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hsCRP and E-Selectin as Markers of Endothelial Dysfunction in Children with Type 1 Diabetes Mellitus

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Methods: Thirty-one T1DM children showing no symptoms of vascular diseases and diagnosed by ultrasound techniques as ED-positive (T1DM-ED) or negative (T1DM), and 58 sex-age-matched healthy children were investigated for circulating levels of E-selectin, s-ICAM and s-VCAM, MMP-9, high-sensitivity C-reactive protein (hsCRP), and IL-6.

Results: No differences were observed in s-ICAM, MMP-9, and IL-6 levels between case and control groups. Significantly higher levels of s-VCAM ($p = 0.0001$) were found in the T1DM (1359.1 ± 273 ng/mL) and T1DM-ED (1358.2 ± 112 ng/mL) groups; (control - 828.5 ± 212 ng/mL). Higher levels of E-selectin ($p = 0.001$) were found in the T1DM-ED group (331.2 ± 77 ng/mL); (control - 222.2 ± 74 ng/mL). The values of hsCRP were higher ($p = 0.002$) in the T1DM-ED group (0.36 ± 0.2 mg/L) relative to control (0.15 ± 0.1 mg/L) and T1DM (0.19 ± 0.2 mg/L). The results suggest that E-selectin and hsCRP can be useful markers of ED in children with T1DM.

Keywords: *type 1 diabetes mellitus, children, endothelial dysfunction, markers, E-selectin, hsCRP.*

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Strictly as per the compliance and regulations of:

hsCRP and E-Selectin as Markers of Endothelial Dysfunction in Children with Type 1 Diabetes Mellitus

Markers Laboratory of Endothelial Dysfunction

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I. Introduction

Type 1 diabetes mellitus (T1DM) is a complex metabolic disorder characterized by chronic hyperglycemia due to a complete lack of insulin secretion [1]. The constant hyperglycemic state associated with the increase in circulating free fatty acids trigger molecular mechanisms that lead to a decrease in nitric oxide (NO) bioavailability, increased oxidative stress, increased expression of pro-inflammatory and prothrombotic factors, and reduced vasodilatation, promoting endothelial dysfunction [2].

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Endothelial dysfunction (ED) precedes the cardiovascular complications that are the leading cause of death in diabetic patients. Nephropathy, retinopathy, and neuropathy are some of the most common microvascular complications, where as cardiovascular disease, peripheral arterial disease, and stroke are the main macrovascular complications. Currently, ED has gained attention as a predictor of T1DM-related vascular diseases [3].However, little is known about the early stages of vascular alterations in children with diabetes.

In recent years, several molecules are being considered new biomarkers of ED, such as VCAM-1 (vascular cell adhesion molecule-1), ICAM-1 (intercellular adhesion molecule-1), and E-selectin. These molecules are involved in the adhesion of leukocytes to the vascular wall and are associated with endothelial dysfunction, retinopathy, albuminuria, and coronary disease [4,5]. Several studies have suggested that these molecules are present in higher levels in patients with diabetes [6,7,8,9,10].

Other important molecules involved in ED are matrix metalloproteinases (MMPs), endopeptidases that are secreted by macrophages and act in the degradation and remodeling of extracellular matrix components, such as collagen, proteoglycans, elastin, fibronect in, and other glycoproteins [10,11,12]. In diabetes, high glucose levels lead to changes in MMP regulation, altering the balance between the synthesis and degradation of matrix components [13]. MMP-9 is an essential molecule for vascular remodeling, and studies indicate that this metalloproteinase is involved in the formation and destabilization of atheromatous plaques [10,14] and in ischemic stroke [15]. Other studies have shown higher levels of MMP-9 in patients with diabetic complications [16], including children [17].

Moreover, C-reactive protein, produced and released by hepatocytes after stimulation by interleukins IL-1 and IL-6, plays an important role in ED: it reduces eNOS messenger RNA transcription and NO formation, increases the release of ET-1 and IL-6, and induces the release of adhesion molecules and

chemotactic cytokines [18]. It has previously been shown that CRP and IL-6 are associated with T1DM-related cardiovascular events and microangiopathy [19].

Circulating biomarkers such as E-selectin, soluble ICAM and VCAM, MMP-9, CRP, and IL-6 play a significant role in ED. Consequently, they may contribute to both the early diagnosis of the inflammatory process involved in ED and the development of new therapeutic strategies [20]. The aim of this study was to investigate the association of these markers with the presence or absence of ED in children with T1DM.

