DISCUSSION

Dengue is an arboviral infection with the largest incidence worldwide and is now endemic in most parts of India. Dengue fever has been recognized for many years in India, since the outbreak of dengue occurred in 1912 in Kolkata (Kennedy, 1912). In south India, all the four serotypes of dengue virus were first isolated from febrile patients in Vellore, Tamil Nadu between 1956 and 1966 (Meyer et al., 1964 and Meyer et al., 1969) and the existence of dengue virus infection was established in Tamil Nadu as early as 1960. Of the 30 districts in Tamil Nadu, dengue cases have been reported from 29 districts between 1998 and 2005 which included DF/DHF outbreaks in Chennai in 2001 (Kabilan et al., 2005), Nagercoil and Tiruchirapalli in 2003 (Victor et al., 2007) and DF outbreaks in Krishnagiri and Dharmapuri districts in 2001 (Victor et al., 2002). Due to increased laboratory diagnostic facilities, there was an increase in the reporting of dengue fever cases in Tamil Nadu. A total of 128 cases and 5 deaths were reported in 1998 which increased to 1600 cases and 12 deaths in 2003 and 1150 cases and 8 deaths in 2005. This increasing trend of dengue fever cases in Tamil Nadu poses a need for the early diagnosis of dengue infection. Clinical expression of dengue virus infection varies widely from asymptomatic to dengue shock syndrome (Gubler, 1998). Despite its clinical variability, the acute phase of dengue begins with fever that is indistinguishable from the initial phase of other acute febrile infectious disease (Kalayanarooj et al., 1997, Gubler 1998). The clinical symptoms of dengue fever mimic much other febrile illness such as typhoid, leptospirosis, rickettsial infections etc. that are commonly prevalent in our state. For the diagnosis of dengue fever, in these circumstances, certain biochemical and haematological
parameters for confirming the clinically suspected cases of dengue and in turn for the management of dengue cases are necessary.

There is direct and indirect evidence of biochemical alteration related to severity of dengue. There are reports showing the elevation of serum level of transaminases such AST and ALT (Wang et al., 1990, Kuo et al., 1992, Kalayanarooj et al., 1997, Nyugen et al., 1997, Wong and Shen 2008), Lactate dehydrogenase (Krippner et al., 1990; Setiawan et al., 1998 and Yusuf et al., 1998) and creatine kinase (Ganscon et al., 1998) in dengue patients. Similarly cross-sectional studies have shown differences in the serum level of cholesterol and triglycerides associated with severe forms of dengue infection (Alvarez et al., 1985; Ray et al., 1999). Hitherto, there is no comprehensive document of biochemical parameters that are altered due to dengue viral infections. Therefore, the present study aims to document certain parameters which could pave the way for the clinicians to effectively manage the cases in time.

For this purpose, the present study has been undertaken in Salem, Tamil Nadu. Salem was chosen as study area because a high incidence of dengue cases has been reported since 2000 and large number cases were diagnosed and treated in major hospitals. To fulfill the purpose of the study, three groups of people were selected. The first group consisted of patients with clinically suspected dengue infection. In general, the diagnosis of dengue is typically made clinically on the basis of reported symptoms and physical examination mainly in endemic area (Whilehoen and Farror, 2010). The clinical cause of classic dengue fever has been well described by Siler (1926) and Sabin (1959). The classic symptoms are abrupt onset of fever along with headache and severe retro orbital pain, severe myalgia and arthralgia in the back and loin. According to WHO (2009), a probable
diagnosis is based on the findings of fever plus any two or more of the following; nausea and vomiting, rash, headache, myalgia, arthralgia, low white blood cell count, positive tourniquet test or any warning sign such as abdominal pain, liver enlargement, mucosal bleeding, high haematocrit with low platelet and lethargy. Therefore, in the present study, 254 patients presented with any two or more of the above mentioned clinical symptoms were clinically diagnosed as dengue patients and they were included for the study as dengue probable patients. To rule out the other infections in these patients, a differential diagnosis of malaria, leptospirosis and typhoid fever was done and none of the patients was found to be positive for any of these diseases.

A second group of study population included 50 patients with other febrile illness. In general, it is difficult to distinguish dengue patients from patients with malaria, leptospirosis or typhoid fever by comparing the clinical symptoms since most of them are overlapping. It is also known that liver dysfunction is common in all these patients. In order to distinguish biochemical pattern of liver injury in patients with dengue from patients with other febrile illness, the patients of other febrile illness were included in the study. The third group of the study consisted of 50 healthy individuals belonging to all age groups as control group.

5.1 Serodiagnosis of dengue infection

Clinical diagnosis is not very reliable in dengue infection as there is a wide variation in the presence of various symptoms. Therefore, the use of clinical case definition may result in inaccuracies. Four leading manifestations in dengue fever are fever, haemorrhagic manifestation (mostly positive Tourniquet Test), headache and leucopenia. In the present study it was found that fever was common in both dengue probable patients and patients with OFI. The occurrence of
headache was surprisingly more common in OFI patients than dengue probable patients. Tourniquet Test, which is considered as a simple clinical tool in assisting the diagnosis of dengue fever and other dengue infections could not be done in the present study which is considered as the limitation of the study. Hence, there was a need in the present study to confirm the clinical diagnosis of dengue infection in the laboratory. The laboratory criteria for confirmation of dengue infection and the disease include the isolation of dengue virus from serum and/or autopsy samples, the demonstration of four fold or greater increase in the titre of IgM or IgG antibody to one or more dengue virus antigens in the paired serum samples or the demonstration of dengue virus antigen in autopsy tissue or serum samples or by detection of viral nucleic acid (Pan American Health Organisation, 1994). There are several studies which reported any two or more of above mentioned techniques to confirm the diagnosis of dengue (Rigau Perez 1997; Rossi et al., 1998; Carlos et al., 2005; Lee et al., 2005). However, it is difficult to apply all these techniques all the time as they require well equipped laboratory and man power. Hence, in order to identify the dengue cases accurately, one should take a help of simple and cheap diagnostic test which helps to diagnose dengue infection accurately in the laboratory. In this context, ELISA is closely very promising for the confirmation of clinical diagnosis.

The detection of IgM antibody to dengue virus by ELISA has become one of the most important and useful methods for dengue diagnosis (Dittman et al., 1979, Lam et al., 1987, Gubler et al., 1989; Cardosa et al., 1992). Anti dengue IgM antibodies are produced transiently during primary and secondary infections and the highest levels of IgM are detected following a primary infection (Chen and Wilson, 2010, Guzman et al., 2010). The antibodies develop rapidly and by day 5
of illness most of the patients have detectable anti dengue virus IgM. On average, IgM antibodies fall to undetectable level between 30 and 60 days after the onset of illness (Pan American Health Organisation, 1994). It has been stated by WHO (2009) that in a person with symptoms, the detection of IgM antibody is considered diagnostic. In this context, in the present study, the dengue infection in clinically suspected dengue probable patients was confirmed serologically by the detection of anti dengue IgM antibodies by ELISA. Care was taken to collect the serum samples by the end of the first week of presentation of symptoms and signs (> 5 days) from all the clinically diagnosed dengue probable patients. The Panbio rapid IgM capture ELISA was used in the study which had a good sensitivity and specificity comparable to other assays as reported by others (Sang et al., 1998).

In the present study, IgM antibodies were detected in single specimen. A second sample for the viral serology could not be taken since most of them were out patients and few inpatients were also discharged from the hospital within five to seven days of their admission. There are several studies which confirmed dengue infection based on the detection of IgM antibodies in single serum samples (Wichmann et al., 2004; Ayyub et al., 2006; Malavige et al., 2006; Souza et al., 2007). According to Souza et al., (2004), immune enzymatic methods are more specific and reliable for the confirmation of dengue infections.

