International Journal of General Medicine

Dovepress
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ORIGINAL RESEARCH

Serum Fibronectin Levels Increased Significantly Following the Administration of an Inactivated SARS-CoV-2 Vaccine: A Prospective Observational Study

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Background: There has been a lack of comprehensive studies on the long-term observation of laboratory values following the administration of SARS-CoV-2 vaccines. The objective of this study is to assess the long-term impact of inactivated SARS-CoV-2 vaccines on coagulation function and other health indicators.

Methods: We enrolled residents of Chengdu who consented to receive inactivated SARS-CoV-2 vaccines, categorizing them into two groups: healthy donors (n=40) and survivors of COVID-19 infection (n=34), based on their SARS-CoV-2 infection status prior to vaccination. Blood samples from the subjects were collected at specific intervals following vaccination.

Results: The levels of Fibronectin (FN) increased significantly in both healthy donors and survivors of COVID-19 infection after receiving two doses of the inactivated SARS-CoV-2 vaccine (both $P < 0.001$), and there was no statistically significant difference in the degree of FN increase between the two groups (153.05 ± 77.19 mg/L vs 172.32±90.42 mg/L, P=0.326). The rate of elevated FN levels was significantly higher six months after vaccination compared to the rate before vaccination, both in healthy donors (85.0% vs 5.0%, P<0.001) and in survivors of COVID-19 infection (94.1% vs 29.4%, P<0.001). Additionally, the levels of FN in healthy donors further increased six months after receiving a booster dose of the inactivated vaccine compared to pre-booster levels (569.90 ±119.44 mg/L vs 467.35±62.04 mg/L, $P < 0.001$).

Conclusion: The study indicates that serum FN levels increased significantly following the administration of the inactivated SARS-CoV-2 vaccine, and these elevated FN levels may persist for more than six months. However, it remains unclear whether this increase could result in any adverse effects.

Keywords: inactivated vaccine, SARS-CoV-2, fibronectin level, laboratory values

Introduction

Over the past four years, vaccines targeting the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) have played a pivotal role in combating the COVID-19 pandemic. These vaccines are categorized according to the various technologies used in their development: live attenuated, inactivated, soluble protein, viral vector, nanoparticle, and nucleic acid vaccines (DNA or RNA).[1] Notably, inactivated SARS-CoV-2 vaccines, such as CoronaVac from Sinovac Life Sciences and BBIBP-CorV from Sinopharm, have been widely administered in the Chinese mainland. Clinical trials have confirmed the safety and efficacy of all World Health Organization (WHO)-approved SARS-CoV-2 vaccines in preventing infections, including those caused by mutated strains, and in managing the severity of the disease.[2–4] Furthermore, research has indicated that vaccinated individuals who contract SARS-CoV-2 exhibit a lower rate of post-infection sequelae compared to the unvaccinated population.[5]

Received: 16 January 2025
Accepted: 21 March 2025
Published: 8 April 2025

A study by Kaytaz M et al[6] has shown that fibrinogen levels significantly decrease (P<0.001), while D-dimer levels markedly increase (P=0.083) following the second dose of the CoronaVac vaccine. Additionally, there have been reports of vaccine-induced thrombocytopenia and thrombosis occurring shortly after the first dose of the ChAdOx1 SARS-CoV-2 vaccine.[7,8] However, long-term observations of these parameters following SARS-CoV-2 vaccination are limited. The aim of the present study is to conduct an extended observation of various laboratory indicators, including coagulation function, in individuals who have been vaccinated to ascertain any potential long-term effects of the inactivated SARS-CoV-2 vaccine on human health. It should be emphasized that fibronectin (FN) was included in this study due to its critical role in thrombosis.

Materials and Methods

Study Population

Our study, initiated in May 2021, was a single-center, prospective observational one conducted at the Public Health Clinical Center of Chengdu. We enrolled residents of Chengdu who consented to receive inactivated SARS-CoV-2 vaccines, categorizing them into two groups: healthy donors and survivors of COVID-19 infection, based on their SARS-CoV-2 infection status prior to vaccination. The inclusion criteria were as follows: 1. Participants over 18 years of age, all of whom were Chinese nationals; 2. No prior COVID-19 vaccination; 3. No other vaccinations administered within the 14 days preceding the study; 4. Absence of chronic infectious diseases, mental illnesses, malignancies, or severe organ dysfunction; 5. Survivors of COVID-19 infection had experienced a single SARS-CoV-2 infection and had been symptom-free for at least six months. The exclusion criteria included: 1. Participants aged 18 years or younger; 2. Individuals with contraindications as per the vaccine's instructions; 3. Individuals with autoimmune diseases or those who are immunocompromised, as well as individuals undergoing treatment with immunosuppressants or immunomodulators; 4. Pregnant women; 5. Those who experienced severe adverse reactions post-vaccination; 6. Healthy donors or survivors who contracted SARS-CoV-2 during the study period; 7. Individuals who are unable to complete the study's follow-up procedures; 8. Those who cannot provide informed consent; 9. Other scenarios deemed inappropriate for study participation by the core members of the research team.

