

## LEVEL OF D-DIMER AND C- REACTIVE PROTEIN AMONG COVID-19 PATIENTS IN RELATION TO DIFFERENT AGES AND GENDERS

SAKAR AHMAD ABDULLAH<sup>\*</sup>, HAREM OTHMAN SMAIL<sup>\*\*</sup>, ALAA AHMED RAHIM<sup>\*</sup>, TANYA KAMIL MUHAMAD<sup>\*</sup>, BANWAN MAJID<sup>\*</sup>, BINAY ROSTAM<sup>\*</sup>, NAZ NAWROZ<sup>\*</sup>, SIVER AWAT<sup>\*</sup> and DYARI KAMAL<sup>\*</sup>

<sup>\*</sup>Dept. of Medical Laboratory, College of health and medical technology, Sulaimani Polytechnic University, Kurdistan Region–Iraq

<sup>\*\*</sup>Dept. of Biology, Faculty of Science and Health, Koya University, Kurdistan Region–Iraq

*(Received: December 7, 2022; Accepted for Publication: January 10, 2023)*

### ABSTRACT

coronavirus disease of 2019 (COVID-19) is a new infectious disease spreading worldwide rapidly. COVID-19 has been associated with a risk of thrombosis, which an increase in the level of D-dimer can detect. D-dimer is a small fragment of protein present in the blood after blood clot degradation. This research aimed to evaluate the level of D-Dimer and C- reactive protein in COVID-19 patients and ascertain the relation of these markers with age and gender. From December 2020 to January 2021, patients with laboratory reports of Covid-19 were retrospectively enrolled in different hospitals and private laboratories. We collected 253 cases of the D-dimer test for different ages and genders and 204 cases of C- reactive protein test with D-dimer to detect the severity of the disease. An increased level of D-dimer of more than 0.5 mcg/ml was reported in 105 patients (41.5%). According to gender, 43.7% of males and 38.79% of females have elevated D-dimer levels. In patients with COVID-19, D-dimer levels greater than 0.5mcg/mL could accurately predict in-hospital morbidity and mortality. Therefore, D-dimer has been proposed as a possible early and effective marker for improved management of COVID-19 patients.

**KEYWORDS:** D-dimer, inflammatory biomarkers, CRP, inflammatory thrombosis and age.

### 1. INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a newly identified transmittable respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that has rapidly become a pandemic in a short duration (Huang et al., 2020; Xu et al., 2020). After the two events of a cross-covering animal beta coronaviruses to humans, the third event occurred in December 2019 in Wuhan-China (Ray et al., 2020).

The hyperinflammatory phase (cytokine storm) in COVID-19 is induced by the activation of both natural and cellular immunity causing the release of large amounts of pro-inflammatory cytokines. This may lead to an increase in the levels of several inflammatory biomarkers which have been reported in patients, including CRP, ferritin, erythrocyte sedimentation rate (ESR), lactate dehydrogenase (LDH), procalcitonin, granulocyte-colony-stimulating factor (G-CSF), and cytokines such as IL-6, IL-2, IL-7, TNF- $\alpha$ , macrophage inflammatory protein-1 $\alpha$  (Yin et

al., 2020). Moreover, the increased level of D-dimer was reported in severe stages of covid-19 patients, indicating poor outcomes and increased risk of adult respiratory distress syndrome (ARDS) (Zhang et al., 2020)

The D-dimer level appears to be elevated in many cases, associated with many disorders where fibrin production and intra/extravascular coagulation occur. Those disorders include pulmonary embolism, community-acquired pneumonia, deep vein thrombosis, chronic kidney disease, acute stroke, disseminated intravascular coagulation (DIC), and many inflammatory conditions (Bayes et al., 2000). Hence, increased D-dimer levels in COVID-19 patients could be a marker for detecting the severity and stage of the disease, pulmonary complications, and risk of venous thromboembolism. This would help in the early introduction of therapeutic measures, which may reduce the risk of morbidity and mortality in COVID-19 patients (Paliogiannis et al., 2020).

