

## **Relationship of Hypocalcemia and Severity of Disease in COVID-19 Patients in Lahore Pakistan**

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**Abstract:** Introduction: In Pakistan, a wave of coronavirus has started in February which was first reported in Wuhun City China. Abnormal calcium blood level is considered one of the important and common electrolyte disturbances in patients with severe infection. The effect of blood calcium level on the severity of COVID-19 and the possible immune regulation mechanism is considered to be an important factor related to severity of disease. Aim of the Study: To find out the correlation of blood calcium levels and related risk factors of patients suffering from COVID-19 and provide a basis for early intervention in patients with relevant clinical characteristics and further reduce the incidence of critical illness and mortality in COVID-19 patients. Method: 180 COVID-19 patients were admitted to Gulab Devi Teaching Hospital, Lahore between February to July 2020. Subject index were tested on real-time fluorescent RT-PCR test. On the basis of the result of RT-PCR, they were divided into two groups according to clinical symptoms as mild (control/placebo group) and critical (experimental group). The patient with the mild symptom were included in the placebo group, whereas patient with the severe symptoms were included in the experimental group. Exclusion criteria: The patients who do not show the detection level of viral infection with the RT-PCR are excluded from the study. Results: The comparison has been carried out between the placebo group and the experimental group on the basis of various parameters. The patients serum calcium level was tested and the value for hypocalcemia was declared as per universal recommendation which is  $< 2.13\text{mmol/L}$ . According to the corrected serum total calcium level, COVID-19 patients can be divided into hypocalcemia group and normal blood calcium group. The result showed that hypocalcemia and immune dysfunction was found high in experimental group as compare to control group which shows a strong correlation between hypocalcemia and COVID-19 disease. Conclusion: It has been concluded from the study that patients with COVID-19 have obvious hypocalcemia and immune dysfunction, especially in experimental group patients. In this study, it is easy to be combined with multiple infections caused by pathogenic bacteria under certain conditions. Close monitoring of blood calcium levels can predict the severity of the disease more effectively.

**Keywords:** Hypocalcemia, COVID-19, Severity of Disease, RT-PCR, Viral Infection, Blood Calcium Level

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## 1. Introduction

In Pakistan, a wave of coronavirus has started in February that was first reported in Wuhun City China. This wave continues to spread coronavirus that has never been detected in humans or animals, called COVID-19 [1]. The World Health Organization (WHO) declared COVID-19 as a pandemic disease which showed reaction of different degrees resulting in severity of symptoms in patients. As of July 31, 2020, a total of 2,79,146 people have been diagnosed in Pakistan, and a total of 5,970 people have died which is in comparison to the Hubei province of China was quite few [2, 3]. Total numbers of death are less as compare to the Wuhun city of China [4].

A study has been carried out regarding the prevalence of hypocalcemia in the patients admitted with COVID-19 infection. It has been found out that the hypocalcemia was present in most of the hospitalized patients with COVID-19 disease [5]. Another study has been carried out about the hypocalcemia in the patients suffering with COVID-19 disease. It was found out that the hypocalcemia is an important feature found in the COVID-19 patients [6].

Another study has been carried out to find out mechanistic basis and therapeutic relevance of hypocalcemia during severe COVID-19 infection. The result of the study showed that the hypocalcemia was found to be important clinical indicator with reference to severity of disease in the COVID-19 patients [7]. Therefore, it is extremely important to accurately and effectively judge the severity and prognosis of COVID-19 infection. Abnormal calcium metabolism is one of the important and common electrolyte disturbances in patients with severe infection, the effect of blood calcium level on the severity of COVID-19 and the possible immune regulation mechanism is yet to be determined [8].

## 2. Background

Corona virus is a new pandemic disease which has detected the persons globally. Recently all the countries have started research and try to share their research with all the countries in order to save the precious lives globally. The relationship between the electrolyte balance and severity of the disease in COVID-19 infected patients has gain importance in terms of its clinical importance.

Guan et al. carried out a research to find out the clinical characteristics of patients suffering from COVID-19. He concluded that the level of calcium and other electrolytes played an important role in carrying out immunological functions in the world. He was of the opinion that the clinical characteristics of the patients must be properly and regularly being monitor in order to find out their proper relevance to the disease [9].

