MEDDOCS Open Access Publisher

ISSN: 2639-4391

Annals of Epidemiology & Public Health

Open Access | Research Article

Cellular and Humoral Factors of Oxidative Burst in COVID-19 Patients with Malaria Parasiteamia

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Received: Jun 28, 2021

Accepted: Jul 21, 2021

Published Online: Jul 23, 2021

Journal: Annals of Epidemiology and Public health

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: COVID-19; Malaria; Innate immunity.

Abstract

Background: Potential impact of malaria parasiteamia on SARS-CoV-2 susceptibility and severity remains unclear. We determined the cellular and humoral factors of oxidative burst (aspect of innate immunity) of COVID-19 patients having malaria parasiteamia as a mechanism to understand the pathogenicity of SARS-CoV-2 in malaria patients.

Methods: This was a case-control study of hospitalized COVID-19 patients with malaria parasitemea (CoV+P), COVID-19 patients without malaria parasiteamia (CoV-P) in an Infectious Diseases Isolation Center, Ibadan, Nigeria and uninfected control (C). Malaria diagnosis was done using microscopy and while COVID-19 diagnosis was done using RT-PCR assay. Total- and differential- white blood cell counts (lymphocytes, monocytes, neutrophils, eosinophils) were done using autoanalyser, neutrophil: lymphocyte ratio was calculated while soluble oxidative burst factors (levels of nitric oxide and hydrogen peroxide, activities of myeloperoxidase, catalase, superoxide dismutase) were determined spectrophotometrically. Data were analyzed using the Student's t-test and p-values<0.05 were considered as statistically significant.

Results: Level of plasma nitric oxide was significantly higher in CoV+P compared with CoV-P or C ($p<0.05$). Myeloperoxidase enzyme activity was significantly higher in CoV+P compared with CoV-P ($p<0.05$). Blood cell counts, hydrogen peroxide, superoxide dismutase and catalase were similar in all groups.

Conclusion: Raised levels of certain mediators of respiratory burst factors were produced by phagocytic cells in CoV+P patients.

Cite this article: Arinola GO, Edem FV, Fashina OA, Olaniyan OA, Alonge TO. Cellular and Humoral Factors of Oxidative Burst in COVID-19 Patients with Malaria Parasiteamia. A Epidemiol Public Health. 2021; 4(1): 1060.

Introduction

By 10th March 2021, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-associated coronavirus disease 2019 (COVID-19) was reported in over 118 million people worldwide and with 2.6 million deaths [1]. COVID-19 spread to all countries in Africa continent following the first reported case in Africa on 14th February 2020, and has caused unprecedented socioeconomic and healthcare systems disruptions [2]. It was proposed that morbidity or mortality of COVID-19 in Africa would be devastating due to limited -financial resources, -infrastructures, -trained health workers [3], poor nutritional status, unhygienic conditions, underlining health conditions and higher burden of other infectious diseases [4].

Despite global spread of SARS-CoV-2 infection, immune response in hosts having COVID-19 with substantial burdens of other infectious diseases is limited. It is also unclear whether immune responses to malaria is beneficial or harmful during SARS-CoV-2 infection [5,6] but malaria was reported to be protective against severe manifestations of some respiratory viruses [7] by suppressing production of cytokines and decreasing recruitment of cellular inflammatory components to the lungs [8]. In addition, a predictive model suggested the possibility of lower mortality from COVID-19 patients in low- and middle-income countries (LMICs) than that in high-income countries [9]. Inverse associations were established between COVID-19 and malaria prevalence [10-13], which was supported by high impact of COVID-19 in African countries historically less affected by malaria [14]. Reasons earlier adduced to a lower risk of COVID-19 in malaria-endemic areas include prophylaxis with chloroquine and hydroxychloroquine [15], shared immunodominant epitopes between SARS-CoV-2 and *P. falciparum* antigens [16], genetic variants connected to blood group O and reduced expression of the ACE2 receptor [17].

Based on the suggested protective effects of malaria against respiratory viruses [7,8] and SARS-CoV-2 [10-15], the burden of COVID-19 in sub-Saharan Africa is expected to be substantially lessened since more than 90% of global malaria cases were reported to be in sub-Saharan Africa [10-12]. Despite these observations, the impact of malaria and SARS-CoV-2 co-infection on host susceptibility and pathogenesis remains largely unknown among Nigerians. It is against this scenario that this study was conducted to characterise innate immune responses using oxidative burst factors in hospitalised COVID-19 Nigerian patients having malaria parasiteamia.

Participants and methods

Subject population

This study comprised of 10 CoV+P, 10 CoV-P and 10 healthy-Nigerian subjects. The COVID-19 cases were confirmed by detection of hCoV nucleic acid using real-time Reverse-Transcriptase Polymerase-Chain Reaction (RT-PCR) assay in nasal and pharyngeal swab specimens following W.H.O recommended guidelines apart from clinical signs of dry cough, high fever, sore throat and shortness of breath. Malaria diagnosis was done using microscopy. For microscopy, thick blood smears were stained with 2% Giemsa and examined by three independent microscopists. Blood smears were considered negative if no parasites were found after reading 100 high power fields. The participants did not have hypertension, diabetes mellitus, cardiovascular disease, cerebrovascular disease, cancer, chronic renal disease, asthma or helminthiasis. Also excluded were those that drink alcoholic beverages or cigarette smokers, with haematological diseases or those with recent history of blood transfusion were excluded from the study.

