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Original Research Article

A study of serum interleukin-10 levels in dengue virus infection in a rural population

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ABSTRACT

Background and Objective: Dengue fever is defined as a febrile individual who has travelled to or resides in a dengue-endemic area and has two clinical symptoms. Extrinsic incubation period changes are influenced by a number of variables, including the extent of daily temperature changes, virus genotype, and starting viral concentration. IL-10 may play a role in dengue pathogenesis by inducing an immune-suppressive function that results in IFN resistance, poor immunological clearance, and a long-term infectious impact for acute viral infections. The goal of this study is to learn more about the role of the cytokine IL-10 in dengue patients in order to get a better understanding of the disease, its treatment, and prevention in the coming years.

Materials and Methods: The research was conducted at the SGT Medical College Hospital and Research Institute in Gurugram, Haryana, in the Department of Microbiology. During the study's duration, 80 patient samples, 40 confirmed dengue positive samples, and 40 healthy controls served as negative controls.

Result: A total of 40 dengue positive serum samples were analysed according to the severity of DENV infection into group 1 and we discovered that 90% of the samples represented non-severe dengue infection, while 10% of the samples represented severe dengue infection.

Conclusion: IL-10 is a post-anti-inflammatory cytokine that represents severity in dengue patients and has immune modulatory properties. This study further underlines the need to study and detect IL-10 in the early stages of severe dengue.

1. Introduction

Dengue fever is defined as a febrile individual who has travelled to or resides in a dengue-endemic area and has two clinical symptoms.[1] Nausea, vomiting, rash, aches and pains, a positive tourniquet test, leukopenia, and other clinical symptoms such as stomach discomfort or soreness, recurrent vomiting, clinical fluid build-up, mucosal bleeding, tiredness, restlessness, and liver enlargement are all warning indicators.[1] The appearance of a warning sign may indicate that a patient is suffering from severe dengue fever.[1] Severe dengue with any of the following symptoms is classified as severe dengue: significant plasma leakage leading to shock or fluid build-up with respiratory distress; severe bleeding; or severe organ impairment, such as increased transaminases 1,000 IU/L, altered consciousness, or heart damage.[1]

Dengue fever is a contagious febrile illness spread by mosquitos.[2] Humans get dengue viruses from infected Aedes species through mosquito bites (Aedes aegypti or Aedes albopictus).[1] It is an 11-kilobyte single-stranded enveloped –positive sense RNA virus belonging to the Flavivirus genus and family Flaviviridae.[1] Dengue fever is caused by one of four antigenically distinct serotypes

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of the virus (DENV-1, -2, -3, and -4), all of which have a 65 percent genomic similarity and are found throughout the world in tropical and subtropical regions.[3] As a result, a person can acquire the dengue virus up to four times throughout their lifetime.[1] There are seven non-structural proteins in the genome: NS1, NS2a, NS2, NS3, NS4a, NS4, and NS5.[2] In October 2013, the fifth variety of the dengue virus serotype was identified in Malaysia, adding to the previous four. DENV-5 does not appear to be present in India.[4] After feeding on a DENV-infected person, the virus replicates in the mosquito's midgut before spreading to secondary tissues like the salivary glands.[5] The extrinsic incubation period (EIP) is the time between eating the virus and transferring it to a new host. The EIP takes 8 to 12 days when the ambient temperature is between 25°C and 28°C.[5]

The magnitude of daily temperature fluctuations, virus genotype, and initial viral concentration are all factors that influence extrinsic incubation period variations.[5] The EIP takes 8 to 12 days when the ambient temperature is between 25°C and 28°C.[5] The magnitude of daily temperature fluctuations, virus genotype, and initial viral concentration are all factors that influence extrinsic incubation period variations.[5] DHF is defined by increased capillary permeability, which causes plasma leakage, reduced blood viscosity, and, in extreme cases, shock.[6] A variety of cytokines that promote permeability have been connected to the pathogenesis of DHF.[6] However, the relative importance of these cytokines in DHF is unknown.[6] The pattern of cytokine response may be connected to the pattern of cross-recognition of dengue-specific T-cells.[6] Cross-reactive T-cells generate TNF-, IFN, Interleukins IL-6, IL-8, IL-10, and chemokines, but they appear to be functionally inadequate in their cytolytic effect. In animal investigations, TNF-a has been associated with a number of significant symptoms, including bleeding.[6] Capillary permeability can be increased when the complement cascade is stimulated. DHF has been discovered to have high quantities of complement fragments.[6] For example, C3a and C5a complement fragments, for example, have been demonstrated to improve permeability. The NS1 antigen of the dengue virus has been shown to regulate complement activation and may have a role in DHF development.[6] The cytokine IL-10, also known as cytokine synthesis inhibitory factor (CSIF), is generated by type 2 helper T-cells.[7] Inhibition of immune mediator release, antigen presentation, and phagocytosis are all anti-inflammatory characteristics of IL-10.[7] The cytokine IL-10 has several functions in immune control and inflammation.[8] IL-10 may play a role in dengue pathogenesis by inducing an immune-suppressive function that results in IFN resistance, poor immunological clearance, and a long-term infectious impact for acute viral infections.[8] T cell apotosis has also been linked to IL-10.[9] Dengue fever affects over half of the world's population, or around 4 billion people. In high-risk areas, dengue fever is a frequent source of illness. Dengue fever infects up to 400 million individuals each year. Approximately 100 million people are infected, with 40,000 people dying from severe dengue fever.[10]

