The relevance of serum complement C3 with fasting blood glucose levels in obese type 2 diabetics

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الخلاصة

صممت الدراسة الحالية للتحقق من العلاقة بين البروتينات C3 و C4 مع مستوى السكر الصيام في الدم (FBGL) لدى الذين يعانون من السمنة من مرضى السكري النوع الثاني.

لانجاز هذا الغرض تم دراسة ٢٢ من مرضى السكري ذوي البدانة (١٢اناث و ١٠ذكور) معدل اعمارهم 45.23±13.83 ومعدل معيار الكتلة الجسمية BMI لديهم 34.52±2.15. $Kg.m^{-2}$ ولغرض المقارنة بين النتائج تضمنت الدراسة ٢٠ شخصا صحيحاً (١٠اناث و ١٠ذكور) معدل اعمارهم 36.55± 5.36 .

قيست مستويات C3 وC4 بطريقة الانتشار المناعي المنفرد بينما قدر مستوى السكرالصيامي بالدم بطريقة Glucose-oxidase .

بينت النتائج زيادة معنوية ($P<0.01$) في تركيز C3 عند مقارنته مع أمثاله في الأشخاص الأصحاء بينما لم يلاحظ مثل هذا التغيير في مستوى C4. اظهر تحليل الارتباط الخطي علاقات ارتباطيه معنوية بين مستوى C3 ($P<0.05$, $r=0.45$) مع FBGL في المصابين بالمرض.

وكذلك مثل هذه العلاقة كانت واضحة مع BMI اذ لوحظت علاقة معنوية موجبة ($r=0.60$, $P<0.001$) لمستوى C3مع BMI في مرضى السكري عند مقارنتهم بالأصحاء.

تشير هذه النتائج إلى إن C3 يوجه مستوى FBG في مرض السكري النوع الثاني وبالأخص المصابين بالسمنة منهم.

Abstract

The aim of the present study was to verify the relevance of complement, C3 & C4 with fasting blood glucose level (FBGL) in type 2 obese diabetics. To achieve this aim, 22 obese diabetic patients (12 females & 10 males) with ages of 45.23±13.83 and body mass index (BMI) of 34.52±2.15 $Kg.m^{-2}$ were enrolled. To compare the results, 20 healthy individuals (10 females & 10 males) of ages 36.55± 5.36 were also included. Serum C3 and C4 levels were measured in the patients & the control group by a single immune-diffusion method. Fasting blood glucose level was estimated by the glucose-oxidase method. In the diabetic patients, serum C3 level was found to be significantly ($P<0.01$) elevated when compared with those of healthy individuals whereas C4 didn't show such variation. The linear regression analysis exhibited a significant positive correlation for C3 ($r= 0.45$, $P< 0.05$) with FBGL in diabetics but not in healthy subjects. Such correlation was also significant ($r=0.60$, $P< 0.001$) with the BMI values. In

conclusion, C3 was found to direct the levels of FBGL in type 2 obese diabetic patients.

Kay words: C3, C4, FBGL, BMI, Diabetes mellitus (DM)

Introduction

The complement (c) system is a set of serum proteins that involved in the inflammatory process after activation due to various stimuli [1]. Activation of the complement cascade produces biologically active peptides capable of increasing vascular permeability, stimulating chemotaxis, enhancing phagocytosis and directly inducing cellular injury [2]. C3 and C4 complements are produced by the liver and a cytokine secreted by activated macrophages in inflammation sites and by adipocytes [3]. It has been widely recognized that the complement system plays a critical role in the pathogenesis of a variety of chronic human diseases including autoimmune diseases, atherosclerosis, and infertility in both males and females [4-7]. Accumulated evidences suggest that the complement system is also involved in the pathogenesis of diabetes mellitus [8, 9]

The synthesis of C3 & C4 is increased in response to inflammation and infection but at slower rate than for traditional acute phase proteins [10,11]. The size and shape of these two proteins appear to be similar, with molecular weights of about 200,000 and gyration radii of approximately the same size [12]. Both C3 and C4 have shown substantial correlations with obesity, in particular complement C3 which probably reflects C3 synthesis by adipocytes [13-15].

