Serum IFN-γ and Electrolyte Dysregulation as Independent Predictors of COVID-19 Severity: A Cross-Sectional Study in Iraqi Patients

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Abstract- COVID-19 pandemic has caused a significant mortality and morbidity in Iraq. Evidence is mounting that immune dysfunction and electrolyte derangements are key factors in disease progression and clinical outcome. The aim of this study was to assess the diagnostic utility of serum interferon gamma (IFN-gamma) along with electrolyte levels (potassium, sodium, chloride and calcium) as markers for the severity of the COVID-19 disease using ROC curve analysis. This cross-sectional study included 200 confirmed COVID-19 (severe, 100 patients; non-severe, 100 patients) admitted to Dar Al-Salam Hospital in Baghdad during January to December 2023. Severity was determined at admission according to the criteria set by the WHO. The level of serum IFN-gamma was measured by sandwich ELISA and electrolytes (Na, K, Ca and Cl) were estimated by biochemical assays with standard chemicals. Mann-Whitney U, chi-square, Spearman correlation, and ROC curve analyses were carried out using SPSS version 26 (IBM Corp., Chicago, IL, USA). Median serum IFN-gamma was significantly higher in non-severe patients than in severe patients (30.71 vs 7.81 pg/ml; P<0.001). Sodium levels were lower in severe cases (139.95 vs 146.80 mmol/L; P<0.001), while calcium was elevated (10.27 vs 9.37 mg/dL; P<0.001). Chloride and potassium were significantly higher in severe patients (P<0.001). ROC analysis demonstrated excellent discriminatory ability for IFN-gamma (AUC=0.917, 95% CI: 0.876-0.957), good ability for calcium (AUC=0.857) and chloride (AUC=0.848), and good ability for sodium (AUC=0.827). Potassium showed fair discriminatory ability (AUC=0.702), with elevated levels associated with disease severity. Elevated IFN-gamma in non-severe cases indicates its association with reduced inflammation and recovery, while low levels correlate with disease progression. Electrolyte imbalance plays a pivotal role in COVID-19 severity. IFN-gamma, calcium, and chloride demonstrate excellent to good predictive performance for disease severity.

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, was first detected in China in November 2019. Approximately 400 million people were diagnosed, and more than 6 million cumulative deaths were reported (1-3). Recently, different studies have reported a high prevalence of electrolyte imbalances in COVID-19 patients, associated with more severe infection (4). Some of these elements affect the immune response and may therefore influence the response to COVID-19 infection (5).

This study aims to investigate the effect of COVID-19 on IFN-γ and macro-element (Na, Ca, K, and Cl) levels. Interferon-gamma is a type II IFN generated by T-cell lymphocytes and natural killer (NK) cells (6). Cytokines are important in the immune response and are essential for antiviral defense, activating cytokine

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production by T-cells, downregulating viral replication, and inducing the killing activity of cytotoxic T-lymphocyte cells (7). Likewise, continuously elevated levels of IFN-γ without proper control and regulation can cause organ failure, tissue injury, and increased inflammation (8). The role of IFN-γ in disease outcomes remains unclear, particularly regarding the serum patterns of this cytokine and other related cytokines in COVID-19. On the other hand, disease progression has been directly associated with dysregulation of the host immune response, culminating in inflammation and cytokine release syndrome, which is known as a cytokine storm (9). This theory gains further support from the fact that the use of steroids as anti-inflammatory agents has appeared to reduce the mortality rate in approximately 29% of patients. However, the exact role of these cytokines in severe cases remains ambiguous. Moreover, the significant immune response dysregulation caused by cytokine release syndrome is not well understood.