The present study revealed that anti dengue IgM antibodies were detected by ELISA in 99 out of 254 clinically diagnosed dengue probable patients. Thus, dengue fever was confirmed in 39% of the dengue probable patients. The importance of laboratory confirmation of clinical diagnosis was reaffirmed, as a number of dengue cases (61%) were misdiagnosed solely on clinical suspicion. However, IgM ELISA also is not sufficient for capturing all positive cases. The
lack of dengue PCR due to the infrastructure constraint stands as a limitation in the present study. Based on the serology, the dengue probable patients were further classified into dengue confirmed (IgM positive) and laboratory negative dengue (clinically diagnosed but IgM negative) patients.

Detection of IgM in 39% of the dengue probable patients in this study was either comparable or higher or lower than the results obtained by other investigators in other parts of Tamil Nadu. Victor et al., (2002) had detected 40% of IgM antibodies in dengue suspected patients in two villages in Dharmapuri district which is geographically adjacent district of Salem, the present study area and their result is in confirmity to the present finding. However, Kabilan et al., (2005), in their study reported that in Chennai, during 2001 epidemic, 74% of the children enrolled in the study had IgM antibodies which was higher than that observed in the present study. In the same period, another study made in Chennai showed that 66% of children had IgM antibodies (Narayanan et al., 2002). On the other hand, Paramasivam et al., (2006) reported only 20% of IgM positive dengue patients in Kanyakumari district during an outbreak of dengue in 2003 which was lower than that observed in the present study. All these studies revealed that dengue infection in Tamil Nadu and the primary or current infection in the state ranged from 20 to 75%.

Like the present study, in many parts of India, detection of IgM by ELISA was widely used as a confirmatory test for dengue fever. Sharma et al., (1999) detected IgM antibodies in 64% of dengue suspected patients in Delhi in 1998 but there was a reduction in the detection of IgM in the subsequent years. In 1999, 2000 and 2001 the percentage of IgM antibodies detected were 27, 24 and
22.5 respectively (Chakravarthy et al., 2002). Shah and Kabra et al., (2005) detected anti dengue IgM in 49 % of patients with suspected dengue in Mumbai.

The detection of IgM antibodies from other parts of the world were 53 % in Japan (Yabe et al., 1996), 46.1 % in Puerto Rico (Rigau-Perez, 1997), 17.5 % in Bangladesh (Amin et al., 2000), 43 % in Japan (Yamada et al., 2000), 60 % in Brazil (Souza et al., 2004), 38.3 % in Thailand (Whichmann et al., 2004), 37.1 % in Bangladesh (Pervin et al., 2004), 78.5 % in Mexico (Espinoza–Gomez et al., 2005), 89.7 % in Philippines (Carlos et al., 2005), 48.75 % in Saudi Arabia (Ayyub et al., 2006), 65.3 % in Srilanka (Wadgue et al., 2006) and 40 % in Colombia (Villar–Centeno et al., 2008).

The age of dengue patients confirmed by serology ranged from 1.5 months to 56 years with a mean age of 21.6± 13.9 years. The children (<15) were 40.4 % and the adults (>15) were 59.5 %. This indicated the involvement of all age groups with the predominance of adults. The finding of involvement of all age groups and mainly adults was consistent with the epidemiological data obtained from other endemic areas. In Kerala, the adjacent state of Tamil Nadu, the mean age of dengue confirmed patients was 42.6 ± 20 years (Daniel et al., 2005). In Lucknow, India, the mean age of the patients was 33 with a range from 7 to 65 years (Itha et al., 2005). Khanna et al., (2004) reported that in Delhi, the mean age of the patients was 35.5 years (range from 20 -67 years). Not only in India, in other parts of the world also, was an involvement of all age groups in dengue infection noticed. In Bangkok, Thailand, Kalayanarooj et al., (1994) reported a mean age as 38 years (range 4- 86 years). In Japan, it was 31.5±10.5 years and it ranged from 18 to 62 years (Itoda et al., 2006). In Karachi, Pakistan, Ahmed et al., (2007) confirmed the dengue infection in patients whose age ranged between 14 and 67 years (mean 31
years). In Taiwan, the mean age of the patients was 63.09 ± 13.48 with a range of 33 – 48 years (Wang et al., 2007). In Srilanka, the mean age of the patients was 30 years (Kularatne et al., 2005). In Brazil, the mean age of confirmed dengue patients was 34.5 with a range of 7 – 78 years (Souza et al., 2004). In Bangladesh, Pervin et al., (2004) reported that the mean age was 29.2±12.9 years. In Singapore Low et al., (2011) observed that the median age of dengue cases was 39 years. It is important to note that in all these studies infants (0-1 year) or young children (1-5 years) were not involved. However, the present study revealed the infection of dengue virus in infants (< 1 year), young children (1-5 years), children (6-15 years) and adults (>15 years). Presumably, this is because in the study area dengue is not yet hyperendemic, thus, older adolescents and adults are still susceptible to new serotype of virus (Halstead, 1997).

The observation of involvement of all age groups in the present study was contrary to most other reported studies, which showed that children were the most affected persons. A study on dengue in Thailand by Kalayanarooj et al., (1997) indicated that all the patients were children with a mean age of 8.01±2.93. In Srilanka, Fernando and Seneviratne (2006) reported suspected dengue infection in a population with a mean age of 2.8±3.5 years. Studies by Guzman et al., (1999) in Cuba and Rojanpithayakorn et al., (1998) in Thailand indicated that young children were mainly affected by dengue. The major burden of the disease in Nicaragua lied in infants and children 5 to 9 years of age (Hammond et al., 2005). In fact, in many countries, it is mainly a pediatric health problem. Interestingly, it was noted in Chennai, Tamil Nadu during 2001 epidemic mainly infants were infected and age group ranged from 1 to 11 months (mean age of 7 months) (Kabilan et al., 2003).
In the present study, the IgM antibodies were more predominant in the age group 0 to 30 when compared to other age groups. This showed that the peak age of contracting dengue was between 1 and 30 years. Kalayanaroooj et al., (1997) and Nimmanitya et al., (1997) reported that peak age lied between 5 and 9 years in Thailand. Ayuub et al., (2006) found that in Saudi Arabia, the commonest age group involved were young adults varying from 20 to 40 years of age. The most susceptible age group of DHF in Bangladesh was 21 –30 years (Islam et al., 2006), 31-40 years in Malaysia (Wito et al., 2006) and 21 – 40 years in Brunei (Osman et al., 2007). According to Hammond, the most affected age group was 5-9 years, which accounted for 58 % of all confirmed dengue cases in the study population in Nicaragua.

It was observed in the present study that male, female ratio of dengue confirmed patients was 0.9:1. This indicated that males and females were equally infected by dengue virus as reported by Ratagiri et al., (2005) in Karnataka. Same observation was made by Daniel et al., (2005) in Kerala who reported that male: female ratio was 1.08:1. Similarly in Thailand, Kalayanarooj et al., (1997) and Witayathawornwong (2004) found that male female ratio was 1:1.03. The male: female ratio was 1.5:1 in Puerto Rico (Rigau -Perez), 1.3:1 in Srilanka (Fernando and Seneviratne, 2006) and 1.5:1 in Saudi Arabia (Khan et al., 2008). However, there are few studies which showed that males were more affected than females (Carlos et al., 2005; Gupta et al., 2005; Ahmed et al., 2008; Ukey et al., 2010; Kumar et al., 2010 ). According to Jain et al., (2011), in India males were affected three times more than females (2.9:1). Garg et al., (2011) also observed a high prevalence of dengue infection among males in Kanpur, India.
Thus in the present study, the diagnosis of dengue by serology documented the occurrence of dengue infection in the study area. It is inferred from the result that all the age groups were infected with a predominance of adults. Both males and females were equally infected. Hence, in the study area, infection with dengue should be highly be suspected in patients presented with fever and other related symptoms. The ELISA for the detection of viral antibodies should be added to the routine investigation to any period with complaints of fever.