II. Material and Methods

a) Study Participants

The study included 31 children with type 1 diabetes mellitus, from 6 to 12 years old (mean age: 8.36±1.8 years), and 58 age- and sex-matched healthy children, also between the ages of 6 and 12 (mean age: 6.9±1.7 years), designated as the control group. Children with T1DM were treated at the Pediatric Endocrinology Outpatient Clinic at the Brasilia University Hospital and at the Pediatric Clinic of the Brasilia Children´s Hospital. The children in the control group were recruited among relatives of hospital staff involved in the study.

All children with T1DM included in the study underwent detailed physical examinations (which included blood pressure and skin sensitivity monitoring), biochemical blood and urine tests (including complete evaluation of renal function and microalbuminuria), and complete ophthalmic examination, all under the supervision of a pediatric endocrinologist. Children in the control group had normal height and weight for their age, according to the Center for Disease Control and Prevention's (CDC) guidelines [21](adapted to Brazilian children) [22]. They all had fasting glucose levels <100 mg/dL, and glycated hemoglobin (HbA1c) ≤5.6%.

Exclusion criteria for both groups were onset of puberty, evidence of hypertension, dyslipidemia (total cholesterol ≥ 200 mg/dL, HDL <45mg/dL, LDL≥130 mg/dL, VLDL ≥ 41mg/dL, triglycerides ≥100 mg/dL for children aged up to 10 years old, or triglycerides ≥130 mg/Dl for children older than 10), family history of primary dyslipidemia and premature death due to cardiovascular or cerebrovascular disease, presence of anemia (hemoglobin <11 g/L and hematocrit <33%), presence of acute infectious conditions or chronic conditions other than diabetes mellitus, and continued use of medications other than insulin.

The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Parents or legal guardians of the children participating in the study signed an informed consent form. The study was approved by the University of Brasilia's College of Health Science's (CEP-FS) Research Ethics Committee.

The children were divided into three groups according to the presence of type 1 diabetes mellitus and endothelial dysfunction: healthy children (control), Children with T1DM and no endothelial dysfunction (T1DM), and children with T1DM and presenting endothelial dysfunction (T1DM-ED).

b) Laboratory Analysis of Biomarkers

Serum samples were collected after 8 h of fasting, according to the H3-A6 standard of the Clinical and Laboratory Standards Institute (CLSI) [23] in serum separator tubes. Samples were centrifuged at 3000 rpm, and the serum was stored at -2°C until used for measuring the levels of high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), endothelial selectin (E-selectin), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and the marker of vascular damage matrix metalloproteinase-9 (MMP-9). The method of measurement and the equipment used are summarized in Table 1.

Table 1: Methods and Equipment used for Determining Serum Levels of each Marker

Marker	Equipment and Manufacturer	Method
sE-selectin	BEST 2000 ELISA System (Werfen group, Barcelona, Spain) IBL International ELISA Kit (IBL, Hamburg, Germany)	Direct Sandwich ELISA
sICAM-1	BEST 2000 ELISA System (Werfen group, Barcelona, Spain) IBL International ELISA Kit (IBL, Hamburg, Germany)	Direct Sandwich ELISA
sVCAM-1	BEST 2000 ELISA System (Werfen group, Barcelona, Spain) IBL International ELISA Kit (IBL, Hamburg, Germany)	Indirect Sandwich ELISA
MMP-9	BEST 2000 ELISA System (Werfen group, Barcelona, Spain) IBL International ELISA Kit (IBL, Hamburg, Germany)	Indirect Sandwich ELISA
hsCRP	BN II System (Siemens®, Marburg, Germany)	Nephelometry
IL-6	BEST 2000 ELISA System (Werfen group, Barcelona, Spain) IBL International ELISA Kit (IBL, Hamburg, Germany)	Indirect Sandwich ELISA

c) Statistical Analysis

We performed statistical analyses using version 5.0 of the Graph Pad Prism software (Graph Pad Software, San Diego, California, USA). One-way ANOVA and Dunnett or Bonferroni post-hoc tests were used for comparisons between control and groups T1DM/T1DM-

ED and between T1DM and T1DM-ED, respectively. Differences were considered statistically significant when $p \leq 0.05$.

III. Results

In a previous study by our research group [24], ultrasonographic techniques were used to measure the flow-mediated dilation (FMD) of the brachial artery and the thickness of the intimal and medial layers (IMT) of carotid arteries in order to investigate the presence of ED in children with T1DM. Results showing decreased vasodilator response, indicative of endothelial dysfunction, were found only in children who were diagnosed with T1DM at least five years prior to the study. These children were included in the T1DM-ED group of the present study, while children without ED were included in the T1DM group.

