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The Potential Role of CD31 in Type 2 Diabetes Mellitus, an Initial Investigation

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ABSTRACT

One of the most common endocrine diseases in the general population is diabetes mellitus (DM). It is a major metabolic disease that leads to significant morbidity and mortality with micro- and macro-vascular consequences, making it one of the major public health concerns in a developing country. Chronic diabetes is not properly managed and often leads to cardiovascular problems and nephropathy. To a higher degree, research suggests that type 2 diabetes patients are a chronic inflammatory disease associated with the immune system. Therefore, the present study aimed to evaluate the CD31 marker to investigate immunological alterations. The subjects were divided into two groups: type 2 diabetes patients (diabetic group) and healthy individuals (control group). Based on our findings, CD31 of the diabetic group was significantly higher than that of the control group. In addition, the WBC and lymphocyte counts of the diabetic group were significantly higher than those of the control group. In conclusion, CD31 analysis showed a significant increase in the HbA1c of diabetic patients compared to the control group.

Keywords: Diabetes mellitus, CD31, Flow cytometry, Endocrinopathy

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Introduction

Among metabolic illnesses worldwide, diabetes is one of the most common conditions, and its prevalence in adults has increased recently [1]. A hereditary condition is type 2 diabetes [2]. Many of the genes involved are complex, and many are yet unknown. Certain chemical processes in the development of β-cells, insulin secretion, and insulin synthesis are normalized by these genes [2, 3]. One general feature of all diabetes illnesses is an insufficiency of the hormone insulin [4]. The inhibition of insulin function is another issue for type 2 diabetes. Because of its many potential causes, insulin deficiency is a complicated disorder. Here, we articulate our current understanding and clarify that many of the ideas may change in the future. CD31 is referred to as a cluster of distinction. For the platelet endothelial cell linkage molecule, it is also known as PECAM-1. This is crucial for the body's removal of aged neutrophils. In their tissues, neutrophils, and macrophages both express CD31, and these CD31 particles work together to fix the two cells all over the analysis process [3].

Epithelioid hemangioendothelioma, epithelioid sarcoma-like hemangioendothelioma, various vascular tumors, histiocytic malignancies, and plasmacytomas are among the cancers that express CD31. It is uncommon in some carcinomas and sarcomas. At the intercellular junctions of endothelial cells, PECAM-1 is also extensively expressed. There, it serves as a mechanosensor, controls leukocyte trafficking, and preserves the integrity of the endothelial cell junction [4]. The measurement of CD31, an endothelial cell marker, determined the mass of the pancreatic microvasculature. Insulin treatment increased the expression of VEGF and VEGFR2, as well as the levels of CD31 protein [5].

In immunological histochemistry, CD31 is primarily utilized to demonstrate the presence of endothelial cells in tissue slices. A fast-developing tumor may be indicated by the degree of tumor angiogenesis, which may be assessed with this method. Various methods, such as the choice of counteracting agent, antigen recovery (AR) procedures, and strategies for vessel thickness appraisal, may have an overall impact on similar immunohistochemistry-based studies, which quantitatively quantify vascularity in tissue segments. The degree of intratumor neovascularization, tumor metastatic potential, and visibility for patients with various types of strong human malignant developments are all correlated with immunohistochemical recoloring of microvessels to assess microvessel thickness (MVD) per unit area. Antibodies that recognize CD31 epitopes are among the immunohistochemical markers that can differentiate endothelial cells [6].

The purpose of flow cytometry criteria was to characterize the levels of cellular content (DNA, protein, surface receptors, calcium) as well as cellular properties (sizes, membrane potential, intracellular pH). Biotechnology depends on assessments that identify how these properties are distributed in cell populations [7]. Moreover, the sorting unit may be combined with flow cytometers, demonstrating the ability to segregate the chosen subpopulation. The cell stream will be broken up into droplets by most sorting machines. Before the droplets turn formative, the cells are electrically charged. After that, the droplets are directed into a collection vessel by an electric field [8].

Human CD31 belongs to the immunoglobulin superfamily and is expressed by distinct populations of circulating lymphocytes as well as endothelial cells, platelets, monocytes, and polymorphonuclear cells [9]. As a union receptor molecule, CD31 is essential for WBC trafficking across the endothelium. Numerous studies have demonstrated that CD31 may participate in both heterophilic (CD31-X) and homophilic (CD31-CD31) links to other network or cell surface proteins [10].

