Original Research Article

Comparative study of derangements in some indices of platelet function among asymptomatic COVID-19 and malaria infected subjects in a tropical setting.

Abstract

The coronavirus (COVID-19) infection is one of the emerging infections that has ravaged the world. The hypercoagulable status reported in severe malaria infection, which implicates the involvement of platelets, has also been reported in COVID-19 infection.This study was carried out to evaluate platelet indices among malaria and COVID-19 positive subjects in Port Harcourt, Nigeria. The cross-sectional, case-control study design was employed for this study, where a total of fifty-five (55) malaria positive subjects, fifty-five (55) COVID-19 positive subjects, fifty-five (55) co-infected subjects and fifty-five (55) control subjects who were within the ages of twenty (20) to sixty-five (65) years old. Five millilitres (5ml) of venous blood was collected aseptically and dispensed into Ethylene Diamine Tetraacetic acid (EDTA) anticoagulant bottle for the assay of platelet indices and malaria parasite detection from the thick blood film that was made. Also nasopharyngeal swab was collected for confirmation of COVID-19 positive subjects using RT-PCR technique. The mean values of the PDW of the subjects were as follows; malaria parasite subjects (15.21 ± 0.22 fL), COVID-19 subjects (15.21 ± 0.22 fL), COVID-19 + malaria subjects (15.61 ± 0.21 fL) and control subjects (13.26 ± 0.17 fL). These results revealed that the mean PDW values were significantly higher in COVID-19 and malaria subjects as well as in co-infection with COVID-19 and malaria. (F-value = 25.850, p = 0.001). No significant changes were observed in the other platelet parameters (platelet count, mean platelet volume and plateletcrit) (p> 0.05). Amongst other platelet parameters, the platelet distribution width appear to possess a potential diagnostic value for mild/asymptomatic COVID-19 and the case is not different in malaria infection in the tropical region of Nigeria.

Keywords: COVID-19, Platelet Indices, Co-infection, Malaria, Nigeria, Port Harcourt

Introduction

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, and is transmitted chiefly by contact with infectious material such as respiratory droplets, objects or surfaces contaminated by the causative virus, and is characterized especially by fever, cough, and shortness of breath and may progress to pneumonia and respiratory failure (Bchetnia *et al.,* 2020). 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 (Rahman *et al.,* 2020).

The COVID-19 disease was detected initially in late December 2021 in Wuhan, Hubei Province, China, and spread worldwide two months later. About 200 countries over the entire 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 (Park, 2020). COVID-19 had caused more than 3.7 million confirmed cases and killed at least 260,000 worldwide as was recorded in April 2020 (Lekhraj *et al.,* 2020).

Malaria remains a highly prevalent disease in more than 90 countries and accounts for at least 1 million deaths every year according to World Health Organization malaria report of 2016. Malaria is a serious infectious disease caused by a peripheral blood parasite of the genus Plasmodium. There are five species of parasite that affect humans - *P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi.* Of these, *P. falciparum* is the most deadly form that can lead to cerebral malaria, and *P. vivax* has a wider distribution than *P. falciparum* because it is able to develop in the Anopheles mosquito vector at lower temperatures (Milner, 2018).

While malaria and COVID-19 can have similar presentation, common symptoms they share include but not limited to: fever, breathing difficulties, tiredness and acute onset of headache, which may lead to misdiagnosis of malaria for COVID-19 and vice versa, particularly when clinician rely mainly on symptoms. Although respiratory signs and symptoms are most pronounced in COVID-19 infection, a typical malarial disease complicated with Acute

Respiratory Distress Syndrome (ARDS) is nearly not differentiable from severe COVID-19 infection (Gutman *et al.*, 2020).

Platelet indices are biomarkers of platelet activation and could be useful for the diagnosis of malaria. 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 (Budak *et al.*, 2016).

Alteration of 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 malaria 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 (Purbiya *et al.*, 2018; Bayleyegn *et al.*, 2021).