To assess the presence of markers indicative of vascular remodeling, serum levels of the MMP-9 enzyme were measured in the control and the groups of children with diabetes. T1DM and T1DM-ED patients presented MMP-9 serum levels similar to the control group (control 35.7±4 ng/mL;T1DM 35.4±2 ng/mL;T1DM-ED 36.5±1 ng/mL), with no statistical differences between them (Fig. 1A).

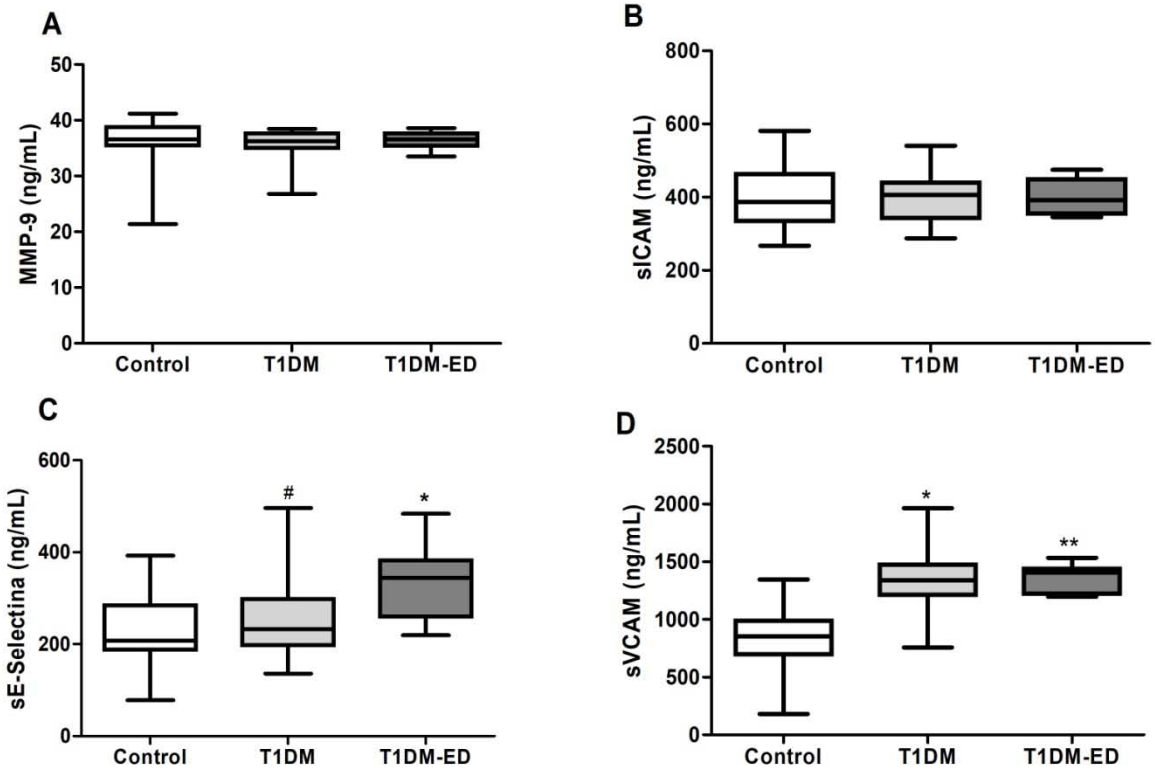

Fig. 1: Serum levels of each biomarker (1A) MMP-9 and (2A) sICAM showed no statistical differences between control and diabetic groups. (1C) E-selectin was significantly higher in T1DM-ED (*p = 0.001) than in the control (Dunnett's post-hoc test) and in T1DM (#p = 0.001): (1D) sVCAM was elevated (* and **p = 0.0001) in both groups of diabetic patients, with and without ED. Results were analyzed using one-way ANOVA and Dunnett's post-hoc test.

Serum levels of E-selectin, sVCAM-1, and sICAM-1 were also investigated. Patients with diabetes had similar sICAM-1 levels to subjects in the control group, with no statistical differences between control (398.1±81 ng/mL), T1DM (394.1±56ng/mL), or T1DM-ED (416.2±44ng/mL) (Fig. 1B).

Significantly higher levels of E-selectin ($p = 0.001$) were found in T1DM-ED (331.2±77 ng/mL) when compared to the control (222.2±74 ng/mL), whereas levels in T1DM (244.4±81ng/mL) did not differ significantly from the control (Fig. 1C). In contrast, increased levels of sVCAM-1 ($p = 0.0001$) were found in both DM groups (T1DM 1,359.1±273 ng/mL; T1DM-ED 1,358.2±112 ng/mL)in comparison to the control (828.5±212 ng/mL) (Fig. 1D).

