Serum Complement 3 (C3) and Complement 4 (C4) Level in Febrile Neutropenia Patients at Dr. Kariadi Hospital, Semarang and Dr. Moewardi Hospital, Surakarta

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ABSTRACT

Aim: *to identify the serum complement 3 (C3) and complement 4 (C4) level in febrile neutropenia and non-febrile neutropenia patients.*

Methods: *this is a cross-sectional prospective study. Samples were collected from patients with febrile neutropenia as sample group and patients with neutropenia but without fever as control. Both groups were tested for serum complement 3 and complement 4 level, and the data were analyzed using student T- test.*

Results: *from 37 neutropenia patients, 23 were classified as febrile neutropenia group and 14 in non-febrile neutropenia as control group. Total mean neutrophil count was 653.22/ ml serum in sample group and 594.36/ ml serum in control group (p = 0.575). Mean C3 level was 95.74 ug/dl in sample group and 130.00 ug/dl in control group, showing significant difference with p= 0.031. The mean serum C4 level was 34.13 ug/ml in sample group and 34.00 ug /dl in control group, the difference is not significant with p = 0.98. When sample C3 and C4 data were combined, the total level was 125.61 ug/ml, which was significantly lower than the total C3 and C4 in control group 184.07 ug/dl. (p = 0,04)*

Conclusion: *in febrile neutropenia there is significant decrease of serum C3 level compared to non-febrile neutropenia. Serum C4 level in febrile neutropenia group is lower than the non-febrile neutropenia group, but the difference is not significant.*

Key words*: neutropenia, serum complement level, sepsis.*

INTRODUCTION

Infectious diseases still remain an essential health problem, especially in some developing countries including Indonesia. Infectious disease is caused by growth of microorganisms in the human body, causing wounds and tissue damage which lead to inflammation. The clinical manifestation of systemic inflammation could be SIRS (systemic inflammatory response syndrome) which consists of: tachypneu (respiratory rate over >20 breaths per minute with PaCO2 < 32 Torr), tachycardia (pulse >100 times/minute), hyperthermia or hypothermia (axillary temperature $\geq 38^0$ C or $< 35,5^0$C), leukocytosis or leukopenia (leukocyte count $\geq$10.000 cells/mm^3 or <6000 cells/mm^3). The inflammatory reaction is related to various body components, such as: vascular, neural tissue, body fluid and cells, especially at the wound site.[1,2]

The body fluid also contains several body components and cells responsible in inflammation, including macrophage and leukocyte, especially neutrophil/ polymorphonuclear neutrophil (PMN) and complement system, especially complement 3 (C3) and complement 4 (C4).

The PMN neutrophil is able to synthesize and to express adhesive receptors, then will attach to and pass through the vascular wall (perdiapedesis) to migrate into related tissue. This neutrophil activity is a response to chemotactic agents produced at the site of inflammation: the chemotactic agents include IL-8, complement-derived factors (C3a, C5a), calcrein, cytokine from TH1 cell and chemotactic factors produced by mast cell.[3-6, 28,35,37]

There are several nonspecific factors that could fortify the antibody effect: phagocyte cell (macrophage cell and neutrophil leukocyte) that could destroy antigen/ bacteria and complement, where besides directly destroying the antigen/bacteria, complement also functions as facilitator through opsonization process to assist phagocytosis by phagocyte cells.[3,4]

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Some patients were found to have neutropenia, these particular people have decreased phagocytosis ability but do not have fever, this is due to another factor that also contributes in phagocytosis, the opsonization. Opsonization is a process to prepare for phagocytosis, where the bacteria is covered by antibody and the complement system before being phagocytosized. Therefore in patients with neutropenia there are complement deficiencies especially C3 and C4, so the phagocytosis ability extremely decreases and the patients become really prone to infection, marked by the presence of fever/febrile neutropenia.[3,6,7,16,22,38]

Neutropenia or neutrophil deficiency is a condition where the absolute neutrophil count (ANC) decreases to below 1500 cells/mm^3 of blood. During its course some neutropenia will also show fever (febrile neutropenia) and some others do not experience fever (non febrile neutropenia). It has been mentioned previously, in some literature, severe neutropenia will cause critical illness, where some of the patients will undergo sepsis which may lead to septic shock.[1,2,30,36]

One must wonder whether there is a difference of immune response in febrile and non-febrile neutropenia. One of immune response system is complement, which then raises the question whether there is a difference of complement level in patients with febrile and non-febrile neutropenia. The aim of this study is to discover the level of C3 and C4 complement in patients with febrile and non-febrile neutropenia admitted to Dr. Kariadi Hospital, Semarang, and Dr. Moewardi Hospital, Surakarta.