Type 2 diabetes mellitus is a chronic disease characterized by insulin resistance of the muscle, liver and adipose tissue and an impaired function of the β- cell of the pancreas. The incidence of type 2 diabetes mellitus has increased dramatically over the last decades [16]. Several inflammatory markers have been associated with the incidence of diabetes including, CRP, sialic acid, white blood cells, and IL-6. It has been proposed that diabetes is a disease of innate immune system [17-23]. However, several studies have reported a non significant relationship between inflammation and incidence of diabetes [24, 25]. It is worthy to mention that patients with diabetes have an increased risk of infection, but information on their immune response is incomplete and contradictory.

The present study is trying to ascertain whether C3 and C4 have a relationship with, fasting blood glucose level, as well as, with BMI, in type 2 diabetic patients.

Method

Twenty-two patients of type 2 diabetes mellitus were selected randomly, in this study. The patients routinely visited the Specialized Center for Endocrinology and Diabetes during May-July 2009. They were 12 females and 10 males. Their mean age was 45.23±13.83 y with a range of 20-63 y. The control group consisted of 20 healthy individuals (10 females & 10 males) of ages 36.55± 5.36 y with a rang 20-45 y. Serum

was collected from each blood sample and preserved at -20 C° until analysis. The complement C3 and C4 were measured by a single immunodiffusion method. Fasting blood glucose level was measured by glucose oxidase method. Body mass index was obtained through the dividing of body weight by the square of height ($Kg.m^{-2}$). Patients with a history of myocardial infarction, stroke, and cancer (determined by questionnaire) were excluded.

Statistical analysis

Data analyses were carried out using the statistical package for social sciences (SPSS version 10). The results were expressed as (mean ± SD) and analyzed by the use of independent t-test was done for the comparison of two groups and the differences were considered significant when P was <0.05. Pearson's correlation analysis of the data was made using the statistical program.

Result and discussion

The results of determination of C3, C4 levels demonstrated significant ($P<0.01$) elevation in diabetic patients when compared with those of the control group. C4 level exhibited non significant elevation in the group of diabetic patients when compared with those of control group (Table1). To clarify the impact of fasting blood glucose level on the complement proteins in diabetic patients, they were classified into two group, those of FBGL ≤ 11.1 mmol/L in the first group, and those of FBGL > 11.1 mmol/L in the second group. The results were evaluated by using independent t-test. Significant ($P<0.05$) elevation of C3 was obtained in the second group when compared with those of the first group. Further conformation of the relationship between C3 and C4 level with the FBGL values was demonstarated by the linear regression analysis. A significant positive correlation ($r =0.45, P<0.05$) for C3 levels was observed with FBGL magnitudes in the diabetic patients, but not in the control group. In contrast, C4 levels didn't show a significant correlation during a comparable evaluation (Table 3 and Fig 1,2).

To understand the relevance of the levels of C3 & C4 changes with obesity, the results were examined in respect with the BMI values of diabetic patients and the control group. As shown in table 4 and fig 3, 4, C3 levels illustrated significant ($r=0.60, P<0.001$) positive correlation with the BMI values in the diabetic patients, but not in the control group. However C4 levels failed to do so.

The relations between diabetes mellitus and inflammation are believed to be associated with hepatic production of glucose. Type 2 diabetes is associated with abnormalities in hepatic glucose production. Most of blood glucose and plasma proteins originate from the liver. IL-6 and other cytokines are involved in the regulation of those macromolecules. Dysregulation of hepatic glucose production in combination with insulin resistance could contribute to the relationship between inflammatory proteins and diabetes [24, 26].

It has been shown that the increased production of C3 and C4 in the liver is secondary to a state of generalized low–grade inflammation [27]. The pathogenesis of type 2 diabetes mellitus has been shown to include a chronic low grade inflammation and the activation of the innate immune system [28]. Thus the inflammatory processes are involved in the pathogenesis of type 2 diabetes mellitus but the relevance of C3 levels in particular in obese patients was unclear.

In the present study C3 levels were found to be significantly correlated with FBGL values in diabetic patients suggesting a role for the C3 complement in directing the glycaemic control. The explanation may be based on the function of insulin in these patients. Insulin is known to inhibit the hepatic synthesis of the complements including C3. Type 2 diabetes mellitus may be associated with elevated levels of insulin with insulin resistance. In such circumstance the inhibitory effect of insulin may be reduced with subsequent increase the of complement production [29]. Further consequences of hyperglycemia in diabetics may involve the non enzymatic glycosylation of the complements which may participate also in the induction of complement synthesis [30].