The goal of this study is to learn more about the role of the cytokine IL-10 in dengue patients in order to get a better understanding of the disease, its treatment, and prevention in the coming years.

2. Materials and Methods

The research was conducted at the SGT Medical College Hospital and Research Institute in Gurugram, Haryana, in the Department of Microbiology. This research was carried out over a six-month period (September 2019 to February 2020). During the study's duration, 80 patient samples, 40 confirmed dengue positive samples, and 40 healthy controls served as negative controls.Based on the severity of the sickness, 40 confirmed dengue positive samples were divided into subgroups I and II. Patients in Subgroup I (Dengue without or with warning signs) have a non-severe dengue infection. According to WHO categorization, patients in Subgroup II had severe dengue infection, which included significant plasma leakage leading to shock, fluid build-up with pulmonary illness, severe bleeding, and severe organ involvement from IPD and OPD.

The "healthy control" group consisted of 40 healthy people who had not had a fever or other sickness in the previous three months. Patients who had already been diagnosed with malaria, enteric fever, or any infection other than dengue were excluded from the research. The WHO 2009 recommendations were used to estimate all of the samples.[11]

2.1. Methods

Approximately 5 mL of blood was taken aseptically through venipuncture and allowed to coagulate in a plain vacutainer.[6] For serum separation, clotted blood was centrifuged for 15 minutes at 3,000 rpm.[12] The separated serum was aliquoted into sterile storage vials, one of which was used for dengue fever (NS1 Ag) confirmation, and the other was kept for further investigation. For the determination of the cytokine IL-10 level, all blood samples positive for dengue virus were kept at -70 degrees Celsius.[6,13] The "Diaclone IL-10 ELISA kit" was used (catalogue number: 950.060.096). All admitted patients were tracked on a daily basis until they were discharged or died in the hospital. Each experiment contained standards, and the standard curves generated were used to estimate cytokine concentrations.[12]

2.2. Statistical analysis

The data was analysed, and the concentrations of cytokines were expressed as mean and standard deviation (mean

SD). Using the student t-test, the statistical significance of differences in IL-10 levels across several groups (healthy, non-dengue, dengue, severe dengue) was determined. Statistical significance was defined as a P-value of less than 0.05 (0.05).

3. Result

The SGT Hospital at SGT University in Gurugram, Haryana, served as the site of this cross-sectional study. This 750-bed, multi-specialty hospital provides tertiary care. All samples of confirmed dengue NS1 were analysed and compared to healthy people. Results are given:

Table 1: Dengue patients are divided into groups based on their clinical symptoms.

Cases	Number of cases (n=40)	Percentage (%)
Subgroup I	36	90%
Subgroup II	04	10%

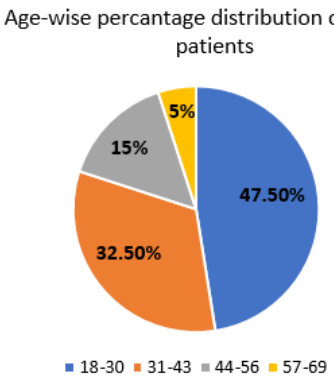

Fig. 1: The percentage of dengue patients by age is seen in this graph

Table 2: Dengue patients are divided into categories based on their age.

Age (Years)	Subgroup I (%) n=36	Subgroup II (%) n=04
18-30	18 (50%)	01 (25%)
31-43	10 (27%)	03 (75%)
44-56	06 (16%)	00 (0%)
57-69	02 (5.5%)	00 (0%)

Table 3: The mean and standard deviation of two subgroups is shown.

