

Serum Ferritin: As a Biomarker in Diagnosis of Dengue Fever

ZIA UL AIN SABIHA¹, HAFSA TARIQ², RABEEA ZIA³, K. SURESH BABU⁴, INAM-U-LLAH⁵, REHANA RASOOL⁶, SUDHAIR ABBAS BANGASH⁷, SAFDAR ALI⁸, MAHNOOR⁹, IRFAN ULLAH⁹

¹Department of Community Medicine, Peshawar Medical College, Peshawar. (Affiliated with Riphah International University, Islamabad. Pakistan)

²Department of Pathology, Pak International Medical College, Peshawar. haf.tariq@gmail.com

³Pakistan Kidney and Liver Hospital (PKLI), Lahore, Pakistan

⁴Professor, Department of Biochemistry, Symbiosis Medical College for Women, Symbiosis International (Deemed University), Pune

⁵Department of Food Science, The University of Haripur, KPK, Pakistan.

⁶Department of Community Medicine, Abbottabad International Medical College, Abbottabad.

⁷Faculty of Life Science, Department of Pharmacy, Sarhad University of Science and Information Technology, Peshawar.

⁸Department of Microbiology, Cholistan University of Veterinary And Animal Sciences, Bahawalpur, Punjab, Pakistan.

⁹Department of Life Sciences, School of Science, University of Management and Technology (UMT), Lahore, Pakistan

Corresponding authors: Irfan Ullah, Email: irfan_btn@hotmail.com

ABSTRACT

Background: Major public health risk was 'Dengue infection' initial discovery is critical to improving the existence in serious infection of dengue. Our objective in this study was to investigate the serum ferritin level to suppose biomarker in the diagnosis of dengue fever.

Method: An observational and prospective study was conducted in the Hayatabad Medical Complex and it included one hundred nineteen cases related to dengue was diagnosed by using NSI Test antigen or serology test related to specific dengue that interment "ELISA". At the time of diagnosis, serum ferritin was measured. Monitoring the clinical and platelets counts was done on daily bases, it also classified into two groups such as non-severe and severe groups by allowing WHO criteria in 2009.

Result: Five out of 119 cases of dengue were severe, dengue patients had lower platelets when they are in severe condition, count the number of platelets (p less than 0.0001); Ferritin level almost high ($p=0.02$) and the value of hospital longer time period (p less than 0.0001) mostly in severe group as compare to non-severe group of dengue. Platelets count had negative value with age factor almost Positive correlations with ferritin levels ($p=0.08$ or $r=0.16$) and a longer hospital stay ($r=-0.427$ and $p=0.0001$) were also found ($r=0.26$, $p=0.004$). These values indicate the severe risk of dengue infection/ disease. Negative value of serum ferritin that are related to the value of platelets ($r=0.51$ and $p<0.001$). On day 4 of clinical infections, an elevated value of ferritin is seen in severe cases.

Conclusion: In Clinical practice, a biomarker for determining the severity of dengue infection is increased serum ferritin concentrations.

Keywords: Biomarkers, Serum ferritin, Severity, Dengue infection, Predicators

INTRODUCTION

"Aedes Aegypti Mosquitoes" that act as a vector causes the highly infectious agent dengue virus. The symptoms of dengue such as Nausea, Vomiting, Abdominal pain, dengue hemorrhagic fever (DHF), Dengue septic shock (DSS), skin itching, and hepatosplenomegaly (1, 2). It is important to diagnose of dengue accurately and rapidly due to this disease's severity. There are many tests performed such as Lipid profile and serum ferritin test are used for diagnosis and prognosis of the disease (3, 4).

