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Original Research Article

Impact of COVID-19 Viral Load on the Biological Profile of Congolese Type 2 Diabetic Patients

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Abstract

Introduction*:** Type 2 diabetes mellitus (T2DM) patients are at increased risk of developing a severe form of COVID-19. And these Patients with a severe form of COVID-19 tend to have a high viral load. ***Objective: Identify biomarkers that show significant variation between different covid-19 viral load groups in T2DM patients in Pointe-Noire. ***Methods*:** We recruited a total of 206 participants for this study. Detailed information on age, gender, and health status of participants was collected from medical records. Biomarkers were quantified from blood samples and sars cov-2 virus was identified using the PCR technique on nasopharyngeal swabs. Viral load results were deduced from threshold cycles (CT) and subjects were grouped into two groups Ct < 25: Strong positive = High viral shedding and If Ct: 30 and 35 inclusive: Weak positive = Low viral shedding. ***Results*:** we found that fever (116/100%), Fatigue (115/99.1%), dyspnea (114/98.2%) and Cough (108/88.8%) were the most common signs in our population. study with high viral shedding. Non-survivors numbered 44 out of 46 for the same group. Biomarkers: CRP, ESR, CBC, GLY, HBA1c, DDI, Creat were significantly disrupted depending on viral load between non-survivors and survivors. ***Conclusion*:** This study showed that a high viral load was a source of disturbance of CRP, VS, CBC, GLY, HbA1c, DDI, Creat, signalling a poor prognosis.

Keywords: Covid-19 Viral Load, Biological Profile, Type 2 Diabetes, Congolese.

1. INTRODUCTION

Three coronaviruses have caused severe, life-threatening illnesses in humans over the last two decades: severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV emerged in China in 2002 and caused a worldwide pandemic in 2003, with a case-fatality rate of around 10% (Drosten C *et al*., 2003). MERS-CoV was first reported in Saudi Arabia in 2012, where it remains a major public health problem and has spread to many countries (Zaki AM *et al*., 2012). In December 2019, an epidemic of pneumonia due to the new 2019 coronavirus, SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) broke out in Wuhan, Hubei, China according to the National health commission of the People's Republic of China in 2020. This beta coronavirus is named COVID-19 by the World Health Organization (WHO). On March 12, 2020, WHO declared COVID-19 a pandemic (Wang L *et al*., 2020; WHO Novel Coronavirus (2019-nCoV) situation reports 2020). In Congo, the 1st case of COVID-19 was reported on March 14, 2020 (MSP: sitrep 130, 2021). COVID-19 is a highly transmissible infection that causes a wide

range of clinical manifestations, from mild influenza-like illness to severe bilateral pneumonia, acute respiratory distress syndrome (ARDS), septic shock and multi-organ failure (Hussain, A *et al*., 2020). Studies have shown that people with type 2 diabetes (T2DM) have an increased risk of developing a severe form of COVID-19, which may be due to a variety of factors, such as chronic inflammation, immunosuppression, hyperglycemia and coagulopathy (Bode, B *et al*., 2020; Huang, C *et al*., 2020; Zhou, Y *et al*., 2021). This concern is also heightened by the fact that higher viral loads are associated with more severe clinical outcomes (Chen, W *et al*., 2020; Makov-Assif, M *et al*., 2021). According to the work of Yang Liu *et al*., 2020, patients with severe COVID-19 tend to have a high viral load and a long period of virus excretion. This suggests that the viral load of SARS-CoV-2 could be a useful marker for assessing the severity and prognosis of the disease. This viral load is detected indirectly by the CT (cycle threshold) values in an RT-PCR (reverse transcription real time polymerase chain reaction) assay. They are defined as the number of cycles required for the fluorescent signal to cross a certain threshold. CT levels are inversely proportional to the amount of target nucleic acid in the sample, and can be an indirect measure of the viral load of samples. Although there is no direct quantification of viral load, it can be extrapolated that each 3.3 increase in CT value corresponds to ~1 log (i.e. 10-fold) less target in the nasopharyngeal sample subjected to PCR. (Martín Eduardo Brizuela *et al*., 2020; Shannon W *et al*., 2023).

