



Article

Biochemical Changes Accompanying Diarrhea in Infected Calves in Tikrit Governorate

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Abstract: The study aimed at some biochemical changes (proteins and electrolytes) and blood changes in calves with diarrhea. The study included (110) calves of the local breed, of both sexes, from different areas of the city of Tikrit, with ages ranging between (1-8) months. The study included two groups, the first group (the group with diarrhea, 100 calves), and the second group (the group Control: 10 healthy calves, and all study animals were subjected to clinical examinations. 110 fecal and blood samples were collected from the calves (100 samples with diarrhea and 10 healthy samples were considered as a control group) and for the period from the beginning of October 2022 until the end of July 2023, the blood samples were drawn. From the jugular vein and placed in test tubes or without anticoagulant (EDTA), it was placed in a centrifuge to obtain blood serum and kept at -20°C until biochemical tests were performed on it using a commercial kit prepared for this purpose. Results of biochemical tests accompanying cases of diarrhea in calves. The study noted a significant decrease ($P<0.05$) in the rates of sodium ions, bicarbonate, total protein, and albumin, and a significant increase ($P<0.05$) in the value of the rising gap rate. As for blood count values in animals suffering from diarrhea, the study noted a significant increase ($P<0.05$) in the values of the total number of red blood cells, hemoglobin concentration, and the size of packed blood cells, while we did not notice any significant differences ($P<0.05$) in the concentrations of each of the following: The average corpuscular volume, the corpuscular hemoglobin rate, and the corpuscular hemoglobin concentration rate.

Keywords: biochemical changes, blood changes, diarrhea

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1. Introduction

Dehydration and metabolic acidosis are among the most important complications accompanying cases of diarrhea in calves, regardless of the cause, as diarrhea results from excessive excretion of water in the feces (as water constitutes approximately 40%-60% of the animal's body weight), which is divided into the external cellular fluid. Extracellular fluid and intracellular fluid, leading to a decrease in the volume of extracellular fluid and a decrease in the resulting plasma volume. This leads to a decrease in the volume of external cellular fluid and a contraction in the volume of plasma, which results in a decrease in arterial blood pressure, shrinkage of the surrounding blood vessels, and a decrease in blood supply to the organs, especially the kidneys, causing a deficiency in renal function and their inability to excrete hydrogen ions, an increase in its level in the blood, and the occurrence of blood acidity [1]. Metabolic acidosis in calves with diarrhea results from the loss of bicarbonate ions in the feces, along with increased absorption of acid resulting from bacterial fermentation of lactose in the large intestine and the formation of L-lactate during the anaerobic glycolysis process resulting from congestion and reduced access of blood

and oxygen to tissues and organs, leading to an increase in the anion gap and a decrease in the level of base excess [2]. Metabolic acidosis leads to cardiac arrhythmia or death due to heart failure resulting from a lack of potassium ion in the cardiac muscle and its high level in the extracellular fluid [3].

2. Materials and Methods

2.1. Blood samples

(110) blood samples were drawn from the study animals (100 samples with diarrhea and 10 healthy samples were considered as a control group). The blood was drawn from the jugular vein using plastic syringes and placed in test tubes without anticoagulant (EDTA), and placed in a centrifuge at high speed. 3000/min for 10 minutes to obtain blood serum and put the serum in 1.5 ml abend off tubes and keep it at -20°C until biochemical tests are performed on it [4].

2.2. Tests to estimate concentrations of electrolyte ions in serum

2.2.1. Estimation of sodium ion concentration in serum

The sodium ion was measured using a kit from Biolab, a French company, according to the Mg- uranylactate method described by [5]. Using a spectrophotometer with a wavelength of 410 nm, and according to the kit manufacturer's instructions.

Table 1. Estimation of sodium ion concentration in serum

Reagents	Plank	Sample	Standarded
The detected	2ml	2ml	2ml
Sample		20 Microlitre	
Standard			20 Microlitre
Mix and incubate the sample for 5 minutes at 37°C, then read it using a spectrophotometer			

Sodium concentration (mmol/L) = (sample absorbance)/(standard absorbance) (standard value) x 5

2.2.2. Estimation of potassium ion concentration in serum

The potassium ion was measured using a Kit from Biolab, a French company, according to the Photometric Turbidimetric method described by [6]. Using a spectrophotometer with a wavelength of 578 nm, and according to the instructions of the kit manufacturer.