Serum levels of IL-6 and hsCRP were measured to verify the presence of an inflammatory state in individuals with diabetes. Serum levels of IL-6 were within reference values and presented no statistically significant differences between control (0.99±0.5 ng/mL), T1DM (1.13±0.6 ng/mL), and T1DM-ED (0.83±0.2 ng/mL) (Fig.2A).

However, elevated hsCRP levels were observed in DM groups. The values were 0.15±0.1 mg/L for the

control, 0.19±0.2 mg/L for T1DM, and 0.36±0.2 mg/L for T1DM-ED. Values were significantly higher (p = 0.002) in the T1DM-ED group than in the control group (Fig. 2B). This result indicates the presence of an inflammatory state in children with endothelial dysfunction.

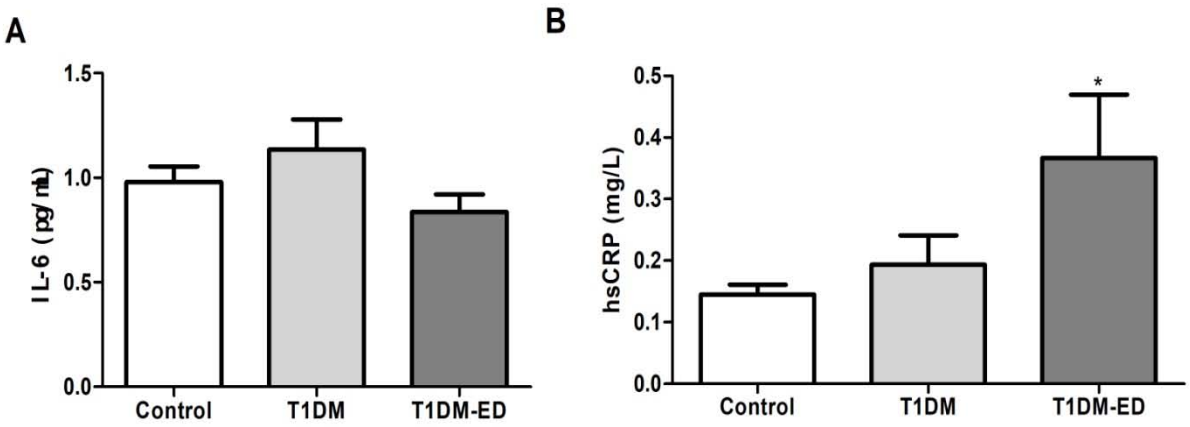

Fig. 2: IL-6 (A) and hs-CRP levels (B) in control and diabetic groups Results were analyzed using one-way ANOVA and Dunnett's post-hoc test, comparing control and T1DM-ED for hs-CRP (*p = 0.0026).

IV. Discussion

Diabetes mellitus is one of the most prevalent chronic diseases affecting children and adolescents. The World Health Organization (WHO) estimates that diabetes will represent the seventh leading cause of death worldwide by 2030 [25]. Vascular alterations are the main cause of morbidity and mortality in patients with Type 1 diabetes mellitus, including children and adolescents [26, 27]. Endothelial dysfunction has been shown to precede vascular alterations and can be used as an early predictor of cardiovascular diseases [28].Endothelial cells actively regulate vascular tonus and reactivity under physiological and pathological conditions, responding to mechanical stimuli and neurohumoral mediators with the release of a variety of vascular relaxing/constricting factors and synthesizing substances that regulate inflammation and homeostasis. As the actions of endothelial cells may affect several systems, either simultaneously or sequentially, a gold standard test for evaluation of endothelial dysfunction has not yet been established [29].Endothelial function is usually estimated by variations in blood flow or arterial vessel diameter in response to mechanical or chemical stimuli, evaluated invasively, through coronary catheterization, or non-invasively, through ultrasound examinations, such as measurement of FMD of the brachial artery and IMT of carotid arteries [29].Despite being non-invasive, these tests require time, equipment, and skilled personnel, not always readily available in health services. On the other hand, laboratory tests based on blood samples are usually faster and cheaper and could serve as tools for the early prevention of cardiovascular complications. Among the various molecules known to participate in ED, hsCRP, IL-6, sICAM-1, sVCAM-1, E-selectin, and MMP-9 were investigated as potential biomarkers in children with Type 1 diabetes mellitus in this study.

The majority of the data available on markers of endothelial dysfunction come from studies in adult patients with Type 2 diabetes mellitus (T2DM). Furthermore, most of theT1DM data available have been obtained from adult patients and/or patients who already showed vascular complications [9,30]. Data on T1DM patients, especially children, are still scarce. The present study evaluated children with T1DM with and without ED who did not present vascular diseases and healthy children as control.