It is important to emphasize that individuals infected with SARS-CoV-2 and their close contacts were strictly monitored in Chinese mainland before December 2022, the subjects in this study underwent nucleic acid testing for SARS-CoV-2 every 1 to 2 months. Therefore, we can determine whether the subjects were infected with SARS-CoV-2 during the study period.

This study was conducted in accordance with the Declaration of Helsinki and received approval from the Research Ethics Committee of Public Health Clinical Center of Chengdu (No.: PJ-K2021-10-01). All participants voluntarily provided their informed consent and agreed to subsequent follow-ups.

Vaccine introduction

The study participants received either the CoronaVac (Sinovac Life Sciences) or BBIBP-CorV (Sinopharm) inactivated SARS-CoV-2 vaccine, both of which were produced in Chengdu. Although the vaccines for each participant were from the same manufacturer, the batch numbers differed.

Follow-up Time

Healthy donors received two doses of the inactivated SARS-CoV-2 vaccine, followed by a booster dose six months after the second dose. The interval between the first and second doses was four weeks. Peripheral venous blood samples were collected for laboratory analysis at specific time points: the day before the initial dose, six months after the two doses (prior to the booster dose), and six months post-booster vaccination. Survivors of COVID-19 infection also received two doses of the inactivated SARS-CoV-2 vaccine, adhering to the same dosing interval as the healthy controls, with blood samples collected the day before the first dose and six months after the two doses.

Observation Index and Data Collection

Every subject had been drawn about 20 mL of fasting venous blood at per sampling period. The blood specimens were sent to the laboratory at the Public Health Clinical Center of Chengdu for testing, and the laboratory values (ie, hematologic and serum chemistry findings) were obtained from these samples. The laboratory values included a complete blood count, liver function tests, renal function tests, cardiac enzyme profiles, blood lipid levels, coagulation function, fibronectin (FN), non-specific immunoglobulins (IgG, IgA, IgM), complement levels (C3 and C4), and lymphocyte subsets. The accuracy, completeness, and authenticity of all data were rigorously monitored by the researchers.

Test Kit for Fibronectin (FN)

The concentration of FN in serum samples was determined using an immunoturbidimetric assay. Skilled laboratory technicians followed the protocols outlined in the FN assay kit supplied by Beijing Strong Biotechnologies, and conducted the assays using a HITACHI LABOSPECT 008 AS fully automatic biochemical analyzer. The normal range for FN levels is 250 to 400 mg/L.

Sample Size

In this study, the subjects were divided into two groups: healthy donors and survivors of COVID-19 infection. The primary observation index was the changes in blood laboratory indicators following inoculation with the inactivated SARS-CoV-2 vaccine. In addition to the before-and-after control within each group, a two independent samples *t*-test was conducted to compare the groups. G*Power (version 3.1.9.7) software was utilized to calculate the minimum sample size required for the study. Due to the lack of relevant literature and the absence of a pre-experiment, we established a two-tailed α level of 0.1, a statistical power (1-β) of 0.8, and selected a moderately large effect size ($d = 0.65$), utilizing a 1:1 distribution ratio. As a result, the sample size for each group was determined to be 30 participants. Taking into account the potential loss of subjects and the available research funding, we planned to recruit a total of 80 individuals, with 40 participants from each group. However, during the actual implementation of the study, only 35 survivors of COVID-19 infection volunteered to participate.

Statistical Analysis

Statistical and cartographic software, including SPSS 26.0 (SPSS, Chicago, IL, USA) and GraphPad Prism 8 (GraphPad, CA, USA), were used for statistical analyses and cartograph production. Continuous variables with a normal distribution were expressed as the mean ± standard deviation, a paired samples *t*-test or a two independent samples *t*-test were utilized for comparison between two groups. Skewed continuous variables were expressed as the median and interquartile range between the 25th and 75th percentiles, and the Wilcoxon signed-rank test was used for comparison between two groups. Categorical variables were expressed as frequencies with percentages, and the McNemar's test was used for comparisons of these data. The Pearson correlation coefficient test was used to assess the correlation between two categorical variables. $P < 0.05$ was considered to define statistical significance.