The normal value of d-dimer is <0.5 mcg/dl (<500ng/ml) (Tang et al., 2020). Suggested

hypotheses of hypercoagulability in COVID-19 include severe inflammatory responses such as cytokine storm, complement activation, and endothelial dysfunction. These mechanisms lead to inflammatory thrombosis and increase D dimer levels during COVID-19 infection (Guan et al. and Campbel 2020). It has been proposed that the virus may directly activate the coagulation cascade (Kastenhuber et al.,2022)

In patients with COVID-19, a significantly increased level of CRP was observed, with an average of 20-50 mg/L (Chen et al.,2019, Gao et al.,2020 ). Also, a positive relation between CRP level and the severity of the disease has been observed. A Higher CRP level indicates a more severe state and lung damage due to a hyperinflammatory response (Matsumoto et al.,2019).

## 2. MATERIAL AND METHODS

### 2.1 Data collection

A total of 253 laboratory test results of D-dimer and 204 C-reactive protein tests of confirmed COVID-19 patients were collected. The data collection was carried out between December 2020 and January 2021 in different hospitals, healthcare centers, and private laboratories in Sulaimani. The patients from which the tests were collected ranged Different ages (16 to 99 years with different genders. Most of these COVID-19 cases were confirmed by polymerase chain reaction (PCR) or immunological methods.

### 2.2 D-dimer and CRP assays

COBAS INTEGRA 400 plus system was used for in vitro testing for the quantitative immunological determination of fibrin degradation products (D-Dimer in human plasma. The test principle was particle-enhanced immunoturbidimetric assay. Latex particles of uniform size are coated with monoclonal antibodies (F(ab)<sub>2</sub> fragments) to the D-Dimer epitope. The antigen/antibody complexes

produced by adding samples containing D-Dimer led to an increase in the turbidity of the test reactants. The variation in absorbance with time depends on the concentration of D-Dimer epitopes in the sample. The residue is determined turbidimetrically at 659 nm.

Cobas c 311 and Cobas c 501/502 were analyzers for in vitro quantitative determination of CRP in human serum. The principle of the assay was a particle-enhanced immunoturbidimetric assay. Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The aggregates are determined turbidimetrically at the wavelength (sub/main) 800/570 nm.

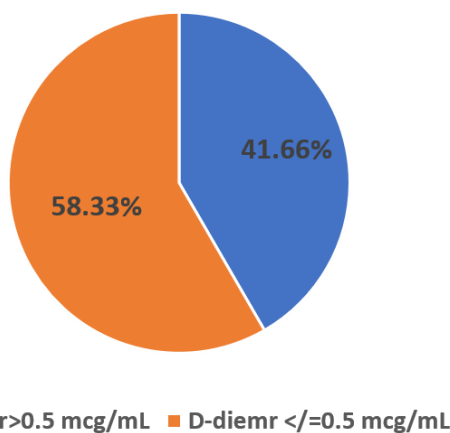
### 2.2 Statistical analysis

Categorical variables (gender) are expressed as percentages, and quantitative variables are defined as the mean value and standard deviation by using Microsoft excel 2016. Furthermore, different groups are classified depending on their ages with 20 years interval (<20 years old, 20-40 years old, 40-60 years old, >60 years old). Finally, the comparison of the D-dimer and CRP levels in different ages and gender are performed.

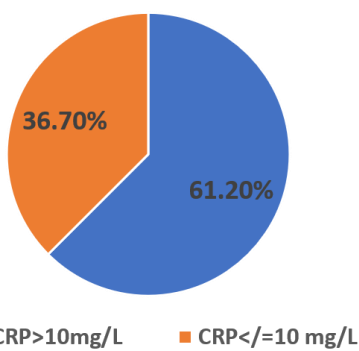
## 3. RESULTS

The study population included 253 COVID-19 patients. The laboratory results of D-dimer and CRP tests were collected to evaluate the hypercoagulable state and inflammatory process in SARS-Cov 2 infections in different ages and genders. The median age was 57 years (Range: 16-99), 54.1% were male, and 45.8% were female.

