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Acute phase proteins levels (C3 , C4 and hsCRP) in type 2 diabetes patients

Nisreen Waleed Mustafa[1], [2]Zaid Nabeel Elia , Sivan Jameel Toma

[1]College of Pharmacy- University of Basrah
[2]Erbil Technical Health College- Erbil Polytechnic University

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Corresponding Author

zaidbio82@yahoo.com

Abstract

Diabetes mellitus is one of public health problem in world. Type 2 diabetes millitus (T2DM) which resistance cell to insulin. more inflammation occur in Patients with T2DM because of elevation of inflammatory marker like C3, C4 & hsCRP. This study was aimed to detect the level of acute phase reactant proteins (C3 , C4 and hsCRP) in T2DM patients.

Blood samples were collected from 60 patients with T2DM and control group was included 30 healthy donors. C3, C4 & hsCRP levels were estimated for all samples. Samples were grouped according to complications of patients.

The results showed that there was a significant increase in C3 level for patients with T2DM (199.0 mg/dl) compared to control group (135.4 mg /dl) while C4 level for T2DM (27.25 mg/dl) showed no significant change compared to control group(30.04mg/dl). C3 level of patients under heart disease (199.6 mg/dl), patients under thyroid disease (230.4 mg/dl) and patients without any disease (188.1 mg/dl) recorded significant elevation compared with control group (135.4 mg /dl) but C4 level did not show any significant change for all groups. hsCRP level recorded significant increasing in all study groups as fallowing : in all included patients (2.506 mg/L), T2DM patients with heart disease (**2**.238 mg/L),T2DM patients with thyroid disease (2.433 mg/L) and T2DM patients without any disease (2.20mg/L),

The conclusion of this study was the inflammation process associated with T2DM where inflammatory markers (C3 and hsCRP) elevated.

Introduction

Diabetes Mellitus is a syndrome of metabolic disease of heterogeneous etiology, characterized by hyperglycemia which is due to deficiency of insulin effect and results in abnormal metabolism of carbohydrate, protein and fat (Behraman et al., 2000)

T2DM is described as a combination of low amounts of insulin production from pancreatic β-cells and peripheral insulin resistance.(Kasuga, 2006) . T2DM is due primarily to lifestyle factors and genetics.(Ripsin et al,.2009).

The WHO has predicted that in the year 2025, the number of people with diabetes will have doubled and that out of 300 million people with diabetes, 90% of them are T2DM (Dikeukwu ,2012) According to International Diabetes Federation, the number of diabetes already reached 451 million in 2017 and estimated that in 2045, 693 million people will have diabetes (Cho et al., 2017).

Acute-phase reactants are known with their involvement as pro-inflammatory molecules in various inflammatory diseases. Most of the APRs generally elevate during various immunologically mediated conditions such as infection, trauma, surgery, burns, tissue infarction, or cancer (Gabay and Kushner,1999). They are being used as clinical markers in the diagnosis and management of some diseases, since they reflect the presence and intensity of inflammation. However, some of the Acute-phase proteins might have also pro-inflammatory properties (Blake and Ridker, 2001).

The complement system was defined as a heat-labile substance that assisted or killing of bacteria by heat-stable antibodies in the blood. Over time, it has come to be recognized as a group of proteins functioning as a humoral immune amplification system in innate immunity as well as a regulator of the adaptive immune response. Besides playing an integral role in host defense against infection, the major functions of the complement system include acting as an interface between innate and adaptive immunity and clearing immune complexes and apoptotic cells. Complement proteins activation occurs in the plasma and extracellular space (Carroll et al., 2012). The complement system is mainly associated with innate immunity in addition to be involved in metabolic events. Certainly, complement components C3 and C4 are associated with diabetes mellitus , cardiovascular risk and the metabolic syndrome. (Essam et al ., 2018).

C-reactive protein (CRP) is an acute-phase reactant produced primarily in the liver under the stimulation of adipocyte-derived proinflammatory cytokines, including IL-6 and TNF-α. CRP is the most commonly measured circulating marker for subclinical inflammation, with widely available, stable and standardised assays for its measurement (Pearson et al .,2003) ,this mediator increased in response to infection and inflammation.(Fronczyk et al., 2014).

