SERUM COMPLEMENT (C_3, C_4) LEVELS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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ABSTRACT

The C3 and C4 levels in the serum of 30 clinically detected Acute Myocardial Infarction (AMI) patients was studied in the present study following the guidelines of Bangladesh Medical Research Council (BMRC). The mean C3 and C4 level ($mg.dl^{-1}$) in the AMI patients was increased significantly at the 5th day (146.88 ± 58.77, 37.08 ± 17.71) in comparison to the 1st day (134.51 ± 43.23, 35.09 ± 13.89) and that of the control subjects (101.55 ± 14.66, 24.77 ± 8.15). A positive correlation was found between the C3 and C4 levels in the AMI patients at 1st day ($r = 0.494$) and 5th day ($r = 0.195$). The difference between the mean of C3 and C4 levels in the AMI patients and that of the control subjects was found significant at 1st day (C3: $t_{58} = 3.956$, $P < 0.001$; C4: $t_{58} = 3.513$, $P < 0.001$) and 5th day (C3: $t_{58} = 4.099$, $P < 0.001$; C4: $t_{58} = 3.458$, $P < 0.001$). The C3 and C4 levels between the 1st day and 5th day of AMI showed an insignificant difference (C3: $F_{2,27} = 2.769$, $P > 0.05$; C4: $F_{2,27} = 2.129$, $P > 0.05$). However, serum C3 and C4 levels were significantly increased in AMI patients in comparison to the healthy control subjects. In the present study it was observed that the activation of complement system occurs after AMI and it is suggestive of an acute phase and inflammatory response.

KEY WORDS: Serum, Complements, Acute Myocardial Infarction.

INTRODUCTION

Cardiovascular diseases such as Coronary Heart Disease (CHD) and strokes are the largest causes of death in developing countries and are one of the main contributors to disease burden (Gaziano *et al.*, 2006). It is estimated that cardiovascular diseases caused 17.5 million (30%) of the 58 million deaths occurred world wide in 2005 (WHO, 2005). By the year 2020, these diseases are expected to increase by 120% for women and 137% for men in developing countries as compared with 30-60% in developed countries (Murray and Lopez, 1997).

Exact data about the incidence and prevalence of Myocardial Infarction (MI) in Bangladesh is lacking. Incidence of Ischemic Heart Disease (IHD - that includes, angina pectoris, unstable angina, myocardial

infarction) was about three per thousand until 1976. A study in 1985 revealed that the incidence of IHD was about 14 per thousand. Prevalence of IHD in urban population was reported to be as high as about 100 per thousand. Myocardial Infarction is the leading cause of death in Bangladesh, mostly in the 4th decade of life (MoHFW, 2005).

IHD is caused by an imbalance between the myocardial blood flow and the metabolic demand of the myocardium. Reduction in coronary blood flow is related to progressive atherosclerosis with increasing occlusion of coronary arteries. Blood flow can be further decreased by superimposed events such as vasospasm, thrombosis, or circulatory changes leading to hypoperfusion (Anversa and Sonnenblick, 1990).

The classic WHO criteria for an acute myocardial infarction require that two of the three elements be present: a) a history suggestive of coronary ischemia for a prolonged period (>30mins), b) evolutionary changes on serial ECGs suggestive of MI, and c) a rise and fall in serum cardiac markers consistent with myonecrosis. Only two out of these three criteria are needed because of the wide variability in the pattern of patient presentation with AMI. (Pedoe-Tunstall *et al.*, 1994).

The complement system, an essential part of the immune system, can be activated by the three pathways: a) classic pathway, b) alternative pathway, and c) Mannose-Binding Lectin (MBL) pathway (Arumugam *et al.*, 2004; Stahl *et al.*, 2003). It plays an important role in the pathophysiology of ischemic heart disease (Iltumur *et al.*, 2005). A single study showed that serum complement levels are predictive of myocardial infarction up to 4 years before the acute event (Muscari *et al.*, 1995b). C3 is powerful predictor of MI in men without previous ischemic events (Muscari *et al.*, 1995a). Serum C3/C4 ratio is a novel marker for recurrent cardiovascular events in acute coronary syndrome (Palikhe *et al.*, 2007).

The C3 is a useful risk factor in coronary (Seifert *et al.,* 1991; Muscari *et al.*, 1998; Széplaki *et al.*, 2004; Ajjan *et al.,* 2005) artery disease and atherosclerosis universally accompanies AMI in vivo. It is initiated within 2 hours after coronary artery obstruction via deposition of C3, which may be due to generation of the alternative pathway C3 convertase in the ischemic area (Väkevä *et al.,* 1994). High C3 levels might have a direct role in the hyperproliferation of vascular smooth muscle cells (Lin *et al.*, 2004) and indicate the chronic complement dependent inflammation as a special marker, and not as a common acute phase reactant (Széplaki *et al.,* 2006).

The mechanisms by which the complement system is activated during AMI are still unclear, although the releases of mitochondrial constituents, reperfusion, and thrombolytic agents have been proposed (Bennett *et al.,* 1987; Hostetter and Johnson, 1989; Kagiyama *et al.,* 1989). However, the experiments in animals have shown that complement activation can enhance infarct size (Maroko *et al.,* 1978; Buerke *et al.,* 1995).