METHODS

This is a prospective study with cross-sectional approach. Stastical analysis was performed using student T-test to compare the level of complement in patients with febrile and non-febrile neutropenia using SPSS for Windows 11.5.[8]

Inclusion Criteria

The patients are 18 years-old or older. Patients with neutropenia admitted to Dr. Kariadi Hospital, Semarang, and Dr. Moewardi Hospital, Surakarta, during February 1st 2005-June 31st 2005, who had had routine blood examination including leucocyte count and differential which determined ANC (absolute neutrophil count), and suffered from fever of ≥38^{o}C (result from two observations within 1 hour). The patients or family representing the patients agreed to be included in the study.

Exclusion Criteria

The patients or family representing the patients were not consent to be included in the study.

Materials and Methods

The material studied are blood specimen from neutropenic patients who fulfill the inclusion criteria.

Sample size includes all samples that fulfill inclusion criteria, based on prevalence of neutropenia in general population, 13%-50%, using Lemeshow and Lwanga formula (1990).[9]

Subjects meeting the study's criteria were questioned for anamnesis, underwent physical examination, blood examination (hemoglobin level, erythrocyte count, leukocyte count, hemogram and thrombocyte count). The ANC is calculated afterwards. The group of subjects with febrile neutropenia became sample group, while subjects with non-febrile neutropenia became control group. Then, body sera were drawn from each subject of both groups for C3 and C4 examination using Immunoturbidimetry assay according to Tina-quant (2003).[10]

Duration of study was done from March 2003 to February 2004.

RESULTS

There were 37 cases of neutropenia being studied from March 2003 to February 2004, consisted of 23 febrile neutropenia as sample group and 14 cases of non-febrile-neutropenia as control group.(Table 1) Of 37 subjects, 19 were male and 18 were female. The youngest were 18 years old and the oldest were 75 years old with mean age of 42.81 years. Mean age for sample group was 42.70 years while in control group 46.86 years, which, statistically, was not significantly different (p=0.43).

Table 1. Characteristics of 37 subjects with neutropenia in Dr. Moewardi hospital, Surakarta, and Dr. Kariadi hospital, Semarang (n=37)

Variables	Sample Group (n=23)		Control Group (n=14)	
	Mean	SD	Mean	SD
Age	42.70	14.90	46.86	15.55
Neutrophil count	653.22	462.85	584.36	476.57
C3 Level	95.74	46.76	130.00	43.36
C4 Level	34.13	17.67	34.00	22.29

Age in years, neutrophil count in mL blood, C3 and C4 level in µg/dL

T-test Result

a. Neutrophil count. Of 23 sample group, mean neutrophil count was 53.22/mL, while in 14 subjects of control group it was 594.36/mL. Both results were not significantly different (p=0.575). (Table 2)

b. C3 Level. Mean of C3 level in sample group was 95.47 µg/dL, while in control group 130.00 µg/dL. Mean of C3 level in sample group is significantly higher compared to in control group with p=0.031.
c. C4 Levels. Mean of C4 level of sample group was 34.13 ìg/dL, while in control group 34.00 µg/dL. Even though C4 level in sample group was higher than in control group, it was not significantly different p=0.98.
d. Total C3 and C4 Level. Even though C3 held a central role in complement system, actually complement system worked synergistically. Thus, we tried to combine the level of C3 and C4. When C3 and C4 complement levels of the sample group were combined the mean result was 125.61 µg/dL, which was lower than normal level, 130 µg/dL. It was significantly lower compared to the level of C3 and C4 in control group, which was 184.07 µg/dL, p=0.040.