5.2 CLINICAL DIAGNOSIS OF DENGUE INFECTION

The clinical signs observed in dengue confirmed patients have included a combination of fever, headache, musculoskeletal symptoms such as arthralgia and myalgia, gastrointestinal symptoms such as nausea, vomiting, diarrhea and abdominal pain, rash, retro orbital pain and hepatomegaly similar to most of dengue fever cases present in endemic areas (Ahmed et al., 2008). Typically, the onset of fever was sudden, with a sharp rise in temperature (39º to 40º C) accompanied by chills and is invariably associated with severe headache. Based on clinical signs and symptoms, all these patients were diagnosed as classic dengue patients. None of the patients developed hemorrhage or shock and hence severe manifestations of dengue such as DHF or DSS were not seen in the study area.

Headache was observed in 47.5 % of dengue confirmed patients. One of the leading clinical manifestation in DF was headache (Sawasdivorn et al., 2001). However, in the present study, only < 50 % of the patients were reported with headache which is lower than usually reported that ranged from 60 to 100 % (Pervin et al., 2004; Daniel et al., 2005; Gonzalez et al., 2005; Khanna et al., 2004; Singh et al., 2005; Itoda et al., 2006; Ahmed et al., 2008). However, there
are reports comparable to the present findings. (Witchman et al., 2004; Ayuub et al., 2006; Wang et al., 2007; Kumar et al., 2008).

The two classic symptoms of dengue namely myalgia and arthralgia were found in 41.4% and 46.5% of dengue confirmed patients. The occurrence of these symptoms are comparatively lower than other reports (Gascon et al., 1998; Haworth et al., 1999; Harris et al., 2000; Pervin et al., 2004; Kularatne et al., 2005; Singh et al., 2005). However, in the present study, about 40% of the dengue confirmed patients were children. In general, these symptoms are mild or asymptomatic in children and children could not verbalize these symptoms. Thus, these clinical features might not be appropriate to apply to children and this might be a reason for observing only < 50% of the patients with these symptoms.

symptoms have been reported previously (Wichmann et al., 2004; Seet et al., 2005; Gonzalez et al., 2005; Ratageri et al., 2005) and highlight the need for medical personnel to consider the possibility of dengue when assessing patients from areas where these symptoms are common. In general, abdominal pain is considered as an early sign of shock in dengue fever (WHO, 1997). In some studies, acute abdominal pain was strongly correlated with DHF and DSS (Guzman et al., 1984; Agarwal et al., 1998.). However, a high proportion of abdominal pain reported in the present study could not be correlated with DHF or DSS since the other important symptoms specific for DHF or DSS such as petechial, gingival bleeding, hematomesis and malena (WHO, 1997) were not observed in the present study. On the other hand, there are reports that show very low incidence of abdominal pain (less than 10%) in DHF epidemics (Agarwal et al., 1999; Pervin et al., 2004).
Retro orbital pain was observed in 42.4% of dengue confirmed patients. This is in accordance with Pervin *et al.*, (2004) who found retro orbital pain in 49.5% of the patients in Pakistan. Khanna *et al.*, (2004) recorded retro orbital pain in 46.6% of the patients in Delhi. Itoda *et al.*, (2006) observed retro orbital pain in 55% of the dengue patients. Kabilan *et al.*, (2003) found retro orbital pain in 27.6% of the infants in Chennai, Tamil Nadu. Even though, the proposed new WHO dengue case definition does not mention the system in its list of clinical features (WHO, 2009), omitting retro orbital pain from the dengue case definition may reduce clinical diagnostic accuracy. It was observed by Gregory *et al.*, (2010) that body aches, joint pain and retro orbital pain were associated with dengue positivity but headache was not associated. Potts *et al.*, (2008) did not observe the relationship between dengue positivity and headache/retro orbital pain. But, when retro orbital pain alone was considered, there was no good correlation between dengue positivity and retro orbital pain. (Nunes *et al.*, 2003; Low *et al.*, 2006). Retro orbital pain has been traditionally considered as non-specific manifestation of dengue fever but according to Gregory *et al.*, (2010), no other acute febrile illness have retro orbital pain listed in the case definition. Retro orbital pain has also been criticized for lacking sensitivity in children as they are unable to express the pain. But, Hammond *et al.*, (2005) documented retro orbital pain in 56% of 1-14 years old children. Thus, it can be inferred that the presence of retro orbital pain in most of the dengue patients was a good predictor of clinical diagnosis in the study area.

In the present study, skin rash was observed in 26.3% of the dengue confirmed patients. In the study by Ahmed *et al.*, (2008) it was noticed in 24% of the DF patients. IraShah and Katira (2005) observed erythematous rash in 14.7%
of the patients. Singh et al., (2005) observed rash in 20% of dengue patients. Kumar et al., (2008) observed it in 14.3% of the patients. Khor et al., (2006) observed rash in 21.4% of the patients. All these findings are comparable to the present observation. There are also studies that reported a high prevalence of rash (more than 82%) in dengue patients (Itoda et al., 2006). In general, high occurrence of rash is correlated with secondary infection.

The nature of rash appeared in the present study was macular or maculo papular. This type of rash is predominantly seen in primary infection whereas petechial rash generally appears frequently in the secondary infection. Thus, it can be concluded that in the present study, all dengue confirmed patients were IgM positive i.e. they all had primary infection. Hence, the rashes were maculopapular. Other studies also reported similar association of rash in dengue fever (Kuberski et al., 1977; Agarwal et al., 1999; Pervin et al., 2004). Petechiae were frequently seen in DHF patients in Combodia (Lam et al., 1998) and Thailand (Cohen et al., 1966).

Liver size is not correlated with disease severity but hepatomegaly is seen more frequently in the cases of shock. In the present study, hepatomegaly was found in 35.4% of the dengue confirmed patients. It is more prevalent in the present study population similar to other studies (Kabra et al., 1999; Mohan et al., 2000; Kabilan et al., 2003; Kalayanarooj et al., 2003). However, it is higher than the results observed in other Indian studies that ranged from 12.1% to 20.4% (Sharma et al., 1998; Daniel et al., 2002; Chhinna et al., 2008).

In a comparison made between males and females in the presentation of clinical symptoms, the present study showed that there was no significant difference in the occurrence of various symptoms between males and females. A
similar report was given by Kalayanarooj et al., (1997) whereas Phoung et al., (2004) in their study found that females were more susceptible to dengue infection than males.

The present study reported variation in clinical features of dengue by patient’s age. Children were more prone to myalgia, nausea, abdominal pain and diarrhea than adults. Differences in occurrence of clinical symptoms between children and adults have been reported earlier (Kalayanarooj et al., 1997; Deparis et al., 1998; Harris et al., 2000; Hammond et al., 2005; Suwandono et al., 2006; Kittigul et al., 2007; Ramos et al., 2008; Gregory et al., 2010; Arshad et al., 2011).

But, WHO case definition makes no distinction between pediatric and adult patients. In the present study, the mechanism for the occurrence of few symptoms in children with high frequency rather than in the adults is not known. However, the other symptoms such as headache, arthralgia, rash, retro orbital pain and hepatomegaly were equally expressed by both adults and children. In contrast, Wichmann et al., (2004) and Hanafusa et al., (2008) observed that headache was more common among adults and the rash with itching was more common in children than adults. Moreover, adults were less likely to have hepatomegaly (Garcia –Rivera and Rigau –Perez, 2003).

