AIJR Preprints
Section: Coronavirus
Article Id: 201, Version: 1, 2020
URL: https://preprints.aijr.org/index.php/ap/preprint/view/201
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NOT PEER-REVIEWED

Version 1: Received: 06 August 2020 / Approved: 08 August 2020 / Online: 10 August 2020

Human Immune Response to COVID-19 Infection and Potential Role of Chloroquine Family of Drugs: A Review

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ABSTRACT

Currently, world is witnessing a massive morbidity and mortality due to COVID-19 pandemic. A novel strain of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the causative agent of coronavirus disease 2019 (COVID-19). The virus enters inside the body and infect the cells through angiotensin-converting enzyme 2 (ACE2) receptor. The S1 protein of SARS-CoV-2 binds to the ACE2 receptor which results in endocytosis and transfer of virus into endosomes of body cells. Entry of SARS-CoV-2 results in activation of innate immune responses first followed by adaptive immune responses. The effective host immune responses are crucial to control and prevent viral infection. However, excessive production of proinflammatory cytokines and decrease in number of T-lymphocytes are the major reasons associated with severity of COVID-19. Therapies and drugs that can modulate the immune responses appropriately may play a crucial role to control and prevent the progression of disease. Chloroquine (CQ) and hydroxychloroquine (HCQ) have anti-inflammatory, immunomodulatory, antitumor, antimicrobial and antithrombotic effects. These drugs have already been registered in many countries to treat arthritis, lupus and malaria. The treatment responses of COVID-19 patients to these drugs have been found positive in some cases and clinical studies are underway for evaluating these drugs for the same. However, there are some serious side effects and health hazards associated. Many regulatory bodies are demanding more conclusive data on efficacy and safety from the clinical studies. Moreover, some regulatory bodies such as Food and Drug Administration (FDA) and European Medicines Agency (EMA) have recommended to use these drugs in emergency and chronic situation to treat critically ill COVID-19 patients under doctor's supervision with all issued guidelines. The national task force (NTF) set up by Indian Council of Medical Research has recommended high risk individuals to take HCQ for prophylaxis. This review summarizes human immune response and various aspects of CQ and HCQ with special reference to COVID-19.

Keywords: Chloroquine, COVID-19, Hydroxychloroquine, Immune response, SARS-CoV-2.

1 Introduction

The SARS-CoV-2 belongs to β-coronavirus family [1] and is responsible for causing COVID-19 disease which mainly attacks human respiratory system [2]. The disease is known to be transmitted through droplets while coughing and sneezing as those come in contact with mouth, nose and eyes of others. Also, this virus can remain stable on inanimate surfaces for many hours or even days. Touching these surfaces

How to Cite:

Sunita Singh *et al.* "Human Immune Response to COVID-19 Infection and Potential Role of Chloroquine Family of Drugs: A Review". *AIJR Preprints*, 201, version 1, 2020.

with subsequent touching of mouth, nose, eyes, may also lead to transmission of disease [3]. The incubation period for this virus varies from 1 to 14 days, however, it was found to spread even during the dormant period [4]. The cough and fever are the most common symptoms of this disease and in severe cases, lung inflammation, acute respiratory distress syndrome (ARDS), cardiac and renal injuries were also observed [5-6]. The pre-symptomatic and asymptomatic carriers have become the main reason for human to human transmission and rapid spread of the disease across the world [7-8]. Originating from Wuhan, China, till date, this pandemic has spread in >212 countries and territories around the world. Total number of confirmed COVID-19 cases reported as on 20th July,2020 are >14million and death toll has reached to >6 lakhs, globally. The frequency of COVID-19 occurrence is higher in elderly people, with compromised immune system and are suffering from diabetes and cardiovascular diseases [9]. The SARS-CoV-2 virus takes entry into body through ACE2 receptors and establish the infection process [10]. T lymphocytes are the major target cells for the virus. In severe COVID-19 cases, drastic reduction in number of natural killer (NK) cells, CD4 and CD8 T lymphocytes and excessive production of cytokines was observed which lead to immune dysfunctioning [5, 11-12]. Until now, there is no proven or registered drugs for COVID-19 [13]. The increased death rate and rapid spread of the disease across the world had created the global emergency and made medical professionals, policy makers to explore all possible, potential treatment strategies to control and eliminate the outbreak [14].

Based on the current understanding about virus pathogenesis and symptoms of the disease, specific drugs with antiviral and immune modulatory properties will be ideal to control the disease. However, several clinical trials are in progress to test antiviral, immunosuppressive or immunomodulating drugs against COVID-19 [15]. The previous experience of handling the diseases caused by viruses belonging to the β-coronavirus family such as Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) causing pneumonia had provided some background understanding for designing the treatment strategy for COVID-19 [16-17]. Anti-malarial drug CQ and its less toxic derivative HCQ, are well known agents for immunomodulation and are being used in rheumatology. For many years, these drugs have been used to treat autoimmune disease like systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). During the outbreak of SARS corona virus, CQ was used in some cases as an antiviral agent to control the infection [18-19]. Many recent studies have suggested the use of these antimalarial drugs to control the COVID-19 infection and several clinical trials are under way [20-22]. This review summarizes the COVID-19 infection process, general immune responses and specific immune responses against COVID-19, mode of action and treatment by CQ and HCQ and global regulatory perspective on usage of these drugs.