In a region with limited resources such as Pointe-Noire, it would therefore seem useful to identify biomarkers that show significant variations between the different covid-19 viral load groups in Congolese T2DM patients.

2. MATERIAL AND METHOD

2.1. Study Population:

We conducted a descriptive cross-sectional study and the study population consisted only of T2DM patients with COVID-19 hospitalized at the Clinique Guenin, Louise Michel and Hôpital Général Adolphe sicé in Pointe-Noire.

2.2. Clinical Survey: Data such as age, sex, BMI, covid-19 symptoms and comorbidities were collected from medical records.

2.3. Biological survey:

Laboratory analyses were performed in the Laboratoire d'Analyses Biomédicales HDL of the Polyclinique Fondation Marie Madeleine Gombes in Pointe noire.

2.3.1. Sampling:

- Blood samples were taken on EDTA, heparinized and citrated tubes and stored at -20°C until use.
- Nasopharyngeal swabs were taken using the virus collections and transport kit type citoswab (W/3ML VTM) supplied by CITOTEST LABWARE MANUFACTURING CO. LTD Haimen city 226100, China.

2.3.2. Blood Biomarker Analysis:

The "Cobas C 311 (Roche Diagnostics, HITACHI, Germany)" automatic biochemistry analyzer was used for biochemical analyses: Fasting blood glucose (Gly); Glycated hemoglobin (HbA1c);Creatinine (Creat);Urea; Uric acid (AU); Lactate dehydrogenase (LDH); D-dimers (DDI); Ultra-sensitive C-reactive protein (CRP us); Transaminases (GOT,GPT); Gamma Glutamyl Transferase (GGT); Lipid profile (TC, TG, HDL, LDL); Blood ionogram (Na+, K+ and Cl-); Creatine phosphokinase(CPK); Creatine phosphokinase-MB (CPK-MB); Lactate The Sysmex XP-300 instrument was used to perform the following blood counts: white blood cell (WBC), neutrophilic polymorphonuclear cell (NPC), eosinophilic polymorphonuclear cell (EPNC), basophilic polymorphonuclear cell (BPNC), lymphocyte (lympho), monocyte (mono): Hemoglobin (Hb) and Platelets (Plqt).

Hemostasis tests were performed using the Stago - STart® Hemostasis Analyzer: Activated partial thromboplastin time (APTT) and Prothrombin rate (PT).

Sedimentation rate (VS): performed using the western green method.

2.3.3 Molecular Analysis:

a) Extractions

We extracted RNA from nasopharyngeal secretions using Total RNA Purification Insert PI12200-37, Norgen Biotek Corp (CANADA), in accordance with the manufacturer's recommendations dehydrogenase (LDH).

b) Amplifications

Extracted RNAs were subjected to PCR using the Covid-19 TaqMan RT-PCR Kit (E/RdRP genes) from Norgen Biotek Corp (CANADA).

Procedure:

- **First Step: Mix Preparation**
  - 10µl 2x One-step RT-PCR (Master Mix Dx)
  - 1.5 µl Enzyme
  - 3.5 µl nuclease free water
  - 5µl total RNA

- **Step 2: Mic Thermocycler Programming**

Amplification parameters were as follows: initial reverse transcriptase at 50°C for 20 minutes, then denaturation at 95°C for 03 minutes followed by 45 cycles of denaturation at 95°C for 15 seconds and 30 seconds of hybridization at 58°C.

- Choice of fluorochromes and targets:
- FAM (E-gene)
- HEX (Internal Control)

c) Interpretation of Results:

Results are expressed as E-gene Ct: Cut of target E SARSCOV2 gene.