On the other hand, macro-element levels were also significantly affected during COVID-19. Sodium (Na), one of the most critical electrolytes, was markedly decreased in the serum of severe COVID-19 patients, a condition known as hyponatremia (10). Hyponatremia was recorded more frequently in severe cases and in patients who required ICU admission. Non-severe cases did not meet any of the above criteria (11-12). Sodium deficiency may increase the risk of disease progression from non-severe to severe COVID-19; therefore, monitoring the level of this element in COVID-19 patients is desirable to help discriminate between severe and non-severe cases (13). Other studies reported hypernatremia associated with increased activity of secondary angiotensin II in response to this virus, which induced downregulation of angiotensin-converting enzyme 2 receptors. In addition, hypernatremia likely reflects dehydration due to water-insensitive losses, such as tachypnea and fever, which represent the main signs and symptoms in severe patients (14). Another macro-element is potassium. Different studies have detected that hyperkalemia (increased potassium level) plays an important role in the increased mortality and severity of COVID-19. Elevated potassium levels are common among patients with congestive heart failure, chronic kidney disease, and diabetic myelitis, and are also common among patients using renin-angiotensin-aldosterone system (RAAS) inhibitors, which disturb potassium homeostasis and could lead to an increased serum potassium level (15).

Various studies have also observed an increased chloride level, known as hyperchloremia, in critical conditions among COVID-19 patients by the third day of admission (16). However, data regarding the role of hyperchloremia in COVID-19 infection remain scarce. The results obtained by different studies indicate that hyperchloremia could significantly evoke and augment the inflammatory mediator response, such as interleukins, and negatively affect COVID-19 patient outcomes through stimulation of the nuclear factor kappa B (NF-kB) pathway, which increases inflammatory factors and exacerbates the severity of COVID-19 (17). This dysregulated immune response ultimately leads to tissue damage and organ dysfunction. Serum chloride acts as one of the most prevalent anions in the extracellular fluid, contributing to almost one-third of extracellular fluid osmolality. It plays a critical role in maintaining homeostasis and body physiology (18). Cl is essential for gastric motility, maintenance of cardiac contractility, and coagulation. Elevated chloride levels are more common in patients requiring ICU admission. In the setting of hyperchloremia, suppression of erythropoietin may result in anemia. Furthermore, renal vasoconstriction may alter kidney perfusion, which contributes to the occurrence of acute kidney injury (AKI) (19).

COVID-19 also affects calcium levels and causes hypercalcemia (elevated calcium level), especially in severe and critical cases (20). Calcium disturbances have been associated with a poor prognosis. In viral infections in general, calcium plays a crucial role in the formation of viral structure and also helps the virus enter the cell, undergo maturation, regulate gene expression, and release viral particles (21). Ca plays a vital role in the pathogenesis of COVID-19, and hypercalcemia has been associated with severe and critical cases, as well as poor outcomes (22). Hypercalcemia may result from dysregulation of vitamin D levels in the bloodstream and parathyroid hormone. The elevated levels of unbound and unsaturated fatty acids observed in severe cases of COVID-19 may be associated with Ca and result in acute hypercalcemia (23). Various viruses use calcium signaling to provide more favorable conditions for their benefit, and changes in ion homeostasis, especially Ca, stimulate conditions favorable for viral growth (24). This element has an essential role in viral replication and fusion through direct interaction with viral fusion peptides (25). Infectious viral progression requires the use of host Ca for viral entry, gene expression, viral structure formation, virion maturation, and release (26). Viruses that use calcium pumps or calcium channels disturb calcium homeostasis in the body, which leads to increased host morbidity (27). Another study revealed that hypercalcemic COVID-19 patients had elevated viral

load and prolonged periods of viral shedding; therefore, hypercalcemia was considered one of the independent risk factors for prolonged hospitalization, and calcium levels are recommended to be monitored in all cases of COVID-19 (28,29). The discrepancy among results requires further investigation to determine the clinical impact of electrolyte elements on COVID-19 mortality and outcomes.

Materials and Methods

This cross-sectional study was conducted on 200 hospitalized patients with RT-PCR-confirmed COVID-19, enrolled between January and December 2023 at ALZahraa Hospital of Imam Al-Hussain Medical City, Karbala/Iraq. All participating patients presented with signs and symptoms of COVID-19 confirmed by RT-PCR. Based on disease severity at the time of hospital admission, and according to guidelines issued by the Iraqi Ministry of Health (30), patients were consecutively classified into two groups: a severe group and a non-severe group, each consisting of 100 patients.

