

## **Evaluation of Some Inflammatory Markers with Calcium Status in the Blood for Diabetic Patients in Al-Samawa City**

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### **Abstract**

**Background:** Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with long-term damage and dysfunction of kidneys, nerves, heart, and blood vessels.

**Materials& Methods:** colorimetric analysis had been applied for glucose concentration, ALP, Ca, CRP test as well as ESR\*.

**Results& Conclusions:** There is a significant increase in (glucose, ESR, ALP) level in diabetic patients as compared to the control group ( $P < 0.0001$ ), but no significant difference in (Ca and CRP levels) in diabetic patients as compared to the control group. There is a significant increase difference between (type1& type 2) diabetic patients & control group in (Glucose and ALP level). There is no significant difference between patients with type1& type 2 in (Glucose, ESR, ALP, Ca, CRP levels). There is no significant correlation between (Glucose, ESR, ALP, Ca, CRP level) between patients duration ( $<5$ ) & duration (5– 10) years. There is a significant positive correlation between (Glucose, ESR, Ca, CRP level) in diabetic patients with duration of the disease (duration  $<5$ ) & (duration  $>10$ ) years, there is a weak significant positive correlation in (Glucose, ESR, CRP level) in diabetic patients with duration of the disease between patients (duration 5-10)& (duration  $>10$ ) years. Also a significant positive correlation between (Ca level) in diabetic patients with duration of the disease between patients (duration 5- 10)& (duration  $>10$ ) years.

**Key words:** Inflammatory Markers, Calcium Status, Blood, Diabetic Patients.

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\* glucose, Alkaline phosphatase(ALP), and Calcium (Ca) ;titer ELISA method for C-reactive protein (CRP) and vertical 20cm Western tube for erythrocyte sedimentation rate ( ESR).

## تقييم بعض الدلائل الالتهابية مع حالة الكالسيوم في مصل المرضى المصابين بداء السكري في مدينة السماوة

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### الخلاصة

بعد جمع النتائج الخاصة بالمتغيرات تم تحليلها إحصائيا لغرض مقارنتها مع النتائج المتحصله من نماذج مجموعتي التحكم، وظهر التحليل الإحصائي ما يلي:

وجود زيادة داله في مستوى ALP و ESR و Glucose وعدم جود فرق معنوي دال في مستوى CRP و Ca بين مرضى داء السكري مقارنة مع مجموعتي التحكم، كما لوحظ وجود زيادة داله في مستوى ALP و Glucose بين مرضى داء السكري للنوعين الأول والثاني مقارنة مع مجموعتي التحكم، وعدم وجود فرق معنوي دال في مستوى CRP و Ca و ESR و Glucose بين مرضى داء السكري للنوع الأول مقارنة مع النوع الثاني، كذلك عدم وجود فرق معنوي دال في مستوى CRP و Ca و ESR و Glucose بين مرضى داء السكري ذوي فترة الإصابة بين (أقل من 5 سنوات و 5-10 سنة)، في حين يلاحظ وجود زيادة داله في مستوى CRP و Ca و ESR و Glucose بين مرضى داء السكري ذوي فترة الإصابة بين (أقل من 5 سنوات و أكثر من 10 سنوات)، بينما يلاحظ وجود زيادة ضعيفة داله في مستوى CRP و ESR و Glucose بين مرضى داء السكري ذوي فترة الإصابة بين (من 5-10 سنة و أكثر من 10 سنوات)، وأخيرا وجود زيادة داله في مستوى Ca بين مرضى داء السكري ذوي فترة الإصابة بين (من 5-10 سنة و أكثر من 10 سنوات).

الكلمات المفتاحية: الدلائل الالتهابية، حالة الكالسيوم، مصل الدم، داء السكري.

## **Introduction**

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with long-term damage and dysfunction of kidneys, nerves, heart, and blood vessels (1; 13; 18). The new classification for DM has been proposed by American Diabetes Association (ADA) and World Health Organization (WHO). It comprises four etiological types: type 1, type 2, other specific types, and gestational diabetes with impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) as stages in the natural history of disorders in carbohydrate metabolism (1; 23):