The high value of Serum ferritin (FT) as compared to any other bacterial or viral disease is shown in dengue fever. The level of FT is greater than 50 ng/ml known as Hyper-ferritinemia (5-7). Serum ferritin is expressed by the Reticulum endothelial system in the reaction of infection and an ideal prognostic marker "hyperferritinemia" for the severity of dengue infection (6-8). Iron is the main component for the proliferation of the pathogenic microorganism. Ferritin binds to iron and regulates its spreading into the circulatory system. Hence, the condition of hyper ferritinemia represent the high and active dengue infection, causing coagulation distress and increase the immunological performance of lymphocytes, neutrophils, and macrophages FT level is high and it indicates a high risk for emerging difficulties. This is also held by the reports that shown the high serum ferritin level with the severity of infection of dengue (3, 7-9). Immunopathogenesis of Dengue causes to increase of cytokines, Antibodies that cause infection, inflammatory cells and finally cause death and organs in failure. During the life cycle, dengue infection changes the metabolism of lipids in the host cells. In critical patients, hyperlipidemia is an independent predictor of the harshness of infection. Dengue virus DNGV is a synthesis of viral organelles recognized as a replication complex RCs in the host endoplasmic reticulum. The replication complex is formed by lipids such as fatty acids, phospholipids, and cholesterol. With the help of RCs, increase the cholesterol level by utilizing the fatty acid synthase (FASN) and 3-hydroxy-3- methyl-glutaryl-CoA reductase (HMGGCR) by Non-structural protein 3 (NSP-3). DNV is involved

in lipid droplet degradation and cause the formation of triacylglycerol. During the viral infection, Low-density lipoprotein Receptor LDP_r is the ideal marker for the identification of the severity of disease and lipid metabolism but some studies are against this statement due to the absence of regularity in the association of DNGV infection and the level of the lipid (10, 11).

For the treatment of DNGV infection, absence of the antiviral therapy is a big challenge. Hence, it is very important to have a consistent prognostic marker to identify the severity of infection of DNGV and the management of diseases. In the acute phase, a study demanded that the high serum ferritin FT worked as severity of disease (hospitalization days) (3) because macrophages are the source of ferritin and it is a primary target in the dengue virus. It is reported that macrophages are actively involved in the severity of infection. The high level of ferritin indicates the hyper activation of the immune system and loss of coagulation. Therefore, in the present study is to investigate the relation between the serum ferritin and platelets with the severity of the disease and it could be used as a marker for the evolution of the serious disease.

METHODOLOGY

This is prospective and observational study was carried out in the Hayatabad Medical Complex, a tertiary care hospital in Pakistan between 2021(April) and 2022 (March). All patients who were analyzed with infection and they were hospitalized either by dengue antigen NSI or specific serology with positive dengue were included. From this study, chronic inflammatory disease patients, excess amount of iron, Thalassemia, sideroblastic anemia, malignancy, and diseases related to liver or kidney were excluded.

This research study based on a prospective and observational process for the collection of data on affected patients who were fulfilled the predetermined requirements. Diagnosis of disease was made by using a various selection of assays or Tests such as " J. Mitra Dengue NSI Ag Microlisa kit" and NSI Ag Test. When the initial stage of dengue fever was less than five days ago or Positive signs in serology test results, all are done by tests such

as ELISA, Dengue test IgG, and IgM, and Panbio dengue IgG, IgM. Serum ferritin was assessed if there had been less than seven days since when dengue fever first appears. After collecting data, approximately, 2ml of blood were drawn for this investigation, by using Electrochemiluminescence immunoassay- COBAS e411, ferritin assays were done at the diagnosis time or at the admission time if it started with positive results of dengue fever from an outside hospital. On the daily basis, clinical trials of the disease and count platelets were observed carefully and they were classified as having non-severe or severe group of infection by the criteria of WHO 2009, severe infection occurs when organ impairment, serious respiratory disorders, shocks, and bleedings occur. During the visit to the hospital, the Mean count of platelet was deliberate in equal groups since all values of platelet attained regularly. For the comparison of both subgroups by calculating the level of mean ferritin FT.

In the statistical analysis, Standard deviation (SD), and Mean were used as descriptive statistics. For the comparison of the count of platelets and level of serum ferritin among the groups of severe and non-severe dengue infection by using Mann- the Whitney U Test. Another test Spearman's Rank was used to determine factors such as patient age, duration in hospital, level of serum ferritin, and count of platelets. For the evaluation of all the data, SPSS version 21.0 was used. This software was used to determine the p-value which is less than 5 percent to be very important in statistically.

