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Interleukin-12 (IL-12) Analysis in Pneumonia Patients of Rewa Region

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Abstract

The present paper deals the analysis of Interleukin-12 (IL-12) with Pneumonia patients in Rewa region. For this work the blood sample of 240 pneumonic patients and 240 controls have been collected. Compared with control in this study, pneumonic patients found a significant higher amount of interleukin-12. The serum concentration of IL-12 in 240 patients and control were measured and the results are presented. Compared with the control in this study, pneumonic patients are found significantly higher amount of interleukin-12. The average concentration of the Interleukin-12 was 35.20±5.68 pg/ml and 12.15±8.15 pg/ml for patients and control respectively. The statistical analysis of these differences in the average concentration of Interleukin-12 between the two groups were analyzed by t-test and the differences were found statistically significant at the level of P <0.0001, with the t-test value t=73.44 was there with the degree of freedom. The value of median was found 36.45 pg/ml and 10.65 pg/ml respectively for patient and control. Standard error of the mean was found 6 pg/ml and 2 pg/ml for patients and control respectively. In current study, the concentration limit of interleukin-12 is 9.84 – 67.54 pg/ml in patients and 8.10-18.96 pg/ml in healthy controls.

Keywords: Pneumonia, Interleukin-12, Inflammation, ELISA

Introduction

A range of pus and fluids in the lungs' alveoli (air sacs) are symptoms of pneumonia, a common respiratory organ infection. The variety of pus in alveoli, which are structures that aid in gas exchange, makes breathing difficult. It is caused by infectious agents called pathogens, which can cause a wide range of illnesses, from minor ones to serious ones. The human body itself is home to thousands of species of bacteria, fungi, and protozoa that are part of its natural flora, so it's crucial to remember that not all microbes are harmful. However, once an infected person coughs or sneezes, these microorganisms become airborne and anyone who has been in close proximity to the contaminated air is at risk of contracting the virus as it is contagious. All age groups can be affected, but the elderly, young children and those with weakened immune systems are more susceptible. The condition varies from a delicate to severe count on the type of organization concerned, age and also the underlying health of the individual.

The pathophysiology of pneumonia and immune regulation of the inflammatory response to lung infection are poorly understood, and few of the factors causing extreme disorder or dying have been identified. The inflammatory response additionally initiate via bodies free radicals like homocysteine mediated inflammation expand the severity and stiffness of the tissues (Xiao, et al. 2013) [1]. The bacterial infection in lungs activates the

immune gadget of which begins defense mechanism against the bacteria and produces multiplied amount of immune cells and immunostimulatory proteins and elements (eg. cytokins and complimentary proteins). Therefore the hematological and immunological profiles of infected folks are changed in compression to wholesome persons (Ewig, et al. 2010) [2]. The goal of the current study was to determine what aspects of the immune system and blood change following a pneumonic infection, as well as how the infection's widespread effects alter the hematological and immunological profiles of those who are infected.

MATERIALS AND METHODS

Patient recruitment

During the year 2017 to 2019, medically diagnosed pneumonic patients were admitted from the Shyam Shah Medical College, Medicine Department (OPD) of Rewa (M.P.), 240 pneumonic patients were recruited for the current investigation.

All of the recruits were of central Indian origin, mostly from Rewa, Satna, Sidhi, Singrauli and Shahdol. Diagnosis of pneumonia was based on measurement of ESR (Erythrocyte Sedimentation Rate) on people suffering from pneumonia.

Healthy controls

240 randomly selected healthy control (HC) was enrolled in the study. The control group included Rewa, Satna, Sidhi, Singrauli, Shahdol, as well as medical staff and healthy volunteers with persons living in the central region of India. Therefore, with the same environmental and social factors as the equal average age and gender ratio, the control group was created from the same area.

Sample collection strategy

About 5 ml Blood samples were collected in 0.5 M EDTA coated vials with healthy palm along with each pneumonia. Other information and clinical profile and matters and control topics was filled in a detailed proforma.