Potts and Rothman (2008), in their systemic review identified 15 studies that have examined the difference in clinical features between dengue and OFI. The result of our study also revealed that there was a difference in the frequency of clinical presentation between these two groups. Myalgia, arthralgia, vomiting, rash and retro orbital pain were more common in dengue confirmed patients than OFI patients. Similarly, Low et al., (2011) found that arthralgia, nausea and vomiting were more common in patients with dengue as compared to OFI patients.
Kalayanarooj et al., (2007) found that patients with dengue virus infection were more likely to report anorexia, nausea and vomiting than patients with OFI. In contrast to the present finding, Wichmann et al., (2006) stated in their study population, rash was the only clinical symptoms significantly more frequently observed in patients with confirmed dengue infection when compared to febrile illness without dengue.

The present study showed difference in the frequency of presenting clinical features between patients with dengue confirmed and laboratory negative dengue patients. Myalgia, rash, retro orbital pain, abdominal pain, diarrhea and hepatomegaly were more frequent in dengue confirmed patients than laboratory negative dengue patients. Similar observation was done by Gregory et al., (2010) who found that retro orbital pain, rash, joint pain and body aches were significantly higher in laboratory negative patients.

Other symptoms such as sore throat, congested pharynx and rhinitis reported by other studies (Nimmanitya et al., 2002; Daniel et al., 2005; Malavige et al., 2006) were not found in the present study. Since these symptoms are very common manifestation of Influenza, absence of these symptoms may be useful in differentiating between the two types of fever. Mucosal bleeding was also not recorded in the present study, which was commonly observed in Thailand (Phoung et al., 2004) and in Philippines (Hayes et al., 1988). However, mucosal bleeding is generally higher in DHF patients than dengue fever patients.

The focus of the present study was to identify clinical observation that could distinguish acute dengue infection from OFI. The analysis and comparison of clinical presentation among dengue confirmed, laboratory negative dengue patients and OFI patients suggested that myalgia, arthralgia, rash and retro orbital pain were
more specific for dengue infection than OFI. Hence, the study suggests that any of these four symptoms along with fever would help clinicians to get a concrete idea about the diagnosis of dengue infection at an early stage along with other laboratory parameters.

5.3 Haematological investigation of dengue infection

One of the key clinical manifestations in dengue disease is thrombocytopenia (Halstead, 1997). It occurs due to decreased production and increased destruction of platelets (Phanichyakam et al., 1977). The degree of thrombocytopenia appears correlated with the clinical severity of DHF (Edelman et al., 1975). However, a significant fraction of dengue fever patients also develop thrombocytopenia (Srikiatkachorn and Green, 2010). The haematological analysis of the present study indicated that 39 % of dengue confirmed patients had thrombocytopenia. Many other studies support the present findings that a significant proportion of classic dengue patients also develop thrombocytopenia. Kalayanarooj et al., (2002) reported thrombocytopenia in 50.2 % of the DF patients. Shah et al., (2005) and Whichmann et al., (2006) also observed it in 50 % of the patients. Llamas et al., (2005) reported thrombocytopenia in 37.7 % of the patients. Itoda et al., (2006) observed it in 57 % of patients. On the other hand, low percentages (< 25 %) of the patients with thrombocytopenia were also reported (Pervin et al., 2004; Banerjee et al., 2008; Villar-Centeno et al., 2008). A significant fraction (39 %) of the dengue confirmed patients with thrombocytopenia in this study is thought to be due to direct infection of megakaryocytes by virus leading to increased destruction of the platelets or the presence of antibodies directed against platelets (Lin et al., 2001).
In the present study, it was found that there was no significant difference in the level of platelets between children and adults. Jain et al., (2011) also revealed the same and they explained that disease severity and platelet count were common in adults and in children. However, Hammond et al., (2005) reported that there was a significant difference in the occurrence of thrombocytopenia between adults (39 %) and children (62 %). Kabilan et al., (2005) in their study reported that mean platelet count for infants was significantly lesser than that for old children. However, in the present study thrombocytopenia was not correlated well with age.

It was observed in the present study there was a significant decrease in platelet count in dengue confirmed patients when compared to laboratory negative dengue patients. It can be stated that IgM positive serology is correlated with thrombocytopenia. Same observation was made by Chadwick et al., (2006) in Singapore who found a positive correlation between thrombocytopenia and IgM positive serology.

Comparison of thrombocytopenia between dengue confirmed and OFI patients indicated that it was more common in dengue infection than OFI. Similar statement was given by Low et al., (2011) who found that the mean platelet count (x 10^3/µl) of dengue patients were significantly lower than those of OFI patients. Similar observation was made by Gregory et al., (2010) in their study on clinical and laboratory features that differentiate dengue fever from OFI in Puerto Rico. Similarly thrombocytopenia along with leucopenia was reported in 40 % of dengue positive cases while only 3.1 % of seronegative OFI had thrombocytopenia (Whichmann et al., 2006). Chau et al., (2011) also observed a significant difference in the mean platelet count between dengue confirmed and OFI patients.
However, Malavige et al., (2006) reported that there was no significant difference in the level of platelet between the dengue confirmed and other study groups.

Leukopenia is one of the manifestations of dengue listed in the current WHO case definition. In the present study, the mean count of WBC less than 1000/µl in dengue confirmed patients was 5.9 ± 1.1 which was significantly lower than the control group. It was also noted that 16 % of dengue confirmed patients developed leucopenia. Similar result was obtained by Rongrungruar and Leelarasamee (2001) in DF patients in Thailand. However, there are many reports with high percentage of dengue fever patients with leucopenia. Qureshi et al., (1997) observed leucopenia in 34% of dengue fever patients. Kalayanarooj et al., (2002) observed in 77.7 % of dengue fever patients in Thailand. Liu et al., (2003) observed leucopenia in 66.1% of dengue fever patients. Ayyub et al., (2006) found it in 58.97 % patients studied. Itoda et al., (2006) reported leucopenia in 71% of the dengue fever patients. Whichmann et al., (2006) found leucopenia in 53.2 % of patients with acute phase of illness. On the other hand, none of the patients with leucopenia was observed by Banerjee et al., (2008) in New Delhi, India. It is generally stated that leucopenia is a predictor of dengue infection in early stages only among adults aged ≥ 20 years. Since, leucopenia is a common clinical finding in many other viral childhood infections ( Karavanaki et al., 2006) and children are tend to acquire an average of 6 to 8 viral infection annually (Monto,1995). Leucopenia may not be a good early predictor of dengue among children. According to the present finding, leucopenia was more common in adults than in children and the decrease in WBC count between these groups was significantly different. Although, a previous study from Thailand did identify leucopenia as a good predictor of dengue infection in children (Kalayanarooj et al., 1997), study
from Nicaragua (Hammond et al., 2005), Puerto Rico (Gregory et al., 2010) and Singapore (Low et al., 2011) found a similar report to the present study that leucopenia was significantly associated with early dengue infection in adults but not in children.

In the present study, a significant difference in the mean value of WBC count was observed between dengue confirmed and OFI patients. A study conducted in Vietnam (Chau et al., 2010) revealed that children with primary dengue had significantly lower WBC count than children with OFI. Similarly observation was made by Whichmann et al., (2006) between travelers with confirmed dengue and OFI. Low et al., (2011) found that the mean WBC in dengue patients was significantly lower than OFI patients. Significant difference in the mean WBC count between dengue confirmed patients and patients with scrub typhus were noticed by Watt et al., (2003).

