Ferritin and LDH as Predictors of Mortality in COVID-19 Infection, Bosnia and Herzegovina Single-center Study

Vahid Jusufovic
Univerzitetski Klinicki Centar Tuzla
Sekib Umihanic
Univerzitetski Klinicki Centar Tuzla
Ervin Alibegovic
Univerzitetski Klinicki Centar Tuzla
Dilista Piljic
Univerzitetski Klinicki Centar Tuzla
Amer Mujkanovic (amer.mujkanovic.18@ucl.ac.uk)
Univerzitetski Klinicki Centar Tuzla https://orcid.org/0000-0002-3915-0140
Asja Bijedic
Univerzitetski Klinicki Centar Tuzla
Emir Tulumovic
Univerzitetski Klinicki Centar Tuzla
Richard Stratton
Royal Free Hospital, UCL Division of Medicine

Research article

Keywords: COVID-19, ferritin, LDH, survival, inflammatory markers

DOI: https://doi.org/10.21203/rs.3.rs-143696/v1

Abstract

Background Since the beginning of the COVID-19 pandemic, several inflammatory markers have been investigated as possible predictors of survival. Ferritin and from recently LDH have emerged as a possible marker that could serve to this purpose, with different cut-off levels than standard. Furthermore, serum elevations of these markers were reported in other viral infections such as dengue fever; while it has not been reported in MERS and SARS outbreaks and possibly making a biochemical distinction between COVID-19 and other similar diseases. The aim of this research was to establish independent admission levels of ferritin and LDH that predict lethal outcome more accurately.

Methods In this prospective cohort study all the patients were hospitalized at UKC, B&H (n=137), between 28th of March and 1st of August 2020. The criteria for hospitalization was based on MEWS score, and all the inflammatory markers were evaluated in the first 72 hours of admission. Cut-off values of serum ferritin levels were set at 1500 ng/mL and LDH 350 U/L.

Results Patients with serum ferritin levels >1500 ng/mL had 7.304 OR (CI95% 1.956-27.277; p=0.003) higher for lethal outcome than the group with <1500 ng/mL. Also, patients with levels of LDH >350 U/I had 5.560 (CI95% 2.480-12.468; p<0.001) higher OR of lethal outcome than patients with LDH <350 U/L. With the significant statistical difference between group means for both ferritin and LDH (p<0.001).

Discussion Serum levels of ferritin >1500 ng/ml and LDH >350 U/L increase OR of lethal outcome. The levels of these inflammatory markers indicate the degree of inflammatory response and severity of the disaese as well as the possible outcome of the disease. Together these two markers could be used as predictors in clinical settings and treatment planning of patients with COVID-19.

Conclusion: Together these two markers could be used as predictors in clinical settings and treatment planning of patients with COVID-19.

Background

In December 2019, a novel β-coronavirus emerged in Wuhan, China that caused pneumonia- and flu-like illness. This virus was later named as the severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV2) and the diseases Coronavirus Disease 2019 - COVID-19. The reported mortality rate ranged from to 2.3% in China to 7.2% in reported in Italy. On August 1st, 2020, the total number of infected people globally passed 20 million, with more than 770 thousand deaths. On the same date, Bosnia and Herzegovina (B&H) reported 16.025 individuals with COVID-19, of which 571 people have deceased (1, 2). The first registered case of COVID-19 at Tuzla University Clinical Center, Northeastern Bosnia and Herzegovina was on the 28th of March 2020 (3).

The vast majority of individuals with COVID-19 (~ 80%) report having no or mild symptoms, whereas minor part develops more severe symptoms that demand hospital admission. Most common first symptoms in confirmed COVID-19 infected patients are cough, dyspnea, and fatigue, fever as well as

myalgia and headache; while gastrointestinal symptoms include nausea, vomiting, abdominal pain, and diarrhoea; some patients report loss of olfactory sensation as well (4).

From the start of the pandemic, several risk factors have that contributed to the development of a severe type of COVID-19 infection were reported such as old age, obesity, chronic kidney diseases, diabetes and black ethnicity among others (5). Nevertheless, the area that is still under research is what are the laboratory and inflammatory markers that could be used in the assessment of the severity of COVID-19 infection and outcome and differentiate COVID-19 from other viral infections.

