

## HYPOCHOLESTEROLEMIA AS A PREDICTOR OF DISEASE SEVERITY IN DENGUE FEVER

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### Abstract

**Objectives:** The objective of this study was to determine the difference between total cholesterol (TC) of dengue fever (DF) and dengue hemorrhagic fever (DHF) patients and also between DHF patients with favourable (DHF I and II) and adverse (DHF III and IV) outcome.

**Methodology:** This comparative cross – sectional study was conducted in the Department of Medicine, Mayo Hospital, Lahore, from August to November 2012. The study comprised of 100 febrile patients with positive dengue serology. TC was measured on the admission day. The patients were observed during admission period for clinical outcome i.e. DF and DHF. DHF patients were graded as I – IV and grouped as favourable (I and II) and adverse (III and IV) outcome.

**Results:** Out of 100 patients included in the study, 64 were males and 36 were females with mean age 33.03

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± 14.5 years and mean TC  $3.02 \pm 0.88$  mmol/L. The 63 DF and 37 DHF patients had mean ages of  $30.21 \pm 12.7$  and  $37.84 \pm 16.21$  years respectively (p-value 0.017) and mean TC  $3.43 \pm 0.79$  and  $2.34 \pm 0.56$  mmol/L respectively (p-value 0.000). M:F for DF and DHF was 2:1 and 1.47:1 respectively (p-value 0.001). Among 37 DHF patients, there were 16 DHF I, 8 DHF II, 10 DHF III and 3 DHF IV patients. For DHF I – IV, mean TC was  $2.77 \pm 0.45$ ,  $2.16 \pm 0.33$ ,  $2.05 \pm 0.35$  and  $1.49 \pm 0.35$  mmol/L respectively. DHF patients with favourable and adverse outcome had mean TC  $2.55 \pm 0.5$  and  $1.92 \pm 0.42$  mmol/l respectively (p = value 0.000).

**Conclusion:** Low serum TC strongly correlated with disease severity in dengue fever patients.

**Key Words:** Dengue Fever, Dengue Hemorrhagic Fever, Total Cholesterol.

### Introduction

Dengue fever is a mosquito borne infectious tropical disease caused by the dengue virus. A relatively geographically restricted disease in the past,<sup>1</sup> it has become the most common mosquito borne viral disease in the world at present.<sup>2</sup> Nearly 100 million cases of dengue fever and between 250,000 and 500,000 cases of dengue hemorrhagic fever are annually reported to the World Health Organization.<sup>3</sup> Pakistan has suffered from five dengue epidemics since 2006.<sup>4</sup> Typically,

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DF has been considered to be a childhood disease and is an important cause of pediatric hospitalization in the tropical countries. However, there is increasing evidence that DF is affecting older age groups.<sup>5</sup>

In 2011, WHO classified symptomatic dengue infection into four categories i.e. undifferentiated febrile illness (viral syndrome), dengue fever, dengue hemorrhagic fever and expanded dengue syndrome / isolated organopathy.<sup>6,7</sup> However, the most commonly detected forms are DF and DHF. The difference between DF and DHF is the absence and presence of plasma leakage respectively.<sup>6,7</sup> DHF is further subdivided on the basis of severity as mild (grade I and II) and severe (grade III and IV). DHF III and IV are characterized by hypovolemic shock and are collectively labelled as dengue shock syndrome. The term expanded dengue syndrome has been introduced in the 2011 WHO guidelines to encompass the unusual and rare symptoms of infection by the dengue virus. There can be involvement of one or multiple organ systems.<sup>6,7</sup> Expanded dengue syndrome can be life threatening when it manifests as fulminant hepatic failure, acute pancreatitis, acute renal failure, myocarditis, acute respiratory distress syndrome and spontaneous splenic rupture.<sup>6,7</sup>

Various laboratory tests are directed either towards establishing the specific diagnosis or towards monitoring the disease severity. Confirmatory tests include serologic testing, isolation of virus, detection of viral genome by PCR and detection of viral protein (NS1 antigen).<sup>6,7</sup>