The sample size was determined based on a previous comparable study, which reported an electrolyte imbalance prevalence of approximately 50% among severe COVID-19 patients. By using a two proportion z-test (80% power, α=0.05), the minimum number of patients per group was determined to be 97, and 100 patients were enrolled in each group to allow for possible incomplete records.

Baseline clinical parameters included at hospital admission were age, sex, days from onset of symptoms to hospital admission and comorbidities (diabetes mellitus, cardiovascular disease, hypertension and immunosuppressed status). Five milliliters (5 mL) of venous blood were collected from each participant at the time of hospital admission, prior to any therapeutic intervention. Blood samples were transferred into plain (serum-separating) tubes, allowed to clot at room temperature, and centrifuged at 3000 rpm for 10 minutes to obtain serum. Serum aliquots were stored at −20° C until the time of analysis.

Serum electrolyte levels (Na, K, Cl, and Ca) were assessed in all study groups using standardized automated biochemical analyzers, while the duration of ICU admission and oxygen therapy requirements (supplemental oxygen flow rate in L/min or FiO2) were recorded from medical charts.

Serum samples were analyzed for IFN-γ determination using a sandwich ELISA kit (R&D Systems, Minneapolis, MN, USA), following the manufacturer's instructions.

Selection criteria

Included patients were hospitalized adults (aged ≥18 years) with RT-PCR-confirmed COVID-19. Patients were classified as either severe or non-severe. Severe cases met at least one of the following criteria: respiratory rate >30 breaths/min, oxygen saturation (SpO_2) <90% on room air, or PaO_2 /FiO_2 ratio <300 mmHg, and/or required ICU admission. Non-severe cases showed evidence of lower respiratory disease through clinical evaluation or imaging and had an oxygen saturation (SpO_2) ≥94% on room air, with various signs and symptoms of COVID-19. Patients with insufficient clinical documentation, incomplete laboratory records, or prior hospitalization exceeding 14 days before enrollment were excluded.

Statistical analysis

Statistical analysis was conducted using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Data normality was assessed using the Shapiro-Wilk test, which indicated a non-normal distribution; therefore, continuous variables are presented as median and interquartile range (IQR). Categorical variables are presented as frequencies and percentages, and were analyzed using the chi-squared test. The Mann-Whitney U test was used to compare continuous variables between the two groups. Spearman's rank correlation was used to assess the relationships between electrolyte elements and IFN-γ within each patient group. Receiver operating characteristic (ROC) curve analysis was used to assess the diagnostic performance of markers in distinguishing between severe and non-severe disease groups. Statistical significance was set at $P<0.05$. Because the study primarily evaluated a limited number of predefined biomarkers based on biological hypotheses, adjustment for multiple comparisons was not performed. Therefore, the findings should be interpreted cautiously and require external validation.

The study protocol was ethically approved by the Iraqi Ministry of Health and by the local ethics committee of the College of Medicine, University of Karbala (document number 41 on 2023/12/21). All subjects gave written informed consent before entering the study, in line with the Declaration of Helsinki.

Results

To check for the association between sex and the severity of the disease, a chi-square test was conducted.

A total of 200 subjects (97 males, 103 females) who were randomly selected into non-severe (n=100) and severe (n=100) groups were analysed. The results indicated that the severity was significantly associated with sex. The majority of males (57 males, 57.0%) were in the non-severe group while 40 males (40.0%) were in the severe group. Conversely, females showed the opposite pattern, with 60 females (60.0%) in the severe group versus 43 (43.0%) in the non-severe group. Odds ratio analysis showed that males had 1.988 times higher odds of being in the non-severe group compared with females (95% CI: 1.133-3.491), indicating a clinically and statistically significant sex difference in disease severity. Regarding age, the median age was 63.50 years (range: 37-95) in the non-severe group, whereas it was 69.50 years (range: 37-95) in the severe group, with no statistically significant difference between the two groups (P=0.084) (Table 1).