So far it has been reported that several laboratory markers are deranged in COVID-19 such as d-dimer, lactate dehydrogenase (LDH), interleukin-6 (IL-6), C-reactive protein (CRP), and ferritin (6). These derangements are also seen in other infections, with certain variations, with potential to provide insight into the disease severity as well as in predicting the outcome. Recent studied have investigated the impact of biomarkers that are used for identification of hyperinflammation syndrome (HI-S) in COVID-19 such as ferritin and CRP. It has been shown that fatal outcome from COVID-19 infection closely associated with HI-S development, suggesting that disease severity is associated with increase in specific inflammatory markers (7). However, other markers of inflammation that are more systemic such as LDH and AST have not been yet extensively researched and used in predictive models. Furthermore, cut-off values have been set on the basis higher than normal rather than establishing a new level that could be more accurate in predicting survival or eventual diseases progress.

Ferritin

The exact origin of elevated serum ferritin seen in infectious diseases (hyperferritinemia) is still matter of debate; macrophage activation syndrome and de nuovo biosynthesis of ferritin could explain these elevated levels, also there is evidence that ferritin is released by necrosis or cell damage and subsequent leakage. Probably both pathways are responsible for the elevated levels of ferritin in inflammatory states and this issue needs further research (8). The role of increased ferritin is another matter of debate; its primary role in a healthy organism is in iron storage. At the same time, in inflammation, it seemingly has a role as a mediator between various cells involved in the immune response. Also, it may prevent over extensive damage to the tissue by free radicals and prevent iron being utilized by the pathogen (9).

In other coronavirus outbreaks in the last twenty years (SARS, MERS) the levels of ferritin have not been reported as a predictor or significant marker of inflammation (10, 11). On the other side, in dengue fever, Ebola and Crimean-Congo hemorrhagic fever, elevated levels of serum ferritin have been observed and reported. Interestingly, patients with influenza virus B infection have a low correlation with serum elevation of ferritin (8).

Recently it has been reported that part of the patients with COVID-19 develop hyperinflammatory syndrome (HI-S) which is associated with increased mortality and morbidity. One of the inflammatory markers that are necessary in order to diagnose HI-S in COVID-19 infection is ferritin, and the cut-off level

of ferritin for diagnosis is set at 1500 ng/mL and higher (12). Additionally, one study reported that in patients who died from COVID-19 infection, ferritin levels were higher upon hospital admission and during the hospital stay; with median values of serum ferritin levels after day 16 of hospitalization surpassing the upper limit of detection, indicating that ferritin levels were elevated throughout hospitalization (13). Patients, in one study, with a severe form of COVID-19 reported elevated levels of serum ferritin when compared to patients in the non-severe disease group. Therefore, it was assumed that serum ferritin levels were closely linked to the severity of COVID-19 infection (14). Lastly, laboratory findings in patients with severe COVID-19 showed data compatible with the development of cytokine storm with raised inflammatory markers, among them elevated serum ferritin levels. (15). This may strengthen the argument that hyperferritinemia is linked specifically with inflammation in SARS-CoV-2 infection, and consequently, ferritin could be a useful parameter to predict disease severity (9).

LDH

Increased serum levels of LDH have been associated with more severe outcome in other virus infections, such as in acute respiratory distress, viral community-acquired pneumonia, and dengue fever (where levels closer to 1000 U/J were correlated with development of a more severe form of the diseases). These findings are consistent with the current medical understanding that high LDH levels are associated with tissue breakdown occurring in various infectious diseases. (16, 17) In previous studies on patients who had been affected in previous coronavirus outbreaks (SARS and MERS), elevated levels of LDH were reported (18). Furthermore, LDH is present in lung tissue and its is one of the primarily affected sites in COVID-19, hence elevated levels of LDH could be the result of lung tissue breakdown (19, 20). Pooled statistical analysis of the impact of increased levels of LDH on severity and outcome in COVID-19 infection have shown an increase of 6-fold in odds of severe COVID-19 disease and a 16-fold increase in a lethal outcome. Elevated levels of LDH were found > 95% In patients with lethal outcome, while < 60% in the survivor group (21). However, the levels of LDH indicative for increased odds of morbidity and mortality have not been thoroughly researched in COVID-19 patients.