Monitoring MMP-9 levels appears to be useful to predict microvascular complications in T1DM. The hyperglycemic medium induces an increase in its levels [31], which are in accordance with the progression of diabetes and the severity of complications [32]. In children with T1DM (5 to15 years old) a substantial increase in MMP-9 levels in lacrimal samples was associated with progression of diabetic retinopathy as well as localized pathological remodeling [33]. In subjects with T1DM (4 to 40 years old) with sub-optimal glycemic control (HbA1c 8.3% ± 1.8),the level of this marker was notably high in urine samples and correlated positively with HbA1c levels and disease duration, suggesting that its levels may function as a marker of latent nephropathy, prior to clinically defined microalbuminuria [34].

However, serum levels of MMP-9 do not seem to be an indicator of the endothelial dysfunction that precedes vascular complications. Lee et al [35] reported no increase in MMP-9 levels in the serum of T2DM adults diagnosed up to 3 years prior to the study and no complications. Our results indicate that, even in children

with T1DM and more than five years of disease duration, there was no change in MMP-9 levels when vascular complications were not present since serum levels of MMP-9 were similar in children with and without ED.

Atherosclerosis is considered an inflammatory disease, because a low-grade inflammatory state contributes to all stages of its development, starting with endothelial dysfunction, up to plaque formation and rupture and the thrombotic complications that follow [28].Cell adhesion molecules mediate the migration of large numbers of leukocytes (selectins) and their adhesion to the endothelium (VCAM-1 and ICAM-1). These molecules are involved in establishing an inflammatory process and can be useful as markers of inflammation [36].

There are, however, inconsistencies in the results of studies with these markers, especially regarding ICAM and VCAM. Some studies found no correlation between ICAM and arterial stiffness or atherosclerosis [37] while others implicated only VCAM-1 [38] or only ICAM-1 [39]. In the present study, we did not find significant differences between sICAM-1 levels in T1DM children with and without ED and the control group. In contrast, sVCAM-1 levels were elevated in all children with diabetes when compared to the control, and, therefore, were associated with the presence of diabetes but not with endothelial dysfunction in our study population. Further studies should clarify the role of sICAM-1 and sVCAM-1 in ED.

On the other hand, more consistent results have been reported for E-selectin, since many studies have associated an increase in its levels and presence of diabetes [40], endothelial dysfunction [41] and elevated diastolic blood pressure values in T1DM children, even in those who had been recently diagnosed [42] or had no vascular disease [43]. Furthermore, the value of E-selectin concentration to evaluate the risk of atherosclerosis was evident by its association with worsening of risk factors, such as high blood pressure, glucose, and lipid levels [30].

In the present study, we demonstrated that elevated E-selectin values were associated with the presence of ED in children with Type 1 diabetes mellitus. The fact that E-selectin is produced exclusively by endothelial cells (EC) makes it an excellent marker for evaluating endothelial dysfunction in comparison to other cell adhesion molecules, such as ICAM-1 and VCAM-1,which are expressed both by EC and leukocytes [44].

The acute hyperglycemia and poor glycemic control in the initial stages of T1DM have been associated with increased inflammation, which persists for at least two hours after the correction of hyperglycemia [45,46].Elevated serum levels of IL-6 and hsCRP were related to high levels of HbA1c [45]. We found the highest levels of hsCRP in children that had shown elevated levels of HbA1c in a previous study ($p = 0.018$) [24] corroborating the association between hyperglycemia and inflammation.

Although serum levels of IL-6 and hsCRP have been used to infer subsequent development of atherosclerosis [47], the most promising marker of inflammation for clinical application appears to be hsCRP [48]because, in comparison with IL-6, it has a longer half-life, more stable serum levels, and no circadian variation [49]. The isolated change in hsCRP levels observed in the present study, with no alteration in IL-6 levels, may be due to the greater stability of the marker or the low-grade inflammation that occurs in stages preceding vascular damage. Nonetheless, the results confirm the utility of hsCRP as an early marker of ED.

V. Conclusions

The results reported herein suggest that hsCRP and E-selectin may be good markers of ED in pediatric patients. Currently, these markers are not routinely analyzed in laboratory tests, at least in Brazil [50]. Their inclusion in routine tests as indicators of ED could serve as a subsidy for early interventions for the prevention of vascular diseases. However, this study presents some limitations due to the small number of participants and lack of patient follow-up. Further studies are required to broaden the results presented herein.