Cardiovascular diseases are the major causes of mortality and disease in the Indian subcontinent causing more than 25% of deaths (Gupta *et al.*, 2008). In Bangladesh the incidence of MI is increasing (Murray and Lopez, 1997) and mortality due to AMI has been reported as 2.54% (MoHFW, 2005). However, there is no reported study on serum C3 and C4 levels in patients with AMI in our country. Therefore, the present study, first of its kind in Bangladesh, was undertaken with a view to evaluate the levels of serum complement C3 and C4 in patients with AMI at 1st day of onset and at 5th day after attack.

Materials and Methods

Data Collection

Data were collected from enrolled patients who fulfill the inclusion criteria and collected by pre-designed questionnaire devised for the study. The questionnaire was pre-tested and face validated by consulting experts and the data were processed, analyzed and interpreted using statistical method.

Enrolment Criteria

All the patients admitted in the Coronary Care Unit (CCU) of Sylhet MAG Osmani Medical College & Hospital with typical ischemic type of chest pain (without having previous history of myocardial infarction, unstable angina, coronary intervention, cardiomyopathy, any other acute or chronic inflammatory conditions, congenital heart disease or valvular heart disease) was considered as the cases. On the other hand, healthy non smoker persons of 35-80 years age without any history of chest pain and allergy was considered for control.

Study Design

A total number of 30 diagnosed patients as cases and 30 as controls were selected according to the inclusion and exclusion criteria.

Sample Collection

Samples were taken from the selected persons after having informed written consent from each patient or attending next to kin. Relevant permission was taken from the concerned authority and ethical committee of Sylhet MAG Osmani Medical College and Hospital. Under all aseptic precautions, 5 ml of venous blood was collected from each patient during the1st day of and 5th day after attack by using a disposable syringe, which was then transferred to a properly labelled test tube. The collected sample was allowed to clot at room temperature. The clotted sample was centrifuged at 2000 rpm for 10 minutes. Serum thus produced was taken into appropriately labelled micro-centrifuge tubes by using micropipettes and kept in -20^{o} C until further analysis.

Estimation of Complements

Serum C3 and C4 level was assayed by using commercially available turbidimetric monoreagent of Human, Germany (Cat. No. C_3: TU-C3, INF 1111001GB, 09-2006-01; C_4: TU-C4, INF 1111301GB, 09-2006-01). The C_3/C_4 antigens in sample or standard react with the anti-C_3/C_4 antibodies in the reagent. The absorbance increase caused by the resulting aggregates was measured by using the ELISA machine (Model: HumaLyser 3000 of Human, Germany) following the turbidimetric end-point method.

Statistical Analysis

Statistical analyses for correlation, t-test and ANOVA were done by using SPSS programme for Windows (version 15.0).

Results and Discussions

In the present study, quantification by ELISA of C3 and C4 was carried out among 30 AMI patients as against 30 control subjects. Among them, 83.33% were male (n = 25) and the rest 16.67% were female (n = 5). The age range of the patients was 38-80 years with a mean ± SD of 55.27 ± 10.31. Out of the total patients

13.33% (n = 4), 40% (n = 12) and 16.67% (n = 5) were found to have positive family history of AMI, hypertension and diabetes mellitus respectively.

The C3 and C4 levels in the AMI patients at different risk factors

The C3 and C4 level ($mg.dl^{-1}$) was found higher in the age group of <59 years. The mean ± SD of the C3 and C4 levels ($mg.dl^{-1}$) in the AMI patients (<59 years) at the 1st day was found as 146.09 ± 39.23 and 38.87 ± 14.05 respectively; while at the 5th day it was found as 162.22 ± 64.16 and 39.96 ± 19.28 respectively. A lower C3 and C4 levels was found with the AMI patients belonging to the group of ≥59 years. The mean ± SD of C3 and C4 level ($mg.dl^{-1}$) in the age group of ≥59 years was found as 120.38 ± 37.25 and 32.10 ± 14.07 respectively (Table 1). The C3 level was higher than the C4 level and the levels of the complements increased with the span of time.

The mean C3 and C4 level ($mg.dl^{-1}$) at both the 1st and 5th day was higher in female (C3: 149.96 ± 49.93, 152.91 ± 48.87; C4: 45.67 ± 12.36, 40.31 ± 9.01) than male (C3: 131.42 ± 42.23, 145.67 ± 61.37; C4: 32.99 ± 13.41, 36.43 ± 19.05). The complements levels increased with time span except the C4 in female (Table 1).

The complement levels in the AMI patients who have positive family history of AMI, was higher at the 1st and 5th day with an exception for the C4 level at the 5th day (Table 1). In the present study, most of the AMI patients were past smokers and they had higher level of C3 and C4 than the present smokers. The mean level of C3 was found higher in the AMI patients with hypertension and vice versa for the C4 (Table 1). Similar characteristics were observed in the AMI patients having the history of diabetes (Table 1).

