# A study of sICAM-1,sVCAM-1 and sE-Selectin Profile in type II diabetes and latent autoimmune diabetes of adult.

**Sawsan M Jabbar Al-Hasnawi; M.B.CH.B.,PH.D. clinical immunology , Alaa Saad Al-Attabi ; F.I.B.M.S.path. clinical immunology and microbiology and Zainab Abdu Al Ruda ; M.SC. histopathology.**

**Lecturer doctors ,faculty of medicine, Kerbela university, Iraq.**

*Key words:* ICAM-1: intercellular adhesion molecule 1, VCAM-1: vascular cell adhesion molecule 1,LADA: Latent autoimmune diabetes of adult,D.M.2 : diabetes mellitus type 2.

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**ABSTRACT:**

**Intercellular adhesion molecule 1( ICAM-1) and vascular cell adhesion molecule VCAM-1 are members of the immunoglobulin superfamily bind to other members of the superfamily, to integrins or to a diverse range of additional counter-receptors. E-selectin which is presented on endothelial cells when its activated acutely. Serum levels of adhesion molecules like ICAM-1 measurement are possible after shedding of these molecules from active surface of endothelial cells and macrophages in diabetes patients following hyperglycemia as in patient with LADA and type II diabetes mellitus .So that this study was done for measuring serum level of ICAM-1,VCAM-1 and E-Selectin Profile in type II diabetes and latent autoimmune diabetes of adult and study their relation with level of HbA1C , gender , age ,BMI and complication of diabetes. In the study the serum of 36 patients with type2 diabetes mellitus ,34 patients with LADA and 20 healthy subjects test by ELISA technique for measuring the level of ICAM-1,VCAM-1 and E-selectin . The study resulted in the mean sVCAM-1level of control group was significantly lower than D.M.2 and LADA group, $P<0.05$. The sE- Selectin level was higher in LADA group than control and D.M. type 2 group & significantly different when compared to control group ($P<0.05$). The sVCAM-1 level had a significant positive correlation ($R=0.25$, $P=0.017$) with BMI in all groups. High level of (sICAM-1,sVCAM-1 and sE-Selectin ) are associated with increased incidence of complication .**

# دراسة sICAM -1، sVCAM - 1 و sE- Selectinفي النوع الثاني من مرض السكري ومرض السكري المناعي الذاتي المتأخر الظهور للبالغين .

د.سوسن محمدجبارالحسناوي ,دكتوراه مناعة سريرية,د.الاء سعد العتابي ,بورد عراقي اختصاص مناعة سريرية واحياء مجهرية ود.زينب عبد الرضا العلي ماجستيراختتصاص نسيج مرضي
اعضاءفي الهيئة التدريسية في كلية الطب ,جامعة كربلاء,العراق

**الخلاصة:**
جزيئات خلوية مثل ICAM-1 VCAM-1 , و E- selectin يمكن قياسها في مصول النوع الثاني من مرض السكريو ومرض السكري المناعي الذاتي المتأخر الظهور للبالغين ودراسة علاقتها مع مستوى نسبة HbA1c، الجنس والعمر ومؤشر كتلة الجسم ومضاعفات مرض السكري.حيث شملت الدراسة مصول 36مريض من النوع الثاني من مرض السكري ,34مريض من مرضى السكري المناعي الذاتي المتأخر الظهور للبالغين وعشرين من مجموعة السيطرة خضعت مصولهم لقياس مستوى ICAM-1، VCAM-1 و E-selectin بواسطة تقنية الELISA. حيث كان متوسط مستوى ا لـ 1 – sVCAM عند مجموعة السيطرة أقل بكثير من مجموعة السكري النوع الثاني والسكري المناعي الذاتي المتأخر الظهور للبالغين ، P < 0.05 . SE- Selectin كان أعلى في مجموعة السكري المناعي الذاتي المتأخر الظهور للبالغين من السيطرة و مجموعة السكري النوع الثاني و ذو مدلول احصائي مقارنة بمجموعة السيطرة (P < 0.05). كان مستوى sVCAM - 1 له علاقة إيجابية هامة مع مؤشر كتلة الجسم( R = 0.25، P = 0.017 ). المستوى العالي من (SICAM-1، sVCAM-1 و SE-Selectin)يؤدي الى زيادة حدوث مضاعفات.