The significant positive correlation of C3 levels with BMI values is an additional factor that confirms the direct relevance between the C3 level and FBGL values in type 2 diabetics. Obese patients are frequently exposed to elevated levels of insulin and insulin resistance [31, 32]. Insulin could be considered as a mean that combines FBGL and BMI values with C3 levels.

It is worthy to mention, that C3 may play specific role in the atherogenesis. it's fragment, C3a-des-Arg exerts important function in the control of lipid and glucose metabolism [29, 30]. It is also found to be the most potent agent stimulating triglyceride synthesis and glucose membrane transport in human adipocytes. Corticosteroids normally antagonize the insulin induced cellular glucose uptake, elevating the blood glucose level. They are known to induce the production of complements from the liver. Corticosteroid secretion is promoted by cytokines through pituitary stimulation [27]. Thus inflammation is associated with elevated cytokine production and subsequent corticosteroid secretion that may elevate C3 concentration.

The reason of the independency of C4 level on FBGL and BMI values seems to be unclear and further investigation may be needed to resolve the problem.

In conclusion, C3 levels is involved in directing the FBGL values in type 2 diabetic patients in particular the obese individuals.

Table 1: Serum complement proteins (C3 & C4) levels in diabetic patients & the control group.

Parameter	group	Mean±SD	Range	P-value
FBGL (mmol/L)	Control	4.92±0.75	3.7 - 6	<0.0001
	Patient	10.61±2.12	7.5 - 13.6	
C3 (mg/dl)	Control	109.07±12.50	92.32 -132	<0.01
	Patient	155.66±30.98	116.7-234.6	
C4 (mg/dl)	Control	21.28±6.88	11 - 32.7	N.S
	Patient	36.38±5.29	23.2 - 43.3	

Table 2: The impact of fasting blood glucose level on complement proteins (C3 & C4)in diabetic patients.

Prameter	FBGL	Mean±SD	Range	P-value
C3 (mg/dl)	≤11.1 mmol/L	137.23±11.62	116.7-161.7	< 0.05
	>11.1 mmol/L	171.03±29.66	121.4 - 234.6	
C4 (mg/dl)	≤11.1 mmol/L	34.30±4.02	23.2 - 42.9	N.S
	>11.1 mmol/L	37.82±2.42	32.7 - 43.3	

Table 3: The correlation of serum C3 & C4 levels with fasting blood glucose level of diabetic patients and the control group

Parameter	Diabetic patients		Control group	
	r	P- value	r	P- value
C3 (mg/dl)	0.45	P<0.05	0.20	N.S
C4 (mg/dl)	0.06	N.S	0.03	N.S