In the present study, a total of nine patients were treated with Streptokinase. The mean C3 level ($mg.dl^{-1}$) at both the 1st day and 5th day in the AMI patients treated with streptokinase was higher (140.65 ± 42.86, 160.10 ± 64.30) than those who were not treated with streptokinase (120.19 ± 43.05, 116.02 ± 25.55). The C4 level exhibited downwards trend at the 5th day in comparison to 1st day (Table 1). The mean C3 and C4 level was found to vary depending on social status (Table 1). The higher frequency of AMI was found to occur in the low-income group (46.67%) following the middle income (36.67%) and upper income group (16.66%).

The C3 and C4 levels in the AMI patients at the 1st day and 5th day

In the present study, the level of C3 ($mg.dl^{-1}$) at the 1st day and 5th day was found to range from 66.06 to 220.47 and 56.62 to 300.14 with a Mean ± SD of 134.51 ± 43.23 and 146.88 ± 58.77 respectively (Table 2). The level of C4 ($mg.dl^{-1}$) at the 1st day and 5th day was found to range from 12.73 to 64.07 and 9.10 to 87.11 with a Mean ± SD of 35.09 ± 13.89 and 37.08 ± 17.71 respectively (Table 2).

There was found a positive correlation between the 1st day and 5th day for C3 (r = 0.539) and C4 (r = 0.337). A positive correlation was also found between the C3 and C4 levels at 1st day (r = 0.494) and 5th day (r = 0.195).

The regression line equation between the C3 and C4 levels was found as Y = 13.760 + 0.159 X at 1st day (Figure 1) and Y = 28.444 + 0.059X at 5th day (Figure 2). The regression line equation between the 1st day and 5th day was found as Y = 48.320 + 0.733X for C3 (Figure 3) and Y = 21.995 + 0.429X for C4 (Figure 4).

A significant difference was found between the mean of each complement in the AMI patients and that of the control subjects at 1st day (C3: $t_{58} = 3.956$, $P < 0.001$; C4: $t_{58} = 3.513$, $P < 0.001$) and 5th day (C3: $t_{58} = 4.099$, $P < 0.001$; C4: $t_{58} = 3.458$, $P < 0.001$).

However, an insignificant difference was found between the 1st day and 5th day for the C3 and C4 complement levels (C3: $F_{2,27} = 2.769$, $P > 0.05$; C4: $F_{2,27} = 2.129$, $P > 0.05$).

In the present study, the incidence of AMI was found to be dependant on various risk factors. Both the C3 and C4 levels were found higher in the age group of <59 years, which might be due to vulnerability to atherosclerotic changes of the aging process. Muscari *et al.* (2000) studied on serum C3 level on 1840 men aged 55–64 years in the San Vitale district of Bologna, Italy and found a lower level of C3 (<59 years: 1.10 ± 0.18 $g.l^{-1}$; ≥59 years: 1.11 ± 0·17 $g.l^{-1}$) than that of the present study (Table 1). They found no relationship of the C3 level with age. Rashid *et al.* (2005) found higher incidence of AMI in the age group 50-59 years. Joshi *et al.* (2007) reported a high rate of AMI in Bangladeshi people with the overall age (mean ± SD) of 51.9 ± 11.0. Anand *et al.* (2008) observed that younger individuals compared to older women and men have a stronger MI risk. Therefore, the present findings correlated with the findings of Rashid *et al.* (2005); Joshi *et al.* (2007) and Anand *et al.* (2008).

The level of C3 and C4 in the present study was found to be a dependant factor of sex. Higher level of complements was observed in the female than male. With the exception of C4 in the female group, the level of the complements was found to increase with time span. Anand *et al.* (2008) observed that women experience their first acute MI on average 9 years later than men. Men were significantly more likely to suffer a MI prior to 60 years of age than were women. Rashid *et al.* (2005) observed a greater risk of MI in man than woman. Others (Yusuf *et al.*, 2001) also observed gender variation of MI. The present study does not correlate with their findings and smaller sample size precludes from drawing any valid conclusion.

There was found no clear relationship of the complements levels in the AMI patients with the positive family history of AMI. Muscari *et al.* (2000) in their study found no relationship of the C3 level (1.11 ± 0.18 g/l) with the positive family history of myocardial infarction. Smoking was found to have a positive relation with the higher level of complements. This finding is in well agreement with the findings of Muscari *et al.* (2000) who found that past smokers had significantly higher C3 levels (1.14 ± 0.18 g/l) than current smokers and those who had never smoked (1.09 ± 0.17 g/l).

Hypertension/Diabetes has a positive relation with the higher level of C3 complement in the AMI patients. A negative relationship was observed for the C4 complement level. Muscari *et al.* (2000) in their study found the C3 levels in the AMI patients with and without hypertension as 1.16 ± 0.18 g/l and 1.08 ± 0.17 g/l respectively. In the AMI patients with and without diabetes, they found the C3 level as 1.20 ± 0.17 g/l and 1.10 ± 0.17 g/l respectively. They also found that hypertension and diabetes, in decreasing order of significance, are associated with higher C3 level. The present study also supports the findings of Muscari *et al.* (2000).

