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Observational Study

Elevated levels of fructosamine are independently associated with COVID-19 reinfection: A 12-mo follow-up study

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Abstract

BACKGROUND

The association between blood levels of fructosamine (FMN) and recurrent coronavirus disease 2019 (COVID-19) is currently unclear.

AIM

To investigate a prospective relationship between blood levels of FMN and COVID-19 reinfection.

METHODS

A total of 146 Chinese hospitalized patients infected with COVID-19 were consecutively collectively recruited and followed up from January 2020 to May 2021. Diagnosis of COVID-19 and reinfection were determined with the diagnostic criteria and treatment protocol in China. The levels of FMN were determined in blood and divided into tertiles based on their distribution in the cohort of COVID-19 patients. Multivariate-adjusted hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated for COVID-19 reinfection across the tertiles of FMN levels. A Cox regression model was used to generate the HR for COVID-19 reinfection in the participants in the top tertiles of FMN levels compared with those at the bottom. Disease-free survival was used as the time variable, and relapse was used as the state variable, adjusted for age, gender, influencing factors such as diabetes mellitus, hypertension and corticosteroid therapy, clinical significance index such as acute liver failure, acute kidney failure, white blood cell (WBC) count, C-reactive protein, prognostic nutritional index (PNI), and blood lipids. Kaplan-Meier analysis with log-rank tests was used to compared the survival rate between patients with elevated FMN levels (FMN > 1.93 mmol/L, the top tertile) and those with nonelevated levels.

RESULTS

Clinical data for the 146 patients with confirmed COVID-19 [age 49 (39-55) years; 49% male] were analyzed. Eleven patients had COVID-19 reinfection. The reinfection rate of COVID-19 in patients with elevated FMN level was significantly higher than in those with nonelevated FMN (17% *vs* 3%; P = 0.008) at the end of 12-mo' follow-up. After adjustments for gender, age, diabetes mellitus, hypertension, corticosteroid therapy, WBC count, PNI, indexes of liver and renal function, and blood lipids, patients with nonelevated FMN levels had a lower risk of the COVID-19 reinfection than those with the elevated FMN levels (HR = 6.249, 95%CI: 1.377-28.351; P = 0.018). Kaplan-Meier analysis showed that the cumulative survival rate of patients infected with COVID-19 was higher in patients with nonelevated FMN levels than in those with elevated FMN levels (97% *vs* 83%; log rank P = 0.002).

CONCLUSION

Elevated levels of FMN are independently associated with COVID-19 reinfection, which highlights that patients with elevated FMN should be cautiously monitored after hospital discharge.

Key Words: Fructosamine; COVID-19; Reinfection; Blood

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Core Tip: Diabetes is a risk factor for coronavirus disease 2019 (COVID-19) which results in increased severity and mortality but has no relationship with reinfection. The present study, for the first time, reported the relationship between COVID-19 reinfection and blood levels of fructosamine (FMN), an index reflecting recent glycemic control. Our results demonstrated that FMN levels may influence the prognosis of

patients with COVID-19, and patients with high FMN levels should be followed up closely to monitor reinfection.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) was identified as an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 2019. It is mainly transmitted by droplets, contact, and aerosols in confined spaces[1,2]. It is highly infectious and widespread[3,4], with more than 505 million patients infected globally, with a cumulative mortality rate of 1.2%[5]. Diabetes is a risk factor for COVID-19 that results in increased severity and mortality[6-8]. A previous study found that of the 570 patients who died or were discharged from hospital, the mortality rate was 6.2% (of 386) for patients without diabetes or hyperglycemia, compared to 28.8% (of 184) for patients who had diabetes and/or uncontrolled hyperglycemia[9]. Hyperglycemia is considered a factor for severity of infection, including severe pneumonia, multiple organ failure, and death. In addition, hemoglobin (Hb)A1c level is an independent risk factor for death and a predictor of COVID-19 severity in patients with diabetes mellitus[10,11].

