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Clinical Severity in Acute Dengue Associates with Increased Plasma Interleukin-10 Levels

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febrile illness.

ABSTRACT:

Background: Dengue viruses, comprising four distinct serotypes, represent the most significant arthropod-borne viral infections globally. As reported by Monath in 1994, these viruses are responsible for more than 260,000 cases of dengue hemorrhagic fever (DHF) and approximately 20,000 deaths annually [1]. Dengue infections can manifest in various clinical syndromes. These encompass an undifferentiated febrile illness, which is more commonly observed in children. Methods: In our study, a subset of participants was chosen from each of the three diagnostic categories: Dengue Hemorrhagic Fever (DHF), Dengue Fever (DF), and Other Febrile Illnesses (OFI) for immunoassay testing. Due to limitations in the volume of available plasma, it was not feasible to conduct all immune response assessments on the same patients' samples. The selection of these subgroup populations was carried out without any knowledge of clinical data, except for their final diagnosis. For each selectedsubject, we included all accessible samples, which ranged from 2 to 6 samples per subject. To serve as healthy controls in the immunoassays, plasma samples obtained during the 6-month follow-up visit from study subjects who had experienced acute dengue virus infection were utilized (n = 25). **Results:** The study involved a total of 55 children who were categorized into three distinct diagnostic groups: 25 children with Dengue Hemorrhagic Fever (DHF), 20 children with Dengue Fever (DF), and 10 children with Other Febrile Illnesses (OFI). Among the 25 children with DHF, the severity was further classified into grades, with 18 children classified as having grade 1 DHF, 2 children with grade 2 DHF, and 5 children displaying grade 3 DHF. This classification allowed for a comprehensive assessment of disease severity within the DHF subgroup. **Conclusion:** Certainly, this study presented compelling evidence of elevated IL-10 levels in children suffering from dengue infection. These heightened IL-10 levels demonstrated a clear correlation with the severity of the disease, effectively distinguishing between Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF).

I. INTRODUCTION

Dengue viruses, comprising four distinct serotypes, stand as the most significant arthropod-borne viral infections ona global scale. Annually, they account for over 260,000 cases of dengue hemorrhagic fever (DHF) and approximately20,000 deaths, as reported by Monath in 1994. These viruses, upon infection, can give rise to various clinical syndromes. These include an undifferentiated febrile illness, more fre- quently observed in children [2], [3]. Additionally, there's dengue fever (DF), characterized by flu-like symptoms such as high fever, headache, retro-orbital pain, myalgias, ab- dominal discomfort, nausea, and vomiting. Lastly, there's dengue hemorrhagic fever

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(DHF), a condition marked by plasma leakage. In its most severe form, DHF can pose a life-threatening risk, as documented by Nimmannitya in 1987.

[4] Plasma leakage represents a prominent clinical feature of Dengue Hemorrhagic Fever (DHF), typically occurring around the time when fever subsides. Our research has fo- cused on understanding the events leading up to this phase to better elucidate its causes. We have discovered that during primary dengue virus infections, specific CD4+ and CD8+ cytotoxic T cells (CTL) are generated. These CTLs have the capability to produce cytokines such as interferon-γ (IFN-γ), interleukin-2 (IL-2), tumor necrosis factors α and β (TNF- α, TNF-β) when exposed to heterologous dengue viruses in vitro. Furthermore, they can effectively destroy dengue virus-infected autologous cells (Kurane et al., 1989, 1990; Mathew et al., 1996; Gagnon et al., 1999)5. CD4+ T cells arecategorized into T-helper (Th) type 1 or 2, depending on theircapacity to produce specific cytokines. Previous research on Dengue Hemorrhagic Fever (DHF) in children has observed elevated levels of TNF-α, soluble TNF receptors, and IFN- γ (Hober et al., 1993, 1996; Bethell et al., 1998; Green et al., 1999). It's noteworthy that IL-10 has been identified as a potent inhibitor of proinflammatory cytokines, including IL-12, IFN-γ, and TNF-α (Trinchieri, 1995). Consequently, the objective of this study was to delve deeper into the rolesplayed by Th1 cytokines like IL-12 and counterregulatory Th2 cytokines such as IL-10. This investigation aimed to shed more light on the potential immunological mechanisms responsible for the onset of plasma leakage observed in DHF.