Measurement interferon gamma and electrolytes elements value among study groups

COVID-19 is considered a global health problem, having caused a pandemic worldwide (27). For this reason, in this study, we decided to explore and describe the behavior of human immunity in terms of IFN-γ levels among patient groups, as a major cytokine affected during COVID-19 progression. IFN-γ is a type II interferon and plays a pivotal role in fungal, viral, and bacterial infections (28). The findings of the present investigation revealed that the severe group had a lower level of IFN-γ compared with the non-severe group (7.81 vs 30.71 pg/ml), with a highly statistically significant difference (P<0.001) (Table 2).

The median value of K in the non-severe and severe groups was 4.15 and 4.52 mmol/L, respectively, with a statistically significant difference (P<0.001). Likewise, the median value of Na in the non-severe and severe groups was 146.80 and 139.95 mmol/L, respectively, with a highly significant difference (P<0.001). There was also a highly statistically significant difference in Ca (P<0.001), with median values of 9.37 and 10.27 mg/dL in the non-severe and severe groups, respectively. Additionally, there was a statistically significant difference between the two groups for Cl (P<0.001), with median values of 97.60 and 107.75 mmol/L in the non-severe and severe groups, respectively (Table 2).

Spearman correlation between electrolytes elements among non-severe group patients

Spearman correlation analysis revealed no significant correlation between any electrolyte elements except between Cl and Ca (r=0.243 and P=0.015) (Table 3). Furthermore, IFN-γ showed no statistically significant correlation with any of the electrolyte elements in the non-severe group, indicating that immune activity and electrolyte balance are relatively independent in non-severe disease.

Spearman correlation between electrolyte elements among severe group

Spearman correlation analysis in the severe group revealed significant correlations of Cl with both Ca and Na (r=0.359, P<0.001 and r=0.733, P<0.001, respectively), while no statistically significant associations were found among the other elements (Table 4). Similarly, IFN-γ showed no statistically significant correlation with any electrolyte element in the severe group, suggesting that IFN-γ dysregulation operates through mechanisms independent of electrolyte imbalance even in severe COVID-19.

Receiver operating curve analysis

Receiver operating characteristic (ROC) curve analysis was conducted to assess the diagnostic performance of the study biomarkers (IFN, Ca, Na, K, and Cl) in discriminating between disease groups. The analysis showed that IFN demonstrated excellent discriminatory ability, with an adjusted AUC of 0.917 (95% CI: 0.876-0.957, P<0.001). Notably, IFN showed an inverse relationship with disease severity, whereby diminished values were significantly associated with the severe disease group. This inverse correlation was reflected in the original AUC of 0.083, which, when adjusted (1-AUC), yielded the reported value of 0.917.

Ca and Cl demonstrated good discriminatory ability, with AUC values of 0.857 (95% CI: 0.807-0.907) and 0.848 (95% CI: 0.794-0.902), respectively (both P<0.001). Increased levels of both biomarkers were associated with severe disease. Na also demonstrated good discriminatory ability (adjusted AUC=0.827, 95% CI: 0.769-0.884, P<0.001), with an inverse relationship similar to that of IFN, where lower values indicated severe disease (original AUC=0.173). K demonstrated fair discriminatory ability (AUC=0.702, 95% CI: 0.631-0.774, P<0.001), with higher values associated with severe disease.

All biomarkers demonstrated statistically significant discriminatory ability compared with random classification (P<0.001), suggesting their potential clinical utility in predicting disease severity. The ranking of biomarkers by discriminatory ability was as follows: IFN (0.917) > Ca (0.857) > Cl (0.848) > Na (0.827) > K (0.702). These findings suggest that IFN, Ca, and Cl may

serve as the most reliable biomarkers for distinguishing between severe and non-severe disease presentations (Table 5, Figure 1).

Multivariable logistic regression analysis

To determine whether serum IFN-γ and electrolyte parameters were independent predictors of COVID-19 severity, rather than markers simply correlated with one another or with demographic factors, a multivariable binary logistic regression model was constructed with disease severity (severe vs non-severe) as the dependent variable. Log-transformed IFN-γ, potassium, sodium, chloride, and calcium were entered simultaneously as covariates, with adjustment for age and sex (Enter method). Variance inflation factors (VIFs) for all predictors ranged from 1.01 to 1.63, well below the threshold of concern (VIF>5), indicating no problematic multicollinearity.