2 Structure of SARS-CoV-2 and Its Pathogenesis

Coronaviruses are pleomorphic, positive stranded RNA viruses (26-32 kb) with 80-120 nm diameter [23]. They are categorized in to four type's α-COV, β-COV, δ-COV and γ-COV [24]. SARS-CoV, MERS-CoV and newly identified SARS-CoV-2 coronaviruses belongs to β-coronavirus type. All these viruses are known to cause respiratory diseases in human beings [25]. The genome of SARS-CoV-2 encodes 4 structural proteins such as Nucleocapsid (N), Membrane (M), Envelope (E) and Spike (S) with various accessory proteins as well [3]. The nucleocapsid or N-protein coats and the viral RNA genome which plays a vital role in its replication and transcription. The M-protein is abundantly present on the viral surface and it is believed to be the central organizer for coronavirus assembly [26]. The E-protein is a small membrane protein which plays an important role in virus assembly and interaction of virus with host cell [27]. Generally, the mechanism of infection starts with the interaction of coronavirus S protein with corresponding receptor on target cell angiotensin-converting enzyme 2. After recognition and attachment

of coronavirus, it injects its RNA in to target cell by protease cleaving and membrane fusion [28]. Once RNA enters in to host cell the replication and transcription of viral RNA are mediated by replication/transcription complex (RTC) [29]. Subsequently the viral envelope glycoproteins are synthesized in the cytoplasm and then viral assembly is established inside the endoplasmic reticulum (ER) [30]. Viral assembly formed by the combination of genomic RNA and nucleocapsid protein in the Endoplasmic Reticulum-Golgi Intermediate Compartment (ERGIC). After the formation of virions assembly, these are transported from Golgi vesicles and ultimately released by exocytosis so that they can infect other healthy cells [31].

The S protein of SARS-CoV-2 plays a major role in initiating the infection. It binds with ACE2 receptor of target cell through its receptor-binding domain (RBD) and have ~ 20 times more affinity than SARS-CoV S protein [10, 32]. S protein of SARS-CoV-2 also has an affinity to bind with host cellular transmembrane serine protease (TMPRSS) [33]. Considering the higher affinity to ACE2 and TMPRSS, these can be considered as potential targets for developing drug to control SARS-CoV-2. It was reported that presence of furin-like cleavage site on S protein of SARS-CoV-2, whereas, this is absent in other β coronaviruses [34]. This cleavage site is believed to be cleaved by furin convertase, for improving the fusion of viral particle with host cell membrane. In addition to this, another furin type recognition site named "RRAR" has been discovered in S1/S2 cleavage site of SARS-CoV-2. Moreover, by molecular modelling studies Fantini et al. (2020), identified a new site at N-terminal domain (NTD) called as gangliosides binding domain (aa 111-158) on S protein of SARS-CoV-2 [35]. It may improve the attachment of virus to ACE2 receptor and lipid rafts by binding to sialic acids (N-Acetylneuraminic acid) linked to host cell surface gangliosides. The mechanism of recognition and attachment of SARS-CoV-2 is considered to be similar as like SARS-CoV.

Although the mechanism of pathogenesis of SARS-CoV-2 is not well understood completely, a probable cellular response is being assumed based on earlier research on SARS-CoV [36]. In an initial 1-2 days of infection virus binds to ACE2 receptor of epithelial cells with a limited innate immune response [37] and this stage of infection can be labelled as asymptomatic state [6]. In second stage of infection (7-14 days) virus travels down the respiratory tract and elicits strong innate immune response. The level of cytokines such as CXCL10, lambda and beta interferon get increased in viral infected cells [38-39]. In third stage of infection virus cause severe disease by infecting lung alveolar type II cells [40]. At this third stage the virus mainly damages alveolar cells with fibrin rich hyaline membranes which leads to the formation of multinucleated giant cells [5].

Still there are significant gaps in understanding the pathogenesis of this virus. Therefore, it's crucial to explore the reasons of failure of immune response in COVID-19 patients. A better understanding on general human immune responses and specific responses during SARS-CoV-2 infection would contribute to design an appropriate therapeutic approach to combat COVID 19.

3 General Immune Response

Immune response is a reaction that occurs within the body of host organism in response to foreign invaders. Also, by this mechanism body recognizes and defends itself against various pathogens such as, bacteria, viruses and other substances that appear foreign and harmful [41-42]. Human beings are exposed to millions of potential pathogenic microbes daily through contact, ingestion and inhalation. These microbes contain a wide range of toxic or allergenic substances which are threat to normal physiology of humans and other mammals. Pathogenic microorganisms such as bacteria and viruses possess various mechanisms by which they infect the host organisms. Mostly, pathogens penetrate through the primary barriers, enter and multiply

in extracellular spaces inside the body of host organism while intracellular pathogens such as viruses spread via extracellular fluids. This lead to alteration in normal body functioning and hence disease [43] occurs. At the same time, immune system recognizes these pathogens as non-self or foreign elements and tries to destroy substances that contain antigen. A general feature of the immune system is to detect structural features of the pathogen or toxin that mark it as distinct from host cells [44]. This discrimination between host and pathogen is very crucial to eliminate or minimize the risk of infection without harming its own tissues [42-45]. In human beings, immune response is an extremely intricate and exceptionally sophisticated system that involves both innate and adaptive mechanisms [46]. These responses consist of network of many cells and organs that work synergistically to protect the body against foreign invaders [47-48]. The cellular components involved in immune response are predominantly produced by bone marrow, which serves both hematopoietic and immunopoietic functions. Bone marrow, a lymphoid tissue is of paramount importance in most of mammalian species it acts as both primary and secondary lymphoid organ to regulate the production, differentiation and maturation of lymphocytes [49-50].