The increased level of d dimer of more than 0.5 mcg/ml (average:  $2.10 \pm 6.28$ ) was reported in 105 out of 253 patients (41.5%). Out of 204 patients, 125 patients (61.2%) have elevated CRP levels above 10 mg/dL (average: 62.95, SD:63.75). (**Figures 3.1 & 3.2**)



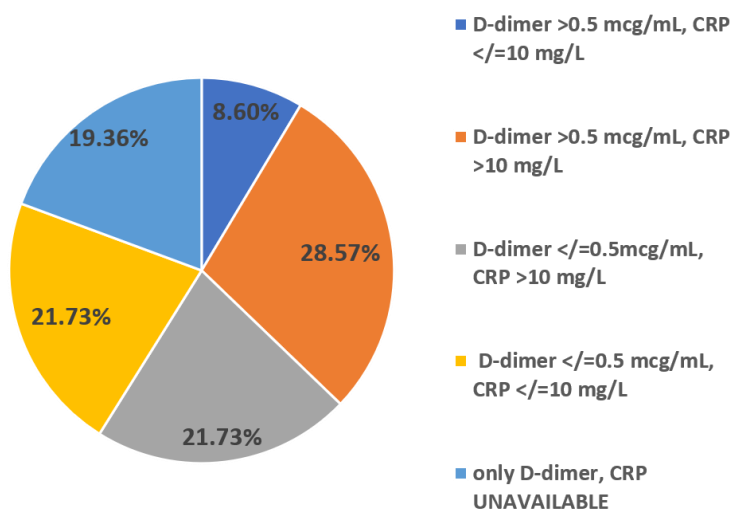
**Fig. (3.1):** The percentage of D-dimer level increased > 0.5 mcg/mL or remained normal.



**Fig. (3.2):** The percentage of C-reactive protein level.

In 8.6% of patients, D-dimer increased, and CRP remained normal. Both D-dimer and CRP increased together in 28.57% of patients. D-

dimer remains normal, with an elevated CRP of 21.73%. And both remain normal in 21.73% of patients. **(Figure 3.3)**



**Fig. (3.3):** The relation between CRP and D-dimer level in percentages.

According to the patient ages, the patients were classified into four groups. The first group involves patients below 20 years old. In this group, 1 patient (0.95%) has elevated D-dimer (0.95 mcg/mL), and two patients (1.6%) have elevated CRP levels (average: 18.86 mg/L). Second group, patients between 20 and 39 years old, 13.33% have increased D-dimer (average:1.27 mcg/ml), and 12.8% have CRP

levels of more than 10 mg/L (average: 81.01 mg/L). The third involves patients between 40 to 60 years old, 46.66% have elevated D-dimer (average: 2.33 mcg/ml), and 50.4% have increased CRP (average:57.28 mg/L). The final group includes patients who are older than 60 years. The D-dimer level increased in 39.4% (average: 2.12 mcg/ml), and CRP increased in 35.2% (average: 66.49 mg/L). (figure 3.4)