Standard widely available laboratory parameters are used for monitoring the disease severity. The most common monitoring tools include hematological tests (hematocrit, total leucocyte count and platelet count) and biochemical tests (liver transaminases).<sup>6,7</sup> Other investigations which are recommended but less commonly used include serum albumin, serum total cholesterol, serum electrolytes (especially sodium and calcium), blood urea nitrogen, serum creatinine, arterial blood gases and coagulation studies.<sup>6,7</sup>

At the onset, the course of dengue infection is often unpredictable until the appearance of features of more severe disease forms. This leads to inadequate or late treatment of this potentially lethal disease. Recurring epidemics of dengue fever in Pakistan demand that we should look for widely available, inexpensive, reliable and reproducible methods to establish disease severity early in the course of illness. This will help the health care professionals to identify high risk patients and focus the available medical resources to improve their clinical outcome.

Several studies have focused on the association of serum TC and disease severity in dengue patients, but as yet, it has not been justified as a screening test; the sensitivity, specificity and predictive values are unknown. There is also scanty data on serum cholesterol values in adult population affected by dengue infection.

Cholesterol has been called "the most highly decorated molecule in biology" due to the numerous Nobel prizes awarded in relation to research on it.<sup>8</sup> Cholesterol is insoluble in water and is present in blood as lipoproteins. These lipoproteins are measurable and include chylomicrons, low density lipoproteins (LDL), very low density lipoproteins (VLDL) and high density lipoproteins (HDL).<sup>9</sup> Both raised and low levels of cholesterol are detrimental to health although most research has been focused on hypercholesterolemia as a risk factor for atherosclerosis.

Hypocholesterolemia is an unusual condition. This term is used when serum TC level is below the 5<sup>th</sup> percentile (after adjustment for age, gender and race).<sup>10</sup> Hypocholesterolemia may be primary or secondary.<sup>10</sup> The primary causes are rare inherited conditions including familial hypobetalipoproteinemia and abetalipoproteinemia.<sup>10</sup>

Secondary causes of hypocholesterolemia include malabsorption, hyperthyroidism, malignancy, chronic liver disease, sepsis, critical illness, adrenal insufficiency, intense diet, primitive life style etc.<sup>10</sup> Low cholesterol levels may also be induced by lipid lowering drugs.<sup>11</sup>

Low cholesterol has been linked to increased mortality due to increased susceptibility to malignancies, hemorrhagic stroke, depression and aortic dissection.<sup>12</sup> Short term mortality is enhanced in elderly patients.<sup>13</sup> Moreover, in critical illness, there are altered cytokine levels and increased oxidative stress.<sup>14</sup> These factors lead to a lowered serum TC level which is increasingly being utilized as a predictor of clinical deterioration and poor prognosis.<sup>14</sup> In 2003, Dunham et al reported that in critically ill trauma patients, mean serum TC was low at admission than the normal population.<sup>15</sup> In addition, the non-survivors showed a significant decline in serum TC levels as compared to the survivors.<sup>15</sup> Similar findings were reported by Combs (1980) in burn patients and by Wilson (2003) in critically ill and injured patients.<sup>16,17</sup>

Cholesterol and lipoproteins bind and neutralize bacterial lipopolysaccharide (LPS) which acts as an endotoxin.<sup>14,17</sup> As a result, the cytokine cascade involving TNF- $\alpha$ , IL-1 and IL-6 is down regulated.<sup>14,17</sup>

Low levels of these lipids which act as neutralizing factors lead to excessive production of these inflammatory cytokines and the consequence is a more severe disease.<sup>14,17</sup>

Experiments have been carried out to raise lipid levels to block the effect of bacterial LPS.<sup>18,19</sup> It has been proposed that raising serum TC level in critically ill patients may be a therapeutic option to prevent and reverse sepsis.<sup>17,19</sup>