This study aims to establish levels of serum ferritin and LDH upon admission and used them as predictors of COVID-19 mortality as well as try to establish independent cut-off levels of ferritin and LDH that have the most significant impact on the survival of COVID-19 patients in Bosnia and Herzegovina (B&H). This is the first study of its kind in B&H that has analyzed these inflammatory markers and tried to make a prognostic model of mortality for COVID-19 patients based serum levels of ferritin and LDH upon admission to the hospital. It is the first study of its kind that has been conducted in B&H and provides information as well as an update on the nature of COVID-19 pandemic in this part of Europe and adds to the body of knowledge regarding COVID-19 that could facilitate further research with providing relevant data.

Methods

In this prospective observational cohort study, 137 patients were observed from the 28th of March to the 1st of August 2020 at Tuzla University Clinical Center, B&H (UKC), all of whom were Caucasian. These patients tested positive for COVID-19 using RT-qPCR, and their clinical condition reacquired hospital admission based on MEWS score. The levels of urea, LDH, ferritin, CRP, full blood count, troponin levels, and d-dimer were analyzed in the first 72 hours of admission and were included in the research. The statistical analysis was performed using SPSS v.25 (IBM Corp., Armonk, NY, US); the difference between group means was established using MANOVA test and Tukey posthoc analyses, while the odds ratio (OR) of lethal outcome was conducted using binary logistic regression. All the values lower than $p<0.05$ were considered significant.

Results

There was a total of 137 patients of which 61.8% (n = 84) were male and 38.2% (n = 52) were female (Fig. 1a). The mean length of hospitalization was 7.76 ± SD 5.029. The average age of patients was 62.34 ± 16.33, with a minimum of 20 and the oldest being 94 years of age (Fig. 1b). In total 32.1% (n = 44) had no known comorbidities; 28.5% (n = 39) had one known comorbidity while two or more in 39.4% (n = 54) of patients.

The overall mean level of ferritin was 636.45 ± 863.11 ng/mL; the group of patients (n = 115) with ferritin levels lower than 1500 ng/mL had a mean ferritin level of 554.95 ± 381.26 ng/mL, with 65 patients discharged from hospital ; on the other side, the group with values of ferritin higher than 1500 ng/mL (n = 25) had a mean of 2301.19 ± 1161.59 ng/mL (MANOVA test $p < 0.001$); with 3 out of 25 patients had a positive outcome and were discharged from hospital. Patient with levels of ferritin > 1500 ng/mL had 7.304 (CI95% 1.956–27.277; p = 0.003) higher OR of the fatal outcome when compared to those with levels of ferritin < 1500 ng/mL. Patient with higher levels of LDH > 350 U/L had OR 5.560 (CI95% 2.480-12.468; $p < 0.001$) than those with LDH lower than 350 U/L (the difference between groups $p < 0.001$, MANOVA test) (table 2.) Hosmer-Lemeshow goodness-of-fit $X^2 = 1.255$; p = 0.534. Mortality in the group with LDH levels < 350 U/L was 32.1% and in the group with LDH > 350 U/L at 74.6% (Fig. 2). Whereas, in the group with ferritin < 1500 ng/mL 42% and 88% in the group of patients with serum ferritin > 1500 ng/mL.

Discussion

Finding biochemical markers that would enable the creation of predicting models in patients inflicted with COVID-19 has been elusive since the start of the pandemic. The role of CRP and other inflammatory markers has not been effective as in other infectious diseases since the knowledge of pathogenesis and mechanism by which COVID-19 operates have been limited. Emphasis on the level of cytokine storm and development of HI-S in severe forms of COVID-19 has been established as one of the hallmarks (12)

