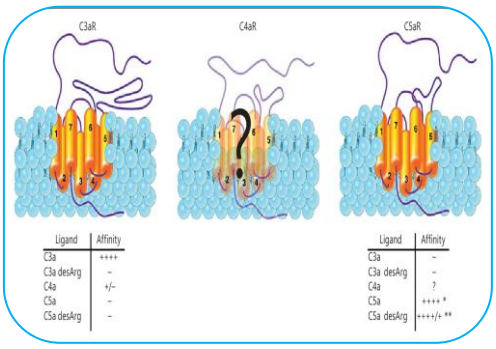
REVIEW OF RESEARCH
ISSN: 2249-894X
IMPACT FACTOR : 5.7631(UIF)
VOLUME - 11 | ISSUE - 6 | MARCH - 2022

"THE ANALYSIS OF ANAPHYLATOXIN C4A WITH PNEUMONIA PATIENTS IN REWA REGION: A REVIEW"

Dr. Sandhya Singh Rathor
Department of Biotechnology, Govt. Girls P.G. College, Rewa (M.P.) India.

ABSTRACT



The present paper deals the analysis of C4a with Pneumonia patients in Rewa region. For this work the blood sample of 240 pneumonic patients and 240 controls have been collected. Mean value of blood C4a concentration was seen 798 ± 3.51 ng/ml and 302 ± 5.51 ng / ml in pneumonic patients and healthy control respectively. Standard error of the mean was found 7 pg/ml and 4 pg/ml respectively for patients and control. The value of t-test of the differences of C4a blood concentration between pneumonic patients and healthy control was found 72.52 with the degree of freedom 998 which is statistically significant at the level of P <0.0001. The median value of C4a blood concentration was found 625pg/ml and 260 pg/ml. The limit of C4a blood concentration was found 263-884 pg/ml the patients and 234-365 pg/ml in the control group. The observation made with the blood concentration of C4a of the case and control measured and differences in the concentration was calculated statistically and the findings show the significantly higher C4a blood concentration in the patient than healthy control.

KEY WORDS: *Pneumonia, Anaphylatoxin C4a, patients and healthy.*

INTRODUCTION

Respiratory infections have become a common problem on these days. With thousands of people dying each year in India and around 4 million deaths worldwide, are attributed to pneumonia alone. With an increase in pollution and use of harmful airborne chemicals, it's becoming increasingly difficult to bring these numbers down. Pneumonia is an infection of respiratory organs within which the lung tissue of an infected person is crammed with fluid or pus. Individuals affected by this condition show symptoms of shortness of breath, fever, chills, chest and abdominal pain, presence of brown, yellow or green colored phlegm and cough **(Grotto *et al.* 2003)**. Pneumonia is classified as: community-acquired, hospital-acquired and ventilator-associated occurring in people (individuals with weakened immune system). Community-acquired pneumonia is one in all the foremost common infectious disease requiring medical aid, and it's the third leading explanation for death worldwide (**Fine *et al.* 1996**). To protect kids from pneumonia, it is important to promote breastfeeding, hand laundry and to reduce indoor air pollution, stopping pneumonia with vaccinations, treating pneumonia and ensuring that each sick kid has access to the proper quite care, either from a community-based medical expert or during a sanatorium if the sickness is severe to get the antibiotics and oxygen **(Brown and Roberts, 2004)**.

Most proteins and glycoproteins that constitute the complement system are synthesized by hepatocytes. But significant amounts are also produced by tissue macrophages, hemoglobin, and

epithelial cells of the genital system and gastrointestinal tract. The three pathways of activation produce all conjugal versions of protease C3-convertases. The classical complement pathway typically requires antigen-antibody complexes for activation (specific immune response), whereas the alternative pathway can be activated by activating 3 component (C3) hydrolysis, foreign materials, pathogens, or damaged cells. The mannose-binding lectin pathway can be activated by C3 hydrolysis or antigen without the presence of antibodies (non-specific immune response). In all three pathways, C3-Convertase cleaves and activates components C3, forming C3a and C3b, and further causing a cascade of cleavage and activation events. C3b binds to the surface of pathogens, leading to greater internalization by phagocytic cells by opsonization.