Quantitative measurement Interleukin-12 (IL-12)

Assay Procedure

Mixed all reagents thoroughly to create any foam within the vials. Determined the number of microplate strips required to test the desired number of samples plus appropriate number of wells needed for controls and standards. Remove sufficient microplate strips from the pouch. Add 100 µl of each standard, including blank controls to the appropriate wells. Add 100 µl of sample and 1X Control Solution to the appropriate wells. Add 50 µl of 1X Biotinylated anti-IL-12 to all wells. Cover and incubate for 1 hours at room temperature (18-25°C). Remove the cover and wash the plate as follows: Aspirate the liquid from each well. Add 300 µl of 1X Wash Buffer into each well. Aspirate the liquid from each well. Repeat for a total of 3 washes. Add 100 µl of 1X Streptavidin-HRP solution into all wells, including the

blank wells. 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 12-15 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. The O.D. values of the plate should be monitored and the substrate reaction stopped before positive wells are no longer accurately readable (maximum ~20 minutes). Add 100 µl of Stop Reagent into each well. Results must be taken immediately after the addition of Stop Reagent or within one hour, if the microplate is stored at 2-8°C in the dark. 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.

Calculations

Calculate the mean absorbance for each set of duplicate standards controls, 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 (Niemann *et al.* 2012) [3].

RESULTS

Clinical profile of patients and control

Clinical profile of patients and control table 1 indicates attributes on enrollment in age, residence and ethnicity of pneumonia and healthy control group. Within the given attribute, the variations between these 2 groups are equally and statistically non-significant, these are vital for keeping an equivalent 2 groups all told the norms apart from the study taken.

Table 1: To show the clinical characteristics of pneumonic patients and control in this study.

S.N.	Characteristic	Pneumonic Patients	Healthy control
1.	No. of subjects	240	240
2.	Male female ratio	88:152	98:142
3.	Children: Adult	210:30	198:42
4.	Mean Age (in year)	14.7	17.2
5.	Age range (in year)	1-26	4-38

6.	Mean weight (in Kg)	18.12	20.34

The number of patients and control for every cluster is 240 for study. The male feminine quantitative relation for case and control severally was 88:152 and 98:142. Children: Adult quantitative relation between groups 210:30 and 198:42 was for case and control. The average age of the case was 14.7 years and it had been adjusted to 17.2 for management. Average weight was 18.12 and 20.34 was for case and control, severally.

Association of Interleukin-12 (IL-12) between pneumonic patients and control

The serum concentration of IL-12 in 240 patients and control were measured and the results are presented in table 2. Compared with the control in this study, pneumonic patients are found significantly higher amount of interleukin-12. The average concentration of the Interleukin-12 was 35.20±5.68 pg/ml and 12.15±8.15 pg/ml for patients and control respectively. The statistical analysis of these differences in the average concentration of Interleukin-12 between the two groups were analyzed by t-test and the differences were found statistically significant at the level of P <0.0001, with the t-test value t=73.44 was there with the degree of freedom. The value of median was found 36.45 pg/ml and 10.65 pg/ml respectively for patient and control. Standard error of the mean was found 6 pg/ml and 2 pg/ml for patients and control respectively. In current study, the concentration limit of interleukin-12 is 9.84 – 67.54 pg/ml in patients and 8.10-18.96 pg/mlin healthy controls.

Table 2: Comparison of IL-12 concentration in blood of pneumonic patients to control using t-test (unpaired).