RESULTS

There were a total 119 subjects with 53 females (44%) and 66 males (56%) (Figure 1). The population's mean age in this research was 28.1±11.4 years. Approximately, the range of 20 to 25 years, 50% population involves in this study. The mean age among the females and males did not differ significantly. By the diagnosis with dengue infection in 61(51.27%) participants based on the presence of antigen NSI and the remaining 58(48.73%) by the presence of positive dengue serology test. Only 5(4.2%) of the 119 participants experienced by severe dengue infection and of those, 1(0.84%) was female and 4(3.36%) were males.

The population that stayed in the hospital for less than three days had lowest mean platelets values in males and females 40426.47×10⁹/L and 45000×10⁹/l; for 4days, 19(16%) and 6 (5%) stayed for five days. The mean value of hospital stay was 3.11 days. Around 6 (5%) of patients required more than 9days hospital stay. On day 03, evaluated serum ferritin level in 13 (10.92%) patients, 35 (29.41%) on day 5, 29 (24.4%) on day 6, 17 (14.28%) on day 4 and 20 (16.80%) subjects had their serum ferritin levels were examined on day 7 (Figure 2). Ferritin had a mean concentration of 1500 ng/ml and a median of 1856. Table 01 demonstrates that individuals who had experienced severe infection had lower median values for platelet counts. During the stay in the hospital, on the daily basis to count the platelets and recorded. The non-severe group value was 29000×10⁹/L and the severe group value was 9000×10⁹/L. The serum ferritin levels in the severe class of dengue infection were greater than those in the non-severe class, as shown in Table 01 as well. The level of serum ferritin between the groups of dengue infection was 3 days (2,3); 15 (10,21), 1500 ng/ml vs 2000ng/ml (2000,2000).

In this study, a negative correlation with count of platelets was seen in upper-age patients by Coefficient of Pearson (r= -0.427 and p is less than 0.0001). Positive correlation between the levels of ferritin (r=0.16, p=0.08) and hospital stay in older patients (r= 0.26, p=0.004). This association with a hospital stay was significant. The platelet count and serum ferritin FT levels had a negative connection, according to Pearson's coefficient (r=-0.51; p=0.001). For the days 3 to 7 of the illness with associated with both groups, to easily evaluate the level of serum ferritin and it was recorded in Table 02. It was seemed that in the severe group of infection shows high ferritin level and from day 4 low in non-severe group of infection.