3.1 Innate Immune Response

Innate immune responses are rapid, nonspecific and of limited duration. Such immune responses are activated by the receptors that are encoded by genes present in the germ line of host organism [51-52]. Innate responses comprise of physical, chemical and cellular barriers that form the first line of defence against pathogenic microbes [51]. Skin, mucus membranes, tears, cough reflexes and stomach acids act as physicochemical barriers. While the chemical barriers include soluble proteins, cytokines, chemokines, complement proteins, defensins and ficolins [49-50, 53-54]. Numerous cells such as phagocytes (macrophages and neutrophils), dendritic cells, mast cells, basophils, eosinophils, natural killer (NK) cells and innate lymphoid cells (Table 1) are cellular barriers involved in innate immune response [55-57] which helps in impeding the infections. The main purpose of the innate immune response is to straightaway prevent the spread of foreign pathogens throughout the body. Penetration of pathogen inside the body of host lead to activation of myeloid and mononuclear phagocytes [52, 58]. These cells react to the chemotactic factors released by either affected cells or by pathogen itself and abolish the pathogens quickly via phagocytosis [56, 59-60]. The myeloid cells, also known as granulocytes or polymorphonuclear leukocytes (PML), are the primary armours to arrive at the site of invasion and engulf the pathogen quickly, but these are short lived. The cellular elements of innate immune responses unable to recognize the epitopes present on an antigen as precisely as observed in adaptive immune responses. These responses to pathogen profoundly relies on the interaction of cell surface receptors known as pattern recognition receptors (PRRs) [61-63]. PRRs permit specific immune cells to identify and respond quickly to an extensive range of pathogens that share common structures with them, such structures are known as pathogen-associated molecular patterns (PAMPs) [63-66]. Examples of PAMPs include, natural antibodies, the complement receptors, mannan binding proteins, lipopolysaccharides (LPS), the cell wall components of bacteria and double stranded RNA that is produced during any viral infections [65-66, 67-69]. Innate immune responses also include soluble factors such as serum proteins which bind to the surface of invading pathogen, referred as opsonins. Opsonins react with pathogens to make them more susceptible to engulfment by immune cells known as phagocytosis. Likewise, cytokines and chemokines are broad category of small proteins which are important in cell signalling and produced by immune cells at site of infection and inflammation [70]. These proteins help in activation of the local cellular immune responses to infection site as well as activate many defence mechanisms throughout the body. The main inflammatory cytokines released during early immune response to pathogen infection consist of tumour necrosis factor (TNF), interleukin 1 (IL-1) and interleukin 6 (IL-6). These cytokines are crucial for initiating the immune cells mobilization, the local inflammation

and development of fever which is required for clearance of pathogens [71]. Additionally, these cells help in processing and presentation of antigen during specific immune responses, intensification and control of immune responses by the release of soluble interleukin mediators. Simultaneously, these cells also help in removal of dead and broken cells which is very crucial for the healing process [72]. Innate immune responses activate adaptive immune responses via activation of antigen-presenting cells (APCs) [59, 73-74].

3.2 Adaptive Immune Response

Adaptive immune response is specific, acquired and act as second line of defence. Contrary to germinal encoded recognition molecules of innate immune response, the adaptive responses are encoded by gene elements that somatically rearrange to assemble antigen- binding molecules with exquisite specificity for individual unique foreign structures [75-76]. The main characteristic feature of adaptive immune response is rapid multiplication and increase of T and B lymphocytes from one or a few cells to millions against an infection [77]. Adaptive responses are primarily based on the antigen-specific receptors expressed on the surfaces of T and B-lymphocytes [78]. Key features of adaptive immune response are; specificity and range of recognition, memory, specialized response, self-restraint and tolerance to components of the organism [70, 75]. The essential components of acquired immune response are T and B lymphocytes, immunoglobulins (antibodies) and antigen presenting cells (APCs) (Table 1) [78-79]. APC's plays a key role in activation of naive T- cells by presenting foreign antigen via major histocompatibility complex (MHC) molecules on cell surface [80]. MHC molecules, also known as human leukocyte-associated antigens (HLA) are the cell surface glycoproteins that bind to peptide fragments of proteins which have been either synthesized inside the cell (class I MHC molecules) or ingested by the cell and proteolytically processed (class II MHC molecules) [81]. Antigen presenting cells includes dendritic cells, B-cells and macrophages which are equipped with MHC molecules and co-stimulatory ligands which are predicted by stimulatory receptors present on helper T-cells [49-50, 82]. When innate immune response become ineffective against growth of microbes, specific immune response gets triggered against the epitopes of pathogen by an interaction with major histocompatibility complexes I /II interleukins (IL), and T-lymphocytes (T_H and $T_{C)}$ [49-50]. The cells produced during adaptive immune response are long lived and remain seemingly in dormant state, however, these cells can re-express their functions swiftly after repetitive encounter with their specific antigen [82]. This results in manifestation of immune memory and make the adaptive immune response more effective against specific pathogens or toxins [83-84]. As adaptive immune response is specific and meant to attack pathogens but can sometimes make mistakes in recognizing the structure of pathogen and attack itself. The capability of the immune response to prevent self-damage is known as self-tolerance and failure of self-tolerance triggers the broad class of autoimmune diseases like lupus and rheumatoid arthritis [45, 69, 85].

The innate and adaptive immune responses usually act together. Components of the innate system contribute to activation of adaptive immune response by stimulating the antigen-specific cells. Synergy between both the immune responses is essential for an intact, fully effective immune response to combat any pathogen [86]. In many cases, innate immune responses or early induced responses are not so effective in controlling the pathogen growth. However, these responses slow down the growth of pathogens and hence, allow time for strengthening of the adaptive immune response to control or eliminate the pathogen [87]. Adaptive immune response is further divided into two: the cell-mediated immune response, which is carried out by T cells, and the humoral immune response, controlled by activated B cells and antibodies [76]. Activated T cells and B cells that are specific to the molecular structures present on the pathogen.

These cells kill pathogens directly by apoptosis or secrete antibodies that enhance the phagocytosis of pathogens and disrupt the infection [88-90].