- If Ct < 25: the result is declared Strong Positive = High viral shedding
- If Ct between 25 and 29: the result is declared Positive = Significant viral shedding
- If Ct between 30 and 35 inclusive: the result is declared Weak Positive = Low viral shedding.

2.4. Ethical Considerations

This study was carried out in accordance with the guidelines of the Declaration of Helsinki and was approved under number 125/CERS/FMMG-2021/PNR by the Health Research Ethics Committee (CERS) of the Marie Madeleine Gombes Foundation in Pointe Noire.

2.5. Statistical Analysis

Categorical data are expressed as numbers (percentage) and quantitative variables as means ± standard deviation. The $\chi 2$ test was used to compare categorical data. A p value < 0.05 was taken to indicate statistical significance. All analyses were carried out using SPSS software (version 26.0; IBM).

3. RESULTS

3.1. Sociodemographic and Clinical Characteristics

- The study population comprised 206 T2DM subjects, with 70% (145) male subjects and 30% (61) female subjects. The sex ratio (M/F) was 2.37% (Figure 1).

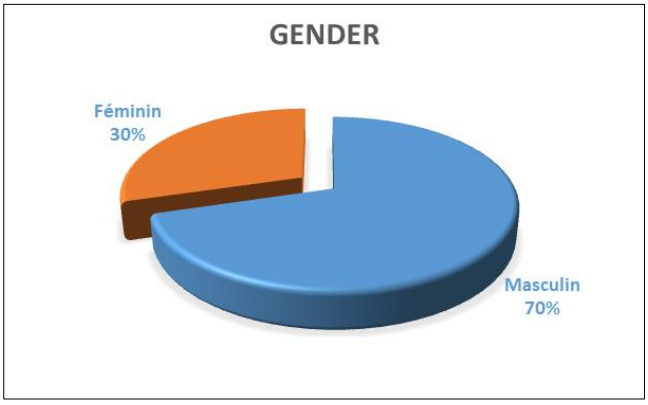
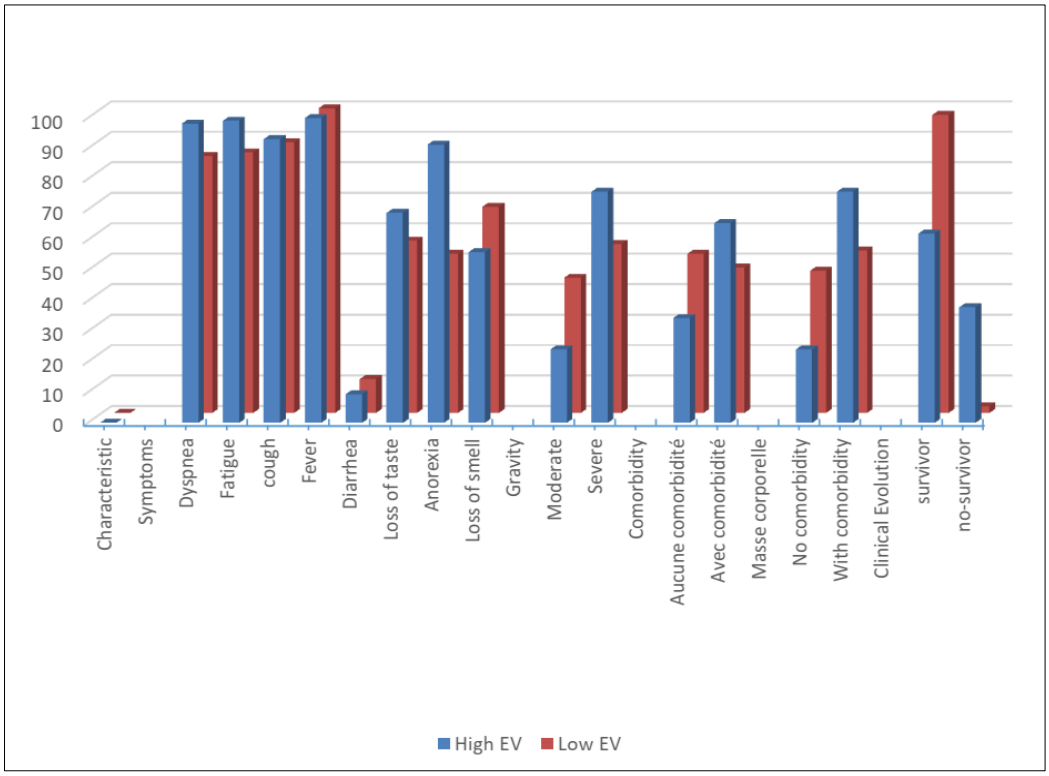