Employing flow cytometry, the present research aimed to evaluate and compare the CD markers (CD31) between untreated patients and normal persons. Assessment of biochemical marker alterations, such as triglycerides, HDL, LDL, cholesterol, HbA1c, and blood glucose.

Materials and Methods

The study was primed at the Clinical Pathology Department, Faculty of Medicine, Minia University. It was conducted on twenty newly diagnosed diabetic patients and twenty healthy individuals as controls.

The selected subjects included in the study were divided into two groups:

- Group I (control group): It included twenty healthy individuals, 13 males and 7 females, their ages ranged from 17 to 53 years old.
- Group II (diabetic group): It included twenty newly diagnosed Diabetic patients; 14 males and 6 females, their ages ranged from 18 to 68 years old.

Exclusion criteria

a. Acute or chronic infection.
b. Acute or chronic inflammatory diseases.

Two groups were under fasting conditions for ten hours before collecting the lipid profile sample.

Patients were subjected to a full assessment of the following:

Laboratory investigations

Routine investigations

a. Random blood sugar using Selective Pro XL (Eli Tech Group clinical systems).
b. Complete blood count (CBC) including the count of white blood cells (total) and percentage of lymphocytes. Using an automated hematology analyzer, Celltac G (Nihon Kohden Corporation).
c. Hba1c using Selective Pro XL (Eli Tech Group clinical systems).

Special investigations

Percentage of expression of CD31 in peripheral blood by Flow Cytometry (BD-FACS Flow Argon laser U.S.A).

Sampling

- Blood was evacuated in an Ethylenediaminetetraacetic acid (EDTA) containing tube for CBC, Flow Cytometry analysis, and HbA1c.

- One ml was evacuated on a plain tube and left to clot in the incubator then centrifuged. The expressed serum was used for the determination of serum glucose.

The methods

Random blood sugar

Blood glucose was determined by using a glucose kit purchased from SPINREACT company, Egypt commercially available kit according to the instruction of the manufacturer.

Complete Blood Count (CBC)

It included the count of white blood cells (total) and the percentage of lymphocytes. It was determined by an automated hematology analyzer, Celltac G (Nihon Kohden Corporation).

HbA1c

Hemoglobin A1C (HbA1c) was determined using the HbA1c kit purchased from SPECTRUM Company, Egypt commercially available kit according to the instruction of the manufacturer.

Assessment of CD31 Percentage

This preliminary study depends on the ability of unequivocal monoclonal antibodies to fix the antigenic determinants expressed by leukocytes. Explicit staining of the leukocytes was executed by incubating the section with the CD31-FITC reagent. The red cells were earlier isolated by lysis and the leukocytes, which were simple by this procedure, were investigated by flow cytometry. The flow cytometer quantifies light dispersion and the fluorescence of cells. The fluorescence of the delimited cells was concentrated to separate the positive recolored occasions from the unstained ones. The results were communicated as a level of positive occasions opposite all the occasions acclimatized by the gating.

Staining process

Whole blood was collected in evacuated tubes containing EDTA, for each sample, two tubes were prepared labeled 1&2 (Control and Test, respectively), 10 µL of specific CD31-FITC conjugated antibody was added to each test tube, and 10 µL of the isotopic control to each control tube, then 100 µL of the test sample was added to both tubes, the tubes were gently shaken then incubated for 15 to 20 minutes at room temperature (18–25 °C) in the dark. The cells were washed by PBS twice to remove any unbound antibodies followed by red cell lysis using 2 ml of lysing solution then incubated for 10 min at room temperature in the dark. After that, the cells were centrifuged for 5 min, the supernatant was discarded and 2 ml of PBS was added then washing by PBS was repeated twice, then the cells were re-suspended in 3 ml of PBS for final flow cytometry analysis.

Analysis of data

The analysis was carried out using a (BD-FACS Flow Argon laser U.S.A) flow cytometry at 505 nm. Data processing was carried out with the software.

Statistical method

The statistical exploration was implemented by Statistical Package for Social Sciences (SPSS Inc. Chicago, IL, V 21.0) and (Graph Pad software V 16). Quantitative variables were expressed by mean ± standard errors (SE). In all tests, a P-value of less than 0.05 was considered statistically significant. Comparisons of the results between the two methods were done using the one-way ANOVA test with *P*-values < 0.05 followed t-test used for comparison of the means.