## Introduction:

**Intercellular adhesion molecule 1(ICAM-1 ) and vascular cell adhesion molecule (VCAM-1) are Members of the immunoglobulin superfamily bind to other members of the superfamily, to integrins or to a diverse range of additional counter-receptors[1,2].The intercellular adhesion molecule 1 (ICAM-1), which are CD54; is present on normal endothelial cells, but expression increased in atherosclerosis early stages [3]. Relevant increment in expression of ICAM-1occur when there is IFN- γ, TNF- α , IL-1 β and endotoxin of bacteria stimulation and this will lead to increased presence of these molecules in the activated endothelium [4]. The vascular cell adhesion molecule 1 (VCAM-1) is a transmembrane glycoprotein. Up regulation of VCAM-1 occur when TNF-α and IL-1stimulated, as when happened in the atherosclerotic lesion [5]. E-selectin (also called endothelial-leukocyte adhesion molecule 1), which is CD62E, is present on endothelial cells when its activated acutely. E-selectin enhances rolling of leukocyte to the inflammatory sites endothelium and is found in atherosclerotic plaques after cytokine stimulation [6]. Serum levels of adhesion molecules like ICAM-1measurment are possible after shedding of these molecules from active surface of endothelial cells and macrophages in atherosclerotic lesions and this is so important because it can give a clue for the**

**endothelial cell activation in atherosclerosis [7]. Elevation of serum levels of sICAM-1 is present in type 2 diabetes mellitus patients. However, increased levels also found in normoglycemic subjects after acute hyperglycemia. These results suggest that increment in the adhesive features of endothelium occurred following hyperglycemia that can be avoided or lowered by insulin therapy [8] serum E-selectin levels will decreased when blood sugar is well controlled, but sVCAM-1 levels will not change. A significant association present between the sE-selectin decrement and insulin sensitivity increment. So microvascular and macrovascular diabetic complications risks in diabetes mellitus type 2 patients can be reduced by these changes [9]. It was found by previous studies lipoprotein oxidation and protein glycation were suggested to increase binding of monocyte to the vessels of diabetic patient [10]. Acute and chronic increment in blood glucose will stimulate expression of adhesion molecules, like VLA-4 and ICAM-1 and lead to activation of endothelium [11].Also diabetic patients have increased serum levels of adhesion molecules such as ICAM-1, VCAM-1, and E-selectin [12].**

**Patients and method:**

**Ninety individual were enrolled in this study at the period from June, 2013 to January, 2014.They were divided into 36 patients with type2 diabetes mellitus ,34 patients with LADA and 20 healthy subjects as control. All patients were selected randomly from Diabetes outpatient clinic in AL-Hussein Teaching Hospital. Descriptive variables of patients included: name, age, gender, type of diabetes, type of treatment (insulin, OHD, diet or mixed), FBS, HA1C, BMI, complications they were suffered due to D.M. and duration of disease. All serum sample were tested by ELISA technique. The ELISA kits were being used Human sICAM-1 platinum ELISA (eBioscience, USA), Human sVCAM-1 platinum ELISA (eBioscience, USA) and Human sE-selectin platinum ELISA (eBioscience, USA).**

**Results:**

**The table (1) showed the mean values of sICAM-1, sVCAM-1 and sE- selectin, it had been noticed that the mean sVCAM-1 of control group was significantly lower than D.M.2 and LADA group, P<0.05. sE- Selectin was higher in LADA group than control and D.M.2 group & significantlly different when compared to control group (P<0.05), while no significant difference had been found among D.M.2 and LADA group, P>0.05 as illustrated in table (1). Table (2) was showing the following statistical result :there was a statistically significant positive correlation between the levels of sVCAM-1 and HA1C, (R = 23, P=0.028), but sE- selectin was inversely correlated (R= -0.20, P=0.047). It had been found that mild to moderate correlation (according to the value of R) exists between sICAM-1,sVCAM-1 and sE-selectin and the duration of D.M., this correlation was not statistically significant, P>0.05. Only sVCAM-1 level had a significant positive correlation (R=0.25, P=0.017) with BMI while other markers had not. P>0.05. sICAM-1 had significantly positively correlated with the age, & the mean sICAM-1, increased with the age, R = 0.29, P=0.006. No statistically significant differences between both genders in the mean values of markers were found, in all correlation tests P>0.05. Some markers are higher in obese patients; others are higher in overweight & normal weight although results are still statistically non significant (p-value>0.05) as show in table3,4. As shown in table 5, no significant differences in mean values of the 3markers(sICAM-1,sVCAM-1 and sE-selectin) were found when those D.M.2 cases were compared with complications against those without, P> 0.05. On the contrary among cases with LADA, the only significant difference was found in the sE-selectin marker, where the mean value in those with complication was higher than those without complication; (74 ± 6) vs. (56±3) respectively, P=0.01, (Table 5). In tables 6& 7 regarding comparison values of markers to different complications in both LADA & D.M.2 cases it shows no statistical significance where p-value<0.05(significant difference).**

