The Association of IL-1 Beta with Pneumonia

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***Abstract*—Pneumonia is the leading cause of death in children worldwide. The present study is aimed to study weather Interleukin-1 beta has a role in pneumonic infection. The 251 patients and 251 controls recruited and the blood level of the Interleukine-1 beta observed. This study found significantly higher concentration of interleukin-1β in the pneumonic patients than control. The mean concentration of the Interleukin-1β was 48 ± 4.2 pg/ml and 23 ± 2.1 pg/ml for patients and control respectively and the differences were found statistically significant at the level of P<0.0001, the value of t-test was t =119.0 with the degree of freedom 998.**

Key words: ***Pneumonia, inflammation, immune system, interleukin, lungs.***

I. INTRODUCTION

Pneumonia is the leading cause of death in children worldwide [1]. Pneumonia kills an estimated 1.4 million children under the age of five years every year accounting for 18% of all deaths of children under five years old worldwide [2]. Pneumonia affects children and families everywhere, but is most prevalent in South Asia and sub-Saharan Africa– more than AIDS, malaria and tuberculosis combined. Every year, pneumonia causes death of 410,000 children in India. Childhood ARI/pneumonia is a significant public health problem in India, although robust epidemiological data is not available on its incidence [3]. Mortality due to pneumonia accounts for approximately one-fourth of the total deaths in fewer than five children, in India. Pneumonia affects children irrespective of socioeconomic status; with higher risk among young infants, malnourished children, non-exclusively breastfed children and those with exposure to solid fuel use [4]. There is a significant gap in the utilization of existing services, provider practices as well as family practices in seeking care [7]. The present study in aimed to study what are the components of blood and immune system changing after pneumonic infection and how much effect of this infection changes the hematological and immunological profile of infected persons.

II. MATERIALS AND METHODS

Sample Collection

A. Patient recruitment

Medically certified pneumonic patients were recruited from medicine department (OPD) and registered pathology of Chhattisgarh during the year 2011 to 2013. 251 pneumonic patients were recruited for present investigation.

B. Healthy controls

251 randomly selected healthy controls (HC) were enrolled in the study. The control group consisted of medical staff and healthy volunteers from same area individuals residing in central region of India.

C. Sample collection strategy

Approximately 5 ml. of blood sample was collected in 0.5 M EDTA tubes from each pneumonic patient as well as from healthy controls.

III. QUANTITATIVE MEASUREMENT INTERLEUKIN-1 BETA (IL-1B)

A. Principle

Abcam's Human IL-1 beta *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of IL-1 beta in Human serum, plasma, buffered solutions or cell culture medium. A monoclonal antibody specific for IL-1 beta has been coated onto the wells of the microtiter strips provided. Samples, including standards of known IL-1 beta concentrations, control specimens or unknowns are pipetted into these wells. During the first incubation, the standards or samples and a biotinylated monoclonal antibody specific for IL-1 beta are simultaneously incubated. After washing, the enzyme Streptavidin- HRP, that binds the biotinylated antibody is added, incubated and washed. A TMB substrate solution is added which acts on the bound enzyme to induce a colored reaction product. The intensity of this colored product is directly proportional to the concentration of IL-1 beta present in the samples. This kit recognize both endogenous and recombinant Human IL-1 beta [1,6,7].

B. Assay Procedure

a. Prior to use, mixed all reagents thoroughly taking care not to create any foam within the vials.

b. Determined the number of microplate strips required to test the desired number of samples, plus appropriate number of wells needed for controls and standards.

c. Added 100 µL of each standard, including blank controls to the appropriate wells.

d. Added 100 µL of sample and 1X Control Solution to the appropriate wells.

e. Added 50 µL of 1X Biotinylated anti-IL-1 beta to all wells.

f. Covered and incubate for 3 hours at room temperature (18-25°C).

Removed the cover and washed the plate as follows:

1. Aspirate the liquid from each well.
2. Add 300 µL of 1X Wash Buffer into each well Aspirate the liquid from each well.
3. Repeat for a total of 3 washes.
4. Add 100 µL of 1X Streptavidin-HRP solution into all wells, including the blank wells.
5. Re-cover and incubate at room temperature for 30 minutes.

Add 100 µL of Chromogen TMB substrate solution into each well and incubate in the dark for 10-20 minutes at room temperature. Avoid direct exposure to light by wrapping the plate in aluminum foil.

Incubation time of the substrate solution is usually determined by the microplate reader performances: many microplate readers record absorbance only up to 2.0 O.D. Add 100 µL of Stop Reagent into each well. Read absorbance of each well on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm (610 nm to 650 nm is acceptable) as the reference wavelength.

