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Original Paper

A Randomized Controlled Study to Assess the Effect of Doxycycline on IL-6 and TNF Levels among the Patients of Dengue

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Abstract
Objective: *To evaluate the ability of doxycycline to modulate cytokines in patients with DF or DHF.*
Methods: *This was a randomized controlled trial conducted in a tertiary care hospital in north India. A total of 36 patients of DF or DHF were included in the study and randomized into 2 groups (18 in each group) by using computer generated random number table. Blood samples were obtained from hospitalized patients 18–55 years of age presenting with symptoms characteristic of DF or DHF. In group A, for patients 18–55 years of age, an initial oral dose of 200 mg was followed by 100 mg administered at 12 hour intervals for 10 days. Patients of Group B were untreated. Cytokine levels were assessed at 0 day and 7 days by standard methods.*
Results: *All patients demonstrated elevated IL-6 and TNF levels at day 0 evidenced by higher mean levels in both the groups. Over the 7-day observation period, IL-6 and TNF remained elevated in the control group (Group B). Treatment with doxycycline resulted in statistically (p=0.0001) lower levels of IL-6 and TNF by day 7, both when compared to that seen at day 0 (intragroup analysis) and when compared to day 0 values in the control group. Decreases in Il-6 and TNF between day 0 and day 7 values were modest in the control group than treated with doxycycline (Group A). Treatment with doxycycline resulted in IL-6 and TNF levels being significantly (p=0.0001) lower than control group by day 7.*
Conclusions: *The findings from the current study indicate that doxycycline could be an effective treatment for dengue patients with high risk of complications.*
Keywords: *Doxycycline, Dengue, Cytokines.*

Introduction

Dengue is an emerging viral hemorrhagic fever with rare severe forms, transmitted by female *Aedes* mosquitoes. Management of clinical dengue is symptomatic because no specific antiviral drug is available (WHO, 2012). Thrombocytopenia due to activation of complex immune mechanisms and direct dengue virus action on bone marrow is one of the hallmark features of dengue (de et al, 2015). In severe

dengue, disseminated intravascular coagulation can contribute to thrombocytopenia ((de et al, 2015). Platelet count is considered to correlate with the severity of dengue (WHO, 2011). Serial platelet counts are a key laboratory investigation parameter in managing dengue patients. In some severe dengue cases, a combination of thrombocytopenia with other factors lead to life-threatening hemorrhages (WHO, 2011), hence a major concern to clinicians.

Cytokines are known to play a role in several viral hemorrhagic fevers including dengue (Marty et al, 2006). Previous studies have shown a correlation between increased levels of several cytokines and disease severity which may have prognostic value (Atrasheuskaya et al, 2003; Bozza et al, 2008). In general, these studies have shown that levels of cytokines adversely affecting coagulation tended to be higher in dengue hemorrhagic fever (DHF) versus Dengue fever (DF) (Suharti et al, 2002).

Given the critical role of cytokines in the inflammatory process and coagulopathies, there have been numerous attempts to suppress their levels in an attempt to control various diseases (Feldmann, 2008). Various classes of antibiotics have been shown to possess immunomodulating properties. Of these, drugs belonging to the tetracycline family were found to benefit patients with multiple sclerosis, Huntington's disease and rheumatoid arthritis presumably by suppressing microglia activity. This, in turn, lowered levels of several proinflammatory cytokines including tissue necrosis factor (TNF) and interleukin (IL-6) (Tauber and Nau, 2008; Lai and Todd, 2006).

The present study was conducted to evaluate the ability of doxycycline to modulate cytokines in patients with DF or DHF.

Material and Methods

This was a randomized controlled trial conducted in a tertiary care hospital in north India. The study was approved by the Ethical committee of the Institute. The consent from each participant was taken before enrolling in the study. A total of 36 patients of DF or DHF were included in the study and randomized into 2 groups (18 in each group) by using computer generated random number table. Group A was treatment group and Group B was control group.

Blood samples were obtained from hospitalized patients 18–55 years of age presenting with symptoms characteristic of DF or DHF. For a presumptive diagnosis of DF or DHF (Day 0), a fever for more than 2 days accompanied by two or more of the following were present severe headache, retro-orbital pain, myalgia, arthralgia, rash, leucopenia, and hemorrhage. Serum was collected, and dengue virus infection was confirmed by PCR testing. DHF was defined as being PCR-positive for dengue virus accompanied by fever with one or more of the following being present positive tourniquet test for petechiae, mucosal hemorrhage, thrombocytopenia, an increase of >20% in hematocrit, or clinical evidence of shock. Patients were randomized within 72 hours of a confirmed diagnosis.In group A,for patients 18–55 years of age, an initial oral dose of 200 mg was followed by 100 mg administered at 12 hour intervals for 10 days. Patients of Group B were untreated. Cytokine levels were assessed at 0 day and 7 days by standard methods.