Table 1. Cellular components/elements involved in human immune responses

Immune Response Type	Type of cells	Primary Location		Target Pathogens	Function	References
Innate	Macrophages	Body cavities and organs		Broad range (Bacteria, viruses, protozoans)	Phagocytosis and antigen presentation to T cells	[116]
	Neutrophils	Blood		Bacteria and Fungi	Phagocytosis and degranulation (discharge of contents of cells)	[117]
	Eosinophils	Blood		Parasites and allergic tissues	Degranulation. Release of enzymes, growth factors, and cytokines	[118]
	Basophils	Blood		Various allergic tissues	Degranulation. Release histamines, enzymes, growth factors and cytokines	[119] [55]
	Mast cells	Connective tissues, gastrointestinal tract, skin and respiratory epithelium		Parasites and various allergic tissues		[120]
	Lymphocytes/ T-cells	Thymus and lymph nodes		Th cells target intracellular bacteria, Cytotoxic T cells target virus infected and tumor cells	T helper (Th) cells, ($CD4^+$): immune response mediators, Cytotoxic T cells ($CD8^+$): Release of cytokines, perforin and granzymes which induce apoptosis	[121]
	Monocytes	Blood		Various microbes	Precursor of mast cells and dendritic cells, Differentiate into macrophages and dendritic cells to elicit an immune response	[122]
	Natural Killer Cells (NK)	Blood, body cavities and tissues		Viruses and Tumor cells	Tumor rejection, Destruction of infected cells, Release of perforin and granzymes which induce apoptosis	[123]
Adaptive	T-Cells	TH -Cell Tc- Cell Treg- Cell	Formed in bone marrow & maturation in Thymus, lymphoid tissues	Bacteria and Viruses	T helper (Th) cells, (CD4+): immune response mediators, Cytotoxic T cells (CD8+): cell destruction	[124]
	B-Cells	Memory B-Cells Plasma B-Cells	Formation and maturation in bone marrow, lymphoid tissues	Bacteria and Viruses	Linear and conformational epitopes on virions and virus infected cells, releases mediator molecules immunoglobulins (Ig)	[125]

3.3 Cell Mediated Immune Response/T-Cell Mediated Immune Response

Cell mediated immune responses are critical in maintaining the normal homeostasis of an individual. These responses are function of T-lymphocytes leading to production of effector T-cells. T-lymphocyte originates from bone marrow, matures in thymus gland, and play a vital role in immune response [91-92]. There are four different types of T cells: helper, cytotoxic, memory and regulatory T cells. Helper T cells help in maturation of B cells and activation of cytotoxic T cells and macrophages via interleukin; cytotoxic T cells or killer T cells destroy virus-infected cells and tumour cells in the cell-mediated immune response [89]. Memory T cells like B cells are antigen specific cells that get converted to effector T cells and provides rapid response when re-exposed to specific pathogen to previous infection [93]. Regulatory or suppressor T cells

deactivate T cells and B cells when needed, thus prevent the immune response from becoming too intense [94-96]. T-cells recognize antigens based on a two-chain protein receptor and most important of these are the alpha-beta T cell receptors. APCs (antigen presenting cells) enzymatically cleaves antigen into smaller pieces, bring them to cell's surface and finally link these fragments with a specific type of antigen-presenting protein known as a MHC molecule [97]. This association of the antigen fragments with an MHC molecule on the surface of a cell is known as antigen presentation and results in the recognition of antigen by a T cell. Antigens from different types of pathogens use different class of MHC molecules that is MHC-I and MHC-II [98]. However, these molecules bring processed antigen to cell's surface through a transport vesicle and present the antigen to the T cell receptor [99]. Immature T cells can express either CD4 (cluster of differentiation 4) or CD8 molecules on their cell surface. These molecules primarily regulate the interaction of T-cell and APC. CD4 cells bind APCs via antigen embedded MHC II molecules and stimulate to form helper T-cells [97, 100]. These helper T-cells further stimulate B-cells or cytotoxic T cells directly or secrete cytokines such as interferons (IFN)-gamma and interleukin to inform target cells about pathogenic threat [101-102]. In contrast, CD8-bearing T cells are associated with cytotoxicity. CD8 molecules interacts with an intracellular pathogen and engage antigen embedded MHC I molecule on APCs. These cells are stimulated to become cytotoxic T-cells (CTLs) which kill the infected cells directly by apoptosis and release cytokines to intensify immune response [103]. During an intense immune response there is rapid increase in cytotoxic T cell production and hence these cells kill the virus infected cells before completion of virus replication cycle [104]. Due to effective antiviral immune response of cytotoxic T-cells, adaptive/active immune response has been evolved at first place.

3.4 Humoral Immune Response/B-Cell Mediated Immune Response

Humoral immune response is a function of B cells and originates in bone marrow, hence it is known as B-cell or antibody mediated response [49-50, 105]. Like T cells, B cells carry antigenic surface receptors known as CD antigens (cluster of differentiation). To date over 80CD antigens have been reported in mammals. Mostly, B cells carry both MHC I and MHC II class antigens, complement receptors, several interleukin receptors and discrete B-cell receptor which is capable to bind with APC processed or free antigen [49-50, 88]. B cells are stimulated by helper T-cells and are differentiated into plasma cells or memory cells [60, 107]. Plasma cells are the immune cells which secrete large amount of antibodies or immunoglobulins. Immunoglobulins are specialized glycoproteins which neutralize the invading pathogens by recognizing the antigen present on cell surface via fragment antigen binding (FAB) variable region and cause phagocytosis [41, 108-109]. Five different classes of antibodies are found in humans: IgM, IgD, IgG, IgA and IgE. Amongst these five classes of immunoglobulins, only IgM and IgD function as antigen receptor for naive B cells [110]. Immune responses are triggered by entry of the foreign elements in body called antigens [82]. These responses to antigens are generally classified into primary and secondary immune responses [111]. Initial encounter of immature B cells to an antigen induces primary immune response. Depending on type of antigen and site of infection primary immune response may take up to 14 days to resolve and leads to generation of memory B cells with a higher specificity against antigen [84]. IgM is mostly stimulated by the primary immune response, however, IgG participates in for the memory response [112-113]. Any kind of subsequent exposure to same antigen lead to the development of secondary immunological response which increases the magnitude of the immune response by production of antibodies at much faster rate [113]. During secondary immune response memory T cells quickly multiply into helper T cells and cytotoxic T cells while memory B cells produces antibodies to counteract the pathogen. As secondary immune response has higher antibody affinity hence, it takes only 2-3 days to counteract with antigen in a very effective way [105]. Unlike T cells, B cells can recognize innate antigen and hence these cells do not entail participation

of MHC molecules and antigen-presenting cells. Most protein antigens require signals from helper T cells (Th2) or interleukin to make antibodies. Th2 cells provide help to B cells in controlling extracellular pathogens and are essential for antibody mediated immune response. The specific receptors present in B cells bind to soluble antigens and engulf them via receptor-mediated endocytosis. The digested fragments of antigen are then displayed on the cell surface of MHC II molecule. Th2 cells with complementary T cell receptors (TCRs) bind with B cells and secrete lymphokines or interleukin which induce B cell differentiation to produce plasma cells [114]. Antibodies produced by B cells destroy the pathogens in extracellular spaces and thus prevent spread of intracellular infection [41, 106]. When B cell binds to a self-antigen, in absence of signal from nearby T helper cells to produce antibody, the cell is summoned to undergo apoptosis and destroyed. The B cells after destroying the antigens, produce memory cells which in turn provide future immunity when the same antigen triggers inside body again [113-115].

