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Original Research Article

The Impact of COVID-19 on Type 2 Diabetic Patients: An Analysis of Cardiovascular Complications Using Biological Explorations

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Abstract: ***Introduction*:** The co-occurrence of diabetes and COVID-19 infection poses a significant medical challenge due to the increased risk of serious complications. Infection with SARS-CoV-2, the virus responsible for COVID-19, exacerbates these risks because of the systemic inflammation it causes and the direct and indirect effects of the virus on the cardiovascular system. ***Objective:*** The aim of this study was to explore cardiovascular risks in diabetic patients testing positive for COVID-19 in Pointe-Noire, Republic of Congo. ***Methods*:** We recruited a total of 206 participants for this study. Biomarkers were quantified from blood samples and sars cov-2 virus was identified using the PCR technique on nasopharyngeal swabs. Results: Analysis of our data shows that the following biological parameters are evidence of cardiovascular complications ESR: OR 1.91(1.84-1.97) p<0.001 DDI: OR 1.01(1.01-1.01) p<0.001, APTT: OR 1.67(1.33-2.09) p<0.001, TG/hdl: OR 1.78(1.48-2.13) p<0.001, CK-MB: OR 1.37(1.17-1.59) p<0.001. There was also a strong correlation between AIP and TG (r =0.86, p<0.001) and inversely with HDL (r= -0.56, p<0.0001). A strong association between AC with LDL (r=0.79, p<0.001) and TC (r=0.68, p<0.001). A strong inverse linear correlation of CRR with HDL (r= -0.79, p<0.001) and positive with TC (r=0.53, p<0.001). A strong correlation coefficient of between CPI with HDL (r=0.69, p<0.001) and negative with CT (r= -0.46, p<0.001) and, an inverse relationship with HbA1c(r= -0.22). ***Conclusion*:** The results of our study show that COVID-19 in T2DM can cause cardiovascular complications or deterioration of coexisting cardiovascular disease by direct or indirect mechanisms.

Keywords: Cardiovascular complications, type 2 diabetes, COVID-19, Pointe Noire.

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1. INTRODUCTION

COVID-19 is an infectious disease caused by a beta coronavirus (SARS-CoV-2) [1], a positive polarity single stranded RNA virus [2], transmissible between humans and animals [3]. It was described for the first time in December 2019, in the city of Wuhan, Hubei province in Continental China, in patients presenting with severe unexplained pneumopathies [4]. The World Health Organisation (WHO) declared the disease a pandemic on 11 March 2020, and considered it a public health emergency of international concern [5] (WHO Novel Coronavirus (2019-nCoV) situation reports 2020). Progressive forms of Covid-19 are often characterised by 'cytokine storm' hyperinflammation, with the development of an acute respiratory distress syndrome-like state. In addition, reports of micro and macro thrombotic phenomena such as microangiopathy and pulmonary embolism are increasingly frequent. The clinical presentation of the disease varies from asymptomatic to severe pulmonary insufficiency. Many patients with Covid-19 have underlying cardiovascular disease (CVD) or develop acute cardiac injury during the course of the disease. The impact of pre-existing cardiovascular disease and the development of new cardiac complications on the clinical outcomes of these patients is under investigation [6].

Biomarkers are substances or molecules that can be measured in blood, urine or other body fluids and are associated with the presence or severity of cardiovascular disease [7]. These are measurable indicators that can be used to diagnose or monitor disease progression or the effects of treatment [8]. In the context of COVID-19 positive type 2 diabetes, there are many different biomarkers that can be used to diagnose and predict the prognosis of cardiovascular disease, each with different levels of specificity and sensitivity. As cardiovascular disease is complex and multifactorial, it is often necessary to test the levels of several biomarkers to improve accuracy.

The aim of this research is to explore cardiovascular risk in diabetic patients testing positive for COVID-19 in Pointe-Noire, Republic of Congo.

2. MATERIAL AND METHOD

2.1. Study Population

We conducted a descriptive cross-sectional study with prospective data collection. The study took place from September 2021 to August 2022, a period of 12 months. The study population consisted of D2T patients with COVID-19 hospitalised at the Guenin and Louise Michel clinics and the Adolphe Sicé General Hospital in Pointe-Noire.