Fructosamine (FMN) reflects the overall glycemic control for the past 2-3 wk[12] and is strongly correlated with glucose and HbA1c levels[13,14]. HbA1c reflects overall glycemic control over the past 2-3 mo, general blood glucose monitoring reflects glucose levels at the point. General blood glucose monitoring and HbA1c levels cannot accurately contribute to a prediction index for recent glycemic control. FMN level can be determined rapidly and better reflects recent glycemic control. It has also been associated with diabetic retinopathy, diabetic nephropathy, and long-term cardiovascular outcomes[15]. In addition, FMN levels are positively associated with the risk of periprosthetic joint infection and negatively associated with cancer risk. A previous study also demonstrated that FMN is a valuable marker for predicting adverse outcomes following total hip arthroplasty[16]. Hence, FMN correlates with diabetic complications, inflammation, and cancer. However, to date, no studies have

demonstrated its association with COVID-19 risk or reinfection. The objective of the present study was to determine whether there was an association between FMN levels and COVID-19 risk and reinfection. This may provide a theoretical basis for the clinical treatment and prognosis of COVID-19 reinfection.

MATERIALS AND METHODS

Study cohort

Between January and May 2020, we enrolled 146 patients from the isolation ward of Yueqing People's Hospital, a designated isolation hospital for COVID-19. Patients met the diagnostic criteria and treatment protocol for COVID-19 (5th edition). Elevated FMN was defined as levels higher than the upper tertile value of 1.93 mmol/L. The study cohort was divided into two groups based on FMN levels (Figure 1), *i.e.*, elevated FMN group (> 1.93 mmol/L; n = 47) and nonelevated FMN group (≤ 1.93 mmol/L; n = 99). All patients were followed up from January 2020 to May 2021, with the average follow-up being 1 year. The study protocol was approved by the Ethics Committee of Yueqing People's Hospital, Affiliated Hospital of Wenzhou Medical University (Number: YQYY202100033).

Laboratory measurements

Venous blood samples were collected after an overnight fast of ≥ 8 h. All laboratory data were obtained from the first serum collection during hospitalization. The peripheral absolute value of white blood cells (WBCs), lymphocytes, serum creatinine, liver function (alanine and aspartate aminotransferases), lipid profiles (total cholesterol, triacylglycerol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol), and albumin were measured using standard methods. FMN levels were measured using the Roche automatic biochemical analyzer (Basel, Switzerland) and HPLC (Roche). The reference range for FMN was 1.15-2.25 mmol/L. Prognostic nutritional index (PNI) reflects the immune–nutritional status of patients and was determined by calculating serum albumin levels plus a fivefold total number of

lymphocytes. PNI is associated with various cancers, such as lung, breast, and gynecological cancers[17].

Diagnostic criteria

Patients were diagnosed using the Chinese Diagnostic Criteria and Treatment Protocol for COVID-19 (5th edition)[18].

Suspected cases: Comprehensive analysis in combination with epidemiological history and clinical manifestations. Epidemiological history: History of travel or residence in Wuhan and surrounding areas, or other communities where cases have been reported within 14 d before onset of illness; history of contact with a SARS-CoV-2-infected patient (positive for nucleic acid test) within 14 d before onset of illness; history of contact with patients with fever or respiratory symptoms from Wuhan and surrounding areas, or from communities where cases have been reported, within 14 d before onset of illness; and aggregation onset. Clinical manifestations: Fever and/or respiratory symptoms; imaging features of SARS-CoV-2 pneumonia; total WBC count normal or reduced, or lymphocyte count reduced during the early stages of the disease. An individual with an epidemiological history and any two of the clinical manifestations. If there is no clear epidemiological history, three of the clinical manifestations should be satisfied.

Confirmed cases: Suspected cases with one of the following etiological factors. SARS-CoV-2 nucleic acid detected by real-time reverse transcription polymerase chain reaction in respiratory tract specimens or blood samples. Genomic sequencing of the respiratory or blood samples with high homology with SARS-CoV-2.

Discharge criteria

Patients were discharged from isolation and transferred to other wards if body temperature returned to normal and was stable for 3 d, respiratory symptoms improved

significantly, lung imaging showed obvious improvement, and two nucleic acid tests were negative (sampling interval was at least 1 d).