Ferritin's primary function is as a serum iron depo; however, during inflammation, it has a potential role in modulating the inflammatory response and could be intermediator between different cells involved in the

process. Another possible role is prevention the utilization of iron by the microorganism (9). Elevation of serum ferritin has been found in other infectious diseases such as Ebola, hemorrhagic fever and dengue fever but have not been reported in other coronavirus caused illness SARS and MERS. The level of ferritin has been found to be correlated with macrophage activation syndrome, but the origin of the serum ferritin remains controversial, is it produced in the cells or it's being leaked through the damaged or dead cells (8). Due to the fact, HI-S is characterized by levels of ferritin > 1500 μg/L; this study used that cut-off value of ferritin. Patients with ferritin > 1500 ng/mL have shown to have 7.304 (CI95% 1.956–27.277; p = 0.003) higher OR of lethal outcome than the group with ferritin < 1500 ng/mL. Also, the difference in the means of ferritin between these groups has proven a statistically significant result ($p < 0.05$). This increase in OR demonstrates that the severity of the illness stimulates greater elevation of ferritin while more moderate forms of the disease do not stimulate to the same extent. Furthermore, the levels of ferritin could be used as a guide to establishing the extent of the infection in a patient without any serious or critical manifestation of the disease. These findings put COVID-19 infection in the group with Ebola and dengue fever but not in the same group with other coronavirus outbreaks such as MERS and SARS; also, the lower association between hyperferritinemia and influenza B infection could be useful toll in upcoming flue season when the distinction between influenza infection and COVID-19 infection is needed (8). Higher OR in the group of patients with ferritin > 1500 ng/mL gives insight into the outcome and enable better planning of patient care.

On the other hand, LDH had been shown to be a more systemic marker of the inflammatory response, the nature of LDH and its ubiquitarian presence does not distinguish it as a suitable candidate as a model predictor (17). However, the nature of COVID-19 infection has proven that even though the primary site of infection could be the lungs or GIT other symptoms of COVID-19 infection such as loss of smell, headache, as well as myalgia and bone pain point out that the virus has its pathological effect on other organs. Increased levels of LDH could be the consequence of more systemic response and virus affliction and point to the general inflammatory response (16) In our study the level of LDH has been set at 350 U/L as a cut off value, while other research has found the levels of 359 U/L to be more significant for prediction of mortality. In our study cohort of patients that had LDH > 350 U/L had shown to have 5.560 (CI95% 2.480-12.468; $p < 0.001$) higher OR for the lethal outcome than the group of patients with LDH < 350 U/L; the difference between groups mean levels of LDH was statistically significant ($p = 0.000$). The increased levels of LDH have been reported in earlier studies on MERS and SARS, as well as dengue fever (17, 18). The levels of LDH > 350 U/L could be used together with levels of ferritin in assessing the severity of COVID-19 patients upon admission and as a predictor of survival.

The combination of LDH and Ferritin in predicting the outcome of COVID-19 could be used in prognostic models as well as a toll in the initial clinical assessment of patients. The admission levels of these two biomarkers, which could become standard practice in initial laboratory work for COVID-19 patients, provide insight in the prognosis of the patient and triage the patients into ICU or high dependency unit regardless of their clinical condition at the moment of presentation to the hospital. Small elevations of LDH and ferritin did not provide significant results when analyzed; however, depending on the point in time of the infections, these markers could increase to a higher level. Therefore, more dynamic

assessment and continues monitoring of these markers based on the time scale from the beginning of the infection could provide signs of deteriorating patients and possible complications of infection.

The combination of LDH and ferritin in predicting the outcome of COVID-19 could be used in prognostic models as well as a toll in the initial clinical assessment of patients. The admission levels of these two inflammatory markers, which could become standard practice in initial laboratory work for COVID-19 patients, provide insight in the prognosis of the patient and triage the patients into ICU or high dependency unit regardless of their clinical condition at the moment of presentation to the hospital. Small elevations of LDH and ferritin did not provide significant results when analyzed; however, depending on the point in time of the infections, these markers could increase to a higher level further down the period of hospitalization. Therefore, more dynamic assessment and continues monitoring of these markers based on the point in time of the infection could provide signs of deteriorating patients and possible complications of the disease.

