Tropical Biomedicine 35(4): 1115–1122 (2018)

Montelukast Reduces the Risk of Dengue Shock Syndrome in Dengue Patients

Ahmad, A.[1], Waseem, T.[2], Butt, N.F.[2], Randhawa, F.A.[2], Malik, U.[2] and Shakoori, T.A.[3*]
[1]Department of Medicine, Allama Iqbal Medical College, Jinnah Hospital, Lahore, Pakistan
[2]Department of Medicine, Mayo Hospital, King Edward Medical University, Lahore, Pakistan
[3]Department of Biomedical Sciences, King Edward Medical University, Lahore, Pakistan.
[*]Corresponding author e-mail: drtaniashakoori@kemu.edu.pk; drtaniashakoori@yahoo.com
Received 20 November 2017; received in revised form 25 April 2018; accepted 4 May 2018

Abstract. A significant percentage of dengue patients develop Dengue Shock Syndrome (DSS) which is characterized by increased vascular permeability, circulatory failure and often death. Montelukast, a cysteinyl leukotriene receptor antagonist regulates vascular permeability and we hypothesized that it may be effective in protecting against DSS. An open label, parallel, randomized controlled trial (RCT) was thus carried out at Mayo Hospital, Department of Medicine, Lahore. A total of 200 patients of dengue fever were recruited and randomized into two groups. The group A was treated with Montelukast 10 mg once daily for 5 days along with general supportive treatment. Group B received the standard supportive treatment and served as the control group. The frequency of DSS was compared in the two groups by Chi square test. A binary logistic regression analysis was conducted to assess the effects of montelukast treatment on onset of DSS after adjusting for gender, age, white cell count, platelet count, haematocrit, serum alanine transaminase (ALT) and aspartate transaminase (AST). Relative risk (RR), absolute risk reduction (ARR), relative risk reduction (RRR) and numbers needed to treat (NNT) were calculated. Significance level was set at $p<0.05$. We found that only 9% of the patients in treatment group developed DSS compared to 31% patients in group B ($p<0.001$). The protective effect of montelukast treatment persisted ($p>0.001$, Odds ratio=5.01, 95% CI=2.17-11.60) even after adjusting for confounders. Montelukast reduced the absolute risk (ARR=22%) and the relative risk (RRR=71%) of DSS in dengue fever. Numbers needed to treat were 4.55. We thus conclude that treatment with oral montelukast may protect patients of dengue fever from DSS and greatly reduce mortality.

INTRODUCTION

Dengue virus is a flavivirus transmitted by the mosquito (Diptera: Culicidae) vectors *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse)(Sarwar, 2014). Each year millions of people get infected with dengue virus and some of them develop potentially serious state of illness, such as Dengue Shock Syndrome (DSS) and Dengue Haemorrhagic Fever (DHF). Both DHF and DSS are characterized by an increased vascular permeability that may lead to haemorrhage within internal organs and leakage of plasma into the tissues. Circulatory failure and death can also occur in severe cases (Halstead, 2007). Approximately, 5-30% of patients diagnosed with dengue fever may develop DSS and the mortality in DSS is fifty times higher than the dengue patients who don't develop DSS (St John, Rathore, Raghavan, Ng & Abraham, 2013).

The first outbreak of dengue fever in Pakistan happened in 1994. Since then dengue virus has become endemic to Pakistan, causing infections throughout the year, particularly since 2005. It is associated with a seasonal peak, mainly in the post-monsoon period, i.e. July – November (Furuta, Murao, Lan, Huy, Huong, Thuy, Tham, Nga, Ha & Ohmoto, 2012).

Mast cells are well-known for their role as regulators of vascular integrity, tone and function. They line blood vessels and produce many vasoactive mediators that induce vascular permeability. Some of these are pre-stored and can act nearly instantaneously on vascular endothelium, including tumour necrosis factor (TNF), proteases and heparin (Abraham & John, 2010; Kunder, St John & Abraham, 2011). Other de novo synthesized vasoactive factors include leukotriene's, prostaglandins, vascular endothelial growth factors or VEGF and TNF. Activation of mast cells derived factors promotes the breakdown of junctions that are present between endothelial cells. This leads to leakage of plasma resulting in oedema inside of tissues (Kunder *et al.*, 2011). High levels of vasoactive factors produced by mast cells have been associated with severe disease in dengue infection (Vitaranal, de Silva, Withanat & Gunasekerat, 1991).