Results and Discussion

As demonstrated in **Table 1** and **Figure 1**, the diabetes group's blood glucose level was significantly higher than that of the control group. Additionally, as indicated in **Table 2** and **Figure 2**, the diabetic group's HbA1c level was significantly higher than that of the control group. Additionally, compared to the control group, WBCs and lymphocytes were dramatically upregulated in the diabetes group, as seen in **Table 3** and **Figures 3 and 4**. The control and diabetes groups' CD31 and other laboratory test results were negatively correlated with each other across all test parameters **(Figures 5-7 and Table 4)**. According to **Table 5** lymphocytes and HbA1C, the diabetic group had the sole positive connection.

Table 1. Comparison of the groups under study in terms of blood glucose levels

Blood glucose	**Control group N = 20**	**Diabetic group N = 20**	**P-value**
Range	80-102	218-370	0.001*
Mean ± SEM	89.5 ± 1.3	282.3 ± 9.01	

Table 2. Comparison between the studied groups regarding HbA1-C

HAb1-C	**Control group N = 20**	**Diabetic group N = 20**	**P-value**
Range	5-6	9-15	0.001*
Mean ± SEM	5.4±0.1	10.5±0.3	

Table 3. Comparison between the studied groups regarding WBCs and Lymphocytes

Parameters Groups		**Control group N = 20**	**Diabetic group N = 20**	**P-value**
WBCs	Range Mean ± SEM	4000-6500 4660 ± 149.6	7800-10100 8975 ± 133.7	0.001*
Lymphocytes	Range Mean ± SEM	22.6-33.1 28.8 ± 0.65	35.6-56.6 45.9 ± 1.4	0.001*

Table 4. Correlation between CD31 and other laboratory investigations among the control group

Variables	**CD31 r (p)**
Blood glucose	-0.05 (0.8)
HbA1C	-0.50 (0.02*)
WBCs	-0.26 (0.2)
Lymph	-0.01 (0.9)

Table 5. Correlation between CD31 and other laboratory investigations among diabetic group

Variables	**CD31 r (p)**
Blood glucose	-0.28 (0.2)
HbA1C	0.13 (0.5)
WBCs	-0.14 (0.5)
Lymph	0.03 (0.8)

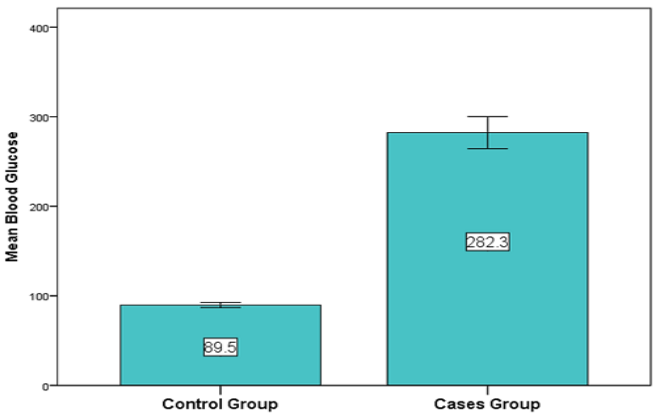

Figure 1. The mean blood glucose readings for the two groups This indicates that the diabetes group's blood glucose level was 215% greater than that of the control group.

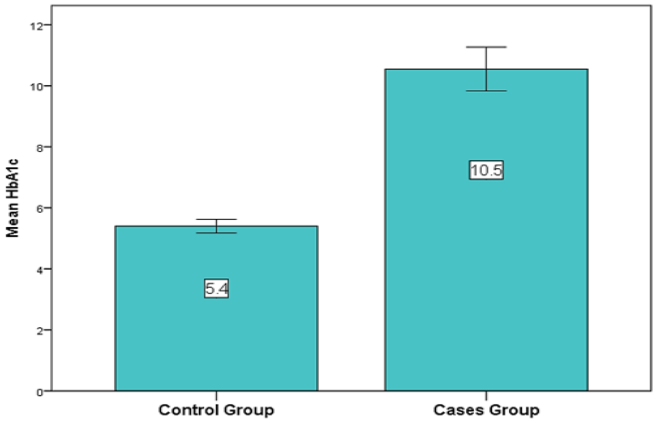

Figure 2. The two groups' average HbA1C readings. This demonstrates that the diabetes group's HbA1C level is 94% greater than the control group.