4 Immune Response Against COVID-19

Clinically, in response to COVID-19 infection, both innate and adaptive immune responses get triggered to kill the virus and protect the body against infection. Immune responses vary at different stages of infection [5]. Previous studies on SARS coronavirus revealed that this virus predominantly targets airway epithelial cells, alveolar epithelial cells, vascular endothelial cells and macrophages in the lungs [126-128]. All these cells express ACE2 host target receptor and the same is being used by SARS-CoV-2 at entry point, hence these cell subsets are specifically targeted by this virus [129-131]. Once the virus breaks the protective barriers or first line of defence it takes over host cell machinery and undergoes rapid multiplication. This lead to huge damage to affected tissues particularly in the organs with high ACE2 expression, such as kidney, liver and intestine. These damaged cells and tissues may further lead to hyper activation of innate response (monocytes and macrophages) that causes hyper inflammation in the lungs. Therefore, in COVID-19, immune hyperactivity is reported to be the main cause of acute lung injury and ARDS at severe stage. It has also been reported that tissue resident macrophages in the lungs found to be associated with the epithelial damage which lead to initiation of ARDS [132-133]. The macrophages are activated by both damage associated molecular patterns (DAMP) such as intracellular contents released by damaged cells or heat shock proteins/hyaluronan fragments released due to tissue injury and PAMPs such as viral RNA or oxidized phospholipids [134-136]. Molecules from DAMPs and PAMPs trigger multiple innate immune responses through TLRs (toll like receptors), NLRP3 (Nod like receptor protein)/inflammasome activation [137-138]. Signal transduction by these molecules initiates production of cytokines and chemokines such as IL-6, IFNγ which activates antiviral gene expression in neighbouring cells and deploy an additional innate and adaptive immune cells to counteract viral infection and maintain tissue homeostasis [11, 139]. Type I and type III IFN production stimulates intracellular antiviral defence in neighbouring epithelial cells, which may restrict viral dissemination. Whereas, release of IL-6 and IL-1β by monocytes triggers mobilization of neutrophils and cytotoxic T cell [5, 139-141]. Activated neutrophils induce pneumocyte and endothelial injury by release of leukotrienes and ROS (reactive oxygen species) which straightway lead to acute lung injury. However, IL-6 promotes maturation of T helper cells as well as naive B cells for antibody production to ensure long-term immunity [142]. Further, by recruiting additional immune cells IL-6 amplifies innate immune responses [143]. In contrast to this, IL-1β got cleaved in response to inflammasome activation and act locally in improving neutrophil cytotoxicity [138]. Available data indicates that enhanced production of cytokines and chemokines create imbalance and dysregulation of innate immune response which becomes the main cause of severity of infection. Such elevations in innate immune cytokines are also known as "cytokine storm," these are similar to the cytokine release syndrome (CRS) which is accountable for the cell toxicity and multiple organ impairment mediated by COVID-19 infections [144-145]. Irrespective of

clinical evidences about dysregulation of innate immune responses is a cause of COVID-19 morbidity and mortality, it is very much clear that viral transmission is a key driver of deadly disease. The histopathologic study performed by clinicians on specimens collected from severely affected COVID-19 patients revealed presence of inclusion bodies with viral persistence [143, 146-147]. The consistent viral persistence may be due to inadequate activation of type I and type III interferons which lead to failure of innate immune response.

Research in immunology have established the fact that to clear and maintain long term suppression of viral infections, activation of T-cell mediated adaptive immune responses are very much needed. T lymphocytes play a vital role in antiviral responses against SARS-CoV-2. One week after beginning of COVID-19 symptoms, presence of both B-cells and T-cells have been detected in the blood samples against SARS-CoV-2. During acute viral infections, the viral peptides activate naive CD4+T cells and CD8+T cells proliferation. CD8+T cells play a significant role in directly killing the virus infected cells, whereas, CD4+T cells are crucial for enhancing CD8+T cell and B cell immune responses [148]. Also, CD4+ T cells are responsible for production of cytokine for immune cell recruitment. Autopsy of patient infected with COVID-19 showed accumulation of mononuclear cells such as monocytes and T cells in lungs. Whereas, presence of low levels of reactive T cells was found in peripheral blood, this state of reduced level of lymphocytes is also known as lymphopenia or lymphocytopenia [5-6, 149-151]. Studies suggest the reason behind decreased level of T cells (CD4+ and CD8+) in peripheral blood is their movement away from blood and mobilization towards the site of infection to kill the infected cells and to prevent the viral dissemination. Amplified exhaustion of T cells or depleted lymphocytes (PLD) contributes to severe outcomes such as viral persistence and mortality in COVID-19 infected patients [152]. However, in case of well-regulated adaptive T cell mediated response and improvement in T cell count both CD4+ and CD8+ directly lead to clinical recovery [143]. Patients recovered from COVID-19 infection shown signs of clonal expansion, T cell activation and memory T cell formation [140].