1. Type1 diabetes (previously named insulin dependent diabetes or juvenile onset diabetes): This form which accounts for only 5-10% of total DM patients (1; 9; 19), is characterized by an absolute deficiency of insulin caused by an autoimmune attack on beta cell of the pancreas; the islets of Langerhans become infiltrated with activated T lymphocytes, leading to insulinitis. Over a period of years, this autoimmune attack leads to gradual depletion of the  $\beta$  cell population, the  $\beta$  cell destroyed leads to absolute insulin openia. The  $\beta$  cell destruction is due to autoimmune diseases or viral infections, and in a few cases due to a genetic reason (16; 4; 11). Markers of the immune destruction of the  $\beta$ -cell include islet cell autoantibodies and autoantibodies to insulin, the rate of  $\beta$ -cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainly adults) (3). The critical mass of the remaining beta cell is unable to sustain insulin secretion at a level sufficient to maintain normal blood glucose values (10), so some patients particularly children and adolescents may show ketoacidosis as the first manifestation of the disease, others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or stress (5; 17; 6).
2. Type2 diabetes (previously named non-insulin dependent diabetes or adult-onset diabetes): This form of diabetes, which accounts for 90-95% of total DM patients those with diabetes (1), results from defects in the insulin molecular form, altered cell receptors for insulin, and represents an impaired insulin function (insulin resistance) than deficiency (2). However, insulin production may be diminished later in the disease and insulin supplementation may

therefore become necessary (12). The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity, women with prior gestational diabetes mellitus and individuals with hypertension or dyslipidemia. It is often associated with a strong genetic predisposition, more than the autoimmune form of type 1. The majority of patients with this form are obese, which itself , causes insulin resistance (1; 22; 7).

3. Other specific types of diabetes: These types could be due to other causes, e.g., genetic defects in  $\beta$ -cell function, genetic defects in insulin action, and diseases of the exocrine pancreas, drug or chemical induced infections (20).

### **Materials and Methods**

One hundred thirty nine patients with diabetes mellitus were seen from Oct. 2009 till May 2010, in Al-Samawa General Hospital. The diagnosis was done by clinical examination. And forty normal healthy persons were considered as normal healthy controls. The blood samples were allowed to coagulate at room temperature and centrifuged for 20 min. The resulting sera were separated and stored at about -20° C. until analyses which didn't exceed a fortnight. The subjects used in this study are:

		<b>Female No.</b>	<b>Male No.</b>	<b>Age/y Rang</b>
<b>Patients</b>	<b>139</b>	<b>74</b>	<b>65</b>	<b>71 -22</b>
<b>Normal healthy control</b>	<b>40</b>	<b>20</b>	<b>20</b>	<b>21-60</b>

Statistical Analysis was done by using student t-test. All the data were presented as mean  $\pm$  standard deviation .P-values <0.05 were considered significant

### **Results and Discussion:**

Diabetes mellitus (DM) is a chronic disease with long-term macro vascular and micro vascular complications, including diabetic nephropathy, neuropathy, and retinopathy. It is a leading cause of disability, and blindness death in the United States for persons 20–74 years of age (1; 23). Approximately 80 percent of blindness in this age group is related to diabetic retinopathy (3; 14).

The results in this study indicate a highly significant increase in glucose concentrations in patients with DM and all types of it compared to the normal control ( $P<0001$ ) as shown in (tables, 1, 2 and 3). These findings are in agreement with the findings reported by (21). No significant differences in glucose concentrations have been observed in patients with diabetic patients according to the type (type1 and type2), duration between (<5 and 5-10) years. A significant positive correlation of glucose concentrations according to the duration between (<5 and >10) years (table, 4) (fig. 1). So the glucose concentration measurement is important in the diagnosis and prognosis of disease and could be potentially useful and specific disease marker (8; 21; 15). A highly significant increase in ESR levels in patients with diabetic patients when compared to the normal control ( $P<.0001$ ) as shown in (table, 5). These findings are in agreement with the findings reported by (15). No significant difference in ESR levels have been observed in patients with diabetic patients according to the type (type1 and type2) and duration between (<5 and 5-10). These findings, as far as we know, have never been reported before .A significant positive correlation of ESR levels according to the duration between(<5 and >10)as shown in (table, 6) (fig. 2). These findings, as far as we checked have never been reported before. Not significant increase in CRP levels in patients with DM and both types of it when compared to the normal control. These findings are not in agreement with the findings reported by (24). No significant difference in CRP levels have been observed in patients with DM according to the type (type1 and type2), and duration between (<5 and 5-10). A significant difference in CRP levels has been observed in patients with DM according to the duration between (<5 and >10), as shown in (tables, 7) (fig. 3). So CRP measurement is not important in the diagnosis and prognosis of DM. A significant increase in ALP levels in patients with DM and both types of it when compared to the normal control ( $P<0001$ ) as shown in (tables, 8, 9 and 10). No significant difference in ALP levels have been observed in patients with DM according to the type (type1 and