Table 2. Estimation of potassium ion concentration in serum

Reagents	Plank	Sample	Standarded
The detected	2ml	2ml	2ml
Sample		200 Microlitre	
Standard			200 Microlitre
Mix and incubate the sample for 15 minutes at 37°C, then read it using a spectrophotometer			

Potassium concentration (mmol/L) = (sample absorbance)/(standard absorbance) x 5 standard value

2.2.3. Estimation of chloride ion concentration

The chloride ion was measured using a kit from Biolab, a French company, according to the colorimetric method described by [7] using a spectrophotometer with a wavelength of 500 nm, and according to the instructions of the kit manufacturer.

Table 3. Estimation of chloride ion concentration

Reagents	Plank	Sample	Standarded
The detected A	1000 Microlitre	1000 Microlitre	1000 Microlitre
Distel water	10 Microlitre		
Sample		10 Microlitre	
Standard B			10 Microlitre

Mix and incubate the sample for 10 minutes at 37°C, then read it using a spectrophotometer

Chloride concentration (mmol/L) = (sample absorbance)/(standard absorbance) x 0.51 standard value

2.2.4. Estimation of bicarbonate concentration

Bicarbonate was measured using a kit from Biolab, a French company, according to the colorimetric method described by [7] using a spectrophotometer at a wavelength of 500 nm, and according to the instructions of the kit manufacturer.

Table 4. Estimation of bicarbonate concentration

Reagents	Plank	Sample	Standarded
The detected A	1000 Microlitre	1000 Microlitre	1000 Microlitre
Distel water	10 Microlitre		
Sample		10 Microlitre	
Standard B			10 Microlitre

Mix and incubate the sample for 10 minutes at 37°C, then read it using a spectrophotometer

Bicarbonate concentration (mmol/L) = (sample absorbance)/(standard absorbance) x concentration of standard solution

2.3. Tests to estimate the concentration of total protein, albumin, and globulin

2.3.1. Estimation of total protein concentration in serum

Total protein was measured using a kit from Biolab, a French company, according to the Biuret method described by [8] using a spectrophotometer at a wavelength of 546 nm.

Table 5. Estimation of total protein concentration in serum

Reagents	Plank	Sample	Standarded
The detected A	1000 Microlitre	1000 Microlitre	1000 Microlitre
Distel water	10 Microlitre		
Sample		10 Microlitre	
Standard B			10 Microlitre

Mix and incubate the sample for 10 minutes at 37°C, then read it using a spectrophotometer

$$\text{Total protein (g/dL)} = (\text{sample absorbance})/(\text{standard absorbance}) \times (\text{standard value})$$

2.3.2. Estimation of albumin concentration in serum

Albumin was measured using a kit from Biolab, a French company, according to the bromocresol green method described by [6]. Using a spectrophotometer with a wavelength of 628 nm.

Table 6. Estimation of albumin concentration in serum

Reagents	Plank	Sample	Standarded
The detected A	1500 Microlitre	1500 Microlitre	1500 Microlitre
Distel water	10 Microlitre		
Sample		10 Microlitre	
Standard B			10 Microlitre

Mix and incubate the sample for 10 minutes at 15-25 °C, then read it using a spectrophotometer

$$\text{Albumin (g/dl)} = (\text{sample absorbance})/(\text{standard absorbance}) \times 3 (\text{standard value})$$

2.3.3. Estimation of globulin concentration in serum

The globulin concentration was calculated by subtracting the albumin concentration from the total protein concentration [9]. The formula:

$$\text{Globulin concentration (g/L)} = \text{total protein concentration} - \text{albumin concentration}$$

3. Results

3.1. Results of biochemical tests accompanying cases of diarrhea in calves

3.1.1. Electrolyte standards in the blood of calves with diarrhea

The results of the current study showed a significant decrease ($P < 0.05$) in the rates of both sodium and bicarbonate ions, as the rates were 127.1 ± 4.225 and 20.4 ± 1.610 mmol/L, respectively, in animals with diarrhea, and 135.4 ± 4.721 and $1.955. 32.5 \pm$ mmol/L, respectively, in healthy animals. The study noted a significant increase ($P < 0.05$) in the value of the ascending gap rate, which was 19.4 ± 2.012 and 15.9 ± 1.833 mmol/L, respectively, in infected and healthy animals, respectively. While no significant difference was observed in the value of potassium and chloride ions between the two groups.