Plasma isolation

Plasma was removed from 10ml whole blood collected in a test tube containing lithium heparin anticoagulant by centrifuging at 1500 ×g for 10 minutes.

Superoxide dismutase (SOD) activity determination

The SOD activity was determined using the method previously carried out [18]. This method is based on the principle that SOD inhibits the autoxidation of epinephrine at pH 10.2.

Catalase (CAT) activity determination

Catalase activity was determined using the method as previously carried out [18]. This method is based on the principle that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as an unstable intermediate. The chromic acetate then produced is measured at 570 nm.

Myeloperoxidase (MPO) activity determination

MPO activity was determined as previously carried out [18]. The rate of decomposition of H_2O_2 by peroxidase, with guaiacol as hydrogen donor, produced tetraguaiacol which was measured at 436 nm.

Hydrogen peroxide determination

Hydrogen peroxide concentration was determined as previously carried out [18]. The assay is based on peroxide-mediated oxidation of Fe^{2+}, followed by the reaction of Fe^{3+} with xylenol orange to form Fe^{3+}-xylenol orange complex with an absorbance maximum of 560 nm. Plasma H_2O_2 was determined by comparing absorbance with standard solutions of H_2O_2.

Nitric oxide (NO) determination

Plasma nitric oxide concentration was determined using Griess reagent (Sulpanilamide and N-1-napthyethylene-diamine dihydrochloride) as previously carried out [18]. The assay is based on a reaction that utilizes sulpanilamide and N-1-napthyethylene-diamine dihydrochloride (NED) under acidic (phosphoric acid) conditions. Nitrite forms coloured chromophore with reagent, with an absorbance maximum at 540nm.

Blood cell counts

An automated haemocytometer (URIT: 5160E-01262, China) was used to estimate the total White Blood Cell (WBC) count and percentages of lymphocytes, neutrophils, eosinophils and monocytes. Thereafter, neutrophil-lymphocyte Ratio (NLR) was calculated as the ratio of neutrophil percentage to lymphocyte percentage.

Statistical analysis

Data obtained were presented as mean (±S.D). Statistical analysis was carried out using SPSS statistical software version 21 for windows. Differences in the mean of variables were determined using the independent and paired Student's *t*-test as appropriate. *p*-value less than 0.05 was considered as statistically significant.

Results

Mean±S.D of total - and differential-white blood cell counts and NLR in CoV+P, CoV-P and uninfected control were presented in Table 1. All the blood cell parameters were similar in all groups (Table 1). Mean±S.D of oxidative burst factors in CoV+P, CoV-P and uninfected control were presented in Table 2. Plasma nitric oxide level was significantly higher in CoV+P compared with CoV-P or C ($p<0.05$). Activity of myeloperoxidase was significantly higher in CoV+P compared with Cov-P ($p<0.05$). However, myeloperoxidase activity was significantly lower in CoV-P compared with control ($p<0.05$).

Table 1: Comparison of total- and differential-white blood cell counts and neutrophil: lymphocyte ratio in CoV+P, CoV-P and uninfected Control.

Variable	CoV+P (n=10)	CoV-P (n=10)	Control (n=10)	t, p[a]	t, p[b]	t, p[c]
WBC ($x10^9$/L)	5.95 ± 1.73	5.28 ± 1.09	4.70 ± 2.00	1.036, 0.314	1.495, 0.152	0.805, 0.431
Lymphocytes (%)	48.08 ± 14.47	47.14 ± 14.45	52.09 ± 7.77	0.145, 0.886	0.772, 0.450	0.954, 0.353
Monocytes (%)	7.39 ± 1.00	7.58 ± 2.46	9.17 ± 3.11	0.226, 0.834	1.723, 0.102	1.268, 0.221
Neutrophils (%)	41.23 ± 14.37	42.40 ± 15.49	35.44 ± 9.50	0.175, 0.863	1.063, 0.302	1.211, 0.242
Eosinophils (%)	5.24 ± 4.06	3.13 ± 2.29	2.68 ± 1.39	1.431, 0.169	1.887, 0.076	0.531, 0.602
NLR	1.00 ± 0.59	1.10 ± 0.79	0.72 ± 0.28	0.321, 0.752	1.356, 0.192	1.434, 0.169

WBC: White Blood Cells; NLR: Neutrophil: Lymphocyte.
a: CoV+P compared with CoV-P; b: CoV+P compared with Control; c: CoV-P compared with Control.

Table 2: Comparison of respiratory burst factors in CoV+P, CoV-P and uninfected Control.