Results

With the assistance of the Chengdu Center for Disease Control and Prevention, we contacted all residents of Chengdu who were infected with SARS-CoV-2 before January 2021. Thirty-five individuals agreed to participate in this study; however, one person was lost to follow-up during the subsequent study phase. We then recruited 40 healthy individuals for matching, all of whom successfully completed the post-vaccination follow-up. The final analysis included 74 subjects: 40 healthy donors with a median age of 50.5 years (range 23 to 71), evenly divided between males and females, and 34 survivors of COVID-19 infection with a median age of 49.5 years (range 20 to 78), consisting of 15 males and 19 females.

Given the study's duration of over a year, metabolic indicators such as blood lipids, uric acid, and blood sugar—variables that can fluctuate with dietary habits—were excluded from the analysis of pre- and post-vaccination laboratory

indicator changes. Paired t-tests and Wilcoxon signed-rank tests indicated that certain indicators related to liver enzymes, cardiac enzymes, coagulation function, and immune function were significantly altered six months after the administration of two doses of the inactivated SARS-CoV-2 vaccine in healthy donors. Specifically, there was a significant increase in levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), ferritin (Fer), FN, thrombin time (TT), and immunoglobulin A (IgA) (all $P < 0.05$), while levels of alkaline phosphatase (ALP), lactated hydrogenase (LDH), creatine kinase isoenzyme (CK-MB), fibrinogen (FBG), D-Dimer, complement component 3 (C3), and B cell counts decreased significantly (all $P<0.05$) (Table 1). Among the survivors of COVID-19 infection, levels of Fer, FN, creatinine (CR), prothrombin time (PT), TT, IgA, complement component 4 (C4), and natural killer (NK) cell counts increased six months after receiving two doses of an inactivated SARS-CoV-2 vaccine. In contrast, levels of fibrin degradation products (FDP) and D-Dimer decreased compared to measurements taken prior to vaccination (all $P < 0.05$) (Table 2). If inactivated vaccination can induce changes in blood laboratory indicators, these changes should be consistent in both healthy donors and survivors of COVID-19 infection, with statistically significant differences. When

Table 1 Variation of Laboratory Values After Two Doses of Inactivated SARS-CoV-2 Vaccine in Healthy Donors (n=40)

Laboratory Parameter	Normal Adult Range	Before Vaccination	Six Months After Vaccination	*t/Z*	*P*
WBC count, 10^9/L	3.50–9.50	5.80±1.37	5.73±1.27	0.455	0.651
LYM count, 10^9/L	1.10–3.20	1.98±0.47	1.84±0.51	1.640	0.109
RBC count, 10^12/L	3.8–5.1	4.68±0.57	4.72±0.48	−0.948	0.349
PLT count, 10^9/L	125–350	198.88±61.60	190.42±54.04	1.799	0.080
ALT level, U/L	0–37	21.50 (15.25, 29.75)	25.00 (17.25, 42.00)	−3.714	<0.001
AST level, U/L	0–37	23.50 (20.00, 27.00)	25.50 (22.00, 31.75)	−2.463	0.014
ALP level, U/L	40–150	91.50±24.01	76.00±19.67	7.138	<0.001
GGT level, U/L	0–50	21.00 (14.00, 31.75)	20.00 (16.00, 31.75)	−0.363	0.717
ALB level, g/L	35–55	44.91±2.73	44.61±2.43	0.890	0.379
TBIL level, μmol/L	0–20.5	6.55 (5.45, 9.38)	8.40 (5.80, 12.22)	−2.897	0.004
Fer level, ng/mL	10–120	94.00 (43.00, 213.00)	119.00 (46.00, 262.75)	−4.302	<0.001
FN level, mg/L	250–400	314.30±67.18	467.35±62.04	−12.540	<0.001
Urea level, mmol/L	2–6.9	5.64±1.40	5.28±1.46	1.747	0.089
CR level, μmol/L	40–130	62.38±14.80	62.22±13.10	0.136	0.893
LDH level, U/L	109–245	195.88±34.57	180.05±37.27	3.038	0.004
HBDH level, U/L	72–182	135.78±23.10	133.85±28.23	0.625	0.536
CK level, U/L	25–196	89.50 (69.25, 122.00)	85.00 (64.00, 105.50)	−1.773	0.076
CK-MB level, U/L	0–24	12.78±4.03	11.02±4.40	2.116	0.041
PT level, sec	10–14	12.15±1.12	12.08±0.62	0.414	0.681
FBG level, g/L	2–4	2.84±0.47	2.67±0.52	2.381	0.022
TT level, sec	14–21	15.56±2.32	16.92±1.78	−2.714	0.010
FDP level, μg/mL	0–5	2.00 (1.82, 2.20)	1.95 (1.80, 2.28)	−1.621	0.105
D-Dimer level, μg/mL	0–1	0.62 (0.55, 0.69)	0.56 (0.52, 0.61)	−3.766	<0.001
IgG level, g/L	8–16	12.95±1.72	12.78±1.71	1.365	0.180
IgA level, g/L	0.7–3.3	2.32±0.98	2.46±0.98	−2.714	<0.001
IgM level, g/L	0.5–2.2	1.18 (0.97, 1.80)	1.18 (0.97, 1.76)	−0.983	0.326
C3 level, g/L	0.8–1.6	1.25±0.16	1.18±0.16	3.497	0.001
C4 level, g/L	0.2–0.4	0.23±0.07	0.22±0.06	1.869	0.069
CD3+T count, cells/μL	770–2041	1260.50±341.44	1178.00±306.90	1.250	0.219
CD4+T count, cells/μL	414–1123	705.00±205.73	629.50±163.53	1.981	0.055
CD8+T count, cells/μL	238–874	431.00 (328.25, 553.50)	402.00 (318.50, 530.50)	−0.342	0.732
B cell count, cells/μL	90–560	239.00 (173.25, 342.00)	194.50 (144.50, 292.50)	−2.491	0.013
NK cell count, cells/μL	150–1100	205.50 (145.50, 324.50)	183.50 (117.50, 289.50)	−0.837	0.402