Author´s Contributions:

Antonella Márcia Mercadante de Albuquerque do Nascimento performed the clinical evaluation of all patients and revised and organized all the laboratory data. Rosane Mansan Almeida performed the laboratory analyses, wrote part of the manuscript and revised it. Inês Jorge Sequeira performed the statistical analysis. Yanna Karla de Medeiros Nobrega conceived the study design, wrote part of the manuscript and revised it. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee of the respective country and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. This article does not contain any studies with animals performed by any of the authors.

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References Références Referencias

1. American Diabetes Association (2014) Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 37 (Supplement 1) S81-S90. doi: 10.2337/dc 14-S08.
2. Yang X, Li Y, Li Y, Ren X, Zhang X, Hu D, Gao Y, Xing Y, Shang H (2017) Oxidative Stress-Mediated Atherosclerosis: Mechanisms and Therapies. Front Physiol 23;8:600.doi: 10.3389/fphys.2017.00600.
3. Ladeia A. M, Sampaio R. R, Hita M. C, Adan L. F (2014). Prognostic value of endothelial dysfunction in type 1 diabetes mellitus. World J Diabetes. 15: 5 (5): 601-605.doi: 10.4239/wjd.v5.i5.601.
4. Joussen A. M, Murata T, Tsujikawa A et al (2001) Leukocyte-mediated endothelial cell injury and death in diabetic retina. Am J Pathol 158: 147-152.
5. Soedamah-Muthu S. S, Chaturvedi N, Schalkwijki C. G. et al (2006) Soluble vascular cell adhesion molecule-1 and soluble E-selectin are associated with micro and macrovascular complications in type 1 diabetic patients. J Diabetes Complications 20: 188-195.
6. Jude E. B, Douglas J. T, Anderson S. G. et al (2002) Circulating cellular adhesion molecules ICAM-1, VCAM-1, P- and E-selectin in the prediction of cardiovascular disease in diabetes mellitus. Eur J Intern Med 13: 185-189.
7. Glowinska B, Urban M, Peczynska J et al (2005) Soluble adhesion molecules (sICAM-1, sVCAM-1) and selectins (sE-selectin, sP-selectin, sL-selectin) levels in children and adolescents with obesity, hypertension and diabetes. Metabolism 54: 1020-1026. doi: 10.1016/j.metabol.2005.03.004.
8. Astrup A. S, Tarnow L, Pietraszek L et al (2008) Markers of endothelial dysfunction and inflammation in type 1 diabetic patients with or without diabetic nephropathy followed for 10 years: association with mortality and decline of glomerular filtration rate. Diabetes Care 31: 1170-1176. doi:10.2337/dc07-1960.
9. Machnica L, Deja G, Polanska J, Jarosz-Chobot P. (2014) Blood pressure disturbances and endothelial dysfunction markers in children and adolescents with type 1 diabetes. Atherosclerosis 237: 129-134. doi: 10.1016/j.atherosclerosis.2014.09.006.
10. Chen Q, Jin M, Yang F, Zhu J, Xiao Q, Zhang L (2013) Matrix metalloproteinases: inflammatory regulators of cell behaviors in vascular formation and remodeling. Mediators Inflamm 2013: 928315. doi:10.1155/2013/9283.
11. Berg G, Miksztowicz V, Schreier L (2011) Metalloproteinases in metabolic syndrome. Clin Chim Acta 412 (19-20): 1731-9. doi:10.1016/j.cca. 2011.06.013.
12. Ketelhuth D. F, Bäck M (2011) The role of matrix metalloproteinases in at herothrombosis. Curr Atheroscler Rep 13 (2):162-9. doi:10.1007/s11883-010-0159-7.
13. Death A. K, Fisher E. J, McGrath K. C, Yue D. K (2003) High glucose alters matrix metalloproteinase expression in two key vascular cells: potential impact on atherosclerosis in diabetes. Atherosclerosis 168 (2): 263-9.
14. da Silva S. H, Moresco R. N (2011) Cardiac biomarkers for assessment of acute coronary syndrome. Sci Med 21 (3): 132-42.
15. Chaturvedi M, Kaczmarek L (2014) Mmp-9 inhibition: a therapeutic strategy in ischemic stroke. Mol Neurobiol 49 (1): 563-73. doi:10.1007/s12035-013-8538-z.
16. Kostov K, Blazhev A, Atanasova M, Dimitrova A (2016) Serum Concentrations of Endothelin-1 and Matrix Metalloproteinases-2, -9 in Pre-Hypertensive and Hypertensive Patients with Type 2 Diabetes. Int J Mol Sci1: 17 (8). doi:10.3390/ijms17081182.
17. Garro A, Chodobski A, Szmydynger-Chodobska J, Shan R, Bialo S. R, Bennett J, Quayle K, Rewers A, Schunk J. E, Casper T. C, Kuppermann N, Glaser N (2017) Circulating matrix metalloproteinases in children with diabetic ketoacidosis. Pediatr Diabetes 18 (2): 95-102. doi:10.1111/pedi.12359.
18. Verma S, Li S. H, Badiwala M. V et al (2002) Endothelin antagonism and interleukin-6 inhibition attenuate the proatherogenic effects of C-reactive protein. Circulation 105: 1890-1896.
19. Schram M. T, Chaturvedi N, Schalkwijik C. G et al (2005) Markers of inflammation are cross-sectionally associated with microvascular complications and cardiovascular disease in type 1 diabetes - the Eurodiab Prospective Complications Study. Diabetologia 48: 370-378.
20. Giannini C, Mohn A, Chiarelli F, Kelnar C. J. H (2011) Macro-vascularangiopathy in children and adolescents with type 1 diabetes. Diabetes Metab Res Rev 27: 436-460. doi:10.1002/dmrr.1195.
21. Kuczmarski R. J, Ogden C. L, Guo S. S, Grummer-Strawn L. M, Flegal K. M, Mei Z, Wei R, Curtin L. R, Roche A. F, Johnson C. L (2002) 2000 CDC Growth Charts for the United States: methods and development. Vital Health Stat 11 (246): 1-190.
22. Silva D. A, Pelegrini A, Petroski E. L, Gaya A. C (2010) Comparison between the growth of Brazilian children and adolescents and the reference growth charts: data from a Brazilian project. J Pediatr (Rio J) 86 (2): 115-20. doi:10.2223/JPED.1975.
23. Clinical and Laboratory Standards Institute (CLSI/ NCCLS) (2008) Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture: Approved Standard - Sixth Edition. CLSI/NCCLS document H3-A6 Vol.27 N°26 (Replaces H3-A5 Vol.23 32). Wayne, PA USA:NCCLS.
24. Nascimento A. M. M. A. D, Sequeira I. J, Vasconcelos D. F, Gandolfi L, Pratesi R, Nóbrega Y.