Montelukast is a cysteinyl leukotriene receptor antagonist (Tintinger, Feldman, Theron & Anderson, 2010) traditionally used in the treatment of asthma(Spector & Tan, 2004). Leukotrienes are important mediators in the vascular leakage produced due to mast cell activation in dengue virus infection. Blocking the leukotriene's receptors with montelukast may help to prevent the vascular leakage and frequency of DSS (St John *et al.*, 2013). A recent study showed that administration of montelukast prevented vascular leakage in an animal model of dengue (Vitaranal *et al.*, 1991). The current study seeks to further investigate the role montelukast as an effective drug which might prevent occurrence of DSS in dengue patients in a randomized control trial study design.

MATERIALS AND METHODS

After obtaining approval from Institutional Review Board, King Edward Medical University, Lahore, an open label, parallel, randomized controlled trial (RCT) was carried out in the Dengue Wards of Department of Medicine, Mayo Hospital, Lahore, from August 2014 to February 2015. The trial is registered at www.researchregistry.com with unique id number 'researchregistry3251'. A total of 200 patients of dengue fever were recruited by non-probability convenience sampling in the study and informed consent was taken. The study protocol is shown in Figure 1. The patients included were 13-65 years old, of either gender, who were diagnosed on the basis of WHO Dengue Guidelines ("Comprehensive Guidelines for Prevention and Control of Dengue and Dengue Haemorrhagic Fever: Revised and Expanded Edition."). In accordance with these guidelines, patients were diagnosed with dengue fever (DF) if they had high grade fever (>100F^{o}) for at least 3 days with two or more of the following symptoms; rash, headache, arthralgia, myalgia, retro orbital pain, haemorrhagic manifestations, leukopenia (<4000/mm^3) and thrombocytopenia (<100,000/mm^3). The diagnosis was confirmed serologically by measuring serum levels of IgM or NS-1 using enzyme-linked immunosorbent assay (ELISA) kits. Dengue haemorrhagic fever (DHF) was labelled if DF patients had platelet counts $\leq$100 ×109/L, any evidence of bleeding and plasma leakage manifesting as either haematocrit change of $\geq$20%, fluid accumulation detected clinically or radio logically in the form of pleural effusion or ascites. DSS was declared when there were signs of circulatory failure in patients with DHF. These signs included weak and rapid pulse with narrow pulse pressure <20 mmHg, or hypotension for age.

Patients with ischemic heart disease, malignancy, co-morbid conditions like positive viral hepatitis serology, chronic liver disease, malaria and typhoid fever, chronic renal disease, history of bleeding disorders, pregnant women, and those admitted in a state of shock were excluded. The patients were randomized into two groups by lottery method. Patients in group A were treated with oral montelukast 10mg once daily for 5 days in addition to general supportive treatment (which comprised of analgesics, fluid replacement, and bed rest). Group B received the above mentioned standard supportive treatment alone and served as control. The primary outcome was onset of DSS. The patients were monitored for changes in

