The correlation between vitamin D 3 and Cathelicidin as a marker of innate immune system in the covid19 patients

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Abstract

Background: Pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection emerged in Wuhan City, Hubei Province, China in December 2019. By Feb. 11, 2020, the World Health Organization (WHO) officially named the disease resulting from infection with SARS-CoV-2 as coronavirus disease 2019 (COVID-19). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) responsible for COVID-19 infection. Vitamin D3 is a fat-soluble vitamin and has immune function, particularly in modulating the inflammatory response to viral infection. vitaminD3 modulates both the adaptive and innate immun at cellular level. Cathelicidin is Antimicrobial peptides and main components of innate immunity.

Aims: To study the association between Cathelicidin and vitamin D3 levels to prove the importance of their role in treatment of Iraqi patients with COVID 19.

Materials and methods This case–control study consisted of 90 samples, 60 of them have COVID-19. Patient divided into three group (20 mild, 20moderate, 20 sever cases of COVID-19). 30 were control individuals and age range of individuals (patient and control) was (20-70). Detection of cathelicidin levels were carried out by Enzyme-Linked Immunosorbent
Assay (ELISA). vitamin D3 levels were carried out by fully automated chemiluminescence immunoassay analyser.

**Conclusion:** Vitamin D3 deficiency is more susceptible to development of COVID-19 diseases by affecting on innate immunity.

1. Introduction

Is Pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection emerged in Wuhan City, Hubei Province, China in December 2019. By Feb. 11, 2020, the World Health Organization (WHO) officially named the disease resulting from infection with SARS-CoV-2 as coronavirus disease 2019 (COVID-19). The global epidemic of coronavirus disease 2019 (COVID-19) has presented a major threat to public health worldwide. COVID-19 is the result of infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that was first isolated and identified in patients who were exposed at a seafood market in Wuhan City (Shi et al., 2020). They were named coronaviruses (Latin: corona = crown) because of their shape as spherical virions with a core shell and surface projections resembling a solar corona. Coronaviruses are divided into four subfamilies: alpha, beta, gamma, and delta. While alpha and beta coronaviruses are thought to have originated in mammals, particularly bats, gamma and delta viruses are thought to have originated in pigs and birds. The genome size ranges from 26 to 32 kb. Beta coronaviruses, one of seven coronavirus subtypes that can infect humans, can cause serious disease and death, whereas alphacoronaviruses induce asymptomatic or slightly symptomatic infections. (SARS Cove) is a beta coronavirus that belongs to the B lineage and is closely related to the SARS virus.
The nucleocapsid protein (N), spike protein (S), small membrane protein (SM), and membrane glycoprotein (M) are the four major structural genes, with an extra membrane glycoprotein (HE) found in the beta coronaviruses as shown in figure(1.1). (Velavan and Meyer, 2020).

Figure (1.1) Coronavirus structure (Mousavizadeh and Ghasemi, 2020)

Although droplet and touch are thought to be the primary modes of transmission for SARS-CoV-2, other coronaviruses have been demonstrated to survive for days on filthy surfaces. In addition, SARS-CoV-2 RNA was found in a stool sample from a person who had symptoms, despite the serum sample being negative. Chinese researchers recently recovered SARS-CoV-2 from a swab sample of a confirmed patient's feces, indicating the possibility of fecal-oral transmission. Even in the context of isolation efforts in medical settings, studies have revealed successful person-to-person transmission of 2019-nCoV. A case study of nine
infected pregnant women found no evidence of third trimester complications. After a caesarian incision, vertical transmission occurs (Jiang et al., 2020). While the SARS-CoV-II virus is only viable for three hours in aerosols, it can survive for three days on polypropylene plastic, a couple of days on stainless steel, twenty-four hours on cardboard, and four hours on copper. COVID-19 transmission can be reduced to some extent by increasing temperature and humidity. Surface disinfectants such as 62–71 percent ethanol, 0.5 percent hydrogen peroxide, or 0.1 percent sodium hypochlorite can inactivate SARS-CoV-II in less than a minute (Gasmi et al., 2020).