Thus, from the present study it can be inferred that the percentage of dengue confirmed with leucopenia was much lesser as compared to other studies and this can be explained by the fact that the infection was caused by less virulent virus. The finding that a significantly higher proportion of adults with leucopenia than children lead to a conclusion that the presence of leucopenia with febrile illness in adults should trigger a differential diagnosis of dengue for further laboratory confirmation.

Given the fact that ESR is maintained within normal limits in dengue patients, this easily performed test could become an important tool to confirm clinical suspicion of dengue. To evaluate the fact, the patients with confirmed dengue and OFI were tested for ESR. It was found that ESR was altered in 11.1 % of dengue confirmed patients and there was no significant difference between
males and females or children and adults in the alteration of ESR. Thus, the present study proved that due to the high frequency of normality, ESR can help in the differential diagnosis of dengue. This statement is also in accordance with Souza et al., (2008) who analysed 1398 cases of dengue and found high frequency of normality in these patients. Supporting evidence was also given by Kalayanarooj and Nimmanitya (1989) who found alteration in ESR only in a low percentage of dengue patients.

The level of haemoglobin was analysed and it was found that there was a reduction in its level in confirmed dengue patients. Anemia (6-9 g/dl) was observed in 25.3 % of the dengue confirmed patients. Banerjee et al., (2008) observed anemia in 11 % of the patients. Daniel et al., (2005) observed reduced haemoglobin only in 6 % of the patients. On the other hand, Schwartz et al., (1996), Kumar et al., (2008) and Arshad et al., (2011) did not observe anemia in the dengue patients. Moreover, in the present study, it was found that there was no significant difference in the percentage of occurrence of anemia between dengue confirmed and OFI patients. Thus, it can be concluded that reduction in haemoglobin content cannot be considered as a significant factor in the diagnosis of dengue infection.

In case of haematocrit, a significant increase was observed in dengue confirmed patients as compared to the control group in the present study whereas Arshad et al., (2011) did not find any change in haematocrit in dengue patients. However, the difference was not significant between confirmed dengue and laboratory negative dengue patients or between confirmed dengue and OFI patients. Low et al., (2011) reported a significant difference between dengue confirmed and OFI patients.
The haematological features of the dengue patients in the present study revealed that thrombocytopenia and leucopenia are the two important parameters in the early diagnosis of dengue infection and other parameters such as haematocrit and anaemia do not play any role in the diagnosis of dengue infection at an early stage.

5.4 BIOCHEMICAL MARKERS IN THE DIAGNOSIS OF DENGUE FEVER.

5.4.1 Liver enzymes

Liver involvement is known to occur in dengue patients (Mohan et al., 2000; Pancharoen et al., 2002). Many workers have reported acute hepatic failure and acute hepatitis in dengue (Alvarez et al., 1983; Lum et al., 1993; Souza et al., 2002; Vinodh et al., 2005). Poovaram et al., (2006) concluded in their study that dengue virus infection is the major cause of acute hepatic failure in the children and all these children were diagnosed by serology. Deepak and Patel (2006) found that in Mumbai, India, 5 children with positive serology had acute liver failure. The degree of liver dysfunction in the patients with dengue fever varies from mild injury with the elevation of transaminase activity to severe injury with jaundice (Souza et al., 2002). The severity of liver dysfunction varies accordingly to the type of clinical presentation of dengue infection and is more common in patients with complicated dengue (Wahid et al., 2000). Liver inflammation due to dengue infection results in the release of liver enzyme such as AST and ALT, the enzymes that involve in amino acid metabolism. In the acute phase of the disease, an increase occurs in amino transferases, the levels of which subsequently decrease as the liver recovers. Other than AST and ALT, alterations in the levels of other liver
enzymes such as ALP, CPK and LDH in dengue patients were also reported previously (Cam et al., 2001; Wang et al., 2007; Kumar et al., 2008; Villar-Centeno et al., 2008). Thus, the use of liver tests to evaluate the degree of liver damage is of great importance.

Hence, in the present study, the levels of aminotransferases and other liver enzymes in the blood samples from serologically confirmed dengue patients were measured in order to evaluate the impact of dengue virus infection on liver function. Further, a comparative analysis of these enzymes was done among serologically confirmed dengue patients, laboratory negative dengue patients and patients with OFI in order to evaluate the role of liver enzymes as biomarkers in the early diagnosis of dengue infection.

It is important to emphasize that none of the patients included in the study had previous active liver disease. The results of present study revealed that there was an increase in the level of AST (68.68 %) and ALT (49.49 %) in dengue confirmed patients. The level of liver enzymes elevation seen in the present study was lower than those described in the patients with dengue belonged to other parts of India. A study carried out in Delhi, India revealed an elevation of AST and ALT in 80 to 87 % of the patients (Mohan et al., 2000). In Kerala, Daniel et al., (2004) recorded 83.9 % of patients with elevated levels of AST. Another study carried out in Lucknow, India showed an elevation of ALT and AST in 96 % of the study population (Itha et al., 2005). In Mumbai, India, IraShah and Katra (2005) reported that 73.5 % of patients had elevated levels of AST and 67.6 % had high levels of ALT. In all these studies, elevation of AST was noticed from 73.5 to 96 % of patients and ALT elevation was noticed from 68 % to 85 %. Thus, in the present study, the elevation of AST (68.68 %) and ALT (49.49 %) were lower than
the findings in other parts of India. It can be inferred from this observation that the severity of infection in the study population was low.

In several countries, an elevation of AST and ALT was noticed in 45% to 90% of dengue confirmed patients; 84% of AST and 70.8% of ALT in Puerto Rico (Perez et al., 1997), 97.7% of AST and 37.3% of ALT in Thailand (Nugyen et al., 1997), 90% of AST and 77% of ALT in northern Queensland, Australia, (Haworth et al. 1999), 24% of ALT in Malaysia (Wahid et al., 2000), 78.5% of AST and 55.3% of ALT in Thailand (Kalayanarooj et al., 2002), 63.4% of AST and 45% of ALT in Brazil (Souza et al., 2004), 52% of AST in Thailand (Wichmann et al., 2004), 88% of both AST and ALT in Srilanka, (Kularatne et al., 2005), 66.7% of both AST and ALT in Saudi Arabia (Ayyub et al., 2006), 78% of ALT in Japan (Itoda et al., 2006), 90.6% of AST and 71.7% of ALT in Singapore (Wong and Shen 2008) and 92% of AST and 85% of ALT in Brazil (Daher et al., 2010). Thus, most of the data on the percentage elevation of AST and ALT in dengue patients obtained from different parts of the world were higher than the values obtained in the present study.

The mean AST level in dengue confirmed patients in the present study was 109.94 U/L. This was higher than that of ALT (71.48 U/L) level. Similar results were reported by others. Villar-Centeno et al., (2008), in their study in Colombia found that the mean levels of AST and ALT were 90.2 U/L and 69.7 U/L respectively. Souza et al., (2004) found that in Brazil the level of AST (93.3 U/L) was higher than ALT (86.0 U/L). Kalayanarooj et al., (2002) reported that, in Thailand AST and ALT levels were 109 U/L and 53 U/L respectively in dengue patients. When complications arise or severity of the disease increases, there will be a very high elevation of these enzymes. Wang et al., (2007) reported that
dengue patients with acute respiratory failure had very high level of AST (1173.18 U/L) and ALT (730.82 U/L). In Lucknow India, there is a study which showed that the mean level of AST was 1326 U/L and ALT was 932 U/L (Kumar 2008). In a prospective case control study in Vietnam, Cam et al., (2001) reported a significant increase in the levels of AST (2751 U/L) and ALT (984 U/L) in dengue patients with encephalopathy. Thus, it is inferred that abnormal increase in the level of aminotransferases occur in dengue patients with complications and the increase in the levels of these enzymes in dengue infection patients were always significantly lower than that in patients with DHF or DSS (Chhinaa et al, 2008). The mean levels of aminotransferases obtained in the present study once again proved that the disease severity in the study area was mild.