Subgroup	Subgroup I	Subgroup II
(Mean ± SD)	32.66 ± 14.22	34.75 ± 8.98

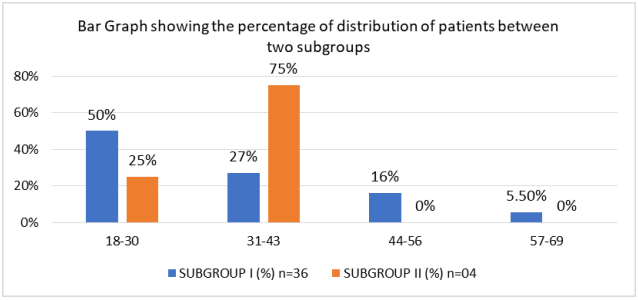

Fig. 2: The proportion of age distribution between groupings is shown.

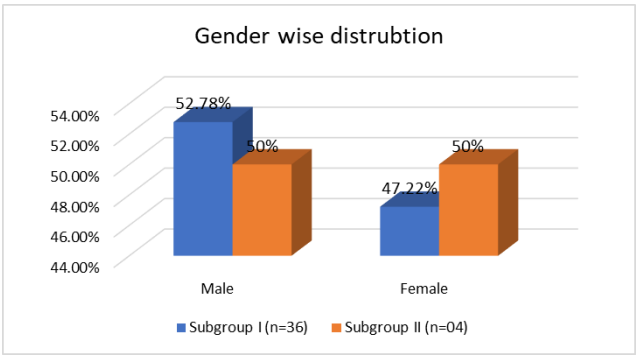

Fig. 3: Gender distribution percentages are shown

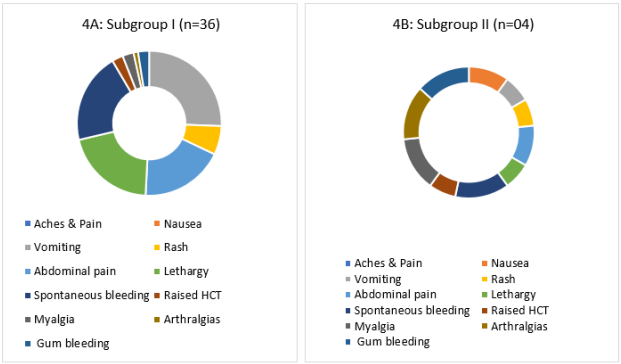

Fig. 4: A and **B** show the clinical appearance of subgroup I and subgroup II, respectively

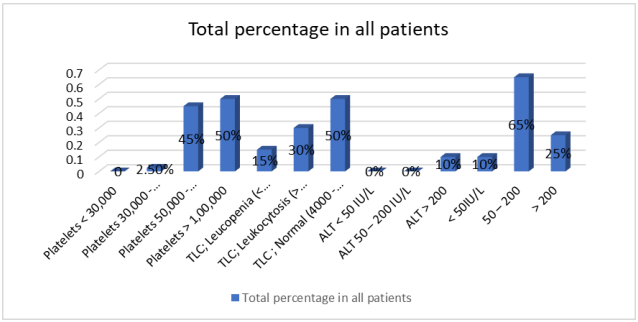

Fig. 5: Displaying a graphical depiction of clinical data in individuals who have been screened

Table 4: Different laboratory findings of patients were distributed amongst the two subgroups

4.1: Platelet count findings		
Clinical Parameters	**Subgroup I**	**Subgroup II**
Platelet count		
< 30,000	00	00
30,000 - 50,000	00	01 (25%)
50,000 - 1,00,000	16 (44.5%)	03 (75%)
> 1,00,000	20 (55.5%)	00
4.2: Total leukocyte count findings		
Clinical Parameters	**Subgroup I**	**Subgroup II**
Total Leukocyte Count		
Leucopenia (< 4000cells/mm3)	03 (8.33%)	03 (75%)
Leukocytosis (> 11,000cells/mm3)	13 (36%)	01 (25%)
Normal (4000 - 11000/mm3)	20 (55.5%)	00
4.3 ALT Findings		
Clinical Parameters	**Subgroup I**	**Subgroup II**
ALT		
< 50 IU/L	04 (11.1%)	00
50 – 200 IU/L	24 (66.6%)	00
> 200	08 (22.2)	04 (100%)
4.4 AST Findings		
Clinical Parameters	**Subgroup I**	**Subgroup II**
AST		
< 50IU/L	04 (11.1%)	00
50 – 200	26 (72%)	00
> 200	06 (16.6)	04 (11.1%)

Table 5: Distribution of IL-10 levels among Subgroup I & II and Groups I & II

IL-10 Range	**Subgroup I (n=36)**	**Subgroup II (n=04)**	**IL-10 Range**	**Group I (Dengue Confirmed) n=40**	**Group II (Healthy Controls) n=40**
<4.8pg/ml	00 (0%)	00 (0%)	<4.8pg/ml	00 (0%)	00 (0%)
4.8-9.8 pg/ml	11 (30.55%)	00 (0%)	4.8-9.8 pg/ml	11 (30.5%)	40 (100%)
>10pg/ml	25 (69.4%)	04(100)	>10pg/ml	29 (72.5%)	00 (0%)

Table 6: Levels of IL-10 (in pg / ml.) in healthy people and people who have been diagnosed with dengue fever.