Low lipid levels are also seen in DHF patients.<sup>20,21</sup> The advancing grades of DHF are inversely related to serum TC levels.<sup>22</sup> Hypcholesterolemia i.e. serum TC < 100 mg/dl (2.59 mmol/l) or reduction of serum TC > 20 mg/dl (0.52 mmol/l) from baseline is taken as an evidence of plasma leakage and entry into critical phase in DHF patients.<sup>22</sup> Villar – Centeno (2008) and Suv-arna (2009) conducted their studies on biochemical markers and lipid profile to determine disease severity.<sup>20,21,23</sup> All these workers reported that serum TC is lower in DHF when compared to DF and among DHF patients, those presenting in DHF III and IV have significantly lower levels.<sup>20,21,23</sup> In 2011, Kalayanarooj, while proposing a new classification for dengue patients, also mentioned serum cholesterol levels of her patients with respect to grade of illness. Mean TC in DF patients was 4.35 mmol/l (168.3 mg/dl) in her study. The mean TC levels for DHF grades I, II, III and IV were 2.82 mmol/l (108.9 mg/dl), 2.11 mmol/l (81.5 mg/dl), 2.13 mmol/l (82.3 mg/dl) and 0.85 mmol/l (33 mg/dl) respectively.<sup>22</sup>

This study was designed to determine the association of serum TC level with disease severity in adult dengue patients. Serum TC is an easily available test.<sup>21</sup> It requires no special preparation and only a blood sample needs to be drawn with minimal patient discomfort.

## Methodology

This comparative cross-sectional study was conducted in Department of Medicine, Mayo Hospital, Lahore from August, 2012 to November, 2012. In accordance with the calculated sample size, 100 febrile patients who were serologically positive for Dengue were selected by purposive non-probability sampling technique. These included both males and females from ages 15 to 60 years. Patients with history of diabetes mellitus, hypertension, ischemic heart disease, cerebrovascular accidents, dyslipidemia, renal failure, liver disease, hematologic disorders, malignancy and thyroid disor-

ders were excluded from the study. Those patients having body mass index > 35 were also excluded.

After taking informed consent and ensuring confidentiality, the purpose of the study was explained to the patients. The patients were initially admitted in Dengue Infection Ward (DIW) which was established in the Department of Medicine and later shifted to HDU if needed.

The basic demographic profile and clinical findings were recorded. The confounding variables i.e. causes of pre-existing dyslipidemia were controlled by following the exclusion criteria. Venous blood samples for analysis of serum TC were obtained on the day of admission. All the patients were given standard treatment. The patients were followed up for the duration of hospital stay and clinical outcome was determined as DF or DHF. The DHF patients were classified into DHF I, II, III and IV. The serum TC of all the patients was recorded. All information was gathered through a specially designed proforma.

The data were entered in SPSS version 13.0 and were analyzed accordingly. The variables were presented as simple descriptive statistics calculating mean and standard deviation of numerical data like age and total cholesterol levels. Qualitative variables like gender were presented in the form of frequency distribution and percentages. The mean TC was calculated for simple DF and for the grades of DHF I – IV. T-test was used to compare the mean age and mean TC and chi-square test was used for gender comparison in both groups i.e., DF and DHF. p-value < 0.05 was considered as statistically significant. For comparison of grades of DHF, the DHF patients were divided into two groups i.e., favorable outcome (DHF I and II) and adverse outcome (DHF III and IV) and similar tests were again applied keeping p-value < 0.05 as statistically significant.

## Results

Out of the 100 patients selected in this study, there were 64 (64%) males and 36 (36%) females with M:F ratio of 1.78:1. The ages ranged from 15 to 60 years and average age (mean  $\pm$  SD) was 33.03  $\pm$  14.5 years. Among these, 39 (39%) were in 15 – 24 years age group, 18 (18%) in the 25 – 34 years group, 16 (16%) in the 35 – 44 years group, 13 (13%) in 45 – 54 years group and the remaining 14 (14%) were 55 years and above. Out of the total patients, 63 (63%) developed dengue fever (DF) and 37 (37%) developed dengue

hemorrhagic fever. In the DHF group, the number of patients developing DHF I, II, III and IV were 16, 8 10

and 3 respectively.

