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Research Article

Elevated Plasma Level of Circulating Immune Complexes in Nigerian Adults infected with Severe Acute Respiratory Syndrome Corona Virus-2

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Abstract
Emerging reports indicate that immune complexes (ICs) play active roles in COVID-19 immunopathology. Since the pathogenic mechanism of Severe Acute Respiratory Syndrome Corona Virus-2 still remains to be fully explored, understanding the changes in circulating IC (CIC) level during the course of COVID-19 could be of clinical importance. The plasma level of CIC was thus, determined in Nigerian adults with SARS-CoV-2. Forty-nine adults consisting of 30 patients with mild COVID-19 and 19 healthy controls were enrolled into this case-control study. The COVID-19 patients were followed up till discharged from the isolation centre. Plasma levels of CIC were determined using the polyethylene glycol precipitation method. The mean CIC level was higher in COVID-19 patients at diagnosis compared with the controls. However, there was no significant difference in the mean plasma levels of CIC at discharge compared with the level at diagnosis in COVID-19 patients. It could be concluded from this study that the plasma level of CIC is elevated in adults with COVID-19 and may be involved in the immunopathology of the disease.

Key Words: *Antibody, Complement activation, COVID-19, Immune complex, Immunopathology*

INTRODUCTION

COVID-19 is a global epidemic with significant morbidity and mortality caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although primarily, it is a disease of the respiratory tract, reports continue to show that it additionally affects multiple organs of the body including the heart, liver, kidney and central nervous system among others (Majumder and Minko, 2021).

The pathogenic mechanism of SARS-CoV-2 remains to be fully explored but reports have unequivocally showed that uncontrolled inflammation contributes centrally to the COVID-19 associated pathologies such as multi-organ failure (Arinola, 2020; Felsenstein *et al.*, 2020; Zhu *et al.*, 2021). This hyperinflammation, usually termed as cytokine storm, is induced via a number of mechanisms. These include strong and poorly controlled inflammatory processes from immune cells such as monocytes, macrophages and neutrophils at the site of infection thereby resulting in systemic inflammation and tissue damage (Chen *et al.*, 2020; Wu and McGoogan, 2020).

Additionally, immune complex (IC) mediated inflammatory responses have been associated with COVID-19 hyperinflammation and have been shown to be involved in the development of vasculitic lesions, occlusion of blood vessels and infarction in patients with COVID-19 (Felsenstein *et al.*, 2020; Vuitton *et al.*, 2020).

Circulating immune complexes (CICs) are macromolecules consisting of antibodies bound to different antigens and circulate in the blood (Arinola *et al.*, 2005). Although formation of CICs is a physiological process constituting an essential part of normal immune response, several reports have shown that CICs especially, when poorly cleared and deposited in tissues or organs, play prominent roles in the pathogenesis of various diseases including systemic lupus erythematosus, rheumatoid arthritis, glomerulonephritis, schistosomiasis, diabetes mellitus and even, cancer (Akinlade *et al.*, 2004; Arinola, 2002; Arinola and Salimonu, 1999; Ezeani *et al.*, 2010; Siddiqui *et al.*, 2016; Thanadetsuntorn *et al.*, 2018; Zhao *et al.*, 2008). In COVID-19, Xu *et al*. (2020) and Yao *et al*. (2020) showed that the histopathological reports from tissue sections of COVID-19 patients had features associated with IC-mediated vasculitis, including monocytes and lymphocytes infiltration within and around blood vessels, wall thickening, and focal hemorrhage. Roncati *et al*. (2020) provided evidence showing that COVID-

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19 vasculitis results from a synergy between the type-2 T-helper immune response and type-3 hypersensitivity; which is immune complex mediated. This IC-mediated process has been identified to be responsible for the severity of COVID-19 in the elderly especially, in those with a history of exposure to seasonal coronaviruses but waning antibody titres (Felsenstein *et al.,* 2020; Gao *et al.,* 2015). Furthermore, Nazy *et al*. (2020) showed that COVID-19 associated coagulopathy (CAC) is mediated by platelet-activating ICs.

