The Association Study of Interleukin-6 with Pneumonia

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Abstract: - **Pneumonia is an inflammatory disease and may be associated with inflammatory interleukin. In this study Interleukin-6 has been analyzed to associated with the Pneumonia. This association study was carried out on 251 case and control. 5 ml blood sample of case and control were analyzed by ELISA reader for Interleukin-6 concentration. The present study found statistically significant at the level of P<0.0001, the value of t test was t=143.2with the degree of freedom 998.**

Key words: Inflammation, Interleukin, pneumonia, ELISA

I. INTRODUCTION

Pneumonia is inflammatory disease of the lung that is caused by bacteria, viruses, or other organisms. Healthy people may usually fight with pneumonia infections. However, people who have a weakened immune system make it easier for bacteria to grow in their lungs.

Pneumonia is one of the leading causes of death in children worldwide. Children under five years old contribute 18 % of 1.4 million deaths by Pneumonia worldwide. Pneumonia causes death of 410,000 children in India every year [2]. Pneumonia affects children and families everywhere, but is most prevalent India- more than tuberculosis, malaria and AIDS combined. Pneumonia in children is a significant public health problem in India, although epidemiological data is not available on its incidence due to low medical registration [3]. Mortality due to pneumonia accounts for approximately one-fourth of the total deaths in children, in India. Pneumonia affects children with higher risk among young infants, malnourished children, those with exposure to solid fuel use and non-exclusively breastfed children [4]. This translates into about 156 million new episodes each year worldwide, of which 151 million episodes are in the developing world. Most cases occur in India (43 million), China (21 million) and Pakistan (10 million), with additional high numbers in Bangladesh, Indonesia and Nigeria (6 million each).

The pathophysiology of pneumonia and immune regulation of the inflammatory response to lung infection are poorly understood, and few of the factors causing severe disease or death have been identified. The inflammatory response also initiate by bodies free radicals like homocysteine mediated inflammation increase the severity and stiffness of the tissues (5). The bacterial infection in lungs activates the immune system of which starts defense mechanism against the bacteria and produces increased amount of immune cells and immunostimulatory proteins and factors (eg. cytokins and complimentary proteins). Therefore the hematological and immunological profiles of infected persons are changed in compression to healthy individuals [22]. 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. MATERIAL AND METHODS

Patient recruitment

Medically certified pneumonic patients were recruited from medicine department (OPD) of Shyam Shah medical college, Rewa, Madhya Pradesh, registered during the year 2011 to 2015. 251 pneumonic patients were recruited for present investigation.

All the recruited patients were Central Indian origin mostly from Rewa, Jabalpur, Bhopal and Indore. The diagnosis of pneumonia was based on measurement of ESR and those who suffering with the pneumonia.

Healthy controls

251 randomly selected healthy controls (HC) were enrolled in the study. The control group consisted of medical staff and healthy volunteers from Rewa, Jabalpur, Bhopal, Indore as well as individuals residing in central region of India. Hence, control group was drawn from same area with similar environmental and social factors with same mean age and sex ratio.

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. The clinical profile and other information of case and control subjects were filled in a detailed Performa**.**

Quantitative measurement Interleukin-6 (IL-6)

Assay Procedure

1. Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.
2. Determine the number of microplate strips required to test the desired number of samples, plus appropriate number of wells needed for controls and standards.
3. Remove sufficient microplate strips from the pouch. Add 100 μL of each standard, including blank controls to the appropriate wells.
4. Add 100 μL of sample and 1X Control Solution to the appropriate wells.
5. Add 50 μL of 1X Biotinylated anti-IL-6 to all wells.

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

1. Remove the cover and wash the plate as follows: Aspirate the liquid from each well.
2. Add 300 μL of 1X Wash Buffer into each well
3. Aspirate the liquid from each well.
4. Repeat for a total of 3 washes. Add 100 μL of 1X Streptavidin-HRP solution into all wells,
5. Including the blank wells.

Re-cover and incubate at room temperature for 30 minutes.

1. Wash as described in Step

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 was 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. Result taken immediately after the addition of Stop Reagent. 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 was noted.