Statistical analysis

Statistical analysis comparing cytokine between controls (untreated) and treatment groups (intergroup analysis) was performed using the unpaired Student t-test. Intragroup analysis (comparing day 0 versus day 7 within the same group) was done using a paired Students t-test. Thep-value $0<0.05$ was considered statistically significant.

Results

There was no significant ($p>0.05$) difference in the age gender between the groups showing comparability of the groups in terms of age and gender (Table-1).

All patients demonstrated elevated IL-6 and TNF levels at day 0 evidenced by higher mean levels in both the groups. Over the 7-day observation

period, IL-6 and TNF remained elevated in the control group (Group B). Treatment with doxycycline resulted in statistically (p=0.0001) lower levels of IL-6 and TNF by day 7, both when compared to that seen at day 0 (intragroup analysis) and when compared to day 0 values in the control group. Decreases in Il-6 and TNF between day 0 and day 7 values were modest in the control group than treated with doxycycline (Group A). Treatment with doxycycline resulted in IL-6 and TNF levels being significantly (p=0.0001) lowerthan control group by day 7 (Table-2& Table-3).

Table-1: Age and gender distribution between the groups

Age and gender	Group A (n=18)	Group B (n=18)	p-value
Age in years, mean±SD	32.23±8.26	31.20±9.56	0.73[a]
Gender, no. (%)			
Male	10 (55.6)	9 (50.0)	0.74[b]
Female	8 (44.4)	9 (50.0)	

[a]Unpiared t-test, [b]Chi-square test

Table-2: Comparison of IL-6 (pg/ml) between the groups

Time period	Group A (n=18)	Group B (n=18)	p-value[1]
Day 0	5.67±0.88	5.33±0.67	0.20
Day 7	3.46±0.23	4.99±0.44	0.0001*
p-value[2]	0.0001*	0.08	

[1]Unpiared t-test,[2]Paired t-test

Table-3: Comparison of TNF (pg/ml) between the groups

Time period	Group A (n=18)	Group B (n=18)	p-value[1]
Day 0	6.78±1.23	6.65±1.11	0.74
Day 7	3.13±0.65	6.11±0.98	0.0001*
p-value[2]	0.0001*	0.10	

[1]Unpiared t-test,[2]Paired t-test

Discussion

Numerous studies have shown that infection with dengue virus leads to increased levels of proinflammatory cytokines, which can lead to profound shock and death (Bozza et al, 2008; Simmons et al, 2007). Doxycycline was selected due to its superior immunomodulation effect and because it has shown to inhibit dengue virus replication in vitro (Rothan et al, 2013). In that sense, daily oral administration of doxycycline was also associated with a significant decline in circulating levels of IL-6 and TNF, confirmed by earlier observations (Castro et al, 2011).

Proinflammatory cytokines, such as IL-6 and TNF, are believed to cause the majority of symptoms, such as fever, malaise, and coagulopathies associated with infections. Indeed, the degree of imbalance between such cytokines and their anti-inflammatory counterparts may be the primary prognostic indicator of disease outcome (Girardin et al, 1992).

In the present study, we investigated the effectiveness of doxycycline to modulate the levels of IL-6 and TNF cytokines in patients with DF or DHF. As has been previously observed (Bozza et al, 2008; Hober et al, 1993), dengue virus infection resulted in a marked increase in serum cytokines levels. Doxycycline drug was able to modulate proinflammatory cytokines levels. Down regulation was rapid being observed within 7 days of treatment. An intragroup analysis did achieve significance indicating that differences in baseline levels between groups at the time of therapy was initiated may have a

substantial influence. Doxycycline appeared to exert the degree of effectiveness in patients with DF or DHF, a much more severe disease syndrome. An additional potential benefit to using doxycycline in the treatment of DF or DHF is its recently discovered ability to inhibit dengue virus multiplication in tissue culture (Yang et al, 2007).
The present study indicates that doxycycline may provide a clinical benefit in the treatment of dengue virus infection by modulating the cytokine cascade. We recommend a study in the near future to determine if doxycycline can provide a clinical benefit to patients with DF or DHF by monitoring disease severity, mortality rates and time of hospital discharge post enrollment.

Conclusion

The findings from the current study indicate that doxycycline could be an effective treatment for dengue patients with high risk of complications. Its low cost, long history of safety, the ability to cross the blood brain barrier, and administration via the oral route appears to be an ideal candidate to be used in patients in rural areas with limited access to healthcare.

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