How to Cite:

The role of NFAT in activation of lymphocytes in COVID 19 patients

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Abstract---After the zoonotic CoV epidemics of SARS (2003) and MERS (2012), SARS-COV-2 was appeared as the human positive sense RNA coronavirus's third and fatal strain that infect birds and a variety of animals and human. It is one of Coronaviridae family. It cause serious respiratory illness. A total number of 68 samples were involved in the current study, the suspected patients infection were confirmed by obtaining nasopharyngeal swab and subjected to PCR test in the molecular biological laboratories accredited by the Iraqi Ministry of Health. The samples were divided into two interval groups: the first period were collected during the first fourteenth day after infection and the second group interval after 15th to 28th of infection. NFAT concentration was tested by ELISA. The study results showed that the concentration of NFAT is increased in the first fourteen days then its concentration decreased at the second fourteen days with significance of (p≤0.03).

Keywords---NAFT, Lymphocyte, COVID-19, Elisa, SARS-COV-2.

Introduction

Coronaviruses are important zoonotic infectious agent. In December of 2019, a new coronavirus was the cause of pneumonia illness cases of in Wuhan (NHCPRC, 2020). The new virus was speared that result in epidemic in China, then it a pandemic, the number of infections increased in other countries around the world (ECDPControl, 2020). From the first case reported of COVID-19, the number was elevated to reach 81,552 cases in China and reach 1,400,000 all over
the world, the WHO declared a health problem January 2020. Then the epidemic increased and had been reported in other countries, except Antarctica. The elevating number of cases in deferent countries, in the USA, Italy and Spain, accessed the rate in China. The virus is named (SARS-CoV-2); and described as 2019-nCoV2. The symptoms appeared after 5 to 14 days (Guan et al., 2020). Actually, there are no specific syndrome that can be adapted to differentiate COVID-19 from the other respiratory viral infections. At the end of the first week, COVID-19 can cause pneumonia (Wang et al., 2020). Pneumonia is an important manifestation of COVID-19 that can be characterized by fever, dry cough and dyspnea. The time to the begging of dyspnea is after 5 days, seven days in hospitalized patients and (ARDS) at eight days. Recovery may begin in the second or third week. The WHO, stated that the recovery time can be two weeks for mild and 3 to 6 weeks for severe COVID-19 cases. The period of hospitalization recovery cases may be after 10 days. Different vaccines have been introduced and have proven to be effective in attenuating the stop spreading the pandemic of COVID-19. In spite of giving vaccines to the populations the disease cases still rise and the virus still infect in the vaccinated individuals, for that reason another strategies are needed to fight SARS-CoV-2 (WHO, 2020).

Through the infection different factors can play different roles on the pathology of COVID-19 infection. The Nfam1 play a vital role in regulating of T cell activation (Crabtree and Olson, 2002; Hogan et al., 2003). It is a member of the other five factors of the NFAT family which can be controlled by Ca2+-calcineurin signaling pathway. Although the NFAT proteins share the main action with DNA, they may undergo more than one expression pattern and function as (Hogan et al., 2003) for example it can involved in wound healing activation of immune cellular components and bone formation (Manabe et al., 2021). Moreover these proteins have the ability react with Fos-Jun (AP-1) to produce NFAT:AP-1 complexes (Chen et al., 1998), than can induce cytokine expression and many genetic activation pathways (Macia´n et al., 2002). Moreover, NFAT have the ability to monomeric interaction with DNA (Giffin et al., 2003; Jin et al., 2010); also it can complexes cooperatively with other transcriptional factors like FOXP3, to regulatory function of T lymphocytes (Bandukwala et al., 2011) or can involved in many transcriptional pathways depending on the type of cell and signaling factors that it is activated (Hogan et al., 2003). Genome analyses of DNA elements may be determined functionally by endogenous function study and protein engineering of NFAT1 proteins in vivo. Transcriptional strategy programs of low responsiveness in helper and cytotoxic T cells which reveals the pivotal NFAT role (Schietinger and Greenberg, 2014). The rationale of the current paper is to highlight the role of NFAT in COVID-19 infection that may give an approach for treating or decrease the severity of the disease.

Materials and Methods

Study Design

A total of 68 samples were involved in the current study, the suspected patients infection were confirmed by taken nasopharyngeal swab which were submitted to PCR test in the molecular biological labortries accredited by the Iraqi Ministry of Health. the samples were divided into two interval groups: the first period were
collected during the first 14th days of infection and the second group interval were from the day 15th to 28th post infection. The level of NFAT activation molecule 1 was determined using Eliza kit from SunLong Biotech Co., LTD (Catalogue Number: SL3395Hu).