2.2. Clinical survey:

Data such as age, sex, 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:

- The blood sample was taken in EDTA and heparinised tubes after a strict fasting period of at least 12 hours; it was stored at -20°C until use.
- Nasopharyngeal sampling was carried out by swabbing by gently pushing the swab deep into the nostril (up to the nasopharynx: approximately half the length from the nose to the ear) and detaching as many cells as possible by scraping the inside of the nostril 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. Analysis of blood biomarkers:

The automatic biochemistry analyser 'Cobas C 311 (Roche Diagnostics, HITACHI, Germany)' was used for the biochemical analyses: Fasting blood glucose (Gly), Glycated haemoglobin (HbA1c), Ultra-sensitive C-reactive protein (CRP-µs), Creatine phosphokinase (CPK); Creatine phosphokinase-MB (CPK-MB), Total cholesterol (TC), Triglycerides (TG), High density cholesterol (HDLc), Low density cholesterol (LDLc). The Plasma Atherogenic Index (PAI): TG/HDLc, the Atherogenic Coefficient (AC): (CT-HDLc)/HDLc, the Cardiac Risk Ratio (CRR): CT/HDL, and the Cardioprotective Index (CPI): HDLc/LDLc were calculated. The Sedimentation Rate (SR) was obtained using the Westerngreen method.

2.3.3. Molecular analysis:

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

b)-Amplifications: The extracted RNAs underwent 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

- Second step: programming the Mic thermal cycler

The 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).

2.4. Ethical considerations

This study was conducted 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). The characteristics of the participants were described by means and standard deviations. We checked the relationship between the indexes (AIP, CA, ICP, RRC) and the glucido-lipid profile, measured using Pearson correlation tests. A univariable and multivariable analysis of the biological poor prognostic factors associated with the risk of death was performed and represented as odds ratios. The statistical analysis was performed using SPSS (version 26.0; IBM). In all calculations, $p < 0.05$ was considered a statistically significant level.

3. RESULTS

3.1 Lipid profil:

Lipid profile disorders are a matter of course, with only AIP influencing the poor prognosis there is a disturbance in carbohydrate balance (Table I).

Table I: Lipid profile

Characteristic	Survivor,	No Survivor,	p	Univariable			Multivariable		
	N = 160	N = 46		OR	IC	P	OR	IC	P
CholT(g/L)	2.18±0.55	2.17±0.39	>0.9	1.15	[0.85, 1.56]	0.4			
HDL-c(g/L)	0.45±0.20	0.48±0.40	0.6	0.95	[0.93, 0.98]	<0.001	0.92	[0.86-0.99]	0.024
LDL-c(g/L)	1,51±0,34	1,64±0,55	0,03	1.00	[0.96, 1.04]	0.058			
TG(g/L)	1.78±0.96	2.05±1.45	0.5	1.08	[1.00, 1.16]	>0.9	1.67	[1.33-2.09]	<0.001
IAP	6.1 ±3.6	7.5 ±4.8	0.044	1.11	[1.04, 1.20]	0.004	1.78	[1.48- 2.13]	<0.001
Cholt/hdl	4.5 ±3.6	7.4 ±8.3	0.007	1.00	[1.00, 1.00]	>0.9	1.09	[1.08- 1.09]	<0.001

3.2 Glucidic profile:

Disturbance in carbohydrate balance (Table II).

Table II: Glucidic profile

Characteristic	Survivor,	No Survivor,	p	Univariable			Multivariable		
	N = 160	N = 46		OR	IC	P	OR	IC	P
GLY(g/L)	2.25±0.93	2.83±1.14	<0.001	1.87	[1.38, 2.60]	<0.001	0.00	[0.00- 0.00]	<0.001
HbA1c (%)	7.89±1.85	7.98±1.86	<0.002	2.00	[1.61, 2.60]	<0.001	0.91	[0.49-1.72]	0.8

3.3 Inflammatory markers

Disturbance of inflammatory markers is more pronounced with the ESR, which influences the poor prognosis (Table III).

Table III: Inflammatory markers

Characteristic	Survivor,	No Survivor,	p	Univariable			Multivariable		
	N = 160	N = 46		OR	IC	P	OR	IC	P
VS (/mm^3)	35 ±31	69 ±45	<0.001	1.02	[1.01, 1.03]	<0.001	1.91	[1.84- 1.97]	<0.001
CRP-µs(mg/L)	226±94	287±112	<0.001	1.01	[1.01, 1.01]	<0.001	0.71	[0.70- 0.72]	<0.001

3.4 Cardiac biomarkers

Analysis of cardiac biomarkers shows an increase in CPK and Ck-mb in deceased subjects, and CK-MB influences the poor prognosis (Table IV).

Table IV: Cardiac biomarkers

Characteristic	Survivor,	No Survivor,	p	Univariable			Multivariable		
	N = 160	N = 46		OR	IC	P	OR	IC	P
CPK(U/L)	71 ±33	119 ±94	<0.001	1.01	[1.01, 1.02]	<0.001	0.84	[0.83- 0.86]	<0.001
CKMB(U/L)	1 ±1	8 ±14	<0.001	2.48	[1.45, 4.90]	0.004	1.37	[1.17- 1.59]	<0.001

3.5 Haemostasis test

DDI and TCA influence poor prognosis (Table V).