In the alternative route, C3B connects to Factor B. Factor D releases Factor B from Factor B to C3B. The complex of C3b (2) Bb is a protease that connects C5 to C5b and C5a. C5 converts are also formed by the classical pathway when C5b is bound to C4b and C2b. C5a is an important chemotactic protein, which helps to recruit inflammatory cells. C3a is a precursor to an important cytokine called adipokine which is cell signaling protein **(Klos, *et al.* 2013)** and is generally rapidly cleaved by carboxypeptidase B. In both C4a and C5a Anaphylatoxin activity occurs, which directly triggers mast degeneration and increasing contraction of cells as well as vascular and muscle permeability. C5b membrane attack pathways activates, resulting in the initiation of membrane attack complex (MAC), including C5b, C6, C7, C8, and the polymer C9 **(Goldman and Prabhakar, 1996)**. MAC is the cytolytic end product of the complement cascade; it forms a transmembrane channel, which causes osmosis in lymphatic system. Kupffer cells and other macrophage cell types help clear complement-coated pathogens.

MATERIALS AND METHODS:

During the year 2020 to 2021, 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 of Anaphylatoxin C4a:

The BD CBA Human Anaphylatoxin Kit (Catalog No. 561418) is used to quantitatively measure anaphylatoxin C4a protein levels in a single EDTA plasma or serum sample **(Loffler *et al.* 2010)**.

Principle:

BD CBA assays provides a way to capture a soluble analyte or set of analytes with beads of known size and fluorescence, making it possible to detect analytes using ELISA Kit Each capture bead in the kit has been conjugated with a specific antibody. The detection reagent provided in the kit is a mixture of phycoerythrin (PE) conjugated antibodies, which provides a fluorescent signal in proportion to the amount of bound analyte. When the capture beads and detector reagent are incubated with an unknown sample containing recognized analytes, sandwich complexes (capture bead + analyte +

detection reagent) are formed. These complexes can be measured using ELISA to identify particles with fluorescence characteristics of both the bead and the detector.

Three bead populations with distinct fluorescence intensities have been coated with capture antibodies specific for C4a plasma proteins and their desArg forms. In plasma and serum, C4a are rapidly converted to their desArg forms (C4a desArg,).

The Human Anaphylatoxin kit measures all C4a and their desArg forms (since this kit will measure both forms of each protein, normal and desArg, this manual will use C4a when referring to the measurement of either form). The three bead populations are mixed together to form the bead array.

During the assay procedure, you will assemble the anaphylatoxin capture beads with standards (purified from human plasma) or test samples (EDTA plasma or serum), incubate, wash and then incubate with the PE conjugated detection antibodies to form sandwich complexes. In this assay, the Human Anaphylatoxin Standards consist of purified C4a desArg.

Antibody and Standard Reagents:

Human Anaphylatoxin PE Detection Reagent (B): An 80-test vial of PE-conjugated anti-human C4a antibodies, formulated for use at 50 µl per test. Human Anaphylatoxin Standards (C): Two vials containing lyophilized human proteins purified from serum. Each vial should be reconstituted in 2.0 ml of Assay Diluent to prepare the top standard. PE Positive Control Detector (E1): A 10-test vial of PE conjugated antibody control that is formulated for use at 50 µl per test. This reagent is used with the ELISA Setup Beads to set the initial instrument compensation settings. FITC Positive Control Detector (E2): A 10-test vial of FITC-conjugated antibody control that is formulated for use at 50 µl per test. This reagent is used with the ELISA Setup Beads to set the initial instrument compensation settings. Buffer reagents Wash Buffer (F): One 260-ml bottle of phosphate buffered saline (PBS) solution (1X), containing protein and detergent used for wash steps and to resuspend the washed beads for analysis. Assay Diluent (G): Two 30-ml bottles of a buffered protein solution (1X) used to reconstitute and dilute the Human Anaphylatoxin Standards and to dilute test samples.

Table : The Human Anaphylatoxin Standard dilutions to the control tubes.

Tube label	Concentration pg/ml)	Anaphylatoxin Standard dilution
1	0 (negative control)	no standard dilution (Assay Diluent only)
2	10	1:256
3	20	1:128
4	40	1:64
5	80	1:32
6	156	1:16
7	312	1:8
8	625	1:4
9	1250	1:2
10	2500	Top Standard

CALCULATIONS

Calculated the mean absorbance for every set ofreproduction standards, controls and samples, and subtractthe average zero standard optical density. Plot the standardcurve on log-log graph paper, with widespread attention onthe x-axis and absorbance on the y-axis. Drawn the best-fitstraight line through the standard points. For samples thathave been diluted, the attention study from the general curvehas to be expanded by using the dilution component todecide the genuine awareness of the goal protein existing(Naito, *et al.* 2006).