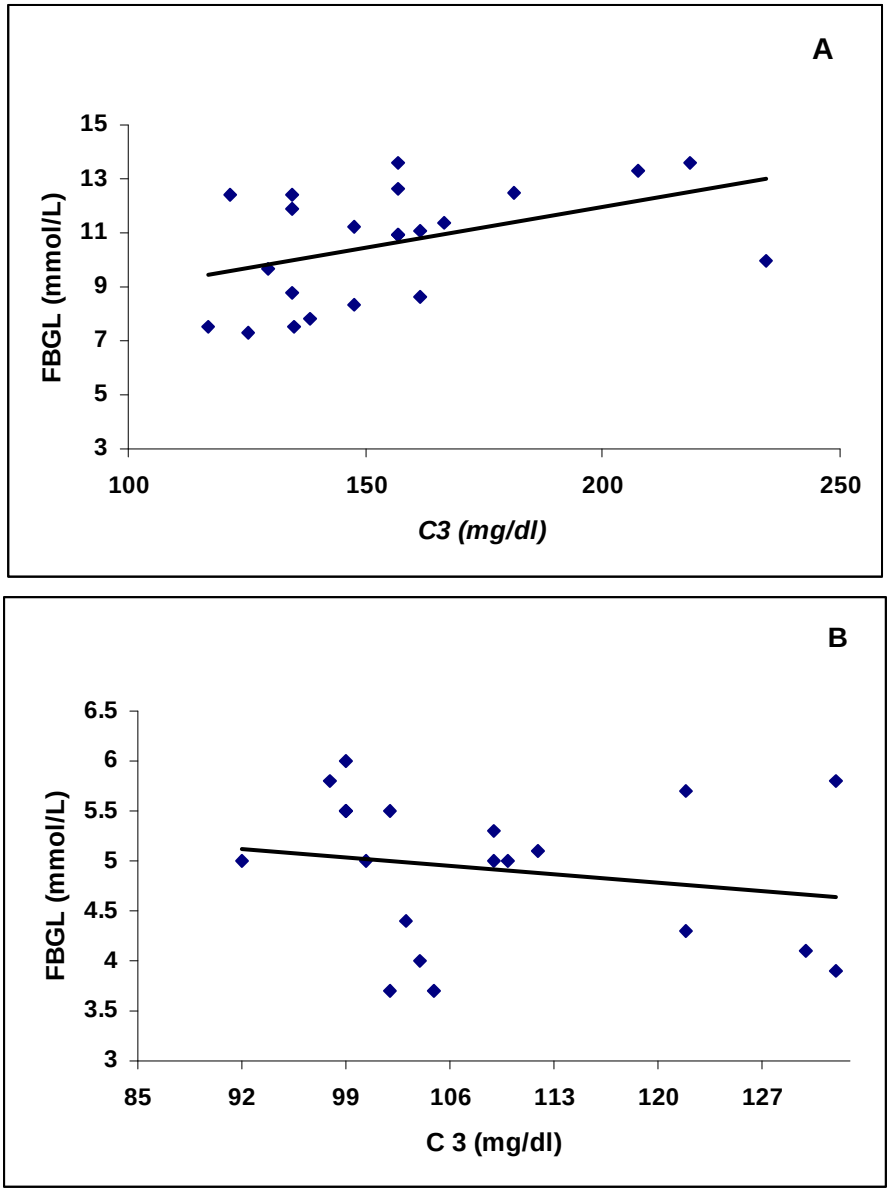
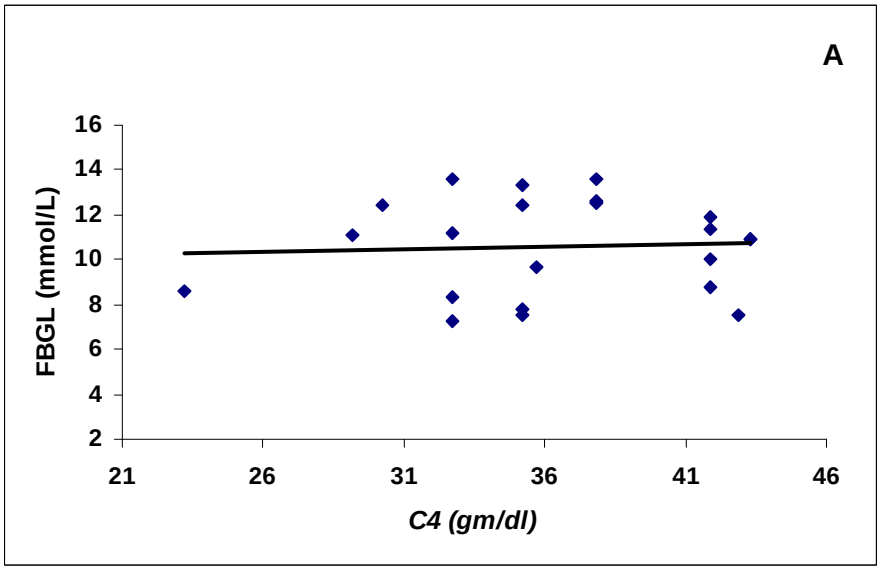


Fig 1: The correlation of serum C3 level with fasting blood glucose level (FBGL) in A:diabetic patients and B: control group