K. M (2017) Endothelial dysfunction in children with type 1 diabetes mellitus. Arch Endocrinol Metab 26:0.doi:10.1590/2359-3997000000271.
25. World Health Organization (WHO) (2011) Global status report on non-communicable diseases in 2010. Geneva, Switzerland.
26. Creager M. A, Lüscher T. F et al (2003) Diabetes and vascular disease: pathophysiology, clinical consequences and medical therapy: party I. Circulation 108: 1527-1532. doi: 10.1161/01.CIR.0000091257.27563.32.
27. Zimmet P, Alberti K. G, Shaw J (2001) Global and societal implications of the diabetes epidemic. Nature 3: 414 (6865): 782-7. Review. doi:10.1038/414782a.
28. Mozos I, Malainer C, Horbańczuk J, Gug C, Stoian D, Luca C. T, Atanasov A. G (2017) Inflammatory Markers for Arterial Stiffness in Cardiovascular Diseases. Front Immunol 31: 8: 1058. doi:10.3389/fimmu.2017.01058.
29. Calles-Escandon J, Cipolla M (2001) Diabetes and endothelial dysfunction: a clinical perspective. Endocr Rev 22 (1): 36-52. doi:10.1210/edrv.22.1.0417.
30. de Almeida-Pititto B, Ribeiro-Filho F. F, Bittencourt M. S, Lotufo P. A, Bensenor I, Ferreira S. R (2016) Usefulness of circulating E-selectin to early detection of the atherosclerotic process in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). Diabetol Metab Syndr 3: 8: 19. doi:10.1186/s13098-016-0133-9.
31. Anderson S. S, Wu K, Nagase H, et al (1996) Effect of matrix glycation on expression of type IV collagen, MMP-2, MMP-9 and TIMP-1 by human mesangial cells. Cell Adhes Commun 4: 89-101.
32. Portik-Dobos V, Anstadt J, Hutchinson M, Bannan M et al (2002) Evidence for matrix metalloproteinase induction activation system in arterial vasculature and decreased synthesis and activity in diabetes. Diabetes 51: 3063-3068. doi:10.2337/diabetes.51.10.3063.
33. Symeonidis C, Papakonstantinou E, Galli A et al (2013) Matrix metalloproteinase (MMP-2, -9) and tissue inhibitor (TIMP-1, -2) activity in tear samples of pediatric type 1 diabetic patients. Graefes Arch Clin Exp Ophthalmol 251: 741-749. doi:10.1007/s00417-012-2221-3.
34. Thrailkill K. M, Moreau C. S, Cockrell G. E et al (2010) Disease and gender-specific dys-regulation of NGAL and MMP-9 in type 1 diabetes mellitus. Endocrine 37: 336-343. doi:10.1007/s12020-010-9308-6.
35. Lee S. W, Song K. E, Shin D. S et al (2005) Alterations in peripheral blood levels of TIMP-1, MMP-2, and MMP-9 in patients with type-2 diabetes. Diabetes Res Clin Pract 69: 175-179. doi:10.1016/j.diabres.2004.12.010.
36. Barac A, Campia U, Panza J. A (2007) Methods for evaluating endothelial function in humans. Hypertension 49: 748-760. doi:10.1161/01.HYP.0000259601.38807.a6.
37. Kilic I. D, Findikoglu G, Alihanoglu Y. I, Yildiz B. S, Uslu S, Rota S, Evrengul H (2015) Circulating adhesion molecules and arterial stiffness. Cardiovasc J Afr 26 (1): 21-4. doi:10.5830/CVJA-2014-060.