The overall model was highly significant (χ^2 likelihood-ratio test, $P<0.001$) and correctly classified 95.0% of patients, with a model-derived AUC of 0.993. After mutual adjustment, IFN-γ (adjusted OR=0.090 per unit increase in log-IFN-γ, 95% CI: 0.023-0.355, $P=0.001$), potassium (aOR=11.89, 95% CI: 1.87-75.79, $P=0.009$), sodium (aOR=0.742, 95% CI: 0.610-0.902, $P=0.003$), chloride (aOR=1.277, 95% CI: 1.113-1.464, $P<0.001$), and calcium (aOR=24.59, 95% CI: 3.71-162.84, $P=0.001$) each remained significantly and independently associated with disease severity. Age (aOR=1.02, 95% CI: 0.95-1.10, $P=0.597$) and sex (aOR=0.24, 95% CI: 0.04-1.30, $P=0.098$) were not independent predictors after adjustment. These findings confirm that the association of IFN-γ and electrolyte disturbances with COVID-19 severity is independent of age, sex, and the other variables included in the model (Table 6).

Table 1. Demographic characteristic among study groups

Variable	Non-Severe Group	Severe Group	Total	*P*
Male	57 (57.0%)	40 (40.0%)	97 (48.5%)	0.016*
Female	43 (43.0%)	60 (60.0%)	103 (51.5%)	
Total	100 (50.0%)	100 (50.0%)	200 (100%)	
Age	63.50 (37-95)	69.50 (37-95)	65.00 (37-95)	0.084
Odds Ratio (Male/Female)		1.988 (95% CI: 1.133-3.491)		

Note: Chi-squared test, *: is significant at 0.01 level were statistical significance was set at $P\leq 0.05$, while $P\geq 0.05$ indicated non-significant results. OR: odds ratio, CI: confidence interval

Table 2. IFN- γ serum level and electrolytes elements value among study populations

Variable	Non-Severe Group (n=100)	Severe Group (n=100)	Total (n=200)	*P*
IFN-γ	30.71 (3.41-176.81)*	7.81 (1.18-56.71)	14.87 (1.18-176.81)	<0.001**
CA	9.37 (7.93-10.32)	10.27 (8.72-11.51)	9.72 (7.93-11.51)	<0.001**
NA	146.80 (135.0-155.0)	139.95 (130.8-152.7)	144.05 (130.8-155.0)	<0.001**
K	4.15 (3.04-5.47)	4.52 (3.40-5.84)	4.33 (3.04-5.84)	<0.001**
CL	97.60 (85.0-111.5)	107.75 (88.9-120.0)	101.45 (85.0-120.0)	<0.001**

Note: Data presented as Median (Minimum-Maximum). **$P < 0.05$ indicates statistical significance. *P* calculated using Mann-Whitney U test

Table 3. Spearman correlation between electrolyte elements among the non-severe group

Variables	Na		K		Cl		Ca	
	r	*P*	r	*P*	r	*P*	r	*P*
Na			0.146NS	0.147	0.115NS	0.254	0.116NS	0.250
K					0.006NS	0.951	0.029NS	0.777
Cl							0.243*	0.015
IFN-γ	0.031	0.757	0.149	0.140	0.118	0.244	0.149	0.139

Note: Spearman correlation test, *: Significant positive correlation, NS: non-significant correlation

Table 4. The correlation between electrolyte elements among severe group

Variables	Na		K		Cl		Ca	
	r	***P***	**r**	***P***	**r**	***P***	**r**	***P***
Na			-0.058^{NS}	0.565	0.733**	0.000	0.092^{NS}	0.363
K					0.141NS	0.161	0.060^{NS}	0.556
Cl							0.359**	0.000
IFN-γ	0.162^{NS}	0.106	0.227^{NS}	0.788	0.135^{NS}	0.181	0.104^{NS}	0.305

Note: Spearman correlation test, **: Significant positive correlation, NS: non-significant correlation