This research, however, does have a specific limitation, the size of the survival group was limited, and in the future studies, a larger sample could be studied and compared. Moreover, in order to have full prognostic model, more factors should be taken into account. Clinical manifestation, as well as imaging modalities, could be used in order to produce a more comprehensive and suitable model of predicting the outcome for a patient with COVID-19 infection. This represents the first analysis of inflammatory markers in B&H of patients with COVID-19 and creation of prognostic models that could enable better clinical evaluation of severity among this group of patients with the intent to provide improved triage and planning of care.

Conclusion

Usage of LDH > 350 U/L and ferritin > 1500 ng/mL and the serum values in the initial clinical assessment of COVID-19 patients could provide deeper insight into diseases severity and predict the possibility of a lethal outcome.

Abbreviations

Acute respiratory syndrome-related coronavirus 2 - SARS-CoV2

Bosnia and Herzegovina – B&H

Coronavirus Disease 2019 - COVID-19

Lactate dehydrogenase - LDH

Interleukin-6 - IL-6

C-reactive protein – CRP

Hyperinflammation syndrome - HI-S

Tuzla University Clinical Center – UKC

Declarations

Acknowledgements

Not applicable

Competing Interest Statement

The authors have declared no competing interest

Funding Statement

This study was retrospective and was based on medical records; no funding was needed

Ethics approval and consent to participate

All relevant ethical guidelines have been followed; approval for data was obtained from ethical committee of Tuzla University Clinical Center. The consent from the patietns was verbal, while medical records were obtained by authorization of ethical committee of Tuzla University Clinical Center

Consent for publication

Not applicable

Availability of data and materials

All the data will be available upon request and will be uploaded online on a later date

Authors contribution

All authors contributed equally to this research since the entire project was led by COVID-19 Research Group Tuzla University Clinical Center and should be considered as group authorship.

References

1. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis. 2020.
2. BiH ZzjzF. COVID-19 2020 [Available from: https://covid-19.ba.
3. A T. COVID-19 in Northeast Bosnia and Herzegovina and Patient's Length of Hospitalization. BMC Infect Dis. 2020.
4. Tjendra Y, Al Mana AF, Espejo AP, Akgun Y, Millan NC, Gomez-Fernandez C, et al. Predicting Disease Severity and Outcome in COVID-19 Patients: A Review of Multiple Biomarkers. Arch Pathol Lab Med. 2020.
5. de Lusignan S, Dorward J, Correa A, Jones N, Akinyemi O, Amirthalingam G, et al. Risk factors for SARS-CoV-2 among patients in the Oxford Royal College of General Practitioners Research and Surveillance Centre primary care network: a cross-sectional study. The Lancet Infectious Diseases. 2020;20(9):1034-42.
6. Gandhi RT, Lynch JB, Del Rio C. Mild or Moderate Covid-19. N Engl J Med. 2020.
7. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506.
8. Kappert K, Jahic A, Tauber R. Assessment of serum ferritin as a biomarker in COVID-19: bystander or participant? Insights by comparison with other infectious and non-infectious diseases. Biomarkers. 2020:1-36.
9. Gomez-Pastora J, Weigand M, Kim J, Wu X, Strayer J, Palmer AF, et al. Hyperferritinemia in critically ill COVID-19 patients - Is ferritin the product of inflammation or a pathogenic mediator? Clin Chim Acta. 2020;509:249-51.
10. Drosten C, Seilmaier M, Corman VM, Hartmann W, Scheible G, Sack S, et al. Clinical features and virological analysis of a case of Middle East respiratory syndrome coronavirus infection. Lancet Infect Dis. 2013;13(9):745-51.
11. Liu CL, Lu YT, Peng MJ, Chen PJ, Lin RL, Wu CL, et al. Clinical and laboratory features of severe acute respiratory syndrome vis-a-vis onset of fever. Chest. 2004;126(2):509-17.
12. Manson JJ, Crooks C, Naja M, Ledlie A, Goulden B, Liddle T, et al. COVID-19-associated hyperinflammation and escalation of patient care: a retrospective longitudinal cohort study. The Lancet Rheumatology.
13. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054-62.
14. Liu T, Zhang J, Yang Y, Ma H, Li Z, Zhang J, et al. The role of interleukin-6 in monitoring severe case of coronavirus disease 2019. EMBO Mol Med. 2020;12(7):e12421.
15. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395(10229):1033-4.