Table 7. Electrolyte standards in the blood of calves with diarrhea

Standard error \pm adjusted		Standards
Healthy animals	Infected animals	
^a 135.4 \pm 4.721	^b 127.1 \pm 4.225	Sodium ion (mmol/L)
^a 4.12 \pm 0.312	^a 3.88 \pm 0.226	Potassium ion (mmol/L)
^a 93.1 \pm 3.325	^a 91.3 \pm 3.633	chloride ion (mmol/L)
^a 32.5 \pm 1.955	^b 20.4 \pm 1.610	Bicarbonate ion (mmol/L)
^b 15.9 \pm 1.833	^a 19.4 \pm 2.012	Rising gap (mmol/L)

Different letters horizontally between the two groups indicate a significant difference at the probability level ($P < 0.05$).

3.1.2. Protein standards in calves with diarrhea

The results of the current study showed a significant decrease ($P < 0.05$) in the rates of total protein and albumin, as they were 5.61 ± 0.334 and 2.40 ± 0.281 g/dL, respectively, in the infected animals, and 7.25 ± 0.464 and 0.251 ± 3.82 g/dL, respectively, in healthy animals, while no significant difference was observed in the value of globulin.

Table 8. Changes in protein parameters in calves with diarrhea and in healthy calves

Standard error \pm the average		Standards
Healthy animals	Infected animals	
^a 7.25 \pm 0.464	^b 5.61 \pm 0.334	Total protein (g/dL)
^a 3.82 \pm 0.251	^b 2.40 \pm 0.281	Albumin (gm/dl)
^a 3.43 \pm 0.145	^a 3.21 \pm 0.131	Globulin (gm/dL)

The horizontally different letters between the two groups indicate a significant difference at the probability level ($P < 0.05$).

3.1.3. Results of blood tests accompanying cases of diarrhea in calves

The results of the study of the blood count in animals with diarrhea showed a significant increase ($P < 0.05$) in the values of the examined blood picture, which included the total number of red blood cells, hemoglobin concentration, and the size of packed blood cells, while no significant differences were observed ($P < 0.05$). In the concentrations of both the average corpuscular volume, the corpuscular hemoglobin rate, and the corpuscular hemoglobin concentration rate when compared with the blood values of healthy animals.

Table 9. Results of blood tests accompanying cases of diarrhea in calve

Standard error \pm the average		Standards
Healthy animals	Infected animals	
^b 8.84 \pm 0.902	^a 15.17 \pm 1.430	Total white blood cell count ($\times 10^3/\mu\text{L}$)
^b 45.1 \pm 3.484	^a 54.4 \pm 4.499	Neutrophils (%)
^a 44.3 \pm 3.378	^b 34.1 \pm 2.288	Lymphocytes (%)
^a 3.8 \pm 0.011	^a 4.3 \pm 0.026	Eosinophils (%)
^a 5.7 \pm 1.041	^a 6.3 \pm 1.095	Mononucleate (%)
^a 0.3 \pm 0.028	^a 0.4 \pm 0.022	Seeds (%)

The horizontally different letters between the two groups indicate a significant difference at the probability level ($P < 0.05$).

3.2. The results of the study on the total and differential counts

The results of the study on the total and differential counts of white blood cells in animals suffering from diarrhea showed a significant increase ($P < 0.05$) in the total count of white blood cells and neutrophil cells, with values of (15.17 ± 1.430 and 54.4 ± 4.499 ($\times 10^3/\text{microliter}$, respectively) in the animals). Infected animals and (0.702 ± 8.84 and 46.2 ± 3.484) And healthy ones, respectively. As for the rest of the types of white blood cells, which included eosinophils, mononuclear cells, and basophils, the study did not notice a significant difference ($P < 0.05$) in their numbers when comparing between the infected and healthy group.

Table 10. Results of total and differential counts of white blood cells accompanying cases of diarrhea in calves

Standard error \pm the average		Standards
Healthy animals	Infected animals	
^b 8.84 \pm 0.902	^a 15.17 \pm 1.430	Total white blood cell count ($\times 10^3/\mu\text{L}$)
^b 45.1 \pm 3.484	^a 54.4 \pm 4.499	Neutrophils (%)
^a 44.3 \pm 3.378	^b 34.1 \pm 2.288	Lymphocytes (%)
^a 3.8 \pm 0.011	^a 4.3 \pm 0.026	Eosinophils (%)
^a 5.7 \pm 1.041	^a 6.3 \pm 1.095	Mononucleate (%)
^a 0.3 \pm 0.028	^a 0.4 \pm 0.022	Seeds (%)

The horizontally different letters between the two groups indicate a significant difference at the probability level ($P < 0.05$).