This study compared some platelet indices among subjects with COVID-19 and malaria infection 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 in 55 COVID-19 confirmed subjects, 55 malaria positive subjects, 55 COVID-19 and malaria co-infected subjects and 55 apparently healthy 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 360km^2 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 (Adeleye *et al.*, 2020). 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 (Sajini and Ijeh, 2021). Port Harcourt has grown by 150,844 since 2015, which represents a 4.99% annual change (Evoh *et al.*, 2021).

Study population

The study was carried out among male and female subjects infected with malaria, COVID-19 and Co-morbidities of the two diseases against a control of apparently healthy individuals. A total of fifty-five (55) malaria positive subjects, fifty-five (55) COVID-19 positive subjects, fifty-five (55) co-infected subjects and fifty-five (55) control subjected were recruited for this study within the ages of twenty (20) to sixty five (65) years old.

Sample Size

Sample size was determined using Cochran's Formula (Kotrlik *et al.,* 2021)

$$n = \frac{z^2(pq)}{e^2}$$

Where n = sample size

z = Z-score (1.96)

p = Prevalence (taken from previous studies)

q = 1-p

e = margin of error (0.05)

The sample size for this study was fifity-five (55) subjects, as calculated based on the prevalence of COVID-19 in Rivers State which was reported as 6% (Chiedozie et *al.*, 2020).

Informed Consent

Oral informed consent were obtained from participants before blood collection. Participants were made to understand the nature of the study and the fact that the participation is voluntary with confidentiality of recovered data maintained at all times during and after the study.

Inclusion Criteria

The participants in this study fulfilled the following inclusion criteria;

1. Subjects between the age range of 20 -65 years.
2. Apparently healthy subjects as control.
3. Confirmed COVID-19 subjects.
4. Confirmed malaria subjects.
5. Confirmed co-infected subjects with COVID-19 and malaria.

Exclusion Criteria

The exclusion criteria include:

1. Subjects below the ages of 20 or above 65.
2. Subjects who refused to give consent.
3. Persons suffering from known thrombotic disorders that is not COVID-19 or malaria related.
4. Persons on any form of anticoagulant therapy.
5. Subjects vaccinated against COVID-19.

Ethical Clearance

For the purpose of this study ethical approval was obtained from Rivers State Hospital Management Board.

Sample Collection and Processing

Five millilitres (5ml) of venous blood was collected aseptically and dispensed into Ethylene Diamine Tetraacetic acid (EDTA) anticoagulant bottle to assay platelet indices and malaria parasite detection from the thick blood film that was made. Then nasopharyngeal swab was collected for confirmation of COVID-19 positive subjects by RT-PCR.

Laboratory Analysis

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.*, 2021).

Venous blood collected from the EDTA bottle will be 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 will be inputted, then the sample will be gently opened and inserted to the probe of the analyzer then after a beep the sample will be removed and then it will take some seconds for the analyzer to process the sample before the result is displayed on the screen and printed out.

Procedure for Thick Blood Film Malaria Parasite Detection using Microscopy as Described by (Hathiwala *et al.*, 2017).

The thick well labeled blood film was prepared on a clean grease free glass slide. whole blood from EDTA bottle was collected within one hour of the collection, a well labeled grease free glass slide was used to make a thick film about 1.5 to 2 cm area in diameter from the EDTA collected blood sample then the slide was allowed to air dry, then the dried thick smear on the slide was dehaemoglobinized by dipping the dried slide into a beaker containing normal water and was removed immediately and allowed to air dry, when dried it was stained using Giemsa stain with buffer of pH 7.2. Fresh working Giemsa stain was prepared by adding 1 ml Giemsa stain stock to 39 ml of working Giemsa buffer; two drops of 5% Triton X-100 was added to the mixture later. The mixture was then poured into a standing 40-ml Coplin jar until full. Thick malaria smears were placed in the Giemsa stain (2.5%) for 45–60 minutes. At the end of the staining period, slides were removed and rinsed by dipping 3–4 times in the Giemsa buffer. The slides were left in the buffer for 5 minutes after which it was dry upright in a rack. A positive smear was included with each new batch of working Giemsa stain for quality control. Examination of the slide were made microscopically first by focusing using the X10 objective

before the x100 oil immersion was used by application of the immersion oil to the middle of the slide and lowering the objective to touch the oil then the examination of the slide was carried out and a minimum of 200 field was examined.