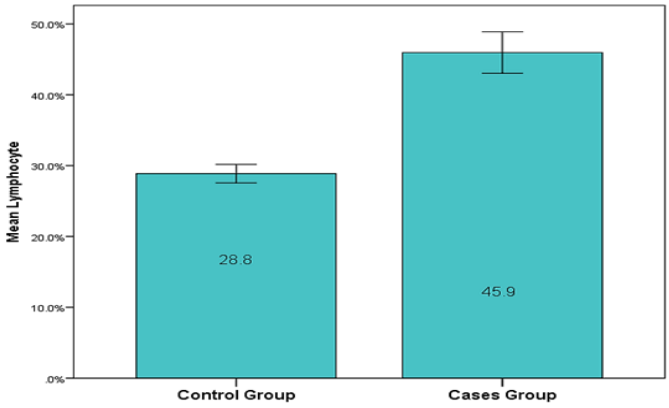

Figure 3. The average lymphocyte numbers between the two groups indicate that the diabetes group has 59% more lymphocytes than the control group.

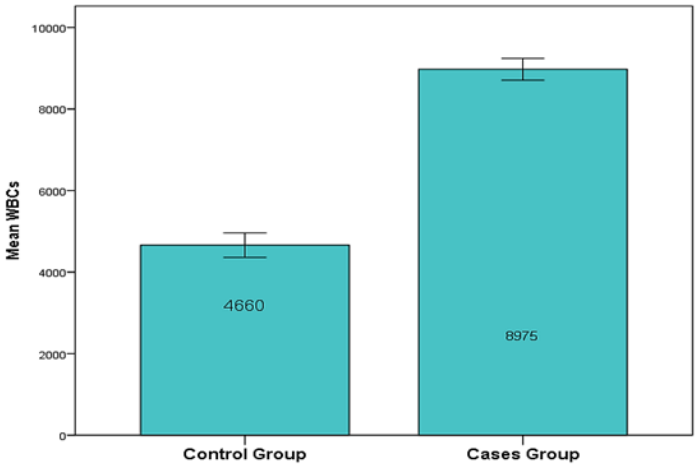

Figure 4. The average WBC levels between the two groups indicate that the diabetes group had 93% more WBCs than the control group.

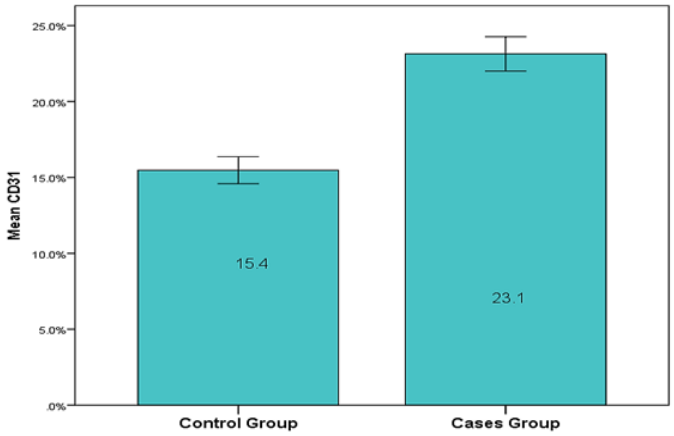

Figure 5. The two groups' average CD31 readings indicate that the diabetic group's CD31 level is 50% greater than the control group's.

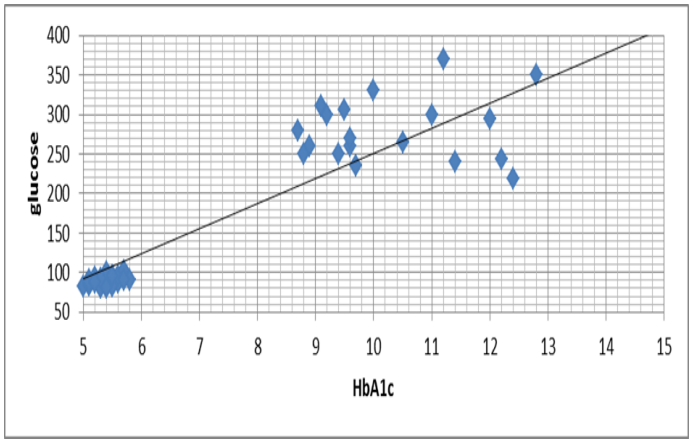

Figure 6. Demonstrates a positive relationship between the two groups' HbA1c and blood glucose, meaning that the higher the blood glucose level, the higher the HbA1c level.