Abbreviations: WBC, white blood cell; LYM, lymphocyte; RBC, red blood cell; PLT, platelet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; ALB, albumin; TBIL, total bilirubin; Fer, ferritin; FN, fibronectin; Urea, urea nitrogen; CR, creatinine; LDH, lactated hydrogenase; HBDH, hydroxybutyrate dehydrogenase; CK, creatine kinase; CK-MB, creatine kinase isoenzyme; PT, prothrombin time; FBG, fibrinogen; TT, thrombin time; sec, second; FDP, fibrinogen degradation products; IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M; C3, complement component 3; C4, complement component 4.

Table 2 Variation of Laboratory Values After Two Doses of Inactivated SARS-CoV-2 Vaccine in Survivors of COVID-19 Infection (n=34)

Laboratory Parameter	Normal Adult Range	Before Vaccination	Six Months After Vaccination	*t/Z*	*P*
WBC count, 10^9/L	3.50–9.50	6.03±1.90	6.24±1.75	−0.730	0.471
LYM count, 10^9/L	1.10–3.20	1.59±0.36	1.65±0.48	−0.949	0.350
RBC count, 10^12/L	3.8–5.1	4.64±0.50	4.61±0.80	0.292	0.772
PLT count, 10^9/L	125–350	189.30±50.52	195.88±62.02	−0.980	0.334
ALT level, U/L	0–37	22.50 (16.00, 33.50)	25.00 (17.00, 41.50)	−1.535	0.125
AST level, U/L	0–37	23.00 (21.00, 29.25)	25.00 (20.50, 31.50)	−0.841	0.400
ALP level, U/L	40–150	75.09±19.12	75.85±18.98	−0.401	0.691
GGT level, U/L	0–50	16.50 (12.00, 42.00)	19.00 (12.00, 43.50)	−0.684	0.494
ALB level, g/L	35–55	44.21±3.34	44.21±3.49	<0.001	1.000
TBIL level, μmol/L	0–20.5	10.15 (6.35, 13.45)	8.00 (6.95, 12.10)	−1.832	0.067
Fer level, ng/mL	10–120	53.00 (29.75, 124.50)	83.00 (48.50, 201.50)	−4.351	<0.001
FN level, mg/L	250–400	375.79±83.66	548.12±122.03	−11.112	<0.001
Urea level, mmol/L	2–6.9	5.22±1.52	5.23±1.46	−0.032	0.974
CR level, μmol/L	40–130	55.31±17.64	61.06±16.93	−2.586	0.014
LDH level, U/L	109–245	189.48±32.69	182.61±35.83	1.349	0.187
HBDH level, U/L	72–182	131.97±20.99	128.12±28.01	1.076	0.290
CK level, U/L	25–196	95.50 (68.75, 124.00)	83.00 (66.50, 128.00)	−1.681	0.093
CK-MB level, U/L	0–24	11.39±2.32	10.64±2.72	1.996	0.054
PT level, sec	10–14	12.62±1.10	13.01±1.09	−2.052	0.049
FBG level, g/L	2–4	2.66±0.52	2.65±0.52	0.191	0.850
TT level, sec	14–21	16.39±1.26	18.50±2.19	−5.331	<0.001
FDP level, μg/mL	0–5	2.05 (1.80, 2.40)	0.70 (0.40, 1.75)	−4.247	<0.001
D-Dimer level, μg/mL	0–1	0.60 (0.54, 0.66)	0.23 (0.15, 0.52)	−3.547	<0.001
IgG level, g/L	8–16	13.01±2.76	13.01±2.95	0.007	0.994
IgA level, g/L	0.7–3.3	1.99±0.77	2.19±0.88	−4.146	<0.001
IgM level, g/L	0.5–2.2	1.17 (0.85, 2.24)	1.22 (0.88, 2.15)	−0.689	0.491
C3 level, g/L	0.8–1.6	1.22±0.20	1.21±0.21	0.173	0.863
C4 level, g/L	0.2–0.4	0.23±0.07	0.24±0.07	−2.579	0.015
CD3+T count, cells/μL	770–2041	1062.42±290.30	1135.09±381.96	−1.447	0.158
CD4+T count, cells/μL	414–1123	550.15±158.59	574.55±202.24	−0.904	0.373
CD8+T count, cells/μL	238–874	402.00 (313.00, 529.50)	405.00 (325.50, 546.50)	−0.616	0.538
B cell count, cells/μL	90–560	172.00 (128.75, 217.75)	178.00 (132.00, 232.50)	−0.911	0.362
NK cell count, cells/μL	150–1100	165.50 (82.75, 240.00)	217.00 (107.00, 319.50)	−2.546	0.011