Procedure for COVID-19 Confirmation by RT-PCR Molecular Method as Described by Arya *et al.* (2005).

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 negative control and specimen, positive control without extraction) was separately added to different reaction plates/tubes then the plates/tubes was 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.

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™ 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 (Non COVID-19 or Malaria (Control), Malaria Positive, COVID-19 Positive and COVID-19 + Malaria 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

Table 1: Demographic Characteristics of Participants in the Study

PARAMETERS	STUDY GROUP (n=55)	CONTROL GROUP (n=55)
NUMBER OF FEMALES	22	7
NUMBER OF MALES	33	17
AGE RANGE (YEARS)	20-65	20-65

Table 2: Mean Values of the Platelet Indices among Study Participants

Treatment/Subject	N	PLT (× 10^3/µL) X ± SD	MPV (fL) X ± SD	PDW (fL) X ± SD	PCT (%) X ± SD
Non COVID-19 or Malaria (Control)	55	246.14± 8.73	10.63±0.18	13.26±0.17 [a]	0.26±0.01
Malaria Positive	55	222.46±15.07	11.35±0.34	15.21 ±0.22[b]	0.23±0.01
COVID-19 Positive	55	239.84±9.21	10.54±0.15	15.21 ±0.22[b]	0.25±0.01
COVID-19+ Malaria Positive	55	213.72±11.98	11.22±0.33	15.61 ±0.21[b]	0.23±0.01
F-value		1.7010	2.4444	25.850	2.345
p-value		0.168	0.065	<0.001	0.074
Remark		NS	NS	S	NS

PLT: Platelets; MPV: Mean Platelet Volume; PDW: Platelet Distribution Width; PCT: Plateletcrit. All levels were compared using Tukey-Kramer HSD

Within parameters, means with different superscripts are significantly different at $p<0.05$.

Table 3: Separation of the Mean Values of the Platelet Indices of the Study Subjects by Sex

Subjects	Sex	Mean ±SD	F-value	p-value
PLT (× 10^3/µL)				
Malaria Positive	Female (n=22)	216.37 ±21.73	0.328	0.744^{ns}
	Male (n=33)	226.21 ±20.57		
COVID-19	Female (n=22)	220.16±15.15	1.683	0.101^{ns}
Positive	Male (n=33)	251.90±11.23		
Co-infection	Female (n=22)	217.26±16.76	1.135	0.262^{ns}
	Male (n=33)	90.83±16.33		
MPV (fL)				
Malaria Positive	Female (n=22)	11.72±0.537	0.865	0.392^{ns}
	Male (n=33)	11.12±0.448		
COVID-19	Female (n=22)	10.36 ±0.250	0.922	0.362^{ns}
Positive	Male (n=33)	10.65 ±0.187		
Co-infection	Female (n=22)	11.22±0.385	0.03	0.998^{ns}
	Male (n=33)	11.22±0.451		
PDW (fL)				
Malaria Positive	Female (n=22)	15.41±0.363	0.720	0.476^{ns}
	Male (n=33)	15.08±0.273		
COVID-19	Female (n=22)	15.66±0.293	1.738	0.089^{ns}
Positive	Male (n=33)	14.93±0.304		
Co-infection	Female (n=22)	15.91±0.217	1.279	0.207^{ns}
	Male (n=33)	15.42±0.337		
PCT (%)				
Malaria Positive	Female (n=22)	0.235±0.018	0.127	0.899^{ns}
	Male (n=33)	0.232±0.013		
COVID-19	Female (n=22)	0.230±0.015	1.790	0.082^{ns}
Positive	Male (n=33)	0.263±0.010		
Co-infection	Female (n=22)	0.224±0.017	0.804	0.427^{ns}
	Male (n=33)	0.240±0.012		

All levels were compared using Tukey-Kramer HSD. Within parameters, means with different superscripts are significantly different at $p<0.05$.

ns = not significant.