The average age (mean  $\pm$  SD) for DF was  $30.21 \pm 12.7$  years and for DHF was  $37.84 \pm 16.21$  years. Most of the DF patients (66.6%) were between 15 and 34 years while DHF patients showed two peaks i.e. 29.7% were aged between 15 and 24 years and 27% were 55 years and above. Both DF and DHF groups revealed an overall majority of males with M:F ratio of 2:1 and 1.47:1 respectively. However, as the severity of DHF worsened, this ratio was reversed i.e., for DHF I, II, III and IV, it was 2.2:1, 1.67:1, 1:1 and 0.5:1 respectively (Figure 1).

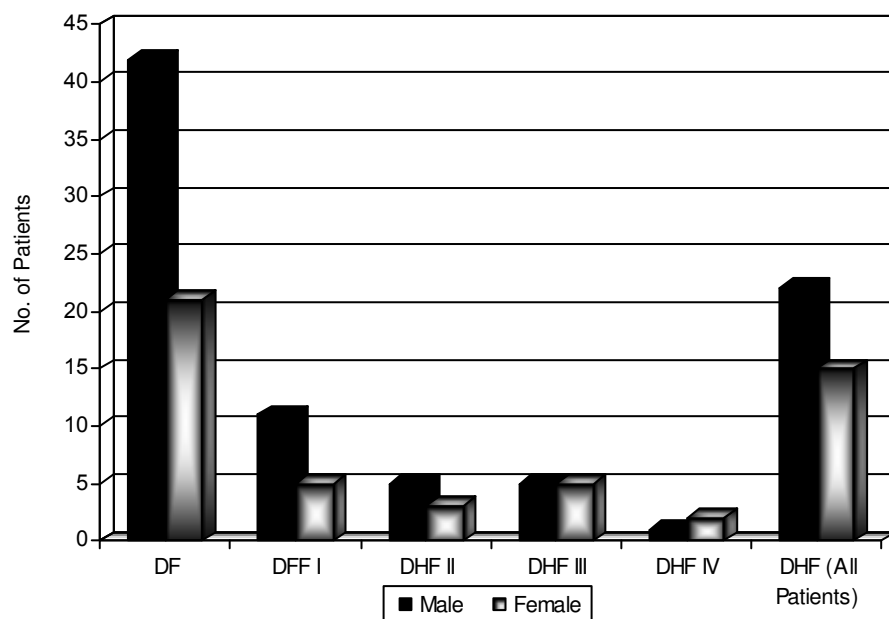
Overall, the mean serum total cholesterol (TC) of the study population was  $3.02 \pm 0.88$  mmol/L. The mean TC for DF & DHF patients was determined as  $3.43 \pm 0.7$  mmol/L and  $2.34 \pm 0.56$  mmol/L respectively (Table 1). Mean TC was also calculated for the different grades of DHF and a downward trend in mean TC was noted with increasing severity of DHF. For DHF I, II, III and IV, values of TC as mean  $\pm$  SD were  $2.77 \pm 0.45$  mmol/L,  $2.16 \pm 0.33$  mmol/L,  $2.05 \pm 0.35$  mmol/L and  $1.49 \pm 0.35$  mmol/L respectively (Table 1).

The comparison of demographics and TC of DF and DHF patients (Table 2) revealed statistically significant difference (p-value  $< 0.05$ ) between mean ages, gender distribution and mean TC. The comparison of favorable outcome & adverse outcome groups of DHF (Table 3) also revealed statistical significance for differences in mean ages and mean TC.

## Discussion

We have observed in this study that dengue fever primarily affects young adults with mean age of 33.03

**Figure 1:** Gender Distribution in DF and DHF.



**Table 1:** Disease Severity and Mean Total Cholesterol.

Clinical Outcome	N = 100	Total Cholesterol in mmol/L (Mean $\pm$ SD)
DF	63	3.43 $\pm$ 0.79
DHF	37	2.34 $\pm$ 0.56
DHF I	16	2.77 $\pm$ 0.45
DHF II	8	2.16 $\pm$ 0.33
DHF III	10	2.05 $\pm$ 0.35
DHF IV	3	1.49 $\pm$ 0.35

**Table 2:**  
Comparison of DF and DHF.

Parameter	DF (n = 63)	DHF (n = 37)	p Value
Mean Age $\pm$ SD (years)	30.21 $\pm$ 12.7	37.84 $\pm$ 16.21	0.017
M:F	2:1	1.47:1	0.001
Mean Total Cholesterol $\pm$ SD (mmol/L)	3.43 $\pm$ 0.79	2.34 $\pm$ 0.56	0.000