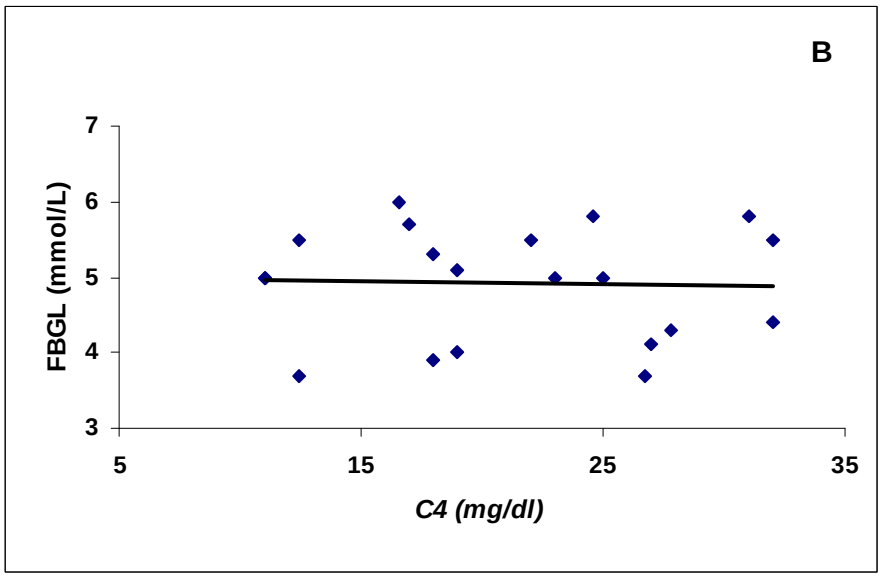


Fig 2: The correlation of serum C4 level with fasting blood glucose level (FBGL) in A:diabetic patients and B: control group

Table 4:The correlation of serum C3 & C4 levels with BMI of diabetic patients and the control group.

Parameter	Diabetic patients		Control group	
	r	P- value	R	P- value
C3 (mg/dl)	0.60	P<0.001	0.28	N.S
C4 (mg/dl)	0.07	N.S	0.24	N.S

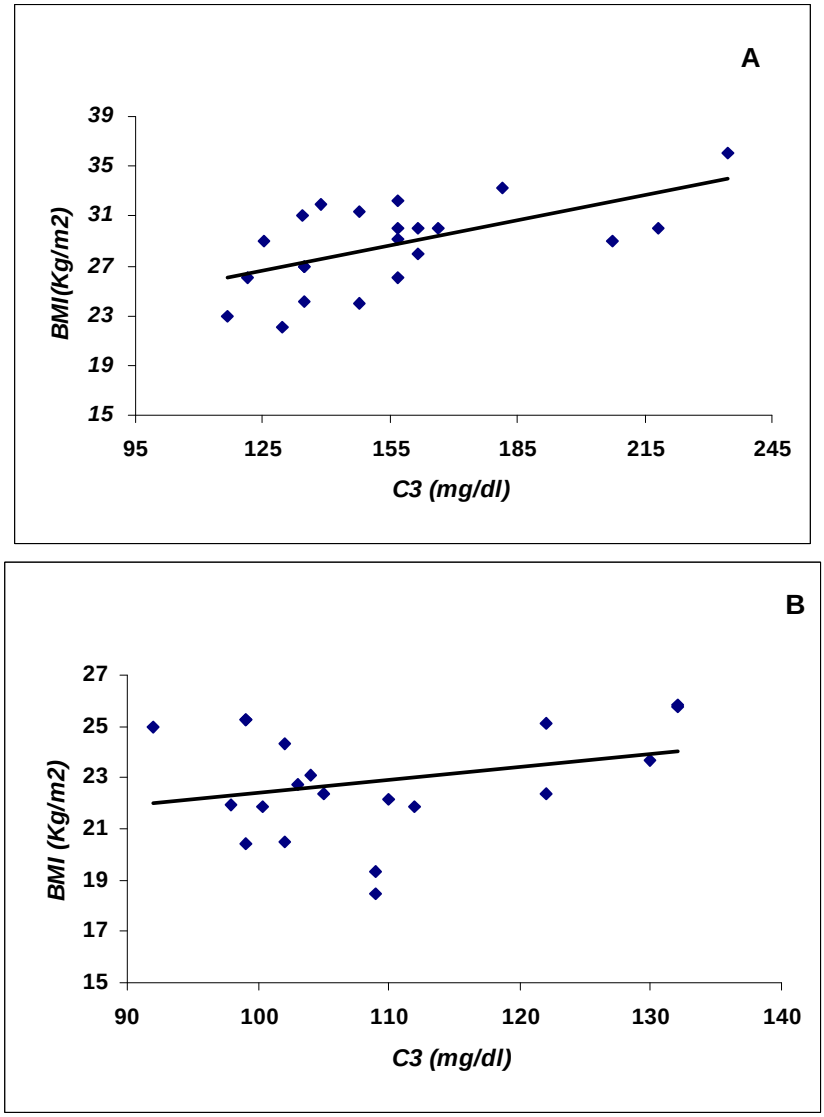


Fig 3: The correlation of serum C3 level with BMI in A:diabetic patients, and B: control group.

