3+ T cells, CD4+ T cells, CD8+ T cells, B lymphocytes and NK lymphocytes), cytokines (IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γ), D-dimer, C-reactive protein, homocysteine, procalcitonin and prognosis.

2.4. Statistical analysis

One hundred twenty-seven patients were divided into Tα1 intervention group (78 patients) and control group (49 patients) according to whether Tα1 was used in clinical treatment. Continuous variables were expressed as Mean value (S.D.) and compared using the T-test. Using χ² tests, we compared lymphocytes, cytokines, D-dimer, C-reactive protein, homocysteine and procalcitonin between females and males in each group. We used multiple logistic regression analysis to calculate unadjusted OR and 95% CI for T lymphocyte subsets and cytokines in all patients and within two age groups and determine whether there was a significant interaction between gender and Tα1 intervention concerning the level of Lymphocyte subsets and cytokines. All comparisons were 2-tailed, with a p value < 0.05 considered statistically significant. Data processing using Statistical Product and Service Solutions 25 software (SPSS 25) and GraphPad Prism 8.

3. Results

3.1. Baseline characteristics

Among the 127 patients, 1 had moderate disease, 107 had severe disease, 19 had critical disease. All the patients were released from quarantine. The average duration from symptom onset to discharge from the hospital was 45 ± 13 days. The average duration from symptom onset to hospitalization was 11 ± 7 days. The average age was 62.08 ± 12.11 years and 92 (72.44%) of cases were community-acquired infection. Respiratory support was provided to 108 patients (71 patients in Tα1 intervention group, 37 patients in control group), of which 85.04% were used nasal cannula or face mask and 7.87% used high flow nasal cannula or non-invasive mechanical ventilation, with only one patient used invasive mechanical ventilation. The main

Fig. 1. Unadjusted risk of the level of T lymphocyte subsets, cytokines, D-dimer, C-reactive protein, procalcitonin and homocysteine were comparing Tα1 intervention group with control group. Red markers are significant statistical difference indexes.
Symptoms were fever (81.10%), cough (77.95%), chest distress (43.31%), fatigue (40.94%). The white blood cell count was decreased by 9.45% of patients, and the lymphocyte count was decreased by 62.99% of the patients. On admission, 81.89% of patients showed pneumonia on chest CT scans. In the control group, the average age was 61.08 ± 13.57 years and the average duration from symptom onset to hospitalization was 13 ± 7 days. Compared with the Tα1 intervention group, these patients were older and the duration was longer (Table 1).

When the 46 patients were released from quarantine, the white blood cell count of 13.04% of the patients was < 3.5 G/L, the lymphocyte count of 60.87% of the patients was < 1.1 G/L, and the absolute counts of white blood cells and lymphocytes were 6.96 ± 3.18 G/L and 0.97 ± 0.46 G/L.

Average levels of CD4⁺ T cells and IL-6 in 127 patients were higher than the normal range. The average levels of CD8⁺ T cells and D-Dimer in Tα1 intervention group were significantly higher than those in control group (CD3⁺ T cells P = 0.003; CD8⁺ T cells P = 0.010; D-dimer P = 0.034), and the average levels of NK lymphocytes, IL-2, IL-4, TNF-α and C-reactive protein were significantly lower than those in control group (NK lymphocytes P = 0.028; IL-6 P = 0.006).

Fig. 2. Unadjusted risk of the level of T lymphocyte subsets, cytokines, D-dimer, C-reactive protein, procalcitonin and homocysteine were comparing males (or females) in Tα1 intervention group with control group. Red markers are significant statistical difference indexes. A: Comparison of Tα1-treated male group with control group; B: Comparison of Tα1-treated female group with control group.
2P = 0.009; IL-4 P = 0.002; TNF-α P = 0.046; CRP P = 0.037). The Tα1 intervention group's cure rate was higher than that of the control group, and the average level of CRP in Tα1 group was significantly lower than control group. There was no significant difference in PCT and IL-6 between the two groups, perhaps Tα1 intervention is not the essential condition for influencing these two indicators (see Tables 2, 3 and Fig. 1).

3.2. Gender differentiation of Tα1 intervention

We first compared male and female differences of Tα1 intervention group and control group. A high degree of gender differences-related variability was observed in the cell counts of many lymphocyte sub-populations in the COVID-19 patients' group after Tα1 intervention. Compared to the control group, these levels of CD3 + T cells (73.75 ± 8.51% vs 68.74 ± 9.65%), CD8 + T cells (28.47 ± 9.46% vs 22.26 ± 6.57%) and D-dimer (3.12 ± 2.95 μg/ml vs

![Fig. 3. Unadjusted risk of the level of T lymphocyte subsets, cytokines, D-dimer, C-reactive protein, procalcitonin and homocysteine were comparing males with females. Red markers are significant statistical difference indexes. A: In the Tα1 intervention group, comparison of males with females; B: Patients older than 65 age in the Tα1 intervention group, comparison of males with females.](image-url)
1.62 ± 2.29 µg/ml) were significantly higher in Tα1-treated male group. Meanwhile, levels of B lymphocytes (14.92 ± 9.64% vs 10.83 ± 6.62%), IL-4 (2.93 ± 2.16 pg/ml vs 2.04 ± 0.85 pg/ml) and CD4 + T cells to CD8 + T cell ratio (2.14 ± 0.90 vs 1.69 ± 0.87) were significantly lower in Tα1-treated male group (Fig. 2A). Compared to the control group, levels of CD3 + T cells (68.74 ± 9.65% vs 74.29 ± 8.66%) and CD4 + T cells (42.60 ± 8.53% vs 46.77 ± 9.31%) were significantly higher in Tα1-treated female group. While levels of NK lymphocytes (10.73 ± 7.11% vs 6.52 ± 4.54%), IL-2 (2.98 ± 1.00 pg/ml vs 2.51 ± 0.44 pg/m), IL-4 (2.93 ± 2.16 pg/ml vs 2.02 ± 0.83 pg/m) and CRP (43.66 ± 43.07 mg/L vs 25.53 ± 30.18 mg/L) were significantly lower in Tα1-treated female group (Fig. 2B).