Humoral response is paramount for clearance of the deadly virus as well as an important part of memory response that prevents virus reinfection [11]. B cell mediated responses act concurrently with T helper cell responses [153]. According to studies, robust B cell responses have been prompted by SARS-CoV-2 virus which is supported by presence of virus specific immunoglobulins such as IgG, IgM, IgA and neutralizing IgG (nAb) in the early days of viral infection [11]. Stimulated B cells lead to production of antibodies that respond to the spiked protein within 2-8 days of onset of symptoms against COVID-19 [154-155] and immunoglobulins IgM are the first antibodies appear in response to an infection followed by IgG. In most of COVID-19 cases seroconversion takes place between 7 to 14 days of onset of symptoms and antibody titres persist for 2-3 weeks or even more than that following the clearance of cytopathic virus [156-162]. Primarily, the immunoglobulins (IgG and IgM) bind to the SARS-CoV-2 internal N protein as well as with S glycoprotein receptor binding domain (S-RBD) that is present externally [163-165]. The antibodies binding with S-RBD domain can trigger the immune responses further and this may lead to neutralize or block the virus interactions with the host entry receptor, ACE2 [161, 164]. In most of the blood samples of COVID-19 cases, presence of virus specific antibodies (IgG, IgM, IgA and anti- RBD nAbs) were detected [161, 164-165] even after 50 days of recovery. Thus, specific immunoglobulins IgG and IgM can serve as immunological marker against COVID-19 and serve as an early detection tool to identify COVID-19 infected host/patient. As severity of COVID-19 is linked with enhanced IgG response, this could serve as a complementary tool to distinguish between mild and severe COVID-19 case. Also, therapeutic approaches to modulate the immunity can be strategized based on both T cell and B cell mediated responses.

In malaria and COVID-19, primarily innate and later, adaptive immune responses are triggered. In both the diseases, Th cells CD4+ and CD8+ play a vital role in pathogen removal by phagocytosis. Decreased levels of T lymphocytes and increased cytokine production contributes for severe pathogenic infection of COVID-19. Since, CQ/HCQ are the approved drugs which have been used previously for treatment of diseases related to ARDS, autoimmune disorders and hyper inflammation. Therefore, in the current situation, the immunomodulatory and anti-inflammatory drugs CQ/HCQ may prove effective in controlling COVID-19.

5 General Use of CQ/HCQ and Mechanism of Action

Hydroxychloroquine is a derivative of chloroquine and was produced with the introduction of hydroxyl group in to chloroquine in the year 1946. CQ and HCQ are derivatives of quinoline and are structurally similar weak bases. HCQ is an aminoquinoline [166], the presence of hydroxyl group influences the conformational changes and water solubility. In animal trials it was found that, the HCQ was nearly 40 % lesser toxic than CQ [167]. HCQ is generally used to prevent or treat uncomplicated malaria in regions where people did not respond to CQ treatment [166]. These medications belong to a class known as disease-modifying antirheumatic drugs (DMARDs). They can reduce skin problems in lupus and prevent swelling/pain in arthritis [166]. Recently, CQ and HCQ are both being investigated for the treatment of SARS-CoV-2 due to their anti-inflammatory and immunomodulatory properties [168].

5.1 Mechanism of Action of HCQ

Several mechanisms of action are proposed to describe therapeutic effects of HCQ, but majority of them are based on in-vitro studies. Notably, the relation between these mechanisms of action and in-vivo clinical efficacy and safety are yet to be fully explained. HCQ is known to have direct molecular effects on lysosomal activity, autophagy and signalling pathways. Based on various therapeutic involvements of the immune system, the mechanism of action is probably dependent on the infection condition, inflammatory conditions and affected tissues or organs.

5.1.1 At Molecular Level

(a) Inhibition of Lysosomal Activity and Autophagy

One of the important mode of action of HCQ is the interfering with lysosomal activity and autophagy. It is commonly accepted that HCQ accumulates in lysosomes and inhibit their function. In-vitro, CQ can destabilize lysosomal membranes and promote the release of lysosomal enzymes inside cells [169]. The ability of CQ and HCQ to interfere with lysosomal activity has been extensively documented [170-172]. This mechanism inhibits the function of lymphocytes and also have immunomodulatory and/or even anti-inflammatory effects. The anti-inflammatory effects could be a result of impaired antigen presentation via the lysosomal pathway. Lysosomes contain hydrolytic enzymes, in association with other vesicles, perform autophagy by digesting materials from inside the cell (endocytosis) or outside the cell (phagocytosis). Lysosomes are engaged in recycling cellular substrates [173], antigen processing and MHC class II presentation, which indirectly promotes immune activation [174-177]. Normally the pH in lysosomes is optimally maintained to keep lysosomal enzymes active for hydrolysis. HCQ might impair the maturation of lysosomes and autophagosomes by increasing the pH of endosomal compartments [178], and inhibit antigen presentation. In assumption, the available studies suggest that HCQ inhibit immune activation by impairing or inhibiting lysosomal and autophagosome functions. One study has identified palmitoyl-protein thioesterase 1 (PPT1) as a potential lysosomal target which bind and inhibit PPT1 activity [179]. PPT1 is

an enzyme involved in the catabolism of lipid-modified proteins [179]. Notably, PPT1 is found to be overexpressed in the synovial tissue of patients with rheumatic arthritis (RA) [180].

(b) Inhibition of Signalling Pathways

Changes in endosomal pH can interfere with toll like receptors, for example TLR9 and TLR7 processing [181] hence, CQ and HCQ might prevent TLR activation upon extracellular stimuli by changing the local pH [181]. HCQ might block TLR9 signalling at the intracellular level by inhibiting TLR–ligand interactions by directly binding to nucleic acids. This hypothesis is backed by an analysis based on fluorescence spectroscopy and surface plasmon resonance which showed that antimalarial drugs directly inhibit CpG–TLR9 communications [182-183]. In addition to TLR9 signalling, CQ can also inhibit RNA-mediated activation of TLR7 signalling [184-185]. Though the exact modes of action for inhibiting TLR7 and TLR9 requires further delineation [186], the inhibition of TLR processing and binding are likely to be the central mechanisms of action. Another potential mode of action of HCQ is interfering with cyclic GMP- AMP (cGAMP) synthase (cGAS) and the activity of cGAS is repressed by ligand binding [187]. The cGAS–stimulator of IFN genes (STING) pathway is the main cause of the type I IFN response. cGAS inhibitors are at present in advancement for the development of treatment of inflammatory rheumatic diseases [190].