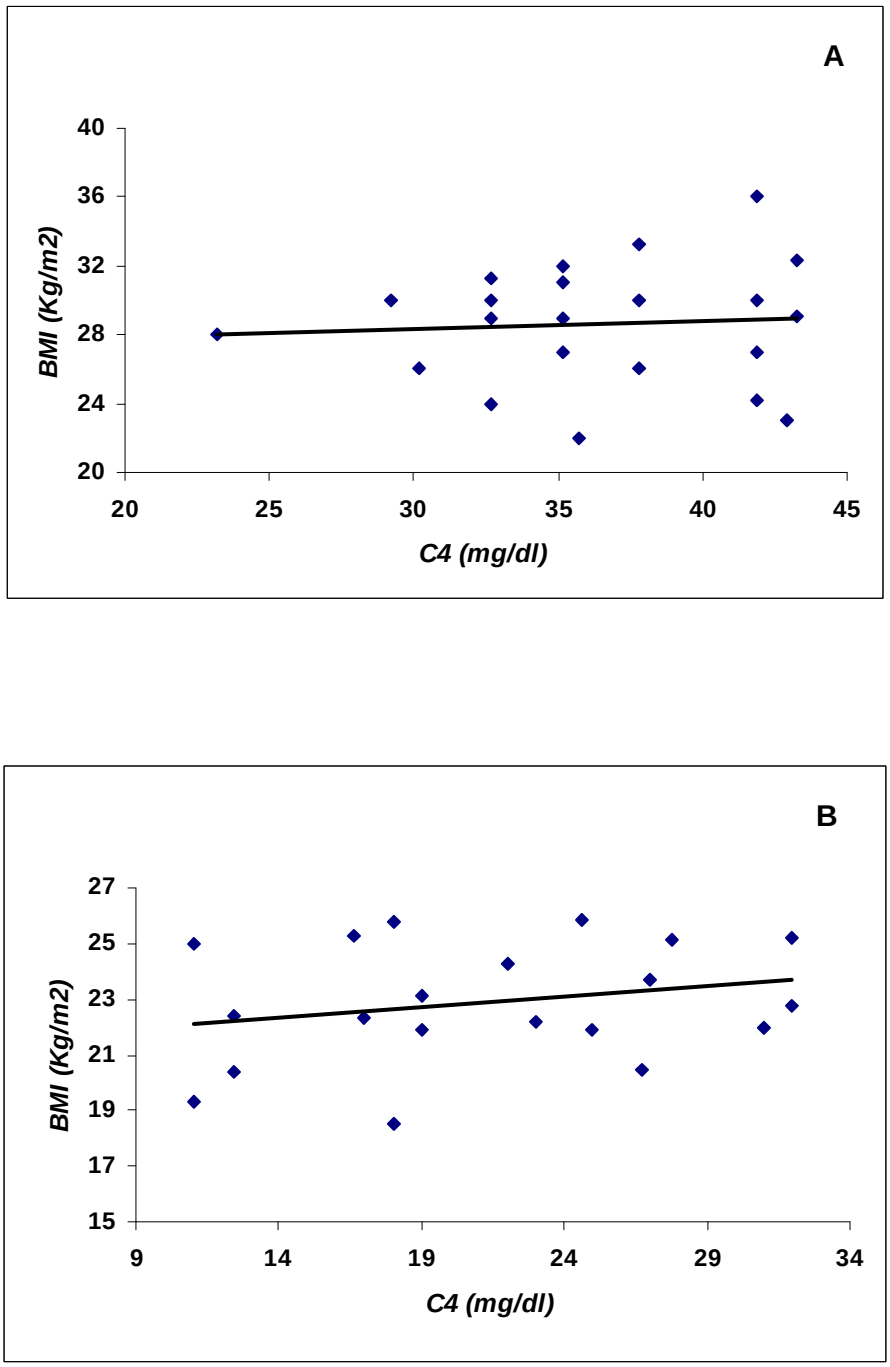


Fig 4: The correlation of serum C4 level with BMI in A:diabetic patients, and B: control group.

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