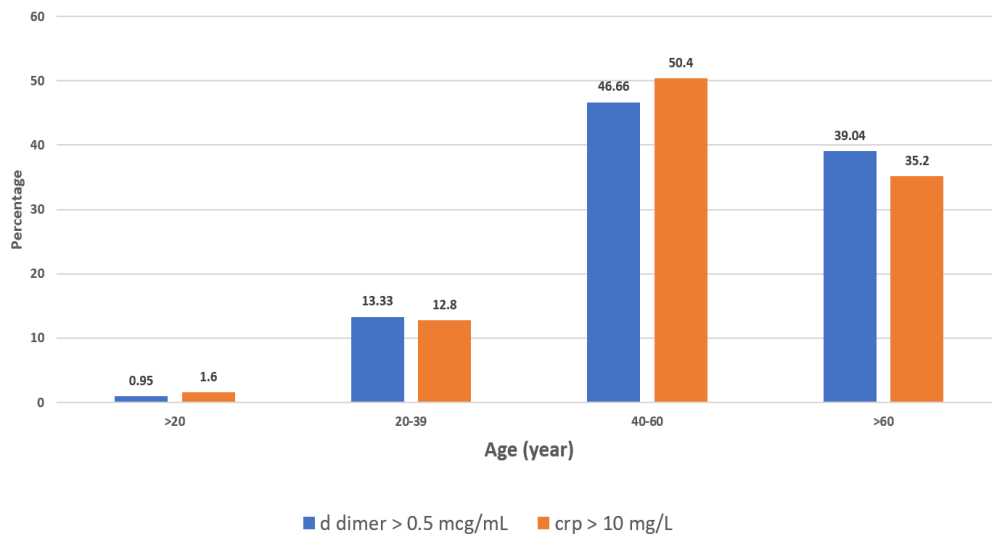


Fig. (3.4): Elevation of D-dimer and CRP in different groups of ages.

Regarding gender, 43.7% of males have elevated D-dimer levels, and 38.79% of females.

CRP increased in 55.47% and 42.4% of males and females, respectively. (figure 3.5)

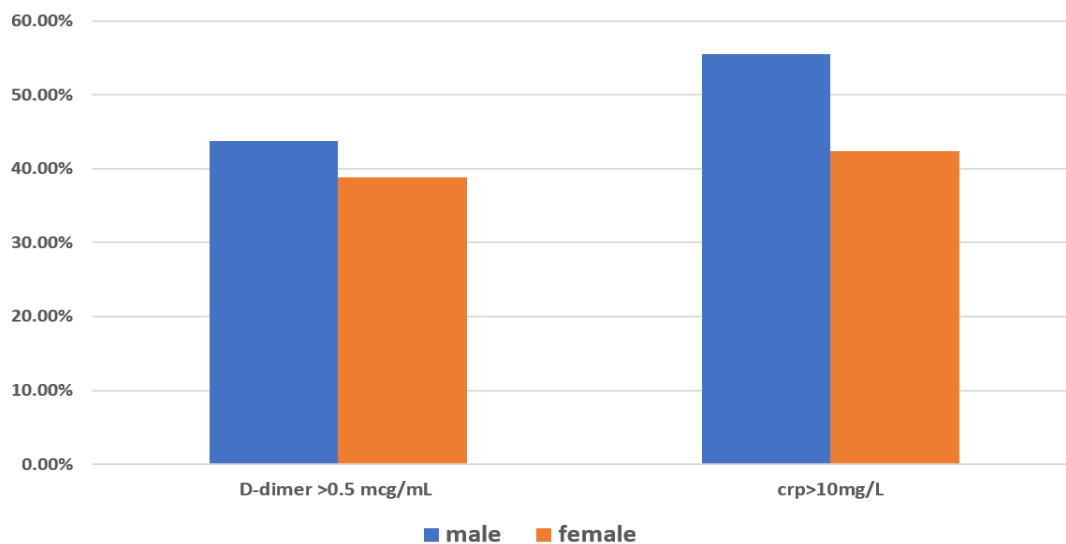


Fig. (3.5): D-Dimer and CRP levels in males and females COVID19- infected patients.

#### 4. DISCUSSION

The major cause of morbidity and mortality in these patients include arterial and venous thrombotic problems and coagulation disorders such as disseminated intravascular coagulopathy (DIC). These thrombotic complications result from endothelial dysfunction, platelet activation, and excessive inflammation. (Gong et al.,2020, Xiong et al.,2020 ). D-dimers are the product of clots broken down and produced from the cleavage of cross-linked fibrin by plasmin. A (D-dimer test) is easily accessible, inexpensive, and rapid. Unfortunately, D-dimer levels elevate in 36% to 43.6% of cases, according to early retrospective cohort studies of COVID-19 patients. (Ray et al.,2020).

