UJMR, Volume 6 Number 2, December, 2021, pp 115 - 120 *ISSN: 2616 - 0668*
https://doi.org/10.47430/ujmr.2162.016

Received: 29th November, 2021 Accepted: 21st December, 2021

Comparative Study and Analysis of Clotting Profiles Between Covid-19 And Non-Covid-19 Patients In Barau Dikko Teaching Hospital, Kaduna

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Abstract
Coronavirus disease 2019 (COVID-19) is associated with coagulation abnormalities- disordered hemostasis and high thrombotic risk. The aim of the study was to compare and analyze the clotting profiles between COVID-19 and non-COVID-19 patients in Barau Dikko Teaching Hospital, Kaduna. Conventional coagulation tests such as Activated Partial Thromboplastin Time (APTT), Prothrombin time (PT) and International normalized ratio (INR) were used to determine the coagulation in the respondents. Fifty (50) samples were collected for the test. Out of these samples collected, twenty five 25 were positive for COVID-19. Age group 61-70 had the highest percentage of positive cases 40% while age group 41-50 had the least percentage of positive cases 4% respectively. Concerning gender, males had the higher number of positive cases 15 than their female counterparts 10. All epidemiological and clinical data were collected; analyzed and comparative results were carried out. The presence of history of previous diseases was more among COVID-19 patients than non-COVID-19 patients. The clinical characteristics between COVID-19 and non COVID-19 patients showed similarities in body temperature, catarrh, headache, sore throat and sputum, but the presence of heavy cough was observed among COVID-19 patients. The clinical laboratory results showed significant difference ($p<0.05$) and positive correlation were found among the PT (0.17), APTT (0.12) and INR (0.08) between COVID-19 and non COVID-19 patients. These results are indications that there is need for more research in this area of study to know the clinical manifestation of COVID-19 virus among patients.
Keywords: Coronavirus, Prothrombin, Patients, Thromboplastin

INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is a respiratory illness caused by the Severe Acute Respiratory Syndrome Corona virus 2 (SARS-CoV-2) that has swept the globe since 2019 and is still ravaging parts of the world in 2021 (Tang *et al.*, 2020; Wool and Miller, 2021). Asymptomatic or mildly symptomatic, SARS-CoV-2 infection affects 80 percent of patients, while 10 percent develop severe respiratory symptoms that progress to Acute Respiratory Distress Syndrome (ARDS). COVID-19 hospitalized patients are admitted to the intensive care unit in almost a third of the cases (Huang *et al.*, 2020).

As a result of the SARS-CoV-2 virus, the lungs become inflamed, leading to a cytokine storm. Alveolar and interstitial inflammation in the lungs of COVID-19 patients is severe (McGonagle *et al.*, 2020). There is an increase in systemic inflammatory response and an imbalance between procoagulant and anticoagulant homeostatic mechanisms when COVID-19 is present (Wool and Miller, 2021). Thrombotic complications can also occur (Giannis *et al.*, 2020). As a result of Coronavirus Disease 2019 (COVID-19), abnormal blood clots, such as pulmonary embolisms in the lungs and deep vein thromboses (DVTs) in the legs, as well as strokes or heart attacks, can occur (Hampton, 2021). Due to COVID-19 infection, cytokines are produced in large quantities, damaging internal organs, especially the lungs (Sayad and Rahimi, 2020). Hypercoagulability is a common complication of severe viral pneumonia. The incidence of venous thromboembolism in COVID-19 patients is like other epidemic coronavirus pneumonias, such as severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS-CoV) (Giannis *et al.*, 2020). It has been found that those with H1N1 influenza had an 18-fold higher risk of developing VTE than those who did not have the virus (Obi *et al.*, 2019).

The risk of developing a VTE in COVID-19 patients hospitalized for more than a week is very high (Bikdeli *et al.*, 2020).