Abbreviations: WBC, white blood cell; LYM, lymphocyte; RBC, red blood cell; PLT, platelet; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transpeptidase; ALB, albumin; TBIL, total bilirubin; Fer, ferritin; FN, fibronectin; Urea, urea nitrogen; CR, creatinine; LDH, lactated hydrogenase; HBDH, hydroxybutyrate dehydrogenase; CK, creatine kinase; CK-MB, creatine kinase isoenzyme; PT, prothrombin time; FBG, fibrinogen; TT, thrombin time; sec, second; FDP, fibrinogen degradation products; IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M; C3, complement component 3; C4, complement component 4.

combined with the statistical results presented in Tables 1 and 2, this suggests that the inactivated SARS-CoV-2 vaccine may lead to increased levels of Fer, FN, TT, and IgA, as well as a decrease in D-Dimer levels, persisting for at least six months.

Compared to the changes in laboratory indicator values, the increase in the abnormal rate of these indicators receives greater attention in clinical practice. We selected the laboratory indicators from Tables 1 and 2 that demonstrated statistically significant differences and analyzed the changes in the rate of abnormality for each indicator before and after vaccination. The McNemar's test revealed that the rate of elevated FN levels was significantly higher six months after vaccination compared to the rate before vaccination, both in healthy donors (85.0% vs 5.0%, P<0.001) and in survivors of COVID-19 infection (94.1% vs 29.4%, P<0.001). In contrast, the differences in the rates of other indicators were not statistically significant (all P>0.05) (Tables 3 and 4). A correlation analysis between FN and other coagulation function

Table 3 Comparison of Abnormal Laboratory Findings Before and After Two Doses of Inactivated SARS-CoV-2 Vaccine in Healthy Donors (n=40)

Abnormal Laboratory Parameter	No. (%) of Healthy Donors with Abnormal Laboratory Findings		*P*
	Before Vaccination	Six Months After Vaccination	
Elevated ALT level	5(12.5)	12(30.0)	0.065
Elevated AST level	3(7.5)	5(12.5)	0.727
Elevated ALP level	1(2.5)	0(0)	1.000
Elevated TBIL level	0(0)	0(0)	1.000
Elevated Fer level	8(20.0)	13(32.5)	0.125
Elevated FN level	2(5.0)	34(85.0)	<0.001
Elevated LDH level	2(5.0)	2(5.0)	1.000
Elevated CK-MB level	1(2.5)	1(2.5)	1.000
Elevated FBG level	1(2.5)	0(0)	1.000
Elevated TT level	0(0)	2(5.0)	0.500
Elevated D-Dimer level	1(2.5)	1(2.5)	1.000
Elevated IgA level	6(15.0)	6(15.0)	1.000
Low C3 level	0(0)	0(0)	1.000
Low B cell count	0(0)	1(2.5)	1.000

Notes: No x^2 value when using McNemar's test.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TBIL, total bilirubin; Fer, ferritin; FN, fibronectin; LDH, lactated hydrogenase; CK-MB, creatine kinase isoenzyme; FBG, fibrinogen; TT, thrombin time; IgA, immunoglobulin A; C3, complement component 3.

