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ORIGINAL ARTICLE

Platelet indices as useful biomarkers in SARS-CoV-2 infection

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Abstract

Introduction: The indices of platelets are biomarkers of platelet activation and could be useful for diagnosis and management of COVID-19 infection. This study was aimed at evaluating the impact of COVID-19 infection on some platelet indices in Port Harcourt.

Materials and Methods: The study population consisted of twenty-two (22) COVID-19 positive and fifty (50) COVID-19 negative (controls). Their ages range from 20-65 years old. Five milliliters of venous blood was collected into EDTA anticoagulant bottles for platelet indices determination using an auto analyzer (Sysmex XP-300), while naso pharyngeal swab was collected for confirmation of COVID-19 by RT-PCR molecular method.

Results: The overall prevalence of COVID-19 in this study population was 30.56%. Chi square analysis revealed that this prevalence rate is very significant. (χ^2 =10.889; P=0.001). The mean PDW values of COVID-19 positive subjects (16.24±0.08fl) was significantly increased when compared with the value of the negative subjects (15.93±0.10fl) (t=2.441, P< 0.01). There was no statistically significant difference in the platelet count when the two groups were compared. However, the platelet count of the positive female COVID-19 subjects (216.27±19.65×10^9/L) was significantly reduced when compared with their male counterparts (247.36±19.65×10^9/L). The reverse is the case in the negative (control) group (F=4.6825, p<0.03). There was a significant negative correlation between MPV and platelets (r= -0.489, p< 0.003). The platelet count of COVID-19 positive subjects was reduced even though not statistically significant.

Conclusion: Platelet indices such as PDW and MPV could serve as surrogate biomarkers in SARS-CoV-2 infection

Introduction

Over the past 2 decades, coronaviruses (CoVs) have been associated with significant disease outbreaks in East Asia and the Middle East. The severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) began to emerge in 2002 and 2012, respectively. Recently, a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease 2019 (COVID-19), emerged in late 2019, and it has posed a global health threat, causing an ongoing pandemic in many countries and territories (1).

The disease is a mild, moderate to severe respiratory illness that is caused by a coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) of the genus Beta coronavirus, it is transmitted mainly by contact with infectious objects such as respiratory droplets, objects or surfaces contaminated by the causative virus. The most common symptoms associated with COVID19 are fever, cough, dyspnea, expectoration, headache, pneumonia, myalgia or fatigue, respiratory failure. In contrast, less common signs at the time of hospital admission include diarrhea, hemoptysis, and shortness of breath (2,3). The virus is a member of the coronavirus family that are zoonotic pathogens, i.e., the viruses cause and transmit illnesses between human and several animal's species such as cattle, camels, cats, and bats (4).

The COVID-19 disease was detected initially in late December 2021 in Wuhan, Hubei Province, China, and spread worldwide two months later. Over 200 countries in the world have reported different numbers of cases; however, the disease has drastically expanded in the United States, Spain, Italy, Germany, France, China, Iran, the United Kingdom, and Turkey (5). COVID-19 had caused more than 3.7 million confirmed cases and killed at least 260,000 worldwide as was recorded in April 2020 (6).

The platelet is a circulating anuclear fragment of a bone marrow megakaryocyte, 3 to 4 µm in diameter, with limited synthetic capability. The mean normal platelet count is between 250,000 and 260,000 cells/mm^3, although there is a wide range of accepted normal values in most laboratories that extend as low as 150,000 to as high as 400,000/mm^3 (7).

The indices of platelets are biomarkers of platelet activation and could be useful for the diagnosis of COVID-19. They allow extensive clinical investigations focusing on the diagnostic and prognostic values in a variety of settings without bringing extra costs. Platelet indices including Plateletcrit (PCT), Mean platelet volume (MPV), Platelet large cell ratio, and Platelet distribution width (PDW) are a group of platelet parameters determined by automatic complete blood count profiles, and they are related to platelet morphology and proliferation kinetics (8).