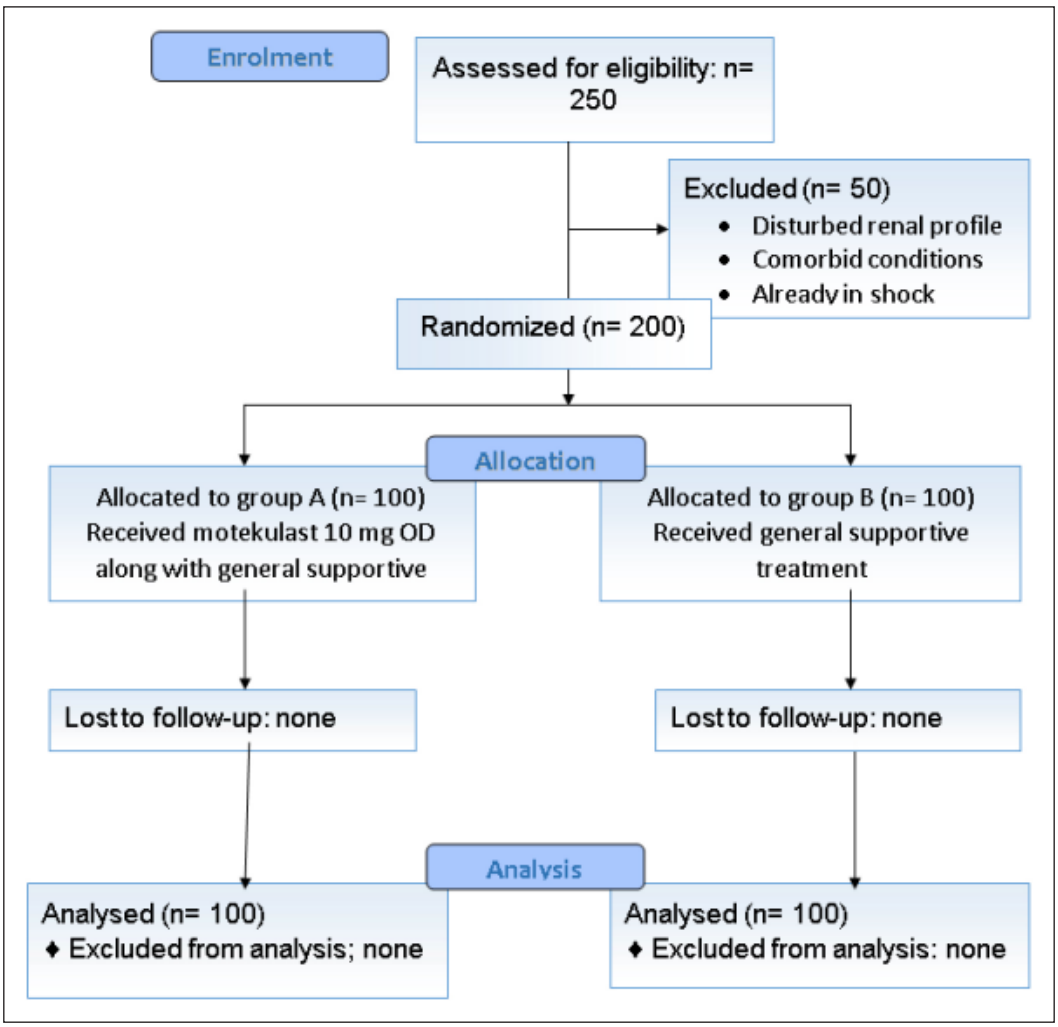

Figure 1. Flow Chart showing protocol of the randomized control trial.

platelets count, leucocytes count and haematocrit daily after admission. Serum aspartate transaminase (AST) and alanine transaminase (ALT) were measured on the day of patients' admission.

The data were collected and analysed using SPSS version 23. The quantitative variables such as age, serum AST and ALT were shown as mean ± standard deviation. On the other hand, the qualitative variables such as shock and gender from each group were shown as simple frequencies and percentages. A binary logistic regression analysis was carried out to assess the effects of montelukast treatment on onset of DSS after adjusting for gender, age, white cell count, platelet count, haematocrit, serum ALT and AST. Furthermore, relative risk (RR), absolute risk reduction (ARR), relative risk reduction (RRR), number needed to treat (NNT) and their respective 95% confidence intervals were calculated by standard formulas. Additionally, changes in blood white cell count, platelet count and haematocrit were investigated for significance by 'repeated measures ANOVA'. Spit plot ANOVA was used to determine if the serial measurements of the aforementioned variables differed significantly between treatment and control groups. Significance was set at level of $p<0.05$. The data were examined by intention to treat analysis.

RESULTS

The average age of the subjects was 27.81±13.58 years (range=13 to 65 years). The average age of patients in the treatment and control groups was 28.08±14.59 and 27.55±12.56 years, respectively. There were 133 male patients and 67 female patients in the study. In Group-A (montelukast treatment group), there were 68 male and 32 female patients, while in Group-B (control group) there were 65 male patients and 35 female patients. The two groups were matched for age (p=0.78) and gender (0.65). Laboratory findings in patients of dengue fever over 5 days in both groups are shown in Table 1.

A total of 40 patients out of 200 (20%) developed dengue shock syndrome (DSS). 9/100 (experimental event rate or EER) and 31/100 (control event rate or CER) patients developed DSS in Groups-A and B, respectively, at p<0.001 (Figure 2). Montelukast reduced the absolute and relative risk of DSS in treatment group substantially as shown in Table 2. Moreover, it was found that treatment of 5 (4.55) patients with montelukast rather than general supportive treatment alone would result in one less patient experiencing DSS (NNT= 4.55).