Complement components such as C3 and C4 change very early after the onset of coronary occlusion in patients with AMI, and concentrations immediately decline, but rise on the following days. This immune response not only depends on the treatment with thrombolytic therapy (streptokinase) of patients in the AMI group but may also reflect immune activation due to myocardial injury (Leinoe *et al.,* 2000). The complement activation caused by streptokinase infusion involves the classic pathway (Frangi *et al.*, 1994). The in vivo effects of streptokinase are not known. Moreover, the consequences of complement activation by fibrinolytic agents have not been studied. Frangi *et al.* (1994) in their study observed only minor and non-significant changes in C4 and C3 levels (mg/dL). They observed C3 level (mean ± SD) before streptokinase treatment, 15 minutes after beginning of streptokinase infusion and the day after as 83 ± 4, 77 ± 6 and 72 ± 5 mg/dl respectively, while the C4 level was 36 ± 3, 35 ± 2 and 33 ± 2 mg/dl respectively.

However, in the present study, Streptokinase infusion depleted native complement component, since C3 and C4 levels were significantly modified.

The present study showed that the socio-economic condition also affects the complements level in the AMI patients. Low income and low level of education have been reported to be associated with premature acute myocardial infarction in South Asians (Ismail *et al.*, 2004). Some authors showed a positive correlation between IHD and economic development (Buyamba-Kabangu *et al.*, 1987; McKeigue *et al.*, 1989; Rosengren *et al.*, 2001; Rashid *et al.*, 2005). However, the present finding is in well agreement with the finding of Ismail *et al.* (2004).

C3 and C4 in the AMI Patients

The mean level of C3 and C4 ($mg.dl^{-1}$) in the AMI patients increased over time. The high level of the complements at the 5^{th} day might be due to the increased synthesis of complement components, decreased consumption, or adequate regulation. Iltumur *et al.* (2005) found a significant higher peak level ($mg.dl^{-1}$) of plasma C3 and C4 in patients with AMI (141 ± 29 and 35 ± 11, respectively) than in patients with the control subjects (114 ± 22 and 22 ± 7, respectively) ($P < 0.01$). The C3 and C4 concentration ($mg.dl^{-1}$) in patients with AMI started to increase in from 1^{st} day (105.5 ± 21.5, 20.9 ± 6.3) to 2^{nd} day (116.9 ± 25.3, 23.8 ± 6.8), reaching a peak on the 3^{rd} day (140.8 ± 28.7, 33.5 ± 7.3) and thereafter decreased on the 7^{th} day (129.2 ± 20.7, 35.2 ± 11.1). The plasma levels of C3 and C4 were significantly different between days in patients with AMI ($P < 0.0001$). Their findings support the present study.

Giasuddin *et al.* (2007) found mean serum C3 and C4 level ($mg.dl^{-1}$) in the AMI patients increasing from 1^{st} day (154 ± 28.5 and 38 ± 13) to 7^{th} day (171 ± 28 and 46 ± 15). Those values were higher than in the control subjects (132 ± 8 and 29 ± 6 respectively). Similar trends were also found with the present study. However, the mean C3 and C4 values reported by Giasuddin *et al.* (2007) were higher than the values found in the present study, which might be due to ethnic or racial difference or more affluent socio-economic conditions of Libyan people. In the present study, a positive correlation was found in the AMI patients between 1^{st} day and 5^{th} day for the C3 ($r = 0.539$) and C4 ($r = 0.337$). Giasuddin *et al.* (2007) also found a significant positive correlation of C3 and C4 elevation in AMI patients ($r = 0.522$ and $r = 0.483$, respectively). Therefore, the present findings are in well agreement with the findings of Giasuddin *et al.* (2007).

The complement C3 normally presents in serum at relatively high concentrations (Charlesworth *et al.*, 1974). It is also an insensitive marker for complement activation (Whaley, 1987). On the other hand, complement C4 is an independent predictor of stroke (Cavusoglu *et al.*, 2007). The complement system plays an important role in the physiopathology of AMI, taking part in myocardial damage and reperfusion injury. Local activation of the classical, alternative and lectin pathways of complement in infarcted myocardium has been observed in animal models for AMI (Amsterdam *et al.*, 1995; Collard *et al.*, 2000). A parallel rises in C3 and C4 was observed in the present study. These results show that complement was activated, particularly via the classical pathway.

In the present study, the lack of an increase of complement anaphylatoxins in non-streptokinase-treated AMI patients does not rule out that complement activation may have occurred in the ischemic area. Such activation might not cause detectable levels of complement catabolic peptides in the peripheral circulation. Previous observations may indicate that ischemia by itself is able to cause enough complement activation to be detected in the circulation (Yasuda *et al.*, 1990; Langlois and Gawryl, 1988). However, one of these publications indicates an activation that did not involve the different complement components proportionally (Langlois and Gawryl, 1988). Differences in the severity of the clinical condition might explain the discrepancies between our data and those of the other study (Yasuda *et al.*, 1990).