Low-grade inflammation is highly associated with (T2DM), which confirmed by an elevation of C-reactive protein (CRP), an inflammatory biomarker in diabetic patients. Several studies suggested that inflammation may be involved in the pathogenesis of long-term complications of diabetes mellitus, especially cardiovascular diseases. (Fronczyk, 2014؛ Pepys, 2003& Garcia, 2010)

Material & methods

A total of 60 blood samples were collected from patients with diabetic types 2 confirmed clinically and by measuring of random blood sugar and HbA1C in blood. All selected patients were adults (34-72 years old). Patients samples were grouped as patients under complication with T2DM. Control group included 30 healthy donors. Sera were separated and kept at – 20 ℃ until used.

Collected sera were subjected to the following tests:

Measurement the level of C3 test (normal value ; 90 – 155 mg/dl. LTA- Italia) , C4 test (normal value; 20 - 50 mg/dl. LTA- Italia) by single radial immunodiffusion & hsCRP :Normal range (>1 mg/L. I.chroma).

Spss program was used for data analysis . Results were considered significant at $p<0.05$.

Result

Figure (1) showed 80 %(n=48) of included patients were female & 20%(n=12) were male.

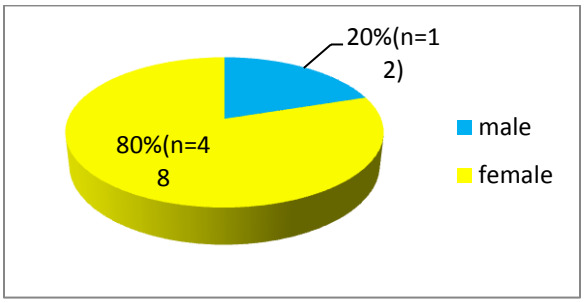

Figure 1: Number & percentage of T2DM according to gender .

Figure (2) Showed that there were 60 %(n=36) of patients had other disease with T2DM and 40 %(n=24) of patients without other disease.

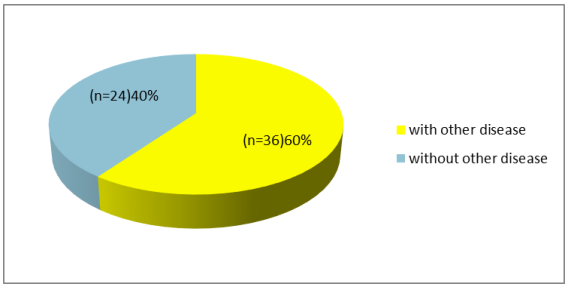

Figure 2: Number & percentage of T2DM patient according to other diseases.

According to BMI , 20% of T2DM patients had normal weight (BMI=18.5-24.9) , 40% of them were overweight (BMI=25-29.9) and 40% were suffering from obesity (BMI≥30) Figure (3)

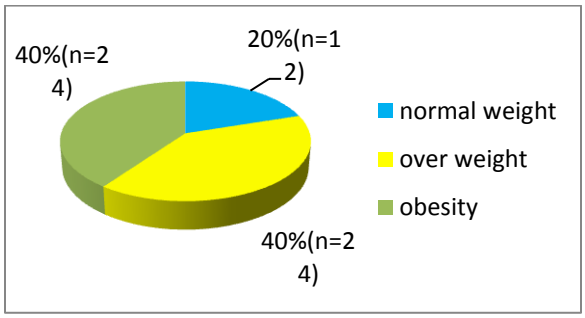

Figure 3: Number & percentage of T2DM patient according BMI.

43% (n=26) of patient did not have family history for T2DM and 57% (n=34) had family history for disease , Figure (4).

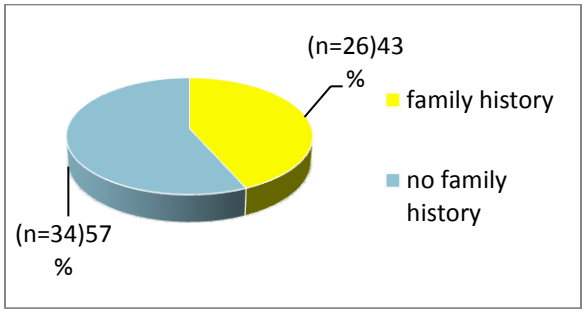

Figure 4: Number & percentage of T2DM patient according family history.