Table 4 Comparison of Abnormal Laboratory Findings Before and After Two Doses of Inactivated SARS-CoV-2 Vaccine in Survivors of COVID-19 Infection (n=34)

Abnormal Laboratory Parameter	No. (%) of Healthy Donors with Abnormal Laboratory Findings		*P*
	Before Vaccination	Six Months After Vaccination	
Elevated Fer level	9(26.5)	13(38.2)	0.125
Elevated FN level	10(29.4)	32(94.1)	<0.001
Elevated CR level	0(0)	0(0)	1.000
Elevated PT level	3(8.8)	4(11.8)	1.000
Elevated TT level	1(2.9)	6(17.6)	0.063
Elevated FDP level	0(0)	0(0)	1.000
Elevated D-Dimer level	1(2.9)	0(0)	1.000
Elevated IgA level	3(8.8)	3(8.8)	1.000
Elevated C4 level	1(2.9)	2(5.9)	1.000
Low NK cell count	16(47.1)	13(38.2)	0.375

Note: No x^2 value when using McNemar's test.
Abbreviations: Fer, ferritin; FN, fibronectin; CR, creatinine; PT, prothrombin time; TT, thrombin time; FDP, fibrinogen degradation products; IgA, immunoglobulin A; C4, complement component 4.

indicators, including PT, FBG, TT, FDP, and D-Dimer, was conducted using the Pearson correlation coefficient test. The results indicated no significant linear correlation between FN and these indicators (all P>0.05).

After discovering that inoculation with an inactivated COVID-19 vaccine may lead to a significant increase in FN levels and a sharp rise in the abnormal rate of elevated FN levels, we collected serum FN results from survivors of COVID-19 infection at the early recovery period (two weeks post-discharge) to analyze the dynamic changes in FN levels among these former COVID-19 patients. Of the 34 survivors of COVID-19 infection, 20 (58.8%) exhibited elevated levels of FN at the early recovery period. Prior to vaccination (ie, 6–12 months post-recovery), the number of individuals with elevated FN decreased from 20 to 10. FN levels in the survivors of COVID-19 infection significantly declined compared to the early recovery period (375.79±83.66 mg/L vs 429.79±106.52 mg/L, t=4.307, P<0.001) (Figure 1). However, these levels remained higher than the baseline FN levels observed in healthy donors prior to

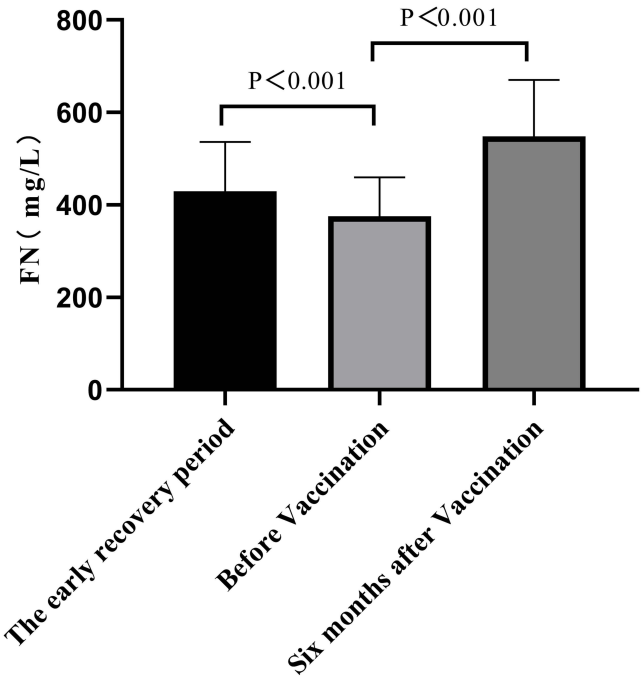

Figure 1 Changes in FN levels among survivors of COVID-19 infection (n=34). A paired samples *t*-test was used for the comparison.

vaccination (375.79±83.66 mg/L vs 314.30±67.18 mg/L, t=−3.507, P=0.001). FN levels in survivors of COVID-19 infection were significantly elevated at 6 months after receiving two doses of the inactivated vaccine compared to pre-vaccination levels (548.12 ± 122.03 mg/L vs 375.79±83.66 mg/L, t=−11.112, P<0.001) (Figure 1). In addition, we compared the difference in FN levels before and after vaccination (post-vaccination minus pre-vaccination) between the two groups. The two independent samples *t*-test indicated that there was no statistically significant difference in the degree of FN increase between healthy donors and survivors of COVID-19 infection following two doses of the inactivated SARS-CoV-2 vaccine (153.05 ± 77.19 mg/L vs 172.32±90.42 mg/L, t=−0.989, P=0.326).