In dengue infection, elevation in serum AST level tends to be greater than ALT level (Souza et al., 2002, Kalayanarooj et al., 1997). This differs from the pattern of viral hepatitis in which ALT levels are usually higher than or equal to AST levels (Gholson et al., 1990). It has been suggested that it may be due to excess release of AST from damaged myocytes during dengue infection (Chung et al., 1992). In the present study also it was noted that a preferential elevation of liver enzyme with AST being significantly high than ALT. Hence, the greater elevation of AST than ALT level is considered to be useful for differential diagnosis of acute hepatitis caused by A, B or C viruses and this abnormality may act as an early indicator of dengue infection. However, there was also a study which stated that the magnitude of elevation of AST and ALT levels were comparable and no preferential elevation of one of these enzymes was observed (Itha et al., 2005). In view of this type of biochemical pattern, it is possible to confuse liver involvement in dengue fever with typical acute viral hepatitis, and if
such situation arises, other diagnosis such as the presence of thrombocytopenia and persistence of fever after the appearance of jaundice should help to make a diagnosis of DF. Moreover, unlike chronic liver damage with Hepatitis B and C viruses the abnormalities in liver enzymes tend to become normal level very soon in dengue patients (Panchaoren et al., 2002). This fact can also be observed in the differential diagnosis of dengue infection.

The present study reported an alteration of AST level alone in 26 % of DF patients and ALT alone in 7 % of the patients. Souza et al., (2004) observed altered levels of at least one of the enzymes in 44.5 % of the patients and they grouped these patients into grade B based on hepatic involvement in these patients. In the present study, rise in transaminases level was mild to moderate in most cases. Greater than two fold increase of AST and ALT was observed in 48.5 % and 30 % of dengue confirmed patients respectively. Kalayanarooj et al., (1997) also reported that about 50 % of the DF patients had greater than two fold elevation of AST and 20 % had > 2 fold elevation of ALT level. The extreme AST level elevation (greater than 10 fold) was observed in only one person (1 %) in the present study. None of the patients was found with > 10 fold elevation of ALT in this study. Whereas Souza et al., (2004) in their study observed > 10 fold elevation of AST in 3.4 % of the patients and ALT in 1.8 % of the patients. Kuo et al., (1992), Kuo et al., (2008) found > 10 fold elevation of AST and ALT in 11.1 % and 7.4 % of the patients respectively. Thus, comparatively a low percentage of extreme enzyme level elevation for AST indicated a mild liver damage and inturn the dengue severity in the study area was mild. As observed in the present study, Souza et al., (2004) and Kuo et al., (2008) also found that an extreme level elevation was greater for AST than for ALT.
In this study it was observed that there was no significant difference in the mean aminotransferase level in males versus females. Similar observation was made by Souza et al., (2004) in Thailand. However, the percentage of females with altered AST and ALT were significantly higher than males. This could be related to the frequency of liver damage. Souza et al., (2004) also found that liver damage was more frequent (74.6 %) among females than males.

Although, the present study reported an elevation of aminotransferase in the patients with DF and the mean values of AST and ALT were higher than normal range, literature on AST and ALT elevation in DF showed that these values were comparatively lower than the values reported by other studies. It is important to emphasize here that none of the dengue confirmed patients had Jaundice as a clinical sign. Hepatic dysfunction in dengue depends upon disease severity. Wahid et al., (2000) noticed hepatic dysfunction in 12 % of DF patients. As there was a possibility of developing hepatic dysfunction when aminotransferases reached very high levels (Petdachai, 2005), here it can be concluded that the comparatively low elevations of AST and ALT must be the reason for not observing jaundice in any of the dengue confirmed patients.

Comparison of aminotransferase levels among study groups indicated that there was a significant difference in both AST and ALT levels between dengue confirmed patients and laboratory negative dengue patients and also between dengue confirmed and patients with OFI and moreover there was no significant increase in AST and ALT level in laboratory negative dengue patients and patients with OFI as compared to control group. Thus, it was proved that liver involvement was seen only in dengue confirmed patients and not in other two study groups. A similar observation was made by Kalayanarooj et al., (1996) in Thailand. A
significant higher level of AST was observed in DF patients (63.07 %) than in patients with OFI (15.1 %). Villar-Centeno et al., (2008) also made a similar observation in Colombia and reported that the AST and ALT levels in DF patients were 90.2 and 69.7 U/L respectively and in OFI patients they were 49.8 IU/L and 33.5 IU/L. The difference in these values among the two groups of these patients was statistically significant. The significant differences observed in the elevation of aminotransferase levels among dengue confirmed, laboratory negative dengue patients, and OFI patients in the present study and also in previous studies tend to conclude that the significant alteration in aminotransferase levels can used as an indicator in the early diagnosis of dengue infection.

The mechanism of liver injury in dengue infection remains unclear. Studies have proved the multiplication of virus in liver cells. The dengue virus has been isolated from liver of fatal cases (Burke, 1968; Sumarmo et al., 1983; Rosen et al., 1989). Dengue virus antigens and nucleic acid were detected in liver tissues by using immunochemistry hybridization and polymerase chain reaction (Kangwanpong et al., 1995; Miagostourch et al., 1997; Jessie et al., 2004). It is unclear whether or not the virus multiplies in the hepatocytes. According to Aurne et al., (2001), the dengue virus is able to replicate in the hepatocytes and Kupffer cells. A report on the accumulation of NSI protein in the liver (Alcon et al., 2005) tempted to speculate that the non structural protein could have a role in liver damage and that the amount of NSI accumulated would depend on the virulence of each particular strain and serotype infecting the liver cells.

Involvement of liver in dengue infection was again proved by observing an alteration in the levels of other liver enzymes such as ALP, LDH and CPK in the present study. The mean value of ALP in dengue confirmed patients was
147.6±106. This is in accordance with the mean value of ALP (122.44±47)
in their study in Brazil reported that the ALP level in one patient was 139 U/L. The
ALP concentration obtained in the present study was lower than the values
reported from Vietnam (279 U/L) and Lucknow, India (957 U/L) (Chhina et al.,
2008; Kumar et al., 2008), but higher than the values observed in Malaysia (93.3
U/L) and in Taiwan (101 ± 46 U/L) (Wahid et al., 2000; Wu et al., 2003). Another
study conducted in Taiwan by Wang et al., (2007) also reported an insignificant
raise in ALP level (78 U/L) in dengue patients.

In the present study, the alteration in the level of ALP occurred in 56.56 %
of dengue confirmed patients. Kuo et al., (2008) found an abnormal level of ALP
in 16 % of the study population in Bangkok, Thailand which was much lower than
the values obtained in the studies. Similarly, Wong and Shen (2008) also found an
increase of ALP in very low percentage (5.51%) of dengue patients. In contrast to
this, an abnormal level of aminotransferases and ALP was observed in 80 and 87%
of dengue patients. However, it is stated earlier that increase in alkaline
phosphatase was unrelated to the severity of the clinical status (Souza et al., 2007).

The mean value of LDH obtained in the present study was 322.50 U/L.
This value was lower that the value obtained by Villar-Centeno et al., in Colombia
(562.3 U/L), but similar results was obtained in Japan (336 IU/L) by Itoda et al.,
(2006). The increase in the level of LDH was observed in 43.43 % of the patients
in the present study which was lower than the percentage obtained in Japan (71 %)
(Itoda et al., 2006). However, elevation of LDH was found only in 20.5 % of
patients in Saudi Arabia (Ayyub et al., 2006). The increase in the level of LDH in
The present study is due to liver damage, which is a frequent problem in dengue (Kuo et al., 2008).