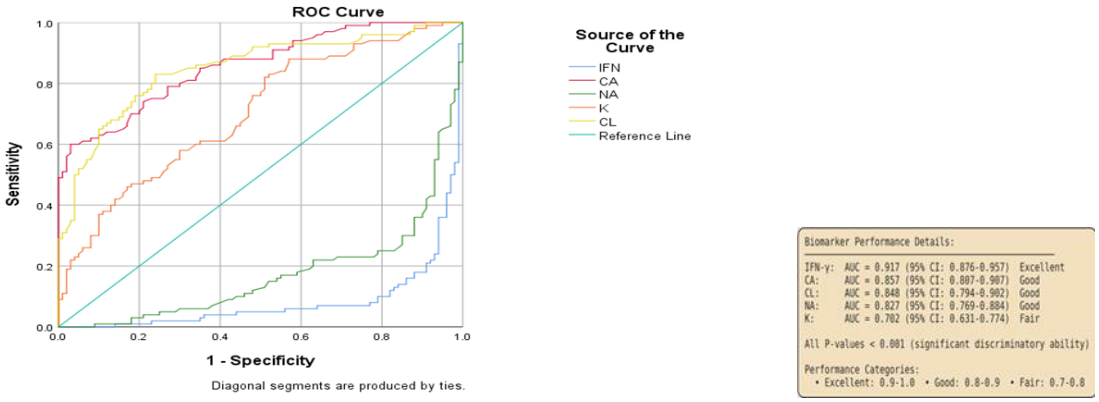

Figure 1. ROC Curve Analysis for Biomarkers Predicting Disease Severity

Note: AUC = Area Under the Curve; CI = Confidence Interval. Discriminatory capacity: Excellent (0.9-1.0), Good (0.8-0.9), Fair (0.7-0.8). All *P*<0.001 reflect significant diagnostic utility relative to random classification (AUC=0.5)

Table 5. ROC curve analysis for biomarkers predicting COVID-19 disease severity

Biomarker	Cutoff	AUC	95% CI (AUC)	Sensitivity (%)	95% CI (Sens.)	Specificity (%)	95% CI (Spec.)
IFN-γ	≤12.33 pg/mL	0.917	0.876–0.957	82.0	73.3–88.3	91.0	83.8–95.2
Ca	>10.10 mg/dL	0.857	0.805–0.910	60.0	50.2–69.1	97.0	91.5–99.0
Cl	>100.70 mmol/L	0.848	0.794–0.902	83.0	74.5–89.1	76.0	66.8–83.3
Na	≤142.60 mmol/L	0.827	0.769–0.885	70.0	60.4–78.1	88.0	80.2–93.0
K	>4.02 mmol/L	0.702	0.630–0.774	88.0	80.2–93.0	43.0	33.7–52.8

AUC: Area Under the Curve; CI: Confidence Interval. Cutoff values represent the optimal threshold determined by the Youden index (sensitivity + specificity − 1). Sensitivity and Specificity are exact values at the stated cutoff, with 95% CIs calculated using the Wilson score method. ***P*<0.001 for all biomarkers (Mann-Whitney/DeLong-based AUC significance)

Table 6. Multivariable Logistic Regression Analysis for Independent Predictors of COVID-19 Severity

Variable	Adjusted OR	95% CI	*P*
IFN-γ (log-transformed)	0.090	0.023–0.355	0.001**
Potassium (K)	11.89	1.87–75.79	0.009**
Sodium (Na)	0.742	0.610–0.902	0.003**
Chloride (Cl)	1.277	1.113–1.464	<0.001**
Calcium (Ca)	24.59	3.71–162.84	0.001**
Age	1.020	0.950–1.100	0.597
Sex (Male vs Female)	0.241	0.045–1.303	0.098