**Serum preparation**

Blood was collected from patients and allowed to clot by left it at room temperature for 10-20 minutes. Then the samples were centrifuged at 2,000-3,000 rpm for 20 minutes.

**Procedure**

After the preparation of the Standards, The dilution factor for the studies samples was 5 (40 sample μl + 10 μl buffer) then the samples were incubated at 37°C. Repeating washing was done before adding the HRP to all wells except blank control followed by incubation and washing. To visualize of the results, Chromogen Solutions were mixed and added to the plate, followed by shaking and incubation avoiding light exposure then the reaction terminated and the O.D absorbance was readed at 450nm and the positivity is indicated by color changing from blue to yellow. The log scale (X-axis) represents the known concentrations while the log scale (y-axis) corresponds the OD reading.

**Results and Discussions**

This study involved 68 infected patients hospitalized with COVID-19. The results show that the NFAT level is increased in the first fourteen days post infection, while, its level decreased after second fourteen days with significance (p≤0.03) as shown in Table 1. These results can explain apart of reasons of sever acute respiratory syndrome cytokine storm during the infection and the severity of symptoms which results from high concentration of some inflammatory cytokines which may cause to mortality of covid-19. NFAT proteins can be expressed by many immune cells and can play an important role in cytokine transcription genes. The NFAT proteins action can be regulated by the calcium/calmodulin-dependent phosphatase calcineurin, that control their translocation from the cytoplasm to the nucleus of activated cells. NFAT proteins DNA-binding domains is look like those of Rel-family proteins, and Rel, the NFAT proteins have the ability to overlap in their action to intract to regulatory factors in cytokine genes. NFATs can bind to transcription factors of the AP-1 to produce NFAT:AP-1 (Jae-Seon et al., 2014). One study results suggested that signaling of NFAT could be a good target to prevent respiratory infections. NFAT role is characterized to control tissue development through embryogenesis, involving vasculogenesis, axonal outgrowth, formation of muscle and bone, also, development of the immune system (Lee et al., 2011). Even though, many studies are done to investigate NFAT signaling in inflammatory processes, such as its role in atherosclerosis (Heim et al., 2002), autoimmune diseases (Nicholas et al., 2007).

The decrease of neutrophil number or targeting specific adhesion molecules is critical to prevent lung injury (Cortes et al. 2007). NFAT proteins act as a key role in neutrophil regulating. Generally, tissue navigation of leukocytes in local of
inflammation is induced by chemokines. The inhibition of NFAT keep T-cell survival and also, cytokine formation. Even though, it is noticed that NFAT inhibition make no differences in T-cell apoptosis and proliferation. The T-cell dysfunction is a result of the overwhelming systemic inflammatory response, as a result T-cell protective effects of NFAT inhibiting signaling are resulted from the attenuated proinflammatory response (Henter et al., 1998). NFAT regulate T-cell function by restoring the number of CD4 T cells and the level of cytokine. NFAT proteins are produced in most immune-system cells and have a vital role in the transcription of cytokine genes. Previous studies suggested that the NFAT1-dependent enhancer activity of conserved noncoding sequence (CNS)-9, a distal cis-acting element (Sulciner et al., 1996). The signaling pathway of both calcineurin and calcium controls the activation of NFAT1-, while, the fifth member of NFAT is responsible of hypertonicity (Ahmad et al., 2021). The role of TCRs triggers intracellular releasing of calcium from endogenous stores causing calcium influx to the cellular membrane which active the calmodulin-dependent serine/threonine phosphatase, calcineurin. NFAT Dephosphorylation by calcineurin result in rapid translocation of NFAT, that cause increase in transcription of NFAT genes (Haataja et al., 1997).

Table 1
FNAT level measured by ELISA

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<tr>
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<th>Levene’s Test for Equality of Variances</th>
<th>t-test for Equality of Means</th>
<th>Std. Error Difference</th>
<th>95% Confidence Interval of the Difference</th>
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<tr>
<td></td>
<td>F</td>
<td>Sig.</td>
<td>t</td>
<td>df</td>
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<td>SMEAN (FNAT)</td>
<td>4.928</td>
<td>.030</td>
<td>1.189</td>
<td>66</td>
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Three transcriptional factors, namely NF-kB, NFAT and AP-1, can induce the transcriptional program that is activate and differentiate T cell. The crucial role of NFAT in the immunity is strengthens by different studies revealed the regulatory transcriptional function of NFAT on different cytokine genes after antigenic stimulation (Cherfils and Zeghouf, 2013).

**Conclusion**

From the current study we conclude that NFAT functions in T cells could be targeted to develop immune modulatory drugs for controlling T cell immunity that may be decrease the cytokine storm in COVID-19 infection.
References