The level of C3 was measured in all study groups sera. Statistical analysis for the results showed significant difference (P<0.05) between C3 concentrations mean of patients with T2DM (199.6 mg/dl) and C3 concentrations mean of control group (135.4 mg/dl), while C4 mean(27.25mg/dl) revealed non-significant (P< 0.05) alteration comparing with control group (30.04 mg/dl). hsCRP significantly elevated (P<0.05) in patients (2.506 mg/L) comparing with control group (0.642 mg/L), table (1).

C3 concentrations mean for DM2 patients with heart disease (199.0 mg/dl) statistically elevated comparing with control group (135.4 mg/dl). Meanwhile C4 result (27.21 mg/dl) didn't show any difference (P<0.05) among the control groups (30.04 mg/dl) . The result of hsCRP gave significant difference between DM2 with heart diseases (2.238 mg/L) and control group (0.642 mg/L),table(2).

Statistical evaluation (P > 0.05) of C3 concentrations mean in T2DM patients with thyroid disease (230.4 mg/dl) when compared with control group (135.4 mg/dl) but C4 results revealed no significant difference among groups. The concentration of hsCRP appeared significant alteration between T2DM with thyroid diseases (2.433mg/L) and control group (0.642 mg/L), table (3).

Statistical elevated (P > 0.05) of C3 concentrations mean in T2DM patients without other disease (188.1 mg/dl) when compared with control group (135.4 mg/dl) but C4 results revealed no significant difference among groups . hsCRP concentration significantly elevated (P<0.05) in T2DM patients (2.20 mg/L) comparing with control group (0.642 mg/L), table (4)

Discussion

Patients with diabetes have an increased risk of infections. It has been suggested that chronic low-grade inflammation may be involved in the pathogenesis of insulin resistance and T2DM (Vozarova et al, 2002).

Type 2 diabetes included in this study were, 20% male and 80% female due biological and psychosocial factors are responsible for sex and gender differences in diabetes risk and outcome. Overall, psychosocial stress appears to have

greater impact on women rather than on men (Kautzky et al.,2016) . T2DM patients included in this study were 40% of included patients have not other diseases while 60% of patients have other diseases with T2DM like kidney disease , heart disease, thyroid disease ,colon disease due each disease have effected on level glucose in body . BMI was normal for 20% of selected patients, whereas 80% have abnormal BMI , Excess weight and physical inactivity are also associated with an increased risk of developing various diseases, particularly T2DM (Guh et al., 2009&Rudra et al., 2007).

This study recorded 57% of patients have family history with T2DM and 43% have not family history . T2DM factors which can be irreversible such as age, genetic and race and revisable such as diet, physical activity and smoking . (Wu et al ., 2014). Insulin binds to receptor on the cell surface, thereby triggering a biological response within the target cell. Mutation in the insulin receptor gene can render the cell resistant to biological action of insulin (Accili et al., 1989).

C3 and C4 are types of acute phase portiens, their concentrations may increase by50% during inflammation (Jayachandran et al., 2016).

In this study C3 concentration significantly elevated in all T2DM patients, patients with heart diseases, patients with thyroid disorders and patients without any disease. There were no significant alteration in C4 concentrations for all study groups . High level of C3 which estimated in T2DM patients with heart disease might interpret as increased C3 deposition within the intima of human atherosclerotic lesions compared with normal vessel intima, provided support for the suggestion that complement may play a direct functional role in atherosclerosis but C4 non-significant.(Bhakdi et al., 1998).

Savas et al. (2016) confirmed that inflammation had an important role in pathogenesis of thyroid dysfunctions regardless of their thyroid dysfunction type. Also they referred to the significant changes in the levels of inflammation markers occur for both autoimmune and non-autoimmune thyroid disorders.

C3 level in hyperglycemia patients with thyroid disease elevated due to thyroid hormones directly control insulin secretion, Insulin resistance states may increase thyroid gland nodularity (Hage et al., 2011).

Level of C3 of T2DM patient without other disease were increased because the secretion of stress hormones (glucagon, catecholamine, cortisol and GH) and especially, cortisol increases during the acute stress and emotional stimuli. Some of these hormones are diabetogenic and might be involved in the development of diabetes during the stress For example, epinephrine inhibits the insulin secretion both in animals and humans (Radahmadi et al .,2006).

Wlazlo et al . (2012) study agree with this study they recorded significant increasing of C3 level and no significant change of C4 level and they referred the complement system is might associated with innate immunity in addition to be involved in metabolic syndrome. Muscari et al. (2007) found C3 was significantly associated with diabetes .