Interference these platelet indices will provide us a more comprehensive insight and probable indicators into potential ethology instead of platelet count alone. Various infections and metabolic disorders cause variations in the platelet counts and platelet indices. It was valuable indicators of illness severity including COVID-19 infection and effective predictors of clinical outcomes. The size of the platelet is decreased as the platelet becomes aged, and an increased MPV indicates an increased proportion of young platelets in the circulation (9,10). Some platelet indices will be compared among subjects with COVID-19 infection in Port Harcourt. In a world where there is an increase in the rate of COVID-19 as a result of contact with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), platelet indices is one of several parameters that can be used to assay for COVID-19, there is an increasing and urgent need for researches to be carried out to tackle what is now a global challenge. The study

intends to evaluate the levels and effects of platelet indices in subjects in order to improve the quality of life as it relates to the virus and the disease. The outcome and results of this study will help to state the significance of the various platelet indices in COVID-19 and why COVID-19 should be properly diagnosed, prevented and managed. Also, the need for platelet levels to be maintained at a normal and reference level among humans. This study was aimed at evaluating the impact of COVID-19 infection on some platelet indices in Port Harcourt.

Materials and Methods

Experimental Design

A cross-sectional, case-control study design was employed to do a comparative study of platelet indices of 72 subjects, 22 COVID-19 positive subjects and 50 negative subjects.

Study Area

The study was carried out at the Rivers State University Teaching Hospital Port Harcourt, Rivers State in the Port Harcourt City Local Government area of Rivers State, Nigeria. Port Harcourt covers a land area of 360 km2 and a population of 1,382,592 at the 2006 census. Port Harcourt is the capital and largest city in Rivers State, Nigeria. It is the fifth most populous city in Nigeria after Lagos, Kano, Ibadan and Kaduna. It lies along the Bonny River and is located in the Niger Delta. As of 2016, the Port Harcourt urban area had an estimated population of 1,865,000 inhabitants, up from 1,382,592 as of 2006 (11). The population of the metropolitan area of Port Harcourt is almost twice its urban area population with a 2021 United Nations estimate of 3,171,076 (12). Port Harcourt has grown by 150,844 since 2015, which represents a 4.99% annual change (13).

Study Population

The study was carried out among male and female subjects infected with COVID-19 against a control of COVID-19 negative individuals. A total of twenty-two (22) COVID-19 positive and fifty (50) COVID-19 negative subjects were recruited for this study within the ages of twenty (20) to sixty-five (65) years old.

Sample Collection and Processing

Five milliliters (5ml) of venous blood was collected aseptically and dispensed into Ethylene Diamine Tetraacetic acid (EDTA) anticoagulant bottle to assay platelet indices. Then nasopharyngeal swab was collected for confirmation of COVID-19 positive subjects by RT-PCR.

Peocedures

The platelet indices were determined using haematology autoanalyzer (Sysmex XP-300) and malaria infection was determined using microscopy while COVID-19 status was determined using RT-PCR technique, the procedures are as follows;**Procedure for platelet indices using haematology autoanalyzer (Sysmex XP-300) as described by Nagy *et al.***

Venous blood collected from the EDTA bottle was mixed for about 10 minute properly using the blood mixer ensuring that no clot is found in the collected blood then on the led screen of the analyzer the patients sample identification number was inputted, then the sample was gently opened and inserted to the probe of the analyzer then after a beep the sample was removed and then it took some seconds for the analyzer to process the sample before the result is displayed on the screen and printed out.

Procedure for COVID-19 confirmation by RT-PCR molecular method as described by Arya *et al.*

The volumes of Super Mix and Enzyme Mix per

reaction multiply with the number of samples, which includes the number of controls and samples prepared. Molecular Grade Water was used as the negative control. For reasons of imprecise pipetting, an extra virtual sample was added then sample was mixed completely and then spun down briefly with a centrifuge then 20µL master mix with micropipettes of sterile filter tips was pipetted to each of the Real Time PCR reaction plate/tubes then 5µL template (nucleic acid extracted from negativecontrol and specimen, positive control without extraction) was separately added to different reaction plate/tubes then the plates/tubes immediately close to avoid contamination then to collect the Master Mix and template in the bottom of the reaction tubes it was Spun down briefly then the instrument of ABI Prism @ 7500/7900 was used to Perform protocols as instructed by the manufacturer, it was ensured that for the ABI Prism® system "none" was selected as passive reference and quencher to avoid any errors.