As shown in figure (1.2), S protein binds to the cellular receptor ACE2 to facilitate the entry of the virus. After the fusion of viral and plasma membranes, virus RNA undergoes replication and transcription. The proteins are synthesized. Viral proteins and new RNA genome are subsequently assembled in the ER (endoplasmic reticulum) and Golgi, followed by budding into the lumen of the ERGIC (endoplasmic reticulum-Golgi intermediate compartment), New virions are released through vesicles (He, Deng and Li, 2020).
Antimicrobial peptides (AMPs) play an essential role in innate immunity in species all over the world (Sitaram and Nagaraj, 2005). AMPs were once thought to be endogenous antibiotics because of their ability to destroy microorganisms by disrupting their membranes. They have wide antibacterial action, which means they can kill both gram-positive and gram-negative bacteria, viruses, and fungi (Reinholz, Ruzicka and Schauber, 2012). Antimicrobial peptides are secreted by epithelial surfaces from both barrier epithelia and glandular structures. In the process of phagocytosis, granules fuse to phagocytic vacuoles containing ingested microorganisms, exposing the microbes to extremely high concentrations of microbicidal and digesting enzymes. Antimicrobial peptides are found in the fluid portion of blood (hemo-lymph) and the granules of phagocytic cells (he- monocytes) in invertebrates (Ganz,
There are about 2,000 of these peptides in the AMP database. They are classified into seven categories: (I) linear peptides; (II) cyclic peptides; (III) glycopeptides; (IV) lipoglycopeptides; (V) lipopeptides and (VII) thiopeptides and chromopeptides. AMPs are small proteins with fewer than 100 amino acids that are found in a variety of cell types. Homologous peptides can be found in vertebrates, invertebrates, and plants, and are generally cationic and amphipathic. Defensin and cathelicidin are two families of AMPs found in mammals (Kuroda et al., 2015). Antimicrobial peptides from the cathelicidin family can be discovered in a variety of mammalian species, including bovine, mouse, rabbit, and humans. They share a highly conserved signal sequence (prorregion) and a cathelin-like prorregion (cathelin = cathepsin L inhibitor), but the C-terminal domain, which encodes the mature peptide, is highly heterogeneous. In humans, rabbits, and mice, the microbicidal C-terminal domain can be 12 to 80 amino acids long, with -helical structures in human, rabbit, and mouse, and -sheet structures in pigs. The sole human cathelicidin, LL-37/hCAP-18, was first isolated from bone marrow (Herr, Shaykhiev and Bals, 2007). In humans, just one cathelicidin gene (CAMP) has been discovered. CAMP codes for the 37-aa peptide LL-37, which has a molecular weight of 18 kDa and starts with two leucine residues at its N-terminus. Human cationic antimicrobial peptide is also known as hCAP-18, FALL-39, or CAMP. LL-37 is found in circulating neutrophils and myeloid bone marrow, It is found in epithelial cells of the skin, as well as the gastrointestinal system, the epididymis, and the lungs, (Strzałkowska and Jo, 2012). With the discovery of the vitamin D receptor (VDR) and major vitamin D metabolizing enzymes expressed by immune cells over the last decade, the role of vitamin D on the control of immune cells has grown in
recognition. In reaction to endogenously produced 1,25(OH)2D, macrophages produce the antimicrobial peptide two Leucine peptide( LL-37), which enhances innate immunity, according to this research (Kamen and Tangpricha, 2010). Monocytes and macrophages are important elements of the innate immune system, and they serve as a first line of defense against microbial intruders. As shown in figure (1.5), 1,25(OH)2D3 has been identified as an important modulator of innate immune responses in this context. Immune cells like monocytes and macrophages have antibacterial properties that can be enhanced. In addition, 1,25(OH)2D3 improves macrophage chemotactic and phagocytic abilities.