III. CALCULATIONS

Calculated the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plotd the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Drawn the best-fit straight line through the standard points. For samples that have been diluted, the concentration read from the standard curve has to be multiplied by the dilution factor to determine the actual concentration of the target protein present [23].

IV. RESULTS

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
	No. of subjects	251	25
	Male female ratio	86:165	91:160
	Children: Adult	230: 21	200:51
	Mean Age (in year)	15.7	18.3
	Age range (in year	1-24	4-36
	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.

Association of Interleukin-6 (IL-6) between pneumonic patients and control

The serum concentration of IL-6 of 251 patients and control were measured and the results are presented in the table-2. This study found significantly higher concentration of interleukin-6 in the pneumonic patients than control. The mean concentration of the Interleukin-6 was 23.3 ± 2.1pg/ml and 5.5 ± 1.82 pg/ml for patients and control respectively. The statistical analysis of this differences in the mean concentration of the Interleukin-6 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=143.2with the degree of freedom 998. The value of the median was found 18.32 pg/ml and 4.9 pg/ml for the patient and control group respectively. The slandered error of the mean was found 6 pg/ml and 2 pg/ml for patients and control respectively. The present study found range of the concentration of the interleukin-6 is 0.7-1.1 pg/ml in patients and 0.7-1.87 pg/ml in healthy controls.

Table- 2 Comparison of blood concentration of IL-6 of pneumonic patients to control using t-test (unpaired) test.

	Parameters	Pneumonic patients	Healthy controls	t-test P value
1.	Mean ± SD	23.3 ± 2.1	5.5 ± 1.82	P<0.0001*** t=143.2 df=998
2.	Median pg/ml	18.32	4.9	
3.	SEM pg/ml	6	2	
4.	Range pg/ml	3.24 – 56.87	3.89-6.21	

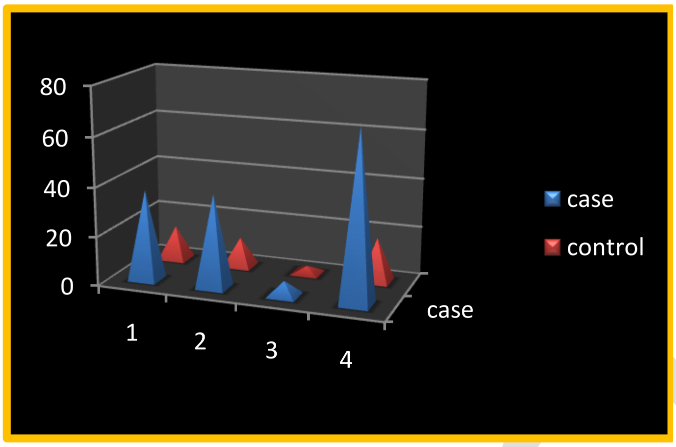

Figure-1 Comparison of blood concentration of IL-6 of pneumonic patients to control

V. DISCUSSION

The data of present study indicate that baseline levels of the inflammatory cytokine IL-6 are significantly elevated among apparently patients of myocardial infarction. The relationship between IL-6 level and risk was not altered in analyses that adjusted for baseline differences in total cholesterol, HDL cholesterol, body mass index, blood pressure, and diabetes, a family history of premature coronary artery disease, alcohol use, or exercise frequency. Prior data evaluating the role of IL-6 among healthy individuals at risk for future pneumonia are sparse [24]. In the setting of acute ischemia, however, it has recently been shown that IL-6 levels increase with the inflammation during pneumonia and that these elevations may be a marker for pneumonia [25]. However, because blood samples in the present study were collected at baseline, we can exclude the possibility that acute ischemia was a cause of IL-6 elevation in these data. Thus, if an enhanced inflammatory response is present among individuals with a propensity for pneumonia, then our data indicate that such effects are present and can be clinically detected. Elevated levels of IL-6 have previously been observed in several autoimmune disorders, including arthritis, Castleman syndrome, psoriasis, mesangial proliferative glomerulonephritis, and inflammatory bowel disease [26]. In this regard, the current data provide support for the hypothesis that pneumonia is a fundamentally inflammatory condition.