For example, Llitjos and colleagues found that 69% of COVID-19 inpatients had thrombotic complications, with the highest incidence occurring due to active ultrasound surveillance for deep-vein thrombosis (69%) in Llitjos and colleagues (2020). Contrary to standard thromboprophylaxis, COVID-19 patients continue to have high rates of thrombosis, both venous and possibly arterial (Klok *et al.*, 2020). ICU and general hospital thromboprophylaxis did not reduce the incidence of VTE, according to COVID-19 researchers (general ward). Ischemic stroke and acute coronary syndrome accounted for 2.5 and 1.1 percent, respectively (Lodigiani *et al.*, 2020). A VTE and pulmonary embolism incidence of 22.5 percent and 10 percent, respectively, was observed in COVID-19 French patients not in intensive care units (ICUs) receiving thromboprophylaxis (Artifoni *et al.*, 2020). In Chinese patients with COVID-19, abnormal coagulation factors were linked to a poor prognosis (Tang *et al.*, 2020). On admission, a high level of D-dimer (> 1g/ml) was associated with in-hospital death in some studies (but not all) (Favaloro and Thachil, 2020). The clinical severity of COVID-19 increases with age in patients aged 60, who have more severe clinical manifestations, a longer disease course, and a higher severity score (Liu *et al.*, 2020). COVID-19 patients admitted to the ICU have a mortality rate ranging from 10 to 44 percent, depending on their age and the presence of co-morbidities that resulted in multi-organ failure (Annunziata *et al.*, 2020; Jamaatia *et al.*, 2020; Pavoni *et al.*, 2020).

MATERIALS AND METHODS

Study Area

The research was conducted in Barau Dikko Teaching Hospital Kaduna. Kaduna North Local Government is home to the Barau Dikko Teaching Hospital. (Long. 100 36' N and Lat. 7027'E) on the Greenwich Meridian is the location of Kaduna North Local Government Area. It has a population of 357,694 and a total area of 72 square kilometers (NPC, 2006). They share a border with the Igabi Local Government Area in the north, Kaduna South Local Government Area in the west, and Chikun Local Government Area in the east (NPC, 2006). As of now, the Kaduna North Local Government Area consists of Unguwan Dosa and Unguwan Rimi among others. Located in Magajin Gari, the Local Government Area's administrative headquarters (Doka) (NPC, 2006).

Study Population

The study population consisted of non-covid subjects (volunteers), primarily health workers at Kaduna State's Barau Dikko Teaching Hospital. In a sterile trisodium citrate tube, fifty samples were collected. Data for the remaining (50) positive subjects were obtained from Kaduna State's BDTH coagulation laboratory between March and September 2020, during the covid-19 pandemic

Sample Size

According to the Nigeria Centre for Disease Control, NCDC, the total laboratory confirmed cases of COVID-19 in Kaduna state as of 6thJuly, 2021 was 9,123 (NCDC, 2021). The prevalence rate of COVID-19 thus is:

$$(Prevalence\ rate = \frac{Number\ of\ cases}{Population\ of\ area}\ X\ 100)$$

$$(Prevalence\ rate = \frac{9,123}{1,133,000}\ X\ 100)$$

= 0.805%

A formula described by Cochran will be used to determine the sample size and prevalence rate from the Nigeria Centre for Disease Control, NCDC (2021), will be used. The Cochran equation is as follows:

$$n = \frac{Z^2pq}{e^2}$$ (Cochran, 1977)

Where n = sample size

Z = standard normal distribution at 95% confidence interval =1.96

p = prevalence rate, which will be taken as 0.805% = 0.00805 (NCDC, 2021).

q = 1-p = 1 - 0.008 = 0.99195

e = allowable error, which is taken as 5%=0.05

Therefore n =

$$\frac{(1.96^2 X\ 0.00805\ X\ 0.99195)}{(0.05^2)}$$

= 12.27

However, = 50 samples will be recruited so as to improve the precision of this research.

Ethical Clearance

Ethical approval was obtained from Kaduna State Ministry of Health.

Inclusion Criteria

Non-covid subjects who passed the exclusion criteria.

Exclusion Criteria

i. Patients on drugs altering coagulation profile.
ii. Patients with liver disease.
iii. Patients with a history of coagulation disorders.
iv. Patients with malignancy, coronary artery disease, cerebro-vascular accident.
v. Patients with clinical evidence of macrovascular and microvascular complications of diabetes mellitus.
vi. Consent not given.

Collection of Samples

Three (3.0) mls of blood were collected from BDTH health workers using sterile vacutainers, needles, and syringes (tourniquet applied) and placed in trisodium citrate tubes (liquid anticoagulant). To keep the blood from clotting, the samples were appropriately mixed with trisodium citrate anticoagulant.

Within one hour (1hr) of collection, samples were taken for centrifugation for 15 minutes at 3000rpm to separate blood cells from blood plasma. The plasma (supernatant) is transferred into microtubes using disposable pipettes. The microtubes were then placed on a rack and analyzed in 2 hours with an EBRA ECL 105 automated analyzer machine.

Preparation of Control

Plasma samples (equal volume) from five non-covid subjects were mixed appropriately in a sample tube using a micropipette (50ml) and analyzed as a single sample. The automated analyser machine's prothrombin time (PT), international normalised ratio (INR), and activated partial thromboplastin time (APTT) values were used as controls.