Figure 1: Distribution of study population by gender

- Fever (116/100%), fatigue (115/99.1%), dyspnea (114/98.2%) and cough (108/88.8%) were the most common signs encountered in our study population with high viral shedding. It had a high proportion of severe cases (88/75.8%), with more comorbidity (76/65.5%), overweight subjects (88/75.8%) and a high number of no survivor (44/37.9%).



EV: Viral Excretion

Figure 2: Clinical characteristics as a function of viral excretion.

3.2. Biomarker Variations According to Viral Load in Deceased and Cured T2DM:

Table I shows considerable biomarker perturbations between decedents and cured patients between the different viral load groups. Biomarkers: CBC, CRP, ESR, GLY, HBA1c, DDI and Créat were significantly perturbed according to viral load.

Table I: Biomarkers according to viral load in deceased and cured T2DM

	High viral Excretion				Low viral Excretion			
Characteristic	**N**	**survivor, N=72**	**No-survivor, N=44**	**p**	**N**	**survivor, N=80**	**No-survivor, N=2**	**p**
WBC(x10^3/mm^3)	116	12,602 ±9,236	12,566 ±8,895	0.7	90	7,900 ±3,361	25,220 ±0	0.016
Hb(g/dL)	116	12.45 ±2.33	11.78 ±2.10	0.15	90	12.47 ±2.06	12.70±0.00	0.8
NPC(x10^3/mm^3)	116	10,466 ±8,464	10,616±8,352	>0.9	90	6,239 ±3,548	22,510±0	0.016
EPNC(/mm^3)	116	20 ±54	54 ±62	<0.001	90	50 ±53	200 ±0	0.008
BPNC(/mm^3)	116	28 ±43	41 ±72	0.6	90	35 ±63	70 ±0	0.12
Lympho (x10^3/mm^3)	116	1,347 ±750	1,238±710	>0.9	90	1,158 ±543	2,011±0	0.094
Mono (x10^3/mm^3)	116	577 ±431	624±448	0.8	90	429±180	610±0	0.054
Plqte (x10^3/mm^3)	116	226,889±126,935	263,000±132,202	0.088	90	204,165±101,256	4,100±0	0.016
ESR(/mm^3)	116	51 ±39	72 ±41	0.002	90	22 ±9	2 ±0	0.016
CRP(mg/L)	116	183 ±97	275 ±105	<0.001	90	162 ±69	99 ±0	0.02
GLY(g/L)	116	2.04 ±0.93	2.83±1.17	<0.001	90	2.46 ±0.93	2.70±0.00	0.6
HBA1c (%)	116	8.12 ±2.48	9.85 ±1.87	<0.001	90	7.68 ±1.24	11.00±0.00	0.015
GPT(U/L)	116	42±21	85 ±59	<0.001	90	52 ±21	98±0	0.031
GOT(U/L)	116	52 ±30	77 ±30	<0.001	90	64 ±31	91 ±0	0.094
GGT(U/L)	116	43 ±25	56 ±3	0.029	90	55 ±38	97±0	0.11
LDH(U/L)	116	296 ±169	357 ±144	0.11	90	268 ±151	552±0	0.017
DDI(µg/L)	116	1,916 ±1,571	5,018±4,243)	<0.001	90	1,934±1,745	5,200±0	0.031
TP(sec)	116	79 ±13	75 ±16	0.6	90	85 ±15)	63 ±0	0.026
TCA (%)	116	32 ±9	29 ±9	0.061	90	26.1 ±5.7	41.0±0.0	0.019
Na+ (mmol/L)	116	140 ±8	139 ±4	0.5	90	138.6 ±4.1	144.0±0.0	0.093
K+ (mmol/L)	116	4.47 ±0.69	4.55 ±0.84	0.8	90	4.10 ±0.58	6.00±0.00	0.016
CL- (mmol/L)	116	101.3 ±5.1	100.8 ±3.6	0.12	90	101.89±2.84	110.00±0.00	0.015
Créat(mg/L)	116	13 ±7	21 ±20	0.006	90	11.17 ±2.48	25.58±0.00	0.016
Urée(g/L)	116	0.53 ±0.61	0.64 ±0.64	0.13	90	0.33±0.28	1.18±0.00	0.019
AU(mg/L)	116	51 ±12	49 ±16	0.3	90	50 ±15	52 ±0	0.4
CholT(g/L)	116	2.15 ±0.71	2.14 ±0.44	0.6	90	2.11 ±0.40	1.80 ±0.00	0.5
HDL(g/L)	116	0.40 ±0.20	0.39 ±0.23	0.4	90	0.43 ±0.21	0.16±0.00	0.016
LDL(g/L)	116	1.43 ±0.69	1.36 ±0.37	0.2	90	1.38 ±0.3	1.60±0.0	0.3
TG(g/L)	116	1.70 ±0.87	2.02 ±1.52	0.4	90	1.74 ±0.80	2.00±0.00	0.4
CHOLT/HDL	116	6.8 ±4.0	8.1±4.8	0.2	90	5.98 ±2.92	11.25±0.00	0.023
TG/HDL	116	5.3 ±5.3	8.4±9.6	0.3	90	4.9±3.3	12.5 ±0.0	0.027
CPK(U/L)	116	79 ±50	110 ±94	0.6	90	75 ±76	45 ±0	0.2
CKMB(U/L)	116	2 ±6	6 ±12	0.13	90	1.28 ±4.75	1.20 ±0.00	0.083