3.8 Data Analysis

Data management and statistical analyses were conducted using SAS 9.4 software and graphical representations were carried out using the JMP statistical discovery TM software version 14.3. The platelet indices of the subjects were initially subjected to descriptive statistics that includes means, standard deviation and 95% confidence intervals. Subsequently, analysis of Variance (ANOVA) was done to determine, if differences exist across the measured parameters by the subject group (COVID-19 Negative and COVID-19 Positive). In addition, interaction effects between the subject group by sex, and subject group by age group were also evaluated where *p*-values less than 0.05 were considered statistically significant

Results

The study was aimed at assessing the impact of COVID-19 infection on some platelet indices. Table 1 shows the association between COVID-19 test results, sex and age group of subjects. Overall, 22 subjects (30.56%) were positive for COVID-19 and this value was highly significant when compared with 50 negative subjects (69.44%) (χ^2 is 10.889, $p<0.001$). A significant association was also found to exist among the older subjects when compared with those of younger age groups (χ^2 is 27.070, $p<0.0001$). There was no association between covid-19 and sex of participants.

Table 2 shows the platelet indices of COVID-19 positive and negative cases. PDW of COVID-19 positive subjects (16.24 ± 0.08FL) was significantly reduced when compared with the values from the negative subjects (15.93 ± 0.10FL) (t= 2.4417, $p<0.01$). There were no significant differences in the values of other platelet indices ($p>0.05$).

In table 3, comparison of the platelet indices of COVID-19 positive and negative cases shows that sex has a significant influence on platelets while the other indices were non significantly influenced. The platelet count of female positive COVID-19 subjects (216.27± 19.65 × 10^9/L) was significantly reduced when compared their male counterparts (247.36 ± 19.65 x10^9/L). Contrastingly the reverse is the case among the among the negative (control subjects) where the female values were highest (259.35 ± 11.70) than the female negative (control) subjects (217.63 ± 14.95 x10^9/L) (F= 4.6825, $p<0.0340$).

Table 4 shows the association of age groups with platelet indices of COVID-19 positive and negative subjects. Age was not found to have a significant influence on COVID-19 infection. ($p>0.05$).

The pairwise correlation of platelet indices and age group amongst subjects with COVID-19 is as shown in Table 5. PCT showed a positive and significant correlation with platelets (r= 0.928, $p<0.001$). There was no significant correlation existing among other

variables. Similarly, the pairwise correlation in COVID-19 negative is shown in Table 6. MPV correlated significantly and negatively with platelets (r= -0.489, p<0.0003).

Figures 1 and 2 show the distribution of the Study subject by sex and age group respectively. The box plots of platelets in COVID-19 positive and negative subjects are shown in Fig. 3. The median value of platelet count in the positive COVID-19 subjects is significantly reduced when compared with the negative. Similarly, the PDW of COVID-19 also shows an elevated value when compared with the negative subjects.

Table 1: Association between COVID-19 Test Results, and Sex and Age Group of

Characteristic	N (%)	COVID-19 Result		Test Statistics	
		Positive n (%)	Negative n (%)	χ^2	P-value
Overall	72 (100.0)	22 (30.56)	50(69.44)	10.889	0.001***
Sex					
Female	42 (58.33)	11 (15.28)	31 (43.06)		
Male	30 (41.67)	11 (15.28)	19 (26.39)	0.905	0.3414 ns
Total	72 (100.0)	22 (30.56)	50 (69.44)		
Age Group (years)					
< 30	39 (54.17)	2 (2.78)	37 (51.39)		
30 – 40	22 (30.56)	12 (16.67)	10 (13.89)		
40+	11 (15.28)	8 (11.11)	3 (4.17)	27.070	<0.0001****
Total	72 (100.0)	22 (30.56)	50 (69.44)		

*Significance Level: ***=p<0.001; ****= p<0.0001, ns=not significant (p>0.05).*

Table 2: Platelet Indices of COVID-19 Positive and Negative Cases

	COVID -19 Result			
	Positive (n=22)	Negative (n=50)	Test Statistics	
Parameter	Mean ± SEM	Mean ± SEM	t-Ratio	Prob > |t|
PLT (10^9/L)	231.52±15.58	243.50±9.09	-0.6476	0.5214 ns
PDW (FL)	16.24±0.08	15.93±0.10	2.4417	0.0173 *
MPV (FL)	10.84±0.25	10.64±0.14	0.7041	0.4859 ns
PCT (%)	0.25±0.02	0.27±0.02	-0.9097	0.3662 ns