5.1.2 At Cellular level

(a) Cytokine Production and Immune Activation

HCQ can indirectly reduce the production of anti-inflammatory cytokines by inhibiting TLR pathways. In in-vitro studies it was found that, HCQ had inhibited the production of IL-1, IL-6, TNF and IFNγ by mononuclear cells [191]. Furthermore, treatment with HCQ had inhibited the production of TNF, IFNα, IL-6 and CCL4 in pDC and NK cells co-cultures stimulated with RNA-containing immune complexes [192-193]. In a study it was found that, IL-1 receptor associated kinase 4 (IRAK4) had reduced the production of cytokines from peripheral blood mononuclear cells (PBMCs) better than HCQ [194]. The IRAK4 exerted the effect by inhibiting the expression of 492 genes, whereas HCQ was found to alter only 65 genes. This study indicated that, HCQ has a remarkable inhibitory effect on cytokine production and gene expression, despite of its effects on few genes [194]. In another study it was found that, treatment with HCQ, reduced IFNα levels in serum of patients with SLE (Systemic Lupus erythrematus) [195]. The long-term treatment of rheumatic arthritis patients with HCQ at the dosage of 200–400mg/day had reduced the circulating levels of IL-1 and IL-6 [196-197]. The anti-inflammatory effects of HCQ could be attributed in part to the inhibition of immune activation including inhibition of lysosomal activity. Indeed, treatment with HCQ is associated with a dose-dependent downregulation of the co-stimulatory molecule CD154 on CD4+ T cells from patients with SLE. The downregulation is accompanied by a decrease in intracellular Ca2+ mobilization and translocation of nuclear factor of activated T cells cytoplasmic 1 (NFATc1) and NFATc2 [198]. However, to be confident on the direct effect of antimalarial drugs on altered cytokine production requires further studies.

(b) Cardiovascular Effects

HCQ is not an anticoagulant molecule, but is generally assumed to have vascular protective effects which can protect from the development of thrombotic complications. This protective effect seems to be most important for patients with a secondary coagulopathy owing to systemic inflammation [199] and in patients with primary Anti-phospholipid syndrome (APS) [200]. Patients with inflammatory rheumatic diseases are at the higher risk of developing cardiovascular complications when compared with the general population

[201-205]. Treatment with HCQ appears to provide long-term benefits by reducing the risk of cardiovascular events, lowering fasting glucose levels [206] and reducing hyperlipidaemia 15 [207]. In a study, patients with SLE were treated with combined low-dose aspirin and HCQ as well as aspirin and HCQ, the results were found superior in combination than aspirin or HCQ alone in terms of preventing cardiovascular complications [208]. However, sufficiently large and controlled studies are needed [209]. HCQ can also potentially inhibit antiphospholipid antibody binding by which, it can reduce the pro-coagulatory state in auto inflammatory diseases [210] or platelet aggregation [211-213].

6 Possible Role of Chloroquine (CQ) and Hydroxychloroquine (HCQ) Against COVID-19

CQ and HCQ were reported to share similar mechanisms of action against COVID-19 [167]. In the earlier outbreak of SARS, HCQ was tested to have in-vitro anti-SARS-CoV activity [214]. But, HCQ becomes safer option than CQ because of narrow therapeutic and safety index margin. Yao et al. 2020, found that HCQ was more potent than CQ in treating SARS-CoV-2-infected Vero cells [147]. It was demonstrated that, the safe dosage (6-6.5 mg/kg per day) of HCQ sulfate concentration possibly produce serum levels of 1.4-1.5 μM in humans [215]. It is recommended to give 400 mg of HCQ orally per day for 7 to 10 days, to efficiently clear the viral nasopharyngeal carriage of SARS-CoV-2, in moderate to severe patient of nCOVID-19. It was observed that 70 % of HCQ-treated cases were found negative comparing with control group (12.5 %) [216]. HCQ was found to inhibit SARS-CoV-2 infection mostly in 3 to 6 days. HCQ individually inhibit the SARS-CoV-2 infection whereas, in combination with other drugs its efficacy was increased. Azithromycin, was known to inhibit Zika and Ebola viruses under in-vitro conditions, Gautret et al. 2020, used azithromycin in combination with HCQ [216] to treat COVID-19 patients. The results were encouraging and on day 6th, 100 % cases were cured [216] hence it is important to understand how HCQ is inhibiting the SARS-CoV-2 infection.

HCQ is a weak base known to raise the pH of acidic organelles (endosomes/lysosomes) [170]. These organelles are essential for membrane fusion. Hence, changes in pH assumed to prevent viral entry, transport and post entry stages as well [217]. In addition to this, it is confirmed that HCQ effectively inhibit the entry of SARS-CoV-2 by changing glycosylation of viral protein [218]. HCQ also inhibit the release of SARS-CoV-2 genome by blocking the transport of virus from early endosome antigen 1 (EEA1)-positive (EEs) to endosomal–lysosomal protein LAMP1^{+} (ELs) [219]. Fantini and colleague 2020, showed that HCQ bind to sialic acid and ganglioside with high affinity and prevents the binding of S protein to gangliosides [120]. Therefore, HCQ treat nCOVID-19 patient by changing pH, protein glycosylation, prevent binding of S protein to gangliosides and blocking transport mechanism. In addition to inhibiting viral infection, HCQ has immunomodulatory property and can suppress the immune response [19, 220]. Therefore, it may also attenuate the inflammatory response in nCOVID-19 cases [167]. Cytokines such as IL-6 and IL-10 have been reported to be increased in response to SARS-CoV-2 infection which may lead to cytokine storm followed by multi-organ failure and death [11, 143]. HCQ was reported to influence the production of these interleukins and hence suppresses the hyper activated immune response [221].