Table V: Haemostasis test

Characteristic	Survivor,	No Survivor,	p	Univariable			Multivariable		
	N = 160	N = 46		OR	IC	P	OR	IC	P
TP (sec)	86 ±12	76 ±17	<0.001	1.00	[1.00,1.00]	<0.001	1.01	[1.01-1.01]	<0.001
TCA (%)	29 ±8	28 ±8	>0.9	0.98	[0.92,1.04]	0.5	515	[383-694]	<0.001
DDI(µg/L)	1,898±1,611	5,310±4,095	<0.001	1.93	[1.28,2.97]	0.003	1.45	[0.33-1.80]	0.005

3.6 Relationship between atherogenic indices and plasma lipid levels

Table VI shows a strong correlation between AIP with TG (r =0.86, p<0.001) and inverse with HDL (r= -0.56, p<0.0001). A strong association between AC with LDL(r=0.79, p<0.001) and TC (r=0.68, p<0.001). A strong inverse linear correlation of CRR with HDL(r= -0.79, p<0.001) and positive with TC(r=0.53, p<0.001). A strong correlation coefficient of between CPI with HDL(r=0.69, p<0.001) and negative with CT(r= -0.46, p<0.001) and, an inverse relationship with HbA1c(r= -0.22).

Table VI: Correlation between atherogenic indexes and lipids

Parameter1	Parameter2	r	95% CI	p
AIP	CT	0.27	[0.13, 0.39]	0.003**
AIP	TG	0.86	[0.81, 0.89]	< 0.001***
AIP	LDL	0.10	[-0.04, 0.23]	0.971
AIP	HDL	-0.56	[-0.65, -0.46]	< 0.001***
AIP	HBA1C	0.14	[0.00, 0.27]	0.793
AC	CT	0.68	[0.60, -0.75]	< 0.001***
AC	TG	0.13	[0.01, 0.26]	0.830
AC	LDL	0.79	[0.73, 0.83]	<0.001***
AC	HDL	0.23	[0.10, 0.35]	0.021*
AC	HBA1C	0.43	[0.31, 0.53]	< 0.001***
CRR	CT	0.53	[0.43, 0.63]	< 0.001***
CRR	TG	0.41	[0.29, 0.52]	< 0.001***
CRR	LDL	0.51	[0.40, 0.60]	< 0.001***
CRR	HDL	-0.72	[-0.78, -0.65]	< 0.001***
CRR	HBA1C	0.03	[-0.10, 0.17]	> 0.999
IPC	CT	-0.46	[-0.56, -0.35]	< 0.001***
IPC	TG	-0.25	[-0.37, -0.11]	0.008**
IPC	LDL	-0.62	[-0.70, -0.53]	< 0.001***
IPC	HDL	0.69	[0.61, 0.75]	< 0.001***
IPC	HBA1C	-0.22	[0.34,-0.08]	0.036*

4. DISCUSSION

Type 2 diabetics (T2DM) have an increased risk of developing a severe form of COVID-19. Biological disturbances during the course of diabetes and COVID-19 provide a dashboard for, among other things, stratifying the severity and monitoring these patients. And identifying risk factors and prognostic mortality rates is crucial for identifying the outcome of patients at an early stage in order to support clinical decision-making [9].

This study also shows an AIP↑ in severe cases and those with a poor prognosis; and there is a strong correlation between AIP with TG (r =0.86, p<0.001) and an inverse linear relationship with HDLc (r= -0.56, p<0.0001). According to the work of Mei-Yueh Lee *et al*., 2018 [10] an AIP↑ ratio was significantly associated with albuminuria, coronary heart disease, Stroke and peripheral arterial occlusive disease (PAOD) in T2DM and is also considered by [11] Nwagha U. I *et al*., 2010 as a highly sensitive predictor of cardiovascular disease risk. There is a strong association between AC with LDL(r=0.79, p<0.001) and TC (r=0.68, p<0.001) in addition to AC↑ in difficult cases with T2DM+COVID-19. A strong inverse linear correlation of CRR with HDL(r= -0.79, p<0.001) and positive with TC(r=0.53, p<0.001). Subjects with CRR↑ are more likely to suffer from stroke or atherosclerosis [12]. We observed a CPI↓ in severe cases, followed by a strong correlation coefficient between CPI with HDL(r=0.69, p<0.001) and negative with CT(r= -0.46, p<0.001) and Hba1c(r= -0.22, p: 0.036), this configuration shows

that our T2+COVID-19 are fragile and susceptible to cardiovascular complications.