Abbreviations: SEM: *Standard error of mean, PLT: Platelet count, MPV: Mean Platelet Volume, PDW: Platelet Distribution Width, PCT: Plateletcrit.*
*Significance Level: *= p<0.05, ns=not significant (p>0.05).*

Table 3: Platelet Indices of COVID-19 Positive and Negative Cases by Sex

Interaction			PLT (x 10 ^{9}/L)	PDW (FL)	MPV (FL)	PCT (%)
COVID - 19 Result	Sex	n	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Positive	Female	11	216.27±19.65 a	16.30±0.18	11.39±0.31	0.25±0.04
	Male	11	247.36±19.65 ab	16.17±0.18	10.29±0.31	0.25±0.04
Negative	Female	31	259.35±11.70 b	15.81±0.11	10.68±0.19	0.30±0.02
	Male	19	217.63±14.95 a	16.13±0.14	10.57±0.24	0.23±0.03
Test Statistics: *F-Ratio (Prob > F)*			4.6825 (0.0340) *	2.1532(0.1469) ns	3.4519(0.0675) ns	1.1490(0.2875 ns

Abbreviations: SEM: Standard error of mean, PLT: Platelet count, MPV: Mean Platelet Volume, PDW: Platelet Distribution Width, PCT: Plateletcrit.
*Within parameter, means ± SEM with different superscript(s) are significantly different (p<0.05). Significance Level: *= p<0.05, ns=not significant (p>0.05).*

Table 4: Platelet Indices of COVID-19 Positive and Negative Cases by Age Group

Interaction		n	PLT (x 109/L)	PDW (FL)	MPV (FL)	PCT (%)
COVID - 19 Result	Age group (years)		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Positive	< 30	2	241.00±48.14	16.20±0.42	10.40±0.77	0.25±0.09
	30-40	12	242.33±19.65	16.16±0.17	10.78±0.31	0.26±0.04
	40+	8	213.75±24.07	16.36±0.21	11.05±0.38	0.23±0.05
Negative	< 30	37	247.76±11.19	15.83±0.10	10.74±0.18	0.29±0.02
	30-40	10	238.10±21.53	16.25±0.19	10.34±0.34	0.24±0.04
	40+	3	209.00±39.30	16.10±0.34	10.33±0.63	0.21±0.08
Test Statistics: *F-Ratio (p - value)*			0.0204(0.9798) ^{ns}	0.5473(0.5811) ^{ns}	0.5283 (0.5921) ^{ns}	0.1314(0.8771) ^{ns}

Abbreviations: SEM: *Standard error of mean, PLT: Platelet count, MPV: Mean Platelet Volume, PDW: Platelet Distribution Width, PCT: Plateletcrit.*
Significance Level: ns=not significant (p>0.05).

Table 5: Pairwise correlation of Platelet Indices and Age Group among COVID-19 Positive Patients

Variable	by Variable	Correlation	Lower 95%	Upper 95%	P-value
PDW (FL)	PLT (10^9/L)	-0.023	-0.441	0.402	0.9177
MPV (FL)	PLT (10^9/L)	-0.288	-0.633	0.152	0.1933
MPV (FL)	PDW (FL)	-0.238	-0.599	0.204	0.2865
PCT (%)	PLT (10^9/L)	0.928	0.832	0.970	<.0001****
PCT (%)	PDW (FL)	-0.105	-0.504	0.332	0.6432
PCT (%)	MPV (FL)	0.071	-0.361	0.479	0.7522
Age (years)	PLT (10^9/L)	0.081	-0.353	0.486	0.7205
Age (years)	PDW (FL)	0.087	-0.347	0.491	0.7002
Age (years)	MPV (FL)	-0.042	-0.456	0.386	0.8516
Age (years)	PCT (%)	0.056	-0.375	0.466	0.8056