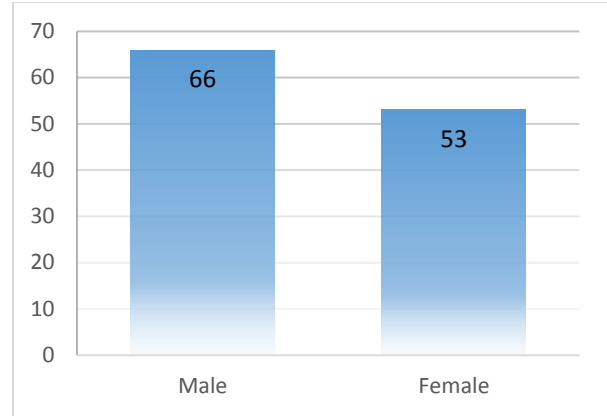


Figure 1: Shows the gender-wise distribution of the dengue patients

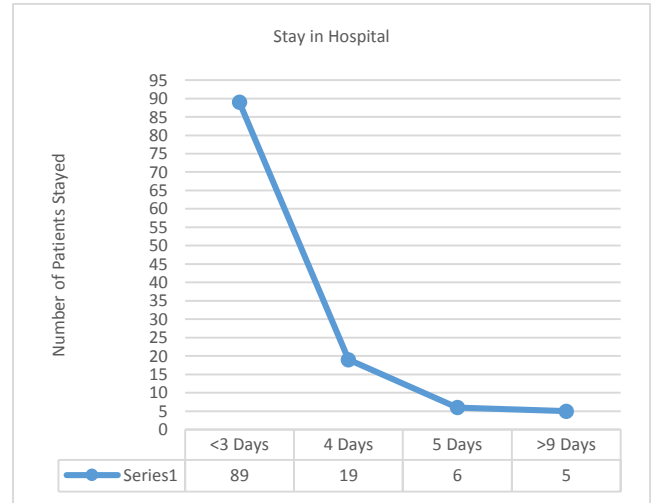


Figure 2: Shows the stay of dengue patients at the hospital.

Table 1: Shows detail of severe and non-severe dengue-infected individuals

	Non-Severe Dengue	Severe Dengue	P value
Platelets Count	29000 (15000-55000)	8500 (7000-9000)	0.001
Serum Ferritin	1500 (750-2000)	2000 (2000-2000)	0.02
Hospital Stay	3 (2,3)	15 (10, 21)	0.001

Table 2: Shows a comparison of severe and non-severe dengue patients regarding ferritin levels

Serum Ferritin Level	Non-severe Dengue	Severe Dengue	P value
3 days	n=13 1485 (1013, 2000)	-	-
4 days	n=17 1000±811 855 (240, 2000)	n=1, 2000±0, 2000 (2000, 2000)	0.4
5 days	n=35 1550±587 2000 (1180, 2000)	n=2, 2000±0, 2000 (2000, 2000)	0.3
6 days	n=29 1232±727 1317 (563, 2000)	n=1, 2000±0, 2000 (2000, 2000)	0.4
7 days	n=20 1514±722 1964 (1105, 2000)	n=1, 2000±0, 2000 (2000, 2000)	0.5

DISCUSSION

Serum Ferritin was monitored all over the study and found positively correlated with the severity of the infection. At that time, there is no proper medication and vaccine present for the dengue infection. The management support of infection of dengue-related

subjects is sympathetic care and careful checking for any difficulties (12, 13). Recovering of the patients approximately five to seven days from starting of the delirious illness while only very few percent move to serious infection of dengue, with 5% study quoting (14). During stage of convalescent, severe dengue often occurs and it shows mortality on the peak. It's hard to identify the form of severity in earlier even previously to appear the threatening marks by common diagnostics marker.

However, we can say that it is a very efficient and proper management process for the identification that would expand the results in infected patients. In acute phase reactants in which expression is increased are severe dengue infection are observed in patients (10). This process to indicate the infection in patients well with the warning signs and symptoms appears clinically. With the help of Reticulum endothelial cells, in initial stage ferritin reactant is formed in case of inflammation and infection. The level of ferritin is increased in these patients than in the other febrile illnesses person (12, 15). Two opposite functions seen in Hyperferritinemia, Early clinical illness phases, increased levels of Serum ferritin and it is used as a defending effect in the location of inflammation by using the free iron radicals which are the toxic chelating agent (16). In case of severe, for the activating of immune cells in cytokine storm to enhance the value of ferritin may use as a role of pathogenic (10). In the present study population 56% were males which were related to the other researches. The mean value of hospital stay was alike to the other studies. In the hospital stay the value of mean was 2.73 ± 1.41 days noted by Ahmad et al and Rawalpindi 3.7 ± 1.02 days in a study conducted (17, 18). This result shows that severe dengue infections are related to hyper-ferritinemia which is also mentioned in other studies (10, 15, 18). In the course of illness, to analyze the value of FT can be predicted earlier, comparison between the level of serum ferritin in both groups on a daily basis; it is shown that FT level was higher in the severe group, not seen in the non-severe group and it was observed from the day 4 of the illness to ahead which determine the FT is higher in the initial step of the dengue infection. During the dengue outbreak, a study was conducted in area (an island in Caribbean Aruba) wherein the level of ferritin in those having additional infectious diseases in addition to dengue and the result that the Ferritin is very useful medical marker for the distinguishing dengue infection from other viral infections; levels of serum ferritin are greater than 1500ng/ml that is linked with severe infections, to measure the serum ferritin on day 4 to 5 was better for predicting the result of dengue infection and the results show that hyperferritinemia patients should be monitored for their complications (15). It is shown that in all severe cases, hyperferritinemia is almost 2000ng/ml and day 4 of the clinical disease is when the Serum ferritin level is increased when it was in non-severe cases. One or more study is arranged in 1077 children of Thai which is used to measure the level of FT during the course of clinical such as febrile, recovery stage, and toxic effect, which showed similarity with these results (11). The higher level of Serum ferritin is related to severe dengue, non-severe dengue infection patients had a 1200ng/ml level of serum ferritin during the whole time of illness, during the febrile stage and the recovery stage, high level of FT was noted. Dengue hemorrhagic fever DHF grades 3 and 4 patients had high levels of ferritin significantly measured on days 5, 6, and 7 of infection than those grade 1, 2 DHF and dengue fever. It was concluded that serum ferritin level is greater than 1200ng/ml from day 4 of illness indicates the fever was present. Similarly, a study conducted which included 48 cases of dengue infection in South India by Soundravally R et al and 48 cases as a controls use for other febrile illness, admission day 4 the level of serum ferritin was observed after the starting of fever and recovery stage (which is median of 4 days after admission day) (10, 14). Compared with the other flushed illnesses, cases of dengue confirmed that the high levels of serum ferritin, severe group revealed the high level of FT throughout the recovery and febrile stages as compare to non-severe group (1264.71 ± 492.59 in severe and in non-severe group is 940.09 ± 568.31), On admission