Although abundant production of neutralizing antibody to SARS-CoV-2 is essential in facilitating favourable outcomes, its involvement in COVID-19 associated pathologies such as antibody-dependent enhancement (ADE) of infection and immune complex mediated immune responses are of clinical concern. Presently, there is the dearth of information on the plasma level of CIC and its interplay with SARS-CoV-2 specific IgG and IgM during the course of infection in Nigerians with COVID-19. These thus, serve as the basis for this study.

MATERIALS AND METHODS

After obtaining approval from the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Review Committee and informed consent, a total of 49 participants were enrolled into this case-control study. They include 30 symptomatic COVID-19 patients enrolled from the Infectious Disease Centre, Olodo, Oyo State, Nigeria and 19 age-and gender matched apparently healthy adults who served as controls. SARS-CoV-2 infection was diagnosed using the real-time reverse-transcriptase polymerase-chain reaction (RT-PCR) assay. All the COVID-19 patients demonstrated viral positivity while the controls, tested negative to SARS-CoV-2 RT-PCR assay.

Sample collection: Venous blood (10 ml) was obtained from the SARS-CoV-2 infected patients at the point of admission to the isolation centre and at discharge but at enrolment only in controls. The blood samples were dispensed into lithium heparin containing sample bottles and plasma was obtained appropriately. All the plasma samples were stored at -20^{0}C until analyzed.

Laboratory analysis: The plasma levels of circulating immune complexes (CICs) were determined using the polyethylene glycol-6000 precipitation method as previously described (Arinola and Salimonu, 1999).

Statistical Analysis: 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

Changes in the plasma levels of CIC in patients with COVID-19 and the controls are shown in Table 1. The mean CIC level was significantly higher in COVID-19 patients compared with the controls ($P = 0.045$). In contrast, the mean CIC level was not significantly different in COVID-19 patients at discharge compared with the controls ($P = 0.098$). At discharge from the isolation centre, there was an insignificant reduction in the mean plasma levels of CICs compared with the levels at diagnosis ($P = 0.466$).

In order to find out if SARS-CoV-2 specific IgG and IgM had equivalent or differential contribution to the observed CIC level, the CIC level was correlated with SARS-CoV-2 specific IgG and IgM which had earlier been reported (Arinola, 2021). There was positive correlation between CIC and IgG as well as IgM. However, the correlations were not statistically significant (Table 2).

Table 1:
Plasma levels of circulating immune complexes in COVID-19 patients and controls

Parameter	Controls (n = 19)	COVID-19 patients at diagnosis (n = 30)	COVID-19 patients at discharge (n = 30)
CICs (mg/dL)	29.25 ± 4.91	32.27 ± 5.04[a]	31.67 ± 4.46

[a] *Significantly different from controls, CIC = Circulating immune complex*

Table 2:
Correlation between circulating immune complexes and selected immunoglobulin classes in COVID-19 patients at diagnosis

CIC (mg/dL)	r-value	*P*-value
SARS-CoV-2 IgG	0.213	0.268
SARS-CoV-2 IgM	0.286	0.132

CIC = Circulating immune complex, IgG = Immunoglobulin G, IgM = Immunoglobulin M

DISCUSSION

The roles of immune complexes in the pathogenesis of COVID-19 and their possible use in the design and time management of immune-based treatments such as plasma therapy and vaccine have recently been elucidated (Vuitton *et al.,* 2020). Felsenstein *et al*. (2020) posited that the early production of neutralizing antibodies against coronaviruses favours the formation of immune complexes which contributes to COVID-19-associated cytokine storm via complement activation, immune cells recruitment and mast cell degranulation resulting in inflammation and damage, as well as vasodilation and oedema.