*Significance Level: ****= p<0.0001*

Table 6: Pairwise correlation of Platelet Indices and Age Group among COVID-19 Negative Patients

Variable	by Variable	Correlatio	Lower 95%	Upper 95%	P-value
PDW (FL)	PLT (10^9/L	-0.114	-0.380	0.170	0.4310
MPV (FL)	PLT (10^9/L	-0.489	-0.675	-0.243	0.0003***
MPV (FL)	PDW (FL)	-0.125	-0.389	0.159	0.3886
PCT (%)	PLT (10^9/L	0.271	-0.008	0.511	0.0572
PCT (%)	PDW (FL)	0.120	-0.164	0.386	0.4053
PCT (%)	MPV (FL)	-0.231	-0.478	0.051	0.1070
Age (years)	PLT (10^9/L	-0.131	-0.395	0.153	0.3657
Age (years)	PDW (FL)	0.161	-0.123	0.421	0.2631
Age (years)	MPV (FL)	-0.166	-0.425	0.118	0.2485
Age (years)	PCT (%)	-0.116	-0.382	0.168	0.4215

*Significance Level: ***= p<0.001*

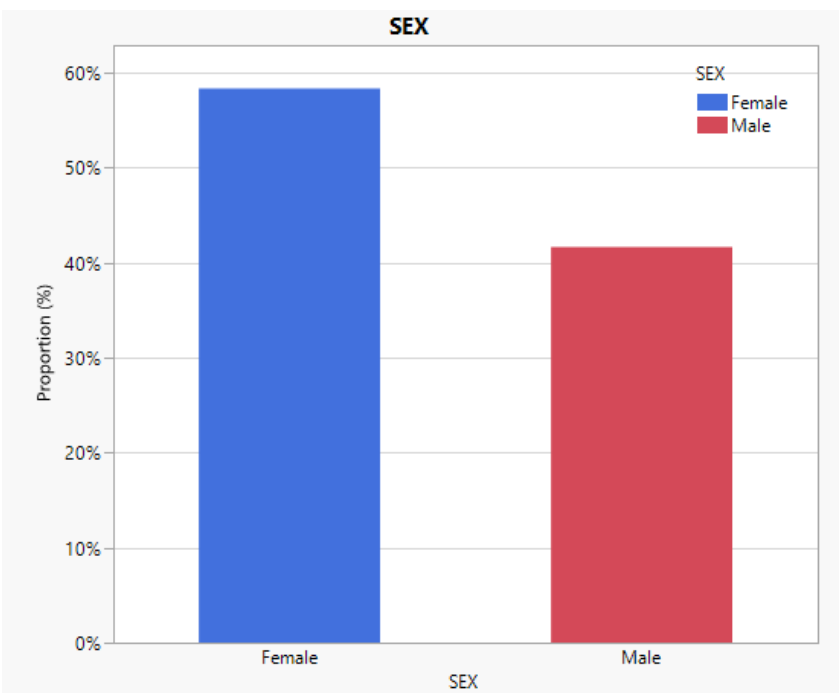

Figure 1: Distribution of Study Sample by Sex

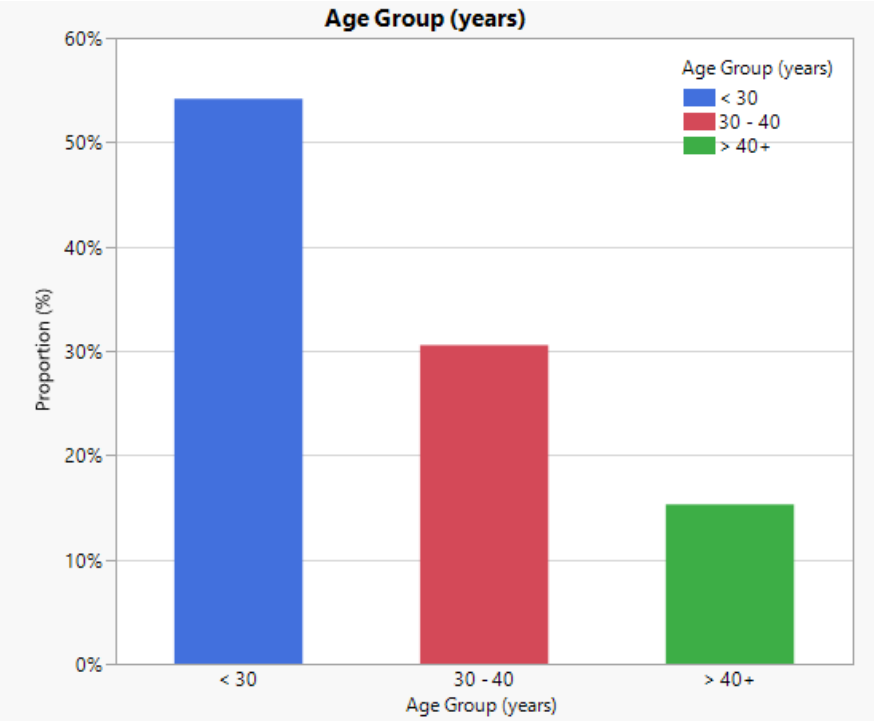

Figure 2: Distribution of Study Sample by Age Group (years)