In addition, results of logistic regression revealed that the protective effect of montelukast treatment persisted (p>0.001, Odds ratio=5.01, 95% CI=2.17-11.60) even after adjusting for gender (p=0.03), age (p=0.10), white cell count (p=0.18), platelet count (p=0.50), haematocrit (p=0.40), serum ALT (p=0.20) and AST (p=0.58). This is shown in Table 3. Interestingly, it was observed that men were more susceptible to develop DSS in both treatment and control groups (p=0.03, odds ratio=2.75, 95% CI=1.12-6.77) even after adjusting for treatment, age and other confounders.

As illustrated in Figure 3 platelet (p<0.001) and white cell counts (p<0.001) reduced from 1st to the 3rd day and rose again by 4th and 5th day. Furthermore, there were no significant differences in the serial changes in WBC and platelet counts between treatment and control groups at p=0.56 and p=0.54, respectively. The haematocrit levels also changed over the course of the 5 days, however, the pattern was not significant (p=0.26). Moreover, there were no statistically significant differences in the serial haematocrit changes over the 5 days between treatment and control groups (p=0.74).

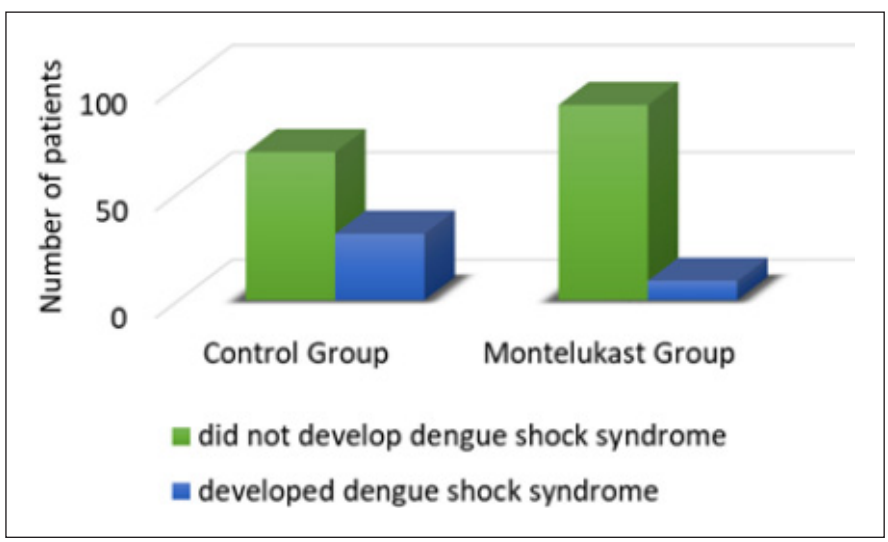

Figure 2. Patients who developed dengue shock syndrome in treatment and control groups.