4. Discussion

The results of the study indicated a significant decrease in the values of the concentrations of both sodium and bicarbonate ions, with a significant increase in the values of the ascending gap, as these changes indicate the occurrence of diarrhea and acidosis in calves suffering from diarrhea [2], [10]. Diarrhea in newborn calves is accompanied by vomiting resulting from the loss of large amounts of fluid in the stool, along with acidosis represented by a decrease in blood pH due to an increase in the excretion of sodium and bicarbonate ions in the stool with an increase in Hydrogen ion level and basal excess in the blood [4], [11], [12] indicated that potassium ions, such as sodium and chloride, are lost with the feces in cases of diarrhea in calves, and this may lead to a decrease in potassium in the body. In severe cases of diarrhea in calves, the concentration of potassium in the blood will increase (hyperkalemia). In cases of metabolic acidity, the Na⁺-K⁺-ATPase pump will operate at its maximum level. In cases of acidemia, this pump will fail to function, and this condition will lead to an increase in sodium inside the cells due to it not being pumped out of the cell. As for potassium, its level will increase outside the cells, which will be more than normal. In cases of chronic and persistent diarrhea, it will lead to a decrease in potassium in the body.

The results of the study showed the appearance of three types of acidosis in calves with diarrhea: decompensated metabolic acidosis, as the molecular pressure concentration of carbon dioxide gas in calves with diarrhea was within the normal range and close to its value in control group animals, followed by the presence of mixed acidosis (metabolic and respiratory), which can This is attributed to the high molecular pressure of carbon dioxide gas, which is accompanied by a significant decrease in the concentration of bicarbonate ions in.

Calves suffering from diarrhea, and finally compensatory metabolic acidosis, which occurred as a result of a decrease in the molecular pressure of carbon dioxide gas, which was accompanied by a significant decrease in the concentration of the bicarbonate ion in the calves that were suffering from diarrhea compared to clinically normal calves. What strengthened these results was the significant decrease in the percentage of the bicarbonate ion: Carbonic acid [4], [11].

The above blood changes and the significant increase in total plasma protein concentration are among the most important changes accompanying cases of depression and acidosis resulting from diarrhea and loss of fluids, which leads to an increase in blood concentration (Hemoconcentration), which leads to an increase in blood viscosity and thus a decrease in blood supply to the various tissues of the body [4]. [1] reported that there was an increase in both the size of packed cells.

The concentration of hemoglobin and the number of red blood cells in calves that suffered from diarrhea and vomiting, which may be due either to the decrease in plasma water or to the release of epinephrine as a result of the stress experienced by the affected animal. Thus, a compensatory mechanism occurs, leading to an increase in the volume of packed blood cells and the number of red blood cells and concentration. Hemoglobin. The increase in hemoglobin concentration may also be due to the body's increasing need for hemoglobin in order to stave off changes in blood levels, as hemoglobin accepts the hydrogen ion from carbonic acid in the event of an injury to the body.

With acidosis [4], [12] also pointed out the decrease in the oxygen extraction ratio from the blood of calves suffering from diarrhea, leading to an increase in the affinity of hemoglobin for oxygen as a result of the decrease in the molecular pressure of oxygen gas, and this reinforces the explanation for an increase in the concentration Hemoglobin in this study due to a significant decrease in the molecular pressure values of oxygen gas in the blood of calves suffering from diarrhea.

5. Conclusion

The study noted a significant decrease ($P < 0.05$) in the rates of sodium ions, bicarbonate, total protein, and albumin, and a significant increase ($P < 0.05$) in the value of the rising gap rate. As for blood count values in animals suffering from diarrhea, the study noted a significant increase ($P < 0.05$) in the values of the total number of red blood cells, hemoglobin concentration, and the size of packed blood cells, while we did not notice any significant differences ($P < 0.05$) in the concentrations of each of the following: The average corpuscular volume, the corpuscular hemoglobin rate, and the corpuscular hemoglobin concentration rate.