Walport (2001) referred to under physiological conditions, complement promotes the clearance of immune complexes, an important way of eliminating antibody-coated bacteria. If, however, immune complexes cannot be eliminated, complement becomes chronically activated leading to increased consumption of the components. This might be the reason for lower level of complement C3 that could affect the formation of membrane–attack complex and lower bactericidal activity. The depressed bactericidal activity might be due to lower activation of the classical pathway complement as evidenced by significantly elevated levels of C4 arising from either low consumption or because of defects in other complement components. Hyperglycemia might have affected complement functions by non-enzymatic glycosylation. However, the cellular and molecular mechanisms which induced inflammation and organ damage in diabetic complications are related to increased activation of complement system via the inhibition of CD59 molecules. High glucose levels in diabetic patients inactivated CD95, prototype death receptor (CD95) molecules. Also, high glucose levels may affect the complement proteins leading to activating more membrane attack complex (MAC) deposition on the cells which developing the inflammatory process in diabetic

patients (Brownlee, 2001 & Kinderlerer et al.,2008).

hs-CRP is a valuable tool in determining the prognosis of systemic inflammatory diseases (Graf et al., 2009) , it's concentration may increase up to 1000 fold during inflammation (Jayachandran et al., 2016).

In present study , hsCRP significantly elevated in all study groups . Effoe et al., (2015) found a positive graded relationship between baseline hs-CRP and incident diabetes.

Temelkova et al. (2002) study reported that serum CRP levels are elevated in patients with impaired glucose tolerance (IGT) or diabetic. A few prospective studies have shown that increased CRP levels are an independent risk factor for future diabetes (Pradhan, 2001 & Barzilay et al .,2001). Muscari et al .(2007) referred CRP did not influence by T2DM .

hsCRP concentration is increased in various types of hyperthyroidism and hypothyroidism (Czarnywojtek et al. 2014).

References

1. Accili, D.; Frapier, C.; Mosthaf, L.; McKeon, C.; Elbein, C.; Permutt, A.; Ramos, E.; Lander, E.; Ullrich, A. and Taylor, I. (1989) . A mutation in the insulin receptor gene that impairs transport of the receptor to the plasma membrane and causes insulin- resistant diabetes. The EMBO journal, 8(9): 2509-2517 .
2. Behraman, E.:, Kliegman, M. and Jenson, B.(2000). Nelson textbook of pediatrics. 2nd ed. Philadelphia : W.B. Saunders.
3. Bhakdi,S. (1998) . Complement and atherogenesis: the unknown connection. Annals of Medicine, 30(6): 503–507.
4. Blake, G. and Ridker, P. Novel clinical markers of vascular wall inflammation. Circ Res., 89: 763-771.
5. Brownlee, M. (2001). Biochemistry and molecular cell biology of diabetic complications. Nature, 3;414:813-820.
6. Carroll m. and Isenman D. (2012) Regulation of humoral immunity by complement. Immunity, 37:199–207.
7. Cho, N.; Shaw, J.; Karuranga, S.; Huang, Y.; da Rocha Fernandes J. ; Ohlrogge, A. and Malanda, B. (2018). IDF diabetes atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract., 138:271–281.
8. Czarnywojtek , A.; Owecki , M.; Zgorzalewicz-Stachowiak , M.; Wolin ´ski ,K.; Szczepanek-Parulska, E.; Budny ,B.; Florek ,E.; ´rska-Stachura , J.; Miechowicz ,I.; Ba˛czyk ,M.; Sawicka ,N.; Dhir ,S. abd Ruchała.M.(2014). The Role of Serum C-Reactive Protein Measured by High-Sensitive Method in Thyroid Disease . Arch. Immunol. Ther. Exp. , 62:501–509.
9. Dikeukwu, R. and Omole, O. (2013) The Awareness and Performance of Appropriate Foot Self-Care Practices among Diabetic Patients Attending Dr. Yusuf Dadoo Hospital. Gauteng Province, South Africa. Journal of Endocrinology, Metabolism and Diabetes of South Africa, 18(2):112-118.
10. Effoe,V.; Correa,A.; Chen,H.; Lacy,M. and Bertoni, A. (2015). High-Sensitivity C-Reactive Protein Is Associated With Incident Type 2 Diabetes Among African Americans: The Jackson Heart Study Diabetes Care., 38:1694–1700.
11. Essam,H.; Norah, S .; Afnan, E. and Toleen, M.(2018).Association between Complement System, Inflammatory Cytokines and Glucose Control in Obese Subjects. Curre Res Diabetes & Obes J., 5(3): 1-5.
12. Fronczyk, A.; Molęda, P.; Safranow, K.; Piechota, W. and Majkowska, L. (2014). Increased concentration of C-reactive protein in obese patients with type 2 diabetes is associated with obesity and presence of diabetes but not with macrovascular and microvascular complications or glycemic control. Inflammation. 37(2):349-357.
13. Gabay, C. and Kushner, I. (1999).Acute-phase proteins and other systemic responses to inflammation. New Engl J Med., 340: 448-454.
14. Garcia, C.; Feve, B.; Ferre, P.; Halimi, S.; Baizri, H.; Bordier, L.; Guiu. G.; Dupuy, O.; Bauduceau, B. and Mayaudon, H.(2010). Diabetes and inflammation: fundamental aspects and clinical implications. Diabetes Metab.,36(5):327-38.
15. Graf, J.; Scherzer, R. ;Grunfeld C et al (2009). Levels of C-reactive protein associated with high and very high cardiovascular risk are prevalent in patients with rheumatoid arthritis. PLoS ONE 4:e6242
16. Guh, D.; Zhang, W.; Bansback, N.; Amarsi, Z.; Birmingham. C. and Anis, A. (2009). The incidence of comorbidities related to obesity and