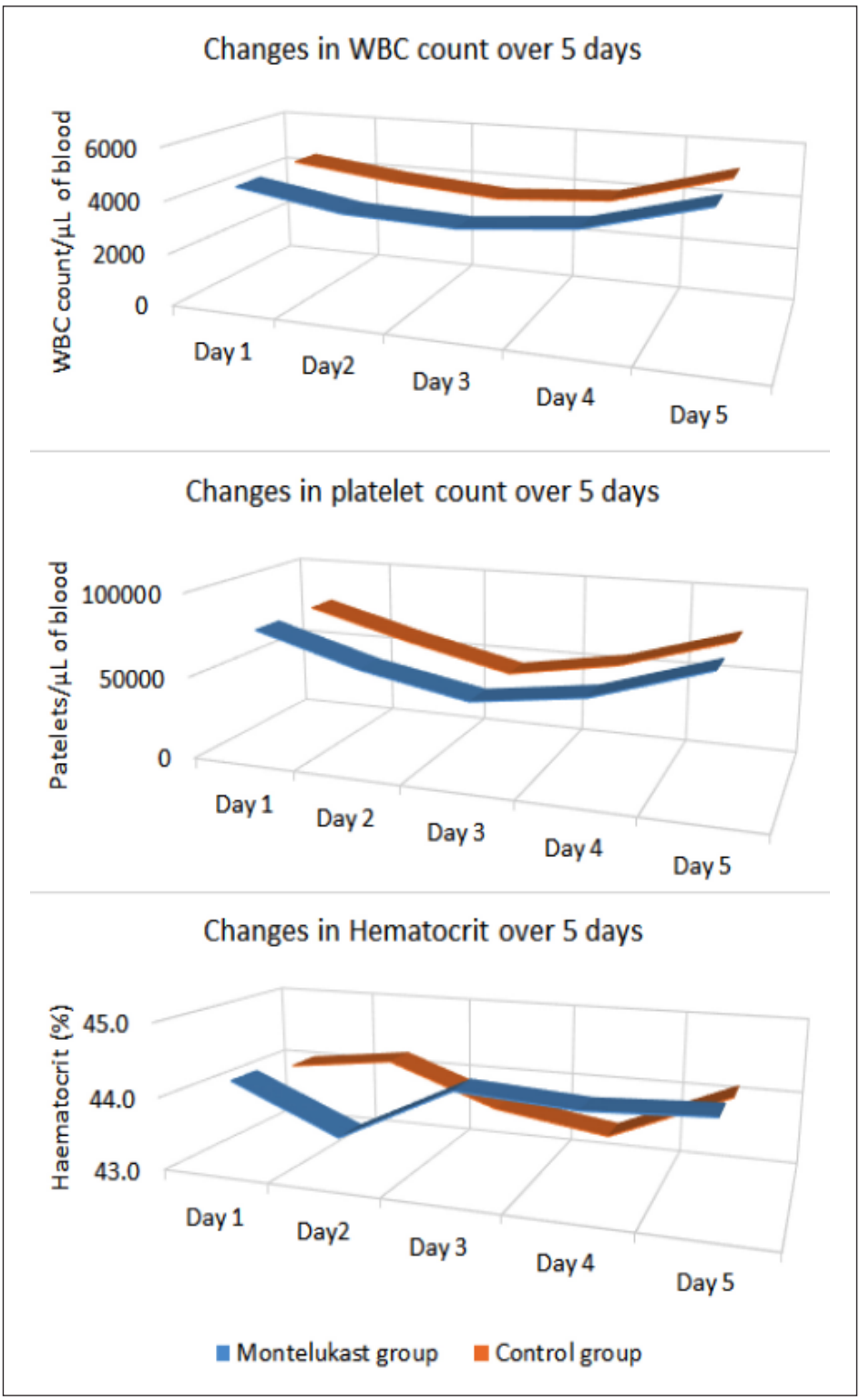
Changes in WBC count over 5 days
WBC count/µL of blood
6000
4000
2000
0
Day 1
Day2
Day 3
Day 4
Day 5
Changes in platelet count over 5 days
Patelets/µL of blood
100000
50000
0
Day 1
Day 2
Day 3
Day 4
Day 5
Changes in Hematocrit over 5 days
Haematocrit (%)
45.0
44.0
43.0
Day 1
Day2
Day 3
Day 4
Day 5
Montelukast group
Control group

Figure 3.

DISCUSSION

Current study, for the first time reports on the efficacy of montelukast in preventing DSS in human subjects. The risk of DSS in the patients treated with oral montelukast in addition to supportive therapy was reduced by 71% (RRR) compared to those treated with the supportive therapy alone. Only 5 patients were needed to be treated with montelukast in order to reduce the occurrence of DSS in one patient as illustrated by NNT of 4.55. Considering the mortality risk associated with DSS, this risk reduction is clinically very significant.

Dengue is amongst the fastest emerging infectious diseases in all tropical areas of the world. In last few years, dengue disease burden has risen considerably with frequent epidemics in subcontinent. This has led to substantial mortality, morbidity and economic burden particularly in poor countries like Pakistan, India and Sri Lanka (Jahan, 2011). Currently, treatments for DSS dengue fever (DF) and dengue haemorrhagic fever (DHF), are bed rest, fluid replacement and supportive care with analgesics (Lai, Lin & Hsieh, 2017).