type2), and duration between (<5 and 5-10). A weak significant difference in ALP levels has been observed in patients with DM according to the duration between (<5 and >10) and (5-10 and >10) (fig. 4). These findings, as far as we know, have never been reported before. So ALP measurement is important in the prognosis of DM and could potentially be useful but nonspecific disease marker. A weak significant increase in total Ca levels in patients with DM type1 when compared to the normal control. No significant difference in total Ca levels have been observed in patients according to the type 1 and 2, and between DM type2 compared to the normal control. A significant difference in total Ca levels has been observed in DM patients according to the duration between (<5 and >10) and (5-10 and >10) as shown in (tables, 11 and 12) (fig. 5). These findings could be used in the prognosis of DM.

**Table (1):** Biostatistical calculation of glucose level (mmol/ l) in sera of diabetic patients and normal healthy controls.

	Normal healthy controls	Patients
Sample size	40	139
Mean $\pm$ SD	6.5450 $\pm$ 1.02155	15.0101 $\pm$ 3.23789
Range	5,0-9.0	10.3-25.1
Standard error of mean	0.16152	0.27463
t- test	16.274	
Probability	<0.0001 (Highly significant)	

**Table (2):** Biostatistical calculation of glucose level (mmol/ l) in sera of type 1 diabetic patients and normal healthy controls.

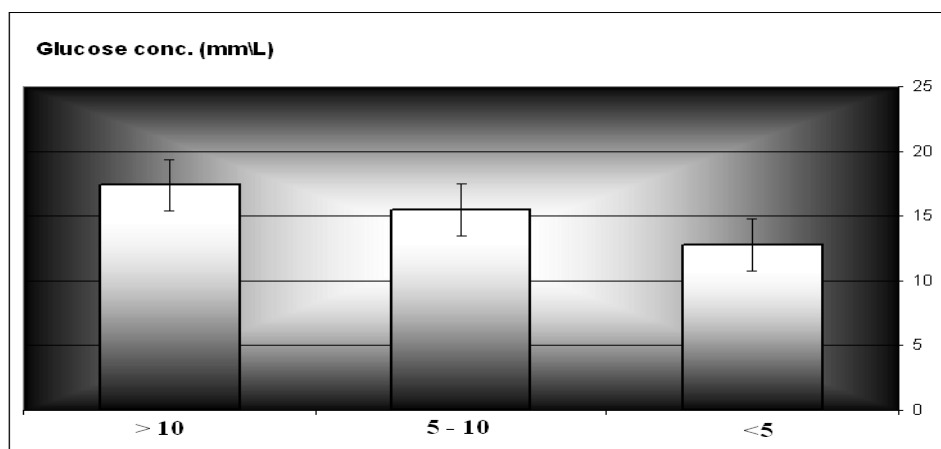
	Normal healthy controls	Patient(type1)
Sample size	40	62
Mean $\pm$ SD	6.5615 $\pm$ 1.02946	15.3629 $\pm$ 3.44275
Range	5,0-9.0	10.4-25.1
Standard error of mean	0.16485	0.43723
t- test	15.509	
Probability	<0.0001 Highly significant	

**Table (3):** Biostatistical calculation of glucose level (mmol/ l) in sera of type 2 diabetic patients and normal healthy controls.

	Normal healthy controls	Patient(type2)
Sample size	40	77
Mean $\pm$ SD	6.5600 $\pm$ 1.01623	14.7364 $\pm$ 3.05295
Range	5,0-9.0	10.6-23.1
Standard error of mean	0.16068	0.34792
t – test	16.442	
Probability	<0.0001 Highly significant	

**Table (4):** Biostatistical calculation of glucose level (mmol/l) in sera of diabetic patients comparing duration between <5 & >10 years.

	Patient (by duration)	
	< 5 years	> 10 years
Sample size	55	42
Mean $\pm$ SD	12.7855 $\pm$ 1.47716	17.4143 $\pm$ 3.88453
Range	10.4-15.1	12.9-25.1
Standard error of mean	0.19918	0.59940
t – test	8.113	
Probability	P<0.0001 Highly significant	



