



Baseline Serum Total Cortisol during the Primary Coronavirus Infection in the Beginning of the COVID-19 Pandemic in Cameroon

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Abstract

Background: Improvement of critically ill patients Covid-19 positive placed on glucocorticoids may suggest impairment of the adrenal function. Our objective was to evaluate baseline serum cortisol in covid+ patients.

Methods: This was a descriptive and analytical cross-sectional study conducted in a population of patients infected with SARS-CoV-2 in Cameroon. Patients receiving corticosteroids irrespective of the route of administration within 3 months prior to recruitment and those with any hypothalamic-pituitary-adrenal axis disease were excluded. We reviewed the medical records of patients to collect socio-demographic and clinical data. Patients were then sampled at 8 a.m. for serum cortisol assay by the competitive ELISA method. Statistical analyses were performed using the Student's test to compare means. The significance level was set at $p < 0.05$.

Results: We included 80 covid+ patients, predominantly males (45, 56.3%) with a mean age of 43 ± 13 years. The comorbidities found were hypertension (9, 11.3%), diabetes mellitus (7, 8.8%), cardiovascular disease (8, 10%) and obesity (4, 5%). The most common symptoms were asthenia (44, 55%), fever (36, 45%) and respiratory symptoms (75, 93.7%). The non-severe form was the most common (70, 87.5%). Only 12.5% (10) of patients received oxygen therapy within our study. The mean baseline serum cortisol in the series was $279.55 \text{ nmol/l} \pm 128.68$ (min 96.1332 nmol/l; max 665.0521 nmol/l). Sixty-nine patients (86.3%) had baseline cortisol $\leq 413.79 \text{ nmol/l}$, suggesting an insufficient response to stress. No statistically significant association was found between serum cortisol and disease severity.

Conclusion: The absence of a marked rise of cortisol during COVID-19 suggests possible involvement of the hypothalamic-pituitary-adrenal axis in this infection.

Keywords

COVID-19, Serum cortisol, Severity

Abbreviations

ACE: Angiotensin Converting Enzyme; ACTH: Adrenocorticotropic Hormone; RNA: Ribo Nucleic Acid; Covid+: Confirmed Case of COVID-19; COVID-19: Disease Caused by the New Coronavirus (2019-nCoV or SARS-CoV-2); ELISA: Enzyme Linked Immuno Sorbent Assay; HTN: Hypertension; MERS: Middle East Respiratory Syndrome; RT-PCR: Real Time Polymerase Chain Reaction; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; SARS: Severe Acute Respiratory Syndrome

Background

Rapidly declared as a pandemic, COVID-19 is a highly contagious disease with a clinical presentation ranging from mild, moderate to severe [1]. The cytokine storm track is cited as the reason for the severity and mortality during COVID-19 [2]. However, the determinants of severity and mortality during COVID-19 are still poorly understood. While it is true that the elemental lesion is thromboembolic and affects the pulmonary parenchyma, it cannot be excluded that the adrenal gland is affected. Moreover, in the recovery trial published in June 2020, the benefit of dexamethasone in the management of COVID-19 has been demonstrated, with a reduction in mortality of critical patients by one third [3]. This beneficial effect of dexamethasone is linked to its action on alveolo-interstitial pulmonary lesions but possibly to a supplementation of the corticotropic axis with respect to severe infection. An induced adrenal response during critical illness is a determinant of survival. During sepsis, the hypothalamus-pituitary-adrenal axis is stimulated by the systemic inflammatory response (SIRS) [4]. The increase in cortisol is proportional to the severity of sepsis. However, in 30 to 70% of sepsis, the adrenal response is insufficient, or there is peripheral resistance to glucocorticoids which is associated with a poor prognosis and requires supplementation [5]. Few data exist on the adrenal response induced by COVID-19 infection. The purpose of this preliminary study was to assess baseline adrenal function during primary infection with the new coronavirus and to correlate it with disease severity.