REFERENCES

- [1] P. R. Scott, G. A. Hall, and P. W. Johnes, "Bovine medicine: diseases and husbandry of cattle," ... *medicine: diseases ...*, 2008.
- [2] O. O. Omole, G. Nappert, J. M. Naylor, and G. A. Zello, "Both L-and D-lactate contribute to metabolic acidosis in diarrheic calves," *J Nutr*, 2001, [Online]. Available: <https://www.sciencedirect.com/science/article/pii/S0022316622143993>
- [3] P. D. Constable, H. R. Stämpfli, H. Navetat, and ..., "Use of a quantitative strong ion approach to determine the mechanism for acid–base abnormalities in sick calves with or without diarrhea," *Journal of Veterinary ...*, 2005, doi: 10.1111/j.1939-1676.2005.tb02731.x.
- [4] E. H. Coles, *Veterinary clinical pathology*. cabdirect.org, 1974. [Online]. Available: <https://www.cabdirect.org/cabdirect/abstract/19760826818>
- [5] P. R. Henry, "Sodium and chlorine bioavailability," *Bioavailability of nutrients for animals*, 1995, [Online]. Available: <https://www.sciencedirect.com/science/article/pii/B9780120562503500421>
- [6] N. W. Tietz and S. Berger, "Fundamentals of clinical chemistry," (*No Title*), 1976, [Online]. Available: <https://cir.nii.ac.jp/crid/1130000795127877248>
- [7] S. E. Winter, P. Thiennimitr, S. P. Nuccio, and ..., "Contribution of Flagellin Pattern Recognition to Intestinal Inflammation during Salmonella enterica Serotype Typhimurium Infection," *Infection and ...*, 2009, doi: 10.1128/iai.01341-08.
- [8] J. C. Young, I. Moarefi, and F. U. Hartl, "Hsp90: a specialized but essential protein-folding tool," *J Cell Biol*, 2001, [Online]. Available: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2150759/>
- [9] T. Bobbo, E. Fiore, M. Gianesella, M. Morgante, L. Gallo, and ..., "Variation in blood serum proteins and association with somatic cell count in dairy cattle from multi-breed herds," *Animal*, 2017, [Online]. Available: <https://www.cambridge.org/core/journals/animal/article/variation-in-blood-serum-proteins-and-association-with-somatic-cell-count-in-dairy-cattle-from-multibreed-herds/62A6FF2BF9FFE60727576F74AA1937E1>
- [10] H. Guzelbektes, A. Coskun, and I. Sen, "Relationship between the degree of dehydration and the balance of acid-based changes in dehydrated calves with diarrhoea," *Bulletin-Veterinary Institute in ...*, 2007, [Online]. Available: https://www.researchgate.net/profile/Hasan-Guzelbektes-2/publication/272790859_RELATIONSHIP_BETWEEN_THE_DEGREE_OF_DEHYDRATION_AND_THE_BALANCE_OF_ACID-BASED_CHANGES_IN_DEHYDRATED_CALVES_WITH_DIARRHOEA/links/58be2dfe92851c471d5c01c6/RELATIONSHIP-BETWEEN-THE-DEGREE-OF-DEHYDRATION-AND-THE-BALANCE-OF-ACID-BASED-CHANGES-IN-DEHYDRATED-CALVES-WITH-DIARRHOEA.pdf
- [11] K. S. Latimer, *Duncan and Prasse's veterinary laboratory medicine: clinical pathology*. books.google.com, 2011. [Online]. Available: [https://books.google.com/books?hl=en&lr=&id=92WVbFZNnEkC&oi=fnd&pg=PR7&dq=duncan+and+prasse%](https://books.google.com/books?hl=en&lr=&id=92WVbFZNnEkC&oi=fnd&pg=PR7&dq=duncan+and+prasse%20)