The antibacterial effects of 1,25(OH)2D3 have been shown to be mediated via the VDR and linked to the overexpression of the cathelicidin hCAP-18 gene (Baeke et al., 2010). Vitamin D also inhibits the pro-inflammatory cytokines IL17 and interferon gamma, while increasing the production of the anti-inflammatory cytokine interleukin10 by CD4+ T cells, with effects that are considerably stronger in female T cells than in male T cells. Similarly, men's anti-CD3 and anti-CD28-stimulated peripheral blood mononuclear cells produced significantly less regulatory CD4+CD25+ FoxP3+ T lymphocytes in response to vitamin D than women's cells, but this gender difference vanished when oestradiol was added. This could explain why men have been observed to have a higher severity of COVID-19 (Griffin et al., 2020).
Figure (1.3) Proposed mechanism for vitamin D’s action on the innate immune system (Kamen and Tangpricha, 2010)

2. Methods:

This case–control study consisted of 90 samples, 60 of them have COVID-19. Patient divided into three group (20 mild, 20 moderate, 20 severe cases of COVID-19). 30 were control individuals and age range of individuals (patient and control) was (20-70). Clinical data collected by use of specific questionnaire including age, gender, BMI and having any current chronic diseases. Detection of cathelicidin levels were carried out by Enzyme-Linked Immunosorbent Assay (ELISA).

3. Result & Discussion

Demographic and clinical characteristics:

The clinical demographic characteristics and laboratory parameters of both patient groups and the healthy control group were summarized in
Table (1). Age of participants which was within the age group of (20-74) years old. Gender distribution among the studied groups were: 35.56% male, 31.11% female for patient group, while 17.78% male and 15.56% female for control group. The descriptive table also shown an adjustment of other risk factors which were collected through the self-reported technique (student questionnaire), these factors included: hypertension, DM, BMI and smoking. DM distribution among the studied groups were: 31.11% DM, 35.56 non DM, while 43.3% with hypertension and 23.3% without hypertension.

**Table (3.1): Demographic characteristics of patient and control**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patient group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean)</td>
<td>50</td>
<td>47</td>
</tr>
<tr>
<td>BMI (Mean Kg/m2)</td>
<td>31.91</td>
<td>30.94</td>
</tr>
<tr>
<td>Gender% (male/female)</td>
<td>n Percent</td>
<td>n Percent</td>
</tr>
<tr>
<td>32/28 35.56%/3.11%</td>
<td>16/ 17.78%/15.56%</td>
<td></td>
</tr>
<tr>
<td>DM(Yes/No)%</td>
<td>28/32 32.22%/34.44%</td>
<td>_</td>
</tr>
<tr>
<td>Hypertension (Yes/No)%</td>
<td>39/21 43.33%/23.33</td>
<td>_</td>
</tr>
<tr>
<td>Smoking state (Yes/No)%</td>
<td>29/31 32.22%/34.44%</td>
<td>20.00%/13.33%</td>
</tr>
</tbody>
</table>
The present study showed that the higher frequency of COVID19 occurs in men (n=32 patient), while the frequency of Women was Lower (n=28). This result is in agreement with (Klein and Morgan, 2020) who showed that (COVID-19) trend to effect man more than woman.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patient (n)=60 Mean ±SD</th>
<th>Control (n)=30 Mean ±SD</th>
<th>P_value</th>
</tr>
</thead>
<tbody>
<tr>
<td>cathelicidin</td>
<td>51.8±8.04</td>
<td>49.4 ± 9.1</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 3.2: Cathelicidin in covid19 patients in comparison with control