Millet and Whittaker carried out a research on the SARS-CoV patients in order to find out the relationship of calcium ion concentration and its physiological and molecular triggers membrane fusion and entry into host cells. He found

out that the concentration of the calcium ion plays an important role for the transportation across the membrane in to host cell as the similarity between the SARS virus and the corona virus is similar. So the detailed study is needed to find out calcium ion concentration to the severity of the disease [10].

Straus et al. carried out a research on concentration of calcium ions in corona virus patients. He found out that  $Ca^{2+}$  ions promote fusion of Middle East respiratory syndrome coronavirus with host cells and increase infectivity. His research concluded that the constriction of calcium ions play an important role in the immune response by the body [11].

Nathan et al. carried out a research on the calcium ions and its relation to Ebola virus fusion peptide and its relationship to infection in infected persons. His study concluded that calcium ions interact with Ebola virus fusion peptide that promotes structure-function changes in the infected persons [12].

Bossoni et al. carried out a study to find out the relationship of hypocalcemia with other comorbid conditions suffering from COVID-19. His study concluded that severe hypocalcemia in a thyroidectomized woman with Covid-19 infection has increase the severity of the disease suffering from corona virus [13].

## 3. Aim of the Study

To find out the correlation between blood calcium levels and related risk factors of patients suffering from COVID-19 and provide a basis for early intervention in patients with relevant clinical characteristics and further reduce the incidence of critical illness and mortality.

## 4. Methods and Material

*Study Type:* Retrospective Study

*Settings:* This study has been conducted in Gulab Devi Teaching Hospital, Lahore, Pakistan.

*Duration of Study:* Six Months from February 2020 to July 2020

*Sampling Technique:* The data has been collected from the subject index coming to the Quarantine Center, CCU and ICU Gulab Devi Teaching Hospital, Lahore, Pakistan through random number table method which were further divided into experimental group and the control group.

*Sample Selection*

*Inclusion Criteria:* All persons coming to the Quarantine Center, CCU and ICU Gulab Devi Teaching Hospital, Lahore, Pakistan.

*Exclusion Criteria:* All persons coming to the Quarantine Center, CCU and ICU Gulab Devi Teaching Hospital, Lahore, Pakistan who were confirmed through RT-PCR technique but not suffering from the COVID-19

*Study Tools:* Questionnaire and Interview

*Sample size* (n)=180 (Control/placebo Group=140, Experimental Group=40; 90 males and 90 females)

#### 4.1. Methodology

The patients which were tested positive by the RT-PCR were divided into control and experimental group. The selected patients who met the diagnostic criteria of COVID-19 as proposed by Chinese Health Commission was adopted [14]. The following signs and symptoms were recorded to find out the improvement in the different levels before and after the therapy in the control and experimental group. Following parameters were tested and compared between the controlled and experimental group:

- 1) blood routine,
- 2) inflammation indicators
- 3) C-reactive protein (CRP),
- 4) various biochemical indicators,
- 5) alanine aminotransferase (ALT),
- 6) aspartate aminotransferase (AST),
- 7) alkaline phosphatase (Alkaline phosphatase (ALP),
- 8) blood phosphorus (phosphorus, P),
- 9) blood calcium,
- 10) ALB, the percentage of T lymphocyte subsets (CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells)
- 11) cytokines
- 12) interleukin 4 (IL-4) Interleukin 17 (IL-17), interferon  $\gamma$  (IFN- $\gamma$ ).
- 13) glomerular filtration rate (eGFR) by the dietary improvement experimental formula for kidney disease,
- 14)  $EGFR=186x[\text{blood creatinine } x0.011]^{-1.154} x[\text{age}]^{-0.203} x0.742 \text{ (female)} x1.233$

The correction formula for serum total calalbumin is: corrected blood calcium value (mmol/L)=measured calcium value (mmol/L)-0.02x [measured value of serum albumin (ALB) g/L-40].

#### 4.2. Data Analysis

Data has been collected in the MS Excel sheet which was

exported to SPSS 26.0 for analysis. For the purpose of analysis of normal distribution, mean and standard deviation was calculated. For non-normal distribution, the median and interquartile range was calculated, t test was performed in order to compare between the groups. For the experimental group *Multivariate Logistic* regression analysis was performed  $P < 0.05$  was considered as the difference which taken as significant.