In the present study, we demonstrated that COVID-19 patients have significantly increased D-dimer levels, which emphasized the possibility of more evidence of activation of the coagulation system. The causes of the Related to age D-dimer levels are not completely explained; however, it is well understood that D-dimer is produced during the breakdown of fibrin and is a marker of fibrinolysis (Young 1991). Derangement in the coagulation and immune response may contribute to this elevation, which is directly or indirectly caused by the SARS-CoV 2. Such include releasing many pro-inflammatory cytokines, over-activation of macrophages and neutrophils, and endothelial cell damage (Avau et al.,2015; Gorbalenya et al.,2020). In our study, D-dimer significantly increased by 41.50% in COVID-19 patients. Our result was approximately similar to Guan et al. result that 46.4% of patients had D-dimer values above the locally defined cut-off ( $\geq 0.5$  mcg/mL) (Guan et al.,2020). Most of the other findings reveal that elevated plasma levels of D-dimer are associated with increased mortality risk.

The D-dimer in COVID-19 patients was related to inflammatory biomarkers, especially with CRP. D-dimer plasma levels decreased synchronously with CRP levels in patients with a good clinical prognosis after treatments (Yu et al.,2020). Also, our study result shows an elevation of CRP 61.20% in COVID-19 patients. Similar to J. Zhang et al. result, 58.5% of patients had increased CRP levels (Zhang et al.,2020). Increased plasma levels of D-dimer and CRP were demonstrated in 28.57% of patients. So, our study supports that a strong

correlation may exist between inflammatory response and hypercoagulation in SARS-Cov 2 infections.

According to our findings, the morbidity is elevated by age, as confirmed in the study of Ying zio in china (Zou et al.,2020), and this severity is caused by age-related physiological changes and possible underlying health conditions, as shown in our result d dimer is also elevated in COVID19 patient With older age 46.66% in the patient between 40-60 years), It may be due to the elderly's presence of comorbidities as many thrombotic diseases that occur in a patient with COVID-19 could be more effective in older age; if we compare our result with Zhang et al.'s study, we could see the same outcome as a result of the inflammatory response and the resulting tissue destruction CRP is elevated in due to age, as it displayed %50.4 in age 40-60 as some critical cases may be done the test lately, so CRP is decreased, and in comparison to L.wang's study both display the same outcome (Wang)

During the Coronavirus Outbreak in China and Europe, several reports explained the severity of SARS-Cov 2 infection In relation to gender. According to data from China, infected men die at a higher rate than infected women, with a fatality rate of 2.8 percent in Chinese men compared to 1.7 percent in women (Rabin 2020). Moreover, sex break-up data for COVID-19 in several European countries appear a like number of cases between the sexes, even so, further hard results in aged men. Men with pre-existing cardiovascular disease have the highest case fatality rate. The scientist reports discussing latent sex-particular mechanisms modulating the route of disease, like the hormone-regulated expression of genes encoding for the (SARS-CoV2) coming receptor angiotensin-converting enzyme (ACE) 2 receptor and TMPRSS2 as well as sex hormone-driven innate and adaptive immune responses and immune ageing (Gemmati et al.,2020). Finally, the scientists elucidate the collision of gender-specific lifestyle, behavior, health, psychological stress, and socioeconomic conditions on COVID-19 and discuss sex-specific aspects of antiviral therapies. The effect of sex hormones, estrogen, and testosterone on immune system reactions has been demonstrated, outcoming in fewer strong immunologic responses in males and following a rise in mortality and morbidity from viral respiratory illnesses (White et al.,2022). In

inclusion, the X chromosome carries the largest number of immune-related genes in the human genome and maybe as well donate to females' higher immune response (as well as a female distinction in autoimmune diseases) (Libert et al.,2010). Angiotensin-converting enzyme 2 (ACE2) and its role in viral transmission and related morbidity have also been a topic of new COVID-19-associated discussion. ACE2 receptors on the pulmonary endothelium serve as the main ingress point for Coronavirus. Several previous animal models have revealed a rise in ACE2 activity in the male or ovariectomized model, hinting at a sex hormone influence (Smail, 2023). Interestingly, the ACE2 receptor gene is also found on the X chromosome and has some generous causes that increase mortality and morbidity in men, such as (smoking) Usually, the smoking rate is much higher in men than in women (Gebhard et al.,2020, Walter et al.,2020). our results showed more elevation of D-dimer and CRP plasma levels in men than in women, indicating an increased risk of severity and mortality of the infection. The risk factor for serious illness and death in patients with COVID-19 has already been identified as older age and male sex(Rostam et al.,2020)