These indices are invaluable in assessing the risk of developing cardiovascular disease; the more precise the increase in AIP, AC, CRR and the decrease in IPC, the greater the predisposition to cardiovascular disease [12].

(VS OR 1.91 IC 95% (1.84-1.97). These results corroborate those of several studies [13-15]. This finding reflects an increased systemic inflammatory response in type 2 diabetic patients infected with COVID-19, which is associated with increased disease severity and mortality. For, a cytokine storm that damages alveolar structures due to a dysregulated immune system may facilitate viral entry into vascular endothelial cells across the blood-air barrier. Endothelial dysfunction increases the stiffness and vulnerability of pulmonary arteries as the disease progresses, ultimately leading to thrombosis and microvessel obstruction in alveolar capillaries, which can result in hypoxemia or pulmonary hypertension [16]. In our study, the group of patients who died had AIP↑ and OR 1.78 95% CI (1.48-2.13) p<0.001]. This finding suggests that AIP is a relevant prognostic biomarker for assessing mortality risk in type 2 diabetic patients infected with COVID-19. Elevated PAI is associated with increased mortality, probably due to the combination of inflammation, oxidative stress and cardiovascular complications that can exacerbate disease progression in these patients. Interestingly, similar results were observed in HIV-positive patients. AIP values were higher in acute HIV infection, demonstrating the relationship between viral load and AIP [17]. Previous reports on the 2002 SARS-CoV epidemic show that a higher viral load of SARS-CoV is associated with a poorer overall prognosis. [18] Based on these results, we suggest that elevated IPA may be associated with increased viral load and a worse prognostic indicator in patients with COVID-19.

CK-MB OR 1.37 95% CI (1.17-1.59) p<0.001, this result suggests that COVID-19-positive diabetic patients are at high risk of cardiovascular complications associated with elevated CK-mb compared to those without this elevation. This disturbance is also indicative of multi-organ failure in the context of COVID-19. Studies suggest that higher levels of CK-MB may be linked to more severe cases of COVID-19 and could be associated with myocardial damage [19].

Haemostasis data DDI OR 1.45 95% CI (1.01-1.80) p<0.001 and APTT OR 1.67 95% CI (1.33-2.09) p<0.001 show that alterations in haemostasis and the resulting state of hypercoagulability are associated with a significant increase in thrombotic complications and have a poor prognosis [20]. Our DDI results showed a level of >5000ug/L in patients who died. In the work of Zhou F *et al*., 2020 [21], it was shown that a D-dimer level greater than 1000 µg/L was associated with a fatal outcome in Covid-19. The excessive inflammatory response is induced by the presence of SARS-COV-2 in the body. This inflammatory response in turn induces disseminated intravascular coagulation (DIC). This state of 'hypercoagulability' essentially includes a marked rise in D-dimer levels, which is associated with an accumulated risk of death [22].

Indeed, SARS-CoV-2 infection is a multifocal disease involving the respiratory, cardiovascular, renal, gastrointestinal and central nervous systems. Hypercoagulability is a frequent haematological alteration in hospitalised patients with COVID-19 and a predictor of worsening of the disease. Venous thromboembolism, and in particular pulmonary embolism, is more common in hospitalised patients with COVID-19 than in patients hospitalised for other acute medical conditions, even when the recommended pharmacological thromboprophylaxis is administered. Disseminated intravascular coagulation may occur in critically ill patients and is a relevant predictor of death. Immunothrombosis with pulmonary intravascular coagulation (PIC) and vascular occlusion in the microcirculation of the lungs are frequent findings reported in autopsies of patients who have died from COVID-19. Finally, patients with COVID-19 also have an increased risk of arterial thrombosis (ischaemic stroke, myocardial infarction, limb ischaemia). Consequently, COVID-19 is a systemic disease involving blood coagulation and vessels. Patients with cardiovascular disease or cardiovascular risk factors are most vulnerable to deterioration of COVID-19 and severe disease following infection with SARS-CoV-19 [23].

It is important to note that the interpretation of cardiac biomarkers in patients with COVID-19 should be made in the context of the patient's clinical condition and other relevant factors, and not rely solely on biomarker values.

5. CONCLUSION

This study shows that our COVID-19-positive diabetics have biological markers indicating increased inflammation, myocardial damage, poor lipid profile, propensity to thrombosis and advanced atherosclerosis. These are all important risk factors for cardiovascular complications.

Thus, this study highlights the need for a multidisciplinary approach to the management of

these patients. In short, proactive, individualized management could significantly improve the cardiovascular prognosis of diabetics with COVID-19.

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