In healthy donors who received the booster dose of inactivated SARS-CoV-2 vaccine, the rate of elevated FN increased from 85.0% (34/40) to 95.0% (38/40) six months post-booster. However, the McNemar's test indicated that this increase was not statistically significant (P=0.219). In contrast, the levels of FN showed a significant increase compared to pre-booster levels (569.90±119.44 mg/L vs 467.35±62.04 mg/L; t=5.704, P<0.001) (Figure 2).

Discussion

In our study, vaccination with an inactivated COVID-19 vaccine was found to increase levels of Fer, FN, TT, and IgA while decreasing D-Dimer levels in both healthy individuals and survivors of COVID-19 infection. These effects may persist for at least six months (all P<0.05). However, in a clinical practice, abnormal laboratory indicators are of greater concern. Our findings indicate that the incidence of elevated FN level post-vaccination was significantly higher than pre-vaccination, which is a notable observation.

FN is a large glycoprotein that is abundant in plasma, cells, and the extracellular matrix, and it plays a role in various cellular functions, including cell adhesion, migration, proliferation, and differentiation.[9] Furthermore, Fn is involved in embryonic development, blood clotting, and wound healing.[10,11] Two primary forms of FN isoforms exist in human. Plasma FN (pFN), secreted by hepatocytes, lacks Extra Domain A (EDA) and Extra Domain B (EDB) segments and has a looped compact conformation. The tissue cellular FN (cFN) is synthesized by various cell types such as fibroblasts, endothelial cells, platelets, and monocytes, bears variable proportions of EDA and/or EDB segments.[9,12] FN has been associated with a variety of inflammatory diseases,[13,14] but research on FN levels in COVID-19 patients is scarce. A large-scale, multi-genomic analysis[15] has shown that the SARS-CoV-2 virus induces an upregulation of the EDA-FN

 https://doi.org/10.2147/IJGM.S517920

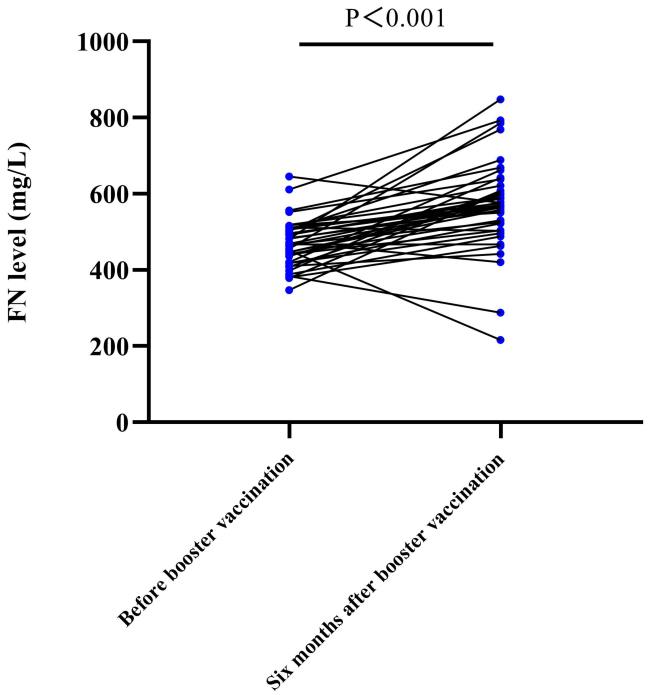

Figure 2 Comparison of FN levels before and after a booster dose of the inactivated SARS-CoV-2 vaccine in healthy donors (n = 40). A paired samples *t*-test was used for the comparison.

form in COVID-19 patients. Further clinical research by Lemańska-Perek et al[16] has shown that the levels of pFN measured in COVID-19 patients were similar to those in healthy adults, while EDA-FN levels were significantly increased. Moreover, the level of both pFN and EDA-FN in severe COVID-19 patients were higher than those in ICU patients with bacterial sepsis. Given that pFN is involved in hemostasis and regulate thrombosis,[17–19] the EDA-FN in plasma can exacerbate stroke by promoting thrombosis post-ischemia.[20] Therefore, it can be inferred from the aforementioned research by Lemańska-Perek et al that the reason severe COVID-19 patients are prone to microthrombus formation and multi-organ damage may be due to the overproduction of FN induced by SARS-CoV-2 infection. However, we have not found any reports on the changes of FN level in recovery COVID-19 patients in the published literature, and it seems that researchers have not paid much attention to this indicator during long-term follow-up of COVID-19 survivors. In this study, 29.4% (10/34) of COVID-19 survivors exhibited elevated levels of FN 6 to 12 months after recovery. The FN levels were slightly higher than the baseline FN levels observed in healthy donors (375.79 ± 83.66 mg/L vs 314.30 ± 67.18 mg/L, P = 0.001). However, FN levels decreased compared to those measured at the early recovery period (two weeks post-discharge) (375.79 ± 83.66 mg/L vs 429.79 ± 106.52 mg/L, P < 0.001). These results suggest that FN levels in COVID-19 survivors may remain elevated for an extended period, but they gradually decline as recovery progresses. The survivors of COVID-19 infection included in this study were all infected with the original strain of SARS-CoV-2 and belonged to a cohort of patients with generally severe disease conditions. Given that patients with pulmonary fibrosis are associated with increased FN deposition in lung tissue,[21,22] it is suggested that some survivors of COVID-19 in this study may have varying degrees of pulmonary fibrosis following recovery.