Methods

Setting and participants

The study was conducted in the isolation unit for the Covid positive patient at the Yaoundé central hospital. This is a unit created as part of the Covid pandemic since March 2020 when Cameroon recorded its first positive case of Covid 19. The department has a capacity of 26 beds with an intensive care unit suitable for critical patients requiring oxygen.

Study period

Patients were recruited from March 2020 to April 2020.

Study design

We conducted a cross-sectional study of Covid-19 management centres in Cameroon.

Inclusion criteria

We included consenting participants followed up in covid-19 management centers with positive virological RT-PCR results.

Exclusion criteria

Patients on corticosteroids, regardless of the route of administration taken during the 3 months prior to recruitment, patients with hypothalamic-pituitary-adrenal axis pathology and pregnant women were excluded.

Procedure

Data were collected from the medical records of the patients concerned. Age, sex, co-morbidities, clinical presentation, ambient oxygen saturation and resort oxygen therapy were recorded.

Specimens collection

RNA Extraction and RTPCR amplification: Nasopharyngeal aspirates samples were collected from patients and frozen at 80 °C before RNA extraction by using the NucleoSpin Dx Virus (Ref: Macherey Nagel 740895.50), according to manufacturer's instructions at the Centre Pasteur, Yaoundé, Cameroon. RNA extracted from 100 µl of original sample, is eluted in 100 µl of elution buffer. RT-PCR was performed by using Invitrogen Superscript™ III Platinum® One-Step qRT-PCR system (ref: 11732-088) in a final volume of 25 µL containing 0.4 µM of each primer, 0.8 mM MgSO₄, 3 mM Mg of reaction mix 2X, 0.2 µM of probe and 5 µL of RNA extract. A Light cycler fi 480 (Roche) system was used with the following cycling conditions: 55 °C for 20 min, 95 °C for 3 min, 50 cycles of 95 °C for 15s, 58 °C for 30 s, and 40 °C for 30s [6].

Quantitative determination of cortisol: Blood samples were then taken at 8 a.m. to respect the nycthemeral cycle of cortisol secretion after curbing the stress of direct venipuncture. The assays were performed by competitive ELISA in a serial manner according to laboratory procedures on a spectrophotometer (UV Mini 1240) using cortisol ELISA from IBL International GmbH, part of TECAN group kits (catalog Number RE52061). The biological data were interpreted as follows: A cortisol threshold ≤ 413.79 nmol/l was considered to be an insufficient cortisolic response to infection and > 413.79 nmol/l normal. Patent adrenal insufficiency was defined by a cortisol level ≤ 137.93 nmol/l.

Statistical analyses: Patients were then classified into 2 subgroups according to severity: Subgroup with oxygen therapy and subgroup without oxygen therapy. Data were compiled and analyzed using SPSS version 23 software. Quantitative variables were reported as means and standard deviation. These were compared using student t-test. Qualitative variables were presented as percentages and these were compared with a chi-square test. The statistical significance level was set at 0.05.

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Table 1: Socio demographic and clinical characteristics of study participants.

Variables	Number (n = 80)	Percentage (%)
Characteristics		
Sex		
Female	35	43.8
Male	45	56.3
Age		
≤ 40 years	36	45
≥ 40 years	44	55
RT-PCR	80	100
Comorbidities		
Diabetes mellitus	7	8.8
Hypertension	9	11.3
Obesity	4	5
Cardiovascular disease	8	10
Symptoms		
Fever	36	45
Asthenia	44	55
Rhinorrhoea	12	15
Cough	49	61.3
Dyspnoea	14	17.5
Diarrhoea	3	3.8
Symptoms		
≤ 3 symptoms	74	92.5
> 3 symptoms	6	7.5
Duration of symptoms		
≤ 5 days	33	50.8
> 5 days	32	49.2
Baseline serum cortisol		
≤ 150 ng/ml	69	86.3
> 150 ng/ml	11	13.8
Oxygenotherapy	10	12.5

RT-PCR: Real Time Polymerase Chain Reaction; SD: Standard deviation

Results

Socio-demographic characteristics

We recruited 80 participants, the majority of whom were male 45/80 (56.3%). The average age was 43 ± 13 years. The most represented age group was over 40 years (44, 55%).