overweight: a systematic review and meta-analysis. BMC Public Health, 9:88–107.
17. Haffner, S . (2003). Insulin resistance, inflammation, and the prediabetic state. Am J Cardiol., 92 (4):18-26.
18. Hage, M.; Zantout, M. and Azar, S.(2011). Thyroid disorders and diabetes mellitus. Journal of thyroid research, 2011:1-7.
19. Jayachandran, C. ; Suchetha, A.; Mundinamane,B.; Apoorva S. M., Bhat,D. and Lalwani,M.(2016). Acute phase proteins . Journal of Chemical and Pharmaceutical Research, 8(2):365-370.
20. Kasuga, M. (2006). Insulin resistance and pancreatic β cell failure. The Journal of clinical investigation, 116(7):1756-1760.
21. Kautzky-Willer, A,; Harreiter, J. and Pacini, G. (2016). Sex and gender differences in risk, pathophysiology and complications of type 2 diabetes mellitus. Endocrine reviews, 37(3):278-316.
22. Kinderlerer, A.; Ali, F.; Johns, M.; Lidington, E.; Leung, V.; Boyle, J.; Hamdulay, S.; Evans, P.; Haskard, D. and Mason, J. (2008). KLF2-dependent, Shear Stress-induced Expression of CD59 a novel cytoprotective mechanism against complement-mediated injury in the vasculature. Jo Biol Chem., 283(21):14636-44.
23. Kriketos, A.; Greenfield, J.; Peake, P.; Furler, S.; Denyer, G.; Charlesworth, J. and Campbell, L. (2004). Inflammation, insulin resistance, and adiposity. A study of first-degree relatives of type 2 diabetic subjects. Diabetes Care, 27:2033-2040.
24. Muscari, A.; Antonelli, S.; Bianchi, G.; Cavrini, G.; Dapporto, S.; Ligabue, A.; Ludovico, C.; Magalotti, D.; Poggiopollini, G.; Zoli , M. and Pianoro Study Group.(2007) Serum C3 is a stronger inflammatory marker of insulin resistance than C-reactive protein, leukocyte count, and erythrocyte sedimentation rate: comparison study in an elderly population. Diabetes Care, 30(9):2362–2368.
25. Pearson,T.; Mensah, G.; Alexander, R.; Anderson, JL.; Cannon, RO.; Criqui,M.; Fadl,YY.; Fortmann, SP.; Hong, Y.; Myers, GL.; Rifai, N.; Smith, SC.;, Taubert, K.; Tracy, RP, and Vinicor, F. (2003). Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation,107:499–511.
26. Pepys M. and Hirschfield, G.(2003). C-reactive protein: a critical update. J Clin Invest, 111:1805-1812
27. Radahmadi, M., Shadan, F., Karimian, S.M. and Nasimi, A. (2006). Effects of stress on exacerbation of diabetes mellitus, serum glucose and cortisol levels and body weight in rats. Pathophysiology, 13(1):51-55.
28. Ripsin, C.; Kang, H. and Urban, R. (2009). Management of blood glucose in type 2 diabetes mellitus. Am Fam Physician,79(1):29-36.
29. Rudra,C.; Sorensen, T.; Leisenring, WM.; Dashow, E. and Williams, MA.(2007). Weight Characteristics and Height in Relation to Risk of Gestational Diabetes Mellitus. American Journal of Epidemiology, 165:302–308.
30. Savas, E.; Sahin,A.; Aksoy, S.; Tascan, A.; Sayıner, Z. and Ozkaya,M. (2016). Serum levels of inflammatory markers in patients with thyroid. Int J Clin Exp Med., 9(2):4485-4490 .
31. Temelkova-Kurktschiev, T.; Henkel, E.; Koehler, C.; Karrei, K. and Hanefeld, M. (2002). Subclinical inflammation in newly detected type II diabetes and impaired glucose tolerance. Diabetologia., 45(1):151.
32. Vozarova, B.; Weyer, C.; Lindsay, RS. ; Pratley, RE.; Bogardus, C. and Tataranni, PA. (2002). High white lood cell count is