Note: OR = Odds Ratio; CI = Confidence Interval. IFN-γ was log-transformed prior to analysis owing to its right-skewed distribution; the adjusted OR for IFN-γ reflects the change in odds of severe disease per one-unit increase in log-transformed IFN-γ (equivalent to an e-fold increase in serum concentration). All variables were entered simultaneously (Enter method), adjusting for age and sex. ***P*<0.05 indicates statistical significance. Model: χ^2 likelihood-ratio test *P*<0.001; overall classification accuracy 95.0%; model-derived AUC = 0.993

Discussion

The present study showed a significant negative correlation between the level of IFN-γ in the serum and the severity of the disease in COVID-19 patients, and showed a definite trend of disturbance in the electrolyte profile in severe patients. Together, these results highlight the synergistic effect of immune response dysfunction and metabolic imbalance as the main mechanisms for clinical deterioration. This study showed that the level of IFN–γ was significantly lower in the severe group than in the non-severe group. Interferon gamma (IFN-γ), a key antiviral cytokine, is mainly produced by activated T lymphocytes (T cells) and natural killer (NK) cells. It has been well established that IFN- γ stimulates activation of macrophages, promotes antigen presentation, and inhibits viral amplification (6,7). Thus, the low level of IFN-γ in the severe group could be explained by a defective early immune response, which led to an inadequate clearance of the virus and eventually to progression of the disease. This is in line with new evidence indicating a defect in the Type II IFN-γ response is linked to critical disease outcomes (31-32). At the mechanistic level, it can be proposed that lowered levels of IFN-γ in severe COVID-

19 cases are due to exhaustion of lymphocytes, very often observed in the context of infection with SARS-CoV-2. (33). Prolonged antigenic stimulation causes functional T-lymphocyte disruption, reducing cytokine synthesis and limiting antiviral defense. Additionally, excessive inflammatory signaling in advanced stages of the disease may paradoxically suppress effective IFN-γ responses, further exacerbating viral persistence and tissue damage (9). Although several studies have reported elevated IFN-γ concentrations during severe COVID-19 as part of the cytokine storm, cytokine responses are highly dynamic and vary according to disease stage, timing of blood sampling, and immune exhaustion. Early antiviral immunity depends on adequate IFN-γ production; however, prolonged antigenic stimulation may induce T-cell exhaustion and functional impairment, resulting in reduced IFN-γ production during advanced disease. Therefore, apparently conflicting findings across studies may largely reflect temporal immune dynamics rather than true biological inconsistency. Our samples were obtained only at hospital admission. Consequently, the present findings most likely reflect immune status at a single clinical time point rather than the complete trajectory of IFN-γ responses throughout disease progression.

Severe cases showed marked electrolyte profile abnormalities, such as hyperkalemia, hypercalcemia, hyperchloremia, and hyponatremia, that were documented in this study. These results may suggest a combined role of renal dysfunction and hormonal imbalance. Although hypocalcemia has been more frequently reported in hospitalized COVID-19 patients, hypercalcemia has also been described in selected cohorts, particularly among critically ill individuals with dehydration, acute kidney injury, prolonged immobilization, or altered vitamin D-parathyroid hormone homeostasis. Therefore, differences in patient characteristics, disease stage, and calcium measurement methodology may explain discrepancies between studies (34).

Hyponatremia, recorded in severe patients, may be linked to inappropriate secretion of antidiuretic hormone (SIADH), exacerbated by elevated cytokine levels (35,36). This results in water retention and dilutional Na reduction, which has been correlated with poor prognosis. The increase in serum K levels in severe patients may be attributed to acute kidney injury (AKI), a prevalent complication of COVID-19 (36,37). In addition, the influence of comorbid conditions and medications modulating the renin-angiotensin-aldosterone system (RAAS) should be considered (15). Hyperkalemia is clinically significant because of its association with cardiac complications and increased fatality rates (38).

In severe patients, hypercalcemia was restarted, which may be related to viral exploitation of Ca signaling pathways. Ca2+ is crucial to viral entry, amplification and membrane fusion (24,25,26). Viral infection has been shown to dysregulate the intracellular Ca homeostasis, which can cause viral amplification, and thus upregulate inflammatory responses through activation pathways such as the NLRP3 inflammasome. (39,40). The link between the increased Ca value with poor clinical outcomes may be explained by this. (2,28,29).