Clinical characteristics

All participants recruited were positive for RT-PCR. The main co-morbidities found were hypertension (9/80, 11.3%), diabetes mellitus (7/80, 8.8%), cardiovascular disease (8/80, 10%) and obesity (4/80, 5%). The symptoms found included asthenia (44/80, 55%), fever (36/80, 45%), cough (49/80,

Table 2: Measures of association.

Variables	Serum cortisol nmol/l (mean ± SD)	P value
Characteristics		
Sex		
Female	235.68 ± 98.74	0.005
Male	313.67 ± 139.55	
Age		
≤ 40 years	232.81 ± 105.01	0.002
≥ 40 years	317.79 ± 134.61	
RT-PCR	279.55 ± 128.68	
Comorbidities		
Diabetes mellitus	305.35 ± 172.11	0.685
Hypertension	282.20 ± 165.80	0.959
Obesity	292.56 ± 145.36	0.864
Cardiovascular disease	313.02 ± 165.39	0.555
Symptoms		
Fever	274.88 ± 130.95	0.772
Asthenia	281.06 ± 133.84	0.908
Rhinorrhoea	234.48 ± 108.63	0.150
Cough	281.51 ± 138.43	0.859
Dyspnoea	232.65 ± 85.56	0.054
Diarrhoea	262.39 ± 125.30	0.830
Symptoms		
≤ 3 symptoms	284.14 ± 129.10	0.274
> 3 symptoms	222.95 ± 118.87	
Duration of symptoms		
≤ 5 days	293.29 ± 115.21	0.458
> 5 days	269.24 ± 142.26	
Oxygenotherapy	316.03 ± 157.28	0.439

RT-PCR: Real Time Polymerase Chain Reaction; SD: Standard deviation

61%), respiratory distress (14/80, 17.5%), rhinorrhea (12/80, 15%) and diarrhea (3/80, 3.8%). Most of the patients presented less than 03 symptoms (74/80, 92.5%). Half of the patients recruited were sampled within an average of 5 days from the onset of symptoms. The non-severe form was the most common (70/80, 87.5%) and 12.5% (10) of the patients received oxygen therapy (Table 1).

Biological data

The mean baseline total cortisol level in the series was 279.5 nmol/l ± 128.68 (min 96.1 nmol/l; max 665.0 nmol/l). Sixty-nine patients (86.3%) had baseline cortisol ≤ 413.8 nmol/l and eleven (11.8%) had > 413.8 nmol/l. The mean cortisol level was higher in the group with oxygen therapy versus no oxygen therapy (316 nmol/l ± 49.7 vs. 124.54 nmol/l ± 14.9). Nine patients had patent adrenal insufficiency with serum cortisol ≤ 137.9 nmol/l (11.2%) (Figure 1).