38. de Faria A. P, Ritter A. M, Sabbatini A. R, Corrêa N. B, Brunelli V, Modolo R, Moreno H (2016) Deregulation of Soluble Adhesion Molecules in Resistant Hypertension and Its Role in Cardiovascular Remodeling. Circ J. 25: 80 (5): 1196-201. doi:10.1253/circj.CJ-16-0058.
39. Kals J, Kampus P, Kals M, Pulges A, Teesalu R, Zilmer K, Kullisaar T, Salum T, Eha J, Zilmer M (2008) Inflammation and oxidative stress are associated differently with endothelial function and arterial stiffness in healthy subjects and in patients with atherosclerosis. Scand J Clin Lab Invest 68 (7): 594-601. doi:10.1080/00365510801930626.
40. Dogruel N, Kirel B, Akgün Y et al (2001) Serum soluble endothelial-cell specific adhesion molecules in children with insulin-dependent diabetes mellitus. J Pediatr Endocrinol Metab 14: 287-293.
41. Pankow J. S, Decker P. A, Berardi C, Hanson N. Q, Sale M, Tang W, Kanaya A. M, Larson N. B, Tsai M. Y, Wassel C. L, Bielinski S. J (2016) Circulating cellular adhesion molecules and risk of diabetes: the Multi-Ethnic Study of Atherosclerosis (MESA). Diabet Med 33 (7): 985-91. 85-991. doi: 10.1111/dme.13108.
42. Maggio A. B, Farpour-Lambert N. J, Montecucco F, Pelli G, Marchand L. M, Schwitzgebel V, Mach F, Aggoun Y, Beghetti M (2012) Elevated E-selectin and diastolic blood pressure in diabetic children. Eur J Clin Invest 42 (3): 303-9. doi:10.1111/j.1365-2362.2011.02583.x.
43. Velarde M. S, Carrizo D. R, Prado M. M et al (2010) Inflammation markers and endothelial dysfunction in children with type 1 diabetes. Medicina (B. Aires) 70: 44-48.
44. Kunutsor S. K, Bakker S. J. L, Dullaart R. P. F (2017) Soluble Vascular Cell Adhesion Molecules May be Protective of Future Cardiovascular Disease Risk: Findings from the Prevend Prospective Cohort Study. J AtherosclerThromb1: 24 (8): 804-818. doi:10.5551/jat.38836.
45. Snell-Bergeon J. K, West N. A, Mayer-Davis E. J et al (2010) Inflammatory markers are increased in youth with type 1 diabetes: the search case-control study. J Clin Endocrinol Metab 95 (6): 2868-2876. doi:10.1210/jc.2009-1993.
46. Rosa J. S, Oliver S. R, Pontello A. M et al (2008) Sustained IL-1α, IL-4 and IL-6 elevations following correction of hyperglycemia in children with type 1

diabetes mellitus. Pediatr Diabetes 9: 9-16. doi:10.1111/j.1399-5448.2007.00243.x.
47. Libby P, Ridker P. M, Maseri A (2002) Inflammation and atherosclerosis. Circulation 105: 1135-1143.
48. Pepys M. B (1981) C-reactive protein fifty years on. Lancet 1: 653-657.
49. Meier-Ewert H. K, Ridker P. M, Rifai N et al (2001) Absence of diurnal variation of C-reactive protein concentrations in healthy human subjects. Clin Chem 47: 426-430.
50. Sociedade Brasileira de Diabetes. Diretrizes da Sociedade Brasileira de Diabetes (2015-2016) Adolfo Milech et al, organização José Egidio Paulo de Oliveira, Sérgio Vencio - São Paulo: A.C. Farmacêutica.

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