This study correlates of IL-6 in these data were C-reactive protein a finding that would be expected because IL-6 is a primary stimulant for hepatic production of acute-phase proteins [27]. The data of this study showing that the effects of IL-6 on subsequent risk remained statistically significant after we controlled for these latter factors. Thus, the current data also help to explain why acute-phase proteins, such as C-reactive protein, have been associated with increased vascular risk.

IL-6 production in healthy men at risk for future lung infection. The IL-6 is a marker rather than a cause of disease. On the other hand, because monocyte-derived macrophages are lungs in inflammation and IL-6 gene transcripts are expressed in human alveolar cells, it is also possible that increased IL-6 production from lungs cells has direct effects on inflammation stability [28]. IL-6 levels also increase with infection [29]. These prospective epidemiological data support a fundamental role for cytokine-mediated inflammation in the early stages of pneumonia, data that suggest and extend the recent finding that IL-6 levels are associated with increased mortality in the elderly [30]. As such, the results of this study believe these data support the possibility that anti-inflammatory therapies might provide a new approach to bacterial pneumonia treatment and prevention.

REFERENCES

[1]. Kolditz, M., Ewig, S., Höffken, G., (2013). Management-based risk prediction in community-acquired pneumonia by scores and biomarkers. Eur Respir J 41:974-84.

[2]. Ewig, S., Torres, A., (2011). Community-acquired pneumonia as an emergency: Time for an aggressive intervention to lower mortality. Eur Respir J 38:253-60.

[3]. Menéndez, R., Martínez, R., Reyes, S., Mensa, J., Filella, X., Marcos, MA., et al. (2009) Biomarkers improve mortality prediction by prognostic scales in community-acquired pneumonia. Thorax 64:587-91.

[4]. Ewig, S., Bauer, T., Richter, K., Szenscenyi, J., Heller, G., et al. (2013) Prediction of in-hospital death from community-acquired pneumonia by varying CRB-age groups. Eur Respir J 41:917-22.

[5]. Xiao, K., Su, LX., Han, BC., Yan, P., Yuan, N., et al. (2013) Analysis of the severity and prognosis assessment of aged patients with community-acquired pneumonia: A retrospective study. J Thorac Dis 5626-33.

[6]. van Vugt, SF., Broekhuizen, BD., Lammens, C., Zuithoff, NP., de Jong, PA., et al. (2013) Use of serum C reactive protein and procalcitonin concentrations in addition to symptoms and signs to predict pneumonia in patients

presenting to primary care with acute cough: Diagnostic study. BMJ 346:f2450.

[7]. Yandiola, PP., Capelastegui, A., Quintana, J., Diez, R., Gorordo, I., et al. (2009) Prospective comparison of severity scores for predicting clinically relevant outcomes for patients hospitalized with community-acquired pneumonia. Chest 135:1572-9.

[8]. Ewig, S., Welte, T., (2008) CRB-65 for the assessment of pneumonia severity: Who could ask for more? Thorax 63:665-6.

[9]. Loke, YK., Kwok, CS., Niruban, A., Myint, PK., (2010) Value of severity scales in predicting mortality from community-acquired pneumonia: Systematic review and meta-analysis. Thorax 65:884-90.

[10]. Chalmers, JD., Singanayagam, A., Akram, AR., Mandal, P., Short, PM., et al. (2010) Severity assessment tools for predicting mortality in hospitalised patients with community-acquired pneumonia. Systematic review and meta-analysis. Thorax 65:878-83.

[11]. Renaud, B., Coma, E., Labarere, J., Hayon, J., Roy, PM., et al. (2007) Routine use of the Pneumonia Severity Index for guiding the site-of-treatment decision of patients with pneumonia in the emergency department: A multicenter, prospective, observational, controlled cohort study. Clin Infect Dis 44:41-9.

[12]. Ewig, S., Woodhead, M., Torres, A., (2011) Towards a sensible comprehension of severe community-acquired pneumonia. Intensive Care Med 37:214-23.

[13]. Menéndez, R., Sahuquillo-Arce, JM., Reyes, S., Martínez, R., Polverino, E., et al. (2012) Cytokine activation patterns and biomarkers are influenced by microorganisms in community-acquired pneumonia. Chest 141:1537-45.