Treatment/Subject	Age Group (years)	n	Mean	SD
PLT (150:00□450:00 × 10^3/µL)				
Non COVID-19 or Malaria (Control)	<25	4	257.33	47.79
	25-34	11	228.50	26.18
	35-44	12	240.45	24.96
	45-54	15	274.43	22.12
	55+	13	230.25	23.90
Malaria Positive	<25	4	237.50	41.39
	25-34	11	256.40	26.18
	35-44	12	215.82	24.96
	45-54	15	202.00	29.27
	55+	13	212.88	20.08
COVID-19 Positive	<25	4	271.25	41.39
	25-34	11	211.70	26.18
	35-44	12	249.45	24.96
	45-54	15	235.38	29.27
	55+	13	244.88	20.08
COVID-19 + Malaria Positive	<25	4	159.50	41.39
	25-34	11	233.40	26.18
	35-44	12	228.36	24.96
	45-54	15	215.25	29.27
	55+	13	204.71	20.08
Test Statistics: F-Ratio (P-value)			0.7806 (0.6700) [ns]	
MPV (8-12.4 fL)				
Non COVID-19 or Malaria (Control)	<25	4	10.97	1.08
	25-34	11	11.22	0.59
	35-44	12	10.26	0.57
	45-54	15	10.47	0.50
	55+	13	10.58	0.54
Malaria Positive	<25	4	11.83	0.94
	25-34	11	10.51	0.59
	35-44	12	11.35	0.57
	45-54	15	12.43	0.66
	55+	13	11.22	0.46
COVID-19 Positive	<25	4	10.15	0.94
	25-34	11	10.73	0.59
	35-44	12	10.29	0.57
	45-54	15	10.15	0.66
	55+	13	10.87	0.46
COVID-19 + Malaria Positive	<25	4	12.13	0.94
	25-34	11	11.02	0.59
	35-44	12	11.05	0.57
	45-54	15	11.03	0.66

	55+	13	11.33	0.46
Test Statistics: F-Ratio (P-value)			0.6736 (0.7754) [ns]	
PDW (9 –14 fL)				
	<25	4	12.07	0.85
	25-34	11	13.09	0.47
Non COVID-19 or Malaria	35-44	12	13.36	0.44
(Control)	45-54	15	13.07	0.39
	55+	13	13.82	0.42
	<25	4	15.98	0.74
	25-34	11	14.91	0.47
Malaria Positive	35-44	12	14.42	0.44
	45-54	15	15.99	0.52
	55+	13	15.35	0.36
	<25	4	16.00	0.74
	25-34	11	14.85	0.47
COVID-19 Positive	35-44	12	15.47	0.44
	45-54	15	15.05	0.52
	55+	13	15.14	0.36
	<25	4	15.15	0.74
	25-34	11	15.37	0.47
COVID-19 + Malaria	35-44	12	15.78	0.44
Positive	45-54	15	15.12	0.52
	55+	13	15.96	0.36
Test Statistics: F-Ratio (P-value)			1.101 (0.3612) [ns]	
PCT (0.22-0.24%)				
	<25	4	0.267	0.038
	25-34	11	0.260	0.021
Non COVID-19 or Malaria	35-44	12	0.245	0.020
(Control)	45-54	15	0.285	0.018
	55+	13	0.254	0.019
	<25	4	0.254	0.033
	25-34	11	0.256	0.021
Malaria Positive	35-44	12	0.219	0.020
	45-54	15	0.230	0.024
	55+	13	0.225	0.016
	<25	4	0.271	0.033
	25-34	11	0.225	0.021
COVID-19 Positive	35-44	12	0.255	0.020
	45-54	15	0.237	0.024
	55+	13	0.265	0.016
	<25	4	0.199	0.033
	25-34	11	0.247	0.021
COVID-19 + Malaria	35-44	12	0.246	0.020
Positive	45-54	15	0.239	0.024