**Table 3:** Comparison of Favorable and Adverse Clinical Outcome in DHF.

Parameter	DHF I and II (n = 24)	DHF III and IV (n = 13)	p Value
Mean Age $\pm$ SD (years)	33.92 $\pm$ 16.29	45.08 $\pm$ 13.86	0.036
M:F	2:1	0.86:1	0.79
Mean Total Cholesterol $\pm$ SD (mmol/L)	2.55 $\pm$ 0.5	1.92 $\pm$ 0.42	0.000

( $\pm$  14.4) years. The mean ages for DF and DHF were 30.21 ( $\pm$  12.7) years and 37.84 ( $\pm$  16.21) years respectively with P value of 0.017.

Similar findings have been reported in other studies conducted in Pakistan. Riaz<sup>24</sup> reported mean age of 31  $\pm$  12.9 years. In the study by Mahboob, the average age of dengue patients was 31.5  $\pm$  15.2 years.<sup>25</sup> According to Hakim the prevalence of DF and DHF was huddled between 20 – 45 years of age.<sup>26</sup>

Among the 100 patients included in this study, 64 (64%) were males with male to female ratio of 1.78:1. The previous workers have found conflicting evidence about the gender distribution of dengue patients. In the study by Villar – Centeno, the gender distribution was almost equal with 49.8% males among the infected patients.<sup>20</sup> However, the study population of Suvarna had a male to female ratio of 2.3:1.<sup>21</sup> In Singapore, males have been reported in higher numbers in different epidemics. Goh found male to female ratio of 1.9:1 in 1987.<sup>27</sup> In 2005 Singapore epidemic, higher incidence was again reported amongst males than females i.e. 324.7 for 100,000 and 272 per 100,000 respectively.<sup>28</sup> The studies conducted in India in different cities during the 1996 and 1999 epidemics had similar results.<sup>29,30</sup>

Serum total cholesterol was measured in all patients on the day of admission. The mean TC values for DF and DHF were 3.43  $\pm$  0.79 mmol/L and 2.34  $\pm$  0.56 mmol/L respectively. This difference was significant with p value of 0.000.

Among the DHF patients, a steady decrease in mean TC was seen with the advancing grades of DHF which declined from 2.77  $\pm$  0.45 mmol/L for DHF I to 1.49  $\pm$  0.35 mmol/L for DHF IV. The grouping of DHF patients with respect to favorable (DHF I and II) and

adverse (DHF III and IV) outcome further highlighted this regression. For these two groups, mean TC was 2.55  $\pm$  0.5 mmol/L and 1.92  $\pm$  0.42 mmol/L respectively with p value of 0.000.

The findings in the present study match with the findings of Kalayanarooj who determined mean TC for DF, DHF I, II, III and IV as 4.35 mmol/L, 2.82 mmol/L, 2.11 mmol/L, 2.13 mmol/L and 0.85 mmol/L respectively.<sup>22</sup> She also proposed the cut off value of 2.57 mmol/L as a marker of plasma leakage. Suvarna et al found mean TC values of 3.64  $\pm$  1.2 mmol/L for DF and 2.76  $\pm$  0.55 mmol/L and 2.41  $\pm$  0.66 mmol/L for DHF without and with shock respectively.<sup>21</sup> In addition, he also linked low cholesterol level to thrombocytopenia and hepatic dysfunction.<sup>21</sup> He has also proposed that high cholesterol level may play a beneficial role on interaction with the immune system.<sup>21</sup>

In 2002, van Gorp also came up with similar findings and reported mean TC for DHF patients with and without shock as 2.35 mmol/L and 3.1 mmol/L respectively.<sup>23</sup> The Indian study on antioxidants and other markers for dengue patients described similar trend of serum cholesterol.<sup>31</sup>

## Conclusion

In this study low serum total cholesterol strongly correlated with disease severity in dengue fever. Serum total cholesterol should be routinely done for all dengue patients to identify those at high risk.

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