Next, we analyzed the effect of Tα1 intervention and age on gender differences-related. CD8 + T cells (28.47 ± 9.46% vs 23.86 ± 8.51%), NK lymphocytes (9.48 ± 5.99% vs 6.52 ± 4.54%), IL-6 (13.70 ± 17.43 pg/ml vs 5.85 ± 3.19 pg/ml) and IL-6 to IL-10 ratios (3.96 ± 5.66 vs 1.83 ± 0.98) were significantly higher in males than females with Tα1 intervention group. While CD4 + T cells (41.61 ± 9.90% vs 46.77 ± 9.31%), PCT (0.20 ± 0.08% vs 0.25 ± 0.09%) and CD4 + T cells to CD8 + T cells ratios (1.69 ± 0.87 vs 2.31 ± 1.16) were significantly lower in Tα1-treated male group (Fig. 3A). The CRP level decreased after the Tα1 intervention both in males and females, and Tα1-treated female group significantly lower than control group. The level of IL-6 decreased in Tα1-treated female group compared to control group, but the difference was not significant. The level of IL-6 was higher in Tα1-treated male group than control group and significantly higher than Tα1-treated female group.

This is how we discovered that age was a factor influencing CRP and IL-6 elevation in males. Levels of CD8 + T cells (30.66 ± 12.23% vs 23.04 ± 8.4%), IL-6 (17.86 ± 19.29 pg/ml vs 6.15 ± 3.32 pg/ml) and CRP (0.19 ± 0.08 mg/L vs 0.24 ± 0.09 mg/L) were significantly higher in Tα1-treated females than their control group. While levels of CD3 + T cells (68.74 ± 9.65% vs 74.29 ± 8.66%) and CD4 + T cells (42.60 ± 8.53% vs 46.77 ± 9.31%) were significantly higher in Tα1-treated female group. While levels of NK lymphocytes (10.73 ± 7.11% vs 6.52 ± 4.54%), IL-2 (2.98 ± 1.00 pg/ml vs 2.51 ± 0.44 pg/m), IL-4 (2.93 ± 2.16 pg/ml vs 2.02 ± 0.83 pg/m) and CRP (43.66 ± 43.07 mg/L vs 25.53 ± 30.18 mg/L) were significantly lower in Tα1-treated female group (Fig. 2B).

4. Discussion

The SARS-CoV-2 virus is one of the seven coronaviruses that cause infections in humans, it became an epidemic in a brief period and had a considerable impact on a global scale [30]. Whenever a new infectious disease emerges, knowledge regarding clinical features, diagnostic tools and treatment options is critical [31]. Tα1 intervention has been recommended for adjuvant immunomodulation therapy in COVID-19 patients, but the efficiency and security of Tα1 treatment and care of patients with COVID-19. Although this study's results can not be confirmed the efficacy of Tα1 intervention in COVID-19 patients, our data suggest that clinical indicators of gender differences in Tα1 interventions must be closely monitored and treatment regimens adjusted accordingly.

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References

[1] Coronaviridae Study Group of the International Committee on Taxonomy of. V. (2020). The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol.
[2] Y. Liu, A.A. Gayle, A. Wilder-Smith, J. Rocklöv, The reproductive number of COVID-19 is higher compared to SARS coronavirus, J. Travel Med. 27 (2) (2020) 1–4.
[3] P. Mehta, D.F. McAuley, M. Brown, E. Sanchez, R.S. Tattersall, J.J. Manson, COVID-19: consider cytokine storm syndromes and immunosuppression, Lancet 395 (10229) (2020) 1033–1034.
[4] National Health Commission, State Administration of Traditional Chinese Medicine. Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 4). (2020).
[5] L. Zhang, Y. Liu, Potential interventions for novel coronavirus in China: A systematic review, J. Med. Virol. 92 (5) (2020) 479–490.
[6] C. Matteucci, S. Grelli, E. Balestrieri, A. Minutolo, A. Argaw-Denboba, B. Macchi, P. Simbaldi-Vellebona, C.F. Perno, A. Martino, E. Garaci, Thymosin alpha 1 and HIV-1: recent advances and future perspectives, Future Microbiol. 12 (2) (2017) 141–155.
[7] Yan-Rong Guo, Qing-Dong Cao, Zhong-Si Hong, et al. The origin, transmission and sequence analysis of an immunologically active thymic polypeptide, PNAS 74 (2) (1977) 725–729.
[8] A.L.Goldstein, T.L.Low, M.McAdoo, J.McClure, et al., Thymosin alpha-1: a polypeptide biological response modifier that plays a significant role in activating and regulating various cells' immune system. Thymosin alpha-1, American J. Health-Syst. Pharm. 58 (15) (2001) 879–885.