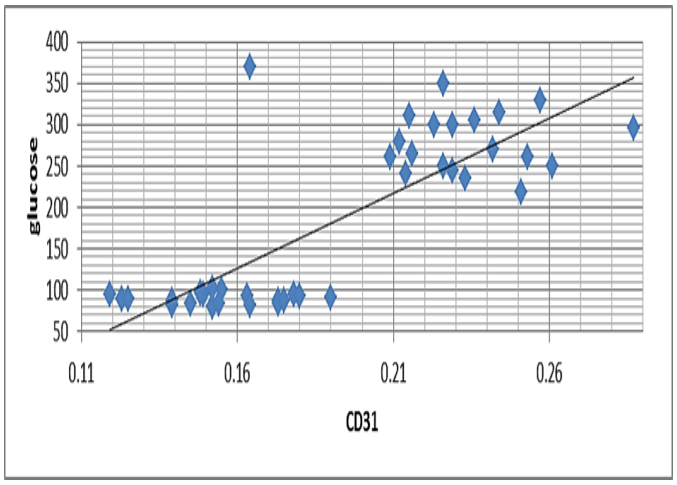

Figure 7. Demonstrates a positive relationship between CD8 and blood glucose in both groups, meaning that the higher the blood glucose level, the higher the CD8 level.

Numerous significant discoveries on the connection between diabetes and other indicators are revealed by the study. Diabetes mellitus is a chronic metabolic disease that is poorly managed and commonly causes cardiovascular problems. Furthermore, research suggests that T2DM is a long-term inflammatory condition that is immune-related and that when it hits the island, there may be a decrease in β-cells and problems with insulin secretion [11]. As seen in **Figure 1**, the findings demonstrated that the diabetic group's HbA1c was 97% greater

than that of the control group, which was consistent with Witczak's research [12]. As seen in **Figure 5**, the findings demonstrated that the diabetic group's CD31 was 49% greater than that of the control group 2, which was consistent with the research of [13].

Additionally, T2DM is associated with a higher level of expression of sticky molecules. Compared to healthy persons, patients with type 2 diabetes have higher levels of platelet activation (CD31, CD49b, CD62P, and CD63). Acute hyperglycemia in healthy individuals causes increased platelet activation and reactivity, as evidenced by raised surface levels of soluble markers (sP-selectin) and P-selectin and CD40 ligand [14].

Greater levels of inflammatory mediators are associated with both diabetes mellitus and WBC. Key indicators for all abnormalities that result in the invasion and inflammation of other agents include insulin, inflammatory agents, and components of human blood [15]. Due to increased inflammatory mediators, there is a correlation between diabetes mellitus and the average WBC count. Together with insulin, these and human blood segments prepare the body for any deviations from the usual, which can lead to inflammation and the invasion of unknown substances. Several factors, such as mean white cell count, mean platelet count, and phagocyte rate, can support defense systems and contribute to variations in blood boundary levels [16]. Based on the scientific evidence now available, T2D is most likely an inflammatory illness [17].

Proinflammatory cytokines, reactive oxygen species, growth factors, and metalloproteinase—all of which are generated by leukocytes (neutrophils, lymphocytes, and macrophages) invading the renal tissues—mediate the inflammatory process in diabetes. The rise in the proportion of lymphocytes in type 2 diabetes is likely due to the homing of leukocytes into renal tissues, which is mediated by intercellular adhesion molecule-1 and the chemokines CX3CL1 and CCL2 [18].

Conclusion

Larger trials will yield more significant results; hence some of the work's constraints were model magnitude. A further constraint was the absence of prior research, as there was a single research study conducted. Further investigation will reveal significant implications in the future. In conclusion, we found that type 2 diabetes mellitus and CD31 are closely related.

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Conflict of Interest: None

Financial Support: None

Ethics Statement: All measures accomplished in this scientific trial containing human supporters remained similar through the ethical principles of the institutional advisory group.

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