Reinfection criteria

COVID-19 reinfection was defined when a discharged patient had a positive result on the SARS-CoV-2 nucleic acid test measured using nasopharyngeal swabs, sputum, lower respiratory tract secretions, blood, feces, and other samples.

Statistical analysis

IBM SPSS Statistics version 26 (IBM, Armonk, NY, United States) was used for statistical analysis. Normality of data distribution was determined by a one-sample Kolmogorov-Smirnov test. Normally distributed data were expressed as mean ± SD, determined using an independent group *t*-test. Non-normally distributed data were expressed as median and interquartile range and were analyzed using the Mann-Whitney *U* test. The c^2 test was used for intergroup comparisons of categorical variables. Cox regression was used to determine the hazard ratio (HR) with 95% confidence interval (CI) for the positive reinfection across the tertiles of FMN levels, with the bottom tertile group as a reference. Kaplan-Meier analysis was used to determine the cumulative survival rate in patients with FMN level higher than the top tertile compared with that in patients with nonelevated levels, tested using log-rank test. A two-sided *P* value < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics of the study cohort

Of the 146 patients with COVID-19, 77 were male (53%) and 74 were female (47%), with an average age of 49 years. Comparison between the nonelevated FMN and elevated FMN groups showed no significant differences in gender, respiratory failure, WBC counts, C-reactive protein, PNI, alanine transferase, aspartate aminotransferase, serum creatinine, triglyceride, total cholesterol, high-density lipoprotein, and low-density

lipoprotein (all $P > 0.05$) (Table 1). The average age of patients with elevated FMN was higher than that of patients in the nonelevated FMN group [53 (43-58) *vs* 47 (35-53) years, $P = 0.008$] (Table 1). There were significant differences in diabetes mellitus, hypertension and corticosteroid therapy (all $P < 0.05$) (Table 1).

Association of FMN levels with COVID-19 reinfection

The COVID-19 reinfection rate for patients in the elevated FMN group was significantly higher than in the nonelevated FMN group (17% *vs* 3%, $P = 0.008$) (Table 1). In the Cox regression model, disease-free survival (DFS) was used as the time variable, and reinfection was used as the state variable. After full adjustment, the elevated FMN group showed an increased risk of reinfection (HR = 6.249, 95%CI: 1.377-28.351, $P = 0.018$; All P for trend 0.05) (Table 2).

Association of FMN with cumulative DFS rate

Kaplan-Meier survival analysis showed that the cumulative DFS rate in the elevated FMN group was lower compared to the nonelevated FMN group (83% *vs* 97%, $P = 0.002$) (Figure 2). The survival rate was determined using the log-rank test, and the P for trend was 0.05.

DISCUSSION

We found that patients with elevated FMN levels were older compared to patients in the nonelevated FMN group. Elevated FMN levels were positively associated with reinfection rate as well as HR for reinfection, while the cumulative DFS rate was lower for patients in the elevated FMN group. These results demonstrate that FMN levels may influence the prognosis of patients with COVID-19. COVID-19 is an acute inflammatory disease. Previous studies have demonstrated that patients with diabetes and severe disease were less likely to experience recurrence of SARS-CoV-2[19]; however, patients with uncontrolled diabetes had an increased risk of reinfection[20]. Blood glucose monitoring reflects glucose levels at the point of testing and does not reflect overall

blood glucose control. Compared to HbA1c, FMN can reflect blood glucose changes more rapidly. Previous studies have demonstrated that FMN is a good predictor of adverse events following total knee arthroplasty. Patients with high FMN levels were more likely to develop prosthetic joint infections compared to patients with low FMN levels. Unlike FMN, HbA1c does not show a significant association with complications[6]. FMN but not HbA1c is a significant predictor of infection in hemodialysis and diabetes patients with acute infections[21]. In our study, we found that FMN was associated with COVID-19 reinfection. Compared to patients with low FMN levels, patients with high FMN levels were found to have a higher reinfection rate. Patients with high FMN levels had a higher HR for reinfection, while patients with low FMN levels had higher cumulative DFS rates. It appears that FMN levels may predispose individuals to reinfection. Thus, the clinical focus should be on maintaining consistent euglycemia, using standard point-of-care glucose checks.