**Figure (1):** Glucose test of diabetic patients comparing duration between (<5 , 5 – 10 & >10) years.

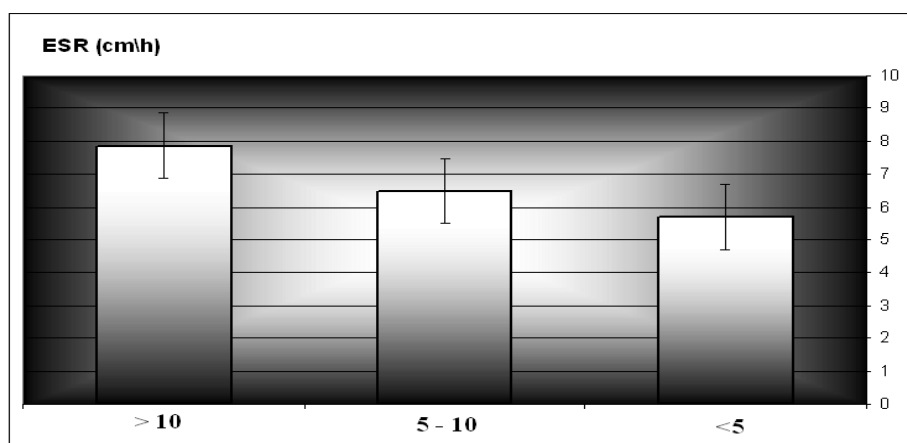
**Table (5):** Biostatistical calculation of ESR level (cm/h) in blood of diabetic patients and normal healthy controls.

	Normal healthy controls	Patient
Sample size	40	139
Mean $\pm$ SD	$3.6575 \pm 2.65223$	$6.5856 \pm 3.27674$
Range	1.2 – 12	2.5 – 14.4
Standard error of mean	0.41935	0.27793
T – test	5.181	
Probability	P <0.0001 Highly significant	



**Table (6):** Biostatistical calculation of ESR level (cm/h) in blood of diabetic patients comparing duration between (<5 & >10) years.

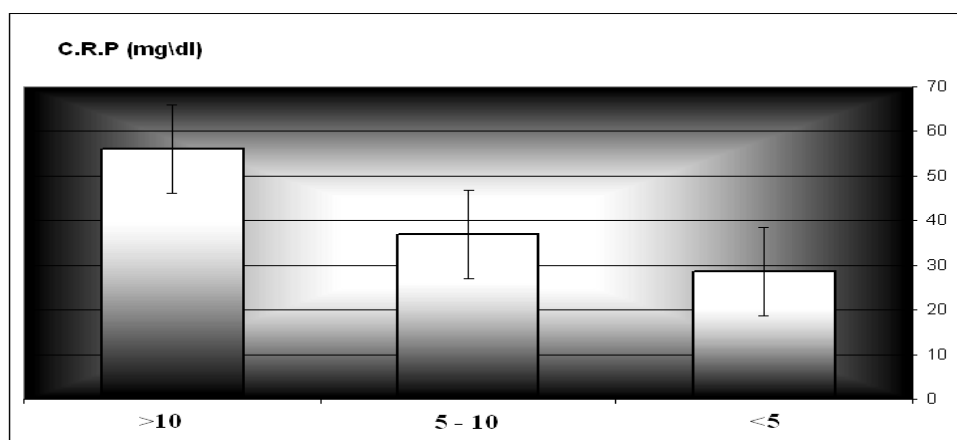
	Patient (by duration)	
	< 5 years	>10 years
Sample size	55	42
Mean $\pm$ SD	5.6945 $\pm$ 2.96881	7.8571 $\pm$ 2.78569
Range	2.5-12.1	4.1-12.7
Standard error of mean	0.40031	0.42984
t – test	3.650	
Probability	0.0001 P< Highly significant	



**Figure (2):** ESR test of diabetic patients comparing duration between (<5 , 5 – 10 & >10) years.

**Table (7):** Biostatistical calculation of C.R.P. Level by ELISA in sera of Diabetic patient comparing duration between (<5 & >10) years.

	Patient (by duration)	
	< 5 years	>10 years
Sample size	55	42
Mean $\pm$ SD	28.5455 $\pm$ 27.08684	56.0000 $\pm$ 35.50060
Range	6-96	6-96
Standard error of mean	3.65239	5.47786
t – test	4.322	
Probability	.00010P < Highly significant	