day (418.19 ± 404.59 vs. 1490.74 ± 359.40). The research result that high level of ferritin could indicate the dengue severity with specificity and sympathy of 83.3% and 76.9% respectively. In this latest study showed comparable results; in severe cases, from illness day 4 to 7 hyperferritinemia was observed. In other study that related to serum ferritin and the infection development on that day of admission (17). Throughout the total of 104 cases of dengue, on the day of admission ferritin serum level was measured and the normal level of ferritin in group A about up to 100 µg/dl and the high level of FT in group B, both groups were followed by the presence of dengue fever.. Other important opinion in this study was conducted that association with age factor had given positive result with the correlated to FT levels and the hospital stay duration; on the other hand, it gives negative response with the platelets counts. This statement shows that the factor of age is very important and it is also considered to predict the severity of dengue infection. As increasing age, the severity of the disease raised with less platelet count, high levels of ferritin, and a lengthy period of time stay in hospital. These observations had shown that developing the severe disease at high risk mostly in adults. During the outbreak with dengue cases about 5336, a study conducted in Southern Taiwan, the hemorrhagic dengue fever cases and syndrome related to dengue shocks is higher in upper-age people as compare to young ones (19). Similar in a Singapore study, 60 aged person had more rate in severe form of dengue (20.3 percent vs. 14.6 percent, $p=0.006$) as compare to younger (20). At the diagnosis time, measurement of serum ferritin at the same time in this study whereas in most of the previous studies, at multiple time down with the serum ferritin during numerous clinical illness stages (10, 15). The present study result that measure the serum ferritin at the diagnosis time (if it referred from outside) within seven days from initiate the fever is linked with the conclusions of the previous studies. It would be simplest way for the measurement of the serum ferritin in a single day in a huge population clinically. Few limitations are also present in this present study. The first one is in this study, a small number of dengue fever was observed; hence we were unable to originate levels of ferritin and also predict the severity of dengue fever. At a definite conclusion, a sample size in high number in severe cases would be appropriate. Second, the limit of serum ferritin was measured and due to limitations of laboratory FT was "2000ng/ml"; so that high values could not be experimental which if present can allow comprehending to its correlation with thrombocytopenia severity and more accuracy in severity in clinically. Serotypes of dengue were not taken into consideration; instead, the secondary or primary type of infection was considered. Future research should focus on finding the serum ferritin cutoff value that can be used to gauge the severity of dengue at the outset of the disease.