FMNs are advanced glycation end products (AGEs) generated when glucose reacts reversibly with amino groups in proteins. Reversible aldehyde imine intermediate is formed by the aldehyde group of carbohydrates and the N-terminal amino acids of proteins. However, irreversible AGEs are generated through a Maillard reaction[22]. Maillard reactions have been shown to impair cellular function[23]. FMN-3-kinase-related protein, designated as a potential longevity gene[24], can catalyze deglycation of Maillard intermediates directly downstream from FMN, thereby reducing AGE levels[25,26]. Several studies have demonstrated that AGE levels increase with age[27]. In our study, we found that older patients had higher FMN levels.

High FMN levels usually reflect hyperglycemia, which may lead to poor outcomes. Hyperglycemia enhances the expression of angiotensin-converting enzyme (ACE)2, which is the major cell entry receptor for SARS-CoV-2. ACE2 is widely expressed in the kidneys, lungs, and intestinal mucosal cells. SARS-COV-2 can replicate abundantly in these sites and may contribute to reinfection[28] (Figure 3). Physiologically, hyperglycemia leads to a significant decrease in lymphocyte count, *i.e.*, $CD3^+$ and $CD4^+$ T cells, which in turn reduces humoral immunity mediated by macrophages and

dendritic cells, and induces interleukin-6 and tumor necrosis factor α, *etc.* to induce a cytokine storm[29]. This immunological disorder may increase the occurrence of antibody-dependent enhancement (ADE). In patients who are positive for coronavirus-specific antibodies or are infected by different virus strains, their antibodies may not neutralize the infection, but instead trigger FCγ-receptor-mediated uptake of the virus, leading to an increase in virus numbers in the body[30,31] (Figure 4). Hence, ADE may be another pathological mechanism of positive securement of SARS-CoV-2.

There were some limitations to the present study, which may have introduced potential bias. First, the study was a prospective, single center, small cohort study. Additional multicenter studies using larger patient cohorts should be performed to validate our findings. Second, HbA1c data for some of the patients were not available, which affected our comparative analysis of HbA1c and FMN levels. Third, diabetes was not excluded in the inclusion criteria, but we adjusted for diabetes.

CONCLUSION

Elevated FMN levels were found to predispose COVID-19 patients to reinfection and hence should be followed up closely to monitor reinfection.

ARTICLE HIGHLIGHTS

Research background

Diabetes is a risk factor for coronavirus disease 2019 (COVID-19) which results in increased severity and mortality but has no relationship with COVID-19 reinfection. No study has reported the relationship between COVID-19 reinfection and blood levels of fructosamine (FMN). The present study for the first time reported this relationship.

Research motivation

We mainly investigate the relationship between blood levels of FMN and COVID-19 reinfection.

Research objectives

We found that FMN levels may influence the prognosis of patients infected with COVID-19, which highlight that the hospitalization patients with elevated levels of FMN should be cautiously monitored at post discharge.

Research methods

A total of 146 inpatients from the designated isolation hospital for COVID-19 patients, who were satisfied based on the diagnostic criteria and treatment protocol of COVID-19 (Fifth Edition). The study cohort was divided into two groups based on FMN levels, elevated FMN was defined as levels higher than its upper tertile value, with the average follow-up period being one year. Cox regression was used to determine the hazard ratios (HRs) with 95% confidence intervals for the positive reinfection across the tertiles of FMN levels. Kaplan-Meier analysis was used to determine the cumulative survival rate in the patients with higher than the top tertiles of FMN levels compared with those with non-elevated levels, tested using log-rank.

Research results

We found that patients with elevated FMN levels were older than the non-elevated FMN group. Elevated FMN levels were positively associated with reinfection rate as well as HR for reinfection, while the cumulative disease-free survival rate was lower for patients in the elevated FMN group. These results demonstrate that FMN levels may influence the prognosis of patients infected with COVID-19.

Research conclusions

Additional multicenter, hemoglobin A1c data available studies using larger patient cohorts should be performed to validate our findings.

Research perspectives

Elevated levels of FMN are independently associated with COVID-19 reinfection, which highlight that the COVID-19 patients with elevated levels of FMN should be followed up closely to monitor reinfection.

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