In this study, it was found that serum C3 and C4 levels were significantly increased in AMI compared with the healthy control subjects. These results show that activation of complement system occurs after AMI. The degree of activated complement system may be associated with the myocardial necrotic size and cardiac dysfunction in patients with AMI. With a better understanding of the inflammatory process subsequent to AMI, we may be able to decrease the ischemic damage and increase the survival rate for patients with AMI.

There are some limitations in the study; one is that it was a small cohort study. The other is the lack of studying complement activation products. However, the latter may be ignored, since it has previously been shown that complement activation products increase in AMI patients in parallel with increase in C3 and C4 (Mollnes *et al.*, 1988 and Yasuda *et al.*, 1989). In addition, since it was an observational design, the findings are hypothesis-generating rather than conclusive. However, the possibility of involvement of other novel inflammatory markers in the development and maintenance of AMI should continue.

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Risk Factors		C3		C4	
		1st day	*5th day*	*1st day*	*5th day*
Age	***<59 yrs***	146.09 ± 39.23	162.22 ± 64.16	38.87 ± 14.05	39.96 ± 19.28
	≥ 59 yrs	114.51 ± 44.21	120.38 ± 37.25	28.58 ± 11.43	32.10 ± 14.07
Sex	***Male***	131.42 ± 42.23	145.67 ± 61.37	32.99 ± 13.41	36.43 ± 19.05
	Female	149.96 ± 49.93	152.91 ± 48.87	45.67 ± 12.36	40.31 ± 9.01
Positive Family History	***Yes***	140.18 ± 22.51	154.15 ± 31.00	39.89 ± 16.54	36.58 ± 12.02
	No	132.91 ± 45.11	147.79 ± 61.99	34.02 ± 13.53	36.74 ± 18.38
Smoking	***Present***	133.80 ± 41.39	145.61 ± 62.69	33.56 ± 13.39	36.39 ± 19.46
	Past	137.37 ± 54.28	151.95 ± 43.78	41.27 ± 15.43	39.82 ± 8.15
Hypertension	***Yes***	141.36 ± 38.69	166.96 ± 71.95	34.79 ± 11.58	33.56 ± 18.46
	No	129.95 ± 46.52	133.49 ± 45.47	35.30 ± 15.57	39.42 ± 17.33
Diabetes	***Yes***	143.99 ± 28.00	199.71 ± 92.11	33.89 ± 13.07	29.92 ± 15.71
	No	133.06 ± 45.36	138.75 ± 49.73	35.28 ± 14.25	38.18 ± 18.02
Streptokinase	***Yes***	140.65 ± 42.86	160.10 ± 64.30	33.25 ± 13.18	36.11 ± 14.82
	No	120.19 ± 43.05	116.02 ± 25.55	39.42 ± 15.34	39.33 ± 24.08
Social Status	***Lower***	140.94 ± 47.22	151.23 ± 59.44	36.09 ± 12.22	37.21 ± 20.60
	Middle	131.72 ± 42.13	149.05 ± 55.78	34.46 ± 15.14	38.00 ± 12.89
	Upper	122.67 ± 39.05	129.91 ± 73.05	33.73 ± 18.29	34.68 ± 21.68

Table 1: The mean C3 and C4 levels ($mg.dl^{-1}$) in the AMI patients at different risk factors.

Complement	Level	Levels of Complements ($mg.dl^{-1}$)*		
		AMI Patients		*Control Subjects*
		1st day	5th day	
C3	Low	66.06	56.62	77.03
	High	220.47	300.14	130.35
	Mean ± SD	134.51 ± 43.23	146.88 ± 58.77	101.55 ± 14.66
C4	Low	12.73	9.10	10.49
	High	64.07	87.11	34.98
		35.09 ± 13.89	37.08 ± 17.71	24.77 ± 8.15
*(C3: Normal range: 75-135 mg/dl, Linear Range: 0-350 mg/dl; C4: Normal range: 9-36 mg/dl, Linear Range: 6-120 mg/dl)				

Table 2: The C3 and C4 levels in the AMI patients at the 1st day and 5th day.

Figure 1: Correlation between C3 & C4 complement level in AMI patients at 1st day