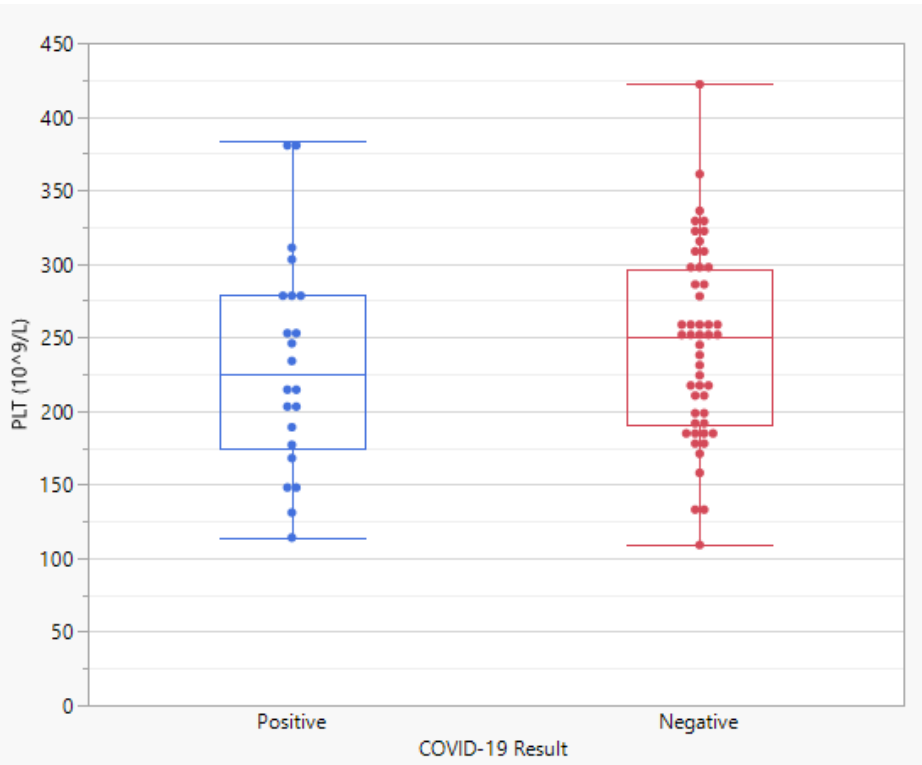

Figure 3: Box Plot of Platelet in COVID-19 Positive and Negative Subjects

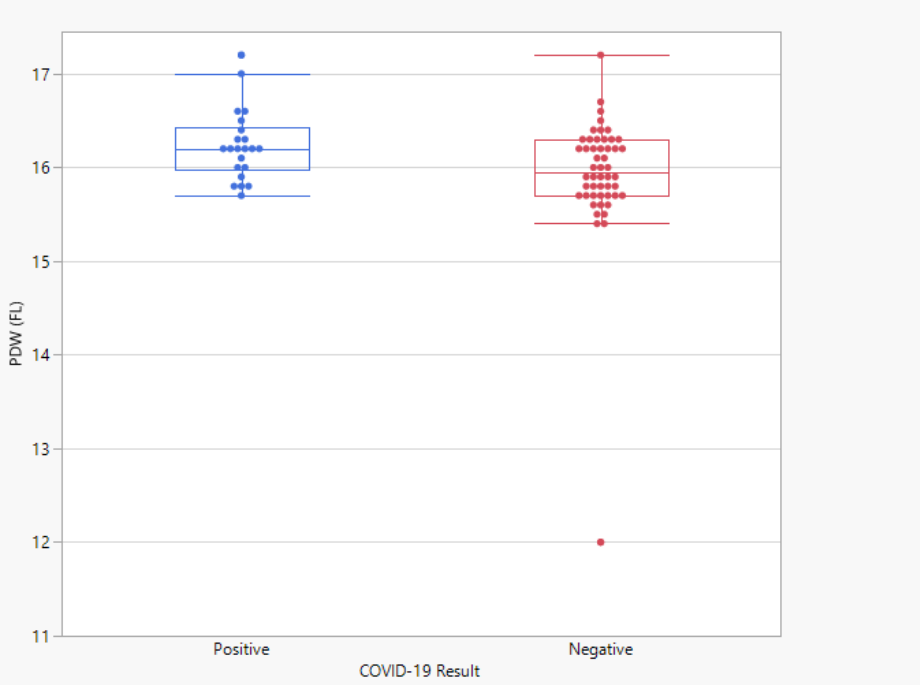

Figure 4: Box Plot of Platelet Distribution