4. DISCUSSION

The presence of diabetes has been identified as an independent factor associated with poor prognosis in recent coronavirus infections, such as severe acute respiratory syndrome (SARS-CoV-1) in 2003 (Yang JK, *et al*., 2006) and Middle East respiratory syndrome (MERS-CoV) in 2012 (Alraddadi BM *et al*., 2016). Very soon after the onset of the SARS-CoV-2 coronavirus 2019 (COVID-19) pandemic, comorbidities, including diabetes, again emerged as associated with severe forms of COVID-19. Thus, in our study we investigated biomarker variations between different covid-19 viral load groups of Congolese T2DM.

In our study, men 145(70%) predominated over women 61(30%), with a sex ratio (M/F) of 2.37. These data are consistent with several studies conducted in Congo by L.M.A. Boumba *et al*., 2022 and Poaty H *et al*., 2021 and worldwide (Palaiodimos *et al*., 2020; Louhaichi S *et al*., 2020; Ketfi A *et al*., 2020). This male predominance is thought to be due to the cumulative effect of risk factors for COVID-19 severity such as smoking in the male population compared with the female population (Plaçais L *et al*., 2020).

In our series, the main clinical signs were dyspnea, fever, fatigue and cough, with significant worsening of signs (Figure 2). These results are in line with existing literature.

(V. Bonny, *et al*., 2020). Indeed, SARS-CoV-2, like SARS-CoV-1, uses angiotensin-converting enzyme 2 (ACE2) as its main cellular receptor to enter the host cell (P. Zhou *et al*., 2020). After an incubation period of

around five days, 70% of infected patients develop cough, fever or dyspnea (W. Guan *et al*., 2020). This phase of viral invasion is followed, in some patients, by an inadequate immune response marked by worsening respiratory symptomatology and inflammatory syndrome, usually eight to ten days after the first symptoms (C. Huang *et al*., 2020). This dysimmune phase, sometimes referred to as a cytokine storm, may be associated with coagulopathy, which some authors have described as viral sepsis (H. Li *et al*., 2020).