## 5. Results

### 5.1. Comparison of Various Indicators Between the Experimental Group and the Placebo Group

There were 40 cases in the experimental group, with an average age of 60.91±18.02 years old, and males accounted for 52.5%. The patients serum calcium level was tested and the value for hypocalcemia was declared as per universal recommendation which is < 2.13mmol/L [15]. Among them, 10 cases of hypertension (accounting for 25%), 17 cases of diabetes (accounting for 42.5%), 9 cases of basic respiratory disease (accounting for 22.5%), 11 cases of cardiovascular disease (accounting for 27.5%); placebo group 140 cases, The average age is 45.23±15.14 years old, and males account for 49.28%. Among them, 21 cases of hypertension (accounting for 15%), 10 cases of diabetes (accounting for 7.2%), 5 cases of basic respiratory disease (accounting for 3.6%), and 7 cases of cardiovascular disease (accounting for 5%); compared with the placebo group, CRP, FPG, IFN- $\gamma$ , IL-17 levels are elevated, the percentage of lymphocytes, ALB, corrected blood calcium, CD4<sup>+</sup> T Cell% and CD8<sup>+</sup> T cell% levels decreased and the difference was statistically significant ( $P < 0.05$ ); the two groups had no composition of gender, proportion of hypertension, ALT, eGFR, CD4<sup>+</sup>/CD8<sup>+</sup> ratio, and IL-4 level significant difference, ( $P > 0.05$ ) (see table 1).

**Table 1.** Comparison of various indicators between the experimental group and the placebo group.

index	Experimental group [ $\bar{x}\pm s/\text{cases} (\%)$ ]	Placebo group [ $\bar{x}\pm s/\text{number of cases} (\%)$ ]	P value
Number of cases (n)	40 (22.22)	140 (77.78)	
Age	60.91±18.02	45.32±15.14	0.001*
Male n (%)	21 (52.5)	69 (49.28)	0.832
Complications			
High blood pressure	10 (25)	21 (15)	0.303
Diabetes	17 (42.5)	10 (7.2)	0.001*
Underlying respiratory disease	9 (22.5)	5 (3.6)	0.001*
Cardiovascular diseases	11 (27.5)	7 (4.5)	0.001*
Systolic blood pressure (mmHg)	131.01±20.03	125.20±15.05	0.101
C-reactive protein (mg/L)	28.1 (7.85, 93.75)	9.07 (2.98, 22.03)	0.003*
Percentage of lymphocytes (%)	27.13±11.99	16.97±9.10	0.001*
Fasting blood glucose (mmol/L)	8.01±3.52	5.99±2.01	0.005*
ALT (U/L)	45.21±12.03	33.96±9.93	0.100
eGFR (ml. min-1. (1.73m2)-1)	106.98±22.97	109.89±19.17	0.380
Corrected blood calcium (mmol/L)	2.11±0.17	2.30±0.22	0.001*
CD4+T cells (%)	45.71±11.03	52.02±14.21	0.002*
CD8+T cells (%)	22.22±11.04	34.13±8.99	0.001*
CD4+/CD8+ ratio	2.20±0.49	2.01±0.79	0.240
IL-4 (pg/mL)	1.10±0.43	0.90±0.30	0.353
IFN- $\gamma$ (pg/mL)	3.13±0.61	1.52±0.29	0.028*
IL-17 (pg/mL)	1.82±0.53	1.31±0.62	0.041*

Note: 1mmHg=0.133kPa; ALT: alanine aminotransferase; eGFR: estimated glomerular filtration rate; IL-4: interleukin 4; IFN- $\gamma$ : interferon  $\gamma$ ; IL-17: interleukin 17; \*  $P < 0.05$ .

### 5.2. Analysis of Influencing Factors in Experimental Group

In the experimental group, the dependent variable and the adjusted blood calcium level, age, gender, presence or absence of comorbidities, SBP, CRP, FPG, ALT, eGFR, CD4

+ T cell%, CD8 + T cell% as independent variables, multi-factor Logistic regression analysis has been done after adjusting for decreased blood calcium levels, age and eGFR increased risk factors for patients suffering from severe COVID-19 (see table 2).