Several antiviral drugs, natural herbs and other medicinal products are being investigated in animal models and human clinical trials for treatment of dengue fever (Lai *et al.*, 2017). In the present study montelukast, a leukotriene receptor antagonist known to modulate the functions of mast cells, was used for the prevention of DSS in patients admitted with dengue fever. Results from this study showed that 40 (20%) out of 200 dengue fever patients suffered from DSS. Only 9 (9%) of the patients who were treated with montelukast developed DSS as compared to 31 (31%) patients who received supportive therapy alone ($p<0.001$). The effect remained significant even after adjusting for confounders as shown in Table 3. These results are supported by the work of St John and colleague who worked on mice as animal models of dengue fever. In the aforementioned study, mice were infected with dengue virus via intra-peritoneal injections. The authors administered mice with 0.4 mg montelukast via oral gavage 1 hour after intra-peritoneal instillation of dengue virus and vascular leakage was assessed 1 day after infection. The authors noticed decreased vascular permeability in DENV-infected mice treated with oral montelukast as compared to the DENV-infected, untreated mice at $p\leq0.05$ (Vitaranal *et al.*, 1991).

To the best of our knowledge there have been no clinical trials prior to the current study which attempted to validate these findings in human subjects. Another closely related drug that is currently being investigated for prevention of DSS in dengue in a clinical trial at National University Hospital, Singapore (NUH) and Singapore General Hospital (SGH), is Ketotifen. The mechanism of action of Ketotifen is closely related to montelukast. It stabilizes mast cells and hence prevents the release of substances, which increase vascular permeability including leukotrienes. Thus it may lead to reduction in vascular leakage. (Makhluf, Kim & Shresta, 2016).

Various other drugs have also been recently investigated to prevent vascular leakage and reduce the risk of DSS (Lai *et al.*, 2017). Celgosivir, an alpha-glucosidase inhibitor, proved effective in animal models, but did not show promising results in clinical trials (Jenny G Low, Sung, Wijaya, Wei, Rathore, Watanabe, Tan, Toh, Chua & Hou, 2014). Similarly, oral calcium was investigated for its possible use in treating of dengue fever. The treatment elevated platelet levels and reduced duration of illness (Cabrera-Cortina, Sánchez-Valdéz, Cedas-DeLezama & Ramírez-González, 2008). Other drugs which have shown some promise include TNF blocker drugs such as Infliximab or Remicade. These drugs alleviated the symptoms of haemorrhage, in a small study of dengue patients (Deligny, de Bandt, Dehlinger, Numéric, Cabié, Lombard, Polomat, JeanBaptiste & Arfi, 2014) Other drugs including Prednisolone, Lovastatin, Chloroquine, Balapiravir and Iminosugars have been tried in management of dengue fever in clinical trials, but have not shown beneficial effects (Jenny GH Low, Ooi & Vasudevan, 2017).

An effective anti-dengue medicine which reduces morbidity and mortality is a dire need of the day. Present study showed that montelukast effectively reduced the risk of DSS in dengue patients and it should be further investigated in larger multicentre trials.

There were several other significant findings in the present study. Firstly, it was noticed that men were 2.75 times (95% CI 1.12-6.77) more likely to develop DSS even after adjusting for treatment group, age and blood counts (p=0.03). There is discrepancy in literature regarding gender as a risk factor for DSS. Various international studies have reported that the female dengue patients are at a higher risk to develop DSS (Organization, 1999; St John *et al.*, 2013). However, current findings are more congruent with Khurram *et al.* (2014), who reported a higher rate of DSS in male as compared to female dengue patients. It was reported that 3/96 (3.1%) men and 1/48 (2.1%) women with primary dengue infection developed DSS (p=0.9). Similarly, 7/59 (11.9) men and none of the women (0/31) with secondary dengue infection developed DSS at p=0.05. The differences in results of Pakistani studies with studies from other countries may be attributed to different genetic back grounds of the cohorts as well as differences in the genotypes of the viruses.

Present results also showed a trend of fall in white cell, platelet counts days 1 to 3 and subsequent rise in these levels, which continued till day 5 at p<0.001 (Figure 3). A similar pattern had been reported by other investigators (Jahan, 2011; "Comprehensive Guidelines for Prevention and Control of Dengue and Dengue Haemorrhagic Fever: Revised and Expanded Edition."). There was no significant pattern of serial changes in blood haematocrit levels (p=0.26). One might have expected the haematocrit levels in the controls to rise due to vascular leakage, however, such a pattern was not observed. This is most likely due to the supportive treatment that these patients were receiving.

There may be several limitations to present study. Firstly, the authors tested only one dose of montelukast in the study. Despite a single daily dose of 10 mg, favourable results were noticed. Future studies should investigate the optimum dose, which produces the maximum effect. Secondly, the researchers do not have data on viral load or serology to classify primary and secondary infections. Thus, it cannot be commented if there was a link between the viral load and infection type with response to treatment. This is another area of possible future investigations.