RESULTS:

Clinical profile of patients and control:

Clinical profile of patients and control table 2 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 : 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 C4a between Pneumonic Patients and Control

C4a is the smaller part of compliment protein that is called C4. The C4a acts as an inflammation mediator protein and helps in initiate and mediate the inflammation process during pneumonia. The observation made with the blood concentration of C4a of the case and control measured and differences in the concentration was calculated statistically and also the results are given in table 4.10. The results of currentstudy show the significant risingof C4a blood concentration in the patient cluster of pneumonia.

Observations of C4a analysis indicated that blood has a normal C4a level in healthy controls. Mean value of blood C4a concentration was seen 798 ± 3.51 ng/ml and 302 ± 5.51 ng / ml in pneumonic patients and healthy control respectively. Standard error of the mean was found 7 pg/ml and 4 pg/ml respectively for patients and control. The value of t-test of the differences of C4a blood concentration between pneumonic patients and healthy control was found 72.52 with the degree of freedom 998 which is statistically significant at the level of P <0.0001. The median value of C4a blood concentration was found 625pg/ml and 260 pg/ml. The limit of C4a blood concentration was found 263-884 pg/mlthe patients and 234-365 pg/ml in the control group.

Table : Comparison of the C4a 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	798 ± 3.51 ng/ml	302 ± 5.51 ng/ml	P<0.0001 t=72.52 df=998
2.	Median pg/ml	625	260	
3.	SEM pg/ml	7	4	
4.	Range pg/ml	263-884	234-365	

DISCUSSION:

In a recent study victimisation enzyme-linked immunosorbant assay technique, it indicates that patients with acute lymphatic disease in C4a had an increase, which would include musculoskeletal and neurological symptoms developed within 96 hours of tick-bite **(Kotton and Morrisey, 2014)**.

This study found that the level of C4a was common in pneumonia patients. Although different studies in two studies can be due to various techniques used to measure C4a, our results were validated by the level of complement activation in patients of our pneumonic patients, which were previously mentioned in this autoimmune disease, Levels were the same. Thus C4a seems to be a helpful marker for both clinical diagnosis and treatment of patients suffering from pneumonia**(O'Garra *et al.* 2004)**.

CONCLUSION:

C4a is the smaller part of compliment protein that is called C4. The C4a acts as an inflammation mediator protein and helps in initiate and mediate the inflammation process during pneumonia. The observation made with the blood concentration of C4a of the case and control measured and differences in the concentration was calculated statistically and the findings show the significantly higher C4a blood concentration in the patient than healthy control.

REFERENCES:

- Brown N. and Roberts C. (2004). Vitamin A for acute respiratory infection in developing countries: a meta-analysis. *Acta Paediatr.*, 93:1437-1442.
- Fine MJ, Smith MA, Carson CA, *et al*. (1996). Prognosis and outcomes of patients with community acquired pneumonia. *A meta-analysis*. JAMA; 275: 134-41.
- Goldman AS, Prabhakar BS (1996). "The Complement System". In Baron S, *et al.* (eds.). *Baron's Medical Microbiology*(4th ed.). Univ of Texas Medical Branch.
- Grotto I., Mimouni M., Gdalevich M. and Mimouni D. (2003). Vitamin A supplementation and childhood morbidity from diarrhea and respiratory infections: a meta-analysis. *J. Pediatr*., 142:297-304.
- Klos, A.; Wende, E.; Wareham, K. J.; Monk, P. N. (2013). "International Union of Pharmacology. LXXXVII. Complement Peptide C5a, C4a, and C3a Receptors". *Pharmacological Reviews*.**65** (1): 500-43.
- Kotton DN and Morrisey EE. (2014). Lung regeneration: mechanisms, applications and emerging stem cell populations. *Nat Med,* 20: 822–832.
- Loffler B., Hussain M., Grundmeier M., Bruck M., Holzinger D., *et al.* (2010). Staphylococcus aureus panton-valentine leukocidin is a very potent cytotoxic factor for human neutrophils. *PLoS Pathog,* 6: e1000715.
- Naito T, Suda T, Yasuda K, Yamada T, Todate A,Tsuchiya T, *et al*. A validation and potentialmodification of the pneumonia severity index in elderlypatients with community-acquired pneumonia. J AmGeriatr Soc, 2006; 54:1212-9.
- O'Garra A., Vieira P.L., Vieira P. and Goldfeld A.E. (2004). IL-10-producing and naturally occurring CD4+ Tregs: limiting collateral damage. *J Clin Invest.,* 114:1372-1378.