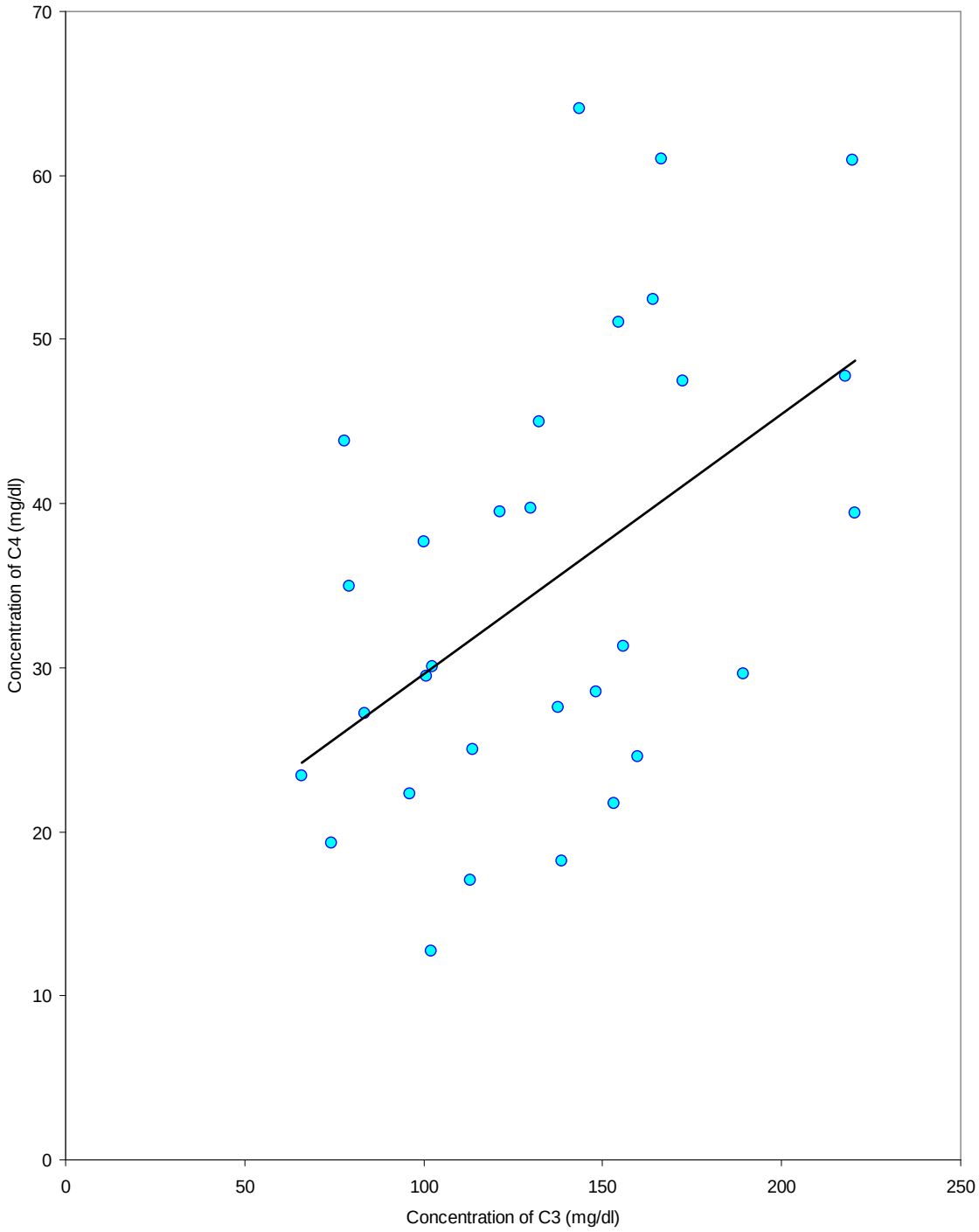
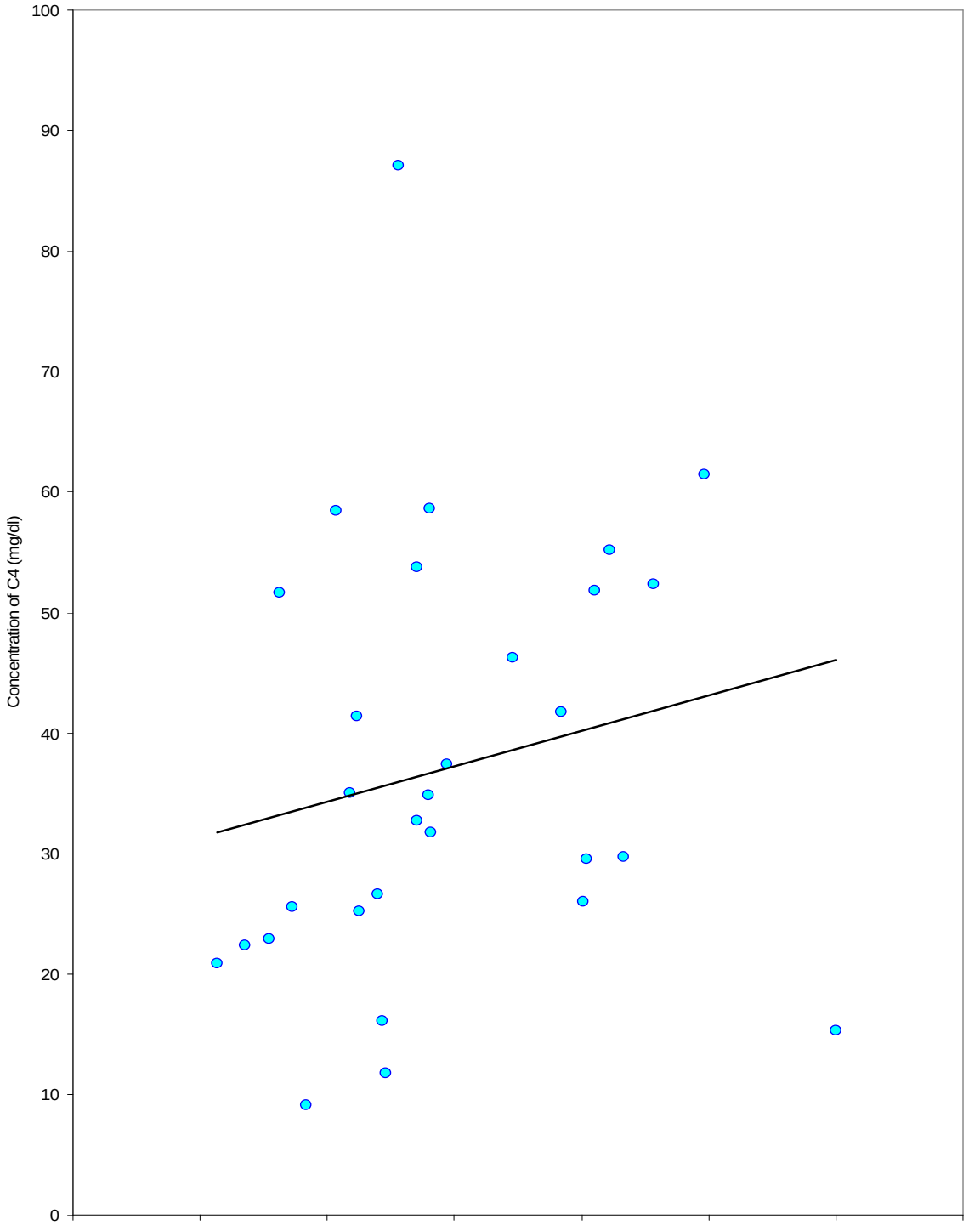