CONCLUSION

This research shows that levels of serum ferritin high during the febrile stage of the illness (3rd to 7th days of illness) and also indicate the dengue infection severity. Moreover, besides serum ferritin, platelets count was also fluctuated during the dengue infection. So, it is then concluded that platelets counts also serve as biomarker for dengue severity as it was also supported from previous literature.

REFERENCES

1. Organization WH. Working to overcome the global impact of neglected tropical diseases: first WHO report on neglected tropical diseases: World Health Organization; 2010.
2. Patro PK, Behera AK, Sahu AN, Murmu AR. Prognostic significance of hyperferritinemia in dengue: A tertiary health care centre study.
3. Organization WH. Global strategy for dengue prevention and control 2012-2020. 2012.
4. Tuiskunen Bäck A, Lundkvist ÅJle, epidemiology. Dengue viruses—an overview. 2013;3(1):19839.
5. Pathak VK, Mohan MJofm, care p. A notorious vector-borne disease: Dengue fever, its evolution as public health threat. 2019;8(10):3125.

6. LOS ENFERMEROS SDDP. MEANINGS ASSIGNED TO DENGUE BY PRIMARY HEALTH CARE NURSES.
7. Zhang H, Zhou Y, Peng H, Zhang X, Zhou F, Liu Z, et al. Predictive symptoms and signs of severe dengue disease for patients with dengue fever: a meta-analysis. 2014;2014.
8. Martina BE, Koraka P, Osterhaus ADJCMr. Dengue virus pathogenesis: an integrated view. 2009;22(4):564-81.
9. John DV, Lin Y-S, Perng GCJJobs. Biomarkers of severe dengue disease—a review. 2015;22(1):1-7.
10. Soundravally R, Agieshkumar B, Daisy M, Sherin J, Cleetus CJL. Ferritin levels predict severe dengue. 2015;43(1):13-9.
11. Chaiyaratana W, Chuansumrit A, Atamasirikul K, Tangnararatchakit KJSAJTMPH. Serum ferritin levels in children with dengue infection. 2008;39(5):832-6.
12. Md-Sani SS, Md-Noor J, Han W-H, Gan S-P, Rani N-S, Tan H-L, et al. Prediction of mortality in severe dengue cases. 2018;18(1):1-9.
13. Biswas A, Pangtey G, Devgan V, Singla P, Murthy P, Dhariwal A, et al. Indian national guidelines for clinical management of dengue fever. 2015;113(12).
14. Alexander N, Balmaseda A, Coelho IC, Dimaano E, Hien TT, Hung NT, et al. Multicentre prospective study on dengue classification in four South-east Asian and three Latin American countries. 2011;16(8):936-48.
15. van de Weg CA, Huits RM, Pannuti CS, Brouns RM, van den Berg RW, van den Ham H-J, et al. Hyperferritinaemia in dengue virus infected patients is associated with immune activation and coagulation disturbances. 2014;8(10):e3214.
16. Torti FM, Torti SVJB. Regulation of ferritin genes and protein. 2002;99(10):3505-16.
17. Ahmed A, Alvi AH, Butt A, Nawaz AA, Hanif AJJCPSP. Assessment of dengue fever severity through liver function tests. 2014;24(9):640-4.
18. Nadeem M, Shafiq MM, Ashfaq MW, Baqai HZ, Ahmed SIJJoIMC. Use of Alanine Aminotransferases Level and Platelet Count to Predict Dengue Fever Severity. 2017;12(3):139-43.
19. Liu CC, Huang KJ, Huang MC, Lin J, Wang S, Liu JJ, et al. High case-fatality rate of adults with dengue hemorrhagic fever during an outbreak in non-endemic Taiwan: risk factors for dengue-infected elders. 2008;4(1):10-7.
20. Diseases PNTDSJPNT. Correction: Challenges in Dengue Fever in the Elderly: Atypical Presentation and Risk of Severe Dengue and Hospita-Acquired Infection. 2014;8(4):e2886.