7 Global Overview on Regulatory Aspects

The Food and Drug Administration (FDA) is a federal agency of the United States (US). Under certain conditions such as public health emergency, FDA may authorize the use of investigational unapproved drugs or therapies. HCQ was an FDA approved anti-inflammatory and antimalarial drug. Whereas, CQ was an approved antimalarial drug. But till date there is no drugs or vaccines against the disease COVID-19, approved by FDA. However, on March 28th, 2020 the FDA has given an emergency use authorization (EUA) for chloroquine phosphate (chloroquine) and hydroxychloroquine sulfate (hydroxychloroquine)

products donated to the strategic national stock pile (SNS) to be distributed and used for certain patients hospitalized with COVID-19. SNS can distribute these drugs to states and doctors can prescribe and treat adults and adolescent patients who weigh ≥50 kg and have been hospitalized with severe COVID-19 infection and for whom a clinical trial or participation is not feasible. EUA states that, fact sheet indicating the information on usage, potential known risks and drug interaction should be made available to health care providers and patients. Patients can access these drugs in two ways. First, they may approach pharmacies with an off-label prescription issued from a licensed doctor. Secondly, the patients may also obtain through SNS. Anticipating the increased demand, FDA had published a product specific guidance's (PSGs) to support the new generic development of these drugs. The PSGs, for these two drugs clearly mention that, these are AA rated (No, bioequivalence problems in conventional dosage forms) and in-vivo studies are not necessary [222-223]. European medical agency (EMA) is involved in the evaluation of medicinal products of European Union (EU). EMA states that, the patients suffering from COVID-19 can use these drugs under doctor's supervision and necessary information about the usage should get from the doctor or pharmacist. EMA also states that, the medical professionals can't prescribe these drugs for more than the required durations and these drugs only should be used in case of chronic disease conditions. The health care professionals can use these drugs only for clinical trials and for outside clinical trials, they should follow the national established protocol [224].

Indian Council of Medical Research (ICMR) is responsible for formulation, coordination and promotion of biomedical research in India. On 23/03/2020, the National Task Force (NTF) constituted by ICMR recommends the use of CQ and HCQ for treatment of high-risk individuals. The task force recommends only high-risk people can be subjected to chemoprophylaxis with HCQ. The high-risk individuals and dosage recommended by NTF is as follows. The asymptomatic health care workers should take 400 mg of HCQ two times on first day, followed by once in a week for next seven weeks. The recommended drug must be taken along with the meals. Whereas, the asymptomatic household contacts of laboratory confirmed cases, should take 400 mg dose two times on day 1, followed by once in a week for another three weeks at 400 mg dose and it should be taken along with meals. The task force restricts the recommendation of prophylaxis in children below 15 years of age and for the person with known cases of retinopathy and having hypersensitive reaction to HCQ and 4-aminoquinoline compounds. ICMR recommends only the above-mentioned people should take recommended dose as preventive measures only. However, they should follow all prescribed public health measures such as maintaining safe social distance (minimum 1 meter), respiratory guidelines, frequent hand washing and also, they should use all kinds of personal protective equipment's [225].

The other regulatory bodies such as Brazilian Health Regulatory Agency (Anvisa), Australia's Therapeutic Good Administration (TGA), Medicines and Healthcare Products Regulatory Agency (MHRA) doesn't recommend CQ and HCQ for treatment of COVID-19 and warns the public about the self-medication due to serious health risks associated with the drug usage. These bodies reinforce on necessary clinical trials on representative human samples to prove the safety and efficacy for approval. World Health Organization (WHO) is following all the clinical trials conducted on control of COVID-19 from various research organizations. However, due to unavailability of efficacy and safety data from clinical trials WHO is not recommending the use of anti-malarial drugs (CQ and HCQ) for the treatment of COVID-19 [226-228].

8 Conclusion

It is clear from research data and clinical trials that controlling of inflammatory immune responses are as crucial as targeting the virus to stop the progression of disease. Doctors and researchers have tried several

drugs alone or in combinations for helping COVID-19 patients. However, till date, there is no approved drug against this newly emerged disease. Historical studies on SARS and MERS suggests that the hosts' immune responses (innate and adaptive) against SARS-CoV-2 are similar to these viruses. Therefore, at present, the vaccines and drugs which were used against SARS and MERS are being tested against COVID-19. Amongst all, antimalarial drugs hydroxychloroquine has received enormous attention due to its immunomodulatory property and could be a potential therapy to treat severely affected COVID-19 patients. However, human immune response and the timing of immunomodulation therapy is crucial to get positive outcomes. As immunosuppressant, HCQ may potentially affect the antiviral immune response, therefore, clinicians should decide the timings to use such medications based on patients' age, medical condition and severity of infection. Moreover, early intrusion of such therapy in infection associated-hyper inflammation is considered as a strategic aspect to get recovery against COVID-19. As HCQ is a broad-spectrum antiparasitic, anti-rheumatic, immunity booster, immunomodulatory and immunosuppressant, several countries have included this therapy for COVID-19 treatment, irrespective of unavailability of enough clinical and scientific data to support the use of HCQ against COVID-19. In India, usage of HCQ drug is recently approved by ICMR to combat against COVID-19 infection as well as to utilize this drug as a prophylactic measure for frontline health workers. However, with current ambiguity regarding usage of HCQ drug to treat COVID-19 patients, it is important to understand the potential risks and benefits of this drug. Ongoing clinical trials will provide significant data for an enhanced understanding of potential role of HCQ in treatment and prevention of COVID-19. Also, additional studies on host immune responses against SARS-CoV-2 are equally crucial to understand the dysfunctional outcome of immune responses, such as cytokine storm and hyper-inflammation in severely affected COVID-19 patients. Understanding of correlation between immune responses and disease severity may also prove to be a game changer strategy to finalize the antiviral drugs and adjuvants to control COVID-19.

9 Declarations

9.1 Acknowledgments

Sincere thanks to Reliance Industries Limited for funding and fellow colleagues for their valuable inputs and help in writing the manuscript.

9.2 Authors Contribution

All authors equally contributed in the work which is reported in the present manuscript.

9.3 Competing Interests

The authors declared that there is no conflict of interest exist in the publication.

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