This study, to our knowledge, is the first to report that the administration of an inactivated SARS-CoV-2 vaccine can significantly increase serum FN levels in humans. This increase can persist for an extended duration (more than six months) regardless of whether individuals were previously infected with SARS-CoV-2 before vaccination. Our research further revealed that there was no statistically significant difference in the degree of FN increase between healthy donors and survivors of COVID-19 infection after receiving two doses of the inactivated SARS-CoV-2 vaccine (P>0.05). Additionally, FN levels further increase following a booster dose of the inactivated vaccine. Unlike attenuated live vaccines, inactivated vaccines do not mimic mild viral infections. Furthermore, aluminum hydroxide is utilized as an

adjuvant in both CoronaVac and BBIBP-CorV, however, we did not find any reports indicating that aluminum hydroxide could lead to elevated FN levels. Therefore, it remains unclear why serum FN levels significantly increase after administration of the inactivated SARS-CoV-2 vaccine.

Previous studies have shown that alterations in FN expression and degradation are associated with several pathologies, including oncogenic transformations and fibrosis. For instance, the mean serum FN level in bladder cancer patients was significantly higher than that in individuals without malignancies (76.794±66.998 ng/mL vs 50.486±25.156 ng/mL, $P = 0.003$),[23] and FN expression was found to increase during liver fibrosis.[24] FN also plays a critical role in thrombus formation.[25] So there is an inevitable concern that persistently elevated serum FN levels may be detrimental to health. However, in our study, we found no significant linear correlation between FN levels and coagulation function indicators measured during the same period (all $P>0.05$).

Upon discovering that the inactivated SARS-CoV-2 vaccine can lead to an increase in FN levels, we intended to extend the follow-up period for the study subjects to further observe the subsequent changes in FN levels. However, due to the relaxation of strict control measures for SARS-CoV-2 by the end of 2022 in our country, all of our study subjects were infected with SARS-CoV-2 one or more times after the last blood collection, making it impossible to continue the study. We hope that other researchers can replicate our study to investigate the long-term dynamics of FN levels in humans after receiving the inactivated SARS-CoV-2 vaccine, particularly the duration required for FN levels to return to pre-vaccination levels.

Limitation of the Study

The sample size included in our study was relatively small, which may limit the generalizability of the results. Additionally, we did not conduct an additional blood draw shortly after vaccination, which prevented us from assessing the trend of FN changes over time following vaccination. Lastly, we observed a significant increase in serum FN levels after administration of the inactivated SARS-CoV-2 vaccine; however, it remains unclear whether this increase led to any adverse effects.

Conclusion

Our findings indicate that the levels of FN increase and remain elevated for an extended period following the administration of the inactivated COVID-19 vaccine. More than 80% of individuals still exhibited elevated FN levels six months after receiving two doses of the inactivated vaccine, and FN levels further increased after booster vaccination. The study's results underscore the necessity for further research to understand the implications of elevated FN levels post-vaccination and their potential impact on individual health. This includes investigating the mechanisms behind the increase in FN levels, the duration of this response, and its correlation with other health outcomes.

Data Sharing Statement

The datasets used during this study are available from the author (Bennan Zhao, E-mail: 993896436@qq.com) on reasonable request.

Ethics Approval and Consent to Participate

This study complies with the Declaration of Helsinki and was approved by the Ethics Committee of Public Health Clinical Center of Chengdu (ethics approval number: PJ-K2021-10-01). All participants provided their informed consent voluntarily and consented to subsequent follow-ups.

Acknowledgment

We thank Zhangjun Wu and Mi Zhou (Public Health Clinical Center of Chengdu, Clinical laboratory).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically

reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This research was supported by key research and development projects jointly implemented by Sichuan and Chongqing in 2020 (cstc2020jscx-cylhX0001), projects from the Chengdu Municipal Health Commission (2021250), and the Chinese Nutrition Society's Nutritionist Empowerment Project (CNS-DNEP2022-34).

Disclosure

The authors declare that they have no competing interests in this work.

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