The observed significant elevation in the mean CIC level in COVID-19 patients at diagnosis compared with the controls is in line with the reported IC-mediated vasculitis and coagulopathy in COVID-19 patients (Nazy *et al.,* 2020; Roncati *et al.,* 2020; Xu *et al.,* 2020; Yao *et al.,* 2020). This observation might be due to rapid proliferation of SARS-CoV-2 which results in the release of viral particles/antigens required for CIC formation. Mahesweri *et al*. (2014) reported that elevation of CIC requires ongoing antigen production. Persistence of IC might thus; be considered a marker of ongoing or persistent infection. In addition, the observed raised levels of CIC in COVID-19 patients might be due to reduced clearance by phagocytes. Emerging reports continue

to show that there is impaired phagocytosis in COVID-19 patients (Arinola *et al.,* 2021; Belchamber *et al.,* 2021; Nomani *et al.,* 2021). Interaction between CICs and the Fc receptors on neutrophils, monocytes and macrophages as well as the mannose receptors on macrophages increases their oxygen (O_2) uptake via the process of respiratory burst. This leads to the activation of membrane-bound NADPH oxidase that reduces O_2 to superoxide (O_2^-) (Chen *et al.,* 2008; Yoshihara *et al.,* 2012). Elevation of intracellular reactive oxygen and nitrogen species (RONS) and redox imbalance have been shown to be responsible for DNA strand breaks and endothelial dysfunction (Chen *et al.*, 2008).

Furthermore, since formation of CIC requires both antigen and antibody, the observed elevated mean CIC level in COVID-19 patients at diagnosis compared with the controls could be a reflection of SARS-CoV-2 specific antibody mediated humoral response initiated upon infection. Studies have established the important roles of antibody mediated response especially, the neutralizing antibodies in controlling viral infections (Klasse, 2014; Zhou *et al.,* 2019). However, early production of neutralizing antibodies against coronaviruses has been hypothesized to favour the formation of immune complexes (Felsenstein *et al.,* 2020). Our observation, together with the earlier reports, indicates that CICs could be one of the main factors driving COVID-19 severity as well as its associated tissue and organ damage, including acute respiratory distress syndrome (ARDS). Also, Mazzitelli *et al*. (2021) reported that immune complexes enhance inflammatory signature acquisition by neutrophils causing worsening of the COVID-19 course. These reports clearly indicate that COVID-19 play prominent role in COVID-19-associated hyperinflammation and can thus; be classified as an immune complex disease.

Circulating immune complexes stimulate different immune responses including complement activation, opsonization, phagocytosis, activation of immune cells (macrophages, neutrophils) and subsequent release of cytokines, chemokines and activation of protease pathways (Manzo, 2020; Mayadas *et al.,* 2009). Similarly, immune complexes activate extravascular infiltration of immune cells such as T-cells, neutrophils, mast cells and macrophages, which release inflammatory mediators capable of activating the endothelium (Stokol *et al.,* 2004). Based on our observation from this study, it could be hypothesized that persistence of circulating immune complexes is a potent source of inflammation and could play a role in COVID-19 associated reduction in the number of certain circulating leucocytes.

The report of Cao *et al*. (2007) showed that the neutralizing IgG against SARS-CoV reached a peak during the convalescent phase and diminished after recovery. In the same vein, rapidly waning antibody response has been posited in SARS-CoV-2 infection due to its usual asymptomatic or mild clinical presentation (Vafaeinezhad *et al.,* 2021). The insignificant reduction in the mean plasma levels of CICs observed at discharge compared with the level at diagnosis probably indicates that CICs persist for weeks during infection and physiologic maintenance of delicate balance in CIC level could determine the course of SARS-CoV-2 infection. Persistent CIC elevation has been shown to be an indicator of poor clearance by phagocytes. This could be the case in this study as we had earlier reported (Arinola *et al.,* 2021) that respiratory burst factors were elevated in COVID-19 patients at discharge compared with the baseline. It could therefore, be suggested that elevated respiratory burst factors in COVID-19 patients could indicate difficulty in clearance of formed CICs. The observed insignificant positive correlation between CIC and IgG as well as IgM might suggest that the two antibodies contributed equally to the formation of immune complexes and that CIC formation increases or decreases based on the corresponding increases or decreases in antibody formation.

It could be concluded from this study that the plasma level of CIC is elevated in patients with COVID-19 and may be involved in the immunopathology of the disease. Thus, COVID-19 may be an immune complex disease

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