## 5. CONCLUSION

1. hypercoagulability in COVID-19 appears as a tremendous pathological event with serious outcomes in mortality and morbidity.
2. D-dimer could be an early and helpful marker for assessing the hypercoagulable state and improving the management of Covid-19 patients.
3. An increased D-dimer level fourfold above the reference range predicts in-hospital mortality.
4. Along with D-dimer, inflammatory biomarkers are frequently used as a marker of COVID-19 severity and increased probability of immune thrombosis.

## REFERENCES

Avau, A., & Matthys, P. (2015). Therapeutic potential of interferon- $\gamma$  and its antagonists in autoinflammation: lessons from murine models of systemic juvenile idiopathic arthritis and macrophage activation syndrome. *Pharmaceuticals*, 8(4), 793-815.

Bayes-Genis, A., Mateo, J., Santaló, M., Oliver, A., Guindo, J., Badimon, L., ... & de Luna, A. B. (2000). D-Dimer is an early diagnostic marker

of coronary ischemia in patients with chest pain. *American Heart Journal*, 140(3), 379-384.

- Campbell, C. M., & Kahwash, R. (2020). Will complement inhibition be the new target in treating COVID-19-related systemic thrombosis?. *Circulation*, 141(22), 1739-1741.
- Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., ... & Zhang, L. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *The lancet*, 395(10223), 507-513.
- Gao, Y., Li, T., Han, M., Li, X., Wu, D., Xu, Y., ... & Wang, L. (2020). Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. *Journal of medical virology*, 92(7), 791-796.
- Gebhard, C., Regitz-Zagrosek, V., Neuhauser, H. K., Morgan, R., & Klein, S. L. (2020). Impact of sex and gender on COVID-19 outcomes in Europe. *Biology of sex differences*, 11(1), 1-13.
- Gemmati, D., Bramanti, B., Serino, M. L., Secchiero, P., Zauli, G., & Tisato, V. (2020). COVID-19 and individual genetic susceptibility/receptivity: role of ACE1/ACE2 genes, immunity, inflammation and coagulation. Might the double X-chromosome in females be protective against SARS-CoV-2 compared to the single X-chromosome in males?. *International journal of molecular sciences*, 21(10), 3474.
- Gong, J., Dong, H., Xia, Q. S., Huang, Z. Y., Wang, D. K., Zhao, Y., ... & Lu, F. E. (2020). Correlation analysis between disease severity and inflammation-related parameters in patients with COVID-19: a retrospective study. *BMC infectious diseases*, 20(1), 1-7.
- Gorbalenya, A. E., Baker, S. C., Baric, R. S., de Groot, R. J., Drosten, C., Gulyaeva, A. A., ... & Ziebuhr, J. (2020). Severe acute respiratory syndrome-related Coronavirus: The species and its viruses—a statement of the Coronavirus Study Group. *BioRxiv*.
- Guan, W. J., Ni, Z. Y., Hu, Y., Liang, W. H., Ou, C. Q., He, J. X., ... & Zhong, N. S. (2020). Clinical characteristics of coronavirus disease 2019 in China. *New England journal of medicine*, 382(18), 1708-1720.
- Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., ... & Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The lancet*, 395(10223), 497-506.
- Kastenhuber, E. R., Mercadante, M., Nilsson-Payant, B., Johnson, J. L., Jaimes, J. A., Muecksch, F., ... & Cantley, L. (2022). Coagulation factors