CONCLUSION

Montelukast along with standard supportive treatment may protect patients from DSS. Future studies can further investigate optimum dosage and duration of this proposed treatment.

REFERENCES

Abraham, S.N. & John, A.L.S. (2010). Mast cell-orchestrated immunity to pathogens. *Nature Reviews Immunology* **10**(6): 440-452.

Cabrera-Cortina, J.I., Sánchez-Valdéz, E., Cedas-DeLezama, D. & Ramírez-González, M.D. (2008). Oral calcium administration attenuates thrombocytopenia in patients with dengue fever. Report of a pilot study. *Proceedings of the Western Pharmacology Society* **51**: 38-41.

Deligny, C., de Bandt, M., Dehlinger, V., Numéric, P., Cabié, A., Lombard, F., Polomat, K., JeanBaptiste, G. & Arfi, S. (2014). Dengue fever in patients under biologics. *Journal of Clinical Virology* **61**(3): 442-443.

Furuta, T., Murao, L.A., Lan, N.T.P., Huy, N.T., Huong, V.T.Q., Thuy, T.T., Tham, V.D., Nga, C.T.P., Ha, T.T.N. & Ohmoto, Y. (2012). Association of mast cell-derived VEGF and proteases in Dengue shock syndrome. *PLoS Neglected Tropical Diseases* **6**(2): e1505.

Halstead, S.B. (2007). Dengue. *The Lancet* **370**(9599): 1644-1652.

Jahan, F. (2011). Dengue fever (DF) in Pakistan. *Asia Pacific Family Medicine* **10**(1): 1.

Kunder, C.A., St John, A L. & Abraham, S.N. (2011). Mast cell modulation of the vascular and lymphatic endothelium. *Blood* **118**(20): 5383-5393.

Lai, J.-H., Lin, Y.-L. & Hsieh, S.-L. (2017). Pharmacological intervention for dengue virus infection. *Biochemical Pharmacology*.

Low, J.G., Ooi, E.E. & Vasudevan, S.G. (2017). Current Status of Dengue Therapeutics Research and Development. *The Journal of Infectious Diseases* **215**(suppl_2), S96-S102.

Low, J.G., Sung, C., Wijaya, L., Wei, Y., Rathore, A.P., Watanabe, S., Tan, B.H., Toh, L., Chua, L.T. & Hou, Y.A. (2014). Efficacy and safety of celgosivir in patients with dengue fever (CELADEN): a phase 1b, randomised, double-blind, placebo-controlled, proof-of-concept trial. *The Lancet Infectious Diseases* **14**(8): 706-715.

Makhluf, H., Kim, K. & Shresta, S. (2016). Novel strategies for discovering inhibitors of Dengue and Zika fever: Taylor & Francis.

World Health Organization. (1999). Prevention and control of dengue and dengue haemorrhagic fever: comprehensive guidelines. *Prevention and control of dengue and dengue haemorrhagic fever: comprehensive guidelines*.

Sarwar, M. (2014). Dengue Fever as a Continuing Threat in Tropical and Subtropical Regions around the World and Strategy for Its Control and Prevention. *Journal of Pharmacology and Toxicological Studies* **2**(2): 1-6.

Spector, S.L. & Tan, R.A. (2004). Effectiveness of montelukast in the treatment of cough variant asthma. *Annals of Allergy, Asthma & Immunology* **93**(3): 232-236.

St John, A.L., Rathore, A.P., Raghavan, B., Ng, M.-L. & Abraham, S.N. (2013). Contributions of mast cells and vasoactive products, leukotrienes and chymase, to dengue virus-induced vascular leakage. *Elife* **2**: e00481.

Tintinger, G.R., Feldman, C., Theron, A.J. & Anderson, R. (2010). Montelukast: more than a cysteinyl leukotriene receptor antagonist? *The Scientific World Journal* **10**: 2403-2413.

Vitaranal, T., de Silva, H., Withanat, N. & Gunasekerat, C. (1991). Elevated tumour necrosis factor in dengue fever and *Ceylon Medical Journal* **36**: 63-65.

World Heath Organisation, Comprehensive Guidelines for Prevention and Control of Dengue and Dengue Haemorrhagic Fever: Revised and Expanded Edition. 2014 (W. R. O. f. S. E. A. 2014, Trans.).