- 27s+veterinary+laboratory+medicine+clinical+pathology&ots=CW8CBCTWrs&sig=L0uc9NOmbJr9m89ipeTINddWc-I
- [12] L. Slanina, A. Bomba, J. Lehocký, S. Paulík, and ..., "Hemoconcentration in calves and its relation to the hematologic, protein, mineral and electrolyte profile," *Veterinarni ...*, 1984, [Online]. Available: <https://europepmc.org/article/med/6437047>
- [13] C. Cambier, T. Clerbaux, B. Moreaux, and ..., "Blood oxygen binding in calves with naturally occurring diarrhea," *American Journal of ...*, 2001, [Online]. Available: <https://avmajournals.avma.org/view/journals/ajvr/62/5/ajvr.2001.62.799.xml>
- [14] H. Tian, "A comprehensive quantification of global nitrous oxide sources and sinks," *Nature*, vol. 586, no. 7828, pp. 248–256, 2020, doi: 10.1038/s41586-020-2780-0.
- [15] J. Hammond, "Oral Nirmatrelvir for High-Risk, Nonhospitalized Adults with Covid-19," *New England Journal of Medicine*, vol. 386, no. 15, pp. 1397–1408, 2022, doi: 10.1056/NEJMoa2118542.
- [16] A. J. Bernal, "Molnupiravir for oral treatment of covid-19 in nonhospitalized patients," *New England Journal of Medicine*, vol. 386, no. 6, pp. 509–520, 2022, doi: 10.1056/NEJMoa2116044.
- [17] C. A. Juan, "The chemistry of reactive oxygen species (Ros) revisited: Outlining their role in biological macromolecules (dna, lipids and proteins) and induced pathologies," *Int J Mol Sci*, vol. 22, no. 9, 2021, doi: 10.3390/ijms22094642.
- [18] E. Drula, "The carbohydrate-active enzyme database: Functions and literature," *Nucleic Acids Res*, vol. 50, 2022, doi: 10.1093/nar/gkab1045.
- [19] C. H. Van Dyck, "Lecanemab in Early Alzheimer's Disease," *New England Journal of Medicine*, vol. 388, no. 1, pp. 9–21, 2023, doi: 10.1056/NEJMoa2212948.
- [20] C. Zhao, "Mechanisms of Plant Responses and Adaptation to Soil Salinity," *Innovation*, vol. 1, no. 1, 2020, doi: 10.1016/j.xinn.2020.100017.
- [21] R. L. Gottlieb, "Early Remdesivir to Prevent Progression to Severe Covid-19 in Outpatients," *New England Journal of Medicine*, vol. 386, no. 4, pp. 305–315, 2022, doi: 10.1056/NEJMoa2116846.
- [22] P. Lee, "Cellular adaptation to hypoxia through hypoxia inducible factors and beyond," *Nat Rev Mol Cell Biol*, vol. 21, no. 5, pp. 268–283, 2020, doi: 10.1038/s41580-020-0227-y.
- [23] Y. Wan, "Molecular mechanism for antibody-dependent enhancement of coronavirus entry," *J Virol*, vol. 94, no. 5, 2020, doi: 10.1128/JVI.02015-19.
- [24] T. Guo, "Cardiovascular Implications of Fatal Outcomes of Patients with Coronavirus Disease 2019 (COVID-19)," *JAMA Cardiol*, vol. 5, no. 7, pp. 811–818, 2020, doi: 10.1001/jamacardio.2020.1017.
- [25] A. M. Jastreboff, "Tirzepatide Once Weekly for the Treatment of Obesity," *New England Journal of Medicine*, vol. 387, no. 3, pp. 205–216, 2022, doi: 10.1056/NEJMoa2206038.
- [26] X. Xu, "Effective treatment of severe COVID-19 patients with tocilizumab," *Proc Natl Acad Sci U S A*, vol. 117, no. 20, pp. 10970–10975, 2020, doi: 10.1073/pnas.2005615117.
- [27] C. Shen, "Treatment of 5 Critically Ill Patients with COVID-19 with Convalescent Plasma," *JAMA - Journal of the American Medical Association*, vol. 323, no. 16, pp. 1582–1589, 2020, doi: 10.1001/jama.2020.4783.
- [28] J. Li, "Ferroptosis: past, present and future," *Cell Death Dis*, vol. 11, no. 2, 2020, doi: 10.1038/s41419-020-2298-2.
- [29] C. Magro, "Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: A report of five cases," *Translational Research*, vol. 220, pp. 1–13, 2020, doi: 10.1016/j.trsl.2020.04.007.
- [30] A. L. Cheng, "Updated efficacy and safety data from IMbrave150: Atezolizumab plus bevacizumab vs. sorafenib for unresectable hepatocellular carcinoma," *J Hepatol*, vol. 76, no. 4, pp. 862–873, 2022, doi: 10.1016/j.jhep.2021.11.030.