The present study showed that the mean levels of cathelicidin was higher when compared with those of control group. This result is in agreement with (Pahar et al., 2020) who showed that AMPs are really expressed in normal skin, but their levels rise when the skin is damaged by external stimuli such as trauma, inflammation, or infection. Human Cathelicidin
(LL-37) possesses antibacterial and antiviral properties. The antibacterial mechanism is most likely based on the peptide's unique membrane penetrating properties. By targeting the steps that occur before the virus enters the cell, LL-37 can prevent viral infection. It has the ability to: (i) produce pores in the viral envelope; (ii) promote extracellular aggregation of viral particles, blocking virus access and increasing virus absorption by phagocytes; and (iii) hinder the virus's attachment to its receptor on the cell surface. LL-37 and defensins can also disrupt viral replication at the intracellular stage. AMPs, on the other hand, do not work in isolation, especially when it comes to their role as powerful immune modulators, where they interact with toll-like receptors (TLRs) and chemokine receptors. Both of these receptors have been implicated in the pathophysiology of COVID-19 (Laneri et al., 2021). Respiratory Viruses are combated by antimicrobial peptides. Bacteria and viruses are common causes of lung illness in humans, with respiratory viruses accounting for a disproportionately higher part of the etiological role. They have a significant impact on mortality and economy around the world, as the World Health Organization has documented and tracked. Influenza A virus (IAV), respiratory syncytial virus (RSV), parainfluenza virus (PIV), metapneumovirus (MPV), human rhinovirus (HRV), human adenovirus (HAdV), bocavirus (BoV), and coronavirus are the most prevalent respiratory viruses (CoV). Antiviral activity of defensins and LL-37 has been proven against a number of viruses, including coronaviruses (Ghosh and Weinberg, 2021).

Table 3.7 showed correlation between vitamin D 3 and Cathelicidin was significant at the 0.05 level in covid19 patients. In patients group( r= - 0.27,p =0.03) negative correlation.
Table 3.3: Correlation vitamin D3 with cathelicidin in COVID19 patients

<table>
<thead>
<tr>
<th></th>
<th>patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>-0.27*</td>
</tr>
<tr>
<td>p</td>
<td>0.03</td>
</tr>
</tbody>
</table>

This result showed correlation between vitamin D3 and Cathelicidin levels in COVID19 patients. This result is in agreement with (Herr et al., 2011) who showed that links vitamin D with innate immunity Activities of Vitamin D3 Many genes, including the hCAP18 gene, which encodes cathelicidin, a precursor to LL37, are transcriptionally controlled in part by vitamin D. LL37 is active against bacteria and viruses after the cathelicidin peptide is cleaved. LL37 also modulates immunological responses and works in tandem with toll-like receptors and other immune system components. LL37 has been shown to inhibit the replication of a variety of viruses, including enveloped RNA viruses like the Corona Virus 2 of the Severe Acute Respiratory Syndrome (SARS-CoV-2) that causes COVID-19 illness (Crane-Godreau et al., 2020)
**Figure (3.2)** correlation between cathelicidin and vitamin D3 in patient group.

**Receiver operating characteristics (ROC):**

Receiver operating characteristics (ROC) curve analysis of cathelicidin, vitamin D3 was performed. The best area under the ROC curve (AUC) was for cathelicidin levels (AUC = 0.39, p <0.001) and for vitamin D3 levels (AUC=0.59, P <0.001).

(Figure 12) ROC analysis indicated that cathelicidin level > 38.11 ng/ml and vitamin D3 levels >8.74 /ml with 93% sensitivity, 96% specificity for cathelicidin while for vitamin D3 95% sensitivity, 96% specificity as shown in Table (3.3).
Table 3.4: Differentiation power (area under the ROC Curve, Sensitivity % and Specificity %) of cathelicidin and vitamin D3 levels in covid19 patients.

<table>
<thead>
<tr>
<th>Test Result Variable(s)</th>
<th>Cut off value</th>
<th>AUC</th>
<th>Asymptotic Sig.</th>
<th>Sensitivity</th>
<th>1 - Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathelicidin</td>
<td>38.11</td>
<td>0.39</td>
<td>&lt; 0.01</td>
<td>0.93</td>
<td>0.96</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>8.74</td>
<td>0.59</td>
<td>&lt; 0.01</td>
<td>0.95</td>
<td>0.96</td>
</tr>
</tbody>
</table>
Conclusions

1. vitamin D 3 and cathelicidin are predictive factors for severity of COVID-19 in the studied subjects.
2. Vitamin D3 deficiency is more susceptible to development of COVID-19 diseases by affecting on innate immunity.

References

- Herr, C., Greulich, T., Koczulla, R. A., Meyer, S., Zakharkina, T., Branscheidt, M., ... and Bals, R., 2011. The role of vitamin D in