Figure 2: Correlation between C3 & C4 complement level in AMI patients at 5th day



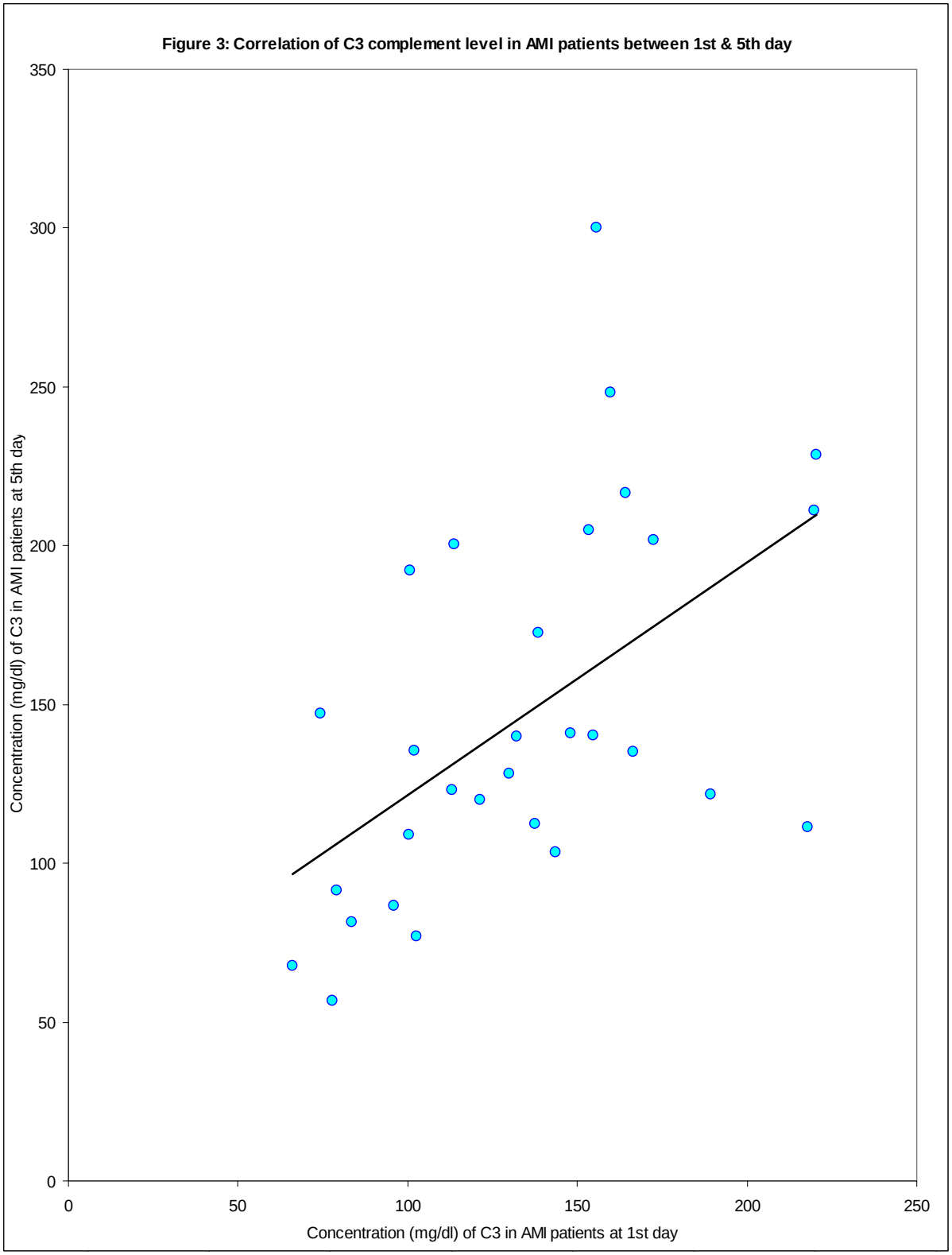


Figure 3: Correlation of C3 complement level in AMI patients between 1st & 5th day
350
300
250
200
150
100
50
0
Concentration (mg/dl) of C3 in AMI patients at 5th day
0
50
100
150
200
250