II. MATERIAL AND METHODS

This project enrolled individuals suspected of having dengue between April 2000 and December 2022. In brief, the study focused on childrenwho presented with fever lasting less than 76 hours and no apparent source of infection [6]. Participants underwent daily venous blood sample collection until the day following defervescence. Additionally, a follow-up blood sample was obtained during an outpatient visit between study days 8 to 14 for serological testing. Furthermore, on the day following defervescence, a right lateral decubitus chest radiograph wasconducted as part of the evaluation process. All procedures were carried out with informed consent obtained from the parents or guardians of the patients. Blood samples were collected into EDTA tubes and they were carefully stored ata temperature of 6°C. Subsequently, the samples were sub- jected to centrifugation at 200g for a duration of 20 minutes. Following this centrifugation step, the resulting plasma was divided into smaller portions, or aliquots, and securely frozen at a temperature of -60°C to preserve them for subsequent analysis [7]. A subgroup of participants from each of the three diagnostic categories-DHF, DF, and OFI-were chosen for immunoassay testing. Due to limitations in the volume of available plasma, it was not feasible to conduct all immune response assessments on the same individuals' samples. The selection of these subgroups was carried out without access to any clinical data aside from the final diagnosis. In each case, we included all accessible samples from each selected subject, ranging from 2 to 6 samples. As a reference group for the immunoassays, we tested plasma samples from the 8-month follow-up visit of study subjects who had experi- enced acute dengue virus infection, and these were treated ashealthy controls (n = 25). Importantly, all sample testing was conducted while maintaining strict confidentiality through acoding system [8].

III. RESULTS

The study encompassed a population of 55 children, dis- tributed across three diagnostic categories: 25 children with Dengue Hemorrhagic Fever (DHF), 20 children with Dengue Fever (DF), and 10 children with Other Febrile Illnesses (OFI). Within the group of 25 children with DHF, 18 were classified as having grade 1 DHF, 2 were identified with grade 2 DHF, and 5 exhibited grade 3 DHF [9], [10]. Ad- ditionally, the study considered factors such as whether the infections were primary or secondary and the serotypes of the isolated dengue viruses. These variables likely played a cru- cial role in understanding the diverse clinical manifestations and outcomes among the studied pediatric population.The study observed that the average plasma levels of IL-10 in children with dengue increased as their illness progressed, reaching their highest point on the day when fever wasat its peak (fever day 0). Interestingly, it was noted that the mean plasma levels of IL-10 were noticeably elevated in children who eventually developed Dengue Hemorrhagic Fever (DHF) compared to those with uncomplicated Dengue Fever (DF) as early as 4 days

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prior to the resolution of fever ($P < 0.05$). This finding suggests a potential association between higher IL-10 levels and the development of DHF in these pediatric patients [11], [12]. Likewise, it was observed that children diagnosed with dengue exhibited significantly higher mean IL-10 levels compared to children with Other Febrile Illnesses (OFI) as early as 1 day before their fever subsided ($P < 0.001$). Furthermore, the maximum levels of plasma IL-10 measured from two days before fever peaked through the fever day itself were found to be correlated with the extent of plasma leakage, as assessed by the pleural effusion index measured one day after fever resolution. This correlation suggests a potential connection between elevated IL-10 levels during the acute phase of dengue and the severity of plasma leakage, which is a critical aspect of the disease. [13], [14]

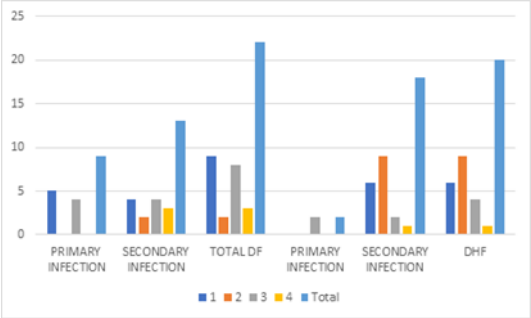

FIGURE 1: Serologic Responses and Virus Serotypes* in Subjects With Dengue Fever and Dengue Hemorrhagic Fever