Table 2. Logistic regression analysis of influencing factors in experimental group.

Variable	$\beta$ value	Standard error	P value
Constant	19.674	16.031	0.216
Corrected blood calcium (mmol/L)	23.321	8.213	0.004*
Age	0.201	0.089	0.031*
Gender	1.702	1.763	0.335
With or without complications	1.212	1.929	0.534
Systolic blood pressure (mmHg)	0.011	0.045	0.789
C reactive protein (mg/L)	0.012	0.019	0.535
Percentage of lymphocytes (%)	0.046	0.098	0.630
Fasting blood glucose (mmol/L)	0.559	0.389	0.149
ALT (U/L)	0.074	0.048	0.107
eGFR (ml•min-1•(1.73m2)-1)	0.166	0.082	0.044*
CD4+T cells (%)	0.081	0.070	0.234
CD8+T cells (%)	0.151	0.109	0.169

Note: ALT: alanine aminotransferase; eGFR: estimated glomerular filtration rate; \*P < 0.05.

### 5.3. Comparison of Various Indexes Between Low Calcium Group and Normal Calcium Group

When the patient was admitted to the hospital without treatment, the serum total calcium level before correction was (2.12±0.23) mmol/L, and the serum total calcium level after serum albumin correction was (2.18±0.19) mmol/L. Among them, 61 patients with low calcium, accounting for 37.22%. In order to analyze the causes of low calcium,

compared with the normal calcium group, the low calcium group has lower levels of serum albumin, CD4 + T cell%, CD8 + T cell%, age, the proportion of experimental group patients, combined diabetes, respiratory diseases and cardiovascular diseases ratio, CRP levels, the difference was statistically significant (P < 0.05). Biochemical parameters were not statistically different (P > 0.05) (see table 3).

Table 3. Comparison of various indexes between low calcium group and normal calcium group.

index	Low calcium group [ $\bar{x}$ ±s/number of cases (%)]	Normal calcium group [ $\bar{x}$ ±s/number of cases (%)]	P value
Number of cases (n)	67 (37.22)	113 (62.78)	
Age	53.45±19.72	44.01±16.22	0.001*
Male n (%)	36 (53.73)	59 (52.21)	0.543
Critical illness n (%)	29 (43.3)	8 (7.08)	0.001*
Complications			
High blood pressure	12 (17.9)	19 (16.8)	0.527
diabetes	13 (19.4)	12 (10.6)	0.043*
Underlying respiratory disease	9 (13.4)	5 (4.4)	0.042*
Cardiovascular diseases	11 (16.4)	6 (5.3)	0.007*
C-reactive protein (mg/L)	12.99 (4.98, 48.01)	9.15 (2.32, 24.97)	0.042*
Percentage of lymphocytes (%)	23.18±11.99	26.03±13.01	0.234
Blood calcium before correction (mmol/L)	1.89±0.21	2.19±0.15	0.001*
Corrected blood calcium (mmol/L)	2.01±0.17	2.32±0.15	0.001*
Blood phosphorus (mmol/L)	1.09±0.29	1.19±0.21	0.297
Alkaline Phosphatase (U/L)	61.03±16.98	62.12±16.13	0.933
ALT (U/L)	43.12±17.01	34.96±8.19	0.380
AST (U/L)	44.98±13.97	30.95±4.90	0.107
eGFR (ml•min-1•(1.73m2)-1)	109.02±19.14	113.02±13.97	0.543
Serum albumin (mmol/L)	33.96±5.38	43.32±3.46	0.001*
CD4+T cells (%)	46.03±13.34	53.16±13.14	0.01*
CD8+T cells (%)	26.32±9.89	33.12±10.08	0.001*
CD4+/CD8+ ratio	2.09±1.91	1.69±0.79	0.129
IL-4 (pg/mL)	1.51±0.49	1.53±0.50	0.265
IFN- $\gamma$ (pg/mL)	2.90±0.71	2.09±0.56	0.446
IL-17 (pg/mL)	2.72±0.48	2.19±0.43	0.275