C. *Calculations*

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

IV. RESULTS

A. *The Clinical profile of the patients and control*

The table-1 indicating the characteristics at enrollment in age, residence, and ethnicity Pneumonia and healthy control group. The differences between these two groups in the given characteristic are similar and statistically non- significant to keep two groups similar in all criteria except the disease taken for the study

TABLE- 1 SHOWING THE CLINICAL FEATURES OF PNEUMONIC PATIENTS AND CONTROL PARTICIPATED IN THIS STUDY

SN	Characteristic	Pneumonic Patients	Healthy control
1	No. of subjects	251	251
2	Male female ratio	86:165	91:160
3	Children: Adult	230: 21	200:51
4	Mean Age (in year)	15.7	18.3
5	Age range (in year)	1-24	4-36
6	Mean weight (in Kg)	19.14	21.43

The number of the patients and control taken for the study are 251 for the each group. The male female ratio was 86:165 and 91:160 for case and control respectively. The children: adult ratio between the groups was 230: 21 and 200:51 for case and control. Mean age for case was 15.7 years and for control it was 18.3 adjusted. Mean weight was 19.14 and 21.43 was for case and control respectively.

B. *Association of Interleukin-1β (IL-1β) between pneumonic patients and control*

The serum concentration of IL-1 β of 251 patients and control were measured and the results are presented in the table-2. This study found significantly higher concentration of interleukin-1β in the pneumonic patients than control. The mean concentration of the Interleukin-1β was 48 ± 4.2 pg/ml and 23 ± 2.1 pg/ml for patients and control respectively. The statistical analysis of this differences in the mean concentration of the Interleukin-1β between two groups were analyzed by t test and the differences were found statistically significant at the level of P<0.0001, the value of t test was t=119.0 with the degree of freedom 998. The value of the median was found 4.2 pg/ml and 4.0 pg/ml for the patient and control group respectively. The slandered error of the mean was found 9 pg/ml and 2 pg/ml for patients and control respectively. The present study found range of the concentration of the interleukin-1β is 0.7-1.1 pg/ml in patients and 0.7-1.87 pg/ml in healthy controls.

TABLE II COMPARISON OF BLOOD CONCENTRATION OF IL-1B OF PNEUMONIC PATIENTS TO CONTROL

	Parameters	Pneumonic patients	Healthy controls	t-test P value
1.	Mean ± SD	48 ± 4.2	23 ± 2.1	P<0.0001 *** t=119.0 df=998
2.	Median pg/ml	4.2	4.0	
3.	SEM pg/ml	9	2	
4.	Range pg/ml	0.7-1.1	0.7-1.87	

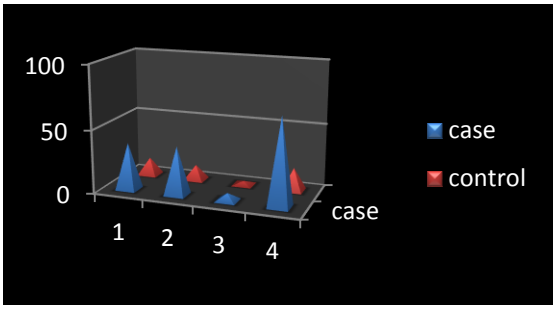

Fig-1 Comparison of blood concentration of IL-1β of pneumonic patients to control

V. DISCUSSION

The inflammatory profile includes the initiator, stimulator, mediators and intermediates of process. The inflammation process is the key event of the pneumonic consequences because inflammation process generated by the immune system is able to reduce the bacterial and viral growth at the infected area therefore differences in inflammatory profile between patients and control may be used as marker and diagnostic parameter.

Pneumonia is the consequence of certain bacterial and viral infections [8]. The main infected sites are lungs. The immune system of the infected person shows protective response

against the pneumonic infections [9]. The principal response of the immune system against the pneumonia is inflammation which helps to protect and check the entry site of the infections [10]. Due to the inflammatory response of the immune system against the pneumonic infections the hematological and inflammatory profile of the patients are changed from the healthy patients [11]. The pneumonic patients showing more amount of the leukocytes, inflammatory mediators and other blood components than healthy individuals [12]. The hematological profiles of pneumonic patients are shown elevated in this study.

VI. CONCLUSION

The interleukin included which directly or indirectly regulate the inflammation response IL-1β. The present investigation found a direct association of Interleukin-1 beta with the pneumonia. The blood concentration of IL-1 beta elevated with the severity of the disease.

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