This study shows that there were 37.9% deaths in the group with high viral shedding. Our results corroborate those of Westblade LF *et al.*, 2020, in their global cohort, using Ct cut-off values specific to the analysis, 38.8% of patients died in the high viral shedding group patients with a high viral load died during hospitalization. In contrast to the series by E. Klement-Frutos *et al*., 2020, whose unfavorable outcome was in the order of 18.2%.

With regard to COVID-19 status, more than half (116/56.3%) of our participants were identified as having high viral shedding. According to Yang Liu *et al*., 2020, the mean viral load of severe cases was around 60 times higher than that of mild cases, suggesting that higher viral loads might be associated with severe clinical outcomes.

Regarding white blood cell status, our results are consistent with several studies that have reported alterations in white blood cells in patients with COVID-19, such as hyper leukocytosis and leukopenia (Moueden *et al*., 2021). These changes may be associated with a dysfunctional immune response to the virus.

Lymphopenia was also observed, with non-significant differences, as well as a considerable increase in CRP and VS with $p<0.05$. Our results are in line with the series by J. Fajnzylber *et al*., 2020, where we found that viral load was associated with a lower absolute lymphocyte count and an increase in markers of inflammation, notably C-reactive protein. The effects of the virus on lymphocytes can be explained by direct and/or indirect mechanisms. Direct action could be linked to viral cytotoxicity, sustained by active viral replication in a pool of infected lymphocytes (H. Li *et al*., 2020).

Our series shows glycemic imbalance (Gly↑ and HbA1c↑) irrespective of viral load. Our results reflect a true pathophysiological reality of diabetes in the context of covid-19. Indeed, the hypersecretion of secondary endogenous glucocorticoids in the context of infection-induced stress or therapeutic use of corticosteroids on the one hand, and the potential viral impairment of pancreatic cell function on the other, could explain the observed hyperglycemia and increase in HbA1c.(Anicet Boumba LM *et al*., 2022).

Our DDI results showed a level >5000ug/L in deceased patients regardless of viral load. In the work of Zhou F *et al*., 2020, it was shown that a D-dimer level above 1000 µg/L was associated with a fatal outcome of Covid-19. The excessive inflammatory response induced by the presence of SARS-COV-2 in the body. This inflammatory response in turn induces disseminated intravascular coagulation. This state of "hypercoagulability", essentially involving a marked rise in D-dimer levels, is associated with an accumulated risk of death. (Mezalek Z. Tazi *et al*., 2021).

Renal function was strongly impacted in our population among those who died (Créât↑). COVID-19 can impact renal function, leading to acute kidney injury and disturbances in electrolyte levels leading to prognostic implications (Benedetti C *et al*., 2020).

By binding to the ACE2 receptor, the virus could unbalance the renin-angiotensin-aldosterone system (RAAS), promoting the negative effects of angiotensin II, which is not balanced by angiotensin 1-7, explains Marco Alifano *et al.*, 2020. In people with diabetes, hypertension or obesity, the RAAS system is already out of balance. The virus could reinforce this phenomenon, and encourage the development of complications. The receptor could also be involved in some of the complications observed in severe forms of Covid-19, such as the development of vascular and myocardial lesions.

5. CONCLUSION

This study showed that a high viral load was a source of disturbance of CRP, ESR, NFS, GLY, HbA1c, DDI, Créat, resulting in a poor prognosis in Congolese type 2 diabetics. In this study, we did not have access to participants' vaccination data, which could influence viremia. In future studies, it would be useful to collect such data to understand how vaccination coverage may affect the impact of COVID-19 viral load on the biological profile of T2DM.

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