16. Yuki K, Fujiogi M, Koutsogiannaki S. COVID-19 pathophysiology: A review. Clin Immunol. 2020;215:108427.
17. Sirikutt P, Kalayanarooj S. Serum lactate and lactate dehydrogenase as parameters for the prediction of dengue severity. J Med Assoc Thai. 2014;97 Suppl 6:S220-31.
18. Assiri A, Al-Tawfiq JA, Al-Rabeeah AA, Al-Rabiah FA, Al-Hajjar S, Al-Barrak A, et al. Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. Lancet Infect Dis. 2013;13(9):752-61.
19. Chiang CH, Shih JF, Su WJ, Perng RP. Eight-month prospective study of 14 patients with hospital-acquired severe acute respiratory syndrome. Mayo Clin Proc. 2004;79(11):1372-9.
20. Tao RJ, Luo XL, Xu W, Mao B, Dai RX, Li CW, et al. Viral infection in community acquired pneumonia patients with fever: a prospective observational study. J Thorac Dis. 2018;10(7):4387-95.
21. Henry BM, Aggarwal G, Wong J, Benoit S, Vikse J, Plebani M, et al. Lactate dehydrogenase levels predict coronavirus disease 2019 (COVID-19) severity and mortality: A pooled analysis. Am J Emerg Med. 2020;38(9):1722-6.

Tables

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

Figures

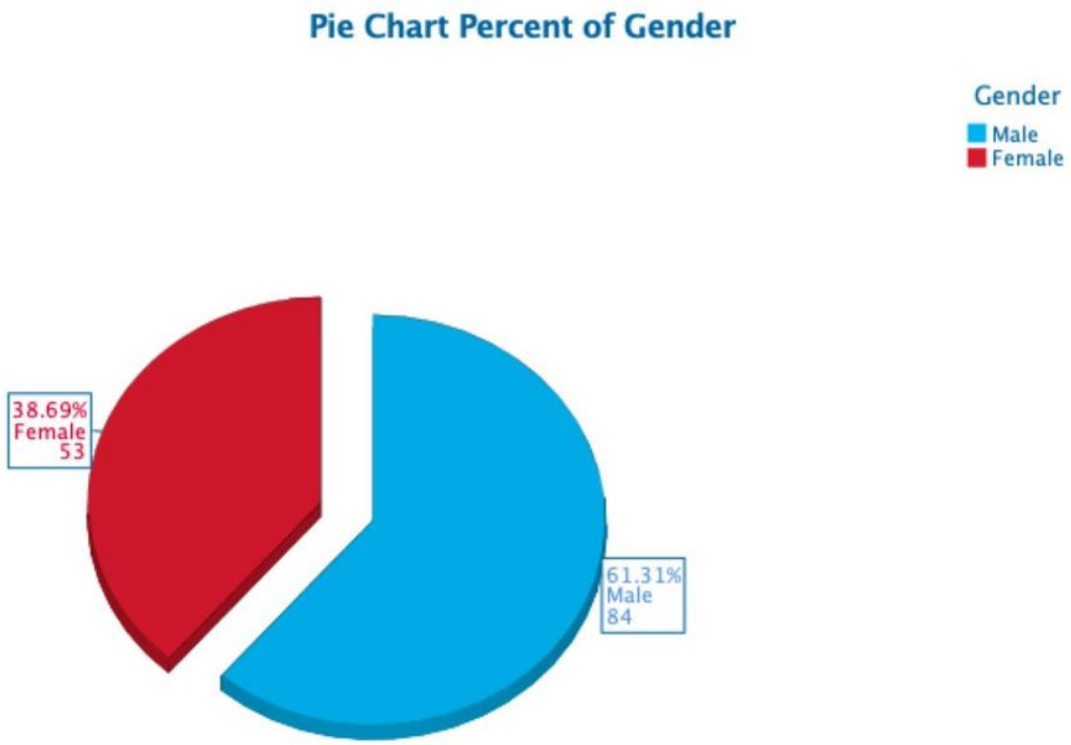

Figure 1

a The distribution between gender. There was 61.31% (n=84) males and 38.69% (n=53) of females.