Similarly, in severe patients, hyperchloremia can be a sign of underlying metabolic acidosis, renal dysfunction, and aggressive fluid therapy. Increased synthesis of inflammatory mediators has also been linked to elevated Cl levels, and poor prognosis in severe cases. (17,18,19), suggesting that Cl dysregulation could play a role in clinical deterioration, not merely a marker of it.

Evaluation of these relationships confirms that there is systemic dysregulating in serious instances. In non-severe cases electrolyte levels appeared relatively independent however significant correlation between electrolyte were recorded in severe cases (30,41). The pattern implies impaired normal homeostatic mechanisms, probably caused by a mix of inflammatory, renal dysregulatory and hormonal dysfunctions.

Crucially, ROC analysis confirmed that IFN-γ exhibited excellent discriminatory accuracy in predicting disease severity, outperforming all evaluated electrolytes (4,42). Ca and Cl also showed significant predictive value, while K and Na demonstrated moderate capacity (43-45). Importantly, these associations were corroborated by multivariable logistic regression analysis, in which IFN-γ, potassium, sodium, chloride, and calcium each remained significant independent predictors of severity after mutual adjustment and adjustment for age and sex, confirming that these findings are not attributable to demographic confounding or intercorrelation among biomarkers (Table 6).

These results indicate that IFN-γ, in combination with selective electrolyte markers, could serve as a practical and accessible tool for early risk stratification in clinical settings.

From a clinical perspective, these results underscore the importance of early immunological and biochemical evaluation in COVID-19 patients. Tracking IFN-γ levels may elucidate the functional status of the immune response, while routine electrolyte monitoring can help identify patients at risk of deterioration. Integrating these markers into clinical practice may improve risk

stratification, inform therapeutic interventions, and optimize resource allocation.

Limitations

Despite the advantages of this study, such as a well-defined cohort and a thorough statistical exploration of the data, some uncertainties must be noted with regards to the results. The cross-sectional study design makes it difficult to establish causal relationships between IFN-γ levels, electrolyte disturbances and severity of COVID-19. The correlations observed are associations at one time and not trends over time as the disease progresses. Secondly, this study was performed in a single healthcare facility, potentially limiting the generalizability of the results to other populations with different demographic and clinical features. Thus, multicenter studies with various populations are recommended to confirm these findings. Third, although other important confounding factors, including age, sex, and comorbidities, were adjusted for in the analysis, the possibility of other unmeasured confounders cannot be excluded. The following potentially important confounding variables were not available for adjustment: corticosteroid use, renin–angiotensin–aldosterone system inhibitor use, renal function measures, hydration states, vitamin D levels, and other inflammatory markers like C-reactive protein and interleukin-6. These parameters could have affected the cytokine responses and electrolyte profiles and should be taken into account in future prospective studies. Finally, unlike the other three groups, an assessment of dynamic changes in IFN-γ and electrolyte levels was not performed, and electrolyte levels were only measured at the point of hospital admission. The longitudinal tracking may give a better understanding of the immunological and biochemical changes throughout the course of the disease and their link with clinical outcomes. Finally, this investigation was limited to the selection of certain parameters and the addition of other inflammatory markers (such as IL-6, TNF-α, and CRP) could have yielded a more detailed picture of the cytokine network and its relationship to electrolyte disturbances in COVID-19. The study offers important insight into the interplay between immune dysfunction and electrolyte imbalance as used to predict the severity of COVID-19, despite the limitations.

This study also found that the amount of IFN-γ in the blood is significantly lower in severe COVID-19 patients, supporting the protective function of IFN-γ in antiviral immunity. Concurrent electrolyte disorders: hypercalcaemia, hyperkalcaemia, hyperchloraemia and hyponatremia, were significantly associated with disease severity and poor prognosis. The ROC curve analysis showed that IFN-γ, Ca and Cl had excellent to good diagnostic power to identify disease severity. Such biomarkers are available and can be used as early risk stratification measures, and to assist in clinical decision-making. Other prospective, multicenter trials with multivariable adjustment are suggested to confirm and extend these results.

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