**Table (1): Comparison of mean values of Adhesion molecules markers of the studied groups.**

| Marker | DM2 (No.=36) | LADA (No.=34) | Control (No.=20) | Compared groups | P-value |
|---|---|---|---|---|---|
| sICAM-1 (ng/ml) | 233.7 ± 90.6 | 234.2 ± 51.4 | 199.3 ± 10.1 | DM2 vs. LADA | 0.99 *NS* |
| | | | | DM2 vs. control | 0.18 *NS* |
| | | | | LADA vs. | 0.17 *NS* |
| sVCAM-1 (ng/ml) | 592.9±88.3 | 651.6±97.5 | 161.9 ± 29..4 | DM2 vs. LADA | 0.82 *NS* |
| | | | | DM2 vs. control | 0.006 *sig* |
| | | | | LADA vs. controls | 0.002 *sig* |
| sE-Selectin (ng/ml) | 56.6 ±24.6 | 59.8 ± 17.3 | 42.9 ± 19.5 | DM2 vs. LADA | 0.81 *NS* |
| | | | | DM2 vs. control | 0.055 *NS* |
| | | | | LADA vs. controls | 0.015 *sig* |

*** NS: not significant, sig : significant. DM :diabetes millets , LADA :latent autoimmune diabetes of adult.**

**Table (2): significant correlation of markers (sICAM-1, sVCAM-1 and sE- selectin) with HbA1c, Duration, BMI ,Age and gender.**

| Markers | Marker vs. HbA1c | | Marker vs. Duration | | Marker vs. BMI | | Marker vs. Age | | Marker vs.gender | |
|---|---|---|---|---|---|---|---|---|---|---|
| | (R) | P-value | (R) | P-value | (R) | P-value | (R) | P-value | (R) | P-value |
| sICAM-1 | 0.01 | 0.37 | 0.11 | 0.38 | 0.020 | 0.85 | 0.29 | 0.006* | 0.16 | 0.88 |
| sVCAM-1 | 0.23 | 0.028* | - 0.084 | 0.49 | 0.25 | 0.017* | 0.003 | 0.96 | - 0.020 | 0.85 |
| sE- selectin | - 0.20 | 0.047* | - 0.87 | 0.48 | - 0.088 | 0.41 | 0.055 | 0.61 | 0.1 | 0.47 |

**Pearson Correlation coefficient (R), * *Correlation is significant at P<0.05.***

**Table (3): Comparison of mean values of the markers sICAM-1,sVCAM-1 and sE-selectin according to BMI categories of 36 DM2 cases. (values represented the mean values of the markers).**

| BMI DM | Normal (18 - 24.9) | | Overweight (25 - 29.9) | | Obese (>=30) | | p-value |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | |
| sICAM-1 (ng/ml) | 264.7 | 55.6 | 226.7 | 20.0 | 229.8 | 6.9 | 0.77 |
| sVCAM-1 (ng/ml) | 770.5 | 256.5 | 669.1 | 115.0 | 886.6 | 181.2 | 0.54 |
| sEselectin (ng/ml) | 75.2 | 17.3 | 54.0 | 4.3 | 49.8 | 4.5 | 0.12 |

**Table (4): Comparison of mean values of the markers sICAM-1,sVCAM-1 and sE-selectin according to BMI categories of 34 LADA cases. (values represented the mean values of the markers).**

| BMI LADA | Normal weight (18 - 24.9) | | Overweight (25 - 29.9) | | Obese (>=30) | | P-value |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | |
| sICAM-1(ng/ml) | 234.8 | 17.9 | 230.8 | 11.3 | 245.0 | 24.8 | 0.87 |
| sVCAM-1 (ng/ml) | 924.6 | 169.2 | 790.1 | 142.2 | 726.8 | 277.8 | 0.78 |
| sE-selectin (ng/ml) | 63.1 | 5.8 | 57.3 | 3.7 | 61.6 | 8.9 | 0.67 |