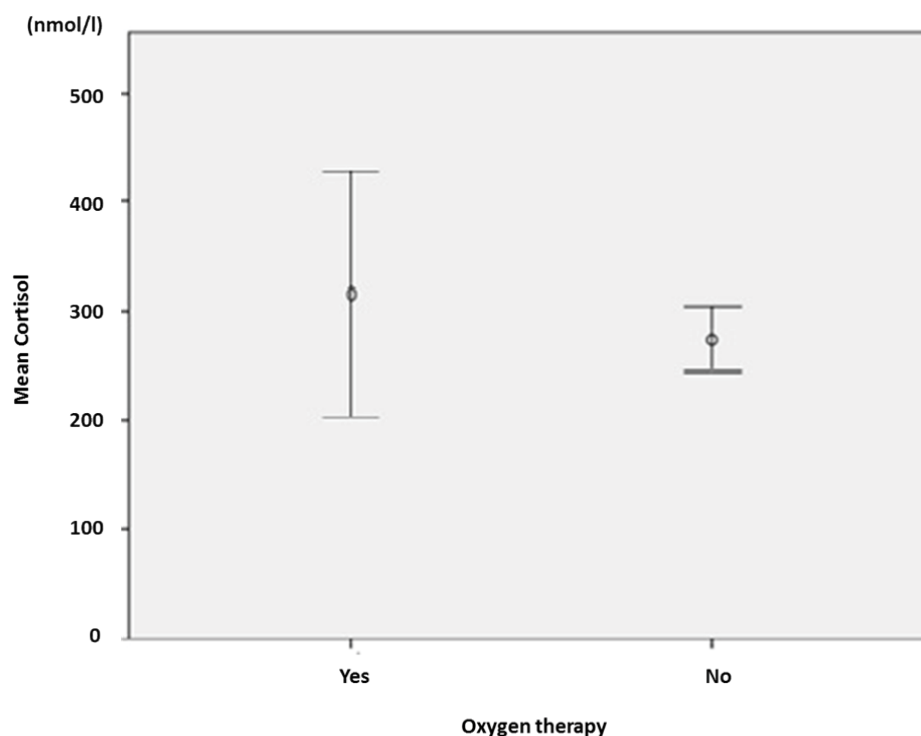


Figure 1: Mean of serum cortisol in the group on oxygen therapy and the group without oxygen therapy. nmol/l: Nanomol per liter.

Association between total baseline cortisol and clinical features of Covid-19

We did not find a statistically significant association between serum cortisol and oxygen therapy, asthenia, comorbidities (hypertension, diabetes mellitus and obesity), number of patient symptoms and duration of symptoms for a $P < 0.05$ value. In contrast, serum cortisol level was associated with patient age (Table 2).

Discussion

The objective of this study was to evaluate baseline total serum cortisol in covid positive patients. We therefore performed an 8 a.m. serum total cortisol in all patient positive to Covid 19 who were hospitalized in the isolation unit for Covid-19 patients. We found that the mean baseline total cortisol level was $279.5 \text{ nmol/l} \pm 128.7$. The mean cortisol level was higher in the group with oxygen therapy versus no oxygen therapy ($316.0 \text{ nmol/l} \pm 49.7$ versus $124.54 \text{ nmol/l} \pm 14.9$). Nine patients had patent adrenal insufficiency with serum cortisol $\leq 137.9 \text{ nmol/l}$. We did not find a statistically significant association between serum cortisol and oxygen therapy. In contrast, serum cortisol level was associated with patient age.

The diagnosis of adrenal insufficiency requires ACTH stimulation test which is the most specific test for the diagnosis of adrenal insufficiency. However, Covid-19 infection in itself could constitute a stimulating stress for the HPA axis by the induced inflammatory systemic response. As a preliminary study, baseline adrenal function should be assessed in all

COVID+ patients regardless of clinical disease status.

The technique used for the bioassay was the competitive ELISA method. Although this is not the reference method, it is easy to perform and suitable for screening. Its threshold of sensitivity is 88% and it is accessible in our context.

The socio-demographic and clinical characteristics of our study population are consistent with those found in the Wuhan cohort in China. Men are the most affected by the disease and have diabetes mellitus and hypertension as the main co-morbidities. The clinical presentation is by far dominated by asthenia and fever [7].

Males are believed to be most vulnerable to SARS-CoV-2 due to the greater expression of ACE receptors in men than in women. In addition, men are more susceptible to co-morbidities (hypertension, diabetes, cardiovascular disease) and therefore to severe forms of the disease [8].

Diabetes is an independent predictor of morbidity and mortality in COVID-19 [9,10]. Regardless of the time of discovery, it predisposes to severe forms through its susceptibility to infection. This is due to impairment of immune mechanisms, dysfunction of immune cells, oxidative stress caused by hyperglycemia and hypersecretion of counter-regulatory hormones [11]. However, SARS-CoV-2 as a risk factor for glycemic dysregulation is yet to be demonstrated. Some authors have raised the hypothesis of viral colonization of the pancreas through ACE receptors expressed in islets of Langerhans, however pancreatic viral particles have not been isolated in the autopsy series [9].