S.N.	**Parameters**	**Pneumonic patients**	**Healthy controls**	**t-test P value**
1.	Mean ± SD pg/ml	35.2 ± 5.68	12.15 ± 8.15	P<0.0001 t=73.44 df=998
2.	Median pg/ml	36.45	10.65	
3.	SEM pg/ml	6	2	
4.	Range pg/ml	9.84-67.54	9.75-18.96	

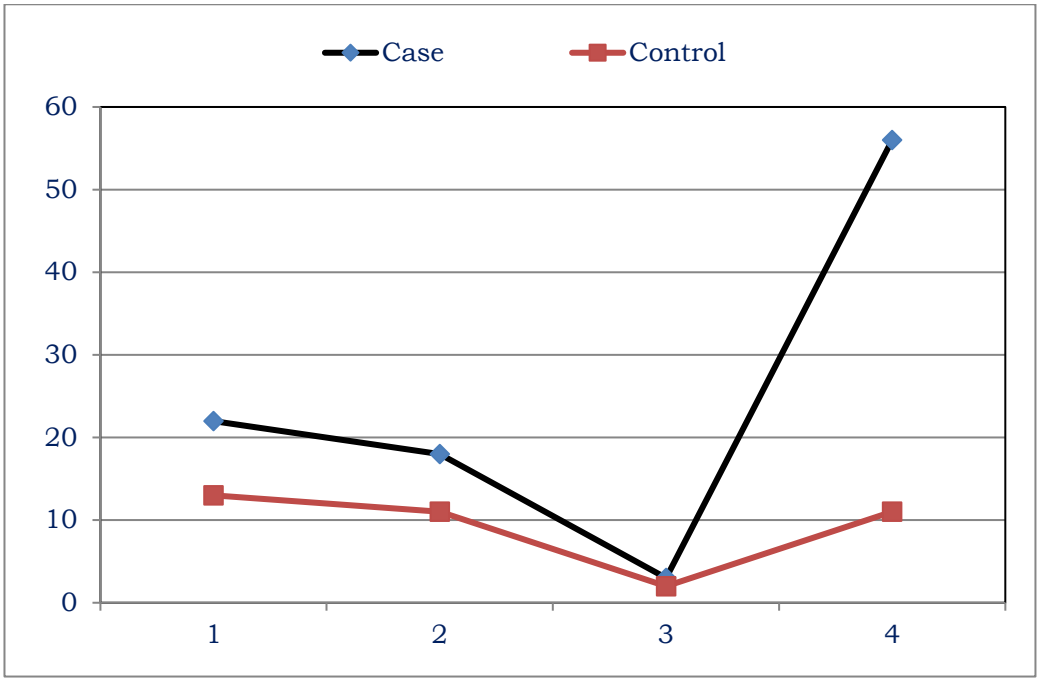

Fig.1: Comparison of IL-12 concentration in blood of pneumonic patients to control.

Discussion

The role of cytokines and interleukins in host defense against respiratory illness has been examined in several previous studies. It is necessary to emphasize that the role of cytokines -within the innate immunologic response to the tract is completely different in those models wherever different pathogens are used. The overall conclusions which will be drawn from these investigations are that ProInflammatory Cytokines, elicited by bacterial Pneumonia likely impaired bacterial clearance from the respiratory organ compartment. Bacterial infections sometimes result from inhalation of contaminated aerosols from environmental sources. Once the bacteria are within the lungs, they preponderantly infect and multiply inside monocytes and macrophages (Horwitz and Silverstein, 1980) [4]. Mortality rates of up to five hundredth are reportable; illustrating the very fact that bacterium respiratory illness remains a difficult communicable disease (Pedro-Botet et al. 1998) [5].

The first member of the Interleukin-12 (IL-12) family has been described (Trinchieri *et al.* 2003)[6]. It includes IL-12p35 and IL-12p40 subunits, and secretion of both subunites in a single cell that is required to secrete bioactive IL-12p70 cytokine (Jalah *et al.* 2013) [7] associated with disulfide bond. Although it is secreted by various types of hematovetric cell types and measured by physiotherapists are antigen-presenting cells (APCs), such as dendritic cell (DC) and macrophages. IL-12 binding for your high-affinity receptor (IL-12R on1 / IL-12R on2) expressed on active T cells, NK cells and Dendritic Cells, TYK2 (Tyrosin Qin 2), JAK2 and STAT pathway (Hunter, 2005) [8]. Although STAT1, STAT3 and STAT4 are activated *in vitro* in different ways, physical responses to IL-12 are primarily mediated through STAT4. IL-12 induces indirect CD4+ T cells to differentiate between Th1 cells, a T-