- directly cleave SARS-CoV-2 spike and enhance viral entry. *Elife*, 11, e77444.
- Libert, C., Dejager, L., & Pinheiro, I. (2010). The X chromosome in immune functions: when a chromosome makes the difference. *Nature Reviews Immunology*, 10(8), 594-604.
- Matsumoto, H., Kasai, T., Sato, A., Ishiwata, S., Yatsu, S., Shitara, J., ... & Daida, H. (2019). Association between C-reactive protein levels at hospital admission and long-term mortality in patients with acute decompensated heart failure. *Heart and vessels*, 34(12), 1961-1968.
- Paliogiannis, P., Mangoni, A. A., Dettori, P., Nasrallah, G. K., Pintus, G., & Zinellu, A. (2020). D-dimer concentrations and COVID-19 severity: a systematic review and meta-analysis. *Frontiers in public health*, 8, 432.
- Rabin, R. C. (2020). Why the Coronavirus seems to hit men harder than women. *The New York Times*, 20.
- Ray, S. K., & Mukherjee, S. (2020). Understanding the Role of Corona Virus based on Current Scientific Evidence-A Review with Emerging Importance in Pandemic. *Recent patents on anti-infective drug discovery*, 15(2), 89-103.
- Rostam, S. R. K., Shekhany, K. A. M., & Smail, H. O. (2020). Comparative study of some biochemical parameters among of COVID-19 symptoms and non COVID-19 symptoms individuals. *BIOVALENTIA: Biological Research Journal*, 6(2), 9-14.
- Smail, H. O. (2023). The Association Between Some Endocrine Conditions and COVID-19: A Review. *Biology, Medicine, & Natural Product Chemistry*, 12(1), 45-53.
- Tang, N., Li, D., Wang, X., & Sun, Z. (2020). Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *Journal of thrombosis and haemostasis*, 18(4), 844-847.
- Walter, L. A., & McGregor, A. J. (2020). Sex-and gender-specific observations and implications for COVID-19. *Western Journal of Emergency Medicine*, 21(3), 507.
- Wang, L. (2020). C-reactive protein levels in the early stage of COVID-19. *Medecine et maladies infectieuses*, 50(4), 332-334.
- White, A. A., Lin, A., Bickendorf, X., Cavve, B. S., Moore, J. K., Siafarikas, A., ... & Leffler, J. (2022). Potential immunological effects of gender-affirming hormone therapy in transgender people—an unexplored area of research. *Therapeutic Advances in Endocrinology and Metabolism*, 13, 20420188221139612.
- Xiong, M., Liang, X., & Wei, Y. D. (2020). Changes in blood coagulation in patients with severe coronavirus disease 2019 (COVID-19): a meta-analysis. *British journal of haematology*.
- Xu, Z., Shi, L., Wang, Y., Zhang, J., Huang, L., Zhang, C., ... & Wang, F. S. (2020). Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *The Lancet respiratory medicine*, 8(4), 420-422.
- Yin, S., Huang, M., Li, D., & Tang, N. (2021). Difference of coagulation features between severe pneumonia induced by SARS-CoV2 and non-SARS-CoV2. *Journal of thrombosis and thrombolysis*, 51(4), 1107-1110.
- Young, B., Gleeson, M., & Cripps, A. W. (1991). C-reactive protein: a critical review. *Pathology*, 23(2), 118-124.
- Yu, B., Li, X., Chen, J., Ouyang, M., Zhang, H., Zhao, X., ... & Tang, J. (2020). Evaluation of variation in D-dimer levels among COVID-19 and bacterial pneumonia: a retrospective analysis. *Journal of thrombosis and thrombolysis*, 50(3), 548-557.
- Zhang, G., Zhang, J., Wang, B., Zhu, X., Wang, Q., & Qiu, S. (2020). Analysis of clinical characteristics and laboratory findings of 95 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a retrospective analysis. *Respiratory research*, 21(1), 1-10.
- Zhang, J., Wang, X., Jia, X., Li, J., Hu, K., Chen, G., ... & Dong, W. (2020). Risk factors for disease severity, unimprovement, and mortality in COVID-19 patients in Wuhan, China. *Clinical microbiology and infection*, 26(6), 767-772.
- Zou, Y., Guo, H., Zhang, Y., Zhang, Z., Liu, Y., Wang, J., ... & Qian, Z. (2020). Analysis of coagulation parameters in patients with COVID-19 in Shanghai, China. *Bioscience trends*.