Variable	CoV+P	CoV-P	Control	t, p[a]	t, p[b]	t, p[c]
NO *(µmol/L)*	20.62 ± 13.10	15.55 ± 1.18	7.85 ± 3.21	1.219, 0.239	2.994, 0.008*	7.120, 0.000*
H_2O_2 *(µmol/L)*	104.05 ± 38.38	94.65 ± 10.48	80.23 ± 22.35	0.747, 0.465	1.696, 0.107	1.847, 0.081
SOD *(U/mL)*	1.99 ± 0.00	1.99 ± 0.01	1.99 ± 0.00	0.000, 1.000	0.000, 1.000	0.000, 1.000
CAT *(U/mg protein)*	4.65 ± 0.99	4.21 ± 0.52	4.68 ± 0.61	1.244, 0.229	0.082, 0.936	1.854, 0.080
MPO *(U/mL)*	1.28 ± 0.85	0.60 ± 0.12	1.16 ± 0.51	2.505, 0.022*	1.212, 0.241	6.398, 0.000*

NO: Nitric Oxide; H_2O_2: Hydrogen Peroxide; SOD: Superoxide Dismutase; CAT: Catalase; MPO: Myeloperoxidase.
a: CoV+P compared with CoV-P, b: CoV+P compared with Control, c: CoV-P compared with Control.
*Significant at $p<0.05$

Discussion

A better understanding of the immune responses in patients with COVID-19 and malaria parasiteamia is of significant public health interest especially in sub-Saharan Africa given the potential for epidemiological overlap. This knowledge could inform response efforts and promote integrated approaches for management. To our knowledge, this study is the first to characterise potential interactions between SARS-COV-2 and malaria parasites in Nigerian patients. The overall trends of raised nitric oxide and myeloperoxidase in CoV+P patients compared with CoV-P was an indication of higher production of mediators of oxidative burst factors by phagocytic cells in CoV+P patients. This could suggest that immunomodulation caused by malaria during COVID-19 may not be deleterious.

The phagocyte oxidative burst is essential for containing SARS-CoV-2 infections [19, 20] and plasmodial infection [21]. To combat infection, immune cells use NADPH oxidase to reduce O^{2-} to oxygen free radical and subsequently to H_2O_2. In neutrophils and monocytes, Myeloperoxidase (MPO) mediates the combination of H_2O_2 with Cl^- to produce hypochlorite, which plays a role in destroying micro-organism [22] This present study observed an increase in plasma MPO activity in CoV+P patients compared with CoV-P or control. Neutrophil Extracellular Traps (NETs) is a mechanism of neutrophil antimicrobial activity [23]. Human Neutrophil Elastase (HNE) and Myeloperoxidase (MPO) are reported to be essential for NET formation [24]. Increased plasma MPO activity in CoV+P patients observed in this study might be a host immune defense mechanism towards production of microbicidal hypochlorite and the formation of NET with COVID-19 patients.

Interferon-γ-induced production of NO is recognized as a potent agent of innate immunity [25]. NO plays a vital role in intracellular killing of pathogens and vascular smooth muscle relaxation, and in addition, modulates gene expression via the agency of transcription factors nuclear factor-κB, which regulates transcription of pro-inflammatory cytokines [26]. This present study observed increased plasma NO level in CoV+P compared with control and CoV-P indicates increased inflammation and higher phagocytic activities in CoV+P group.

Both NO and MPO have been implicated in cardiovascular events [27,28]. Myeloperoxidase (MPO) has several potential effects that increase the development of coronary plaque such as decreases nitric oxide, oxidizes LDL forming oxLDL and oxidizes HDL making it dysfunctional [27]. Thus, MPO might be

associated with adverse cardiovascular events experienced by COVID-19 patients. Nitric Oxide (NO) produced by endothelial Nitric Oxide Synthase (NOS) is a powerful vasodilator and as such plays a critical role in the regulation of vascular tone. Additionally, NO suppresses binding of circulating cells to the endothelium and inhibits proliferation of smooth-muscle cells in the vascular wall [28]. Taken together, the findings indicate that raised NO in CoV+P is a critical element in vascular homeostasis, thus might be proposed to regulate adverse cardiovascular episodes of MPO in CoV+P patients.

This study had limited sample size as its limitation. Despite this, the strengths of this study is that it gives additional understanding of innate immune responses in patients with COVID-19 and malaria parasiteamia. This knowledge could inform response efforts and promote integrated approaches for management of COVID-19 patients. In conclusion, co-infection of SARS-CoV-2 and *Plasmodium* is not deleterious, though additional studies are required to further explore the observation of this study.

Author's contributions

GOA conceived and designed the study. OAF and OAO were involved in study implementation. FVE did data collection and statistical analysis. GOA and TOA drafted the first version of the manuscript. All authors contributed to data review and interpretation, and finalised and approved the manuscript.

Declaration of interest

All other authors declare no competing interests.

Acknowledgements

Special gratitude to the patients for their cooperation during the study. The authors wish to appreciate the Oyo State COVID-19 Task Force ably led by the Executive Governor, Engineer Seyi Makinde FNSE for the comprehensive program set up to combat the COVID-19 pandemic.

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