helper subset that is implicated in many types of etiology of human autoimmune diseases. The high levels of IL-12 and Th1 cells are found in the aquatic humor of autoimmunity eveitis and *in vitro* patients, which is one of the IL-12inspired proliferations of Th1 cells in the group of such vision-intractable peristalsis inflammatory diseases. Multiple sclerosis (MS) is another old CNS autoimmune disease that plays an important role in the proliferation of IL-12 induced Th1cells (Balashov *et al.* 1997) [9], (Comabella *et al.* 1998) [10]. In conclusion, the current study shows that IL-12 is part of the general host reaction to infection in pneumonia, and it is necessary but is not essential for optimal clearance of pulmonary infection. IL-12 therapy can increase host resistance to infection. A study in this support also shows that a treatment effect of IL-12 is mediated through increased inflammatory cell recruitment into lung tissues and an increase in tissue concentrations of pro-inflammatory cytokines. These results support a potential role as adjunctive therapy for pneumonia for IL-12.

Conclusion

Evidence from existing studies indicates that baseline levels of the inflammatory cytokine IL-12 are significantly elevated in patients with myocardial infarction. Statistical analysis of these differences in mean IL-12 concentrations between groups was analyzed using Student's t test, and statistically significant changes were also identified.

References

1. Xiao K, Su LX, Han BC, Yan P, Yuan N, et al. Analysis of the severity and prognosis assessment of aged patients with community-acquired pneumonia: A retrospective study. J Thorac Dis, 2013; 5626-33.
2. Ewig S, Welte T, Chastre J, Torres A. Rethinking the concepts of community-acquired and healthcareassociated pneumonia. Lancet Infect Dis, 2010; 10:279- 87.
3. Niemann S., Ehrhardt C., Medina E., Warnking K., Tuchscherr L., *et al.* (2012). Combined action of influenza virus and Staphylococcus aureus panton-valentine leukocidin provokes severe lung epithelium damage. *The Journal of infectious diseases,* 206: 1138-1148.
4. Horwitz MA and Silverstein SC. (1980). Legionnaires' disease bacterium (*Legionella pneumophila*) multiples intracellularly in human monocytes. *J. Clin. Invest.*, 66:441-450.
5. Pedro-Botet ML, *et al.* (1998). Role of immunosuppression in the evolution of Legionnaires' disease. *Clin. Infect. Dis.*, 26:14-19
6. Trinchieri G, Pflanz S and Kastelein RA. (2003). The IL-12 family of heterodimeric cytokines: new players in the regulation of T cell responses. *Immunity*, 19:641-644.
7. Jalah R, Rosati M, Ganneru B, Pilkington GR, Valentin A, Kulkarni V, *et al.* (2013). The p40 subunit of interleukin (IL)-12 promotes stabilization and export of the p35 subunit: implications for improved IL-12 cytokine production. *J Biol Chem.*, 288: 6763-6776.

8. Hunter CA. (2005). New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions. *Nature reviews.* 5:521-531.

9. Balashov KE, Smith DR, Khoury SJ, Hafler DA, Weiner HL. (1997). Increased interleukin 12 production in progressive multiple sclerosis: induction by activated CD4+ T cells via CD40 ligand. *Proc Natl Acad Sci U S A.* 94:599-603.

10. Comabella M, Balashov K, Issazadeh S, Smith D, Weiner HL, Khoury SJ. (1998). Elevated interleukin-12 in progressive multiple sclerosis correlates with disease activity and is normalized by pulse cyclophosphamide therapy. *J Clin Invest.*, 102:671-678.