Covid 19 Rapid test Antigen

Test procedure

The buffer was held vertically, and the extraction tube was filled with the buffer (300l), the extraction tube was then placed on a tube rack, the patient's head was tilted backwards, and the swab was inserted through the nostrils, gently rubbed 3-4 times, and slowly removed, the swab was then inserted into the extraction tube, and homogenized, and the cotton swab was then squeezed with a swab was then detached from the extraction tube's cap. The tube's nozzle cap was then dropped. After that, 5 drops of the extracted specimen were extracted by holding it vertically. Following the extraction, the nozzle of the tube containing the extracted fluids was disposed of in accordance with biosafety procedures.

A timer was then set for 20 minutes, and recordings were taken; the used device was then disposed of following biosafety purposes.

PROCEDURE FOR PROTHROMBIN TIME (PT)

The PT for each sample was determined twice according to the manufacturer's instruction (recoplastin)

1. Switch on the ERBA ECL 105 Automated machine
2. Place the test cuvette on the machine
3. Pipette micro litter of the sample (plasma) into the test cuvette
4. Allow to incubate for 120 seconds
5. Add 100microlitter of PT reagent into the test cuvette
6. Then allow the machine to read and display the result on the screen

INTERNATIONAL NORMALISED RATIO (INR)

The automated machine reads and displays both the result of PT and INR at the same time

ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT)

Place the test cuvette on the ERBA ECL 105 Automated machine. Pipette 100 microlitter of the sample (plasma) into the test cuvette and 100 microlitter of the APTT reagent were added into the same test tube. Incubate for 180 seconds, 100microlitter of Calcium chloride ($CaCl_2$) reagent were then added into the test cuvette and the result were read.

The result APTT of each sample were determined twice according to the manufacturers' instruction (SP-Unicellin)

RESULTS

A total of 50 samples were collected out of which 25 were positive for covid-19 and 25 were negative, for those positive 8% were between the ages of 30-40 years, 4% were between 41-50 years, 16% were between the ages of 51-60, while 40% were between the ages of 61-70 and finally 32% were between the ages of 71-80.

The socio demographic factors between the covid-19 and non covid-19 patients can be observed, it shows that there were more male in the covid patients than in the non covid patients, Also (n=27) of all the patients examined were less than 60 years of age, while the mean age of the covid patients was 69.68 compared to the 60.54 of the non covid patients, Finally 30 out of the 50 patients totally examined had no history of any previous sickness, while 10 patients had hypertension, 5 had diabetes, 3 had stroke and 2 had kidney diseases.

The clinical characteristics of the covid and non covid-19 patients, from the results could be observed that both categories had normal temperatures of between 36º to 39º C. 14 of the covid-19 patients had intense cough while only 2 non covid-19 patients experienced coughing, Only 2 covid-19 patients produced sputum, and 1 patient had sore throat, while 6 covid patients had severe headaches, finally catarrh was only observed on one non covid-19 patient.

The Prothrombin time of the covid patients (33.42) was higher than that of the non covid patients (13.44), the Activated partial thromboplastin time showed that the covid-19 patients had a higher mean (77.83) compared with the non-covid (37.21).

The bivariate correlations showed no significant relationship between the two variables ($p<0.12$). Finally, the international normalized ratio showed slight difference in the mean of the two patients and it also showed that there was no significant relationship between the INR of the covid and non-covid patients as ($p<0.08$).

Table 1: Distribution of Covid-19 Among Patients Attending Barau Dikko Teaching Hospital With Respect To Age Group

Age Group	No. of samples	Number of Negative cases	Number of Positive cases	Percentage Positive (%)
30-40	9	7	2	8
41-50	6	5	1	4
51-60	10	6	4	16
61-70	14	4	10	40
71-80	11	3	8	32
Total	50	25	25	100

Table 2: The Demographic Characteristics of the Covid-19 and Non-Covid-19 Patients

Variable		Covid-19 patients (n=25)	Non-Covid-9 Patients (n=25)
Gender	Male	15	10
	Female	10	15
Age	Above 60 years	13	10
	Less than 60 years	12	15
Weight		69.68	60.54
Previous diseases	None	9	21
	Hypertension	9	1
	Diabetes	4	1
	Stroke	2	1
	Chronic Kidney disease	1	1