The mean baseline cortisol level ($279.5 \text{ nmol/l} \pm 128.7$) with 69 patients (86.3%) with baseline cortisol $\leq 413.79 \text{ nmol/l}$ and 09 patients with cortisol less than 137.93 nmol/l represent an insufficient adrenal response to the stress induced by 2019-n CoV infection. Tricia Tan, et al. in 2020 found an association between baseline cortisol threshold value $\geq 744 \text{ nmol/l}$ and worsening morbidity/mortality in COVID-19. They compared the value of the patient's baseline cortisolaemia within 03 days following the onset of symptoms and its fate during the following 42 days. This suggests integrity and an adapted response of the corticotropic axis in the acute phase of infection [12].

Nevertheless, these results are not applicable in our context since our patients were enrolled later in the infection (beyond 03 days). The cytokine storm characteristic of SARS-CoV-2 infection and predictor of disease severity occurs from day 6 [2,13]. This overly inadequate inflammatory response to viral aggression would also be incriminated in the syndrome of multivisceral failure with damage to the corticotropic axis in COVID-19 by destruction of the adrenal parenchyma [4]. This suggests an alteration of the cortisolic response later in the infection. In our series, it is clear that the mean baseline cortisol level decreases with the duration of symptoms ($293.3 \text{ nmol/l} \pm 115.2$ vs. $269.24 \text{ nmol/l} \pm 142.2$).

The cortisolic response is inadequate in both the oxygen therapy group and the non-severe group. This suggests an insufficiency of basal cortisol secretion in response to stress in all phases of the disease, from less symptomatic patients to patients on respiratory assistance. During the SARS outbreak in 2003, adrenal insufficiency was described in patients infected with SARS-CoV-1 [4,14,15]. Three mechanisms were incriminated:

1. Necrosis of the adrenal tissue found in autopsy series by viral colonization of the adrenals through ACE receptors expressed in this tissue and the cytokine tornado triggered by the viral infection and directed to the adrenals.
2. Viral infiltration of the hypothalamo-pituitary axis through ACE receptors expressed in this tissue and resulting in hypopituitarism.
3. Corticotropic insufficiency caused by antibodies to ACTH. This is explained by the molecular analogy between viral proteins and ACTH [16].

SARS-CoV-2, a virus of the same family as SARS-CoV-1, could also trigger the same mechanisms and be responsible for adrenal insufficiency.

Preliminary results from the RECOVERY study published in June 2020 showed a significant reduction in mortality in severe patients with dexamethasone [10]. This potent corticosteroid is believed to be effective in reducing the cytokine storm characteristic of severe and lethal forms of COVID-19. Hence the benefit of corticosteroid therapy in severe forms of the disease which will act as an anti-inflammatory and could also have a benefit in hormone supplementation if there is associated cortisol deficiency. In our cohort, there is evidence of an insufficient and inadequate cortisol response that may require supplementation for effective control of COVID-19.

Conclusion

The absence of a marked rise in serum cortisol during COVID-19 would suggest possible involvement of the hypothalamic-pituitary-adrenal axis during this infection.

The need to perform cosyntropin stimulation tests and ACTH assays keeps its place to better characterize organ damage in the covid+ patient.

Declarations

Ethics approval and consent to participate

Before starting the study, an authorization was obtained from authorities of the Yaoundé Central Hospital, and an ethical clearance was delivered by the Institutional ethic committee of the Université des Montagnes (CIE-UdM) N° 2020/057/UdM/PR/CIE. All the procedures used in the present study were in conformity with the current revision of the Helsinki Declaration. All participants were informed of the various aspects of the study, and they were enrolled only after providing a signed consent form.

Consent for publication

All the authors approved the final version of the manuscript and consented for its publication.

Availability of data and materials

Data will be made available by the corresponding author upon request.

Competing interests

All the authors declared no competing interest.

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None.

Authors' contributions

Study conception: ES, MCEE, AHI; Data collection: AHI, JANM; Laboratory analysis: AHI, MGF, VAM; Data analysis and Manuscript drafting: MCEE, AHI, ES; Critical revision of manuscript: ES; ABO, MD, MGF, LKM, CK, PJF, VAM; All authors read and approved the final manuscript.

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We declared that no patients have been reported in a previous study in our context.

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