Cytokine	**Group 1**	**Group 2**	***p Value**
IL-10	Subgroup I =38.60±44.61 Subgroup II =371.75±53.75	1.22± 1.40	$^{\#a}$=<0.0001 $\$b$=<0.00015

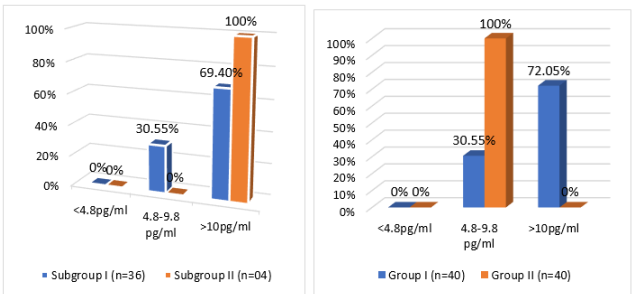

Fig. 6: A and **B** show the distribution of IL-10 in subgroups and groups, respectively

4. Discussion

In our study, a total of 40 dengue positive serum samples were analysed according to the severity of DENV infection into group 1 (subgroup I & subgroup II) as shown in Table 1, and we discovered that 90% (36/40) of the samples represented non-severe dengue infection, while 10% (04/40) of the samples represented severe dengue infection, indicating that non-severe dengue infection is the most common type of dengue infection in the population. Rosenberger et al reported 84.4% (1463/1734) of patients with non-severe dengue and 15.6% (271/1734) of patients with severe dengue in a study published in 2020.[14] Another study published in the year 2020 by Hegazi et al in Saudi Arabia found that 87.6% (15,244/17646) of non-severe and 91.8 percent (223/17,646) of severe dengue cases.[15] According toFigure 1, the highest prevalence of dengue infection based on severity was observed among patients aged 18–30 years (47.5%), whereas the lowest infection rate was observed among senior patients aged 57–69 years

(5%). Haroon et al. from Pakistan published research in 2018 that matched our findings. The largest frequency was found in the age range of 21–40 years, with 38%, while the lowest prevalence was found in elderly patients over 60 years, with 9%.[16] In research published in 2013, Mandal et al. from Eastern India observed a greater frequency of dengue infection in the age group of 18–40 years (67.30%) and a lower prevalence of 32.70 percent in patients over 40 years (32.70%) in research published in 2013.[17]

In our investigation, the prevalence of dengue infection was found to be greater in non-severe dengue between the ages of 18 and 30, at 50%.[18], and severe dengue between the ages of 31 and 43, at 75% [03], as shown in Table 2. In research published in the year 2022 by Pratt et al. from Bangladesh, the prevalence of non-severe dengue fever was found to be 100% in elderly patients above the age of 60, and severe dengue fever was observed to be 97.9% among those aged 41 to 60.[18] In research published in the year 2020 by Rafi et al. from Bangladesh, the prevalence of non-severe dengue was found to be 100% among those aged 41 to 50, and severe dengue was recorded to be 15.4% among those aged 60 and above.[19] The mean and SD of age in severe dengue patients (34.75±8.98 years) were substantially higher in our research (Table 3). Another study based on a retrospective analysis in Saudi Arabia by Hegazi et al in the year 2020 found that the mean and SD of age in severe dengue patients (38.7 ± 13.9 years) were substantially higher than in non-severe cases (35.9 ± 9.8 years).[15] Pereira et al. observed in 2018 that the mean and SD of age in severe dengue cases (34.39±12.0 years) were substantially higher than in non-severe dengue cases (31.96±11.51years).[16] In our study (Figure 3), male patients were found to have a greater prevalence of non-severe dengue infection (52.5%) than female patients (47.5%) in our study (Figure 3). According to research published by Wang et al in 2022, male patients had a greater prevalence of severe dengue (63.2%) than female patients with non-severe dengue (55.2%).[17] According to a study published by Hegazi et al. in 2020, male patients had a greater prevalence of non-severe dengue infection (79.3%) than female patients with severe dengue (22.6%).[15] Another finding from our research was the signs and symptoms that patients in Group 1 had (Subgroup I and Subgroup II). All 40 patients involved in our trial had a high temperature, and nearly half of them complained of aches and pains, nausea, and vomiting.