Width (PDW) in COVID-19 Positive and Negative Subjects

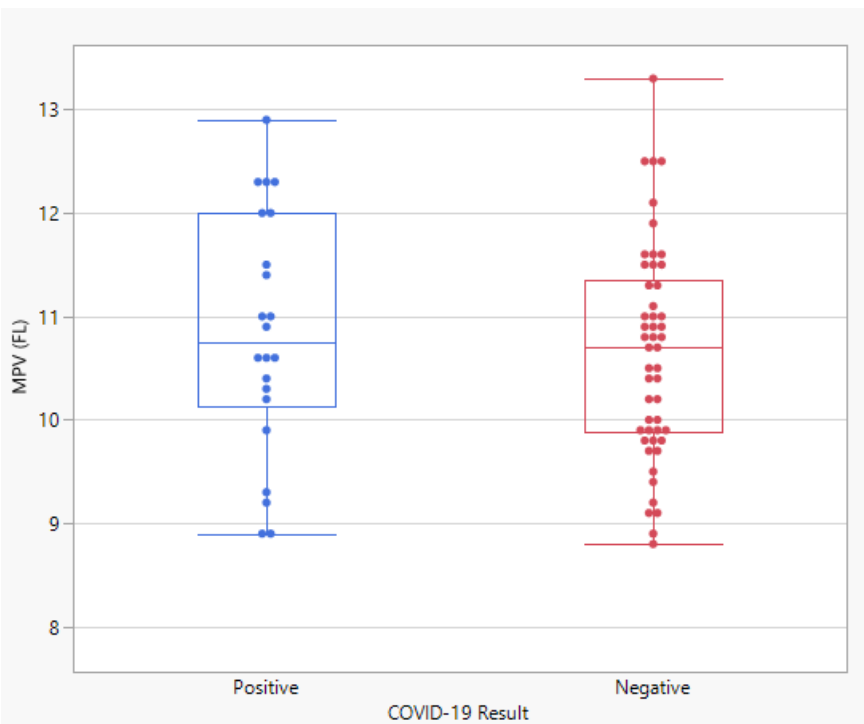

Figure 5: Box Plot of Mean Platelet Volume (MPV) in COVID-19 Positive and Negative Subjects