**Table (5): Comparison of mean values of markers sICAM-1,sVCAM-1 and sE-selectin according to presence of complications among the 36 DM2 cases and the 34 LADA cases.**

| Markers | DM2 cases no.36 | | | | | LADA cases34. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | With complication No.=11 | | Without complication No.=25 | | P-value | With complication No.=7 | | Without complication No.=27 | | p-value |
| | Mean | SD | Mean | SD | | Mean | SD | Mean | SD | |
| sICAM-1(ng/ml) | 268 | 30 | 218 | 17 | 0.13 | 236 | 19 | 234 | 10 | 0.92 |
| sVCAM-1(ng/ml) | 652 | 189 | 567 | 98 | 0.66 | 733 | 214 | 631 | 111 | 0.68 |
| sE-selectin(ng/ml) | 48 | 7 | 60 | 5 | 0.18 | 74 | 6 | 56 | 3 | 0.010* sig. |

**P. value is significant at <0.05 level**

**Table (6): Comparison of mean values of markers sICAM-1,sVCAM-1 and sE-selectin according to complications among DM2 cases. (values represented the mean values of the markers).**

| Marker | Complications | | | | | P-value |
|---|---|---|---|---|---|---|
| | Retinopathy | diabetic foot | IHD | CVA | Nephropathy | |
| sICAM-1 (ng/ml) | 197.2 | 268.0 | 371.5 | 346.0 | 341.0 | 0.189 |
| sVCAM-1 (ng/ml) | 738.0 | 1521.5 | 472.0 | 279.0 | 426.0 | 0.44 |
| sE-selectin (ng/ml) | 39.4 | 56.5 | 66.0 | 42.0 | 47.0 | 0.82 |

*** P. value is significant at <0.05 level**

**Table (7): Comparison of mean values of the markers sICAM-1,sVCAM-1 and sE-selectin according to complications among LADA cases. (values represented the mean values of the markers)**

| Markers | Retinopathy | diabetic foot | IHD | CVA | nephropathy | p-value |
|---|---|---|---|---|---|---|
| sICAM-1 (ng/ml) | 240.7 | 208.0 | None | None | None | Non significant p>0.05 |
| sVCAM-1 (ng/ml) | 794.0 | 366.0 | | | | |
| sE-selectin (ng/ml) | 77.7 | 54.0 | | | | |

## Discussion:

**In our study we noticed that the mean sVCAM-1 of control group was significantly lower than DM2 and LADA group, P<0.05. Also, sE- Selectin was higher in LADA group than control group (P<0.05). All mean levels of adhesion molecules were higher in diabetic patients (D.M.2 & LADA) than in control group, (see table 1) These results are in line with other studies that showed an increment in VCAM-1 concentrations in D.M. type 2 compared to normal individuals (Matsumoto *et al.*, 2002; Pham *et al.*, 2012; Albertini *et al.*, 1998)[9,12,13] Also another study done by Antonio Ceriello, (2004) [14] confirms that ICAM-1, VCAM-1, and E-selectin plasma levels are lower significantly in control cases compared with type 2 diabetic patients (144.6±22.7 vs. 225.5±18.4, 630.8±11.5 vs. 934.6±32.7& 35.8±18.5 vs. 75.9±12.3 respectively). On the contrary, Boulbou *et al.*, 2005[15] revealed no difference in VCAM-1 concentrations in type 2DM patients when compared to control. Our results show that adhesion molecules increased in patients with LADA compared to control group, (see table1). This result is in agreement with previous studies that show that these concentrations are associated with the development of autoimmune diabetes compared to control subjects (Davies *et al.*, 1994; Hathout *et al.*, 2003; Soltesz *et al.*, 2007; Lee *et al.*, 2011)[16,17,18,19]. However, another study done by M. N. Pham in Germany failed to certain this finding (Pham *et al.*, 2012)[13]. All mean levels of adhesion molecules were higher in LADA than in D.M.2 group, although levels did considerably overlap between them. This result is not statistically significant and these findings are likely due to different**