Note: ALT: alanine aminotransferase; AST: aspartate aminotransferase; eGFR: estimated glomerular filtration rate; IL-4: interleukin 4; IFN- $\gamma$ : interferon  $\gamma$ ; IL-17: Interleukin 17; \*P < 0.05

## 6. Discussion

COVID-19 is a lung inflammation caused by a new type of coronavirus. It is highly contagious and changes rapidly. Severe cases can cause breathing difficulties, organ dysfunction, and even death. With a global outbreak, its high pathogenicity and the mortality rate has attracted worldwide attention. For the purpose of COVID-19 patient diagnosis, we are currently using indicators to assess the condition and prognosis of patients such as: CRP, procalcitonin (Procalcitonin, PCT), white blood cell count, etc. that have advantages and disadvantages of diagnosis, so more indicators are needed to comprehensively evaluate the prognosis of the disease.

Calcium in the body not only constitutes teeth and bones, but also participates in neurotransmitter release, hormone secretion, maintenance of blood coagulation and internal environment stability. The vast majority of blood calcium exists in plasma, including diffuse calcium and non-diffuse calcium. The former is mainly physiologically active. The latter can be combined with plasma albumin, accounting for 40%-50% of the total, and the two can be converted to each other to achieve dynamic balance.

Booth *et al.* conducted a retrospective multicenter study of 144 patients with severe acute respiratory syndrome (SARS) in the Toronto area, the incidence of hypocalcemia is as high as 60% [16]. Comprehensive clinical and laboratory characteristics can help improve disease diagnosis and poor prognosis analysis capabilities.

Qing *et al.* carried out a study of 25 untreated SARS patients showed that hypocalcemia was found in the experimental group in great numbers [17].

Our study was a retrospective study. This study included 180 patients with COVID-19 in Gulab Devi Teaching Hospital, Lahore. Among them, 37.22% of them had lowered blood calcium levels, and the blood calcium level was significantly negatively correlated with the severity of the disease, indicating that hypocalcemia is more common in COVID-19 patients, which is partly consistent with previous studies on other types of coronavirus infections in China and USA and may be related to differences in the degree and types of diseases included in this study [18].

Due to the relatively insidious manifestations of hypocalcemia, clinical work is often ignored [19]. Therefore, hypocalcemia as the basis of infection in COVID-19 patients or the clinical manifestations after infection is still inconclusive.

Early effective evaluation of the clinical and laboratory characteristics of experimental group patients as compare to control group with proper intervention can effectively reduce the incidence of severity of disease in the experimental group patients.

Compared with the placebo group and experimental group suffering from COVID-19, the proportion of diabetes, underlying respiratory disease and cardiovascular disease and the level of FPG were found significantly higher. Age and eGFR independent of other influencing factors can effectively predict

the severity of COVID-19, suggesting that elderly patients are often accompanied by a decline in glomerular filtration function hence are more susceptible to the coronavirus, and are more likely to worsen after onset. Gender differences such as female X chromosome and sex hormone levels may reduce the protective effect of virus susceptibility [20]. It is not reflected in this study, and it is considered that it has caused by regional differences and limited sample size.

Studies have shown that both total calcium and adjusted calcium ion concentrations in ICU experimental group patients can assess disease severity to a certain extent [21]. To further analyze the causes of hypocalcemia in COVID-19 patients, the specific analysis is as follows:

1. We use the corrected total calcium level as the observation index. As the age increases, the proportion of chronic comorbidities increases and patients with low calcium are more likely to suffer from COVID-19. The higher the CRP level, the more is severity of disease. Severe stress or increased consumption, decreased synthesis and absorption and increased capillary permeability, the loss of albumin will increase, leading to calcium binding protein and non-diffusible calcium levels decrease, resulting in hypocalcemia. Because of low calcium, it is unable to perform normal physiological functions and there are serious complications such as the cardiovascular system [22]. Giustina and Formenti carried out a research on the relationship of hypocalcemia and its impact on the preventing a covid-19 disease in Italy. The result of his find were found in consistence with our study [23].
2. The level of liver ALT enzyme increased and the level of eGFR reflecting glomerular function decreased. Although the difference is not statistically significant, it still suggests a certain degree of liver and kidney damage, which may lead to decreased liver and kidney hydroxylase activity and activity of hydroxyvitamin D3 (1,25 (OH)2D3). Hypocalcemia occurs due to insufficient synthesis of hydroxyvitamin D3 (1,25 (OH)2D3). Thomas *et al.* carried out a research and found of the impairment of renewal function in COVID-19 patients. The finding of his study was found to be in consistent with our present study [24].
3. The disease broke out in winter and spring. People's outdoor activities and UV exposure time were relatively reduced, and vitamin D synthesis in the body decreased, resulting in insufficient 1,25 (OH)2D3 synthetic substrates and induced hypocalcemia. This is similar to the results of a recent study published on medRxiv. The study found that it may be related to latitude and longitude, light, skin pigment, and eating habits [25].
4. This study found that there was a certain degree of decline in the level of T lymphocyte subsets in the low calcium group, suggesting that there may be a certain correlation between the decline in immune function and the deficiency of trace elements, but the specific mechanism is not yet clear [26].

T lymphocytes and their subgroups play an extremely important role in maintaining immune function. According to function and molecular phenotype, T lymphocytes can be divided into CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells. The former are mainly differentiated into Th1, Th2, Th17 and Regulatory T cells (regulatory T cells, Treg) four types of helper T cells, through the synthesis and secretion of cytokines to assist and regulate the immune response. Among them, Th1 cells secrete pro-inflammatory response factors mainly IFN- $\gamma$ , Th2 cells secrete anti-inflammatory cytokines mainly IL-4 and pro-inflammatory cytokines mainly IL-17 secreted by Th17 cells. We have observed decreased levels of lymphocytes, CD4<sup>+</sup> T cells, and CD8<sup>+</sup> T cells in COVID-19 patients and increased levels of CRP, IFN- $\gamma$ , and IL-17 factors, suggesting that in the acute phase of infection in experimental group patients with COVID-19. It cannot be ruled out that the coronavirus replicates in lymphocytes in large numbers or even directly destroys lymphocytes, causing continuous responses of cytokines and chemokines. Th1 and Th17 pro-inflammatory helper T cell subsets may be the main functional groups involved in immune disorders, and then increased inflammation, produce a "cytokine storm", resulting in deterioration of the degree of tissue damage. The results were similar to SARS moderate respiratory syndrome (Middle East respiratory syndrome, MERS) part [27, 28].

The mechanism of hypocalcemia in experimental group patients has not been fully elucidated. Some scholars believe that some cytokines and adhesion molecules may affect cell membrane permeability and calcium pump opening, leading to abnormal calcium ion transport inside and outside the cell [29]. As an important metabolite that regulates calcium and phosphorus metabolism, 1,25 (OH)<sub>2</sub>D<sub>3</sub> can also inhibit the production of Th1 and Th17 factors in the body, and has a certain immunomodulatory effect on T cell-mediated autoimmune diseases and sequelae of cerebral infarction [30]. Therefore, COVID-19 experimental group patients with hypocalcemia may also be widespread due to the lack of immunosuppression of active vitamin D and further research is urgently needed.

## 7. Conclusion

It has been concluded from the study that the blood calcium level is an important parameter to check the severity of the disease in COVID-19 patients. It has been found out that the monitoring of blood calcium levels can provide us about the severity of disease in the COVID-19 patients. The patients serum calcium level are hypocalcemia as per universal recommendation is < 2.13 mmol/L. On the basis of this serum calcium level the deficiency of serum calcium level as shown sever impact on the progression of the disease. It is therefore concluded that the hypocalcemia and immune dysfunctions are correlated in COVID-19 disease. The patients with COVID-19 have obvious hypocalcemia and immune dysfunction, especially in experimental group patients. In this study, it is easy to be combined with multiple infections caused by pathogenic bacteria under certain

conditions. Close monitoring of blood calcium levels can predict the severity of the disease more effectively.

## 8. Limitation

This study has been carried on a very limited scale in the city of Lahore which has included only one quarantine center in a teaching hospital of Lahore. More studies with large number of subject index should be conducted in order to find out more reliable and generalized results.

## Conflict of Interest

All authors declare that there is no conflict of interest.

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