	55+	13	0.225	0.016
Test Statistics: F-Ratio (P-value)			0.8041 (0.6459) [ns]	

Table 4: Separation of the Mean Values of the Platelet Indices of the Study Subjects by Age Group (years)

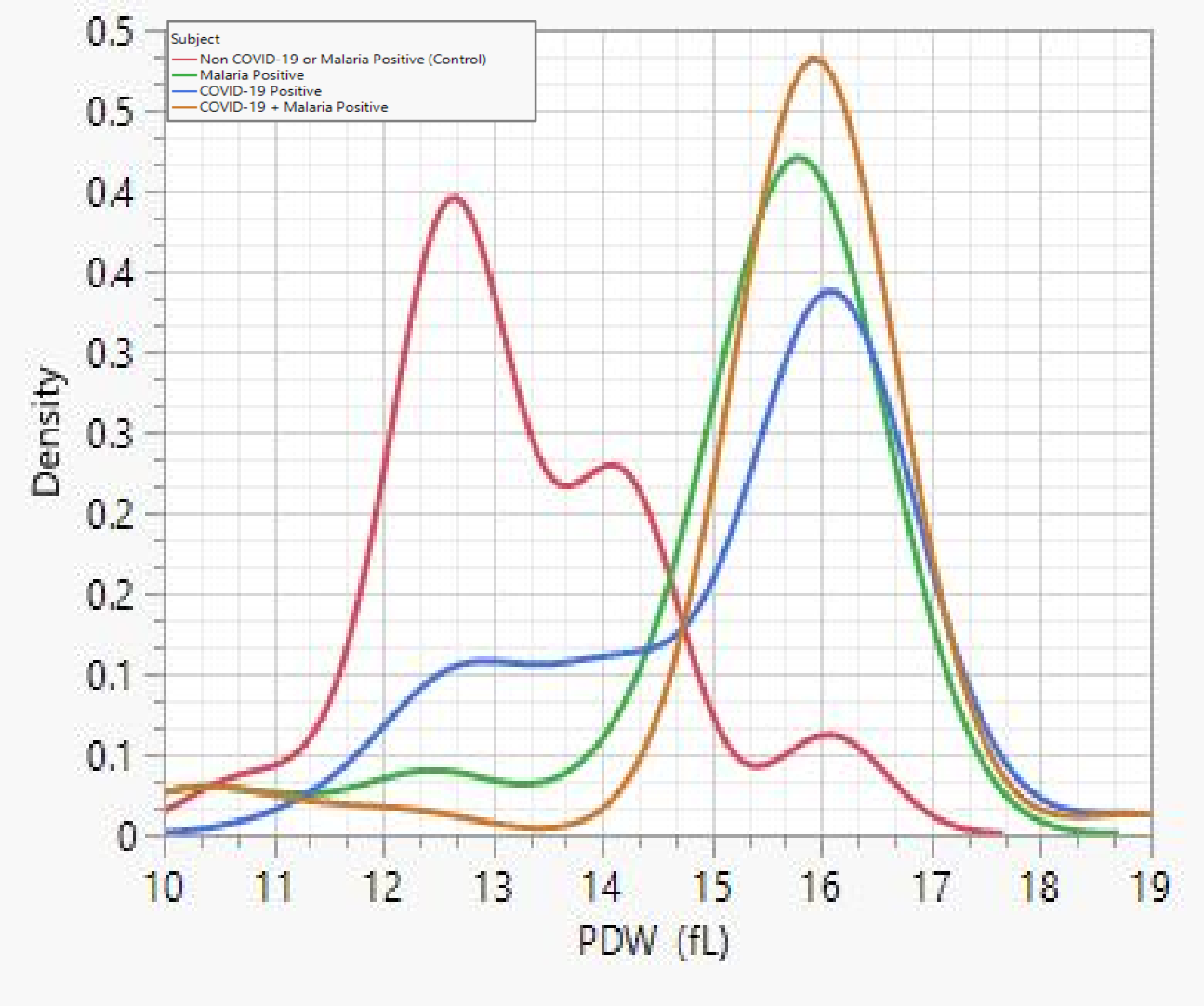
Subject
Non COVID-19 or Malaria Positive (Control)
Malaria Positive
COVID-19 Positive
COVID-19 + Malaria Positive
Density
0.5
0.5
0.4
0.4
0.3
0.3
0.2
0.2
0.1
0.1
0
10
11
12
13
14
15
16
17
18
19
PDW (fL)