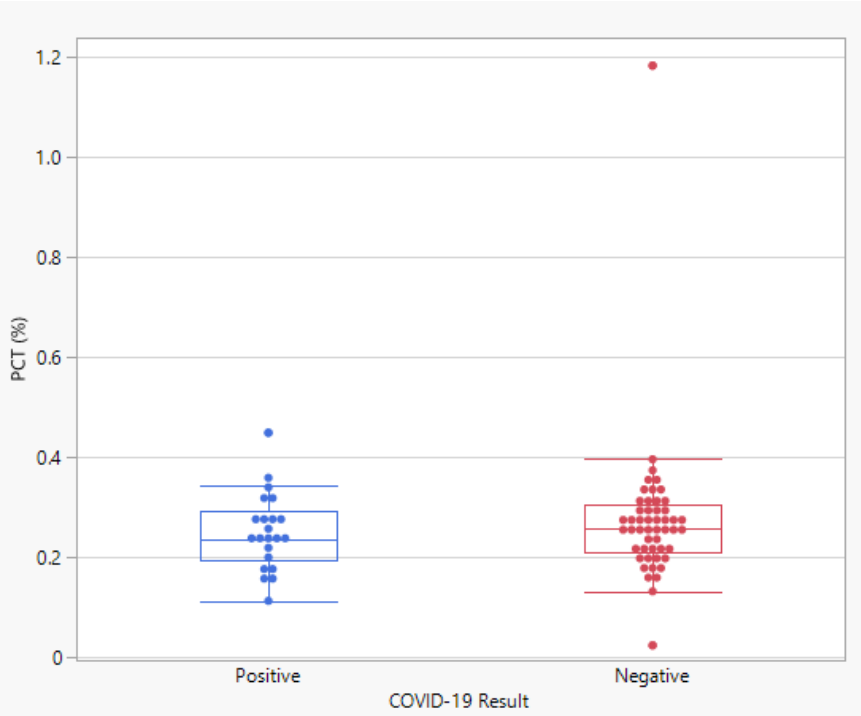

Figure 6: Box Plot of Plateletcrit. (PCT) COVID-19 Positive and Negative Subjects

Discussion

The study was carried out to evaluate and assess the platelet indices in covid-19 subjects in Port Harcourt. Coronavirus disease (COVID-19) is a mild to severe respiratory illness that is caused by a coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) of the genus betacoronavirus.

From a total of 72 subjects that were tested, 22 (30.56%) tested positive for covid-19 and 50 (69.44%) tested negative for covid-19 showing a disease prevalence of 30.56%, this value for positive subjects was significant in relation to the value of negative subjects. This is similar and in consonance to the study of Chadeau-Hyam *et al.*, (15) in which they tested 4509 subjects and 1325 (29.4%) were positive and 70.6% were negative. However the prevalence is largely contradictory to studies carried out in Qatar and the USA where the prevalence of covid-19 was 10.6% and 19% respectively (16,17). The reason for such descripancies will likely be the environmental differences and access to healthcare.

The PDW reduced significantly in the covid-19 positive patients when compared to their negative counterparts. This is in agreement with the research work of Wang *et al.* (18), their research comprised of 40 covid-19 patients and 40 healthy subjects which served as the control group. The PDW of the covid-19 positive group reduced significantly (p=0.0003) against covid-19 negative patients. I therefore conclude that PDW changes occur in COVID-19 patients and demonstrate an association between the PDW and COVID-19 infections. However it is not in consonance with the study of Ozcelik *et al.*,(19) as there was a mild to higher levels of PDW in

Covid-19 subjects. PDW value is normally expected to be higher due to a cytokine storm leading to platelet production and destruction mechanisms. The disparities in both studies might be a result difference in sampling size and again the influence of environment.

There was a significant reduction in the platelet counts of female covid-19 positive patients when compared to their male counterparts. Contrastingly, a reduction was observed in the male negative covid-19 subjects as opposed to their female counterparts. This study is in contrast with the study of Ahmed *et al*. (20) where there was no significant relationship of covid-19 positive patients with gender. The reason for this disparity may be the difference in sampling size, environment and state of the subjects and patients at the time of the study.

A significant positive correlation existed between platelets and PCT in covid-19 positive patients but a significant negative correlation between platelets and other platelet indices including MPV in covid-19 negative subjects. This is in line with the study of Shankaralingappa *et al*., (21) in which the platelet count of positive covid-19 patients significantly correlates positively with PCT but significantly correlates negatively with other platelet indices.

Conclusion

Conclusively, COVID-19 infection causes significant negative impacts on the affected subjects as the platelet counts and PDW values are deranged. Platelet indices could serve as markers for COVID-19 diagnosis and management.

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