The concentration of CPK found in the present study was 321 IU/L in dengue patients and the abnormal level of CPK was observed in 43.43 % of the patients. The CPK level obtained in the present study was comparable to the value obtained by Villar-Centeno et al., (2008). In their study, CPK level was 298.7 U/L in the DF patients but it was significantly higher (549.6 U/L) in DHF patients. It was due to myositis in DHF patients. The high prevalence of elevated CK level was reported by Haworth et al., (2004) in Queens Land, Australia. The alteration in the levels of CPK and LDH may be caused not only by liver damage and also by the damage of the skeletal muscle in DHF patients (Alvarez et al., 1985; Kuo et al., 1992; Ray et al., 1999).

Even though, there were no previous reports on the pattern of elevation of these liver enzymes (ALP, LDH and CPK) among different age groups or between males and females in the present study, an age wise and gender wise analysis of these enzyme was carried out and found that that there was no significant difference in the mean level of enzyme between the groups. However, the percentage of children with altered ALP and CPK was significantly higher than adults. There was no significant difference in the ALP, CPK and LDH levels in male versus female.

The difference in the levels of these three enzymes between dengue confirmed patients and OFI patients were significantly different. Similar observation was made by Villar-Centeno et al., in Brazil (2008).

The alteration in liver enzymes found in the present study indicated that dengue fever is associated with mild to moderate elevation of aminotransferases.
and other enzymes with or without acute hepatitis. These elevations were significantly higher as compared to OFI or laboratory negative dengue patients. Therefore, the use of liver tests to evaluate the degree of liver damage is of great importance and markers such as AST and ALT may be used as early predictors of dengue infection.

5.4.2 Lipid profile

Lipoproteins are thought to have the ability to modify inflammatory immune function and modify the host immune response during infection (Rauchhaus et al., 2000). Hypolipidemia occurs in critically ill patients (Gordon et al., 1996; Wilson et al., 2005) and is an independent predictor of clinical outcome (Gordon et al., 2001). In viral infections, lipoproteins are postulated to bind to viruses, thus neutralizing their ill effects (Sernatinger et al., 1988; Superti et al., 1992). Certain viruses use LDL receptors to enter the cell, thus LDL may compete with viruses for these cellular receptors (Hofes et al., 1994; Agnello et al., 1999). In order to know the lipoprotein status in dengue patients, lipid profile was performed in the present study.

The mean values of TG, HDL, LDL, VLDL and Cholesterol in dengue patients were found to be 215.1, 25.06, 61.32, 29.34 and 150.3 mg/dl respectively and the mean values obtained by Suvarna and Rane (2009) in Mumbai, India were 254.6, 33.04, 56.0, 50.7, and 140.7 (mg/dl) which were highly comparable to the present observation. These values were also comparable to the mean values of TG (128.9 mg/dl), HDL (36.1mg/dl), LDL (33.1 mg/dl and cholesterol (144.8mg/dl) obtained by Villar-centeno et al., (2008) in their study conducted in Colombia. In the present study, > 2 fold decrease of cholesterol, HDL and LDL level were found in 20.2 %, 27.3 % and 10 % of dengue confirmed patients respectively. On the
other hand, >2 fold increase of triglycerides, cholesterol and VLDL were found in 24.2 % and 2% of these patients respectively.

Higher TG, VLDL levels and lower HDL, LDL levels in dengue patients may require further studies to understand the lipid changes in dengue patients. The proposed mechanism for these changes by Von Gorp et al., (2002) explained that cytokines such as tumor necrosis factor (TNF α) and Interleukin L (IL-1) decrease serum cholesterol levels probably by influencing the enzyme hydroxyl methyl glutaryl (HMG) co enzyme A (CoA) reductase. The decrease in HDL levels observed during infection is probably enzyme mediated. TNF may decrease the plasma activity of lecithin cholesterol acyl transferase (LCAT), the enzyme responsible for esterifying free cholesterol in HDL (Ly et al., 1995). The increase in triglyceride levels observed during infection may be the result of an increase in lipolysis (Feingold et al., 1992) and denovo fatty acid synthesis in the liver (Feingold et al., 1989; 1992) or by the interaction between lipids and free radicals which are increased in patients with dengue (Kuo et al., 1992) or accumulation of lipid is a common feature in hepatic injury associated with changes at cellular level that may lead to increase in triglycerides.

There was a significant decrease of cholesterol, HDL and LDL and a significant increase in triglycerides and VLDL patients in dengue confirmed patients as compared to the control group or patients with OFI. However Vilar – Centeno et al., (2008) in their study did not find any significant difference in these values between DF patients and OFI patients. According to them, changes in lipid are related to severity of dengue infection and significant differences in these values were observed only between DHF patients and OFI patients. In other studies also these biochemical markers have been associated with severe form of dengue
such as DHF and DSS but not with classic dengue fever (Ray et al., 1999; Van Gorp et al., 2002; Suvarna and Rane 2009). But, the present study documented the alteration of lipid profile in the classic dengue infection but the mechanism of alteration was not clear. This may be due to nutritional factors (Thisyakorn and Nimmannitya, 1993) or an elevated level of TNF α and IL-1 (Hober et al., 1993 and Van Gorp et al., 2002). But, all these factors were not evaluated in the present study and the present study further suggests to carry out study on evaluation of nutritional aspects, dietary habits and indicators of oxidative stress in classic dengue patients.

Thus, from the analysis of lipid parameters in the present study, it can be inferred that lipid profile changes accompany dengue infection. Cholesterol, HDL and LDL levels could be used as potential predictor of clinical outcome. The role of each lipid parameter in the pathogenesis of dengue infection requires further studies.

5.4.3 Minerals

Hyponatremia (serum sodium level <130meq/L) is commonly found in dengue patients (Varavitha et al., 1973; Ibrahimin et al., 1995; Torres et al., 2004). It may cause convulsions, especially in infants (Pancharoen and Thisyakorn, 2001). Hence, in the present study the mean sodium level of the study population was measured. The mean sodium level of dengue confirmed patients in the present study was found to be 129.38meq/l. Similarly, Mekmullica et al., (2005) found 132.7 meq/l of sodium in dengue patients in Thailand. Lampaopong et al., (2010) also found that the serum sodium level in dengue patients in Thailand was 133meq/L.
There was a significant decrease in the mean value of sodium in dengue confirmed patients when compared either to the control group or to the patients with OFI. Similarly, Mekmullica et al., (2005) also observed that the mean serum sodium levels were significantly lower among the dengue patients when compared to the non dengue patients.

The prevalence of dengue patients with hyponatremia was 58 % in the present study. This value was much higher than the value (18.4 %) obtained by Mekmullica et al., (2005) but almost similar results was obtained by Lumpaopong et al., (2010) who found that it was 61 %. In the present study, the prevalence of hyponatremia was 5 times higher in dengue patients than in OFI patients (58 % Vs 12 %) Similarly, Mekmullica et al., (2005) reported that it was 9.7 times more common in dengue patients than in non dengue patients.

The reason for hyponatremia in classic dengue fever patients was uncertain. However, it might be the consequence of salt depletion, excess water from increased metabolism, decreased renal excretion, transient inappropriate antidiuretic hormone or the influx of sodium into the cells as a result of dysfunction of sodium potassium pump.