Table 2. Analysis of student T-test

Variables	Sample Group (n=23)		Control Group (n=14)		p
	Mean	SD	Mean	SD	
Age	42.70	14.90	46.86	15.55	0.43
Total Neutrophil Count	653.22	462.85	584.57	476.26	0.58
C3 Level	95.74	46.76	130.00	43.36	0.03
C4 Level	34.13	17.67	34.00	22.30	0.98
Total C3 & C4 Level	125.51	55.10	164.07	61.41	0.04

DISCUSSION

Complement system was first discovered as thermolabile substance in serum, involved in perfecting antibody in eliminating bacteria or immune complex.

Almost all complement component produced by liver is in inactive form, thus, in order to function, must be activated—usually by infectious stimuli or immunostimulant.[3-7,11-15,29,33,34]

Kirschfink and Mulines (2003) stated that complement is activated through 3 pathways: classical pathway, alternative pathway, and Lectin pathway (MBL). All three pathways require activation of complement 3 (C3) as central/main component, which will generate a Cytolitic Membrane Attack (MAC) complex derived from C5b-9, or the fourth or terminal pathway.[4,12,24,29]

Defect in complement system will increase susceptibility to infection; especially when classical pathway is affected, the disease manifested will be correlated with immune system disorder. Furthermore, for protection against tissue damage due to overactive complement mediator, complement system is equipped with regulation of soluble protein and protein in the cell membrane.[3,5,31,32]

The study found that mean level of C3 in the sample group (94.74 µg/mL) was below normal level (105 µg/mL); while mean level of C3 in control group was 130.00 µg/mL, which was above that of the sample group. The result was significantly different in statistical perspective with p=0.031 (p<0.05).

The mean level of C4 in sample group (n=23 subjects) was 34.13 µg/dL, which was under normal C4 level (25 mg/dL). Meanwhile, mean level of control group was 34.00 µg/ml, which was also above normal level. Mean level of C4 in sample group was higher than control group, however this margin was not statistically different (p=0.984).

Complement *in vivo* works as an integrated system (complement system). When C3 and C4 level of sample group was added, the mean level was 125.61 µg/dL. This result was lower than C3 and C4 total in normal person (130 µg/dL). Mean of total C3 and C4 in control group was 184.07 µg/dL, which was higher than mean in the sample group with significant statistical difference (p=0.040).[14,15]

The decrease of C3 in patients with febrile neutropenia (sample group) in comparison to non-febrile neutropenia group (control) was in accordance with the opinion of previous researcher (Chapel H et al [2001]; Kirschfink and Mulines [2003]; Atkinson [2003]), that C3 had central role in several process of complement activation in assisting phagocytosis process.[3,12,18]

The result of this study is almost similar to Guntur (2000), however, in this study, from immunocompromised (IC) patients (14 subjects) and non-immunocompromised (NIC) patients (14 subjects), the mean of C3 level was 1.050 and 1.449, respectively. Statistically, there was significant difference of both group (p=0.016). Meanwhile, mean levels of C4 in IC group and NIC group were 24.289 and 27.929, respectively, with no significant difference in statistics (p>0.05).[17]

In other study by Guntur (2002), it was reported that out of 27 sepsis cases being studied, 22 cases were improving and 5 cases deteriorated into septic shock. The study group were sepsis group and septic shock group, who underwent examination for several variables: IL-10, IFN-γ, TNF-α, IL-Iβ, IgG, C3 and C4. The result of mean level of C3 in septic shock group was lower than sepsis group with statistical significant difference (p<0.05). However, even though the mean level of C4

in septic shock group was lower than sepsis group, the difference was not statistically significant.[18,19]

Our study, along with other previous research, has shown that mean level of C3 in sample and control group are significantly different. Such condition showed the important role of C3 physiologically in assisting phagocytosis component by preparing opsonization process toward foreign matters, whether bacteria, virus or other matters, to be eliminated in complete phagocytosis by macrophage or neutrophil.