**Figure (3):** C. R. P test of diabetic patients comparing duration between (<5 , 5 – 10 & >10) years.

**Table (8):** Biostatistical calculation of ALP (U\L) in sera of diabetic patients and normal healthy controls.

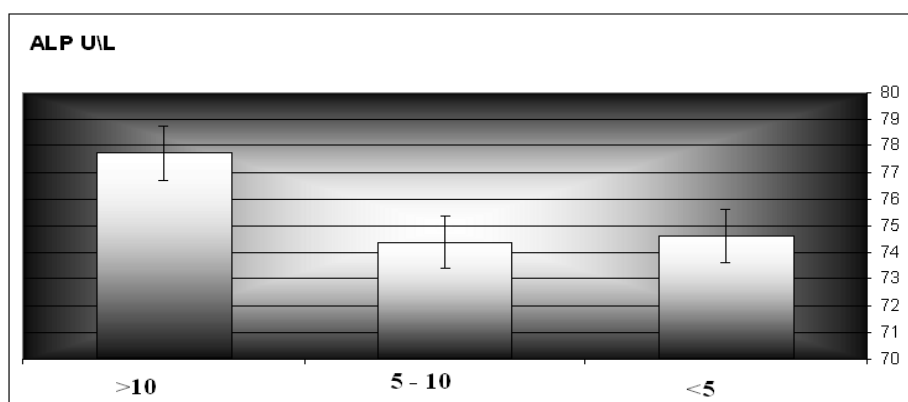
	Normal healthy controls	Patients
Sample size	40	139
Mean $\pm$ SD	66.6316 $\pm$ 11.11712	75.3525 $\pm$ 8.47491
Range	87 - 45	89 - 48
Standard error of mean	1.80343	0.71883
T – test	5.237	
Probability	.00010P < Highly significant	

**Table (9):** Biostatistical calculation of ALP (U\L) in sera of type 1 diabetic patients and normal healthy controls.

	Normal healthy controls	Patients (type1)
Sample size	40	62
Mean $\pm$ SD	66.5750 $\pm$ 10.83178	75.7419 $\pm$ 8.55634
Range	47-77	67-89
Standard error of mean	1.71266	1.08666
t – test	4.754	
Probability	.00010P < Highly significant	

**Table (10):** Biostatistical calculation of ALP (U\L) in sera of type 2 diabetic patients and normal healthy controls.

	Normal healthy controls	Patients (type2)
Sample size	40	77
Mean $\pm$ SD	66.5750 $\pm$ 10.83178	75.7419 $\pm$ 9.24034
Range	47-77	55-90
Standard error of mean	1.71266	1.05303
T – test	4.651	
Probability	.00010P < Highly significant	



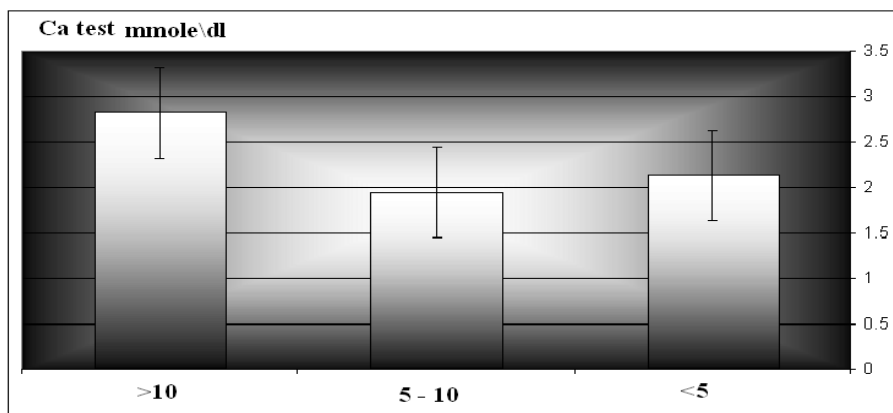
**Figure (4):** ALP test of diabetic patients comparing duration between (<5 , 5 – 10 & >10) years.

**Table (11):** Biostatistical calculation of Ca (m mole/dl) in sera of diabetic patient comparing duration between (<5 & >10) years.

	Patient (by duration)	
	< 5 years	> 10 years
Sample size	55	42
Mean $\pm$ SD	2.1382 $\pm$ 0.31298	2.8262 $\pm$ 0.47063
Range	10.4-15.1	12.9-25.1
Standard error of mean	0.04220	0.07262
t – test	8.583	
Probability	P<0.0001 Highly significant	

**Table (12):** Biostatistical calculation of Ca (m mole\dl) in sera of diabetic patient comparing duration between (5-10 & >10) years.

	Patient (by duration)	
	5 – 10 years	> 10 years
Sample size	42	42
Mean $\pm$ SD	1.9500 $\pm$ 0.46499	2.8262 $\pm$ 0.47063
Range	12.9-23.5	12.9-25.1
Standard error of mean	0.07175	0.07262
t– test	8.583	
Probability	P<0.0001 Highly significant	



**Figure (5):** Ca (m mole\dl) test of diabetic patients comparing duration between (<5, 5–10 & >10) years.

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