**glycemic control on one hand and on the difference of ethnicity, age and diabetes duration on the other hand. Our results shows a statistically significant positive correlation between the levels of sVCAM-1 and HbA1C, (R = 23, P=0.028). On the other hand, sE- selectin was inversely correlated. (R= -0.20, P=0.047), table 2. The sICAM-1 levels have a weak positive correlation with HA1C. High levels of VCAM-1 may be due to poor controlling of diabetes related to insulin resistance. This result goes well with Morigi study (1998)[20] that clarifies the role of high blood glucose on levels of ICAM-1, VCAM-1, and E-selectin in samples taken from human umbilical vein endothelial cells (HUVEC) and found an increment in these concentrations. Also, high blood glucose can cause increment in production of AGEs (advanced glycation end products) and enhancement of inflammation in vesseles and VCAM-1 secretion (Tarek *et al.*, 2013 ; Md Isa *et al.*, 2006)[21,22]. In a study done in Helsinki HbA1 was correlated positively with sE-selectin concentrations in diabetic men only (r =0.29, p < 0.05), but not in diabetic women (r = -0.07, NS), ( Leena , 2001)[23]. No statistical significant correlation was found between the duration of disease and the adhesion molecules, (see table 2) .This finding is in line with previous observation reported by Pham *et al.*, (2012)[13], (-0.15, -0.01 & -0.04 for sVCAM-1, sE-Selectin & sICAM-1 respectively). Only the sVCAM-1 level had a significant positive correlation (R=0.25, P=0.017) with BMI, sICAM-1 level correlate weakly with BMI (see table 2). Our finding goes well with a study undertook in Germany which pointed out a the association between sVCAM-1, sICAM-1, sE-Selectin and BMI among patients with LADA, diabetes mellitus type 2 and normal individuals (sICAM-1: b= 0.34; sVCAM-1: b= 0.15; sE-Selectin:b= 0.38) (Pham *et al.*, 2012)[13]. Moreover, a study by Leinonen, 2003[24] approved this result. Concentrations of sICAM-1 associate positively with age of the studied groups (p=0.006), concentrations of sVCAM-1, and sE-selectin has a weak positive correlation with age (not significant statistically) (see table 2). This result is in agreement with other studies such as Leinonen *et al.*, (2003) [24]and Pham *et al.*, (2012)[13]. No correlation between gender & adhesion molecules (see table 2). This result fits report of Pham *et al.*, 2012[13]. A significant difference was found in the sE-selectin marker among LADA cases, where the mean value in those with complications was higher than those without complication, although the other 2 marker levels also increased in patients with complications but the result**

**is not significant statistically (see table 5). Levels of sVCAM-1 & sICAM-1 in patients with D.M.2 also increased in complicated cases, (see table 5). Levels of sICAM-1 were higher in patients with macrovascular complications (IHD & CVA) sE-selectin levels also increased in IHD, (see table 6). Diabetes mellitus is an atherosclerosis-prone, hyper- coagulable condition resulting in macrovascular disease with considerable morbidity and premature mortality [25,26]. An increment in concentration of E-selectin is benifitial for early impairment of endothelial function and this level is better than the rest endothelial adhesion molecules due to exclusive secretion of E-selectin from cells of endothelium, whereas other adhesion molecules secretedfrom different cells [27]. In addition, E-selectin concentration measurement is more sensitive than other adhesion molecules in reflection the exact membrane-bound form level. levels of adhesion molecules like sICAM, sVCAM and sE-Selectin were increased in patients with high BMI, CVD and diabetes mellitus, especially in type 1 and type 2 diabetes as it was seen by many studies (Aukrust *et al.*, 2008[28]; Meagher *et al.*, 2007[29]; Boulbou *et al.*, 2005[15]; Schram *et al.*, 2003)[30]. Our results are in agreement with Edward *et al.* (2002)[31] study which reported significant high levels of sICAM-1 in diabetic patients with macrovascular complications(CVA & IHD) and elevated mean levels for both sVCAM-1& sE-selectin in complicated cases. V. Mohamed *et al.* (2001)[32] reported an increase level of sE-selectin [61.23 vs. 43.25 ng /mL] and sICAM-1 [336.48 vs. 262.35 ng/mL)] for diabetic patients with macrovascular complications versus non complicated cases respectively.**

## Conclusion:

**In the current study all patients with LADA and D.M.2 showed increase in the serum level of sICAM-1 ,sVICAM-1 and E-selectin and the increment level of these adhesion molecules in LADA more than D.M2 and illustrated high percent of patients with LADA show complication of diabetes . Thus measuring adhesion molecules can be used to prevent complication by control of blood suger level and by reduction of weight from the results that showed in the study which illustrated a significant positive correlation between the sVCAM-1 level**

**and BMI in all groups. So can use of monoclonal antibody against adhesion molecules to prevent complication of diabetes.**

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