Subgroup I symptoms include lethargy, stomach discomfort, and spontaneous bleeding. Higher HCT levels, rash, nausea, vomiting, stomach discomfort, and tiredness were noted in more than half of the patients, whereas 100% of the patients in subgroup II had myalgia, arthalgia, gum bleeding, aches and pains, and spontaneous bleeding (Figure 4 A & B respectively). Pereira et al. published research in 2018. Fever was the most common symptom, with 547 (99.5%), followed by headache 263 (47.8%), myalgia 241 (43.8%), vomiting 227 (41.3%), and abdominal discomfort 165 (30%); Petechiae 35 (6.4%); melena 18 (3.3%); hematemesis 8 (1.5%); gum bleed 13 (2.4%); epistaxis 4 (0.7%); haematuria 9 (1.6%); and menorrhagia 7, all symptoms of haemorrhage (4.8%). Some of the individuals had multiple haemorrhagic manifestations.[18] In 2022, Faridah et al. discovered that fever, nausea/vomiting (66.97%), headache (35.79%), and stomach discomfort were the most prevalent symptoms of dengue patients after hospital admission (21.71%). The percentage of DHF patients with nausea/vomiting and stomach discomfort was substantially greater than the DF patients. Fatigue was found to be substantially more common in the DF group than in the DHF group. Haematocrit is a measurement of the amount of red blood cells in the body (50.24%).[19] In our study, platelet counts were normal, i.e. > 1,000,000, in only 5.5 percent of subgroup I patients, while four of the patients in subgroup II had platelet counts between 30,000 and 1,00,000. (Table 4.1). In 2022, Wang et al reported that the platelet count was normal, i.e., 1,00,000 in 52.99 percent of non-severe dengue cases and 30,000 in 63.3 percent of severe dengue cases.[17] In 2021, Reddy et al. published research from Telangana. Platelet counts below 50,000 are found in 10.3% of non-severe dengue cases and 59.3% of severe dengue cases.[20]

The majority of subgroup I patients (55.5%) had normal TLC, whereas 75% had leukopenia (Table 4.2). This is comparable to research by Laul et al., who found leukopenia in 70% and 71% of cases, respectively.[21] Ageep et al. found leukopenia in 90% of patients in another investigation.[22] ALT levels were found to be relatively high; >200IU/L in 100% of patients in subgroup II, while 66.6% of patients in subgroup I had ALT levels ranging from 50 to 200 IU/L (Table 4.3). The AST level revealed comparable results, with 100% of patients in Subgroup II having an AST level greater than 200 IU/L and 72 percent of patients in Subgroup I having an AST level between 50 and 200 IU/L. (Table 4.4). According to research by Pereira et al., severe dengue patients have higher levels of AST (93.1%) and ALT (69.3%) than non-severe dengue patients.[18] Another study by Laul et al. found a similar outcome to ours, with 57 percent of patients having increased ALT and 49% having elevated AST.[21] When compared to the patients in subgroup I (69.44%), serum IL-10 levels were considerably higher (> 10pg/ml) in all (100%) of the subgroup II patients, while none of the group 2 patients demonstrated this level of IL-10. While all group 2 people had normal blood IL-10 levels, just 30.55% of subgroup I patients did. Of the 29 patients in our research with higher serum IL-10 levels, 5/36 were from group I, while 04/04 were from subgroup II (Table 5). Subgroup II patients had a larger mean SD than subgroup I and group 2;371.75±53.73, 38.60±44.61 and 1.22±1.40, respectively, and a significant p value was discovered across

the groups (Table 6). In research published in 2021 by Puc et al., the mean S.D. of severe dengue patients was found to be greater than that of non-severe dengue patients and healthy controls; 1.22±1.40, respectively, with a significant p value.[23] Another study indicated that severe dengue patients had a greater mean SD than non-severe dengue patients and healthy controls 2.1±8.2, 1.0±4.3,0.4±0.6.[24]

5. Conclusion

IL-10 is a post-anti-inflammatory cytokine that represents severity in dengue patients and has immune modulatory properties. 90% of the dengue patients had conventional dengue fever, and ten percent had severe dengue fever. In the study, higher levels of IL-10 were found in dengue patients than in healthy people. This study further underlines the need to study and detect IL-10 in the early stages of severe dengue since this allows for faster disease treatment and reduces morbidity, death, and the global health and social impact of dengue. As a result, it's known as a disease diagnostic predictor.

6. Source of Funding

None.

7. Conflict of Interest

There is no conflict of interest in this study.

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