Similar to the concept proposed by Atkinson (2003) that complement activation for opsonization process and MAC (membrane attack complex) formation must encompass C3b component, which was synthesized from C3.[20] Markiewski MM *et al* (2004) and Suresh M *et al* (2003) reported that C3a and C3b, synthesis of C3, played an important role in hepatocyte regeneration due to intoxication. It was predicted that C3b/iC3b facilitated phagocytocis of necrotic and apoptotic cells by macrophages.[21,23]

As reported by Guntur (2002), patients with sepsis underwent increase of C3 level because in sepsis there was drastic increase of IL-10, which will accelerate B lymphocyte maturation, causing plasma cell differentiation and increasing IgG level. IgG will bind immunogenic chemical substances forming a complex which will activate and increase C3 level. C3 derives to the synthesis of C3a and C3b. C3a will accelerate C5a synthesis; both substances are named anaphylatoxin compound which will cause vascular dilatation, decrease vascular resistance and increase vascular permeability causing plasma extravasation.[24] (Figure 1)

The importance of C3 role could also be seen in the study by Shauna et al (2004), which stated that *Burkholderia pseudomallei* often cause septicemia because *B. pseudomallei* contain polysaccharide capsule capable to block CR1 or CR3 receptor and decrease C3b level, causing inadequate phagocytosis process.[25]

Prevention of over-activation of C3/C3a was also stated by Girardi G et al (2003) and Atkinson C et al (2005). Atkinson C et al stated that Intestinal ischemia/reperfusion injury (IRI) was the main complication of abdominal surgery, cardiopulmonal bypass, rupture of abdominal aneurism, and cardiac arrest. Decrease in abdominal circulation causes hemorrhagic shock and intestinal IRI leading to bacteria translocation and sepsis. IRI causes intestinal dysfunction indicated by decrease in motility, increase of permeability and mucosal wall injury. All condition are partly mediated by complement activation and neutrophil infiltration. IRI can be prevented by activating CR2, which will inhibit

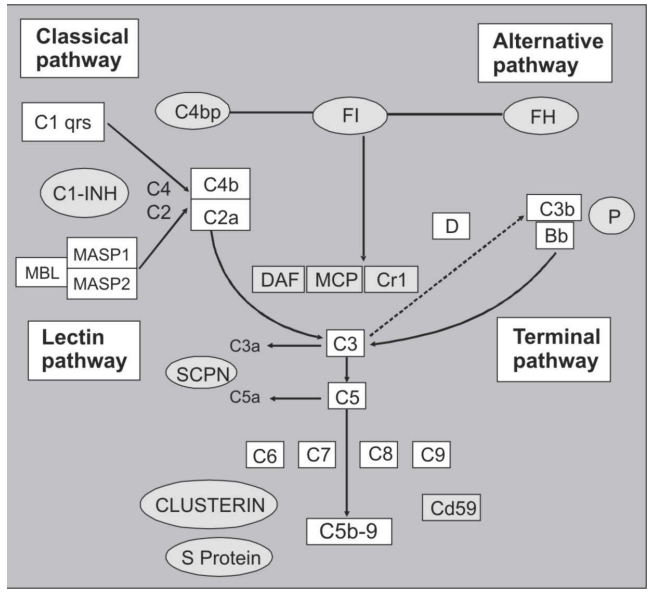

Figure 1. Schematic diagram of complement cascade reaction. Protein regulator of complement is in circles (fluid phase regulators) and boxes (membrane-associated regulators). MASP=MBL-Associated Serine Protease; C1 INH=C1 Inhibitor; SCPN=Serum Carboxypeptidase N; DAF;CD55; MCP=Membrane Co-factor Protein (CD46); CRI=complement receptor 1 (CD55).

accumulation of C3d/C3 that leads to accumulation of C3a. C3a accumulation will accelerate synthesis of C5a, tissue destroyer. Girardi et al recommended C5-antibody medication—a peptide of anti-C5aR, anti-granulocyte and anti-neutrophil ability which can prevent pregnancy failure in APL (antibody anti-phospholipid) cases.[26,27]

CONCLUSION

C3 plays a central role in complement activation and function in opsonization process in assisting phagocytosis by macrophage and/or neutrophil.

C3 is the first complement component in the immune system that can react to external immunogen/microorganism. Thus, decrease in C3 will increase the body's susceptibility to infection.

For better justification of the study result, more samples are required.

In the future, C3 levels can be considered as a predictor for prognostic factor of infection, especially in sepsis.

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