[14]. Zobel, K., Martus, P., Pletz, MW., Ewig, S., Prediger, M., et al. (2012) CAPNETZ study group. Interleukin 6, lipopolysaccharide-binding protein and interleukin 10 in the prediction of risk and etiologic patterns in patients with community-acquired pneumonia: Results from the German competence network CAPNETZ. BMC Pulm Med 2012;12:6.

[15]. Martínez, R., Menéndez, R., Reyes, S., Polverino, E., Cillóniz, C., et al. (2011) Factors associated with inflammatory cytokine patterns in community-acquired pneumonia. Eur Respir J 37:393-9.

[16]. Kruger, S., Ewig, S., Marre, R., Papassotiriou, J., Richter, K., et al. (2008) Procalcitonin predicts patients at low risk of death from community-acquired pneumonia across all CRB-65 classes. Eur Respir J 31:349-55.

[17]. Schuetz, P., Suter-Widmer, I., Chaudri, A., Christ-Crain, M., Zimmerli, (2011) Prognostic value of procalcitonin in community-acquired pneumonia. Eur Respir J 37:384-92.

[18]. Berg, P., Lindhardt, BO., (2012) The role of procalcitonin in adult patients with community-acquired pneumonia--a systematic review. Dan Med J 59:A4357.

[19]. Huang, DT., Weissfeld, LA., Kellum, JA., Yealy, DM., Kong, L., et al. (2008) Risk prediction with procalcitonin and clinical rules in community-acquired pneumonia. Ann Emerg Med 52:48-58.

[20]. Ramirez, P., Ferrer, M., Marti, V., Reyes, S., Martínez, R., et al. (2011) Inflammatory biomarkers and prediction for intensive care unit admission in severe community acquired pneumonia. Crit Care Med 39:2211-7.

[21]. Corrales-Medina, VF., Musher, DM., Wells, GA., Chirinos, JA., Chen, L., Fine, MJ., (2012). Cardiac complications in patients with community-acquired pneumonia: Incidence, timing, risk factors, and association with short-term mortality. Circulation 125:773-81.

[22]. Ewig, S., Welte, T., Chastre, J., Torres, A., (2010). Rethinking the concepts of community-acquired and health-care-associated pneumonia. Lancet Infect Dis 10:279-87.

[23]. Naito, T., Suda, T., Yasuda, K., Yamada, T., Todate, A., Tsuchiya, T., et al. (2006). A validation and potential modification of the pneumonia severity index in elderly patients with community-acquired pneumonia. J Am Geriatr Soc 54:1212-9.

[24]. Niu, WY., Wan, YG., Li, MY., Wu, ZX., Zhang, LG., Wang, JX., (2013). The diagnostic value of serum procalcitonin, IL-10 and C-reactive protein in community acquired pneumonia and tuberculosis. Eur Rev Med Pharmacol Sci 17:3329-33.

[25]. Paats, MS., Bergen, IM., Hanselaar, WE., Groeninx van Zoelen, EC., Hoogsteden, HC., Hendriks, RW., et al. (2013). Local and systemic cytokine profiles in nonsevere and severe community-acquired pneumonia. Eur Respir J 41:1378-85.

[26]. Demartini, G., Esposti, D., Marthyn, P., Lapidari, A., Fraschini, F., Scaglione, F., (2004). Effect of multiple doses of clarithromycin and amoxicillin on IL-6, IFN gamma and IL-10 plasma levels in patients with community acquired pneumonia. J Chemother. 16:82-5.

[27]. María-José, L., Inés, M., Benjamín, S., Enrique, C., Julio, C., (2015). Lung inflammatory pattern and antibiotic treatment in pneumonia. Respiratory Research. 16:15; 1-9.

[28]. Varelias, A., Gartlan, KH., Kreijveld, E., (2015). Lung parenchyma-derived IL-6 promotes IL-17A–dependent acute lung injury after allogeneic stem cell transplantation. Blood 125 :2435-2444.

[29]. Takanori, T., (2015). The primacy of IL-6 in IPS? Transplantation. Blood: 15; 2320 – 2322.

[30]. Bacci, MR., Leme, RC., Zing, NP., Murad, N., Adami, F., et al. (2015). IL-6 and TNF-α serum levels are associated with early death in community-acquired pneumonia patients. Braz J Med Biol Res. 45:427-32.