associated with a worsening of Insulin sensitivity and predicts the development of type 2 diabetes. Diabetes, 51(2): 455-461.
33. Walport, MJ. (2001). Complement—Second of Two Parts. N Engl J Med., 344(15): 1140–1144.
34. Wlazlo, N.; van Greevenbroek, M.;, Ferreira, I.; Jansen, EJ.; Feskens, EJ. ; van der Kallen, CJ.; Schalkwijk, CG.; Bravenboer, B. and Stehouwer, CD. (2012). Low-grade inflammation and insulin resistance independently explain substantial parts of the association between body fat and serum C3: the CODAM study. Metabolism, 61(12): 1787-1796.
35. Wu, Y.; Ding, Y.; Tanaka, Y. and Zhang, W. (2014). Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. International journal of medical sciences, 11(11): 1185-1200.

Table (1): C3 , C4 and hsCRP concentrations in T2DM patients and control group.

	C3		C4		HSCRP
Groups	Mean (mg/dl) ± SD	Range (mg/dl)	Mean (mg/dl) ± SD	Range (mg/dl)	MEAN (MG/L)± SD
Patients(n=60)	199.6 ± 76.53	75.40-337.7	27.25± 11.94	6.400-58.0	2.506±3.21
C(n=30)	135.4± 21.67	114.5 - 159.6	30.04 ± 8.338	16.10 - 41.90	0.642±0.31
P value	0.0031		0.2565		0.004

Table(2): C3 , C4 and hsCRP concentrations in T2DM patients with heart diseases and control group.

	C3		C4		HSCRP
Groups	Mean (mg/dl) ± SD	Range (mg/dl)	Mean (mg/dl) ± SD	Range (mg/dl)	MEAN (MG/L)± SD
Patients(n=26)	199.0 ± 68.97	94.20-337.7	27.21 ± 10.65	16.1 -58	2.238±2.60
C(n=30)	135.4 ± 21.67	114.5 - 159.6	30.04 ± 8.338	16.10 - 41.90	0.642±0.31
P value	0.0023		0.5177		0.04

Table(3): C3 , C4 and hsCRP concentrations in T2DM patients with thyroid diseases and control group.

	C3		C4		HSCRP
Groups	Mean (mg/dl) ± SD	Range (mg/dl)	Mean (mg/dl) ± SD	Range (mg/dl)	MEAN (MG/L)± SD
Patients(n=6)	230.4 ± 92.31	149.6-331	31.53 ± 13.63	16.10 - 41.90	2.433±0.60
C(n=30)	135.4 ± 21.67	114.5 - 159.6	30.04 ± 8.338	16.10 - 41.90	0.642±0.31
P value	0.0015		0.8634		0.000

Table (4): C3 , C4 concentrations and hsCRP concentrations in T2DM patients without any diseases and control group.

	C3		C4		HSCRP
Groups	Mean (mg/dl) ± SD	Range (mg/dl)	Mean (mg/dl) ± SD	Range (mg/dl)	MEAN (MG/L)± SD
Patients(n=24)	188.1 ± 87.91	75.4-337.7	23.11 ± 12.32	6.400 - 41.9	2.20±2.36
C(n=30)	135.4 ± 21.67	114.5 - 159.6	30.04 ± 8.338	16.10 - 41.90	0.642±0.31
P value	0.0342		0.0941		0.043