In general, dengue infection leads to mild hypokalemia due to poor intake and an increase in renal excretion due to activation of rennin - angitension and aldosterone system secondary to volume depletion. Mild hypokalemia (serum potassium less than 3.5meq/L) in DF patient was reported by Lumpaopong et al., (2010). However, in the present study hypokalemia was not observed. Mean value of potassium in dengue confirmed patients were 5.4 meq/L. The reason for the significant increase in potassium level in dengue patients was not known. Waduge et al., (2006) reported an elevation of serum potassium level in only one patient out
of 26 patients studied in Srilanka. The present study confirms only the hyponatremia in dengue confirmed patients but not hypokalemia. The study also reported a significant increase in chloride level and a significant decrease in calcium level in dengue confirmed patients as compared to OFI patients or control groups.

In conclusion, mild hyponatremia is a common electrolyte disturbance and renal involvement is mild in patients with DF patients. Careful monitoring of electrolytes, acid-base status, and renal function are necessary for the early diagnosis of dengue infection.

5.4.4 OTHER BIOCHEMICAL PARAMETERS

5.4.4.1 Proteins, Bilirubin, Glucose, Creatinine, Urea, Uric Acid and Antioxidants

Hypoalbuminemia is an indicator of severity of dengue infection (Gubler, 1998; Rigau - Perez et al., 1998). Hence, the levels of albumin, globulin and total proteins were measured in the study population. The mean level of albumin found in the dengue confirmed patients was 3.49 ± 1.14 mg/dl and the value was very similar to the value given by Wang et al., (2007) in DF patients. The albumin level was significantly lower as compared to the control group. However Villar – Centeno et al., (2008) did not observe a significant difference in the mean value of albumin between the group of patients with OFI and classic DF patients. But, they observed a significant difference in the albumin level between DHF patients and patients with OFI. In general, albumin levels less than 4 g/dl will be an early indicator of vascular permeability alteration. Thus, hypoalbuminemia observed in the present study might be an indicator of vascular permeability alteration. However, none of these patients in the study had DHF/DSS.
In the present study, 22% of the patients with confirmed dengue had hypoalbuminemia. Espinoga – Gomez et al., (2005) observed hypoalbuminemia in 10% of classic dengue patients. Similarly, Wong and Shen (2008) found that low albumin level in 16.5% of the patients. Several previous studies reported hypoalbuminemia mainly in DHF or DSS patients. Wills et al., (2004) demonstrated among children with DSS markedly reduced plasma concentration of different sized proteins. Khan et al., (2008) showed hypoalbuminemia in 21.9% of DHF patients. Similarly, Daher et al., (2010) observed a significant decrease in the level of serum albumin during the course of illness in DHF patients and they observed hypoalbuminemia in 66% of DHF patients. Rigau – Perez (1997) along with Puerto Rico association of epidemiologist reported low serum albumin in 67.3% of the DHF patients. Thus, it is confirmed from the literature and the results of the present study that only a small proportion of classic dengue patients (<30%) generally show hypoalbuminemia and a marked decrease in albumin is common in dengue severe cases such as DHF and DSS.

The present study reported a significant decrease in mean level of total protein in DF patients as compared to either OFI or control group. Acute hepatic failure is indicated by a defect in the synthesis of proteins (Wang et al., 2007). However, previous studies on the level of total protein in dengue patients are scanty but there was a report stating the low protein level in DHF patients (Rigau – Perez, 1992). In the present study, there was no significant difference in the level of globulin between dengue confirmed patients and control group. Similarly Pachareon et al., (2002) reported an alteration on globulin level in DHF and DSS patients but not in DF patients. Moreover, Wong and shen (2008) reported low globulin level in severe forms of dengue infection only.
Thus, the present finding of very few percentages of DF patients with hypoalbumunemia indicates that this factor could not be the biomarkers of early predictors of classic dengue infection. As stated by Wang et al., (2007) and Villar - Centeno et al., (2008) hypoalbuminemia could be an early indicator of severe plasma leakage and thus development of severe forms of dengue infection such as DHF and DSS.

Dengue induced acute kidney injury is a poorly studied complication. Renal insufficiency was defined as an increase in blood urea nitrogen and serum creatinine. In order to find out the degree of renal injury in classic dengue infection, an attempt was made to assess the level of serum creatinine, urea and uric acid. A significant increase in the mean level of all these three parameters was seen in dengue confirmed patients, thus proved renal insufficiency even seen in classic dengue infection. Jain et al., (2010) reported raised serum creatinine levels in 61 % of the DF patients. However, only a very low percentage DF patients showed increase in creatinine (35.35 %), urea (16.16 %) and uric acid (10.1 %) levels and hence, these parameters cannot be considered as early predictors of dengue infection.

In the present study, liver dysfunction was observed in the form of raised level of liver enzymes. Jaundice was not observed in any of the confirmed patients, However hyperbilirubinemia was observed in 22 % of the dengue confirmed patients and the mean level of bilirubin was 0.653± 0.276 which was significantly higher than the control group. Jain et al., (2010) reported hyperbilirubinemia in 15 % of the dengue cases. Ayyub et al., (2006) found it in 13 % of the DF patients in Saudi Arabia. Wang et al., (2007) reported an increase in bilirubin level in a significant proportion of the patients. Thus it can be inferred from the present study
that in addition to the measurement of liver enzymes level, bilirubin should be measured to increase the accuracy of the laboratory diagnosis of dengue infection.

Thus, study on other biochemical parameters such as Albumin, globulin, urea, uric acid, creatinine and bilirubin revealed that although a small proportion of dengue patients showed alteration in the level of these parameters they cannot be assessed to predict the early diagnosis of classic dengue infection. They are mainly useful to predict the severity of dengue infections.

Viral infection activates the immune system, neutrophils and other cells to produce reactive oxygen species (ROS) as a mechanism of signal amplification for protection (Peterhans et al., 1987). Oxidative stress arises when the balance between oxidants and antioxidants is tipped in favour of oxidants (Sahnous et al., 1997). This phenomenon may be influenced not only by exogenous agents but also by endogenous agents such as virus. There are more evidences that antioxidants play a complex role in viral disease, starting from influences on host cell metabolism, viral replication and extending to desirable inactivating effects on viruses and less desired toxic effects on hosts tissues. (Schwartz 1996; peterhans 1997). Oxidative damage may affect all biochemical compounds including lipids, nucleic acids, carbohydrates and macromolecules of connective tissues (Zwart 1999). The process might cause the loss of fluidity which leads to the destruction of cell membrane because of structural deformity and the production of lipoproteins and their products such as (MDA) and 4–hydroxyl alkenes (4-HAD). Inactivation and removal of these ROS depends on the relations involving a wide spectrum of anti oxidative mechanisms. The capacity of defense mechanism is determined by a dynamic interaction between individual components which
includes vitamins and metabolites such as glutathione and antioxidant enzymes such as superoxide dismutase and glutathione peroxidase (Toyokuni 1999).

There are very few studies which explain the antioxidant status in dengue infected patients, so an attempt was made to evaluate the antioxidant status in dengue infected patients and a comparative analysis was made with laboratory negative dengue patients, OFI and healthy control groups. The present study showed that there was an alteration in antioxidants SOD, GPX, TAS, MDA in the dengue confirmed patients than the other study groups but the difference was not much when compared to laboratory negative dengue patients. There was a significant difference between the confirmed cases and OFI patients, confirmed cases and control group. The study was in accordance with the previous study made by Gill et al., (2004). The level of SOD in the present study was more in confirmed cases suggesting that the generation of superoxide species is greater. This enzyme produces the oxidant species known as hydroperoxides which damages the biomolecules. Serum level of MDA was less in the present study suggesting that lipid peroxidation was higher in dengue infected patients. The level of TAS was low in the present study which is in acceptance with the study made by Klassen et al., (2004). The present study on antioxidants was performed with only few samples as the availability of the samples was less.

The present findings suggest slight turbulence of the antioxidant system that may be a response to or a consequence of the viral inflammatory process. The study may be extended with more number of samples to confirm the antioxidant status in dengue.