IV. DISCUSSION

This study provided evidence of elevated levels of IL-10in children affected by dengue infection, and these elevated levels were associated with the severity of the disease, dif- ferentiating between Dengue Fever (DF) and Dengue Hem- orrhagic Fever (DHF), as well as with the extent of plasma leakage quantified by the size of pleural effusions. IL-10 has previously been shown to have inhibitory effects on TNF-α production by human monocytes (de Waal Malefyt et al., 1991) [15], and it also suppresses TNF production in animal models of endotoxemia (Gerard et al., 1993; van der Poll et al., 1997) [16]. It's important to note that the administration of IL-10 to healthy adults has not resulted in clinically signifi- cant adverse reactions (Chernoff et al., 1995) [17]. Therefore, the study suggests that IL-10 itself is unlikely to be the direct cause of the observed plasma leakage in the studied popula-tion. Instead, it highlights the complex interplay of cytokines and immunological factors in the pathogenesis of dengue infection and its associated complications. Our hypothesis re- volves around the idea that in cases of Dengue Hemorrhagic Fever (DHF), IL-10 functions as part of a negative feedback mechanism for proinflammatory cytokines like TNF-α and IFN-γ. However, in DHF, this anti-inflammatory response might be insufficient either due to the quantity of IL-10 produced being inadequate or because its timing in response to the infection is delayed.

It's worth noting that elevated IL-10 levels have been detected in other infectious diseases that are characterized by high levels of proinflammatory cytokine production. This suggests that IL-10 may play a role in regulating the immune response in a way that is both disease- specific and context-dependent. Indeed, there is evidence from other infectious diseases that supports the notion of elevated IL-10 levels being associated with disease severity and outcomes. For instance, in a study on meningococcemia, elevated IL-10 levels were linked to the presence of shock and an increased likelihood of a fatal outcome (Lehmann et al., 1995) [18]. Similarly, in cases of Plasmodium falciparum malaria, high levels of IL-10 were detected in individuals with parasitemia, as opposed to healthy control subjects (Wenisch et al., 1995) [19]. These findings suggest that IL- 10's role in modulating the immune response can vary across different infectious diseases and may have implications for the clinical course and prognosis of these illnesses. IL-10 is known to possess multiple roles impacting the humoral immune response, particularly affecting B-cell differentiation and boosting immunoglobulin secretion from activated B lymphocytes (Rousset et al., 1992) [20]. In our cohort, a no- table observation was the higher prevalence of primary cases of Dengue Fever (DF) compared to Dengue Hemorrhagic Fever (DHF). Given the potential influence of the antibody response

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on our results, we conducted a thorough analysis. To mitigate the potential impact of antibody response, we performed linear regression analyses comparing maximal IL- 10 levels with the pleural effusion index [21]. This analysis was adjusted for various factors, including maximal IgM and IgG levels, hemagglutination inhibition antibody levels, and serologic diagnosis (primary vs. secondary). Despite these adjustments, we observed a persistent and significant relationship between IL-10 levels and the pleural effusion index ($P < 0.01, P < 0.05, P < 0.01, P < 0.01$,
respectively), underscoring the significance of IL-10 in the
context of plasma leakage in dengue infection [22]–[25].

V. CONCLUSION

Indeed, this study provided evidence of heightened IL-10 levels in children afflicted by dengue infection, and these elevated levels correlated with the severity of the disease, distinguishing between Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF), as well as with the extent of plasma leakage quantified by the size of pleural effusions. It's noteworthy that IL-10 has demonstrated its capability to inhibit the production of TNF-α by human monocytes and also to suppress TNF production in experimental models of endotoxemia involving mice and monkeys [26], [27]. These findings suggest that IL-10 plays a pivotal role in regulating the immune response in the context of dengue infection, im- pacting the disease's severity and associated complications.

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CONFLICTS OF INTEREST

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors equally contributed to preparing this article.

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