Figure 1: Comparison of Densities of Platelet Distribution Width (PDW) in COVID-19 and Malaria Positive Subjects

Discussion

This study evaluated the platelet markers among subjects with COVID-19 and malaria infections in Port Harcourt. The major finding in this study is the significant increase in platelet distribution width in COVID-19 as well as malaria subjects. This finding is consistent with the findings of Maina et al. (2010). During COVID-19 and malaria infection platelets are excessively consumed, the bone marrow produces large amount of immature platelets, which have larger volume than the mature ones. Platelet distribution width is an indicator of variability of volume in the platelet size and is increased in the presence of platelet anisocytosis. It is used to directly measure variability in platelet size, changes with platelet activation, and reflects the heterogeneity of platelet morphology. Under a normal physiological condition, there is a direct relationship between PDW and MPV. Meanwhile, the relationship between platelet volume and platelet count is not concluded, which suggests that they are affected by different mechanisms (Budak *et al.*, 2016). According to Gupta et al. it was stated that there is a relationship between platelet count and plateletcrit (PCT) this agrees with a study that demonstrated that the increasing level of parasitemia is correlated with the decreasing of both PCT and platelet count (Tangvarasittichai *et al.*, 2016).

From the comparison of some platelet indices of subjects infected with COVID-19 and malaria in Port Harcourt by age group, it was observed that sex exerted significance influence on the parameters than age this agrees with a study by Onosakponome and Wogu, (2020) on the role of sex in Malaria-COVID19 co-infection and some associated factors in Rivers State, Nigeria. Where it was observed that there was a significant association of the co-infection with sex, and males had a higher value than females ($P < 0.05$).

The density plot of PDW comparison for the control subjects, malaria positive subjects, COVID-19 positive subjects and co-infected subjects showed a right skew for the control subject and a left skew for the other parameters but among the left skewed parameters, the subjects with the co-infection had the highest peak when compared to the malaria positive or COVID-19 positive subjects, this indicates that persons with the co-infections had a compensatory production of immature platelets that may have been consumed due to its aggregation in the lungs, which leads to the variability in volume of platelet sizes as a result of the coagulation process that must have occurred in participants co-infected by these two hypercougulable diseases. This agrees with a study by Güçlü *et al.* (2020) stating that an increased number of young platelets which are also functionally more active than older platelets causes these changes, this explains the increase in PDW for COVID-19 patients.

Conclusion

From this comparative study of the platelet indices of subjects infected with malaria and COVID-19, PDW was significantly raised among the co-infected subjects. PDW possesses a potential diagnostic value for mild COVID-19 and malaria infection.

Recommendation

PDW should be incorporated as a surrogate diagnostic tool in mild asymptomatic COVID-19 and malaria infected patients.

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