Table 3: The Clinical Characteristics between Covid-19 and Non-Covid-19 Patients

Variable		Covid 19 patients (n=25)	Non-Covid 19 Patients (n=25)
Temperature	36º	6	10
	37º	8	6
	38º	8	5
	39º	3	4
Cough		14 (56%)	2 (8%)
Sputum		2 (8%)	0 (0%)
Sore throat		1 (4%)	0 (0%)
Headache		6 (24%)	4 (16%)
Catarrh		0 (0%)	1 (4%)

Table 4: The Clinical Laboratory Results of Covid19 and Non-Covid 19 Patients

Variable	Covid 19 patients	Non-Covid 19 Patients
PT (Prothrombin time)	33.42	13.44
APTT(activated partial thromboplastin time)	77.83	37.21
INR (international normalized ratio)	2.57	1.01

Table.5: Correlation between the Clinical Laboratory Results of Covid-19 and Non-Covid-19 Patients

Variable	Covid 19 patients	Non-Covid-19 Patients	P value
PT (Prothrombin time)	33.42	13.44	-0.17
APTT(activated partial thromboplastin time)	77.83	37.21	-0.12
INR (international normalized ratio)	2.57	1.01	-0.08

DISCUSSION

After SARS and MERS, Covid-19 is currently the fourth large-scale outbreak of coronavirus, affecting Nigeria and dozens of other countries (Huang *et al* 2019). Statisticians estimate that Nigeria could lose $12 billion in the first quarter of 2020, while the world could lose over $280 billion (CNN Business, 2020). In order to prevent and control disease, it is crucial to compare the clinical differences between those with and without Covid-19. In this study, the patients with Covid-19 had advanced aging symptoms and were older than average. In light of this finding, Covid-19 may be more susceptible to older people with more severe symptoms that could lead to negative outcomes. Males are more susceptible to contracting covid-19 than females, according to the results of the study. Patients with severe chronic diseases were admitted. It is possible that obesity played a major role in the development of Covid 19. These findings were consistent with the earlier epidemiological conclusions about Covid-19 virus.

Some patients examined had upper respiratory tract symptoms, such as sore throat (4%) and catarrhal symptoms, but this was not common (4%). SARS and MERS-like clinical characteristics were found in Covid-19 (Killerby *et al.*, 2020).Covid-19 patients had fewer catarrhal symptoms (4%) than non-Covid-19 patients, and both had lower rates of severe headache (24 vs 16%), while the temperature (36–39%) was similar in MERS and SARS patients (Assiri *et al.*, 2013).

This study showed that patients with Covid-19 had significantly longer Prothrombin times. This suggest Covid-19 might primarily affect thrombocytes, causing the blood cells to clot earlier.

The APTT of covid 19 patients were higher than that of the non covid patients, this test measures the number of seconds it takes for a clot to form in a sample of blood after substances (reagents) are added, it simply means that the covid virus infects the blood so fast that it increases the blood clotting time by more than half its normal rate in very few seconds. Finally the International normalised ration of the two types of respondents showed different but variations in the figures, the INR for covid-19 patients was higher than that of non covid patients , this was also confirmed by there being no correlation between the two various types of covid patients, this results rejects the null hypothesis which states that there was prolonged clotting time in the non covid and covid patients, meanwhile there was a higher value of PT, APTT and INR values from the study above, this result is similar to the works of Li *et al.*, 2020 who performed a similar study in southern China, he observed that the clinical results of the covid patients were way higher than that of the non covid patients. Furthermore the socio-demographic factors of the patients didn't really show any relationship to the increase in blood clotting profile, as more covid 19 patients were seemingly healthier than the non covid patients.

Finally the clotting profiles of each individual varied across the study, some individuals showed a higher PT and APTT profile in covid 19 patients while some showed lowered PT time, this phenomenon isn't fully understood and much more research is needed to ascertain the virulence factor of covid 19 that may cause this variations amongst individuals.

CONCLUSION

The result of this study showed that there was prolonged clotting period amongst the Covid-19 patients. When compared with non-Covid-19 patients, patients with Covid-19 had previous medical conditions and showed some clinical manifestation. Finally, to investigate the disparities amongst clotting profile in Covid patients, the current mutations and changes in the SARS-COVID virus could be a reason for the unstable differences in the clotting profile of various individuals, as the virus uses the genetic composition of individuals to carry out its virulence amongst individuals.

Future research should be done in order to understand the virus and its pathogenicity, and therefore understand the clotting profile of individual from the different parts of the world, as the virus is a current epidemiological problem in the world as a whole.

RECOMMENDATION

Future research should be conducted to understand the virus and its pathogenicity, and therefore understand the clotting profile of individuals from the different parts of the world, as the virus is a current epidemiological problem in the world.

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