Concentration (mg/dl) of C3 in AMI patients at 1st day

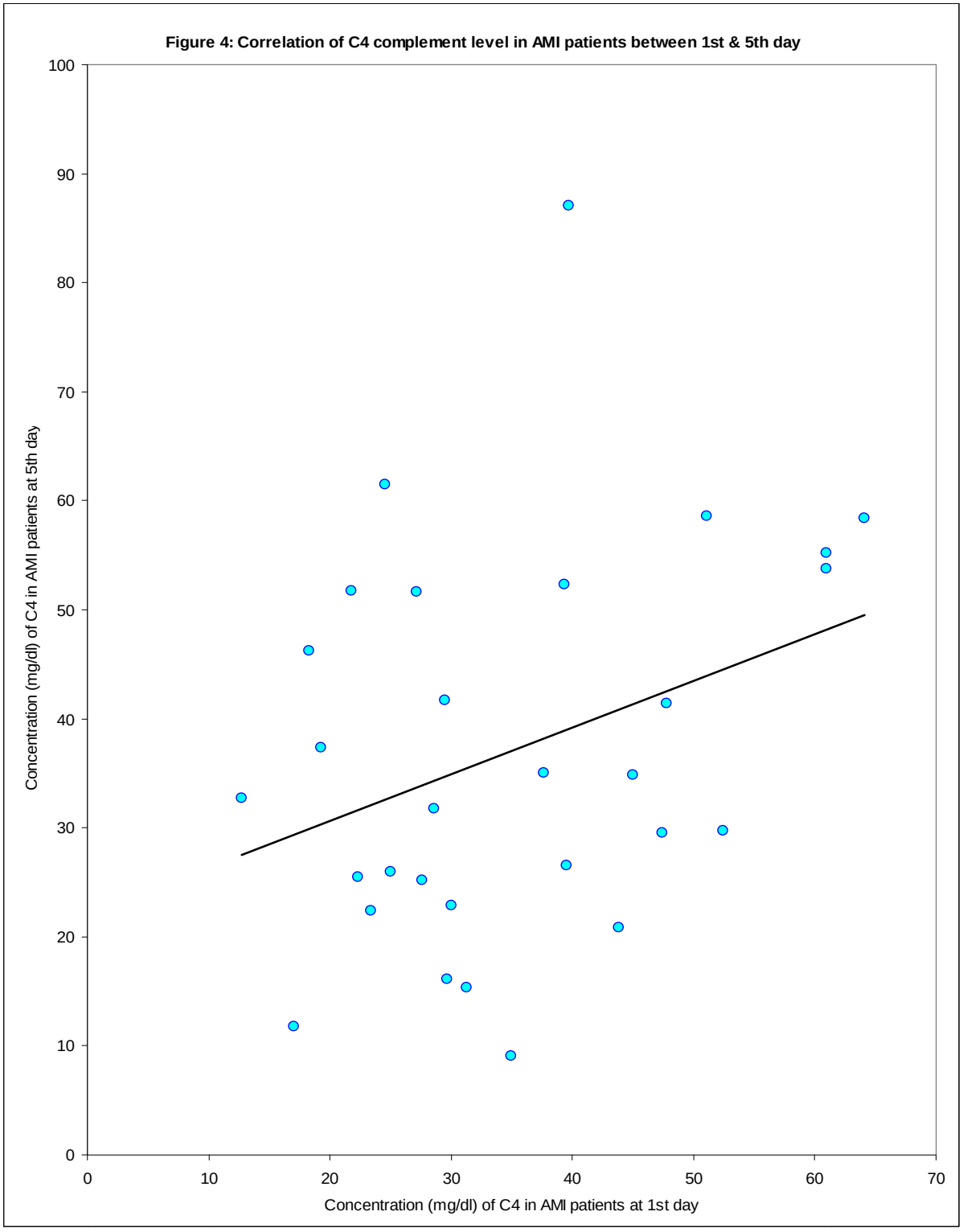


Figure 4: Correlation of C4 complement level in AMI patients between 1st & 5th day
Concentration (mg/dl) of C4 in AMI patients at 5th day
Concentration (mg/dl) of C4 in AMI patients at 1st day
0
10
20
30
40
50
60
70
80
90
100